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Biosynthesis of amino acids; Degradation of amino acid; Transamination; Oxidative deamination; Urea cycle; Amino acid oxidases; Excretion of ammonia; Catabolism; Genetic disease; Nitrogen fixation; Nitrogen assimilation; Inborn error
What are amino acids?

Amino acids are the basic structural units of proteins. Amino acids consist of an amino group, a carboxyl group, a hydrogen atom and a distinctive R group bonded to a carbon atom, which is called the α-carbon atom. Amino acids in solution at neutral pH (7.0) are predominantly dipolar ions (or zwitterions) rather than unionized molecules. In the dipolar form of an amino acid, the amino group is protonated (\(-\text{NH}_3^+\)) and the carboxyl group is dissociated (\(-\text{COO}^-\)). The ionization state of amino acid varies with pH. In acid solution (e.g., pH 1), the carboxyl group is unionized (\(-\text{COOH}\)) and the amino group is ionized (\(-\text{NH}_3^+\)). In alkaline solution (e.g., pH 11), the carboxyl group is ionized (\(-\text{COO}^-\)) and the amino group is unionized (\(-\text{NH}_2\)).

\[
\begin{align*}
\text{NH}_3^+ & \quad \text{NH}_3^- \quad \text{NH}_2^- \\
\text{R} - \text{C} - \text{COOH} & \quad \text{R} - \text{C} - \text{COO}^- \\
\text{H} & \quad \text{H} \quad \text{H}
\end{align*}
\]

(Predominant form at pH 1) (at pH 7.0) (at pH 11.0)

Fig. 1: Structure of amino acids in three ionization states

All the standard amino acids except glycine have an asymmetric carbon atom, α-carbon atom, to which four different substitute groups, i.e., a carboxyl group, an amino group, an R-group, and a hydrogen atom, are attached. When a carbon atom has four different substitute groups, they can arrange in two ways that represent non-superimposable mirror images of each other. Such a carbon atom is asymmetric and is called chiral atom or center. Compounds with an asymmetric carbon occur in two different isomeric form, which are identical in all chemical and physical properties except one, the direction in which they cause the rotation of plane polarized light in a polarimeter. With the single exception of glycine, which has no asymmetric carbon atom, all of the 20 amino acids obtained from the hydrolysis of proteins under sufficiently mild conditions are optically active, i.e., they can rotate the plane polarized light in one direction or the other. These two forms are called optical isomers, enantiomers, or stereoisomers. A solution of one stereoisomer of a given amino acid will rotate plane-polarized light to the left (counterclockwise) and is called the laevorotatory isomer; the other stereoisomer will rotate plane polarized to the same extent but to the right and is called the dextrorotatory isomer. An equimolar mixture of both isomers will not rotate plane-polarized light.

Classification of amino acids

Amino acids can be classified on the basis of their

i). R-group
ii) requirement
iii) degradation product.

Classification of amino acids based on their R-groups

Based on the R-groups, there are four main families of amino acids.
a). **Nonpolar or hydrophobic amino acids**
Eight amino acids have nonpolar R-groups. The R-groups in this class of amino acids are hydrocarbon in nature and thus hydrophobic. This group includes five amino acids with aliphatic R-groups (alanine, valine, leucine, isoleucine and proline), two with aromatic rings (phenylalanine and tryptophan) and one containing sulphar (methionine). Of this group, proline requires special mention because its \( \alpha \)-amino group is not free but is substituted by a portion of its R-group to yield a cyclic structure.

b). **Polar but uncharged R-groups**
Seven amino acids have polar but uncharged R-groups. The R-groups of these amino acids are more soluble in \( \text{H}_2\text{O} \), i.e. more hydrophilic, than those of the nonpolar amino acids, because they contain functional groups that form hydrogen bonds with \( \text{H}_2\text{O} \). This class of amino acids includes, glycine, serine, threonine, cysteine, tyrosine, asparagines and glutamine. The polarity of serine, threonine and tyrosine is contributed by their hydroxyl groups, that of asparagines and glutamine by their amide groups and that of cysteine by its sulphhydryl or thiol group. The R-group of glycine, a single hydrogen atom, is too small to influence the high degree of polarity of the \( \alpha \)-amino and \( \alpha \)-carboxyl groups.

c). **Negatively charged R-groups**
Two amino acids have negatively charged R-groups. The two amino acids whose R-groups have a negative charge at pH 7.0 are aspartic acid and glutamic acid, each with a second carboxyl group. These amino acids are the parent compounds of asparagines and glutamine respectively.

d). **Positively charged R-groups**
Three amino acids have positively charged R-groups. The amino acids in which the R-groups have a net positive charge at pH 7.0 are lysine, arginine and histidine. Lysine has a second amino group at the \( \epsilon \)-position on its aliphatic chain, arginine has a guanidine group and histidine contains the weekly-ionized imidazole group.

**Classification of amino acids on the basis of their requirement**
Amino acids can be classified into two categories on the basis of the requirement by man and albino rats.

a). **Essential amino acids**: Many vertebrates, including man lack the ability to manufacture a number of amino acids. They require these amino acids preformed in the diets, they are called essential amino acids. Lysine, tryptophan, histidine, phenylalanine, leucine, isoleucine, threonine, valine, methionine, and arginine are essential amino acids.

b). **Non-essential amino acids**: Man and albino rats can make only 10 of the 20 amino acids require as building blocks of proteins. These amino acids are known as non-essential amino acids. Glycine, alanine, serine, cysteine, tyrosine, aspartic acid, glutamic acid, proline, asparagines and glutamine are non-essential amino acids.

**Classification of amino acids on the basis of product form on degradation**

**Ketogenic amino acids**: Five amino acids (phenylalanine, tyrosine, lysine, tryptophan and leucine) on degradation are converted into acetoacetyl-CoA. These amino acids can yield
ketone bodies in the liver, since acetoacetyl-CoA can be converted into acetoacetate and β-hydroxybutyrate. These five amino acids are called ketogenic.

**Glucogenic amino acids:** The amino acids that can be converted into pyruvate, α-ketoglutarate, succinate and oxaloacetate, 15 altogether, can be converted into glucose and glycogen. They are called glucogenic amino acids.

The division between ketogenic and glucogenic amino acids is not sharp since two amino acids (phenylalanine and tyrosine) are both ketogenic and glucogenic. Some of the amino acids that can be converted into pyruvate, particularly alanine, cysteine and serine can also potentially form acetoacetate via acetyl-CoA, particularly in diabetes mellitus.

**Nonprotein amino acids**
In addition to the 20 common and several rare amino acids of protein, over 150 other amino acids are known to occur biologically in free or combined form but never in proteins. Most of these amino acids are derivatives of the L-α-amino acids found in proteins, β-, γ- and δ-amino acids are also known. Some of nonprotein amino acids serve as important precursors for the biosynthesis of important molecules and intermediates in metabolism. For example, β-alanine is a building block of the vitamin pantothenic acid; homocysteine and homoserine are intermediates in amino acid metabolism; citrulline and ornithine are intermediates in the synthesis of arginine. Other nonprotein amino acids function as chemical agents for the transmission of nerve impulses, such as γ-aminobutyric acids.

**Amino acid degradation**
In higher animals amino acids serve as building blocks of proteins and as precursors of many other important biomolecules, such as hormones, purines, pyrimidines, porphyrins and some vitamins. Most of the metabolic energy generated in the tissues comes from the oxidation of carbohydrates and fats, which together furnish up to 90 per cent of the energy requirement of the adult human. Only 10 to 15 percent of the total energy requirement is furnished by the oxidation of amino acids. Although amino acids function primarily as building blocks for the biosynthesis of protein, they can undergo oxidative degradation in three different physiological conditions and serves as major source of energy. (i). During the normal dynamic turnover of body proteins the amino acids released, if not needed for synthesis of new body proteins, undergo oxidative degradation; (2) When amino acids are ingested in excess of the body’s needs for protein synthesis, surplus amino acids are used as metabolic fuel since amino acids cannot be stored. (3). During fasting or in diabetes mellitus, when carbohydrates are either unavailable or not properly utilized, body proteins are called upon as fuel. Under these different circumstances, when they are used as fuel, amino acids undergo loss of their amino groups, the keto acids so formed then have two major fates: (1) conversion into glucose in the process of gluconeogenesis, or (2) oxidation to CO₂ via the tricarboxylic acid cycle.

The first stage in catabolism of most of the amino acids is removal of the α-amino group by two enzymatic pathways: (i) transamination and (ii) oxidative degradation. The resulting carbon skeletons of the twenty standard amino acids are funneled into only seven molecules: pyruvate, acetyl-CoA, acetoacetyl-CoA, α-ketoglutarate, succinyl-CoA, fumarate and oxaloacetate, by twenty different multienzyme sequences (Fig.1).
**Transamination**

In this process the α-amino group of most of amino acids is transferred to α-ketoglutarate to form glutamate and the corresponding α-keto acids.

\[
\text{α-amino acid} + \text{α-ketoglutarate} \leftrightarrow \text{α-keto acid} + \text{glutamate}
\]

The enzymes that catalyze these reactions are called transaminases (aminotransferases). The α-amino group of 12 amino acids (ala, arg, asp, cys, ileu, leu, lys, phe, tryp, tyr and val) is enzymatically removed by transamination. A large number of transaminases are now known. Most require α-ketoglutarate as one amino group acceptor; however they are less specific for the other substrate, the L-amino acid that donates the amino group. As an example, A prominent transaminase in animal tissues is aspartate aminotransferase, more commonly called aspartate transaminase, which catalyzes the reversible reaction:

\[
\begin{align*}
\text{HOOC -CH}_2\text{-CH-COOH} & \quad + \quad \text{HOOC-CH}_2\text{-CH}_2\text{-CO-COOH} \\
\text{(Asp)} & \quad \text{NH}_2 & \quad \text{(α-Ketoglutarate)} \\
\downarrow & \quad \text{Aspartate transaminase} \\
\text{HOOC-CO-CH}_2\text{-COOH} & \quad + \quad \text{HOOC-CH-CH}_2\text{-COOH} \\
\text{(Oxaloacetate)} & \quad \text{(Glu)} & \quad \text{NH}_2
\end{align*}
\]

Although the enzyme is most active with aspartate as amino group donor in the forward direction, it will accept a number of other α-amino acids as donors. In addition to aspartate transaminase, animal tissues contain other transaminases requiring α-ketoglutarate as amino group acceptor, such as alanine transaminase, leucine transaminase and tyrosine transaminase, catalyzing, respectively.

**Alanine transaminase**

\[
\text{L-Alanine} + \text{α-ketoglutarate} \rightarrow \text{Pyruvate} + \text{L-Glutamate}
\]

**Leucine transaminase**

\[
\text{L-Leucine} + \text{α-ketoglutarate} \rightarrow \text{α-Ketoisocaproate} + \text{L-Glutamate}
\]

**Tyrosine transaminase**

\[
\text{L-Tyrosine} + \alpha \text{-ketoglutarate} \rightarrow \text{p-Hydroxyphenylpyruvate} + \text{L-Glutamate}
\]

It will be noted that there is no net deamination, i.e. loss of amino groups, in such reaction since the α-ketoglutarate becomes aminated as the α-amino acid is deaminated. The whole point of transamination reactions is to collect the amino groups from many different amino acids in the form of only one, namely, L-glutamate. The amino group catabolism converges into a single product.

Transaminases are found both in the mitochondria and in the cytosol of eukaryotic cells. In mammals the collection of amino groups from other amino acids takes place in the cytosol, catalyzed by the cytosol form of aspartate transaminase, with the formation of glutamate. The glutamate so formed then enters the mitochondrial matrix via a specific membrane transport system. In the mitochondrial matrix glutamate is either directly deaminated or becomes the amino group donor to oxaloacetate by the action of mitochondrial aspartate
transaminase to yield aspartate, one of the immediate amino-group donors in the formation of urea.

**Role of pyridoxal phosphate in transamination**

All the transaminases have the same prosthetic group, pyridoxal phosphate (PLP) that is derived from pyridoxine (vitamin B₆) and share a common reaction mechanism. Pyridoxal phosphate functions as an intermediate carrier of amino group on the active site of transaminases.

![Structure of pyridoxine (vitamin B₆), pyridoxal phosphate and pyridoxamine phosphate](image)

**Fig. 2: Structure of pyridoxine (vitamin B₆), pyridoxal phosphate and pyridoxamine phosphate**

During the catalytic cycles it undergoes reversible transition between its aldehyde form, pyridoxal phosphate, which can accept amino groups, and its aminated form, pyridoxamine phosphate which can donate its amino group to α–ketoglutarate. In this way prosthetic group acts as a reversible, transient carrier of amino groups from an α–amino acid to α–ketoglutarate. In the absence of substrate, the aldehyde group of pyridoxal phosphate forms a covalent Schiff base linkage (imine bond) with the ε-amino group in the side-chain of a specific lysine residue in the active site of the enzyme. On addition of substrate, the α-amino group of the incoming amino acid displaces the amino group of the active site lysine and a new Schiff base linkage is formed with the amino acid substrate. The resulting amino
acid-pyridoxal phosphate-Schiff base that is formed remains bound to the enzyme by multiple non-covalent interactions.

The amino acid is then hydrolyzed to form a α-keto acid and pyridoxamine phosphate, the α-amino group having been temporarily transferred from the amino acid substrate on to pyridoxal phosphate (Fig. 3). These steps constitute one half of the overall transamination reaction. The second half occurs by a reversal of the above reactions with a second α–keto acid reacting with the pyridoxamine phosphate to yield a second amino acid and generate the enzyme-pyridoxal phosphate complex (Fig. 3)

**First stage**
\[
\begin{align*}
R_1\text{-CH-NH}_2 & + [\text{E}]-\text{C}=\text{O} \rightarrow R_1\text{-CH-----N} = \text{C-}[\text{E}] + \text{H}_2\text{O} \rightarrow R_1\text{-C = N} \rightarrow \text{CH}_2-[\text{E}] \\
\text{COOH} & \quad \text{H} & \quad \text{COOH} & \quad \text{H} & \quad \text{COOH} \\
\alpha\text{-Amino acid}_1 & \quad \text{Pyridoxal phosphate-} & \quad \text{Aldimine} & \quad \text{Ketimine} & \quad +\text{H}_2\text{O} \\
\text{Enzyme} & & & & \\
\text{H}_2\text{N-CH}_2-[\text{E}] & + R_1\text{-C-COOH} & \quad \text{Pyridoxamine phosphate} & \quad \alpha\text{-Keto acid}_1 & \quad \text{enzyme}
\end{align*}
\]

**Second stage**
\[
\begin{align*}
R_2\text{-C-COOH} & + H_2\text{N-CH}_2-[\text{E}] \rightarrow R_2\text{-C = N} = \text{CH}_2-[\text{E}] \rightarrow R_2\text{-C} = N = \text{C} \rightarrow \text{[E]} \rightarrow \text{H}_2\text{N-CH}_2-[\text{E}] \\
\text{COOH} & \quad \text{COOH} & \quad \text{COOH} & \quad \text{H} \\
\alpha\text{-Keto acid}_2 & \quad \text{Pyridoxamine phosphate} & \quad \text{Ketimine} & \quad \text{Aldimine} & \quad +\text{H}_2\text{O} \\
\text{enzyme} & & & & \\
\text{R}_2\text{-CH-NH}_2 & + [\text{E}] - \text{C} = \text{O} & \quad \text{COOH} & \quad \text{H} & \quad \text{COOH} \\
\alpha\text{-Amino acid}_2 & \quad \text{Pyridoxal phosphate} & \quad \text{enzyme} & \quad \text{O}
\end{align*}
\]

**Fig. 3: Intermediate steps in the transaminase reaction** [The pyridoxal phosphate enzyme complex is symbolized H-C-[E]; the pyridoxamine phosphate enzyme complex as H₂N-CH₂-[E]. The pyridoxal phosphate prosthetic group O is the intermediate amino group carrier between amino acid and keto acid.]
**Oxidative deamination**

The α-amino groups that have been funneled into glutamate from the other amino acids are then converted into ammonia by the action of glutamate dehydrogenase, which is present in both the cytosol and mitochondria of the liver. The enzyme is unusual in being able to utilize either NAD⁺ or NADP⁺. In the biosynthesis of glutamate, the NADP⁺ form of the coenzyme is used, whereas NAD⁺ is used in its degradation.

\[
\text{HOOC-CH}_2\text{-CH}_2\text{-CH-NH}_2 + \text{NAD}^+ (\text{NADP}^+) + \text{H}_2\text{O} \rightarrow \text{Glutamate dehydrogenase} \\
\text{HOOC-CH}_2\text{-CH}_2\text{-C-COOH} + \text{NH}_4 + \text{NADH (NADPH)}
\]

Glutamate dehydrogenase is responsible for most of the ammonia formed in animal tissues, since glutamate is the only amino acid whose α-amino group can be directly removed at a high rate in this manner. Glutamate and glutamate dehydrogenase therefore have a unique role in amino group metabolism. Glutamate dehydrogenase is a complex allosteric enzyme and consists of six identical subunits, each containing a single polypeptide chain of 500 amino acids residues. GTP and ATP are allosteric inhibitors, whereas GDP and ADP are allosteric activator. Hence, when the energy charge of the cell is low glutamate dehydrogenase is activated and the oxidation of amino acids increases. The resulting carbon skeletons are then utilized as metabolic fuel, feeding into citric acid cycle and ultimately giving rise to energy through oxidative phosphorylation.

**Amino acid oxidases**

The major route for the deamination of amino acids is transamination followed by the oxidative deamination of glutamate. However, a minor route also exists that involves direct oxidation of the amino acid by L-amino acid oxidase. This enzyme utilizes flavin mononucleotide (FMN) as its coenzyme with resulting FMNH₂ being reoxidized by molecular oxygen, a process that also generates the toxic H₂O₂. The H₂O₂ is rendered harmless by the action of catalase. Kidney and liver are also rich in the FAD-containing D-amino acid oxidase. However, the function of this enzyme in animals is unclear, since the D-isomer of amino acids are rare in nature, only occurring in bacterial cell walls.

\[
\text{R-CH-NH}_2 + \text{FMN} + \text{H}_2\text{O} \rightarrow \text{R-C=O} + \text{FMNH}_2 + \text{NH}_4
\]

For the L-isomer:

\[
\text{FMNH}_2 + \text{O}_2 \rightarrow \text{FMN} + \text{H}_2\text{O}_2 \downarrow \text{Catalase} \\
\text{H}_2\text{O} + \frac{1}{2} \text{O}_2
\]
Excretion of ammonia

There is no store for nitrogen-containing compounds as there is for carbohydrates (glycogen) or lipids (triacylglycerol). Higher organisms tend to salvage and reuse ammonia derived from the catabolism of amino acids by reversing the glutamate dehydrogenase reaction

$$\alpha\text{-ketoglutarate} + \text{NH}_3 + \text{NADH} (\text{NADPH}) + \text{H}^+ \leftrightarrow \text{glutamate} + \text{NAD}^+(\text{NADP}^+) + \text{H}_2\text{O}$$

However, a certain fraction of the ammonia formed from amino acid is ultimately excreted by vertebrates in one of three forms: urea, ammonia itself, or uric acid. Most aquatic animals such as fishes excrete amino nitrogen as ammonia itself directly into the surrounding water. Birds and terrestrial reptiles, whose water intake is very limited, excrete the amino nitrogen in a semisolid form as suspension of solid uric acid. Most terrestrial vertebrates have evolved the ability to excrete nitrogen as the nontoxic compound urea. These three classes of organisms are called: ammonotelic, uricotelic and ureotelic, respectively. The amphibia occupy a midposition. The tadpole, which is aquatic, excretes ammonia. After metamorphosis, during which the liver acquires the necessary enzymes, the adult frog forms and excretes urea.

Urea cycle

In ureotelic animals the ammonia resulting from the deamination of amino acids is converted into urea in the liver by a cyclic mechanism known as Urea Cycle. The urea cycle was the first metabolic cycle to be discovered by Hans Krebs and Kurt Henseleit in 1932, 5 years before Krebs discovered the citric acid cycle. The overall reaction of the pathways is:

$$\text{NH}_4^+ + \text{HCO}_3^- + 2\text{H}_2\text{O} + 3\text{ATP} + \text{aspartate} \rightarrow \text{urea} + 2\text{ADP} + \text{AMP} + 2\text{Pi} + \text{PPi} + \text{fumarate}$$

One of the nitrogen atoms of urea comes from ammonia, the other is transferred from amino acid aspartate, while the carbon atom comes from CO$_2$. Ornithine, an amino acid that is not in the standard set of 20 amino acids and is not found in proteins, is the carrier of these nitrogen and carbon atoms. Five enzymatic reactions are involved in the urea cycle. These reactions are compartmentalized. 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 three reactions of the urea cycle, which lead to the formation of urea, take place in the cytosol.

Enzymatic reactions in mitochondrial matrix

1. Carbamoyl phosphate synthetase catalyzes the condensation and activation of ammonia (formed from oxidative deamination of glutamate by glutamate dehydrogenase) and CO$_2$ (in the form of bicarbonate, HCO$_3^-$) to form carbamoyl phosphate.

$$\text{CO}_2 + \text{NH}_4 + 2\text{ATP} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{N}–\text{C}—\text{O}—\text{P}—\text{OH} + 2\text{ADP} + \text{Pi}$$

(Carbamoyl phosphate)
2. The second reaction also occurs in the mitochondria and involves the transfer of the carbamoyl group from carbamoyl phosphate to ornithine by ornithine transcarbamoylase. This reaction forms another nonstandard amino acid citrulline, which then has to be transported out of the mitochondria into the cytosol where remaining reactions of the cycle take place.

\[
\text{COOH} \quad \text{O} \\
\text{HC} - \text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2 + \text{H}_2\text{N} - \text{C} - \text{O} - \text{P} - \text{OH} + 2\text{ADP} + \text{Pi} \\
\text{NH}_2 \quad \text{O} \quad \text{OH} \quad \text{Ornithine} \quad \text{transcarbamoylase} \\
\]

\[
\begin{align*}
\text{COOH} \\
\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-NH-C-NH}_2 + \text{HO} - \text{P} - \text{OH} \\
\text{NH}_2 \quad \text{O} \quad \text{OH} \\
\end{align*}
\]

\text{Citrulline} \quad \text{Carbamoyl phosphate} \\

\text{Enzymatic reactions in cytosol}

1. The citrulline is then condensed with aspartate, the source of the second nitrogen atom in urea, by the enzyme argininosuccinate synthetase to form argininosuccinate. This reaction is driven by the hydrolysis of ATP to AMP and PPI with subsequent hydrolysis of the pyrophosphate by pyrophosphatase. Thus both of the high-energy bonds in ATP are ultimately cleaved.

\[
\text{H}_2\text{N-CH-CH}_2\text{-CH}_2\text{-NH-C-NH}_2 + \text{NH}_2\text{-CH-CH}_2\text{-COOH} + \text{ATP} \\
\text{COOH} \quad \text{O} \quad \text{COOH} \quad \text{(Aspartate)} \\
\text{(Citrulline)} \quad \text{Argininosuccinate synthetase} \\
\text{COOH} \\
\text{H}_2\text{N-CH-CH}_2\text{-CH}_2\text{-NH-C-NH-CH-CH}_2\text{-COOH} + \text{AMP} + \text{PPI} \\
\text{COOH} \quad \text{NH} \quad \text{(L-Argininosuccinic acid)} \\
\]

2. In the next reaction argininosuccinate undergoes a β-elimination reaction by the action of argininosuccinate lyase to form free arginine and fumarate:

\[
\text{L-Argininosuccinic acid} \rightarrow \text{H}_2\text{N-CH-CH}_2\text{-CH}_2\text{-NH-C-NH}_2 + \text{Fumaric acid} \\
\text{COOH} \quad \text{NH} \quad \text{(Arginine)} \\
\]
The arginine formed in this reaction becomes the immediate precursor of urea, whereas the fumarate returns to the pool of tricarboxylic acid cycle intermediates.

3. Up to this point the reaction sequence is that employed by all organisms capable of the biosynthesis of arginine. However, only ureotelic animals possess large amounts of arginase, which cleaves urea from arginine and regenerates ornithine.

\[
\text{Arginine} + \text{H}_2\text{O} \rightarrow \text{Ornithine} + \text{H}_2\text{N-C-NH}_2 + \text{O} \quad \text{(Urea)}
\]

Ornithine is thus regenerated and can enter the mitochondria again to initiate another round of the urea cycle. The formation of one molecule of urea therefore requires the hydrolysis of four high-energy phosphate groups provided by ATP.

**Fig. 4: Urea cycle**

*Genetic defects in urea cycle*

Urea cycle disposes off two waste products, ammonia and CO\(_2\) from our body. Therefore, a deficiency of any of the enzymes of the urea cycle has devastating consequences because there is no alternative pathway for the removal of ammonia from our body. Several inherited defects of the urea cycle have been discovered. People with heritable genetic defects in one enzyme or another of the urea cycle have an impaired ability of to form urea from ammonia; consequently blood level of ammonia is significantly elevated. This
condition is known as hyperammonemia. These patients show mental deficiency, lethargy and retard development of nervous system.

Some of these genetic defects become evident a day or two after birth, when the afflicted infants becomes lethargic and vomits periodically. Some of these patients can be treated by replacing dietary protein with a mixture of α-keto acid analogs of the essential amino acids as the essential parts of the indispensable amino acids are their carbon skeleton not their amino groups. The α–keto acid analogs of essential amino acids can accept amino groups from excess nonessential amino acids. In this way the nonessential amino acids are kept off from delivering their amino groups to the blood in the form of NH₃.

**Treatment of urea cycle defects**

Novel strategies have been developed to treat these patients on the basis of thorough understanding of the biochemistry of formation of urea. For example, patients with defects in argininosuccinate lyase will not be able to form urea but able to form argininosuccinate, which will be excreted, as a result there is a deficiency of arginine. But this defect can be partly bypassed by providing a surplus arginine in the diet and restricting the total protein diet. In treated patients, argininosuccinate substitute for urea in carrying out nitrogen out of the body and blood level of NH₃ will be lowered. Patients with defects in carbamoyl phosphate synthetase or ornithine transcarbamoylase will also not be able to form neither urea or argininosuccinate from NH₃ and blood level of NH₃ of these patients will be significantly higher. The excess NH₃ of these patients are utilized for biosynthesis of glycine and glutamine by the following pathways:

1. \[ \text{Glycine synthase} \]
   \[ \text{CO}_2 + \text{NH}_3 + \text{N}^5,\text{N}^{10}-\text{Methylene-FH}_4 + \text{NADH} + \text{H}^+ \]
   \[ \text{Glycine} + \text{FH}_4 + \text{NAD} \]

2. \[ \text{Glutamine synthetase} \]
   \[ \text{Glutamine} + \text{ATP} + \text{NH}_3 \rightarrow \text{Glutamine} + \text{ADP} + \text{P}_i \]

As a result excess nitrogen accumulates in glycine and glutamine in the blood. The challenge then is to get rid the body of these two amino acids. This can be achieved by supplementing a protein-restricted diet with large amount of benzoate and phenylacetate, which are activated to form benzyl-CoA and phenylacetyl-CoA respectively. Benzyl-CoA reacts with glycine to form hippurate:

\[ \text{Benzoate} + \text{CoA} + \text{ATP} \rightarrow \text{Benzoyl-CoA} + \text{AMP} + \text{PP}_i + \text{Glycine} \]
\[ \text{Hippuric acid} + \text{CoA} \]

Similarly, phenylacetyl-CoA reacts with glutamine to form phenylacetylglutamine.

\[ \text{Phenylacetate} + \text{ATP} + \text{CoA} \rightarrow \text{Phenylacetetyl-CoA} + \text{AMP} + \text{PP}_i + \text{Glutamine} \]
\[ \text{Phenylacetetylglutamine} + \text{CoA} \]

Thus nitrogen is excreted in the form of hippurate and phenylacetylglutamine.
**Fate of carbon skeleton of amino acids after removal of the α–amino groups**

Thus far, we have considered a series of reactions that removes the α–amino group from amino acids and the conversion of NH$_3$ into urea. We now turn to the fates of the resulting carbon skeleton of the amino acids. The strategy of amino acid degradation is to form major metabolic intermediates that can be converted into glucose or to be oxidized by the TCA cycle. There are 20 different catabolic pathways for the catabolism of 20 different amino acids found in proteins. The 20 catabolic pathways converge to form only five products: acetyl-CoA, oxaloacetate, α-ketoglutarate, succinyl-CoA and fumarate, all of which enter the TCA cycle for complete oxidation to CO$_2$ and H$_2$O. These amino acids can be grouped according to common degradation products formed.

**Fig. 5: Fate of the amino acid carbon skeleton**

**Pathway to acetyl-CoA via pyruvate**

The carbon skeleton of five amino acids (alanine, glycine, serine, cysteine, threonine) converted to acetyl-CoA via formation of pyruvate. Alanine is converted to pyruvate directly by transamination reaction with α–ketoglutarate catalyzed by alanine transaminase.

Glycine has two pathways of degradation. The major route, which does not lead to acetyl-CoA is by reversible oxidative cleavage to form CO$_2$, ammonia and N$^5$N$^{10}$-methylene-FH$_4$ by the action of glycine synthase. In this oxidative cleavage pathway the two carbon atoms of glycine do not enter the TCA cycle. One carbon is lost as CO$_2$ and the other becomes the methylene group of N$^5$N$^{10}$-methylene-FH$_4$, a one-carbon group donor in certain biosynthetic pathways.
Fig. 6: Catabolic pathways for alanine, glycine, serine and threonine

Fig. 7: Metabolic fate of glycine (a) conversion to serine; (b) breakdown to CO$_2$ and NH$_3$
Glycine may also be converted to serine by serine hydroxymethyltransferase, a pyridoxal enzyme. Serine in turn is dehydrated and deaminated to pyruvate by serine dehydrase, also a pyridoxal phosphate enzyme. Serine may also be converted to glycine and N5, N10-methylene-FH4 through reverse action of serine hydroxymethyltransferase, which is reversible under intracellular condition.

Threonine can also be converted to pyruvate by a pathway in which serine hydroxymethyltransferase catalyzes aldol cleavage of threonine, forming aldehyde and glycine (Fig. 6). Acetaldehyde can be oxidized by aldehyde dehydrogenase to acetate, which can then be converted to acetyl-CoA. Glycine produced can be converted to serine as discussed above and serine can be converted to pyruvate by dehydration and deamination.

![Fig. 8: Conversion of threonine to succinyl-CoA](image)
The most important pathway for catabolism of threonine in most organisms is conversion of threonine to α-ketobutyrate and ammonia by pyridoxal phosphate containing enzyme serine-threonine dehydratase, also known as threonine dehydratase. α-Ketobutyrate can then undergo oxidative decarboxylation to form propionyl-CoA, which can be converted to succinyl-CoA, a TCA cycle intermediate.

The major route of cysteine catabolism is a three-step pathway from cysteine to pyruvate. First cysteine is oxidized by iron-sulfur protein called cysteine dioxygenase to cysteinesulfinate. Transfer of an amino group from cysteinesulfinate to α-ketoglutarate forms β-sulfanylpyruvate and glutamate. Loss of sulfur dioxide, a process similar to decarboxylation of β-ketocarboxylic acids, leads to formation of pyruvate. Cysteinesulfinate is also a biosynthetic intermediate. Decarboxylation followed by dehydration produces taurine, a component of some bile acid.

Another pathway, cysteine transaminates with α-ketoglutarate to form β-mercaptopyruvate, which is converted to pyruvate by desulfuration reaction catalyzed by β-mercaptopyruvate transsulfurase.
Pathway to oxaloacetate

The two amino acids asparagine and aspartic acid, lead to formation of oxaloacetate. Asparagine is first hydrolyzed to aspartic acid and NH₃ by asparaginase. Asparaginase has wide distribution in animal and plant tissues. Injection of asparaginase into the blood stream has been found to be effective in combating leukemia in some patients, presumably the enzyme functions by limiting the availability of asparagine to the malignant white blood cells.

![Conversion of asparagine and aspartic acid to oxaloacetate](image)

Aspartic acid undergoes transamination with α–ketoglutarate to form oxaloacetate. In this way all four carbon atoms of these two amino acids can enter the TCA cycle.

In plants and some microorganisms aspartate undergoes direct elimination of NH₃ to yield fumarate, catalyzed by aspartate ammonia-lyase, also called aspartase, which is not present in animal tissues.

Pathways to α–Ketoglutarate

The carbon skeletons of five amino acids, proline, arginine, Histidine, glutamine and glutamate, enters the TCA cycle via α-ketoglutarate. The first four of these amino acids are converted to glutamate, which then undergoes transamination or oxidative deamination by glutamate dehydrogenase to yield α–ketoglutarate.
Fig. 11: Conversion of aspartic acid to fumaric acid

\[
\text{HOO} - \text{C} - \text{CH}_2 - \text{CH} - \text{COOH} \quad \text{Aspartic acid}
\]

Aspartate ammonia lyase

\[
\text{H} \quad \text{COOH} \quad \text{C} = \text{C} + \text{NH}_3 \quad \text{Ammonia}
\]

(Fumaric acid)

Proline        Arginine      Histidine     Glutamine

Glutamate

Transamination or oxidative deamination

\(\alpha\)-Ketoglutarate

Fig. 12: Proline, arginine, histidine and glutamine are first converted to glutamate, which is then converted to \(\alpha\)-ketoglutarate by transamination or oxidative deamination

Proline is converted to glutamate by three steps. First proline is dehydrogenated to pyrroline-5-carboxylic acid, by proline oxidase, which undergoes ring opening spontaneously to yield glutamic acid semialdehyde. An NAD-dependent dehydrogenase oxidizes the semialdehyde to glutamic acid.

Arginine is converted into ornithine by the action of arginase. This step is also employed in the synthesis of urea via urea cycle which occurring in the liver of mammals. Ornithine is then converted to glutamic acid semialdehyde by transamination reaction with \(\alpha\)-ketoglutarate catalyzed by ornithine transaminase. Oxidation of semialdehyde by NAD-dependent glutamate semialdehyde dehydrogenase yields glutamate.
Glutamine is converted to glutamate and ammonia in a single step. This reaction, which requires water, is catalyzed by glutaminase.
Fig. 14: Conversion of arginine to glutamate

Fig. 15: Conversion of glutamine to glutamate
The major pathway for the conversion of Histidine to glutamate is shown in figure. In the first step, α-amino group of histidine is removed by histidine ammonia lyase, also known as histidase, to form urocanate. Urocanase catalyzes 1,4-addition of water to urocanate to yield 4-imidazolone 5-propionate. The amide bond in the ring of this intermediate is hydrolyzed by imidazolone propionate hydrolase forming N-formimino derivative of glutamic acid. The tetrahydrofolate-dependent enzyme glutamate formiminotransferase shifts the formyl group to the coenzyme, producing N5-formiminotetrahydrofolate and glutamate.

Fig. 16: Conversion of histidine to glutamate
Pathways to acetyl-CoA via acetoacetate

Three amino acids, leucine, lysine and tryptophan are converted to acetyl-CoA via acetoacetate. The pathways of degradation of these amino acids are multistep process and only outline are given in the Figs 17 and 18.

Fig. 17: Conversion of leucine to acetoacetic acid and acetyl-CoA
Pathways to fumarate

Two amino acids phenylalanine and tyrosine are converted to fumarate and acetoacetate. The first step in the catabolism of phenylalanine is its conversion to tyrosine by phenylalanine 4-monooxygenase, also known as phenylalanine hydroxylase. This enzyme requires molecular oxygen and a cofactor, tetrahydrobiopterin. Hence the catabolic fate of these two amino acids is identical. Tyrosine is converted to p-hydroxyphenylpyruvate by tyrosine transaminase. This keto acid then reacts with molecular oxygen to form homogentisic acid. The enzyme catalyzing this reaction, p-hydroxyphenylpyruvate hydroxylase, is also called a dioxygenase because both atoms of O2 become incorporated into the product. This enzyme contains copper. This oxidation step is very complex and involves hydroxylation of the phenyl ring and decarboxylation, oxidation and migration of the side chain. The aromatic ring of homogentisic acid is cleaved by O2, which yields maleylacetoacetate. This reaction is catalyzed by homogentisate 1,2-dioxygenase, another dioxygenase. In fact, nearly all cleavage of aromatic rings in biological systems are catalyzed by dioxygenases. Maleylacetoacetate is then isomerized to fumarylacetoacetate by maleylacetoacetate isomerase. Cleavage of the fumarylacetoacetate yields fumarate, a TCA cycle intermediate, and acetoacetate by a reaction that resembles aldol cleavage. The
acetoacetate formed can be converted to acetoacetyl-CoA, which can be converted to acetyl-CoA.

**Fig. 19: Conversion of phenylalanine and tyrosine to acetoacetate and fumaric acid**

**Pathways to Succinyl-CoA**

Three amino acids methionine, valine and isoleucine are converted to succinyl-CoA. The carbon skeletons of these amino acids are degraded via propionyl-CoA and methylmalonyl-CoA to succinyl-CoA. The outlines of these pathways are given below in Fig. 20.

**Biosynthesis of amino acids**

Different organisms vary considerably in their ability to synthesize amino acids required for protein synthesis and in the forms of nitrogen utilized for this purpose. Some organisms, such as *E.coli*, can synthesize the necessary amino acids using a carbohydrate such as glucose as the sole carbon source. Certain photosynthetic bacteria are even more resourceful and can manufacture all of their cellular molecules from carbon dioxide. In contrast, the bacterium *Leuconostoc mesenteroides* cannot grow unless it is supplied with a total of 16 different amino acids. Vertebrate are not able to synthesize all the common amino acids, e.g. man and albino rats can make only 10 of the 20 amino acids required for protein synthesis. Higher plants can also make all the amino acids required for protein synthesis. There is also significant variation in the forms of source of nitrogen utilized for this purpose. For example, higher animals can utilize only reduced form NH3 as the nitrogen source. On the other hand higher plants are able to utilize ammonia, nitrate or nitrite as nitrogen source. The leguminous plants, which harbor symbiotic nitrogen-fixing bacteria in their root nodules, can utilize even atmospheric nitrogen as a source of nitrogen for biosynthesis of amino acids.
Nitrogen fixation

Atmospheric nitrogen is the source of nitrogen for the biosynthesis of all nitrogenous biomolecules for all living organisms. The atmosphere contains approximately 78% N₂, but this nitrogen is chemically unreactive or inert and therefore cannot be utilized by most living organisms. Certain bacteria and many cyanobacteria can reduce atmospheric nitrogen.
to ammonia (NH₃), which is chemically reactive. Symbiotic Rhizobium can invade the roots of leguminous plants and form root nodules in which N₂ is converted into NH₃. This conversion of atmospheric N₂ to ammonia is called nitrogen fixation. These microorganisms then either utilize this ammonia in the synthesis of nitrogen compounds, or they excrete it into the water or soil. Most ammonia in the soil is then converted into nitrate by oxidation. Plants take up nitrogen in the form of nitrate or ammonia and utilize it in the synthesis of amino acids and other organic nitrogen compounds. Animals obtain their nitrogen predominantly through the ingestion of plants. Through the expulsion of urea by animals and through the degradation of dead plants and animal materials, nitrogen is returned to the soil and eventually recycled.

The enzyme complex nitrogenase catalyzes the six-electron reduction of atmospheric nitrogen to ammonia. The nitrogen complex consists two proteins: a reductase, which provides electrons with high reducing power, and nitrogenase, which uses these electrons to reduce N₂ to NH₃. The reductase is also called iron protein, is a dimer of identical 30 Kd subunits bridged by a 4Fe-4S clusters. The nitrogenase is also called iron-molybdenum protein, is a tetramer of 220 Kd mol wt, with an αβ₂ ratio stoichiometry. The nitrogen fixation requires NADH and ferridoxin and is accompanied by the hydrolysis of 12 molecules of ATP per molecule of N₂ that is reduced.

\[
\text{Nitrogenase} \quad \begin{align*}
N_2 + 6H^+ + 6e^- + 12ATP + 12H_2O & \rightarrow 2NH_3 + 12ADP + 12Pi
\end{align*}
\]

The electron donor in this process is NADH, however the electron is transferred from NADH to oxidized ferridoxin. Reduced ferridoxin transfer these electrons to the iron protein of nitrogenase. Binding to ATP increases the reducing power of this protein, which enables it to pass electrons to the iron-molybdenum protein of nitrogenase, which is the center for nitrogen fixation. The iron-molybdenum protein stores electrons until there is sufficient electrical potential for electron transfer to N₂ and reduced it to NH₃. Nitrogenase is strongly inhibited by ammonia and can function only when the cell requires reduced nitrogen.

**Assimilation of nitrogen into biomolecules**

Two amino acids glutamate and glutamine play important role in the assimilation of nitrogen into biomolecules. Ammonia is incorporated directly into these two amino acids and these amino acids then serve as source of nitrogen in many biosynthetic pathways. Most amino acids receive their amino groups from glutamate by transamination reaction. Glutamine contributes its amido nitrogen atom in the biosynthesis of many important biomolecules including several amino acids.

The glutamate dehydrogenase catalyzes the incorporation of NH₃ into α-ketoglutarate to form glutamate. The NADPH is the reductant in this reaction.

\[
\text{Glutamate dehydrogenase} \quad \begin{align*}
\text{NH}_3 + \alpha\text{-ketoglutarate} + \text{NADPH} + \text{H}^+ & \rightarrow \text{Glutamate} + \text{NADP} + \text{H}_2\text{O}
\end{align*}
\]

The direct amination of \(\alpha\)-ketoglutarate by ammonia occurs only under condition of high ammonia concentration. But when the ammonia concentration is limited the formation of
the amino group of glutamate occurs not from ammonia but from the amide group of glutamine. In such case, the primary conversion from NH$_3$ to organic nitrogen is catalyzed by glutamine synthetase. The reactions catalyzed by glutamine synthetase require ATP hydrolysis. During this reaction, the γ-carboxylate group of glutamate attacks the γ-phosphoryl group of ATP, displacing ADP and producing the energy-rich, mixed anhydride γ-glutamyl phosphate as an enzyme bound intermediate. Ammonia then displaces phosphate from γ-glutamyl phosphate, forming glutamate.

\[
\text{Glutamine synthetase} \quad \text{Glutamate} + \text{ATP} + \text{NH}_3 \rightarrow \text{Glutamine} + \text{ADP} + \text{Pi}
\]

Glutamate dehydrogenase and glutamate synthetase are present in all living organisms. Most microorganisms also contain another enzyme, glutamate synthase, which catalyzes the reductive amination of α-ketoglutarate with the use of side-chain amide of glutamine as the nitrogen donor. Loss of the side-chain amide group converts glutamine to a second glutamate.

\[
\alpha\text{-Ketoglutarate} + \text{glutamine} + \text{NADPH} + \text{H}^+ \quad \text{Glutamate synthase} \quad 2 \text{Glutamate} + \text{NADP}^+
\]

When the supply of ammonia is limited, glutamine synthetase and glutamate synthase acts sequentially to provide a way to synthesize amino acids. However this is an energy requiring process on the other hand assimilation of ammonia to α-ketoglutarate by glutamate dehydrogenase does not require any supply of energy.

**Pathways for the biosynthesis of amino acids**

The pathways for the biosynthesis of amino acids are diverse and often vary from one organism to another. However, they all have an important feature in common: their carbon skeleton comes from key intermediates in central metabolic pathways like glycolysis, TCA cycle or the pentose phosphate pathway. The primary amino group usually comes from transamination of glutamate.

The amino acids can be grouped together into six biosynthetic families depending on the intermediate from which they are derived.

**i). Glutamate family:** Four amino acids belong to this family. These are glutamate, glutamine, proline and arginine. These are synthesized from α–ketoglutarate, which is the intermediate of TCA cycle. We have already discussed the biosynthesized of glutamate and glutamine.

**Biosynthesis of proline:** Proline is synthesized from glutamic acid (Fig. 21). In the first step of proline biosynthesis, the γ-carboxyl group of glutamate is phosphorylated by ATP catalyzed by γ-glutamyl kinase producing γ-glutamyl phosphate. The proline biosynthesis is regulated through negative feedback inhibition of γ-glutamyl kinase by proline. The γ–glutamyl phosphate is then reduced by NADPH to glutamate γ-semialdehyde. Glutamate γ-semialdehyde cyclizes nonenzymatically with loss of water to form pyrroline 5-carboxylate and is then reduced further to yield proline. This reaction is catalyzed by pyrroline 5-carboxylate reductase that require NADPH as a reductant.
Biosynthesis of arginine: It is synthesis from glutamate by a multistep pathway via ornithine (Fig. 22) and the urea cycle in animals. In the first step, the α-amino group of glutamate is blocked by an acetylation requiring acetyl-CoA to form N-acetylglutamate.
This reaction is catalyzed by N-acetylglutamate synthase. Arginine is a feedback inhibitor of this step. Phosphorylation of N-acetylglutamate, catalyzed by N-acetylglutamate kinase, yields N-acetylglutamyl phosphate and reduction of this product yields N-acetylglutamate \( \gamma \)-semialdeyde. Because of presence of acetyl group in this compound, it is not cyclizes to proline. Transamination followed by hydrolysis produces ornithine and releases acetate. Arginine is synthesized from ornithine as described in the urea cycle.

**Fig. 22: Biosynthesis of arginine from glutamate**

**ii). Aspartate family:** Six amino acids belong to this family. These are aspartate, asparagines, lysine, methionine, threonine, and isoleucine. These are synthesized from oxaloacetate, which is a intermediate of TCA cycle. Some microorganisms posses a second asparagine synthetase in which ammonia rather than glutamine is the amino donor fro the second step.
**Biosynthesis of aspartate and asparagine:** Aspartate is synthesized from oxaloacetate by the transfer of amino group from glutamate to the $\alpha$–carbon of oxaloacetate (Fig. 23). This reaction is catalyzed by aspartate transaminase. Asparagine is synthesized from aspartate by the addition of an amino group to the $\beta$–carboxylate group of aspartate from amido nitrogen of glutamine. This reaction is catalyzed by asparagine synthetase and requires input of energy through ATP. Some microorganisms possess a second asparagine synthetase in which ammonia rather than glutamine is the amino donor to the $\beta$-carboxylate group. This pathway is probably useful only when the concentration of ammonia is high.

![Fig. 23: Biosynthesis of aspartic acid and asparagine from oxaloacetate](image)

**Biosynthesis of lysine, threonine and methionine:** Aspartate gives rise to these three amino acids. Aspartate is first converted to aspartate $\beta$-semialdehyde, which is a two steps process. These reactions are catalyzed by aspartate kinase and aspartate $\beta$–semialdehyde dehydrogenase. Aspartate $\beta$–semialdehyde is a precursor for biosynthesis of lysine. Homoserine dehydrogenase catalyzes the NADPH-dependent reduction of the aldehyde to yield homoserine, which is the precursor of threonine and methionine (Fig. 24).

**Biosynthesis of threonine from homoserine:** The threonine is synthesized form homoserine by two enzymatic steps (Fig. 25). First, homoserine kinase catalyzes phosphorylation of hydroxyl group of homoserine from ATP producing O-phosphohomoserine. Threonine synthase, which is pyridoxal phosphate containing enzyme, catalyzes the movement of hydroxyl group to the adjacent $\beta$–position and converts O-
phosphoserine to threonine. Homoserine kinase is inhibited by threonine in most organisms.

**Fig. 24: Biosynthesis of homoserine from aspartate**

**Biosynthesis of methionine from homoserine**: Four steps are required to form methionine from homoserine (Fig. 26). In the first step, homoserine succinyltransferase catalyzes the transfer of succinyl group from succinyl-CoA to the hydroxyl group of homoserine to form O-succinylhomoserine. Higher plants use acetyl-CoA as the acyl donor in this step. In the
next step, cystathionine γ–synthase catalyzes the displacement of succinyl group by the sulphydryl group of cysteine producing cystathionine. Cystathionine β–lyase, which is pyridoxal phosphate containing enzyme, then catalyzes the hydrolysis of cystathionine to produce homocysteine and pyruvate an releasing of ammonia. Finally methionine is produced by methylation of sulphydryl group of homocysteine. This reaction is catalyzed by homocysteine methyl transferase and N5-methyltetrahydrofolate serves as the methyl group donor. In human, the coenzyme methylcobalamin, a derivative of vitamin B12, is required for the homocysteine methyltransferase reaction.

![Fig. 25: Biosynthesis of threonine from homoserine](image)

In bacteria and higher plants, the biosynthesis of lysine from aspartate β–semialdehyde and pyruvate occurs in eight steps as shown in Fig. 27.
Fig. 26: Biosynthesis of methionine from homoserine in certain bacteria
**Fig. 27: Biosynthesis of lysine from aspartate β-semialdehyde**

**Biosynthesis of Isoleucine:** The threonine is the precursor of isoleucine. Threonine is deaminated by a pyridoxal phosphate containing enzyme threonine deaminase to form α-ketobutyrate. Next four enzymes are responsible for the synthesis of isoleucine from α-
ketobutyrate and pyruvate are shown in Fig. 28. Four enzymes responsible for the biosynthesis of isoleucine are also required for the biosynthesis of valine.

Fig. 28: Biosynthesis of isoleucine from threonine and pyruvate
iii). Serine family: Three amino acids belong to this family. These are serine, glycine and cysteine. 3-Phosphoglycerate, a glycolytic intermediate, provides the carbon skeleton of serine. Glycine and cysteine are then derived from serine.

Biosynthesis of serine and glycine: Serine is synthesized from 3-phosphoglycerate in three steps (Fig. 29). In the first step, the hydroxyl group of 3-phosphoglycerate is oxidized by a NAD-dependent 3-phosphoglycerate dehydrogenase to form 3-phosphohydroxypyruvate. Phosphoserine transaminase then catalyzes the conversion of 3-phosphohydroxypyruvate to 3-phosphoserine, which is hydrolyzed to free serine by phosphoserine phosphatase. The end product, serine, inhibits 3-phosphoglycerate dehydrogenase, the first enzyme in the pathway.

![Fig. 29: Biosynthesis of serine from 3-phosphoglycerate](image)

Serine is the precursor of glycine. Glycine is synthesized for serine by a reversible reaction catalyzed by the pyridoxal phosphate containing enzyme serine hydroxymethyltransferase. This enzyme also requires the coenzyme tetrahydrofolate. Tetrahydrofolate accepts the β-
carbon of serine, which forms a methylene bridge between N-5 and N-10 to form N\textsuperscript{5},N\textsuperscript{10}-methylenetetrahydrofolate.

![Diagram of glycine biosynthesis](image)

**Fig. 30: Biosynthesis of glycine from serine**

Glycine is also synthesized from CO\textsubscript{2} and NH\textsubscript{3} by the action of glycine synthase, with N\textsuperscript{5}, N\textsuperscript{10}-methyltetrahydrofolate as methyl group donor.

\[
\text{CO}_2 + \text{NH}_3 + \text{NADH} + \text{H}^+ \rightarrow N_5^5, N_10^10-\text{methylenetetrahydrofolate}
\]

**Glycine synthase**

Glycine + NAD + tetrahydrofolate

---

**Biosynthesis of Cysteine:** Cysteine is also synthesized from serine by two steps (Fig. 31a). In the first step, serine acetyltransferase catalyzes acetyl group transfer from acetyl-CoA to the hydroxyl group of serine, producing O-serine. Next sulfide displaces the acetate group of O-acetylserine to produce cysteine. Only bacteria and plants produce the reduced sulfur required for synthesis of cysteine. Cysteine is a negative-feedback inhibitor of serine acetyltransferase. In addition to this allosteric regulation, both cysteine and sulfide repress the synthesis of serine acetyltransferase.

In mammals, cysteine is made from two amino acids methionine and serine, methionine provides sulfur atom and serine provides the carbon skeleton (Fig. 31b). Methionine is activated to S-adenosylmethionine by methionine activating enzyme methionine adenosyl transferase. S-Adenosylmethionine can lose its methyl group to any number of acceptors by methyltransferase to form S-adenosylhomocysteine. This dimethylated product, S-adenosylhomocysteine, is hydrolyzed to free homocysteine by adenosyl homocysteinase (Fig. 32). Homocysteine undergoes a reaction with serine to form cystathionine. This reaction is catalyzed by cystathionine \(\beta\)-synthase. Cystathionine \(\gamma\)-lyase then catalyzes removal of ammonia and cleavage of cystathionine to form cysteine and \(\alpha\)-ketobutyrate.
iv). Pyruvate family: Alanine, valine and leucine belong to pyruvate family. Pyruvate, a glycolytic intermediate, provides the carbon skeleton of these amino acids.

Biosynthesis of alanine: Alanine is synthesized from pyruvate in a single step. In this step amino group of glutamate is transferred to pyruvate by transamination reaction catalyzed by alanine transaminase.

\[
\text{Pyruvate + Glutamate} \xrightarrow{\text{Alanine transaminase}} \text{Alanine} + \alpha\text{-ketoglutarate}
\]

Pyridoxal phosphate

Fig. 31: Biosynthesis of cysteine from (a) serine; and (b) methionine and serine
**Biosynthesis of valine and leucine:** Valine is synthesized from pyruvate. The four enzymes are responsible for the biosynthesis of valine (Fig. 33), which is very similar to the biosynthesis of isoleucine as described earlier (Fig. 28.)

An intermediate in the valine pathway, α-ketoisovalerate, is the starting point of leucine biosynthesis. In the first step, α-isopropylmalate synthase catalyzes the addition of an acetyl group from acetyl-CoA to α–ketoisovalerate to from α–isopropylmalate. The β–hydroxyl group of α–isopropylmalate is then transferred to the α–carbon to form β-isopropylmalate catalyzed by α–isopropylmalate isomerase. Oxidation followed by decarboxylation of β–isopropylmalate catalyzed by isopropylmalate dehydrogenase produce α–ketoisocaproate. α–Ketoisocaproate is then converted to leucine by a transamination reaction with glutamate catalyzed by branch-chain amino acid transaminase.

**v). Aromatic family:** Phenylalanine, tyrosine and tryptophan are the aromatic family of amino acids. These amino acids are synthesized from two metabolites.
phosphoenolpyruvate, which is the intermediate of glycolytic pathway, and erythrose 4-phosphate, which is the intermediate of pentose phosphate pathway, respectively.

**Fig. 33:** Biosynthesis of valine from pyruvate and leucine from α–ketoisovalerate
Biosynthesis of Phenylalanine, tyrosine and tryptophan: Phenylalanine, tyrosine and tryptophan are synthesized by a common pathway. First a common intermediate, chorismate, is formed in a seven steps pathway from phosphoenolpyruvate and erythrose 4-phosphate as shown in Fig. 34. The pathway then diverted at chorismate and three different amino acids are synthesized by three different pathways from common precursor chorismate.

Fig. 34: Biosynthesis of chorismate from phosphoenolpyruvate and erythrose-4-phosphate

Chorismate is converted to prephenate, the immediate precursor of aromatic ring of phenylalanine and tyrosine, by chorismate mutase. Prephente can then be converted to
phenylalanine by two steps (Fig. 35). First, prephenate dehydrase catalyzes a 1,4-elimination with loss of carbon dioxide and water to form phenylpyruvate. Second, phenylalanine transaminase catalyzes transfer of an amino group from glutamate to phenylpyruvate to form phenylalanine.

Prephenate can also be converted to tyrosine in plants and bacteria in two steps (Fig. 35). Prephenate dehydrogenase catalyzes oxidative decarboxylation of prephenate to form 4-hydroxyphenylpyruvate. 4-Hydroxyphenylpyruvate can be converted to tyrosine by a transamination reaction with glutamate catalyzed by tyrosine transaminase. In some organisms, including humans, tyrosine can be synthesized from phenylalanine by hydroxylation reaction catalyzed by phenylalanine hydroxylase.

Chorismate can be converted to tryptophan in five steps as shown in Fig. 36.

**vi). Histidine family:** Histidine is the only amino acids in this family. This amino acid is synthesized from three precursors, ribose 5-phosphate, which is the intermediate of pentose phosphate pathway, which contributes five carbons, the purine ring of ATP contributes a nitrogen and a carbon, and glutamine supplies the second ring nitrogen. Histidine is
synthesized form ribose 5-phosphate in 11 steps as shown in Fig. 37. The key steps are condensation of ATP and PRPP, in which N-1 of the purine ring is linked to C-1 of the ribose of PRPP (step 1 or 2) in figure; purine ring opening that ultimately leaves N-1 and C-2 of adenine linked to the ribose (step 3); and formation of the imidazole ring, a reaction in which glutamine donates a nitrogen atom (step 5).

Inborn error of amino acid metabolism

Inborn error of amino acid metabolism is inherited metabolic disorder caused by the absence of an enzyme in a metabolic pathway. Several inborn errors of metabolism of amino acids have been discovered in human. In most such diseases specific intermediates accumulate, causing defective neural development and mental retardation. For example, alcaptonuria is an inherited metabolic disorder caused by the absence of homogentisate oxidase. In this patience a large amount of homogentisic acid is accumulated in the blood and is excreted in the urine due to absence of its degradation, which turns black on standing.

Fig. 36: Biosynthesis of tryptophan from chorismate in bacteria and plants
as homogentisate is oxidized and polymerize to a melanin-like substance. This condition produces few ill effects and individuals with acaptonuria are prone to develop a form of arthritis. In 1902, A Garrod first discovered that this condition is inherited, and he traced the caused to the absence of a single enzyme.

Fig. 37: Biosynthesis of histidine from ribose-5-phosphate on bacteria and plants
Phenylketonuria is another inheritable disease of phenylalanine catabolism and is perhaps the best known of the diseases of amino acid metabolism. Phenylketonuria is caused by an absence or deficiency of phenylalanine hydroxylase, or more rarely, of its tetrahydrobiopterin cofactor. A. Folling first discovered this disease in 1934. In the absence of this enzyme, phenylalanine is not converted to tyrosine and a secondary pathway of phenylalanine metabolism that is normally little used comes into play. In this minor pathway, phenylalanine undergoes transamination with α–ketoglutarate to yield phenylpyruvate, which accumulate in the blood and excreted in the urine. In childhood excess circulating phenylpyruvate or one of its metabolic byproducts, impairs normal development of brain, causing severe mental retardation. The worst effects of phenylketonuria can be avoided if the disease is detected at birth and the diet of the afflicted infant is regulated to provide only the minimum amount of phenylalanine necessary for development.

In maple syrup urine disease there is a block in the oxidative decarboxylation of α–ketoacids derived from valine, isoleucine and leucine caused by an absence or deficiency of branched-chain dehydrogenase. The urine of the patients has the odor of maple syrup—hence the name of the disease due to elevated level of branched chain amino acids and its α–ketoacid analogs in the blood and urine. These patients also suffer from mental and physical retardation unless the patients are placed on a diet low in valine, isoleucine and leucine early in their life.

A few of the many molecular diseases that result from inborn error of amino acid metabolism are summarized in Table.

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<th>Disease</th>
<th>Clinical symptoms</th>
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<td>Argininemia</td>
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<tr>
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<td>Tyrosine</td>
<td>Tyrosinase</td>
<td>Albinism</td>
<td>Absence of pigmentation</td>
</tr>
<tr>
<td>Lysine</td>
<td>α-Aminoadipic semialdehyde dehydrogenase</td>
<td>Hyperlysinemia</td>
<td>Seizures, mental retardation, lack of muscle tone, ataxia.</td>
</tr>
</tbody>
</table>
Suggested Reading

1. Principles of Biochemistry by Lehninger
2. Biochemistry by L. Stryer