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THE BIOSYNTHESIS OF AMINO ACIDS

Amino acids are the building blocks of proteins and the nitrogen source for many important molecules such as nucleotides, neurotransmitters, prosthetic groups like porphyrins etc. Organisms vary greatly in their ability to synthesize the 20 common aminoacids. Most bacteria and plants can synthesize all 20, mammals can synthesize only about half of them; the others are required in the diet (essential amino acids). So amino acid biosynthesis is intimately connected with nutrition.

Nitrogen is an essential component of amino acids. Nitrogen in the form of ammonia is the source of nitrogen for all the amino acids. The carbon backbones come from the glycolytic pathway, the pentose phosphate pathway, or the citric acid cycle.

An important problem that we encounter during amino acid biosynthesis is stereochemical control. Because all aminoacids except glycine are chiral, therefore biosynthetic pathways must generate the correct isomer. In each of the 19 pathways for the generation of chiral amino acids, the sterochemistry at the α-carbon atom is established by a transamination reaction that includes pyridoxal phosphate(PLP).

The biosynthetic process starts with the reduction of N_2 to NH_3 (ammonia), a process called nitrogen fixation carryout mainly by diazotropic (nitrogen-fixing) microorganisms such as Rhizobium present in the root nodules of leguminous plants. The nitrogenase complex, which carries out this fundamental transformation, consists of two proteins: a reductase, which provides electrons with high reducing power, and nitrogenase, which uses these electrons to reduce N_2 to NH_3.

In most nitrogen-fixing microorganisms, the eight high-potential electrons required for each molecule of N_2 to be reduced come from reduced ferredoxin, which inturn is generated by photosynthesis or oxidative processes. Two molecules of ATP are hydrolyzed for each electron transferred and in total atleast 16 molecules of ATP are hydrolyzed.

The overall reaction can be represented as follows,

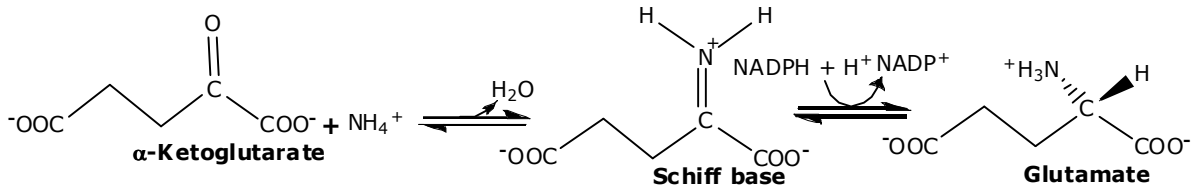
$$N_2 + 8\,e^- + 8H^+ + 16\ ATP + 16\ H_2O \longrightarrow 2NH_3 + H_2 + 16\ ADP + 16\ Pi$$

Assimilation of Ammonium into Glutamate and Glutamine

The next step in the assimilation of nitrogen in to biomolecules is the entry of NH_4^+ into amino acids. Glutamate and glutamine play important roles in this regard. The α-amino group of most amino acids comes from the α–amino group of glutamate by transamination. Glutamine contributes its side-chain nitrogen atom in the biosynthesis of amino acids tryptophan and histidine. Glutamate is synthesized from NH_4^+ and α-ketoglutarate, a citric acid cycle intermediate, by the action of glutamate dehydrogenase. NADPH is the reductant in biosynthesis.

$$NH_4^+ + \alpha\text{–Ketoglutarate} + NADPH + H^+ \rightleftharpoons \text{Glutamate} + NADP^+ + H_2O$$

This reaction proceeds in two steps. First, a shiff base forms betweem ammonia and α-ketoglutarate . The formation of a schiff base between an amine and a carbonyl compound is a key reaction that takes place at many stages of amino acid biosynthesis and degradation. Schiff bases are easily protonated. In the second step, the protonated schiff base is reduced by the transfer of a hydride ion from NADPH to form glutamate.



This reaction is crucial because it establishes the stereochemistry of the α-carbon atom in glutamate. The enzyme binds the α-ketoglutarate substrate in such a way that hydride transferred from NADPH is added to form the L isomer of glutamate. This stereochemistry is established for other aminoacids by transamination reactions that depend on pyridoxal phosphate.

A second ammonium ion is incorporated into glutamate to form the glutamine by the action of glutamine synthetase. ATP participates directly in the reaction by phosphorylating the side chain of glutamate to form an acyl- phosphate intermediate, which then reacts with ammonia to form glutamine.

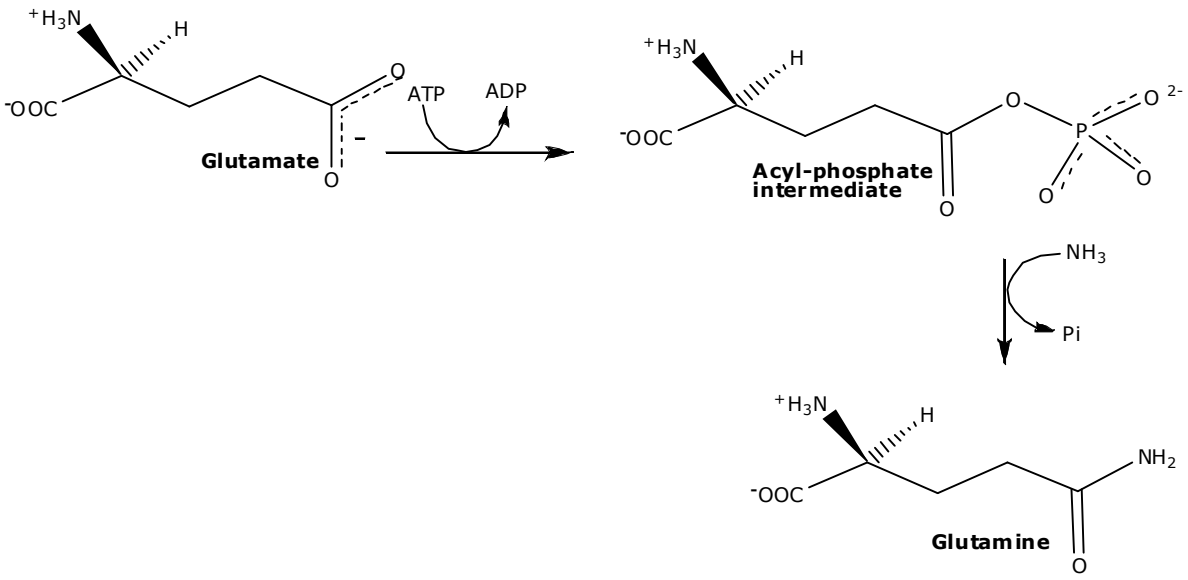
The regulation of glutamine synthetase plays a critical role in controlling nitrogen metabolism. Glutamate dehydrogenase and glutamine synthetase are present in all organisms. Most prokaryotes also contain an evolutionarily unrelated enzyme, glutamate synthase, which catalyzes the reductive amination of α-keto glutarate to glutamate. Glutamine is the nitrogen donor.

α-Ketoglutarate + glutamine + NADPH + H^+ ⇌ 2 Glutamate + $NADP^+$

Amino Acids are Made from Intermediates of the Citric Acid Cycle and Other Major Pathways

Based on the starting materials from which the carbon skeletons are derived, aminoacids can be grouped into six biosynthetic families in bacteria and plants.

Human beings cannot make 9 out of 20 amino acids. The amino acids that must be supplied in the diet are called essential amino acids, whereas the others are termed nonessential amino acids.



The non-essential aminoacids are synthesized by quite simple reactions most often in a single step, where as the pathways for the formation of the essential aminoacids are quite complex and require 5 to 16 steps.

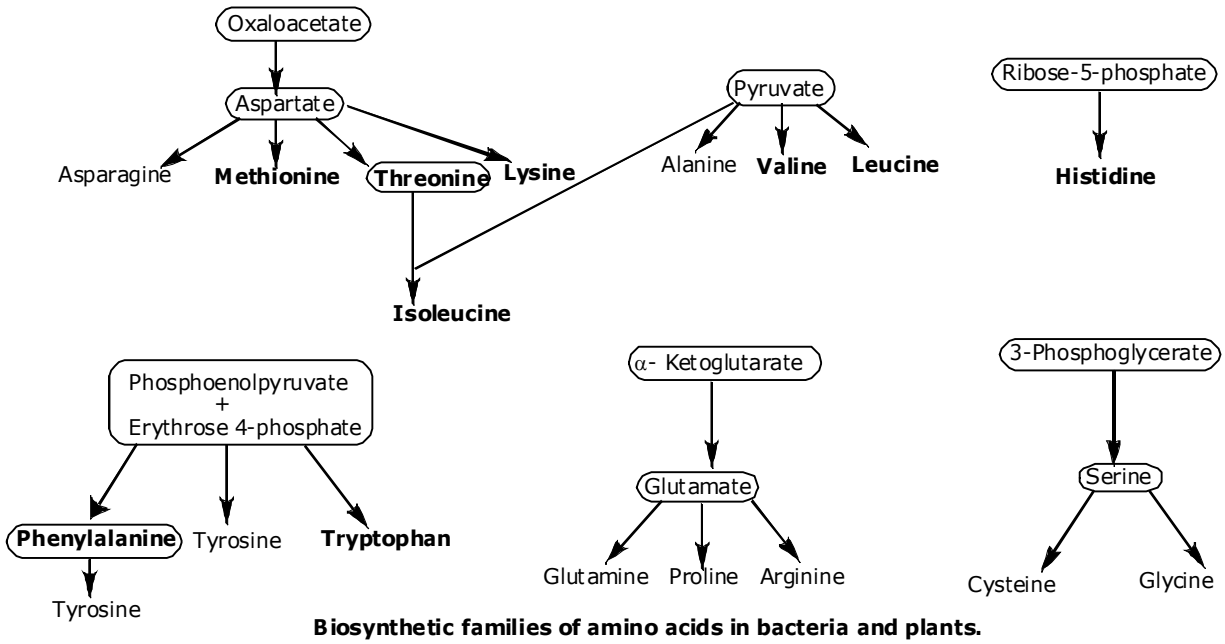
4.1.1 Biosynthesis of Non essential Aminoacids

Synthesis of Aspartate, Alanine and Glutamate

Three α-ketoacids, α-ketoglutarate, oxaloacetate and pyruvate can be converted into amino acids such as glutamate, aspartate and alanine respectively in one step through the addition of an aminogroup. we have already seen that α-ketoglutarate can be converted into glutamate by reductive amination. The aminogroup from glutamate can be transferred to oxaloacetate and pyruvate by transamination reactions to form aspartate and alanine respectively.

These reactions are carried out by pyridoxal phosphate-dependent transaminases. Most amino acids are synthesized by transamination reactions.

The reaction pathway begins with pyridoxal phosphate in a schiff-base linkage with lysine at the transaminase active site, forming an internal aldimine. An amino group is transferred from glutamate to form pyridoxamine phosphate(PMP), the actual amino donor, in a multistep process. Pyridoxamine phosphate thEn reacts with an incoming α-ketoacid to form a ketamine. Proton loss forms a quinonoid intermediate that then accepts a proton at a different site to form an external aldimine. The chirality of the amino acid formed is determined by the direction from which this proton is added to the quinonoid form. The newly formed amino acid is released with the concomitant formation of the internal aldimine.



Biosynthetic families of amino acids in bacteria and plants.

The formation of Aspargine from Aspartate through an adenylated Intermediate

The formation of asparagine from aspartate is chemically analogous to the formation of glutamine from glutamate. Both transformations are amidation reactions and both are driven by the hydrolysis of ATP.

Oxaloacetate + glutamate $\rightleftharpoons$ aspartate + α-ketoglutarate

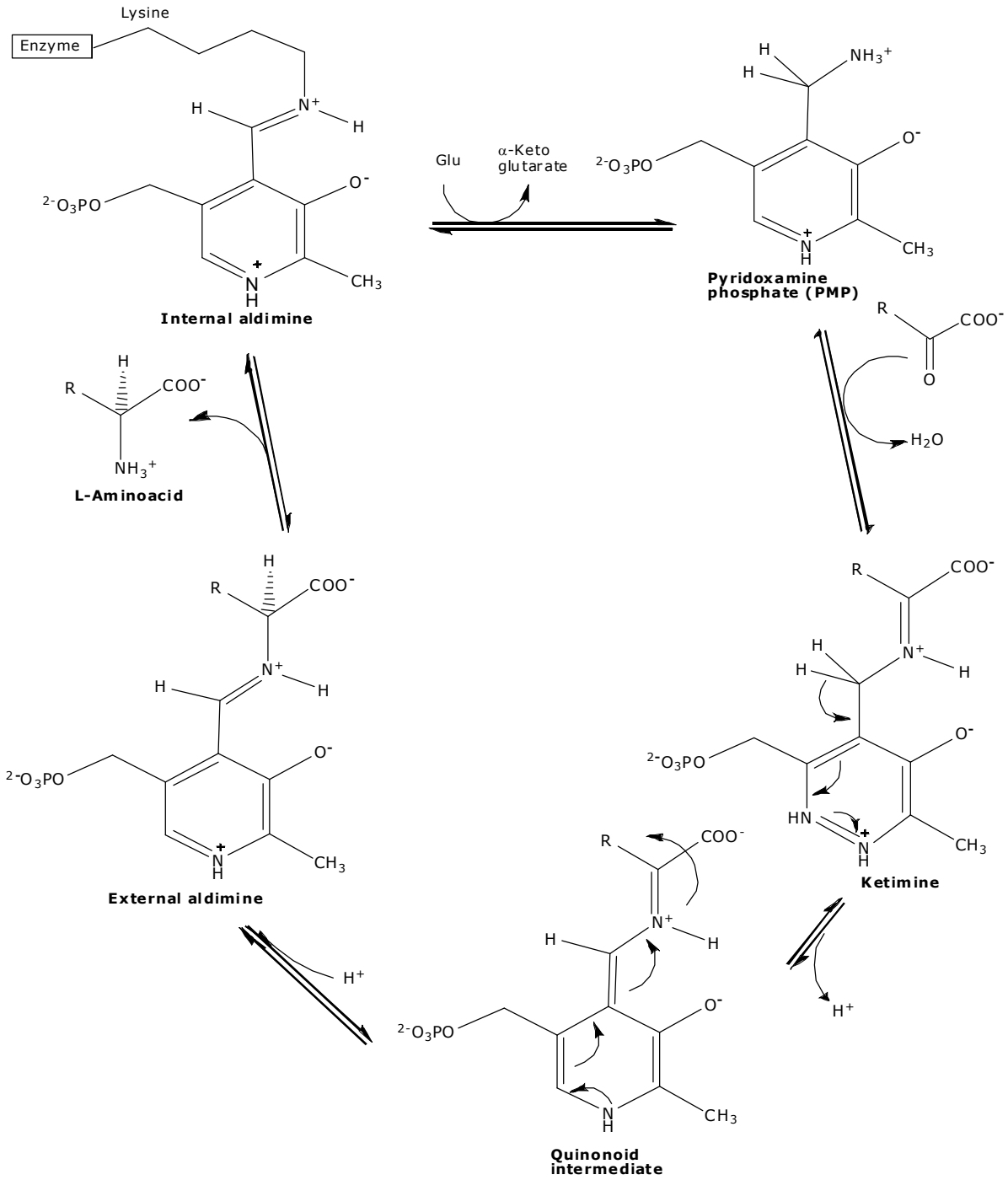
Pyruvate +glutamate $\rightleftharpoons$ alanine +α–ketoglutarate

In bacteria, the reaction for the asparagine synthesis is

$$\text{Aspartate} + NH_4^+ + ATP \longrightarrow \text{asparagine} + AMP + PP_i + H^+$$

Aspartate is activated by adenylation rather than by phosphorylation. The intermediate formed in this reaction is acyl-adenylate intermediate.

In mammals, the nitrogen donor for asparagine is glutamine rather than ammonia as in bacteria. Ammonia is generated by hydrolysis of the side chain of glutamine and directly transferred to activated aspartate, bound in the active site. An advantage is that the cell is not directly exposed to NH_4^+, which is toxic at high levels to human beings and other mammals.

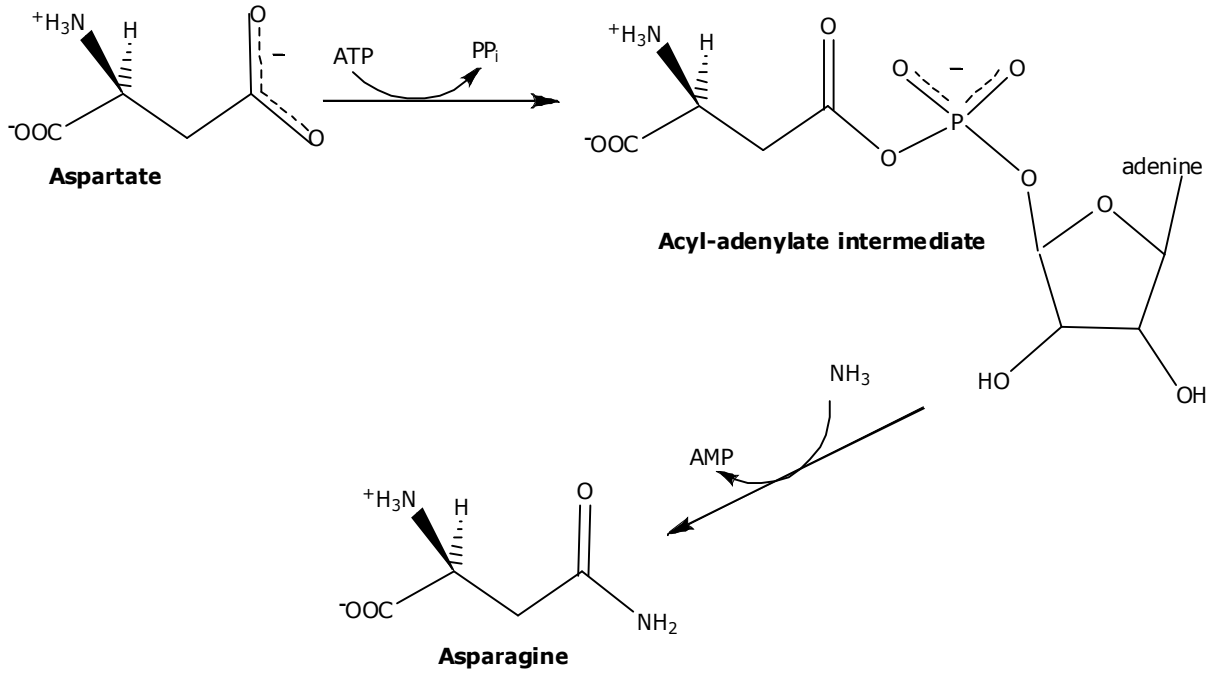


Chirality determining steps of amino acid biosynthesis

Glutamate is the Precursor of Glutamine, Proline and Arginine.

The conversion of glutamate into glutamine has already been discussed. Glutamate is the precursor of two other nonessential amino acids-proline and arginine. The γ-carboxyl group of glutamate reacts with ATP to form an acyl phosphate. This mixed anhydride is then reduced by NADPH to an aldehyde known as glutamic γ-semialdehyde.

Glutamic γ-semialdehyde cyclizes with a Loss of H_2O in a nonenzymatic process to give Δ^1-pyrroline-5-carboxylate, which is reduced by NADPH to proline. Alternatively, the semialdehyde can be transaminated to ornithine, which is converted into arginine by urea cycle.

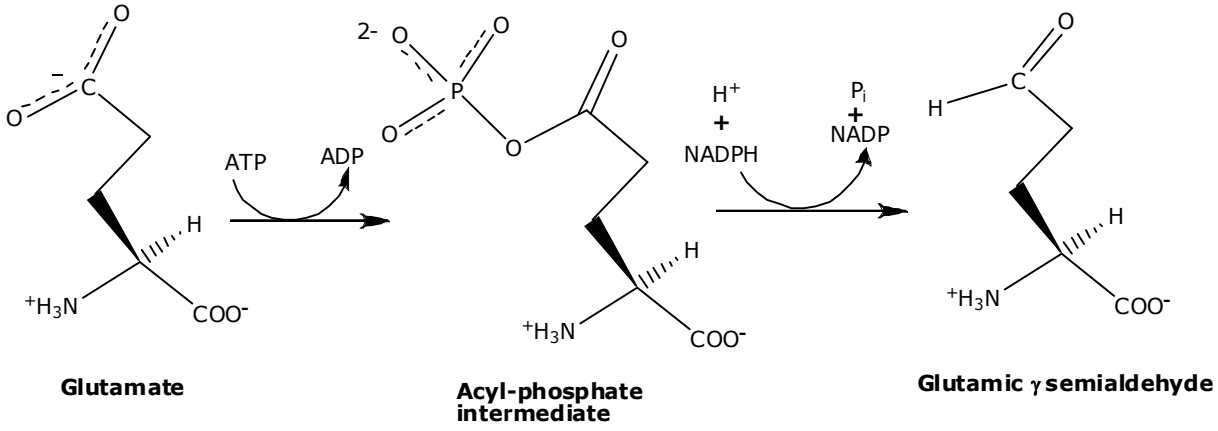


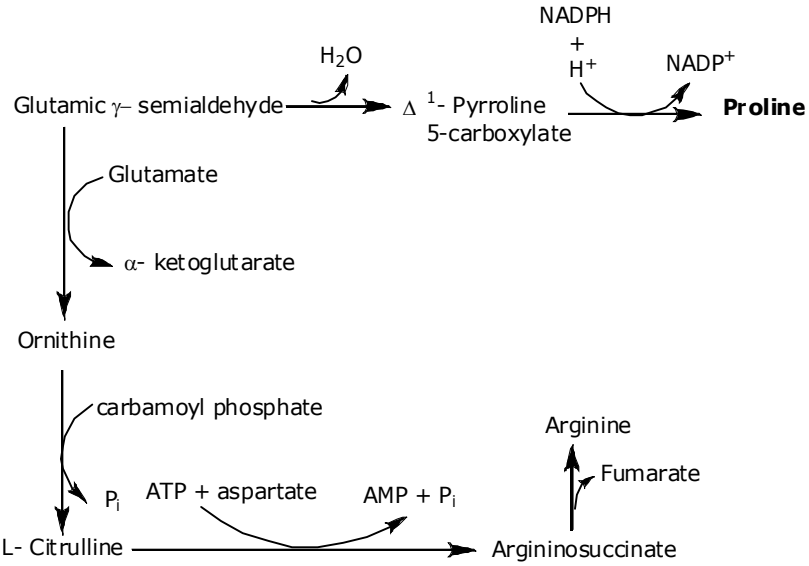
Synthesis of Serine , Cysteine and Glycine from the Precursor 3- Phosphoglycerate

Serine is synthesized from 3- phosphoglycerate, an intermediate in glycolysis. In the first step, the hydroxyl group of 3- phosphoglycerate is oxidized to yield 3-phosphohydroxy pyruvate. This α-ketoacid is transaminated to 3- phosphoserine, which is then hydrolyzed to serine.

Serine is the precursor of glycine and cysteine. In the formation of glycine, the side- chain methylene group (C-3) of serine is transferred to tetrahydrofolate (a carrier of one-carbon units), which forms a methylene bridge between N-5 and N-10 to yield N^5,N^{10}-methylenetetrahydrofolate. This interconversion is catalyzed by an enzyme serine hydroxymethyltransferase, which requires pyridoxal phosphate.

In the synthesis of Cysteine; serine and homocysteine condense to form cystathionine, which is then deaminated and cleaved to cysteine and α-ketobutyrate;Sulfur atom of cysteine is derived from homocysteine , whereas the carbon skeleton comes from serine.



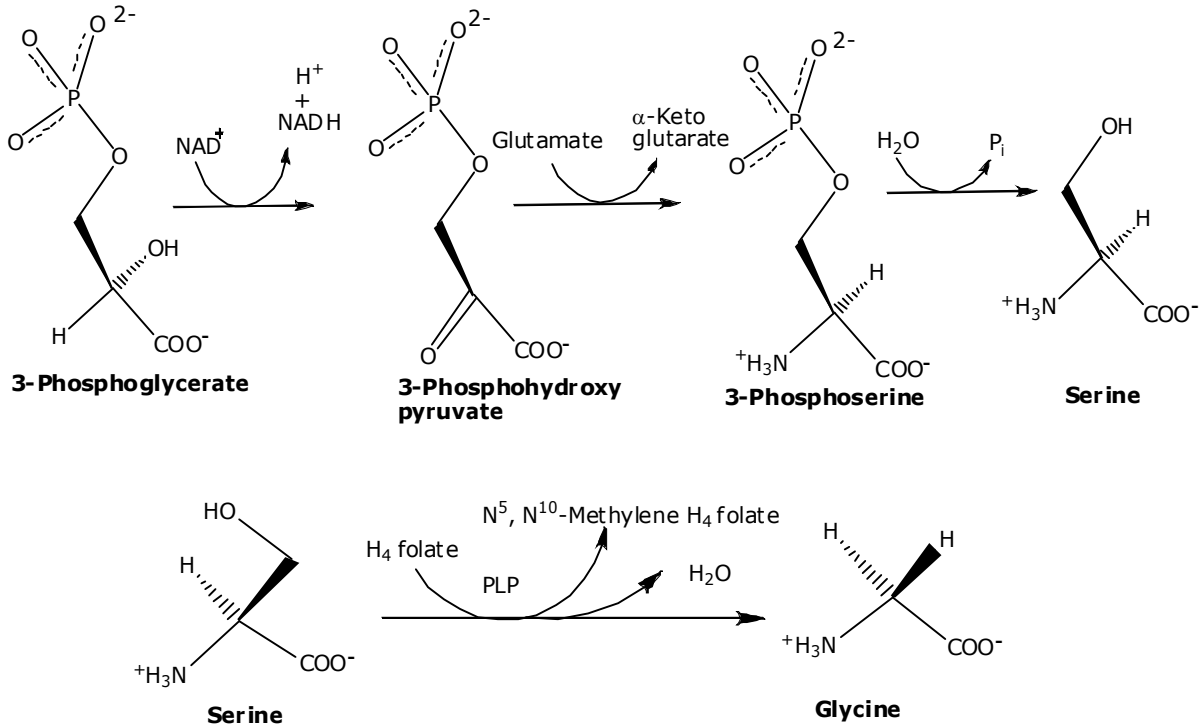


4.1.2 Biosynthesis of Essential Amino Acids

Synthesis of Aromatic amino acids

Phenylalanine, tyrosine and tryptophan are aromatic amino acids. They are synthesized by plants and microorganisms, and those in the human diet are ultimately derived primarily from plants. Tyrosine is classified as a nonessential amino acid, which can be synthesized in one step from phenylalanine. But it becomes essential if phenylalanine is not abundant in human beings

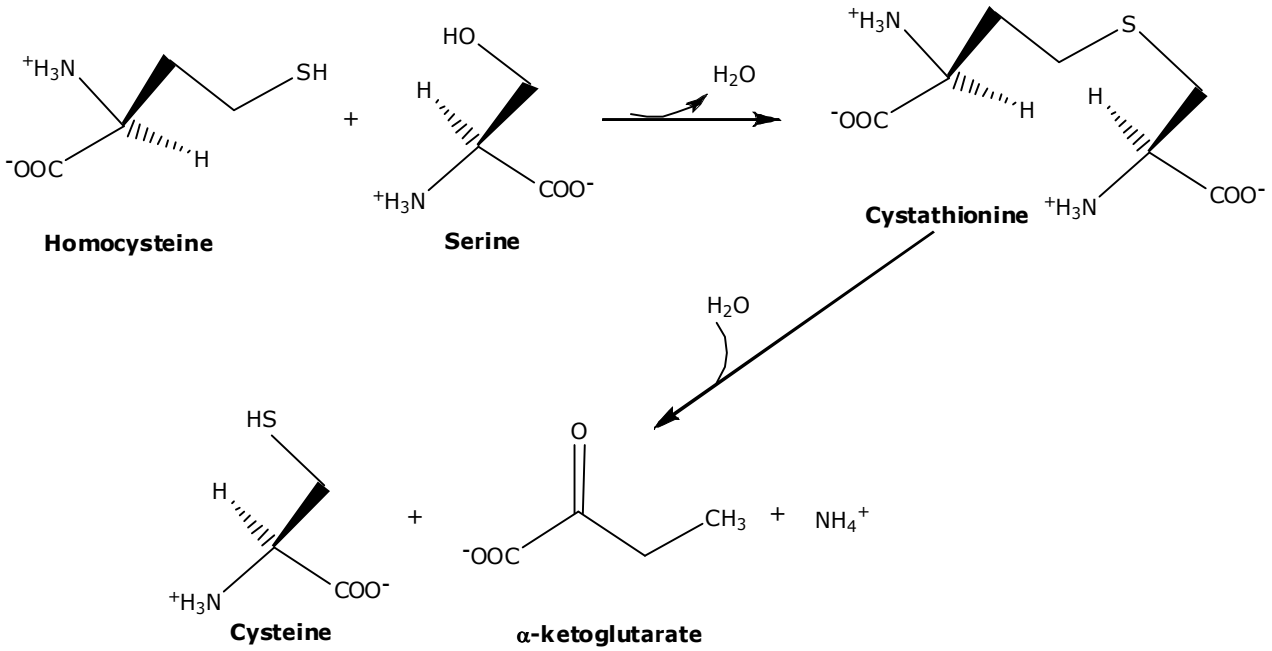
The pathways for the synthesis of aromatic amino acids in bacteria have been selected for discussion here because they are well understood. Aromatic aminoacids are synthesized by a common pathway in *E.coli.* The initial step is the condensation of phospho-enolpyruvate (a glycolytic intermediate) with erythrose 4- phosphate (a pentose phosphate pathway intermediate). The resulting seven carbon open-chain sugar is oxidized, loses its phosphoryl group, and cyclizes to 3-dehydroquinate. Dehydration then yields 3-dehydroshikimate, which is reduced by NADPH to shikimate. The phosphorylation of shikimate by ATP gives shikimate 3-phosphate, which condenses with a second molecule of phospho-enolpyruvate. The resulting 5-enolpyruvyl shikimate 3-phosphate loses its phosphoryl group, yielding chorismate, the common precursor of all three aromatic amino acids.



The pathway for the synthesis of aromatic aminoacids divides at chorismate into prephenate branch and anthranilate branch. In the starting reaction of prephenate branch; a mutase converts chorismate into prephenate , the immediate precursor of the aromatic ring of phenylalanine and tyrosine. Dehydration and decarboxylation of prephenate yield phenylpyruvate. Alternatively,prephenate can be oxidatively decarboxylated to p-hydroxyphenylpyruvate. These two α-ketoacids are then transaminated to form phenylalanine and tyrosine respectively.

Animals can produce tyrosine directly from phenylalanine through hydroxylation at C-4 of the phenyl group by phenylalanine hydroxylase, which requires the co-factor tetrahydrobiopterin to carry electrons from NADH to O_2.

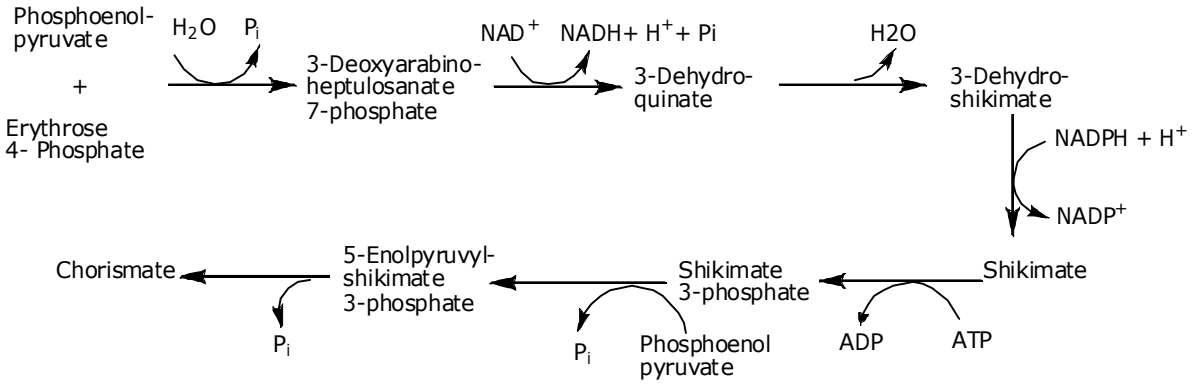
In the starting reaction of anthranilate branch for the synthesis of tryptophan, chorismate acquires an amino group derived from the hydrolysis of the side chain of glutamine and releases pyruvate to form anthranilate. Then anthranilate condenses with 5-phosphoribosyl-1-pyrophosphate (PRPP), an activated form of ribose 5-phosphate becomes bonded to the nitrogen atom of anthranilate in a reaction that is driven by the release and hydrolysis of pyrophosphate. The ribose moiety of phospho- ribosyl anthranilate undergoes rearrangement to yield 1-(*o*-carboxyphenylamino)-1-deoxyribulose 5-phosphate. This is dehydrated and then decarboxylated to indole-3-glycerol phosphate, which is cleaved to indole. Then indole reacts with serine to form tryptophan. In the above mentioned last two steps, the side reaction of indole-3-glycerol phosphate is removed as glyceraldehyde-3-phosphate and replaced by the carbon skeleton of serine.



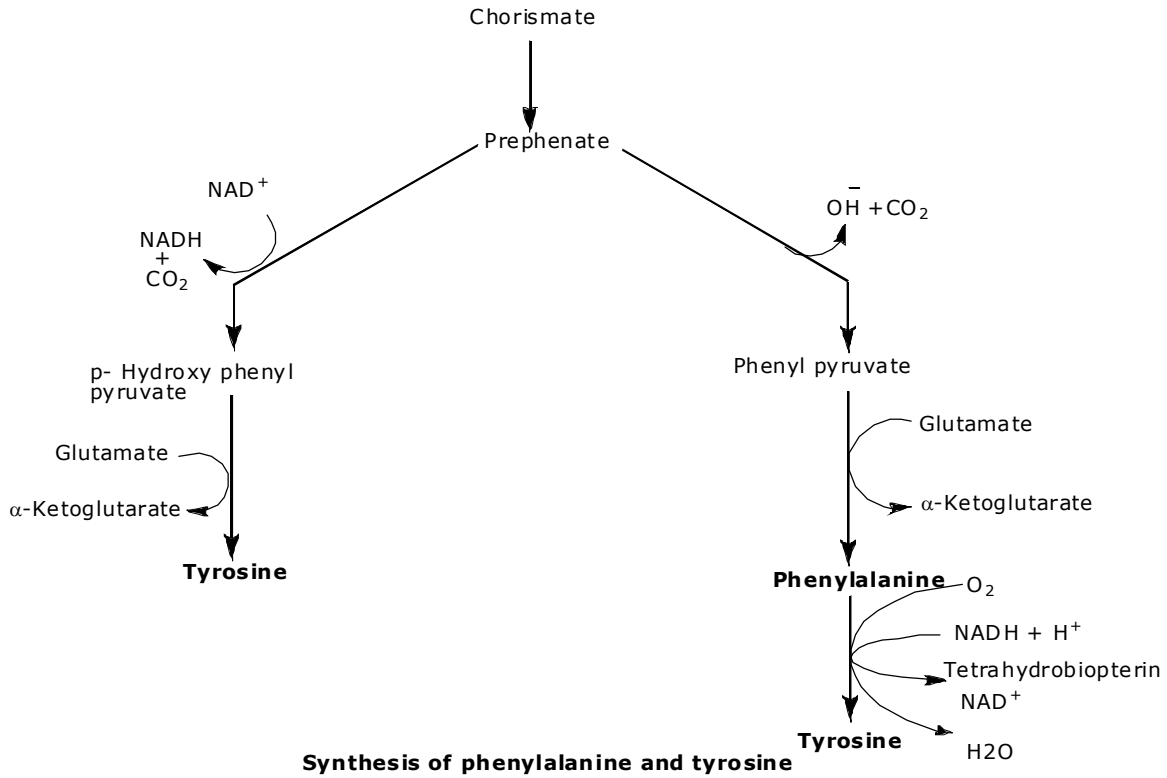
Synthesis of Methionine, Lysine, Threonine and Isoleucine from Aspartate

Their biosynthetic pathways are complex and interconnected. Aspartate gives rise to methionine, threonine, and lysine. Branch points occur at aspartate β-semialdehyde, an intermediate in all three pathways, and also at homoserine, a precursor of threonine and methionine. Threonine and pyruvate are the precursors of isoleucine.

The pathway for the synthesis of isoleucine begins with the condensation of two carbons of pyruvate (in the form of hydroxy ethyl thiamine pyrophosphate) with α-ketobutyrate derived from threonine in a reaction that requires pyridoxal phosphate, the prosthetic group of amino transferases.



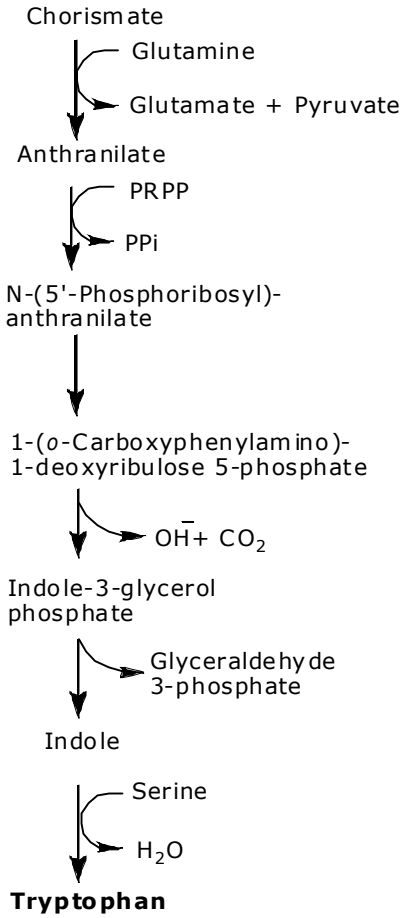
Pathway to chorismate



Synthesis of phenylalanine and tyrosine

Synthesis of Valine and Leucine from Pyruvate

Synthesis of valine begins with condensation of two carbons of pyruvate (in the form of hydroxyethyl thiamine pyrophosphate) with another molecule of pyruvate. An intermediate in the valine pathway, α-ketoisovalerate, is the starting point for a four-step branch pathway leading to the synthesis of leucine.



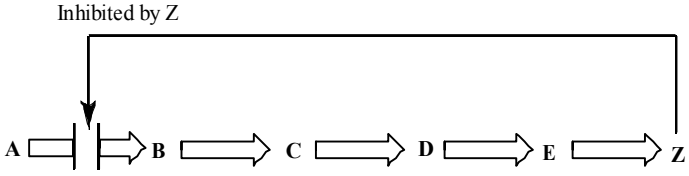
Synthesis of tryptophan

Synthesis of Histidine from Ribose 5 phosphate

Histidine is derived from three precursors: 5-phosphoribosyl-1-pyrophosphate (PRPP) contributes five carbons, the purine ring of ATP contributes a nitrogen and a carbon, and glutamine supplies the second ring nitrogen. PRPP inturn is synthesized from ribose-5 phosphate (derived from the pentose phosphate pathway), in a reaction catalyzed by ribose phosphate pyrophosphokinase. The key steps in the biosynthesis of histidine are condensation of ATP and PRPP, purine ring opening and formation of the imidazole ring, a reaction in which glutamine donates a nitrogen. The remnant of ATP, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) formed during this reaction is recycled to purine biosynthesis.

4.1.3 Regulation of AminoAcid Biosynthesis

The rate of synthesis of amino acids depends mainly on the amountS of the biosynthetic enzymes and on their activities. In a biosynthetic pathway, the enzyme that catalyzes the first irreversible reaction, called the committed step (A $\rightarrow$ B) is often allosterically inhibited by the final product of the pathway (Z). This kind of control is known as feed back inhibition and is essential for the conservation of building blocks and metabolic energy.

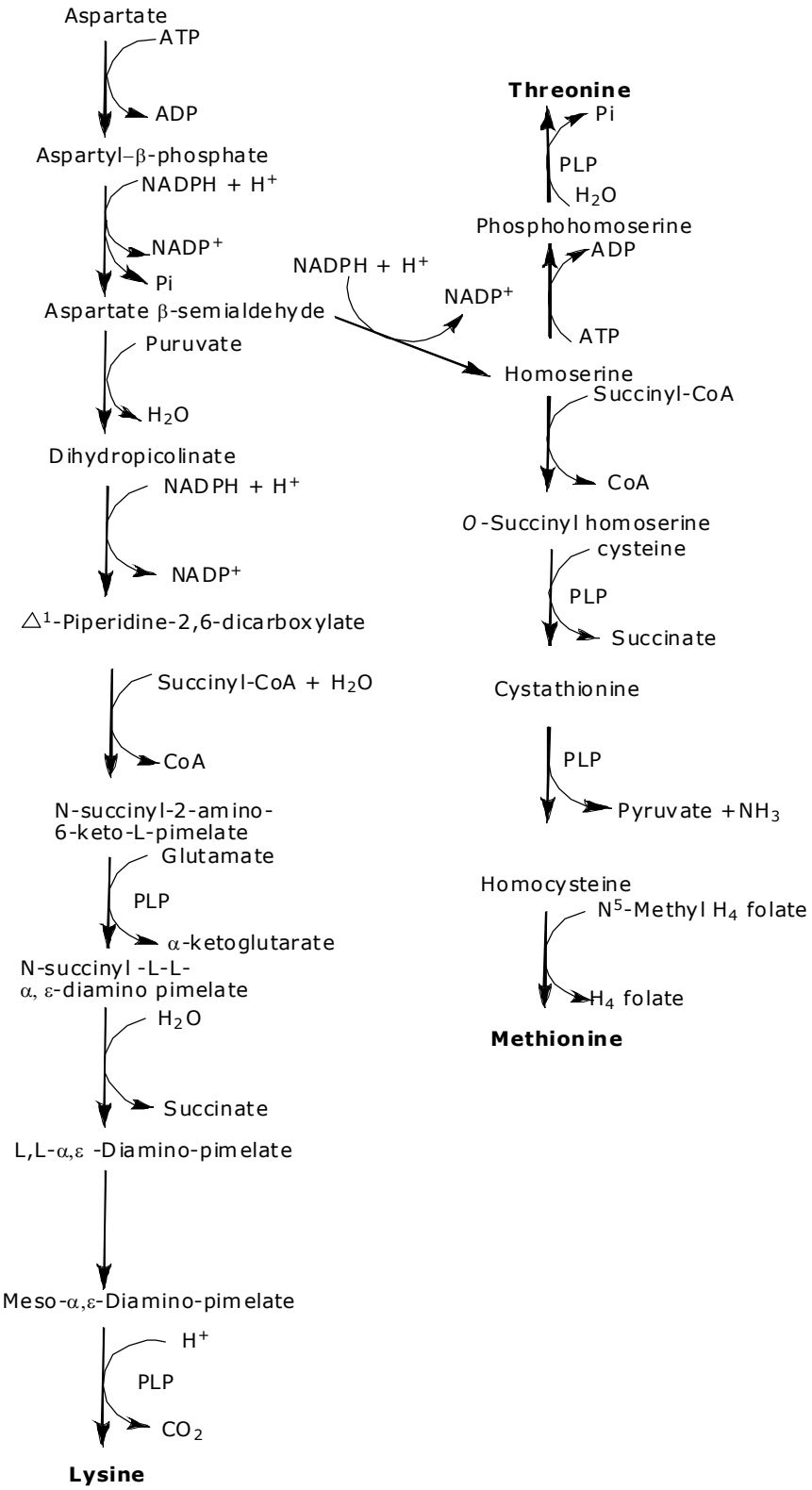


The comitted step in the biosynthetic pathway of serine is the oxidation of 3-phospho glycerate, catalyzed by the enzyme 3- phosphoglycerate dehydrogenase. The E.coli enzyme is a tetramer of four identical subunits, each comprising a catalytic domain and a serine binding regulatory domain. The binding of serine to a regulatory site reduces the value of V_{max} for the enzyme. So an enzyme bound to four molecules of serine is essentially inactive.Thus, if serine is abundant in the cell, the enzyme activity is inhibited.

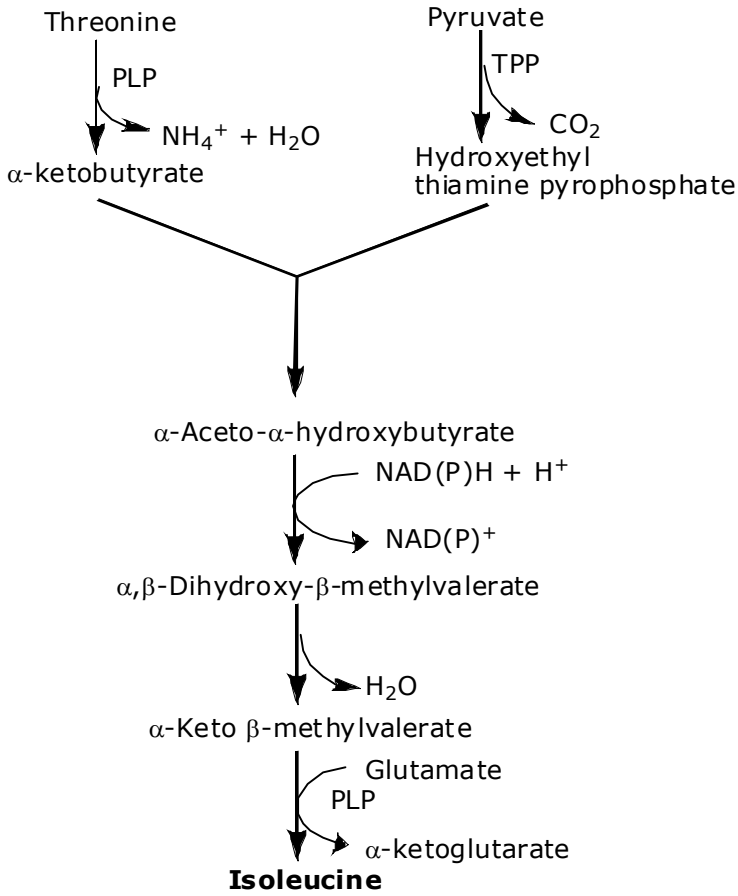
Regulation of Branched Pathways

(1) Feed Back Inhibition and Activation

Two pathways with a common initial step may each be inhibited by its own product and activated by the product of the other pathway. The biosynthesis of the aminoacids valine, leucine and isoleucine is an example for feed back inhibition and activation. Hydroxy ethyl thiamine pyrophosphate is the common intermediate for the synthesis of all three of these aminoacids. Hydroxyethyl-TPP reacts with α ketobutyrate in the initial step for the synthesis of isoleucine. Alternatively, hydroxyethyl-TPP reacts with pyruvate in the committed step for the pathways leading to valine and leucine. Threonine deaminase, the PLP enzyme that catalyzes the formation of α–ketobutyrate, is allosterically inhibited by isoleucine and is also allosterically activated by valine. This mechanism balances the amounts of different amino acids that are synthesized



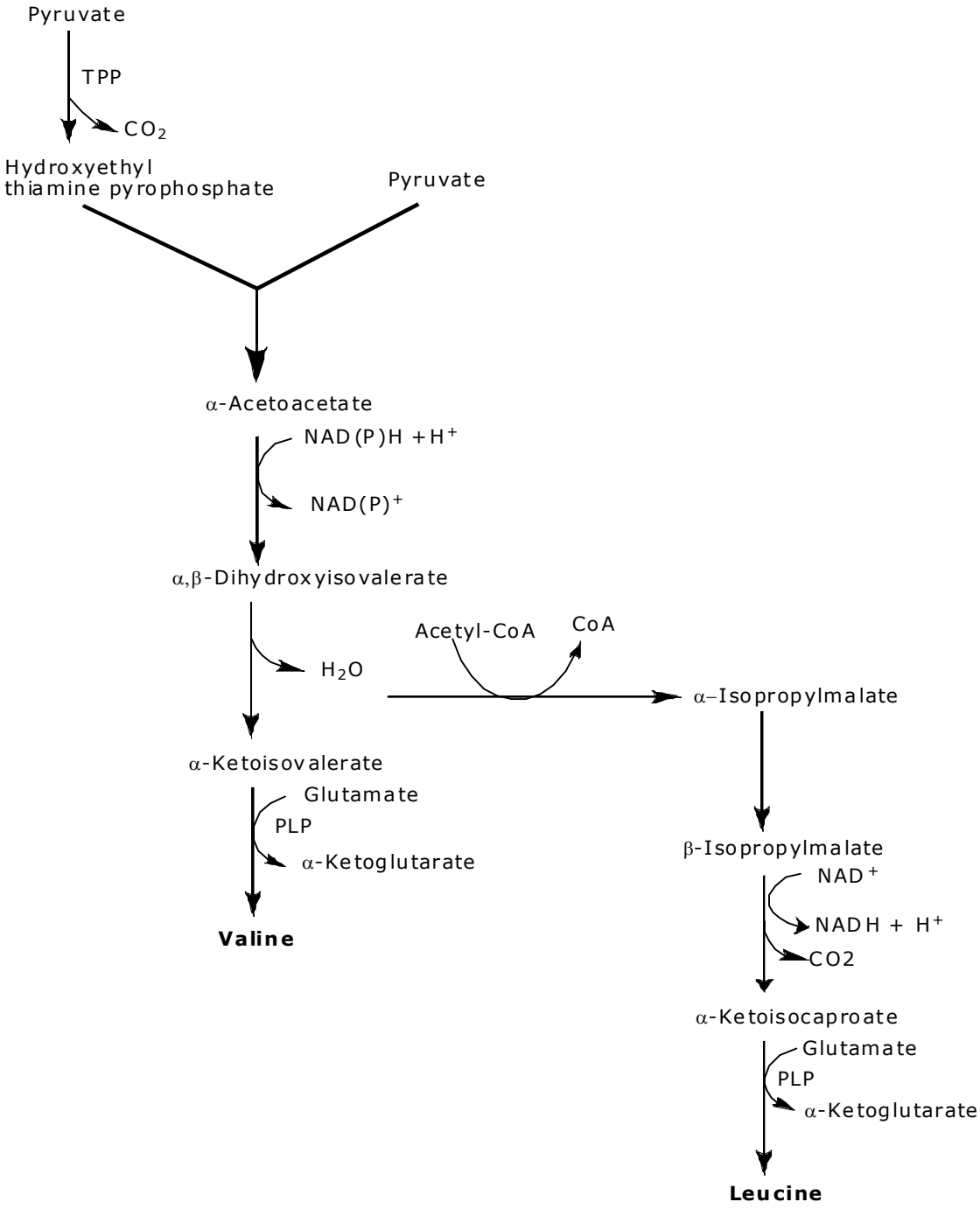
Synthesis of methionine, lysine and threonine



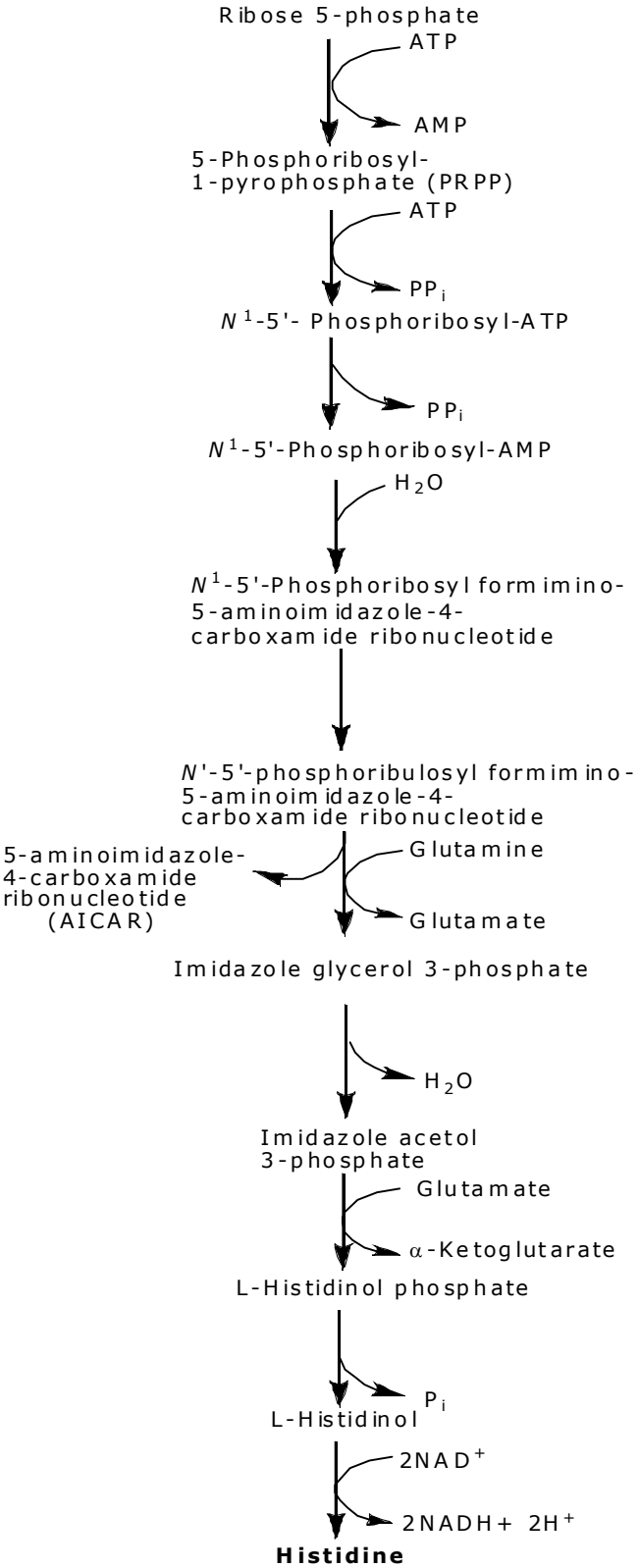
Synthesis of isoleucine

(2) Enzyme Multiplicity

The committed step can be catalyzed by two or more enzymes with different regulatory properties. For example, the phosphorylation of aspartate is the committed step in the biosynthesis of threonine, methionine, and lysine. Three distinct aspartokinases catalyze this reaction in E.coli. The catalytic domains of these enzymes show approximately 30% sequence identity. Although the mechanisms of catalysis are essentially identical, their activities are regulated differently: one enzyme is not subject to feedback inhibition, another is inhibited by threonine, and the third is inhibited by lysine.



Synthesis of valine and leucine



Synthesis of histidine

(3)Cumulative Feedback Inhibition.

A common step is partly inhibited by each of the final products, acting independently. The regulation of glutamine synthetase in E.coli is a striking example of cumulative feedback inhibition. Glutamine synthetase consists of 12 identical 50-kd subunits arranged in two hexagonal rings that face each other. The amide group of glutamine is a source of nitrogen in the biosynthesis of a variety of compounds., such as tryptophan, histidine, carbamoyl phosphate, glucosamine 6-phosphate, cytidine triphosphate and adenosine monophosphate. Glutamine synthetase is cumulatively inhibited by each of these final products of glutamine metabolism, as well as by alanine and glycine. In cumulative inhibition, each inhibitior can reduce the activity of the enzyme, even when other inhibitors are bound at saturating levels. The enzymatic activity of glutamine synthetase is switched off almost completely when all final products are bound to the enzyme.

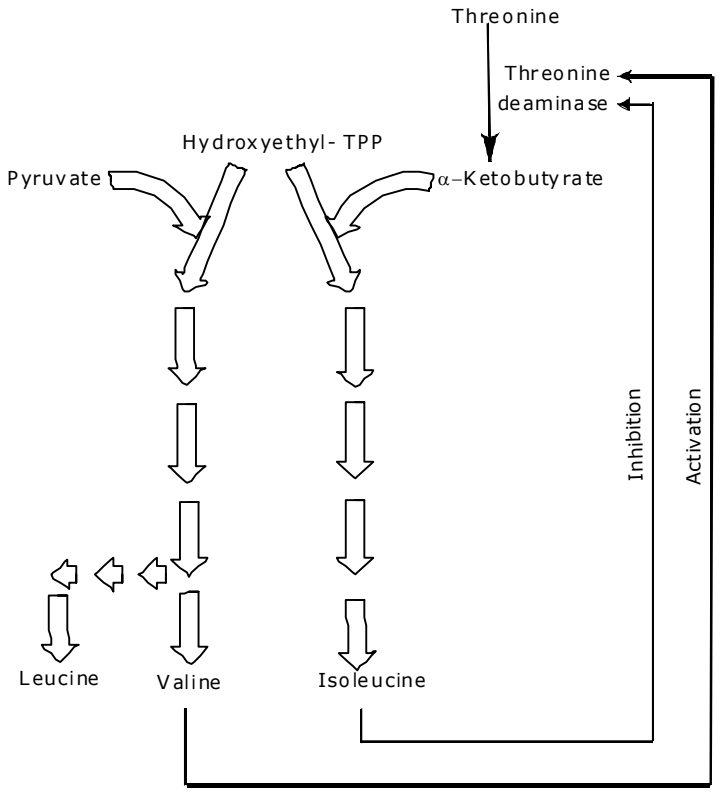
4.2 AMINO ACID DEGRADATION

Amino acids in excess of those needed for biosynthesis can neither stored nor excreted, rather used as metabolic fuel. The α-amino group is removed , and the resulting carbon skeleton is converted into a major metabolic intermediate. Most of the aminogroups removed from exess amino acids are converted into urea through the urea cycle, whereas their carbon skeletons are transformed into acetyl CoA , pyruvate, or one of the intermediates of the citric acid cycle. The principal fate of carbon skeletons is conversion into glucose and glycogen. The major site of amino acid degradation in mammals is the liver, although muscles readily degrade the branched-chain amino acids (Leucine, and Isoleucine, Valine)

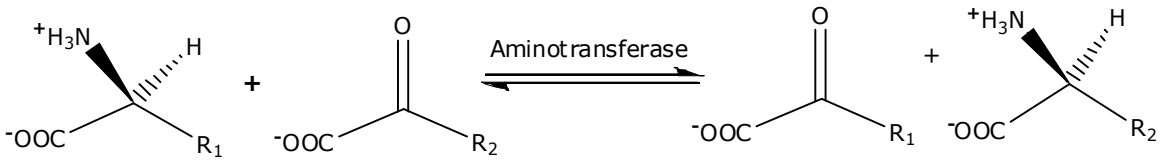
4.2.1 Removal of Alpha Amino Group is the First step in Aminoacid Degradation

Alpha-Amino Groups Are Converted into Ammonium Ions by the Oxidative Deamination of Glutamate.

The α amino group of many amino acids is transferred to α- ketoglutarate to form glutamate, which is then oxidatively deaminated to yield ammonium ion (NH_4^+) Aminotransferases, also called transaminases are the enzymes which generally catalyze the transfer of α-aminogroups from a variety of amino acids to α-ketoglutarate, an α-ketoacid, for conversion into NH_4^+.



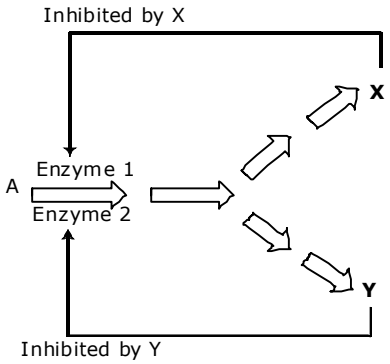
Regulation ot threonine deaminase



For example, Aspartate aminotransferase, one of the most important of aminotransferases, catalyzes the transfer of the amino group of aspartate to α-ketoglutarate.

Aspartate + α-ketoglutarate $\rightleftharpoons$ oxaloacetate + glutamate

The nitrogen atom in glutamate is converted into free ammonium ion by oxidative deamination. This reaction is catalyzed by glutamate dehydrogenase, which can utilize either NAD^+ or $NADP^+$, at least in some species. The reaction proceeds by dehydrogenation of the C-N bond, followed by hydrolysis of the resulting schiff base.



Glutamate dehydrogenase is located in mitochondria, as are some of the other enzymes required for the production of urea. This compartmentalization sequesters free ammonium ion, which is toxic. In most terrestrial vertebrates, NH_4^+ is converted into urea, which is excreted.

All aminotransferases contain the prosthetic group pyridoxal phosphate (PLP), which is derived from pyridoxine (Vitamine B_6) .The reactions catalyzed by PLP enzymes other than transamination are decarboxylations, deaminations, raecemizations, aldol cleavage, etc. Three common features of PLP catalysis are

(1) A schiff base is formed by the amino acid substrate and PLP
(2) The protonated form of PLP acts as an electron sink to stabilize catalytic intermediates that are negatively charged. Electrons from these intermediates are attracted to the positive charge on the ring nitrogen atom of PLP.
(3) The product schiff base is cleaved at the completion of the reaction.

Serine and Threonine Can Be Directly Deaminated.

The α-amino groups of serine and threonine can be directly converted into NH_4^+, without first being transferred to α-ketoglutarate. These direct deaminations are catalyzed by serine dehydratase and threonine dehydratase, in which PLP is the prosthetic group.

Serine $\longrightarrow$ pyruvate + NH_4^+

Threonine $\longrightarrow$ α-ketobutyrate + NH_4^+

These enzymes are called dehydratases because dehydration precedes deamination. The presence of a hydroxyl group attached to the β-carbon atom in Serine and Threonine permits the direct deamination.

Peripheral Tissues Transport Nitrogen to the Liver

Amino acid degradation is also takes place in tissues other than liver. For instance, muscle uses branched-chain amino acids as a source of fuel during prolonged exercise and fasting. As

in the liver, the first step is the removal of the nitrogen from the amino acid. Muscle lacks the enzymes of the urea cycle. So the nitrogen must be released in a form that can be absorbed by the liver and converted into urea.

Nitrogen is transported from muscle to the liver in two principal trnasport forms-as alanine and as glutamine. During the transport of nitrogen as alanine, glutamate is formed by transamination reactions. But the nitrogen is then transferred to pyruvate to form alanine, which is released into the blood. The liver takes up the alanine and converts it back into puruvate by transamination. The pyruvate can be used for gluconeogenesis and the amino group is converted into urea. This transport is referred to as the glucose-alanine cycle.

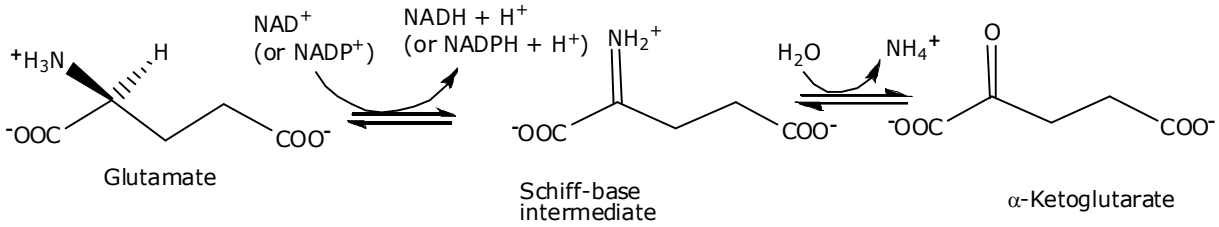
During the transport of nitrogen as glutamine, glutamine synthetase catalyzes the synthesis of glutamine from glutamate and NH_4^+ in an ATP-dependent reaction.

$$NH_4^+ + \text{glutamate} + ATP \xrightarrow{\text{Glutamine Synthetase}} \text{glutamine} + ADP + P_i$$

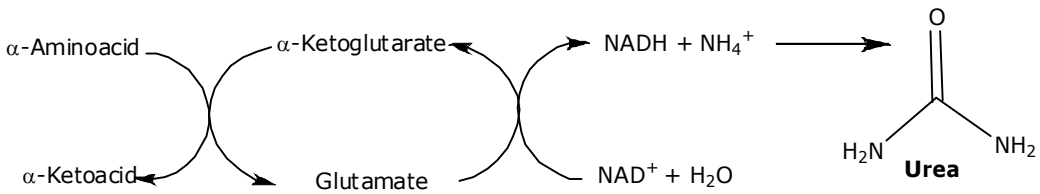
The nitrogens of glutamine can be converted into urea in the liver.

Conversion of Ammonium Ion into Urea

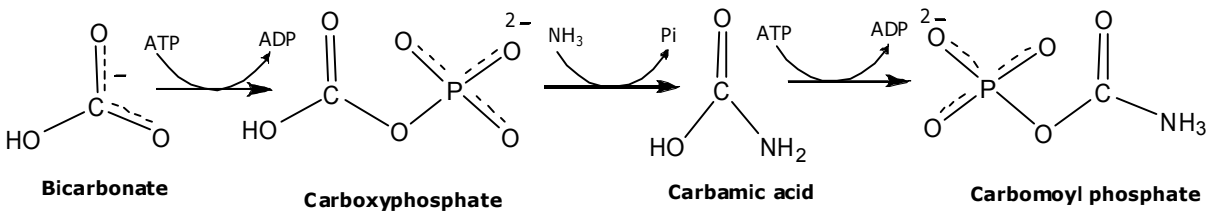
In most terrestrial vertebrates, the exess $NH4^+$ is converted into urea and then excreated . They are referred to as ureotelic. In these organisms, urea is synthesized by the urea cycle.The urea cycle, proposed by Hans Krebs and Kurt Henseleit in 1932, was the first cyclic metabolic pathway to be discovered. One of the nitrogen atoms of urea is transferred from an aminoacid, aspartate. The other nitrogen atom is derived directly from free NH_4^+ and the carbon atom comes from HCO_3^-.



The Urea Cycle Begins with the Formation of Carbamoyl Phosphate



The urea cycle begins with the coupling of free NH_3 with HCO_3^- to form carbamoyl phosphate catalyzed by carbamoyl phosphate synthetase. The synthesis of carbamoyl phosphate begins with the phosphorylation of HCO_3^- to form carboxyphosphate, which then reacts with NH_3 to form carbamic acid. A second molecule of ATP phosphorylates carbamic acid to form carbamoyl phosphate.



The carbamoyl group of carbamoyl phosphate is transferred to ornithine to form citrulline, in a reaction catalyzed by ornithine transcarbamoylase. Ornithine and citrulline are amino acids, but they are not used as building blocks of proteins. The formation of NH_4^+ by glutamate dehydrogenase, its incorporation into carbamoyl phosphate as NH_3, and the subsequent synthesis of citrulline take place in the mitochondrial matrix.

Citrulline is transported to the cytoplasm where it condenses with aspartate to form argininosuccinate catalyzed by argininosuccinate synthetase. This reaction is driven by the cleavage of ATP into AMP and pyrophosphate. Argininosuccinase cleaves argininosuccinate into arginine and fumarate. Finally, arginine is hydrolyzed to generate urea and ornithine in a reaction catalyzed by arginase. Ornithine is then transported back into the mitochondrion to begin another cycle. The urea is excreted.

The overall reaction of urea synthesis is

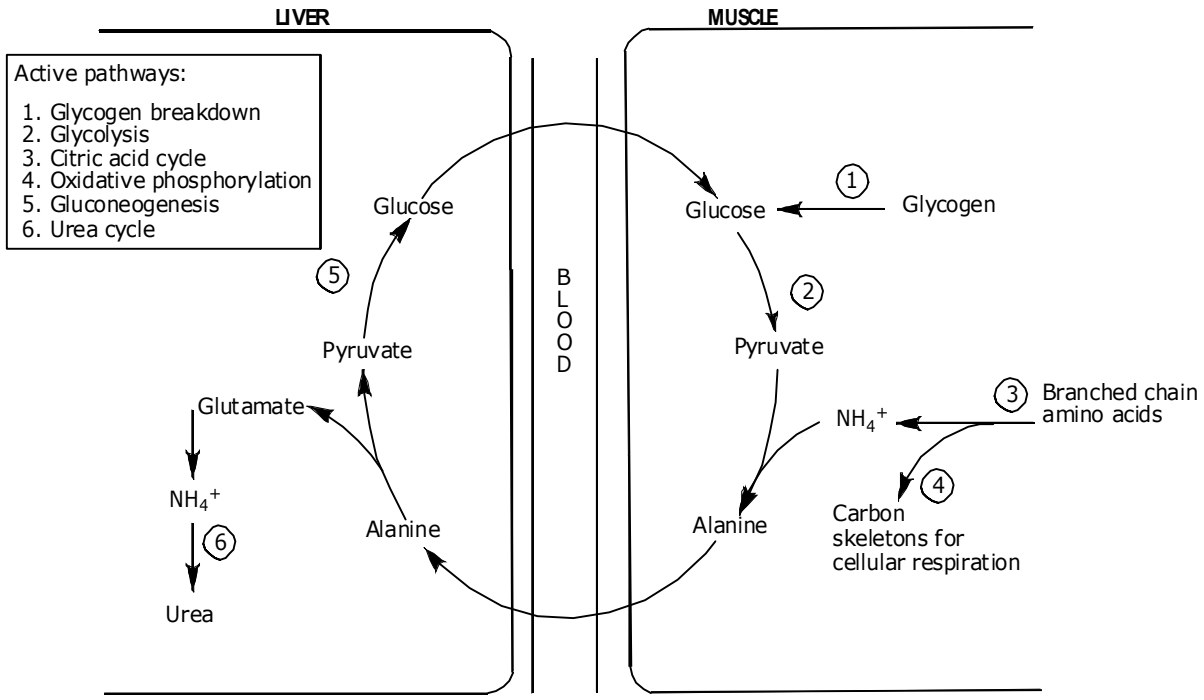
$$CO_2 + NH_4^+ + 3\ ATP + aspartate + 2H_2O \longrightarrow urea + 2ADP + P_i + AMP + PP_i + fumarate$$

The Urea Cycle is Linked to Gluconeogenesis

The fumarate synthesized by urea cycle is a precursor for glucose synthesis. Fumarate is hydrated to malate, which in turn is oxidized to oxaloacetate. Oxaloacetate can be converted into glucose by gluconeogenesis or transaminated to aspartate.

Different means of Disposing of Excess Nitrogen

Urea is not the only excretable form of nitrogen. As already stated, most terrestrial vertebrates excrete excess nitrogen as urea. So they are known as ureotelic organisms. Ammonotelic organisms, such as aquatic vertebrates and invertebrates, release nitrogen as NH_4^+ and rely on the aqueous environment to dilute this toxic substance, Lung fish, which are normally ammonotelic, become ureotelic in time of drought, when they live out of the water. Both ureotelic and ammonotelic organisms depend on sufficient water, to varying degrees, for nitrogen excretion. In contrtast, uricotelic organisms, such as birds and reptiles, excrete nitrogen as the purine uric acid. Uric acid is excreted as almost solid slurry requiring little water. The excretion of uric acid also has the advantage of removing four atoms of nitrogen per molecule.



The glucose-alanine cycle

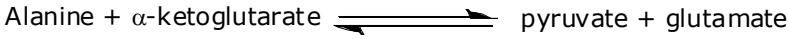
4.2.2 Carbon Atoms Of Degraded Amino Acids Emerge As Major Metabolic Intermediates

The strategy of amino acid degradation is to transform the carbon skeletons into major metabolic intermediates that can be converted into glucose or oxidized by the citric acid cycle. The carbon skeletons of the diverse set of 20 fundamental aminoacids are funneled into only seven molecules: Pyruvate, acetyl CoA, acetoacetyl CoA, α -Ketoglutarate, succinyl CoA, fumarate, and oxaloacetate.

Amino acids that are degraded to acetyl CoA or acetoacetyl CoA are termed ketogenic amino acids because they can give rise to ketone bodies or fatty acids. Amino acids that are degraded to pyruvate , α -ketoglutarate, succinyl CoA, fumarate, or oxaloacetate are termed glucogenic amino acids. The net synthesis of glucose form these amino acids is feasible because these citric acid cycle intermediates and pyruvate can be converted into phosphoenolpyruvate and then into glucose. Of the basic set of 20 amino acids, only leucine and lysine are solely ketogenic. Isoleucine, phenylalanine, tryptophan and tyrosine are both ketogenic and glucogenic. Some of their carbon atoms emerge in acetyl CoA or acetoacetyl coA, where as others appear in potential precursors of glucose. The other 14 aminoacids are classified solely as glucogenic.

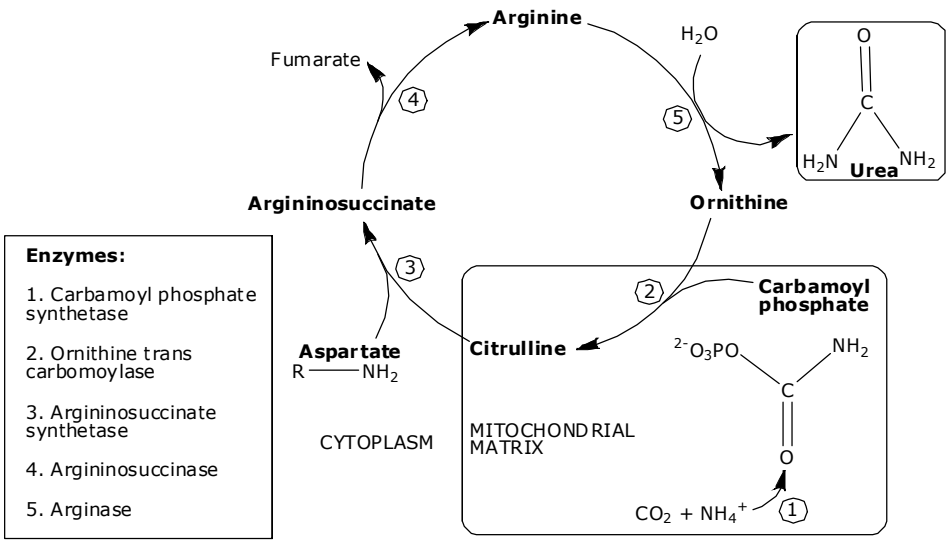
Pyruvate is the Entry Point into Metabolism for Six Amino Acids

Pyruvate is the entry point of the three-carbon amino acids such as alanine, serine and cysteine into the metabolic mainstream. The transamination of alanine directly yields pyruvate.



Deamination of serine by serine dehydratase yields pyruvate

Cysteine can be converted into pyruvate by several pathways, with sulfur atom emerging in H_2S, SCN^- ,or SO_3^{2-}. The carbon atoms of three other amino acids also can be converted into pyruvate. Glycine can be converted into serine by the enzymatic addition of a hydroxymethyl group or it can be cleaved to give CO_2, NH_4^+, and an activated one-carbon unit. Threonine can give rise to pyruvate through the intermediate 2-amino-3- ketobutyrate. Three carbon atoms of tryptophan can emerge in alanine, which can be converted into pyruvate.



The urea cycle

Aspartate and Asparagine Enter into Metabolism through Oxaloacetate.

Aspartate and asparagine are converted into a citric acid cycle intermediate,oxaloacetate. Aspartate, a four-carbon amino acid, is directly transaminated to oxaloacetate.

$$\text{Aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}$$

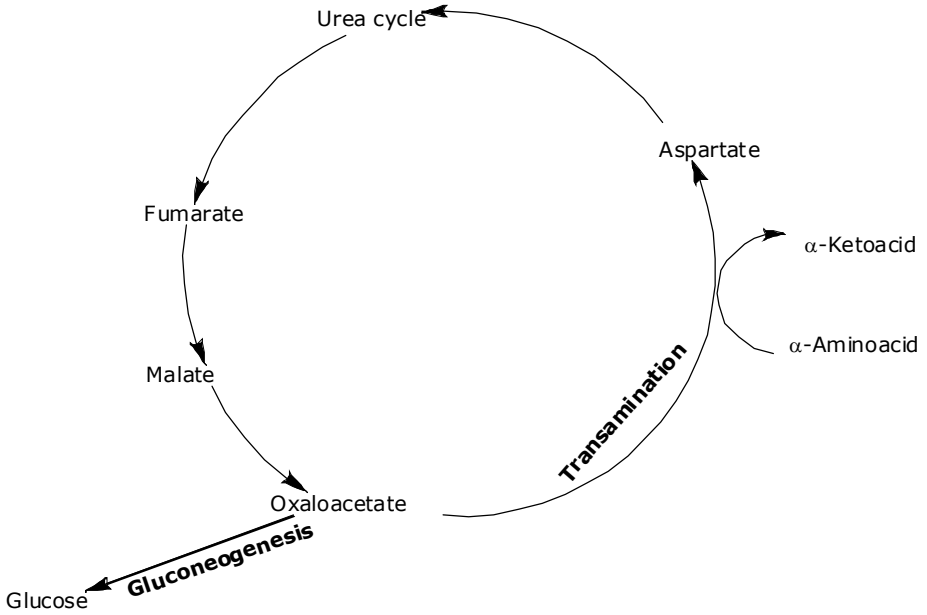
Asparagine is hydrolyzed by asparagin are to NH_4^+ and aspartate, which is then transaminated.

Aspartate can also be converted into fumarate by the urea cycle. Fumarate is a point of entry for half the carbon atoms of tyrosine and phenylalanine.

Five carbon Amino Acids Enter into Metabolism through Alpha-Ketoglutarate

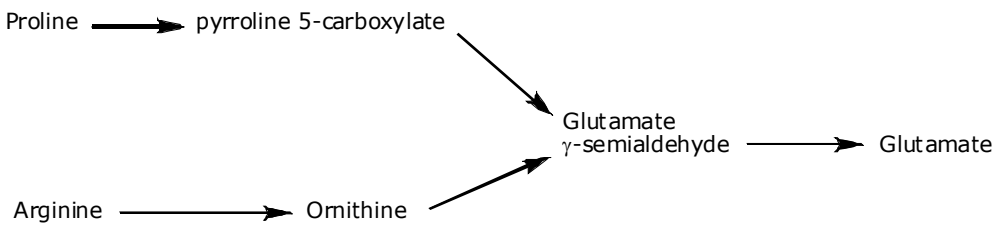
Five-carbon amino acids are first converted into glutamate, which is then oxidatively deaminated by glutamate dehydrogenase to yield α-ketoglutarate, a citric acid cycle intermediate.

Histidine is converted into 4-imidazolone 5- propionate.This is hydrolyzed to the N-formimino derivative of glutamate, which is then converted into glutamate by the transfer of its formimino group to tetrahydrofolate, a carrier of activated one -carbon units.



Histidine → Urocanate → 4-Imidazolone 5-propionate → N-Formiminoglutamate → Glutamate

Glutamine is hydrolyzed to glutamate and NH_4^+ by glutaminase. Proline and arginine are each converted into glutamate γ- semialdehyde, which is then oxidised to glutamate.



Succinyl Coenzyme A is the Point of Entry for three Nonpolar Amino acids and Threonine

Propinoyl CoA and and methylmalonyl CoA are intermediates in the breakdown of non-polar amino acids such as methionine, isoleucine, valine and polar amino acids threonine to form succinyl CoA.

The first step in the conversion of methionine to Succinyl CoA is the adenylation of methionine to form S-adenosylmethionine(SAM), a common methyl donor in the cell. Loss of the methyl and adenosyl groups yields homocysteine, which is eventually processed to α-ketobutyrate. This α-ketoacid is oxidatively decarboxylated by the α-ketoacid dehydrogenase

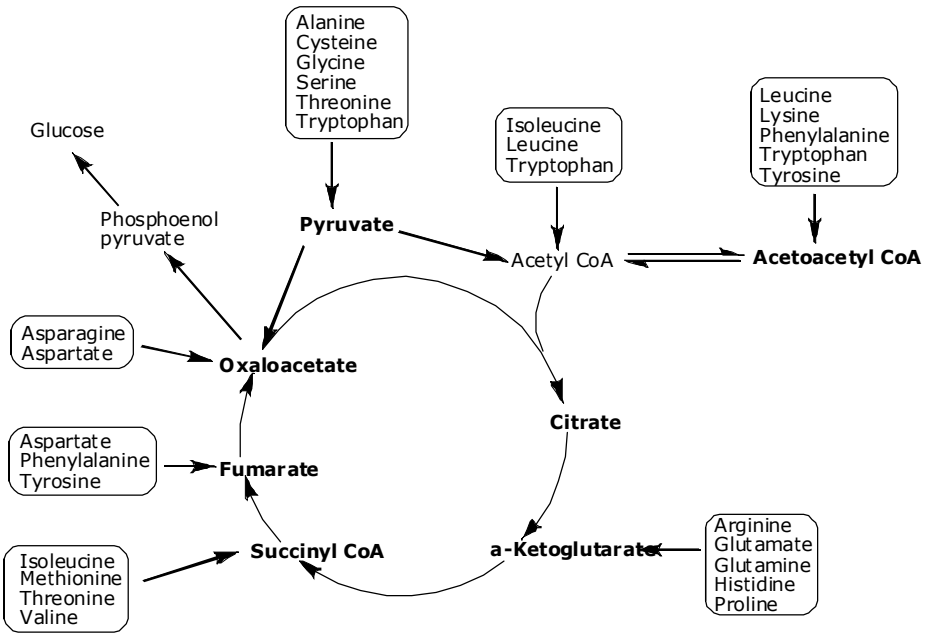
complex to propionyl CoA, which is converted to Succinyl CoA.

Isoleucine undergoes transamination, followed by oxidative decarboxylation of the resulting α-ketoacid. The remaining five carbon skeleton is further oxidized to acetyl-CoA and propionyl-CoA. Valine undergoes transamination and decarboxylation, then a series of oxidation reactions that convert the remaining four carbons to propionyl-CoA.

Threonine is also convertd into propionyl CoA through α-ketobutyrate. This is the primary pathway for threonine degradation in humans. The pathway we already discussed for the degradation of threonine to form pyruvate is a relatively minor pathwway in humans, but is more important in some other mammals.

The Branched-Chain Amino Acids Yield Acetyl CoA , Acetoacetate, or Propionyl CoA.

Carbon skeleton degradation of three branched-chain aliphatic amino acids-leucine, valine and isoleucine are discussed here. Leucine is transaminated to the corresponding α-ketoacid, α-ketoisocaproate. This is oxidatively decarboxylated to isovaleryl CoA by the branched-chain α-ketoacid dehydrogenase complex. Isovaleryl CoA is dehydrogenated to yield β-methylcrotonyl CoA.



Fates of the carbon skeletons of amino acids

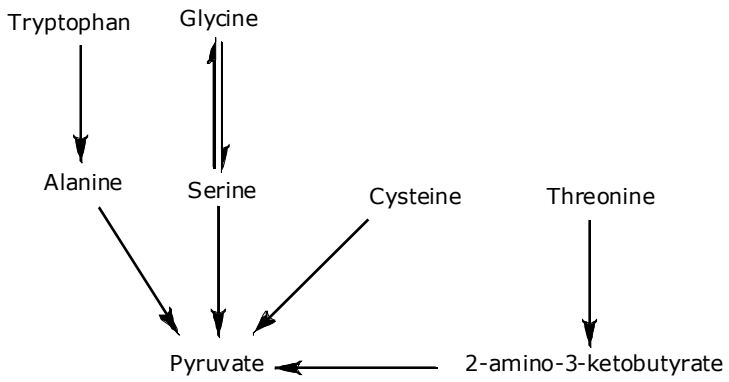
β–Methylglutaconyl CoA is then formed by the carboxylation of β-methylcrotonyl CoA at the expense of the hydrolysis of a molecule of ATP. β-Methylglutaconyl CoA is then hydrated to form 3-hydroxy-3-methylglutaryl CoA, which is cleaved into acetyl CoA and acetoacetate.

The degradative pathways of valine and isoleucine resemble that of leucine. The α-ketoacids of valine and isoleucine are also substrates of the branched-chain α- ketoacid dehydrogenase complex.

Isoleucine yields acetyl CoA and propionyl CoA, whereas valine yields CO_2 and propionyl CoA.

Degradation of Aromatic Amino Acids Require Oxygenases

The degradation of the aromatic amino acids such as phenylalanine, tyrosine and tryptophan yield the common intermediates acetoacetate, fumarate, and pyruvate. Molecular oxygen is used to break aromatic ring of these aminoacids.

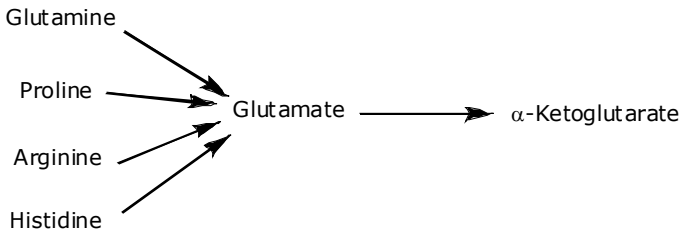


Pyruvate formation from amino acids

The degradation of phenylalanine begins with its hydroxylation to tyrosine catalyzed by phenylalanine hydroxylase. This enzyme is called a monooxygenase (or mixed-function oxygenase)because only one atom of O_2 appears in the product and the other in H_2O. The reduc tant here is tetrahydrobiopterin, an electron carrier. The quinonoid dihydrobiopterin is produced in the hydroxylation of phenylalanine. It is reduced back to tetrahydrobiopterin by NADPH in a reaction catalyzed by dihydropteridine reductase. The reactions catalyzed by phenylalanine hydroxylase and dihydropteridine reductase are

The next step in the degradation of phenylalanine and tyrosine is the transamination of tyrosine to p-hydroxy phenylpyruvate. 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. 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. Finally, 4-fumarylacetoacetate is hydrolyzed to fumarate and acetoacetate.

Tryptophan degradation starts with the cleavage of pyrrole ring by tryptophan 2,3 dioxygenase to form N-formylkynurenine. It is then converted to kynurenine. Kynurenine-3 monooxygenase hydroxylates the remaining benzene ring to form 3-hydroxykynurenine. Alanine is removed from it and the remaining 3-hydroxyanthranilic acid is cleaved by another dioxygenase and subsequently processed to acetoacetyl CoA. Nearly all cleavages of aromatic rings in biologi cal systems are catalyzed by dioxygenases.



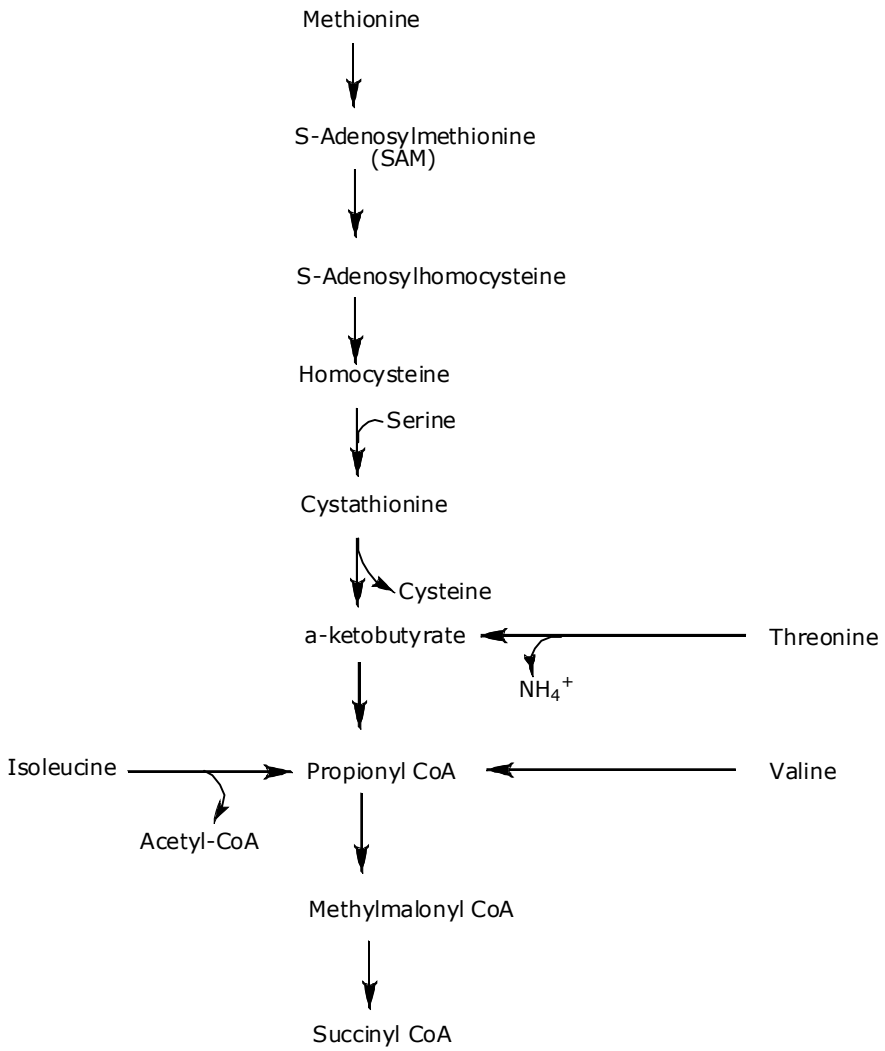
4.2.3 Amino Acid Degradation Can be Disrupted by Inborn Errors of Metabolism

In 1902, Archibald Garrod showed that **alcaptonuria** is transmitted as a recessive mendelian trait. It is an inherited metabolic disorder caused by the absence of homogentisate oxidase, which is the enzyme for the degradation of homogentisate, an intermediate in the degradation of phenyl alanine and tyrosine. So homogentisate accumulates and is excreted in the urine, which turns dark on standing as homogentisate is oxidized and polymerized to a melanin-like substance.

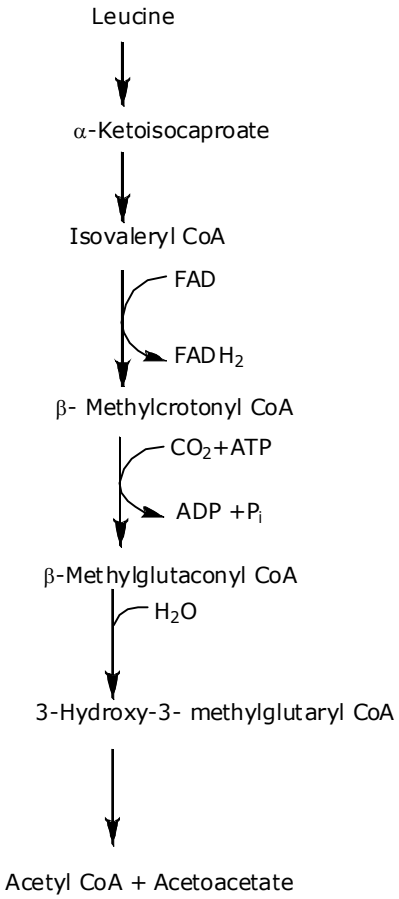
In **maple syrup urine disease**, the oxidative decarboxylation of α-ketoacids derived from valine, isoleucine, and leucine is blocked because the branched -chain dehydrogenase is missing or defective. Hence, the levels of these α-ketoacids and the branched chain amino acids that give rise to them are markedly elevated in both blood and urine. The urine of pa-

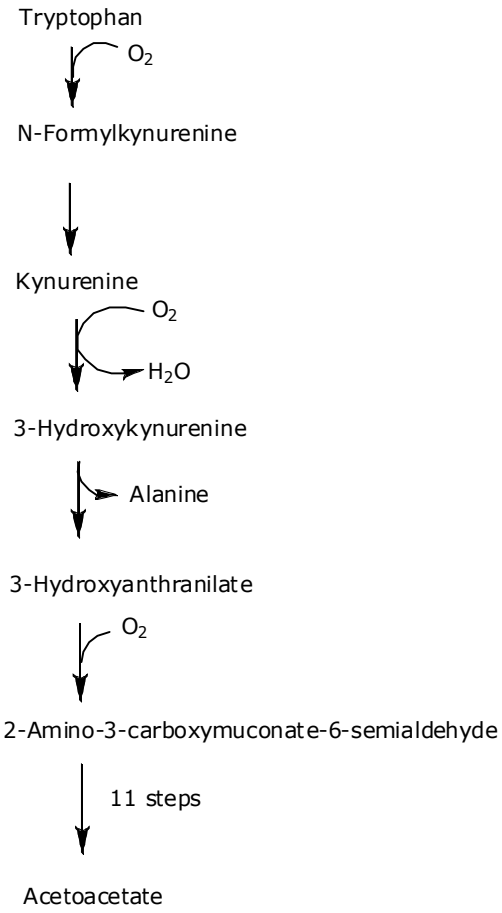
tients has the odour of maple syrup-hence the name of the disease. Maple syrup urine disease usually leads to mental and physical retardation unless the patient is placed on a diet low in valine, isoleucine, and leucine early in life. Diagnosis of the disease can be made by mass spectrometry.

Phenylketonuria is perhaps the best known of the diseases of aminoacid metabolism. This is an autosomal recessive disease. It is caused by an absence or deficiency of phenylalanine hydroxylase or, rarely of its tetrahydrobiopterin cofactor. Phenylalanine accumulates in all body fluids because it cannot be converted into tyrosine. Because the major outflow pathway is blocked in phenylketonuria, the blood level of phenylalanine is typically atleast 20-fold as high as in normal people.Minor fates of phenylalanine in normal people, such as the formation of phenylpuruvate, become major fates in phenylketonurics.The phenyllanine level in the blood is the preferred diagnostic criterion.Almost all untreated phenylketonurics are severely mentally retarded. The biochemical basis of their mental retardation is not known. The life expectancy of untreated patients is drastically shortened. The therapy for phenylketonuria is a low-phenylalanine diet.

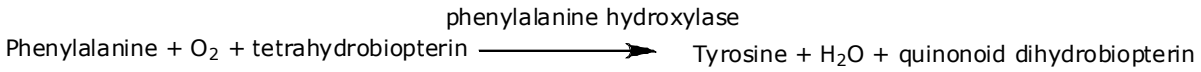


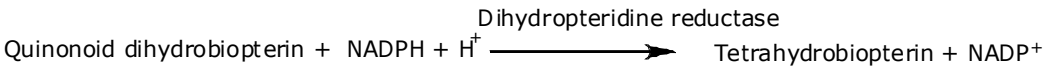
Degradation of methionine, isoleucine,valine and threonine

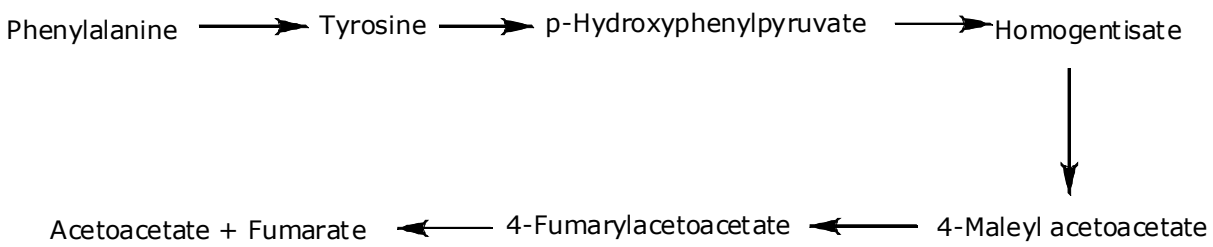




Tryptophan degradation







Phenylalanine and tyrosine degradation