UNIT 8 AMINO ACID AND NUCLEOTIDE METABOLISM

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8.1 INTRODUCTION

In Unit 2, we studied about the chemistry of proteins and amino acids. We studied that the amino acids are used for protein synthesis. Subsequently, in Unit 5 we studied about the digestion, absorption and transport of proteins and amino acids. We learnt that proteins do not immediately diffuse into the surrounding tissues of the blood stream, but first undergo a series of steps of biochemical reactions in the digestive tract. These reactions reduce the protein into its individual amino acids which are rapidly absorbed into the blood stream. Amino acids contain nitrogen, which cannot be stored, and therefore amino acids, which are in excess of biosynthetic needs of the cell, are degraded. This involves the removal of α-amino groups (amino groups attached to carbon atom next to the carboxyl carbon) by two processes. What are these processes? How are amino acids catabolized in the body? What are the specialized products formed from amino acids and what are their biological functions? These are a few aspects related to amino acid metabolism discussed in this unit.

The second part of the unit focuses on nucleotide metabolism. Nucleotides are important for being precursors of nucleic acids (DNA, RNA). Here in this unit we will learn how the purine and pyrimidine bases found in the nucleotides are synthesized and degraded. However, to understand the metabolism of nucleotides, it is essential to be thoroughly familiar with the nomenclature and the essential structures of the purine bases-adenine and guanine; the pyrimidine bases-cytosine, uracil and thymine; the nucleosides and deoxynucleosides derived from these; and the nucleotide and deoxynucleotide derivatives. We have already studied about these aspects earlier in Unit 2. We strongly suggest that you must revisit Unit 2, section 2.8 before reading this unit.

Objectives

After studying this unit, you will be able to:

- explain how amino acids are catabolized in the body,
- describe the synthesis of urea,
- discuss how α-keto acids are used for the production of energy,
- explain how non-essential amino acids are synthesized in the body,
- relate the specialized products formed from amino acids with their biological functions,
- describe how purines and pyrimidines are synthesized in the body,
- discuss the degradation process of purines and pyrimidines, and
- explain how gout occurs.

8.2 AMINO ACIDS METABOLISM

Nitrogen enters the body through a variety of compounds present in the food, the most important being amino acids present in dietary proteins. The primary role of amino acids is in the synthesis of tissue proteins and other biosynthetic reactions. Amino acids contain nitrogen, which cannot be stored, and therefore amino acids, which are in excess of biosynthetic needs of the cell, are degraded. This involves the following:

- the removal of α-amino groups (amino groups attached to carbon atom next to the carboxyl carbon) by two processes called *transamination* and *oxidative deamination*, forming ammonia and the corresponding α-keto acids, and
- the conversion of ammonia to urea in the Urea Cycle. A portion of the free ammonia is excreted in urine, and the remaining is excreted in urine after getting converted to urea.

The carbon skeletons (structure left after the removal of amino group) of the α-keto acids are converted to common intermediates of energy producing metabolic pathways. Lastly, the body has the capacity to produce certain specialized products from amino acids. A summary of nitrogen and amino acid metabolism is presented in Figure 8.1.

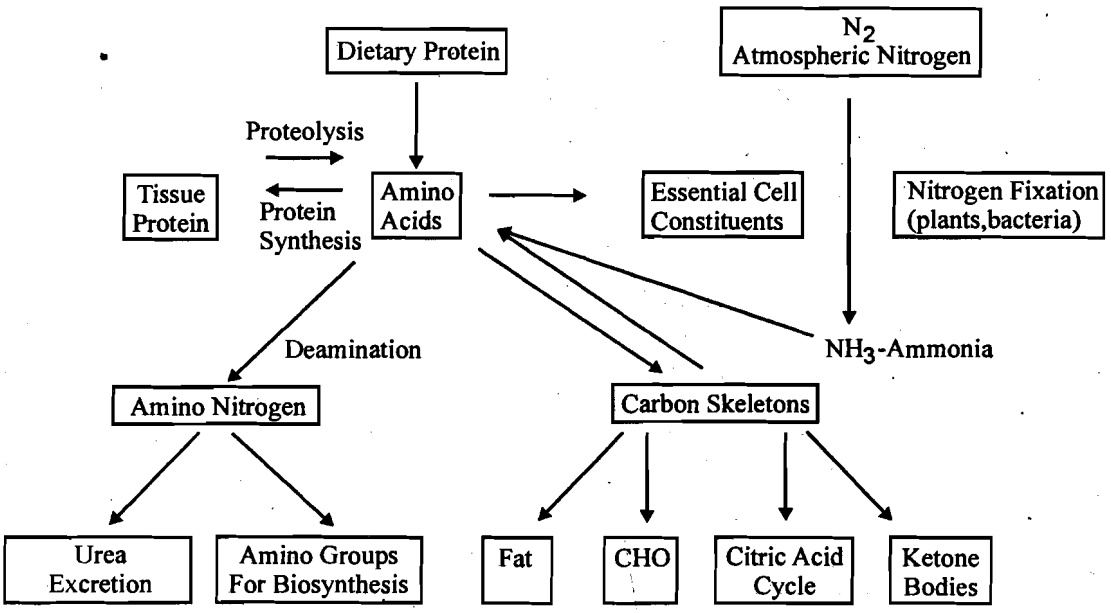


Figure 8.1: Nitrogen and amino acid metabolism

The amino acids that are released by the hydrolysis of dietary and tissue protein, mix with other amino acids distributed throughout the body. This is called as *amino acid pool*. The amino acid pool contains 100 g of amino acid and is very small in comparison to the amount of protein in the body. The concentration of free amino acids in the extracellular fluids is significantly lower than that within the cells of the body. The movement of amino acids from the extracellular space to the interior of the cells is by active transport mechanism which requires carrier molecule and energy from ATP, as you may recall reading in Unit 5.

The first step in the catabolism of all amino acids is the *removal of α-amino group*. Once removed, nitrogen can be incorporated into other compounds or excreted.

We learnt earlier in this unit that the removal of the α-amino group can be achieved by the two processes, *transamination* and *oxidative deamination*. We shall get to know about these processes next.

8.2.1 Transamination Reaction

Most common amino acids can be converted into the corresponding keto acid by *transamination*. In this reaction, there is a transfer of α-amino group of amino acid to keto acid (keto group present next to the carboxyl group) –R-CO-COOH. As a result, the amino acid becomes α-keto acid and the keto acid is converted into amino acid, as shown in Figure 8.2.

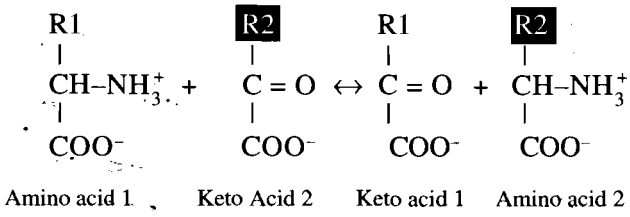
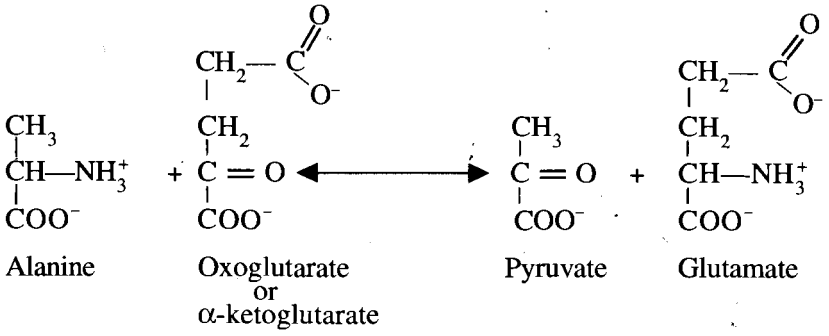


Figure 8.2 : Transamination reaction

α-ketoglutarate plays a significant role in amino acid metabolism by accepting the amino groups from other amino acids thus forming glutamate. The glutamate formed can be oxidatively deaminated (oxidized coupled with removal of ammonia) or can be used as an amino group donor in the synthesis of non essential amino acids. The reaction of transamination is catalyzed by *aminotransferases* (*transaminases*). The two most important transferases are:

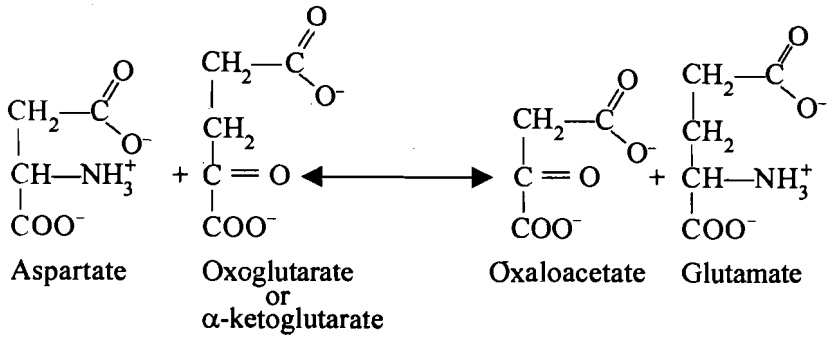
a) *Alanine aminotransferase*

Alanine + α-ketoglutarate ⇔ glutamate + pyruvate

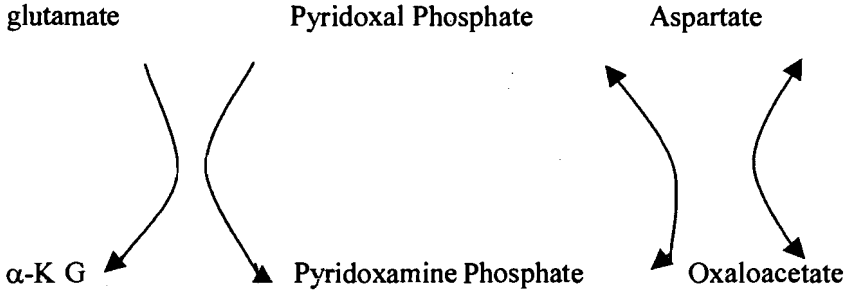


b) *Aspartate aminotransferase (AST)*

Asparate + α-ketoglutarate ⇔ oxaloacetate + glutamate



There are at least 13 different aminotransferases. All aminotransferases require the coenzyme pyridoxal phosphate (derived from vitamin B_6). You have already learnt about coenzymes, particularly about pyridoxal phosphate in Unit 4. Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid and regenerates back the coenzyme. The details of transamination reactions are given in the section on biochemical role of vitamins in Unit 10 later. Thus you can see that by transamination, one amino acid can be converted into another amino acid as shown herewith.



You may recall that the diagnostic value of plasma aminotransferases is discussed in Unit 4, section 4.11.

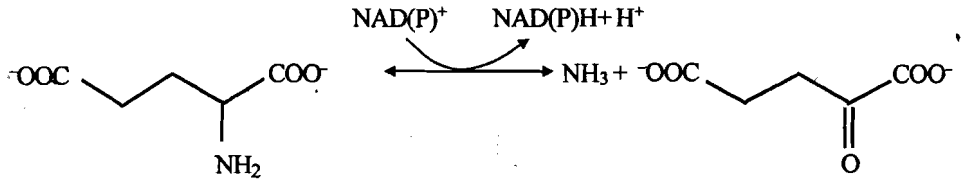
Next, let us get to know the deamination reaction.

8.2.2 Deamination Reaction

Deamination is *a process by which N of amino acid is removed as ammonia (NH_3).* These reactions occur primarily in liver and kidney. The reaction is catalyzed by the following two enzymes:

a) *Glutamate dehydrogenase*

Glutamate, as we have just seen above, is the major end product of transamination reactions. Further breakdown of glutamate occurs through the process of oxidative deamination. This is catalyzed by the enzyme *L-glutamate dehydrogenase* to form α-iminoglutaric acid, which on addition of a molecule of water forms NH_3 and α-ketoglutarate. (This enzyme requires NAD(P) provided by vitamin B_3(niacin) and is present in the mitochondrion). It is a reversible reaction as shown herewith :



$$\text{Glutamate} + \xrightarrow[\text{NAD(P)}^+]{H_2O} \alpha\text{-Ketoglutarate} + NH_3 + \text{NAD(P)H} + H^+$$

b) *Amino acid oxidase*

D-amino acids present in the diet are efficiently metabolized by the liver by the enzyme *amino acid oxidase*. Amino acid oxidases are of two types. *D-amino acid oxidase* (breaks down D-amino acid) and *L-amino acid oxidase* (which acts on L-amino acids). D-amino acid oxidase requires FAD (provided by vitamin B_2) as the cofactor. It liberates NH_3 and α-keto acids, which can enter the general pathway of amino acid metabolism.

However, the tissue proteins contain L-amino acids. These are catabolized by L-amino acid oxidases of liver and kidney which uses FMN (provided by vitamin B_2) as the coenzyme and once again as earlier, liberates NH_3 and α-keto acids. However, the activity of L-amino acid oxidase in the body is very little and hence this type of oxidative deamination is not the major pathway of amino acid catabolism. Then, how are the amino acids broken down? Primarily by the transamination process. The amino acids are converted to glutamate as you have already learnt and then the glutamate is catabolized by L-glutamate dehydrogenase. The activity of this enzyme is very high in the body.

The discussion so far centered on the removal of amino groups. The end product formed being ammonia and the corresponding α-keto acids. What happens to this ammonia in the body? The next section focuses on the conversion of ammonia into urea. Let us see how this is done.

8.2.3 Urea Cycle

From our discussion above, it is clear that the amino group of all amino acids is ultimately converted to ammonia (NH_3). Ammonia is highly toxic to the nervous system. Hence, it must be removed. How is this done? Basically, ammonia combines with CO_2 to form *urea*, which is not toxic to the body. Hence, one of the major end products of protein metabolism is *urea*.

Urea is the major disposal form of amino groups derived from amino acids and accounts for 90% of the nitrogen containing compounds of urine. One of the nitrogen of the urea molecule is supplied by free NH_3 and the other one by aspartate. Glutamate is the immediate precursor of both ammonia and aspartate nitrogen. The carbon and oxygen of urea are derived from CO_2. Urea is produced by the liver and is then transported in the blood to the kidneys for excretion in the urine. The steps involved in converting ammonia to urea include:

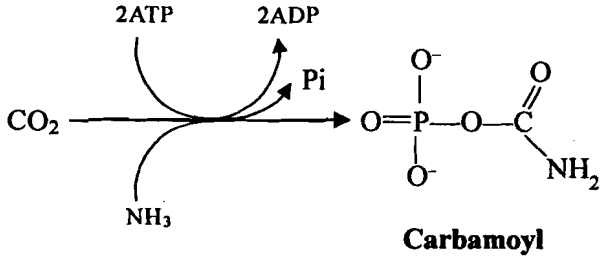
1) Ammonia + CO_2 + 2ATP ⟶ Carbamoyl-Phosphate
2) Carbamoyl-P + Ornithine ⟶ Citrulline
3) Citrulline + Aspartate ⟶ Fumarate + Arginine
4) Arginine ⟶ Urea + Ornithine

Most of our nitrogenous waste comes from the breakdown of amino acids. This occurs by deamination. Deamination of amino acids results in the production of ammonia (NH_3) as we learnt above. Ammonia is an extremely toxic base and its accumulation in the body would quickly be fatal. However, liver contains a system of carrier molecules and enzymes which quickly converts the ammonia (and carbon dioxide) into urea. This is called the 'urea cycle'. This entire sequence of urea cycle is discussed below, along with the enzymes involved in the synthesis of urea.

Carbamoyl Phosphate Synthetase (CPSI)

In the first step, the mitochondrial enzyme *Carbamoyl Phosphate Synthetase 1* converts the ammonia produced by glutamate dehydrogenase into carbamoyl phosphate. See Figure 8.3 which illustrates the entire urea cycle. The formation of carbamoyl phosphate requires 2 molecules of ATP and takes place in the matrix of mitochondria. The ammonium may come from glutamate (as learnt earlier by the action of L-glutamate dehydrogenase) or in free form (as NH_3) from blood, and the HCO_3^-

comes from respiration (CO_2 is hydrated to form carbonic acid, H_2CO_3 which ionizes to $H^+ + HCO_3^-$). NH_3 (as NH_4^+, ammonium ion) directly takes part in this reaction. Hence, this reaction is also called *ammonia-fixation reaction.*



Carbamoyl phosphate

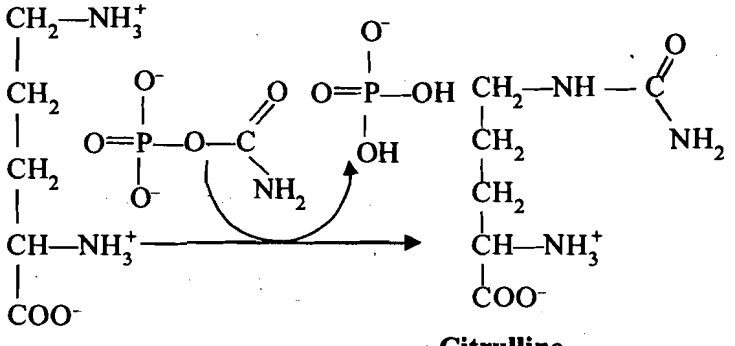
$$NH_4^+ + HCO_3^- + 2\ ATP \rightarrow NH_2\ COOPO_3^{2-} + 2\ ADP + Pi$$
(carbamoyl phosphate)

Pi is inorganic phosphate. ATP, as usual, functions as a magnesium-ATP complex.

Carbamoyl phosphate is next transferred to ornithine. Let us learn about this in the next step.

Ornithine Carbamoyl Transferase

In this reaction, carbamoyl moiety is transferred to ornithine to generate citrulline. *Ornithine transcarbamoylase* (OTC) catalyzes the condensation of ornithine with carbamoyl phosphate, producing citrulline. The energy for the reaction is provided by the high-energy anhydride of carbamoyl phosphate. The product, citrulline is then transported to the cytosol, where the remaining reactions of the cycle take place. Citrulline leaves the mitochondria by the same transport system that facilitates ornithines' entry from the cytoplasm.

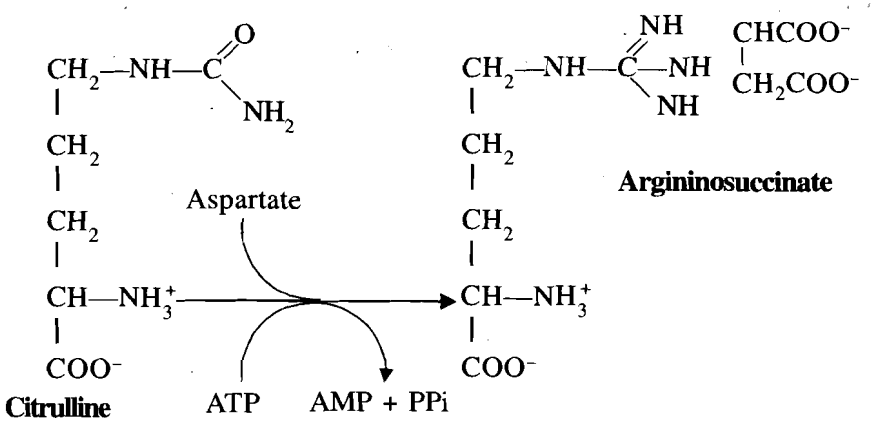


Ornithine **Citrulline**

Once in the cytosol, citrulline condenses with aspartate, as can be seen in the next step. Please note the enzymes for the remaining three steps are located in the cytoplasm (cytosol) of the cell. Hence, the citrulline formed in the mitochondria is transferred across the mitochondrial membrane. The next three enzymes functioning in the cytosol are as follows:

Argininosuccinate Synthetase

In a 2-step reaction catalyzed by the cytosolic enzyme *argininosuccinate synthetase*, citrulline and aspartate are condensed to form argininosuccinate. This reaction involves the addition of AMP (from ATP) to the amido carbonyl of citrulline, forming an activated intermediate on the enzyme surface (AMP-citrulline), and the subsequent addition of aspartate to form argininosuccinate as shown herewith.



Citrulline + Aspartate + ATP → Argininosuccinate + AMP + PPi

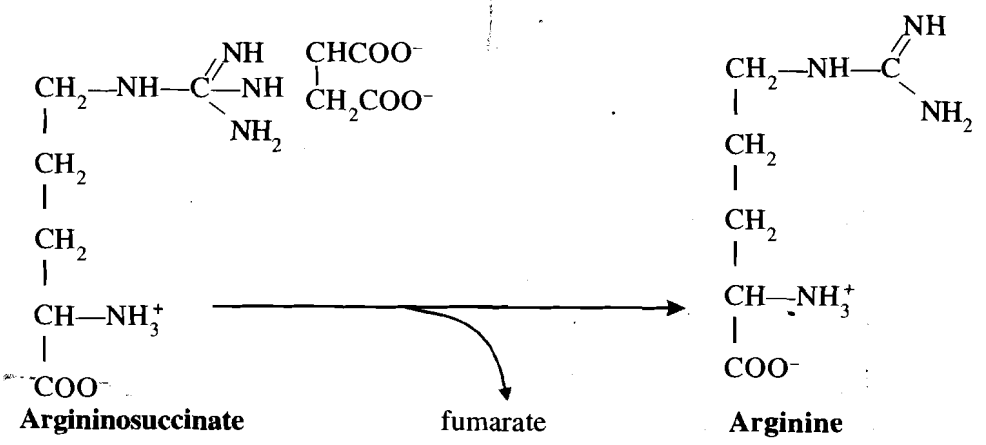
Inorganic pyrophosphate (PPi) consists of 2 phosphate groups. It is very unstable and is hydrolyzed by inorganic pyrophosphatase. This means breakdown of 2 phosphate groups releases energy for the reaction. Hence, this may be considered as a utilization of 2 ATP molecules.

$$PPi + H_2O \longrightarrow 2Pi$$

Elimination of fumarate from argininosuccinate then yields arginine, which is the next step in the urea cycle.

Argininosuccinate Lyase

Arginine and fumarate are produced from argininosuccinate by the cytosolic enzyme *argininosuccinate lyase* (also called *argininosuccinase*). It reversibly catalyzes the cleavage of argininosuccinate to fumarate and arginine.

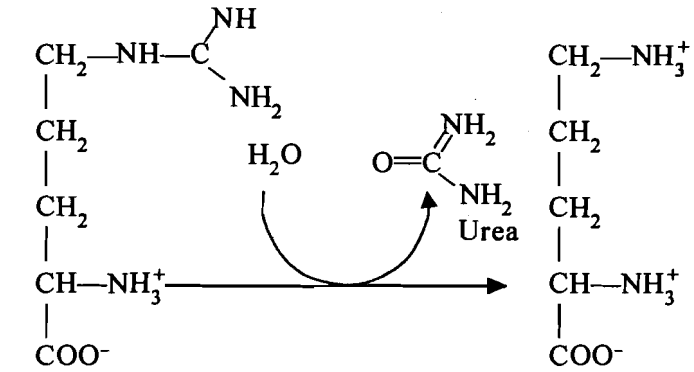


Argininosuccinate ⇔ Arginine + fumarate

This reaction also supplies arginine for protein synthesis. The fumarate, generated via the action of *argininosuccinate lyase*, is reconverted to aspartate for use in the *argininosuccinate synthetase* reaction earlier.

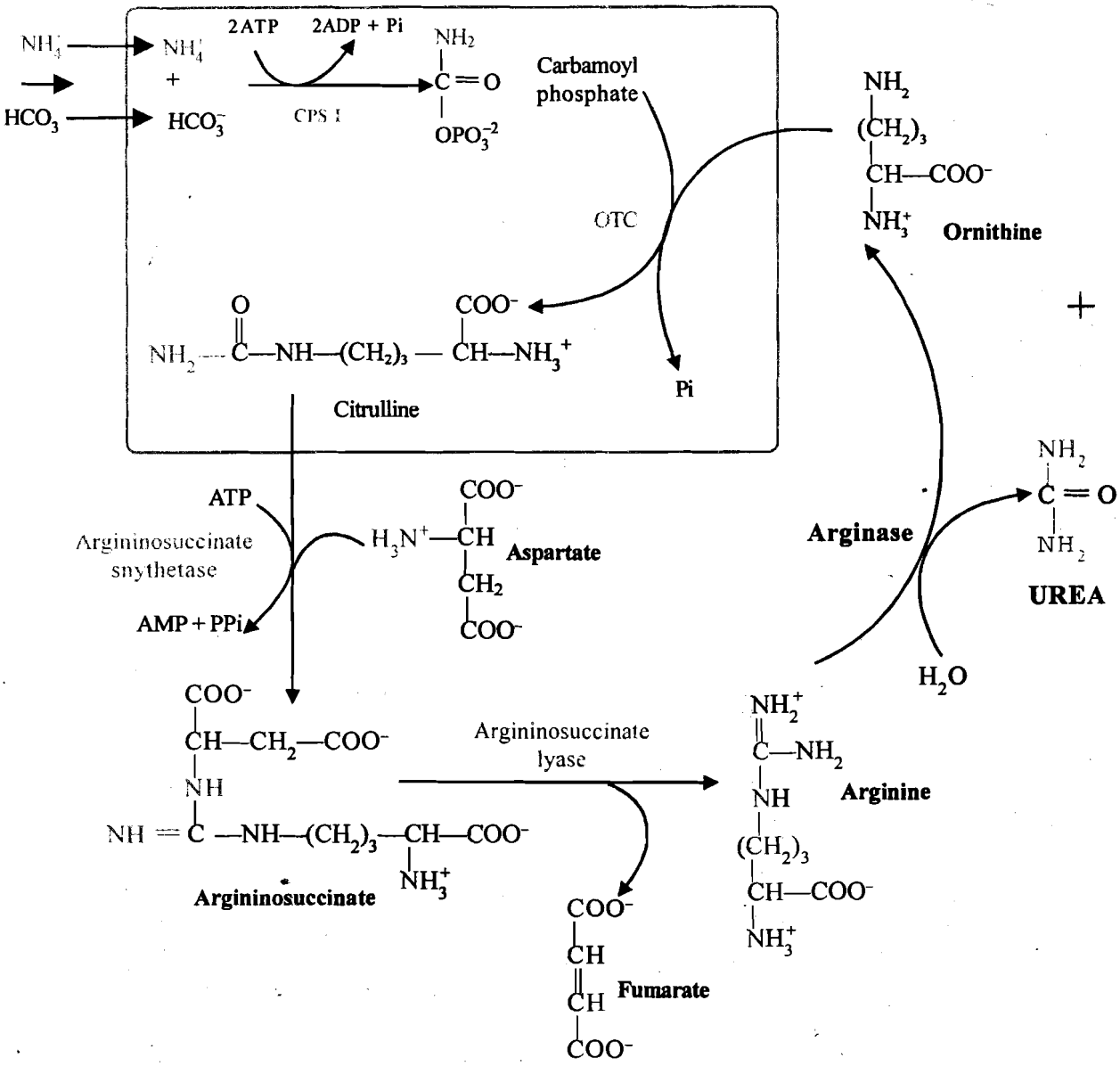
Arginase

In the final step of the cycle, the cytosolic enzyme *arginase* cleaves arginine (from the diet or from protein breakdown), generating urea and ornithine. The enzyme *arginase* cleaves urea from arginine, regenerating cytosolic ornithine, which can be transported to the mitochondrial matrix for another round of urea synthesis. Hence, this last enzyme of urea cycle catalyzes the hydrolytic cleavage of arginine to urea and ornithine. Ornithine, as you can see, is regenerated to be used again.



Arginine + H_2O ⟶ Urea + Ornithine

The urea passes via a transport protein into the blood and is carried to the kidneys where it enters the glomerular filtrate, from which it is excreted in the urine. The entire urea cycle is presented in Figure 8.3.



CPS-I is carbamoyl phosphate synthetase-I, OTC is ornithine transcarbamoylase.

Figure 8.3: The urea cycle

With our discussion on the urea cycle, we come to an end of our study about the catabolism of amino acids. In Figure 8.1 you recall reading about the carbon skeletons of the α-keto acids (the structure left after removal of the amino group). What happens to this carbon skeleton of the amino acids? The next section deals with the metabolism of the carbon skeleton of amino acids. But first let us recapitulate what we have learnt so far.

Check Your Progress Exercise 1

1) List the two processes involved in the degradation of amino acids in our body.

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2) What are the transamination reactions? Give any one example.

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3) Name the two enzymes involved in deamination reaction. Where do these reactions occur in our body?

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4) How is ammonia removed from our body? What is this process called?

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5) Indicate the various enzymes and coenzymes involved in the urea cycle.

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8.2.4 Metabolism of Carbon Skeletons of Amino Acids

The catabolism of 20 amino acids found in proteins involves, as seen above, the removal of α-amino group. This is followed by the breakdown of the remaining product, which is referred to as the carbon (C) skeletons. The catabolism of C skeletons results in the formation of seven different products. These are *oxaloacetate, α-ketoglutarate, pyruvate, fumarate, acetyl CoA, acetoacetyl CoA* and *succinyl CoA*. Table 8.1 gives the products formed by the amino acids.

Table 8.1: Products formed by the amino acids

Product formed	Amino acid forming the product
Oxaloacetate (OAA)	Asparagine, Aspartate
α-ketoglutarate (α-KG)	Glutamine, Glutamate, Proline, Arginine, Histidine
Pyruvate	Alanine, Serine, Glycine, Cystine, Threonine
Fumarate	Phenylalanine, Tyrosine
Succinyl CoA (Succ-CoA)	Methionine, Valine, Isoleucine, Threonine
Acetyl CoA or acetoacetyl CoA	Leucine, Isoleucine, Lysine, Tryptophan

These products can enter the various metabolic pathways as shown in Figure 8.4.

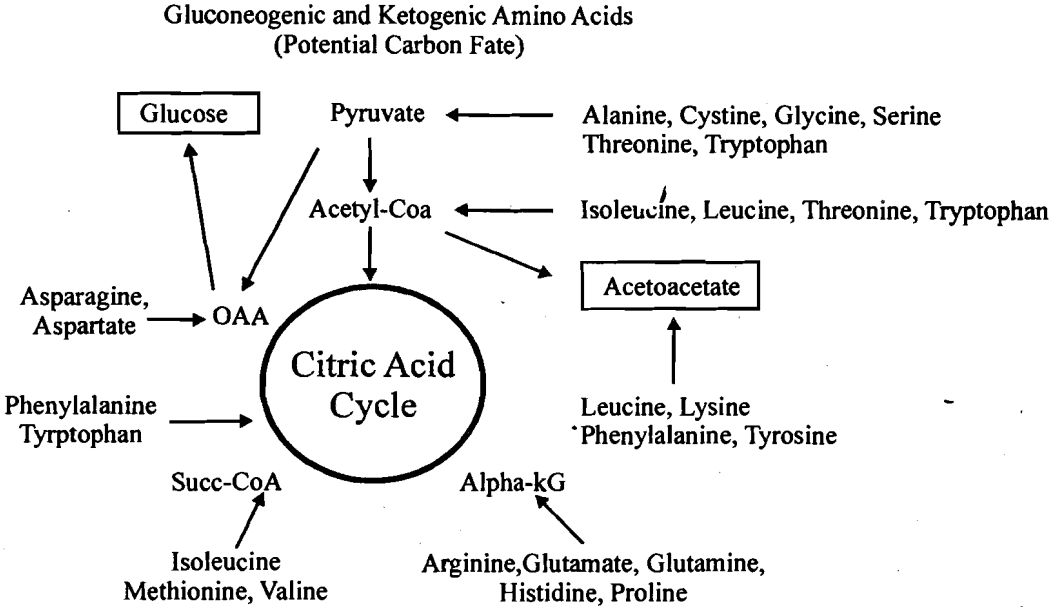


Figure 8.4 : Metabolic pathways of products of carbon skeleton

The 20 amino acids can be classified as either *glucogenic* or *ketogenic* amino acids. Some fall into both categories. Let us understand the terms glucogenic and ketogenic in relation to amino acid metabolism.

Ketogenic and Glucogenic Amino Acids

Amino acids can be classified as ketogenic or glucogenic, according to the nature of their metabolic end products as described herewith:

a) *Ketogenic*: Amino acids whose catabolism yields either acetoacetate or one of its precursors – acetyl CoA or acetoacetyl CoA – are termed *ketogenic*. This is because acetoacetate is commonly referred to as *ketone body*. The ketogenic amino acids are degraded to intermediates that can be utilized in the formation of ketone bodies. These products are: acetyl CoA and acetoacetyl CoA.

b) *Glucogenic*: Amino acids whose catabolism yields pyruvate or one of the intermediates of the citric acid cycle are termed 'glucogenic' or 'glycogenic'. This is because these intermediates can act as substrates for gluconeogenesis (synthesis of glucose) and therefore can give rise to the net formation of glycogen in liver and muscle. The glucogenic amino acids are degraded to intermediates that can be utilized in the formation of *glucose*. These products are: pyruvate, oxaloacetate, succinyl CoA, α-ketoglutarate and fumarate.

Table 8.2 gives the classification of amino acids into glucogenic and ketogenic amino acids.

Table 8.2 : Glucogenic and ketogenic amino acids

Glucogenic		Glucogenic + Ketogenic	Ketogenic
Alanine	Proline	Tyrosine	Leucine
Asparagine	Serine	Isoleucine	Lysine
Aspartate	Arginine	Phenylalanine	
Cysteine	Histidine	Tryptophan	
Glutamate	Methionine		
Glutamine	Threonine		
Glycine	Valine		

Next, what is the fate of the products (as highlighted in Table 8.1 earlier) formed by the catabolism of the carbon skeletons? How are these products further metabolized? Let's find out. *Oxaloacetate*, *α-ketoglutarate*, *fumurate* and *succinyl CoA* are intermediates of the citric acid cycle as you may have noticed in Figure 8.4. Hence they can be oxidized to CO_2 and H_2O, or if the blood glucose level is low, they can undergo gluconeogenesis to form glucose. Similarly, *pyruvate* can go through the reversal of glycolysis and form glucose or get converted to acetyl CoA which can be further oxidized to CO_2 and H_2O in the citric acid cycle. Thus, the nutritional state of the individual (well-fed or starvation) will determine the intermediary metabolic pathway taken by these products. *Acetoacetyl CoA* is oxidized to acetyl CoA. Acetyl CoA is further oxidized to CO_2 and H_2O in the citric acid cycle.

With this, we have completed the full circle of amino acid catabolism. To summarize what we have learnt so far : the potentially toxic nitrogen of amino acids is eliminated via transaminations, deamination and urea formation, the carbon skeletons are generally conserved as carbohydrate, via gluconeogenesis, or as fatty acid via fatty acid synthesis pathways. In this respect, amino acids fall into three categories: *glucogenic, ketogenic* or *glucogenic* and *ketogenic*. Glucogenic amino acids are those that give rise to a net production of pyruvate or TCA cycle intermediates, such as α-ketoglutarate or oxaloacetate, all of which are the precursors to glucose via gluconeogenesis. Lysine and leucine are the only amino acids that are solely ketogenic, giving rise only to acetyl CoA or acetoacetyl CoA, neither of which can bring about net glucose production.

A small group of amino acids comprised of isoleucine, phenylalanine, threonine, tryptophan and tyrosine give rise to both glucose and fatty acid precursors and are thus characterized as being glucogenic and ketogenic. Finally, it should be recognized that amino acids have a third possible fate. During times of starvation, the reduced carbon skeleton is used for energy production, with the result that it is oxidized to CO_2 and H_2O.

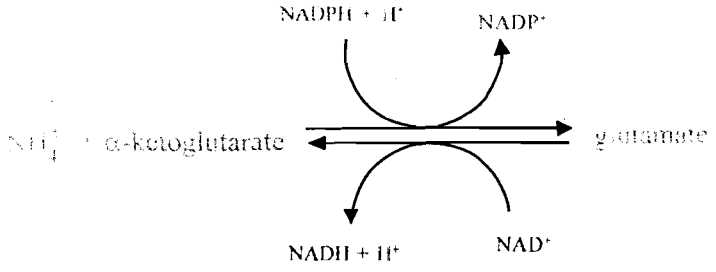
So far we have studied about the catabolism of proteins, amino acids. Next we shall study about the anabolic activity i.e. biosynthesis of amino acids. In fact, the next few sections focuses on the synthesis of amino acids and specialized products from amino acids, formation of biogenic amines from various amino acids and lastly, the non-protein functions of amino acids. We start with biosynthesis of nonessential amino acids.

8.2.5 Biosynthesis of Nonessential Amino Acids

This label 'nonessential' may mislead some people into believing that we don't need it. But, in essence, "non essential" as you may already know, means only that the body can synthesize this amino acid. It does not mean "unimportant." The nonessential amino acids can be synthesized in sufficient amounts from the intermediates of metabolism or from essential amino acids. These synthetic reactions are given next.

a) *Synthesis from α-keto acids*

Alanine, aspartate and glutamate are synthesized by the transfer of an amino group to the corresponding α-keto acids which are pyruvate, oxaloacetate and α-ketoglutarate, respectively, as discussed in transamination reaction earlier in sub-section 8.2.1. Glutamate and aspartate are synthesized from their widely distributed α-keto acid precursors by the simple 1-step transamination reactions. The former is catalyzed by *glutamate dehydrogenase* and the latter by *aspartate aminotransferase.*



Reactions of glutamate dehydrogenase

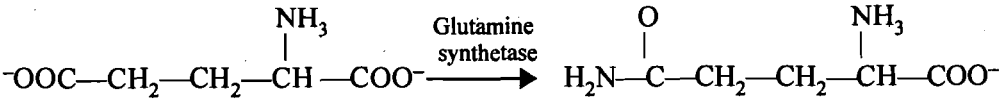
There are 2 main pathways to the production of muscle alanine: directly from protein degradation and via the transamination of pyruvate by *glutamate-pyruvate aminotransferase* as shown herewith

glutamate + pyruvate <·····> α-ketoglutarate + alanine

b) *Synthesis by amidation (introduction of NH_3 into COOH group)*

Glutamine, asparagine, proline, serine, glycine, cysteine and tyrosine is formed by amidation. Let us learn how.

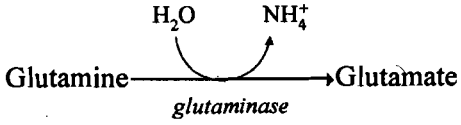
i) Glutamine is synthesized from glutamate by *glutamine synthetase.* NH_3 combines with glutamate to form glutamine catalyzed by the enzyme glutamine synthetase as presented in the reaction below:



L-glutamate L-glutamine

In this reaction, NH_3 is substituted in the carboxyl (COOH) group of glutamate giving rise to the amide ($CONH_2$) group, thus glutamine is the amide of glutamate. In this way, glutamine synthase fixes NH_3 as glutamine. Hence, this reaction is also called '*ammonia-fixation reaction*'. You may recall reading about this earlier in the urea cycle. *Note, glutamine is the transport form of NH_3.*

The *glutamine synthetase* reaction is also important in several respects. First, it produces glutamine, one of the 20 major amino acids. Its role is to carry ammonia to and from various tissues but principally from peripheral tissues to the kidney, where the amide nitrogen is hydrolyzed by the enzyme *glutaminase* as can be seen in the reaction below. This process regenerates glutamate and free ammonium ion (NH_4^+) which is excreted in the urine.



Note that, in this function, ammonia arising in peripheral tissue is carried in a nonionizable form which has none of the neurotoxic or alkalosis-generating properties of free ammonia.

ii) Asparagine is formed from aspartate by *asparagine synthetase*.

iii) *Proline*: Proline is formed from glutamate.

iv) *Serine, glycine* and *cysteine* are formed as discussed herewith:

1) Serine: It is formed from 3-phosphoglycerate which is an intermediate of glycolysis.

2) Glycine: It is synthesized from serine. The main pathway to glycine is a 1-step reaction catalyzed by *serine hydroxymethyltransferase*. This reaction involves the transfer of the hydroxymethyl group from serine to the cofactor tetrahydrofolate (THF), producing glycine. In fact serine and glycine are readily interconvertible.

3) Cysteine: It is formed from methionine. In fact the sulfur for cysteine synthesis comes from the essential amino acid methionine. Thus while methionine is an essential amino acid, cysteine is a nonessential amino acid.

v) *Tyrosine*: It is formed from phenylalanine by the enzyme *phenylalanine hydroxylase*. Tyrosine is produced in cells by hydroxylating the essential amino acid phenylalanine. This relationship is much like that between cysteine and methionine. Half of the phenylalanine required goes into the production of tyrosine; if the diet is rich in tyrosine itself, the requirements for phenylalanine are reduced by about 50%. Note, tyrosine can be readily synthesized in the body while phenylalanine cannot be synthesized and must be provided in the diet.

After the study of the biosynthesis of nonessential amino acids, we move on to learn about the synthesis of specialized products from amino acids. Amino acids function as precursors for many nitrogen containing compounds. Which are these compounds and what is the role of amino acids in their formation? Let's find out. But, first let us check how much we have grasped from our study so far.

Check Your Progress Exercise 2

1) What is the metabolic fate of amino acids after the removal of α-amino group?

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2) What is meant by 'ketogenic' and 'glucogenic' amino acids? Give two examples of each type.

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3) Explain how the nutritional state of an individual affects the metabolic pathway of products formed by the catabolism of C-skeletons.

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4) What are the ways by which nonessential amino acids can be synthesized? Give examples.

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8.2.6 Synthesis of Specialized Products from Amino Acids

In addition to serving as building blocks for proteins, amino acids are precursors of many nitrogen containing compounds that have important biological functions. These are:

a) ***Synthesis of porphyrins*** - Porphyrins are *the cyclic compounds that readily bind metal ions*, usually Fe^{2+} or Fe^{3+}. The most common metalloporphyrin in humans is *heme*, which is the prosthetic group for the following compounds:

- *Haemoglobin* - You may already know that haemoglobin is present in the red blood cells (RBC) and transports oxygen from lungs to tissues.
- *Myoglobin* - It is present in muscles and stores oxygen as a reserve against oxygen deprivation.
- *Cytochromes* - They take part in oxidation-reduction reactions since they contain iron which can accept electrons to exist in the ferrous (Fe^{2+}) state or give up electrons to exist in ferric (Fe^{3+}) state. Thus several cytochromes b_1, c_1, c, a and a_3 are present in the mitochondrial respiratory chain acting as carriers of electrons. You shall read more about this later in Unit 11.
- *Catalase* - It is an enzyme found in blood, bone marrow, mucous membrane, kidney and liver. The function is to destroy the highly toxic hydrogen peroxide (H_2O_2) formed during various oxidation reactions in the body to the non-toxic water (H_2O)

$$2\ H_2O_2 \longrightarrow 2H_2O + O_2$$

- *Tryptophan pyrrolase* - It is an enzyme which catalyses the first step in the degradation of tryptophan, which is an oxidative reaction requiring oxygen. Hence, this enzyme is also called tryptophan oxygenase.

b) ***Synthesis of creatine*** – Creatine phosphate, *the phosphorylated derivative of creatine* is found in muscles. It is a high energy compound that can reversibly donate a phosphate group to ADP to form ATP. It is synthesized from glycine.

c) ***Synthesis of histamine*** – Histamine is a powerful *vasodilator* (causing relaxation of the smooth muscle of the vascular system) and is formed by the decarboxylation of the amino acid histidine. It is secreted by *mast cells* (present in the connective tissue) as a result of allergic reactions or trauma. It is a chemical messenger that mediates a wide range of cellular responses.

d) ***Synthesis of serotonin*** – It is also called 5-hydroxytryptamine and is synthesized from *tryptophan* (the aromatic amino acid) and involves decarboxylation reaction. Serotonin functions as a neurotransmitter.

e) ***Synthesis of catecholamines*** – Dopamine, norepinephrine (noradrenalin) and epinephrine (adrenalin) are biologically active amines (containing amino groups) that are collectively termed as *catecholamines*. They are classified as *hormones*. Dopamine and norepinephrine function as neurotransmitters in the brain and the autonomic nervous system. Norepinephrine and epinephrine are synthesized in the *adrenal medulla* (the inner, reddish-brown portion of the adrenal glands that synthesizes, stores, and releases epinephrine and norepinephrine). The catecholamines are synthesized from tyrosine (tyrosine → dopamine→ norepinephrine → epinephrine).

f) ***Synthesis of melanin*** – Pigment melanin is also synthesized from tyrosine in a multi-step pathway.

8.2.7 Decarboxylation Reaction and Biogenic Amines

What are biogenic amines? Any of a group of naturally occurring, biologically active amines, such as norepinephrine, histamine and serotonin, that act primarily as neurotransmitters and are capable of affecting mental functioning and of regulating blood pressure, body temperature and other bodily processes are called *biogenic amines*. Amines are usually formed by the *decarboxylation* of amino acids. *Decarboxylation* is the reaction by which CO_2 is removed from COOH group of an amino acid. As a result, an amine is formed. The reaction is catalyzed by the enzyme *dicarboxylase*, which requires pyridoxal phosphate (vitamin B_6) as a coenzyme. Biogenic amines formed from various amino acids and their biologic importance is given in Table 8.3.

Table 8.3: Biogenic amines and their functions

Amino acids	Amine	Biologic importance
Tyrosine	Tyramine	• Increases blood pressure (Vasoconstriction) • Contracts uterus
Tryptophan	Tryptamine	• Tissue hormone: a derivative of 5-OH Tryptamine (Serotonin) • Vasoconstriction
	5-methoxy Tryptamine (Melatonin)	• Increases blood pressure • Hormone of pineal gland
Histidine	Histamine	• Vasodilator, decreases blood pressure • HCl↑ • Pepsin ↑
Serine	Ethanolamine	• Forms choline by three methylations • Constituent of phospholipids like cephalin
Threonine	Propanolamine	• Constituent of vitamin B_{12}
Cysteine	β-mercaptoethanolamine	• Constituent of coenzyme A
Aspartic acid	β-alanine	• Constituent of pantothenic acid (coenzyme A) • As a constituent of dipeptide carnosine and anserine (they activate myocin, the muscle protein, ATP-ase activity and also enhance copper uptake)
Glutamic acid	γ-amino butyric acid (GABA)	• Presynaptic inhibitory neuro-transmitter in brain • Forms a bypass in citric acid cycle (GABA-shunt)
3,4,di-OH-phenylalanine (DOPA)	Dopamine	• Precursor of the hormones epinephrine and norepinephrine
Cysteine	Taurine	• Constituent of bile acid, taurocholic acid

Next, in our study of amino acid metabolism, we shall look at the non-protein functions of amino acid. Yes, the non-protein functions of amino acids. The well-known function of amino acid is as the building block or the basic unit of proteins. Additionally, amino acids perform other functions as well without being a part of the protein molecule. Let us learn about these functions next.

8.2.8 Non-protein Functions of Amino Acids

The non-protein functions of amino acids are presented in the following section. It is interesting to note that the non-protein functions of amino acids are suppressed when the diet is low in protein. The functions include:

1) *Immunity:* Amino acids are involved in giving immunity by maintaining the vulnerable surfaces of the body in such a way so as to resist infections. Most of the external agents that cause damage to the human system enter through either the a) lung or b) gastrointestinal tract. Both these organs are protected by the mucus membrane, which offers resistance against the invasion of microorganisms and has the ability to stop the growth of microorganisms. The amino acid which is of importance and found in the mucous membrane is *threonine.* The mucus protein synthesis is reduced in *Protein Energy Malnutrition (PEM).* It is estimated that about 60% of the adult requirements for threonine is involved in maintaining the mucus protein synthesis to optimum. If threonine is deficient in diet, then the mucus membrane has a compromised immune system, where sufficient immunity is not provided against the invasion of foreign organisms by the mucus membrane.

2) *Acute phase proteins (fighting functions):* When there is an infection in the body, the acute phase proteins are released by the liver in higher amounts into the blood circulation, which will provide immunity against these infections, e.g. serum ferritin is an acute phase protein. During infections, the ferritin levels go up.

3) *Synthesis of Glutathione (GSH):* Glutathione is a tripeptide consisting of three amino acids: (1) Glutamine (2) Cysteine and (3) Glycine. This is a very important agent in inactivating free radicals like *peroxides, superoxide anion, fatty acids* etc. If these free radicals are not removed by glutathione, then they damage the cell membrane and alter the cell membrane permeability. Thus, GSH prevents the release of free radicals. If superoxide anion (O_2^-) (it is generated when a single electron is transferred to oxygen) has access to lipids, it can oxidize poly unsaturated fatty acids (PUFA) in cell membrane and one PUFA oxidized can trigger the oxidation of other PUFAs, thus giving rise to free radical chain reaction. PUFA can take part in immediate chain reactions and cause damage. So the free radicals have to be scavenged or quenched (destroyed). Many of the age-related functions like loss of mental functions, memory occurs because of the oxidative damage. Also, cardio vascular disease (CVD), cancer etc. may be caused earlier in individuals with oxidative damage. *Glutathione* is an antioxidant. Erythrocyte glutathione is reduced in children with kwashiorkor. It is also lower in low birth weight infants.

4) Glutamine, which is an amide of *glutamic acid,* as you learnt earlier, is needed for the regulation of protein turnover in muscle. It is also needed for muscle function. During trauma and infections, glutamine levels are lower and provision of glutamine helps in recovery. As already discussed earlier, glutamine is also the transport form of NH_3.

5) Taurine derived from cysteine is a β-amino acid. Taurine provides resistance against the peroxides that are formed. So, some toxic products can be inactivated by taurine. The levels of taurine in breast milk are higher, providing a protective function from free radicals.

6) Creatine is a compound that chiefly arises from glycine, arginine and a source of methyl group and is catabolized to creatinine. Creatine is present as creatine phosphate in muscle. The function of creatine is to provide quick energy to muscles. Some of these functions are impaired in PEM.

7) Nitric oxide (NO) is the metabolic product of arginine. It has gained importance as a lot of functions have been discovered, involving virtually every tissue of the body. They are:

   - maintenance of vascular tone and blood pressure
   - inhibition of adhesion (sticking together), activation and aggregation of platelets
   - involvement in the higher level cognitive functions, and
   - participation in immunoprotection.

It appears that NO acts through macrophages, moves towards the place, where the

organism is present and then brings about macrophage killing. Nitric oxide stimulates macrophage killing. The level of arginine required to produce level of nitric oxide should be such that it will provide the most benefits e.g. DHEA (n_3 fatty acid).

With this, we come to an end of our study on amino acid metabolism. Next we shall learn about nucleotide metabolism.

Check Your Progress Exercise 3

1) What specialized products are synthesized from amino acid?

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2) What are biogenic amines? Name any two physiologically important biogenic amines.

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3) Which coenzyme is required for decarboxylation reaction?

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4) Enumerate the non-protein functions of amino acid. Explain the role of GSH as an antioxidant.

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5) What is glutathione? Which amino acids are present in it?

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8.3 NUCLEOTIDE METABOLISM

The nucleotides i.e. ribonucleoside and deoxyribonucleoside phosphate are essential for all cells. Without them, DNA and RNA cannot be produced, with the result the cells cannot proliferate and proteins cannot be synthesized. Further, nucleotides serve as carriers of activated intermediates in the synthesis of carbohydrates, lipids and proteins. They are also structural components of a number of essential coenzymes such as coenzyme A, FAD, FMN, NAD^+ and $NADP^+$. Nucleotides also play an important role as "energy currency" of the cell. Lastly, nucleotides are important regulatory compounds for many of the pathways of intermediary metabolism by either inhibiting or activating key enzymes.

We learnt earlier in Unit 2 that nucleotides consist of a *nitrogenous base* which may be either a purine (adenine or guanine) or a pyrimidine (cytosine or uracil or thymine) base, The purine and pyrimidine bases found in the nucleotides can be synthesized de novo or can be obtained through salvage pathways and reuse those we already have. The salvage pathways, in fact, are a major source of nucleotides for synthesis of DNA, RNA and enzyme co-factors. We shall learn about purine and pyrimidine synthesis next.

8.3.1 Purine Nucleotide Synthesis - De Novo Synthesis

Look at Figure 8.5. This is the structure of purine ring. The atoms of the purine nucleotide ring are contributed by a number of compounds including amino acids such as aspartic acid, glycine and glutamine, CO_2 and derivatives of tetrahydrofolate as can be seen in Figure 8.5.

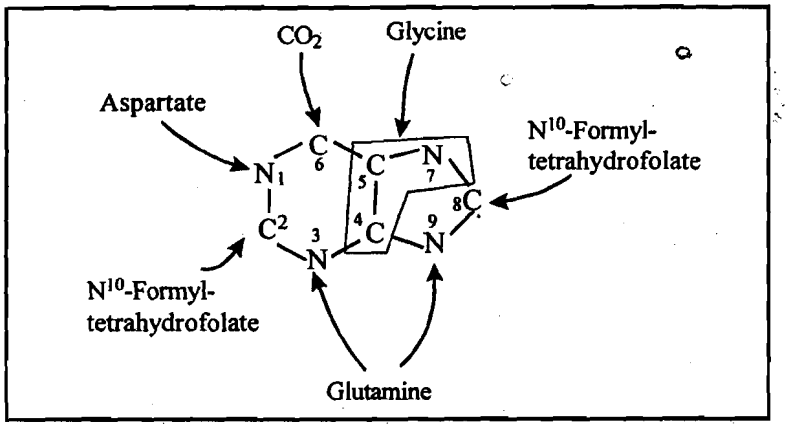


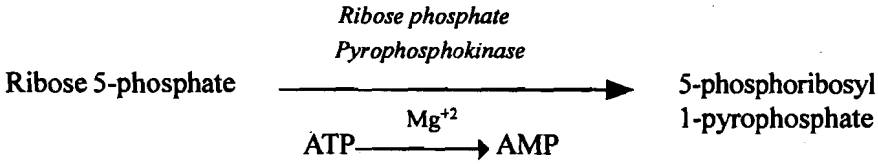
Figure 8.5 : Origin of atoms in the purine ring structure

The purine ring is formed by a series of reactions, as presented in Figure 8.5, that add the donated carbons and nitrogen to a preformed ribose 5-phosphate, which is synthesized by the HMP shunt, about which you may recall reading in Unit 6 on carbohydrate metabolism.

The major site of purine synthesis is in the liver. Synthesis of the purine nucleotides begins with *5-phosphoribosyl-1-pyrophosphate* (PRPP) and then follows a series of reactions and leads to the first fully formed nucleotide, *inosine 5'-monophosphate* (IMP) and AMP and GMP are subsequently derived from IMP. The purine base of IMP is hypoxanthine. The de-novo pathway starting from PRPP to the formation of IMP is diagrammed in Figure 8.6. Let us learn about each of these reactions one by one.

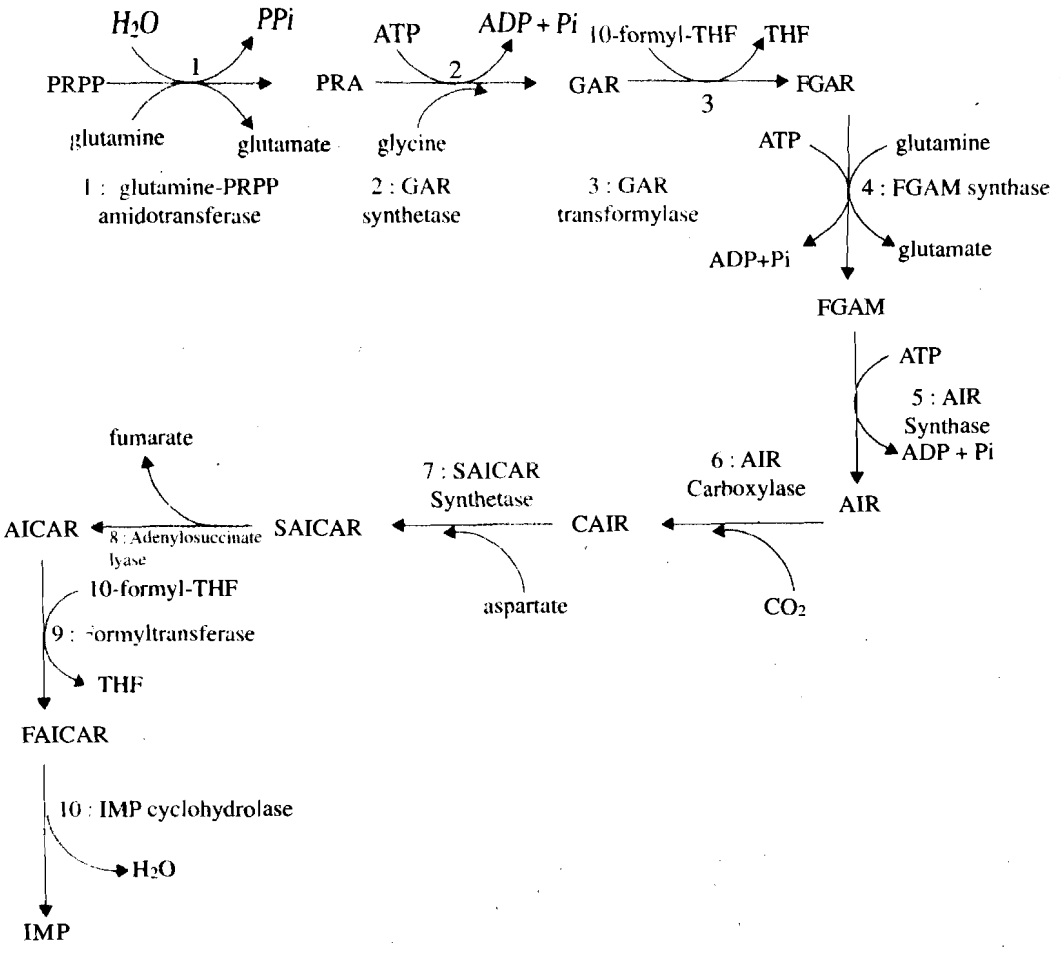
1) Synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP)

PRPP is synthesized from ATP and ribose-5-phosphate. The reaction is catalyzed by the enzyme ribose 5-phosphate pyrophosphokinase. The enzyme is activated by Pi and inhibited by purine nucleoside and triphosphates.



2) Synthesis of 5' - phosphoribosylamine

5'-phosphoribosylamine is synthesized from PRPP and glutamine. In this, the amide group of glutamine replaces the pyrophosphate group attached to C1 of PRPP. The enzyme catalyzing the reaction is glutamine phosphoribosyl pyrophosphate amidotransferase. The enzyme is inhibited by the purine 5' nucleotides AMP, GMP and IMP, which are the end products of this pathway (feedback inhibition). This is the committed step in purine nucleotide biosynthesis.

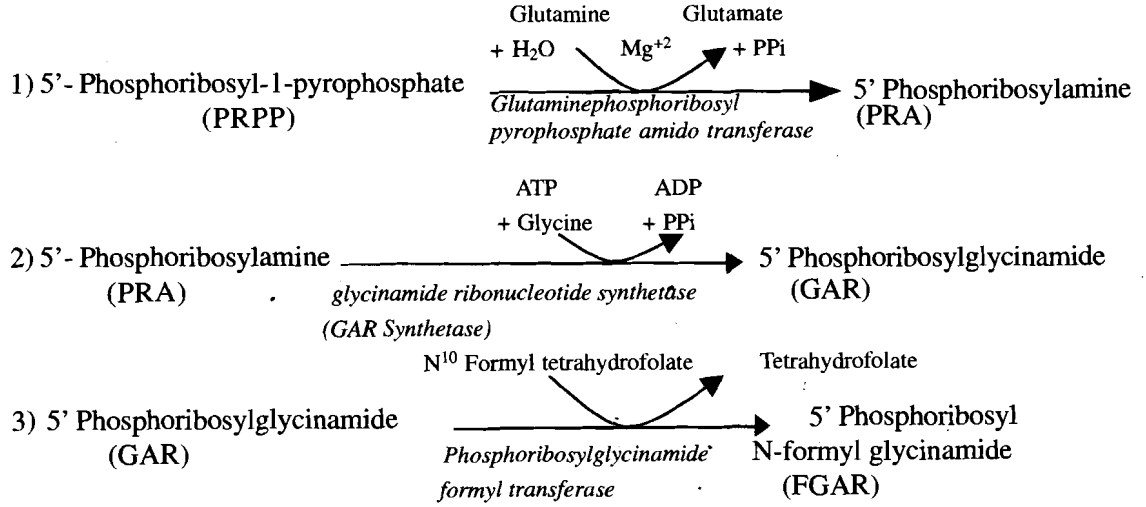


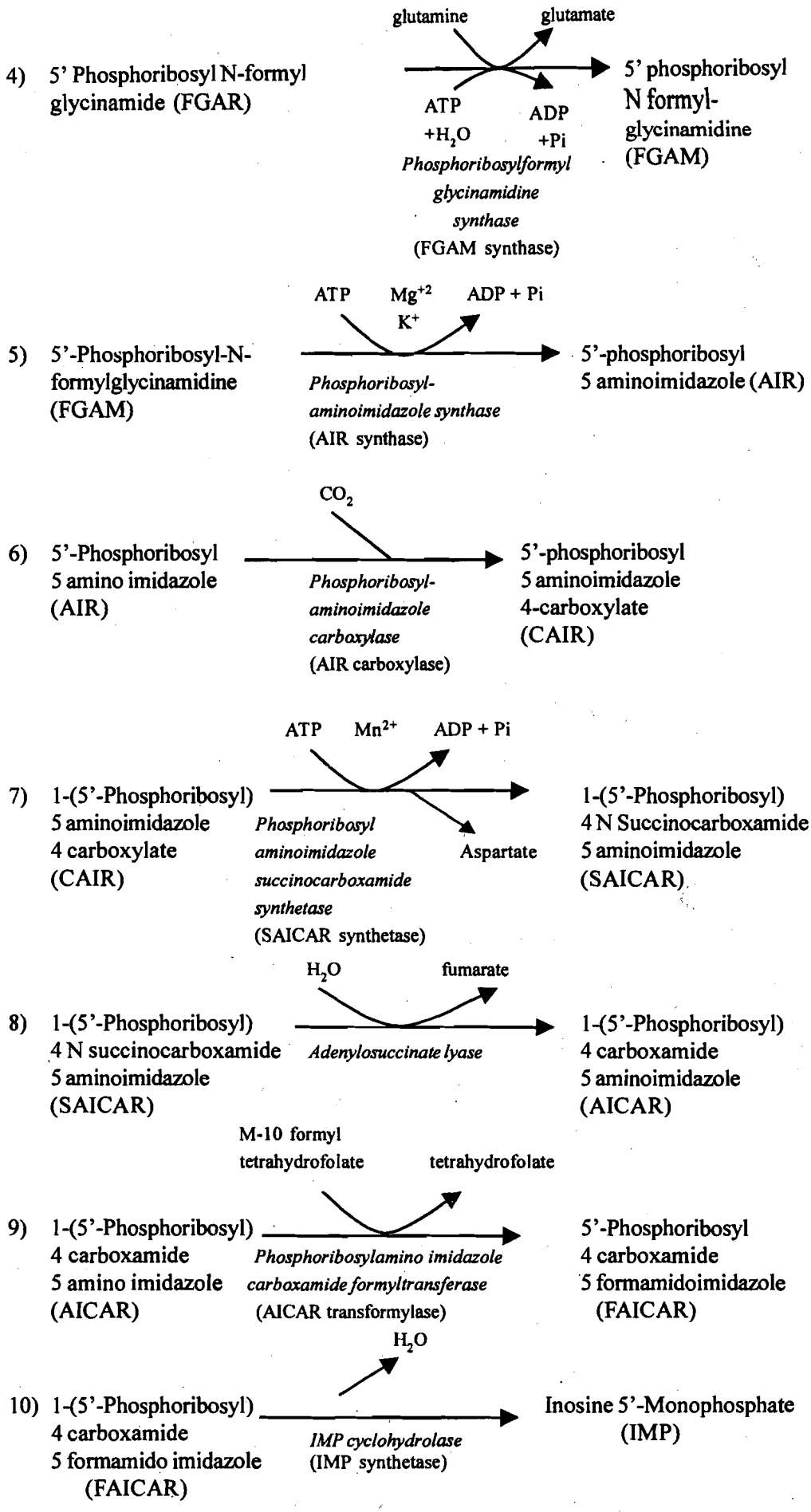
PRPP: 5-phosphoribosyl-1-pyrophosphate
PRA: 5-phosphoribosylamine
GAR: 5-phosphoribosylglycinamide
FGAR: 5-phosphoribosyl-N-formylglycinamide
FGAM: 5-phosphoribosyl-N-formylglycinamidine
AIR: 5-phosphoribosylaminoimidazole
CAIR: 1-(5-phosphoribosyl)-5-amino-4-carboxyimidazole
SAICAR: 1-(5-phosphoribosyl)-4-(N-succinocarboxamide)-5-aminoimidazole
AICAR: 1-(5-phosphoribosyl)-5-amino-4-imidazolecarboxamide
FAICAR: 1-(5-phosphoribosyl)-5-formamido-4-imidazolecarboxamide
IMP: inosine-5-monophosphate

Figure 8.6 : Pathway for synthesis of purine nucleotide

3) Synthesis of inosine monophosphate

This pathway requires 4 ATP molecules as an energy source. In all, 10 steps are involved in the purine nucleotide biosynthesis as shown in Figure 8.6 and enumerated herewith :





Earlier Figure 8.6 illustrated the formation of IMP, the first fully formed nucleotide. IMP can then become either AMP or GMP as discussed in the next step. It is important

for us to understand that nucleotides are important for reasons besides being precursors of nucleic acids. Most of them provide energy used to drive biochemical reactions. In fact, IMP represents a branch point for purine biosynthesis, because it can be converted into either AMP or GMP through two distinct reaction pathways. We shall learn about this next.

4) **Conversion of IMP to AMP and GMP**

It is clear that besides being precursors of nucleic acids, most nucleotides provide energy used to drive biochemical reactions. You have frequently heard ATP referred to as the "universal energy currency" of the cell. ATP is a nucleotide and it is also called a *nucleoside triphosphate*. Adenosine monophosphate (AMP) indicates that a single phosphate is in ester linkage to the hydroxyl groups of an adenosine molecule. Guanosine monophosphate (GMP) would indicate that a phosphate is in ester linkage to the hydroxyl group of a guanosine.

The conversion of IMP to either GMP or AMP utilizes a two-step, energy-requiring pathway. The synthesis of AMP requires GTP as an energy source, whereas, the synthesis of GMP requires ATP. The first reaction in each pathway is inhibited by the end product of that pathway as shown in Figure 8.7.

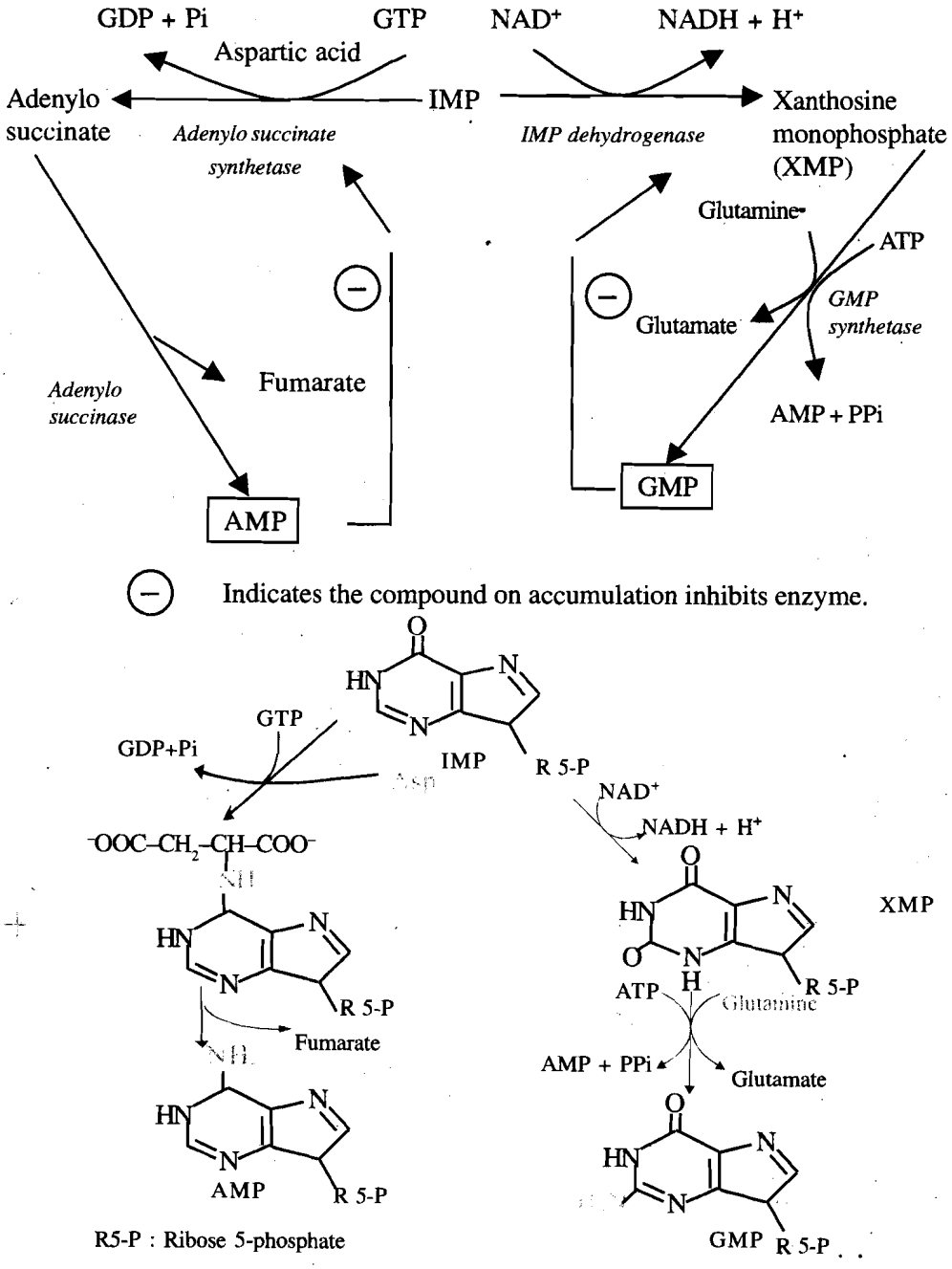


Figure 8.7 : Conversion of IMP to AMP and GMP

5) **Interconversion of nucleotides: Conversion of nucleoside monophosphate (NMP) to nucleoside diphosphates (NDP) and triphosphates (NTP)**

You already know that nucleotides with one phosphate are also called nucleoside monophosphates (NMP), those with two phosphates are nucleoside diphosphates (NDP) and those with three phosphates are nucleoside triphosphates (NTP). ATP, you learnt in the above section, is a *nucleotide* and it is also called a *nucleoside triphosphate*.

NDP are synthesized from the corresponding NMP by base specific *nucleoside monophosphate (NMP) kinases*. These *kinases* do not discriminate between ribose or deoxyribose in the substrate. ATP is the source of transferred phosphate.

$$AMP + ATP \xrightarrow{\textit{adenylate kinase}} 2\ ADP$$

$$GMP + ATP \xrightarrow{\textit{guanylate kinase}} GDP + ADP$$

Adenylate kinase is particularly active in liver and muscle, where the turnover of energy from ATP is high. Its function is to maintain equilibrium among AMP, ADP and ATP.

$$2\ ADP \longrightarrow AMP + ATP$$

NDP and NTP are interconverted by *nucleoside diphosphate kinase*.

$$GDP + ATP \Leftrightarrow GTP + ADP$$

$$CDP + ATP \Leftrightarrow CTP + ADP$$

The discussion above focused on the de novo synthesis of purine nucleotides, starting from PRPP leading to IMP and finally to GMP and AMP. Before we end our study on purine synthesi we would also like to look at the inhibitors of purine synthesis. These are discussed next.

Inhibitors of purine synthesis

Some inhibitors of purine synthesis are specific for inhibiting the growth of rapidly dividing microorganisms for e.g. PABA analogues– *sulfonamides*. Sulfonamides are *structural analogs of PABA that competitively inhibit bacterial synthesis of folic acid.* Since purine synthesis requires THF as a coenzyme, the sulpha drugs slow down this pathway in bacteria. Humans cannot synthesize folic acid, and must rely on external sources of this vitamin. Therefore sulfa drugs do not interfere with human purine synthesis.

Folic acid analogues: Methotrexate and related compounds inhibit the reduction of dihydrofolate to tetrahydrofolate, catalyzed by dihydrofolate reductase (reaction 9 of purine synthesis). These drugs limit the amount of tetrahydrofolate available for use in purine synthesis, and thus slow down DNA replication in mammalian cells. These compounds are therefore useful in treating rapidly growing cancers, but are toxic to all dividing cells.

You may recall reading earlier that there is yet another salvage pathway for purine synthesis. Let us learn about this pathway next.

8.3.2 Salvage Pathway for Purines

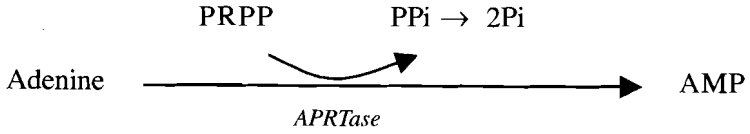
Purines that result from the normal turnover of cellular nucleic acids (i.e. from the degradation of nucleic acid) or those that are obtained from the diet and not degraded can be reconverted into NTP and used by the body. This is referred to as the "*salvage pathway*" for purines. As expected, this would be a very short pathway since purines are already available. The free purine bases–adenine, guanine and hypoxanthine–can be reconverted to their corresponding nucleotides by phosphoribosylation. Following two key transferase enzymes are involved in the salvage of purines:

a) Adenine phosphoribosyl transferase (APRTase); and

b) Hypoxanthine-guanine phosphoribosyl transferase (HGPRTase)

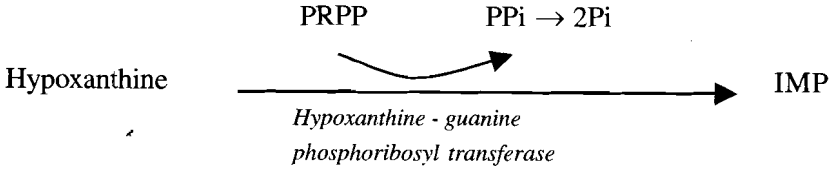
Both the enzymes utilize phosphoribosyl pyrophosphate (PRPP) as the source of the ribose-5-phosphate group. The release of pyrophosphate and further hydrolysis to inorganic phosphate makes these reactions irreversible. A deficiency of HGPRT causes the *Lesch-Nyhan Syndrome.*

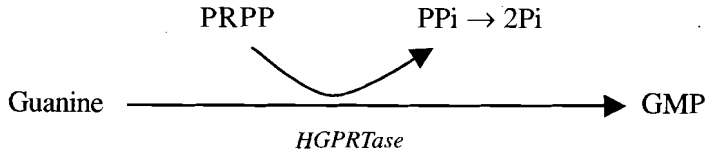
Adenosine phosphoribosyltransferase (APRTase) catalyzes the following reaction:



Thus ribose-5-phosphate is transferred from PRPP to the purine base hypoxanthine to form the nucleotide, IMP (inosine monophosphate).

Hypoxanthine-guanine phosphoribosyl transferase (HGPRTase) catalyzes the following reactions:





When guanine adds on ribose-5-phosphate, GMP is formed while adenine gives AMP as you may have noted above. Salvage pathways are important, in those tissues which cannot synthesize purines and purine nucleotides. For example, the human brain has a low level of the enzyme PRPP glutamyl amido transferase, while erythrocytes cannot synthesize 5-phosphoribosyl amine. Hence, liver the major site of synthesis, provides purines and purine nucleotides from salvage pathway in these tissues. This concept i.e. the salvage pathway, you would agree is like that of 'recycled paper'.

With the understanding of salvage pathway, we come to an end of our study about purine synthesis. After synthesis, it is the turn of the degradation of nucleotides. In the next sub-section, we shall learn about the degradation process of purine nucleotides.

8.3.3 Degradation of Purine Nucleotides

Purines are degraded to uric acid, a relatively water insoluble compound in the acid form and only slightly more soluble as the anion. Most of purine degradation takes place in all tissues ; the last steps, however, the oxidation of hypoxanthine and xanthine to uric acid, are restricted to the liver.

The end product of purine catabolism in humans is *uric acid*, which is itself a purine. The steps involved in degradation of purine to uric acid are diagrammatically presented in Figure 8.8 and a summary is presented herewith.

AMP → Inosine → Hypoxanthine → Xanthine → Uric acid

GMP → Guanosine → Guanine → Xanthine → Uric acid

The steps in uric acid synthesis include:

1) An amino group is removed from AMP to produce IMP or from adenosine to produce inosine.

2) IMP and GMP are converted into their nucleoside forms.

3) Purine nucleoside phosphorylase converts inosine and guanosine into their respective purine bases, hypoxanthine and guanine.

4) Guanine is deaminated to form xanthine.

5) Hypoxanthine is oxidized by xanthine oxidase to xanthine, which is finally oxidized to uric acid.

Uric acid is excreted in the urine. Chronic elevation of uric acid in blood occurs in 3% of the population. What is the outcome? *Gout* results from excess uric acid in various fluids. It is somewhat insoluble. One result is arthritic pain in joints due to deposition in cartilaginous tissue. The big toe is most susceptible.

Gout is characterized by hyperuricemia, with recurrent attack of acute arthritic joint inflammation caused by the deposition of uric acid crystals. Primary gout is due to an inborn error of metabolism, such as overproduction of uric acid. Secondary gout may be caused by other diseases such as cancer, chronic renal insufficiency, HGPRTase deficiency etc.

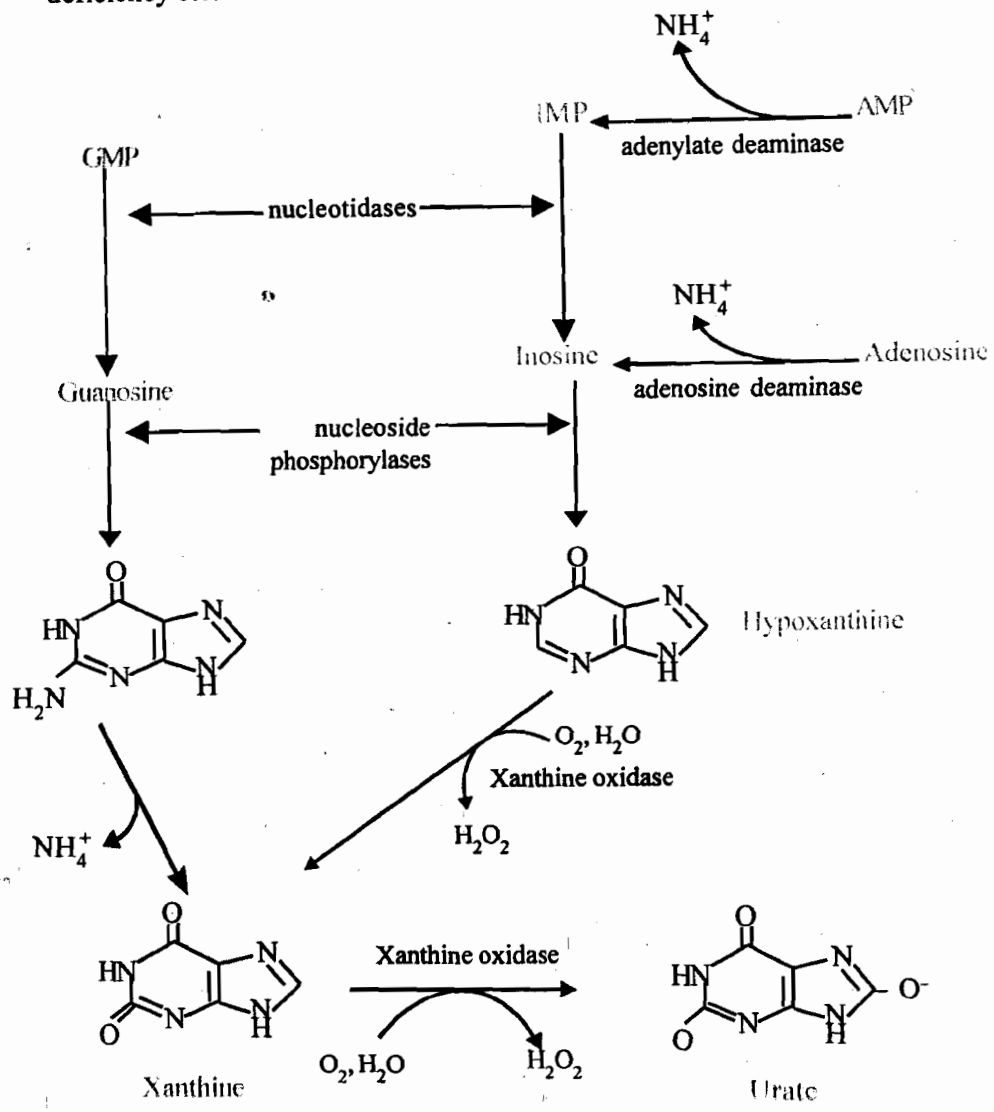


Figure 8.8 : Steps in uric acid synthesis

Check Your Progress Exercise 4

1) Give the physiologically important roles of nucleotides in our body.

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2) How many ATP molecules are required for the synthesis of IMP?

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3) What do you understand by the term de novo synthesis for purine synthesis? Give the steps involved in de novo synthesis of purine nucleotides.

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4) How does sulphonamides inhibit purine synthesis?

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5) Explain how NMP can be converted into NDP and NTP.

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6) What do you understand by the term 'salvage pathway' for purine nucleotide?

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7) What is the end-product of purine degradation? What is the disease caused due to the accumulation of the end-product in the body?

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Our study of nucleotide metabolism, so far, has focussed on purine nucleotide synthesis and degradation. Next, let us look at pyrimidine synthesis. Pyrimidine you know is the other nitrogeneous base from which nucleotides are formed.

8.3.4 Pyrimindine Synthesis

The synthesis of pyrimidines is much simpler. Only two molecules contribute to the pyrimidine skeleton, *carbamoyl phosphate* and *aspartate* as can be seen in the structure of the pyrimidine ring in Figure 8.9. The sources of the C and N atoms of viz. the pyrimidine ring are glutamine, CO_2 (from which carbamoyl phosphate is synthesized) and aspartic acid. Here, the pyrimidine base is first synthesized to which ribose-5-P is added. You would realize that this is different from purine synthesis, where the purine nucleus is built on ribose-5-P.

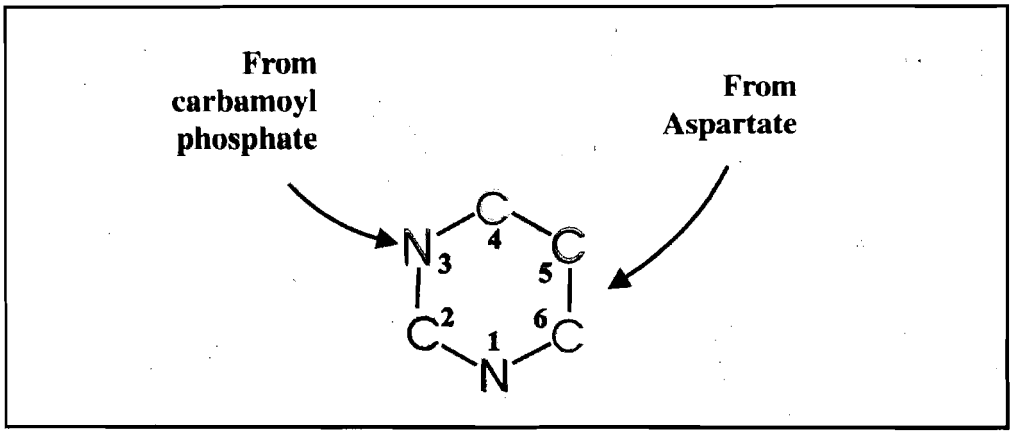

Figure 8.9 : Origin of atoms in the pyrimidine ring

Let us get to know about the pyrimidine synthesis and the steps involved next.

1) ***Synthesis of carbamoyl phosphate***

As mentioned above, the pyrimidine skeleton is synthesized from carbamoyl phosphate and aspartate. The synthesis of carbamoyl phosphate from glutamine, CO_2 and two ATPs occurs in the cytoplasm of the cell, catalyzed by carbamoyl phosphatase synthetase II (CPS II). *This is the committed step (rate-limiting) in pyrimidine biosynthesis.* The CPS II does not require biotin. It is inhibited by UTP and activated by ATP and PRPP. You may recall reading about CPS I), earlier in sub-section 8.2.3. The differences between carbamoyl phosphate synthetase I and II are given herewith.

	CPS I	**CPS II**
Cellular location	Mitochondria	Cytosol
Pathway	Urea cycle	Pyrimidine synthesis
Source of N	Ammonia	γ-Amide group of glutamine

2) ***Synthesis of orotic acid***

From carbamoyl phosphate and aspartate, carbamoyl aspartate is formed by the action of *aspartate transcarboxylase*. The pyrimidine ring is then closed through a loss of a molecule of water by *dihydroorotase*, to form dihydroorotate. This is then oxidized by *dihydroorotate dehydrogenase* to produce orotic acid as shown in Figure 8.10. At this point, the pyrimidine ring is synthesized. The 3 enzymes: *CPS II, aspartate transcarbamoylase* and *dihydroorotase* are all domains (regions) of the same polypeptide chain which facilitates the ordered synthesis of the compound. That is, the protein is a multienzyme complex.

Pyrimidine biosynthesis

Points to remember:

1. Synthesis occurs at the free base level. The ribose is added later in the form of PRPP to form the nucleotide
2. The first 6-member ring system is dihydroorotate, which needs to be oxidized (removal of electrons) to orotate.

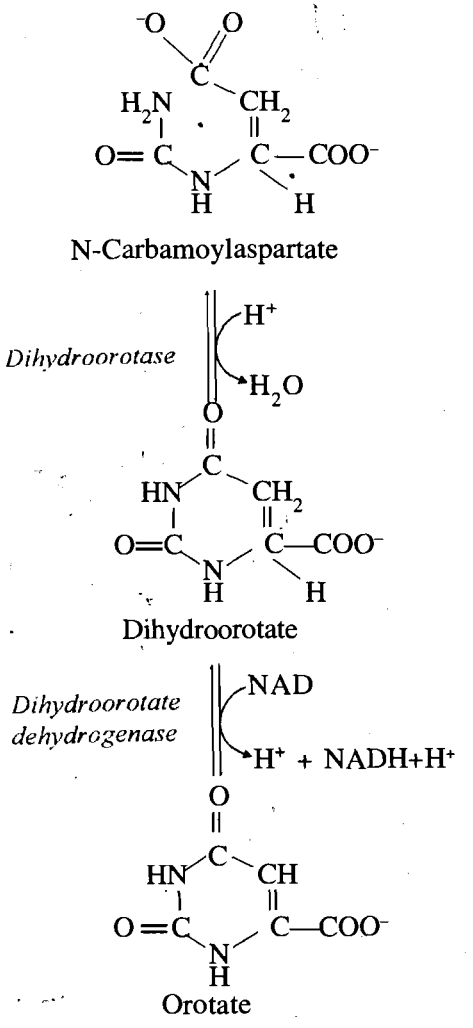

Figure 8.10 : Synthesis of orotate

3) *Formation of pyrimidine nucleotide*

The first pyrimidine nucleotide, as seen in the last step, is orotate. Once the pyrimidine ring is synthesized, it is converted to the nucleotide orotidine-5'-monophosphate (OMP), which involves addition of ribose and phosphate. PRPP (you recall reading about it earlier in sub-section 8.3.1) is the ribose-5-phosphate donor. The reaction is irreversible. However, OMP is not a pyrimidine present in nucleic acid. Hence, OMP is converted to UMP by orotidylate decarboxylase by removing acidic carboxyl group. Thus UMP (uridine monophosphate) has uracil as the pyrimidine base. Figure 8.11 illustrates these steps in the pyrimidine synthesis. Deficiency of *orotate phosphoribosyl transferase* and *orotidylate decarboxylase*, results in accumulation of orotic acid which is excreted in the urine. This condition is called *orotic aciduria.*

Besides UMP, nucleic acids also contain 2 other pyrimidine nucleotides which are CMP (cytidine monophosphate having cytosine) and TMP (thymidine monophosphate with thymine). So next, we shall see how CMP is synthesized.

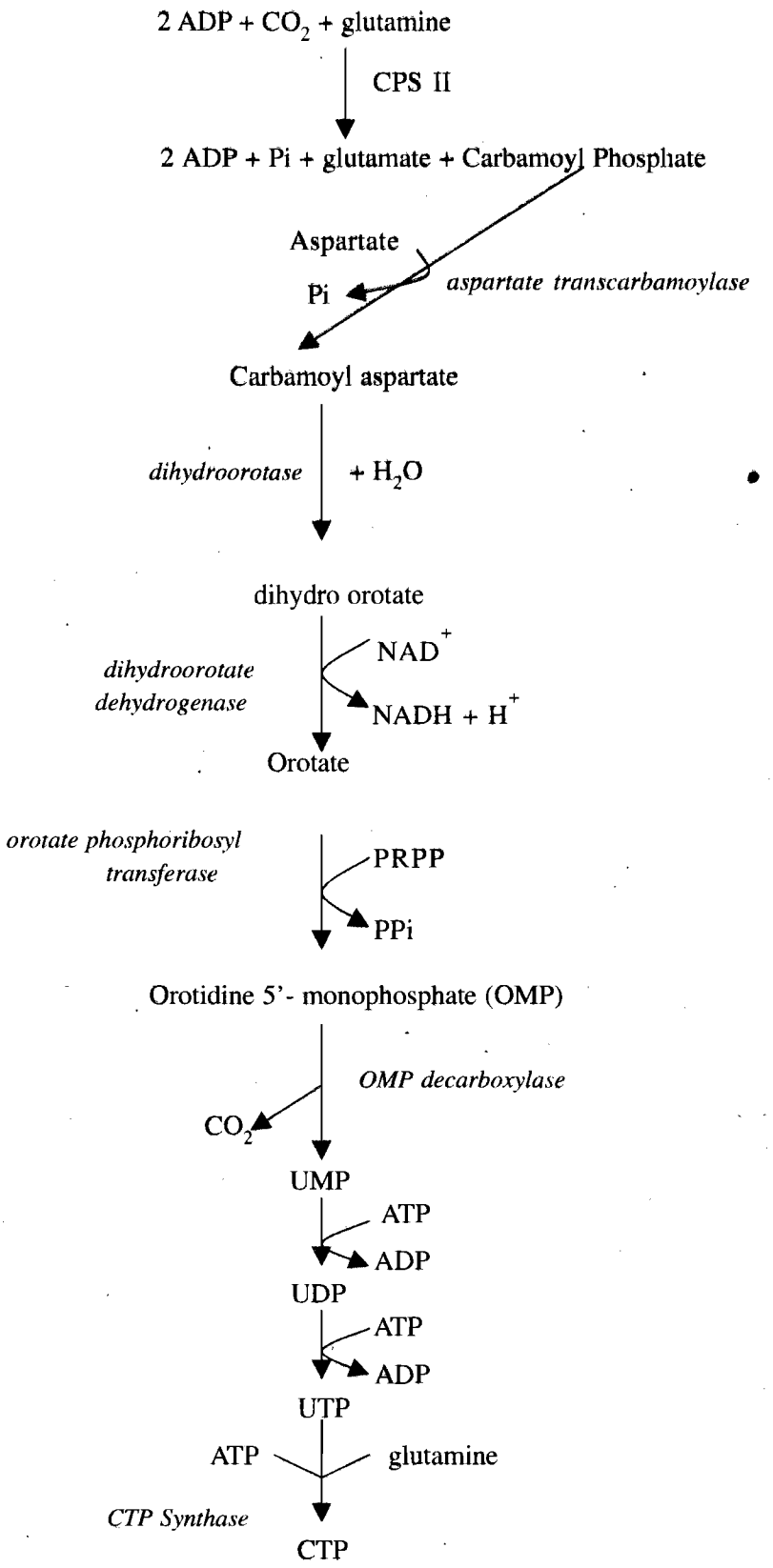

Figure 8.11 : Pyrimydine synthesis

4) ***Synthesis of uridine triphosphate UTP and cytidine triphosphate (CTP)***

UMP is first phosphorylated using ATP to form UDP (uridine diphosphate). A further phosphorylation by ATP form UTP (uridine triphosphate). The pyrimidine base cytosine is next aminated i.e. oxygen atom at position 4 is replaced by amino group obtained from

glutamine. This converts uracil into cytosine forming CTP (cytidine triphosphate). Thus CTP is produced by amination of UTP catalyzed by the enzyme *CTP synthase*. This step requires energy, which is provided by ATP. The entire pyrimidine synthesis reactions starting from ATP, CO_2 and glutamine to UMP and CTP is presented in Figure 8.11.

5) *Synthesis of deoxyribonucleotides from ribonucleotides*

The nucleotides synthesized are all ribonucleotides, which can be used as building blocks in RNA synthesis or as nucleotide carriers of other compounds, such as sugars (e.g. UDP-glucose used in glycogen metabolism). The nucleotides required for DNA synthesis are 2'-deoxyribonucleotides (i.e. instead of ribose, there is 2'-deoxyribose where in 2nd position of ribose sugar, oxygen atom is absent), which are produced from ribonucleoside diphosphates. The enzyme *ribonucleotide reductase* is specific for the reduction of nucleoside diphosphates (ADP, GDP, CDP and UDP) to their deoxy forms (dADP, dGDP, dCDP and dUDP). Since oxygen is removed, it is a reduction reaction.

6) *Synthesis of TMP from dUMP*

The fourth pyrimidine nucleotide commonly present in nucleic acids is thymidine monophosphate (TMP). Figure 8.12 illustrates the biosynthesis of TMP. TMP is present only in DNA which has deoxyribose. Hence technically it is dTMP, however, it is also generally referred to as TMP. dUMP is converted to dTMP by *thymidylate synthetase*, which utilizes N5, N10 – methylene tetrahydrofolate as the source of the methyl group when the pyrimidine base uracil is methylated at position 5, it becomes the base thymine (6-methyl uracil). THF not only transfers a C unit but also 2 H atoms resulting in the oxidation of THF to dihydrofolate (DHF). Inhibitors of thymidylate synthetase include thymine analogs (compounds structurally similar to thymine) such as 5-fluorouracil, which serve as antitumor agents. In addition, dihydrofolate reductase, the enzyme that reduces DHF to THF, is inhibited in the presence of drugs such as *metrotrexate*. By decreasing the supply of THF, these folate analogs inhibit purine synthesis and prevent methylation of dUMP to dTMP. They lower the cellular concentration of dUMP, which is an essential component of DNA. In this way, DNA synthesis is inhibited and cell growth is arrested or slowed down. Therefore, these drugs are used to decrease the growth rate of cancer cells.

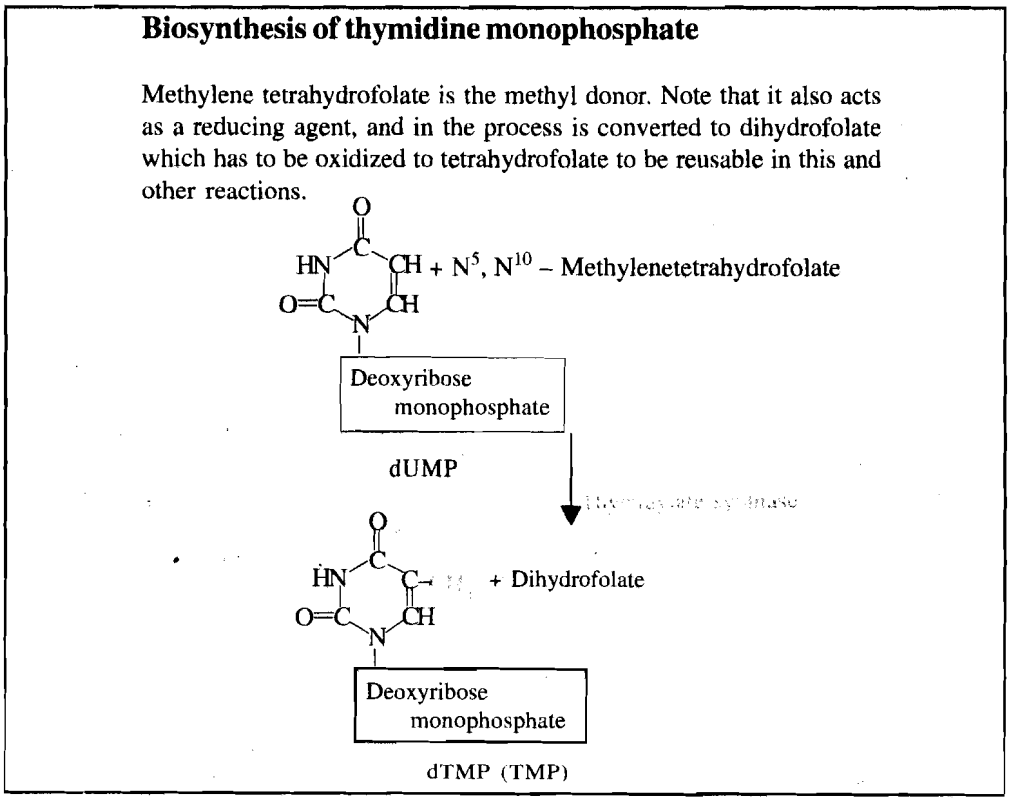

Figure 8.12 : Biosynthesis of TMP

We shall end our study of pyrimedine metabolism by learning about degradation and salvage reactions of pyrimidine nucleotide.

Degradation of Pyrimidine Nucleotides

The pyrimidine ring can be opened and degraded to highly soluble structures such as β alanine and β amino isobutyrate. They serve as precursors of acetyl CoA and succinyl CoA respectively.

Salvage Reaction of Pyrimidine nucleotide

The pyrimidines, like the purines we saw earlier, can be salvaged and converted into nucleotides by the enzyme *pyridine phosphoribosyl transferase* wherein again it utilizes PRPP as the source of the ribose-P. In addition, orotate phosphoribosyl transferase (an enzyme of pyrimidine nucleotide synthesis) salvages (i.e. reuses) orotic acid by converting it to OMP.

8.3.5 Regulation of Deoxyribonucleotide Synthesis

Ribonucleotide reductase is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis. The regulation of the enzyme is complex. In addition to the single active site, there are 2 sites on the enzyme involved in regulating the activity as given below:

a)	Activity site	ATP combines with this site and activates enzyme
		d ATP inhibits enzyme
b)	Substrate specificity site	ATP, dATP, dTTP or dGTP
		regulate reduction of specific ribonucleotide

Check Your Progress Exercise 5

1) Name the two molecules that contribute to the pyrimidine skeleton.

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2) Enumerate the steps involved in the pyrimidine synthesis.

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3) How is synthesis and degradation of pyrimidine nucleotides different from purine nucleotides?

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4) Which is the enzyme responsible for regulating deoxy ribonucleotide synthesis and what are its two sites?

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8.4 LET US SUM UP

In this unit we focussed on amino acid and nucleotide metabolism. We learnt that amino acids play an important role in the synthesis of tissue proteins and have a variety of important biological activities. Transamination, deamination and decarboxylation are some of the important reactions of amino acids. The ammonia formed is converted to urea in the liver and is excreted by the kidneys. The biogenic amines formed through the decarboxylation process have important physiological functions.

The nucleotides are necessary for the synthesis of DNA and RNA. Purine and pyrimidine nucleotides can be synthesized in the body. The end product of purine catabolism in humans is uric acid, the accumulation of which can result in gout. Pyrimidines are degraded to highly soluble structures such as α-alanine and α-amino isobutyrate which serves as precursors of acetyl CoA and succinyl CoA. The nucleotides synthesized are ribonucleotides. The ribonucleotides reductase enzyme reduces the nucleoside diphosphates to their deoxy forms. This enzyme is also responsible for maintaining a balance supply of deoxyribonucleotides for DNA synthesis.

8.5 GLOSSARY

Acute phase proteins : proteins needed during stress, immunoglobulins, leukocytes, lymphocytes, haemoglobin, albumin and enzymes necessary for protein synthesis.

Amino acid Pool : amino acids coming from exogenous and endogenous sources found in circulation.

Analogues : structural similarity.

Hyperuricemia : increased levels of uric acid in circulation.

Melanin : any of a group of naturally occurring dark pigments, especially the pigment found in skin, hair, fur and feathers.

Protein Energy Malnutrition (PEM) disorder : also referred to as protein calorie malnutrition; it is a potentially fatal body-depletion disorder, which develops in children and adults whose consumption of protein and energy (measured by calories) is insufficient to satisfy the body's nutritional needs.

Urea : the chief nitrogenous end product of protein metabolism and the chief nitrogenous constituent of urine.

8.7 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) The two processes involved in the degradation of amino acids in our body are :

   - the removal of α-amino groups (amino groups attached to carbon atom next to the carboxyl carbon) by two processes called *transamination* and *oxidative deamination*, forming ammonia and the corresponding α-keto acids, and
   - the conversion of ammonia to urea in the Urea Cycle. A portion of the free ammonia is excreted in urine, and the remaining is used in the synthesis of urea.

2) In transamination reaction, there is a transfer of α-amino group of amino acid to keto acid (keto group present next to the carboxyl group) –R–CO–COOH. As a result, the amino acid becomes α-keto acid and the keto acid is converted into amino acid. Aspartate aminotransferase (AST) catalyzes the reaction. One example of transamination reaction is :

   Asparate + α-ketoglutarate ⇔ oxaloacetate + glutamate

3) The two enzymes involved in deamination reaction are glutamate dehydrogenase and amino acid oxidase. These reactions occur in liver and kidney.

4) Ammonia is removed from our body as urea. The conversion of ammonia into urea is carried out by a system of carrier molecules and enzymes present in liver. This process is referred to as urea cycle.

5) Carbamoyl phosphate synthase I, Ornithine transcarbamoylase, Arginase, Argininosuccinate lyase, Argininosuccinate synthetase are the various enzymes involved in the urea cycle. Coenzymes include pyridoxal phosphate, NAD, FAD and FMN.

Check Your Progress Exercise 2

1) After the removal of α-amino group, there is a breakdown of the remaining products, that is, carbon skeletons. The products formed are OAA, α-KG, pyruvate, fumarate, Succ-CoA and acetyl CoA. These enter the various metabolic pathways and are oxidized to CO_2 and H_2O.

2) Amino acids whose catabolism yields either acetoacetate or one of its precursors are termed as ketogenic. These are degraded to intermediates that can be utilized in the formation of ketone bodies, for example, leucine and lysine.

   Amino acids whose catabolism yields pyruvate or one of the intermediates of the citric acid cycle are termed glucogenic, for example, alanine and asparagine.

3) The nutritional state of the individual determines the intermediary metabolic pathway taken by the catabolism of the carbon skeletons. For example, in case of starvation, these undergo gluconeogenesis to form glucose, and pyruvate can go through the reversal of glycolysis and form glucose/get converted to acetyl CoA which can be oxidized to CO_2 and H_2O in the citric acid cycle.

4) Nonessential amino acids can be synthesized from the intermediates of metabolism or from essential amino acids by the reaction with α-keto acids and by amidation process. For example, glutamate and aspartate can be synthesized from α-keto acid by transamination reaction and glutamine from glutamate by action of glutamine synthetase.

Check Your Progress Exercise 3

1) Porphyrins (haemoglobin, myoglobin, cytochromes, catalase), creatinine; histamine, serotonin and catecholamines are synthesized from amino acid.

2) Any of a group of naturally occurring, biologically active amines, such as norepinephrine, histamine and serotonin, that act primarily as neurotransmitters and are capable of affecting mental functioning and of regulating blood pressure, body temperature and other bodily processes are called biogenic amines. For example, any two of the following: tyramine, tryptamine, histamine, ethanolamine, propanolamine, β-mercaptoethanolamine.

3) Pyridoxal phosphate is the coenyzme required for decarboxylation reaction.

4) Immunity, synthesis of glutathione, regulation of protein turnover, muscle function, quick energy, maintenance of vascular tone and blood pressure, inhibition of adhesion, activation and platelet aggregation, cognitive functions are the non-protein functions of amino acid. GSH acts as an antioxidant and prevents the release of free radicals.

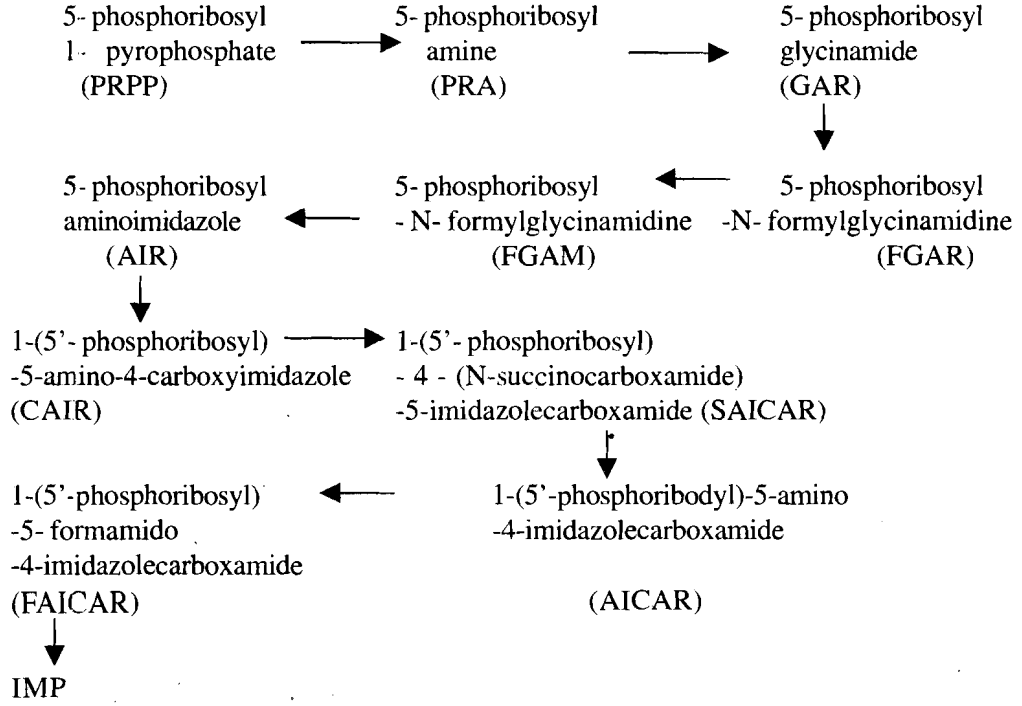
5) Glutathiane is an antioxidant. It is a tripeptide consisting of amino acids glutamine, cysteine and glycine.

Check Your Progress Exercise 4

1) Without nucleotides, DNA and RNA cannot be produced with the result the cells cannot proliferate and proteins cannot be synthesized. Further, nucleotides serve as carriers of activated intermediates in the synthesis of carbohydrate, lipids and proteins. They are also structural components of a number of essential coenzymes such as coenzyme A, FAD, NAD^+ and $NADP^+$. Nucleotides also play an important role as "energy currency" of the cell. Lastly, nucleotides are important regulatory compounds for many of the pathways of intermediary metabolism by either inhibiting or activating key enzymes.

2) Four ATP molecules are required for the synthesis of IMP.

3) De novo synthesis is the afresh synthesis. The steps involved are :



4) Sulfanomides are the structural analogs of PABA and competitively inhinbit bacterial synthesis of folic acid. Since purine synthesis requires THF as a coenzyme, the sulpha drugs slow down pathway in bacteria.

5. NDP (ADP) and NTP(ATP) are synthesized from corresponding NMP (AMP) as under:

$$AMP + ATP \xrightarrow{\textit{adenylate kinase}} 2\ ADP$$

$$GMP + ATP \xrightarrow{\textit{guanylate kinase}} GDP + ADP$$

$$2\ ADP \longrightarrow AMP + ATP$$

$$GDP + ATP \Leftrightarrow GTP + ADP$$

$$CDP + ATP \Leftrightarrow CTP + ADP$$

6) Purines that result from the normal turnover of cellular nucleic acids (i.e. from the degradation of nucleic acid) or those that are obtained from the diet and not degraded can be reconverted into NTP and used by the body. This is referred to as the salvage pathway for purines.

7) Uric acid is the end-product of purine degradation. The disease caused due to the accumulation of end-product in the body is gout.

Check Your Progress Exercise 5

1) The two molecules that contribute to the pyrimidine skeleton are carbamoyl phosphate and aspartate.

2) The steps involved in pyrimidine synthesis are:
   1) Synthesis of carbamoyl phosphate
   2) Synthesis of orotic acid
   3) Formation of pyrimidine nucleotide, and
   4) Synthesis of uridine triphosphate and cytidine triphosphate.

3) In pyrimidine synthesis, pyrimidine base is first synthesized to which ribose 5-P is added whereas in purine synthesis, purine nucleus is built on ribose 5-P. The degradation product of pyrimidines serve as precursors of acetyl CoA and succinyl CoA whereas purine degradation product is uric acid, which is a purine.

4) Ribonucleotide reductase is the enzyme responsible for regulating deoxyribonucleotide synthesis. Its two sites are activity site and substrate specificity site.