

UNIT 4

PROTEIN METABOLISM



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Learning Objectives

After studying this unit the students will be able to

- Understand the general reactions concerned with the catabolism of dietary amino acids
- Comprehend the various ways in which the amino groups of the amino acids are removed
- Reciprocate the removal of carboxyl groups and fate of carbon skeletons of the amino acids
- Emulate (Reproduce) the reactions of Urea cycle and realize that the amino groups of amino acids are removed in the form of urea by liver
- Understand that the amino acids also serve as precursors for many biologically important compounds

INTRODUCTION

We already know that proteins are made up of amino acids and have many functions in all living system. The 20 amino acids are obtained from the diet or from turnover of proteins. The major part of amino acid content of the cell exists in the form of proteins which is constantly being synthesized and degraded. Amino acids cannot be stored in an analogous form of glycogen (glucose) or triglycerides (fatty acids) leaving a small pool of amino acids in the cells as per the needs for protein synthesis. Excess amino acids are broken down into the carbon skeleton and the nitrogen amino group. The carbon skeleton is converted into common metabolites that can be used for the synthesis of other biomolecules or for further oxidation to generate ATP, while their amino group is converted to urea.

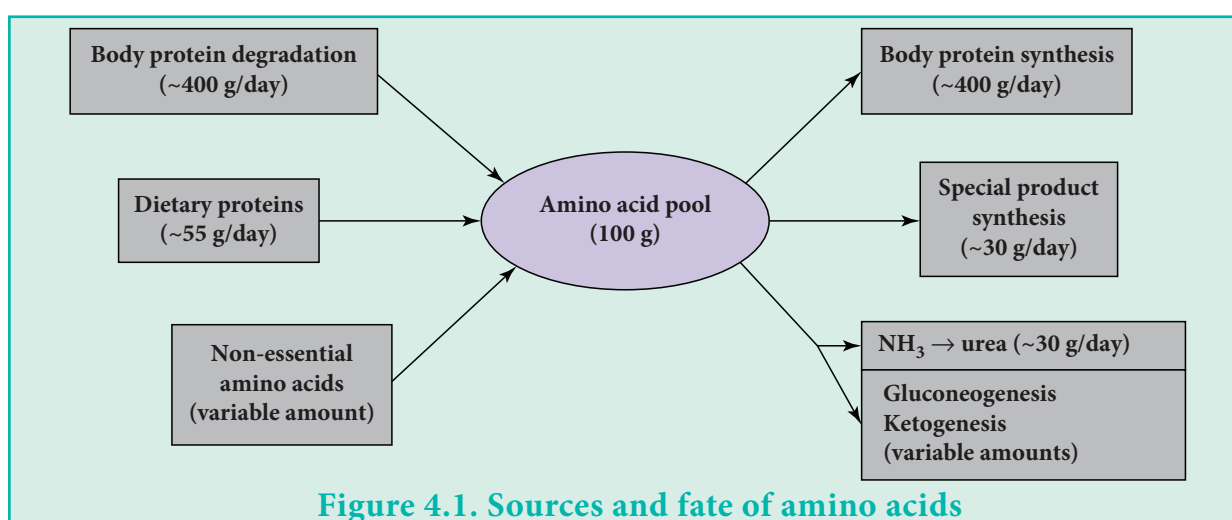


Figure 4.1. Sources and fate of amino acids

Amino acids are not only used to synthesize proteins, but also they act as precursors for specialized products of amino acid derivatives that play biologically significant roles in metabolism and homeostasis (Figure 4.1).

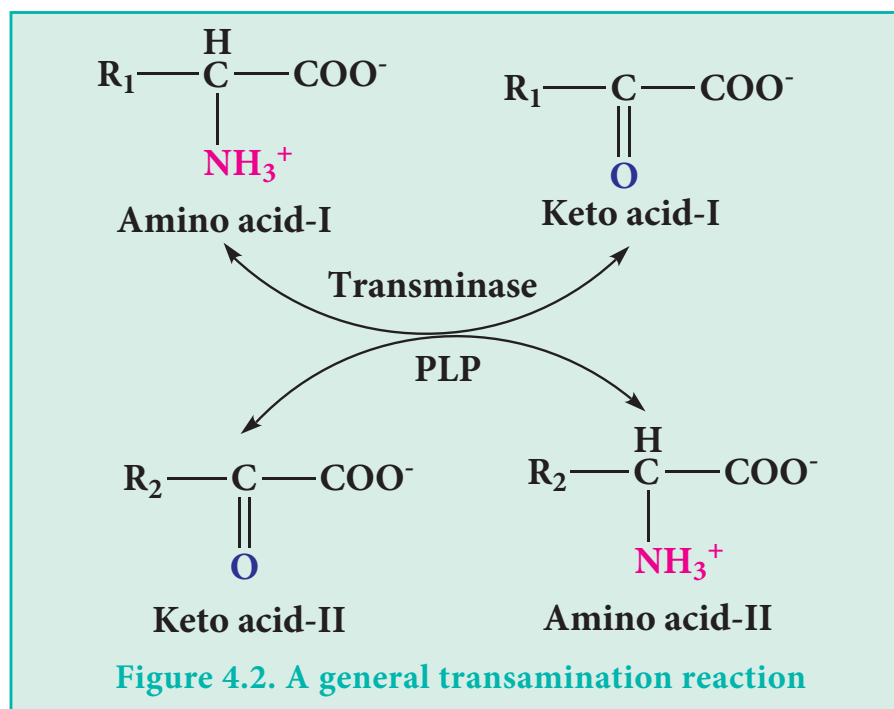
4.1 GENERAL REACTIONS OF AMINO ACID

Non-essential amino acids are synthesized from α -keto acids by transfer of amino groups with the help of transaminases. However, essential amino acids cannot be synthesized from α -keto acids. Transfer of amino groups also occurs during degradation of amino acids. Amino groups can be removed from amino acids by transamination as well as by deamination. Removal of carboxyl group (Decarboxylation) from amino acids results in the production of biologically active amines. The carbon skeletons of amino acids are degraded on entry into TCA cycle.

4.1.1 Catabolism of amino acid

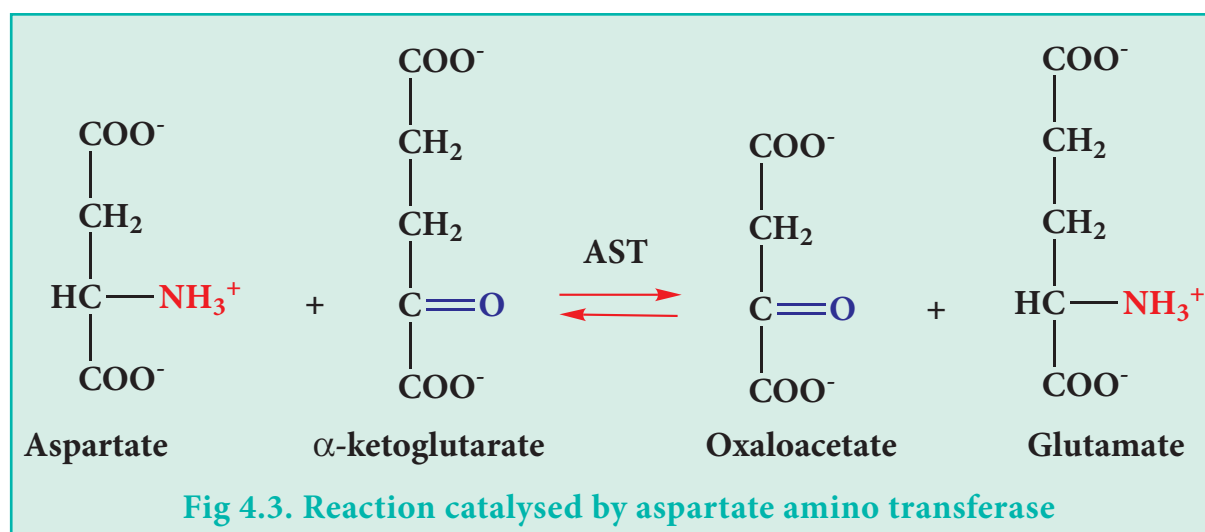
Even though, each amino acid has a specific pathway for its catabolism, the first step in the catabolism of amino acids is the removal of its amino group. This can happen by two different ways namely, transamination and deamination.

4.1.2. Transamination (AST and ALT)

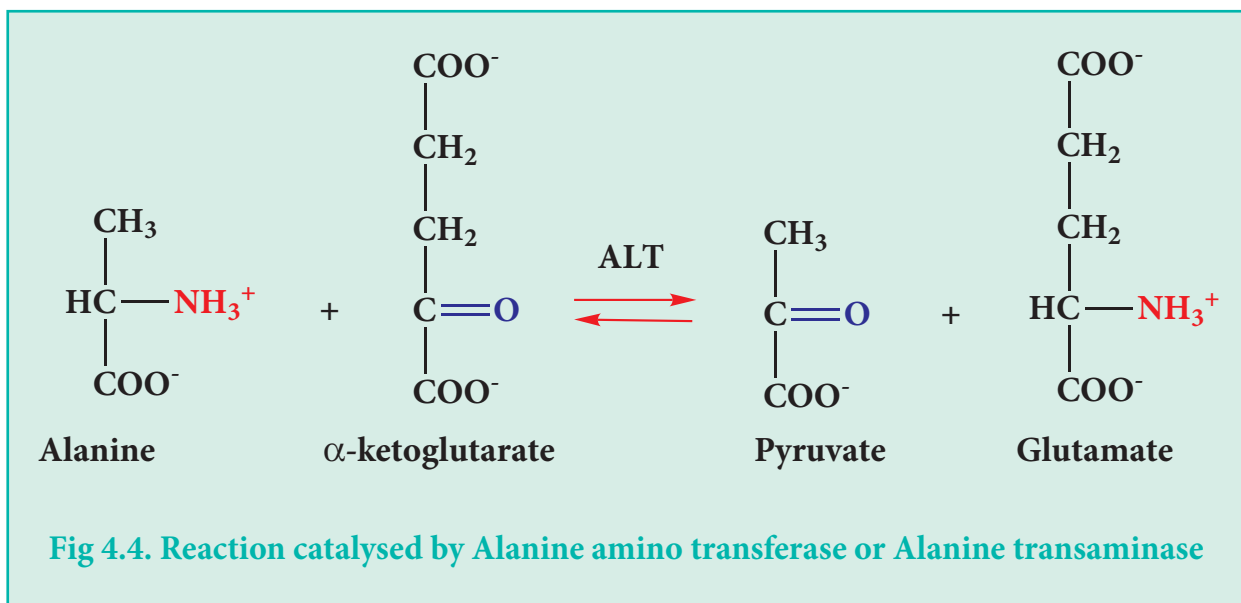


Transamination is a process, in which transfer of amino groups occur between a keto acid and an amino acid. It is just shuffling of amino groups and not removal of amino groups. Each amino acid has a specific transaminase (Figure 4.2). However, Alanine transaminase (ALT) and Aspartate transaminase (AST) are the important transaminases.

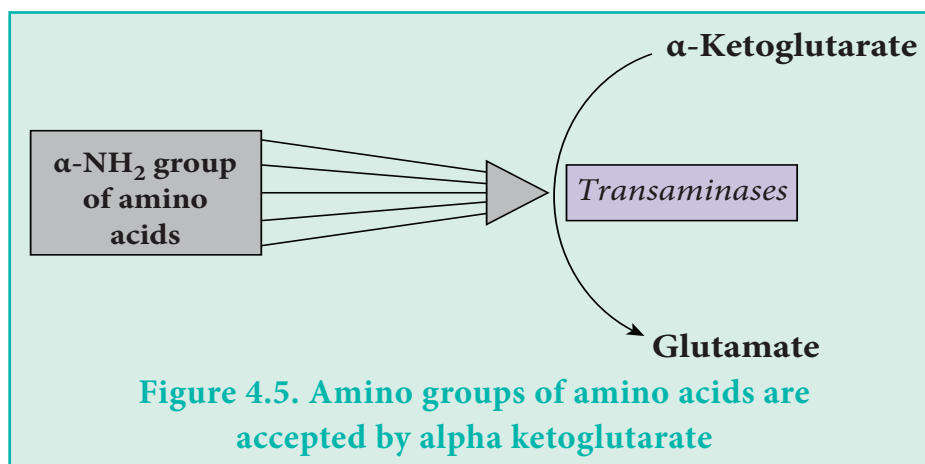
The reactions catalysed by ALT and AST are :



Aspartate transaminase catalyses the inter-conversion of aspartate and α -ketoglutarate to oxaloacetate and glutamate (Figure 4.3), while alanine transaminase catalyses the conversion of alanine and α -ketoglutarate to pyruvate and glutamate (Figure 4.4).



Transamination involves the coenzyme pyridoxal phosphate for the shuttling of amino groups. During this process, pyridoxamine phosphate is formed as an intermediate. The amino group of the amino acid reacts with pyridoxal phosphate bound to the enzyme to form a Schiff's base. As the α ketoglutarate is involved in all the transamination reactions, glutamate acts as a sink for the amino groups for most of the amino acids (Figure 4.5).



Features of Transamination

- Transamination reactions are reversible reactions.
- All amino acids except lysine, threonine, proline and hydroxyl proline undergo transamination.
- Serum transaminases are significant markers for diagnosis of liver and heart diseases.
- Many non-essential amino acids are synthesized using transamination reactions.

4.1.3 Deamination

Deamination is the process of removal of amino groups from amino acids as ammonia. Deamination may be or may not be coupled with oxidation. Depending upon this, deamination is classified as oxidative deamination and non-oxidative deamination.

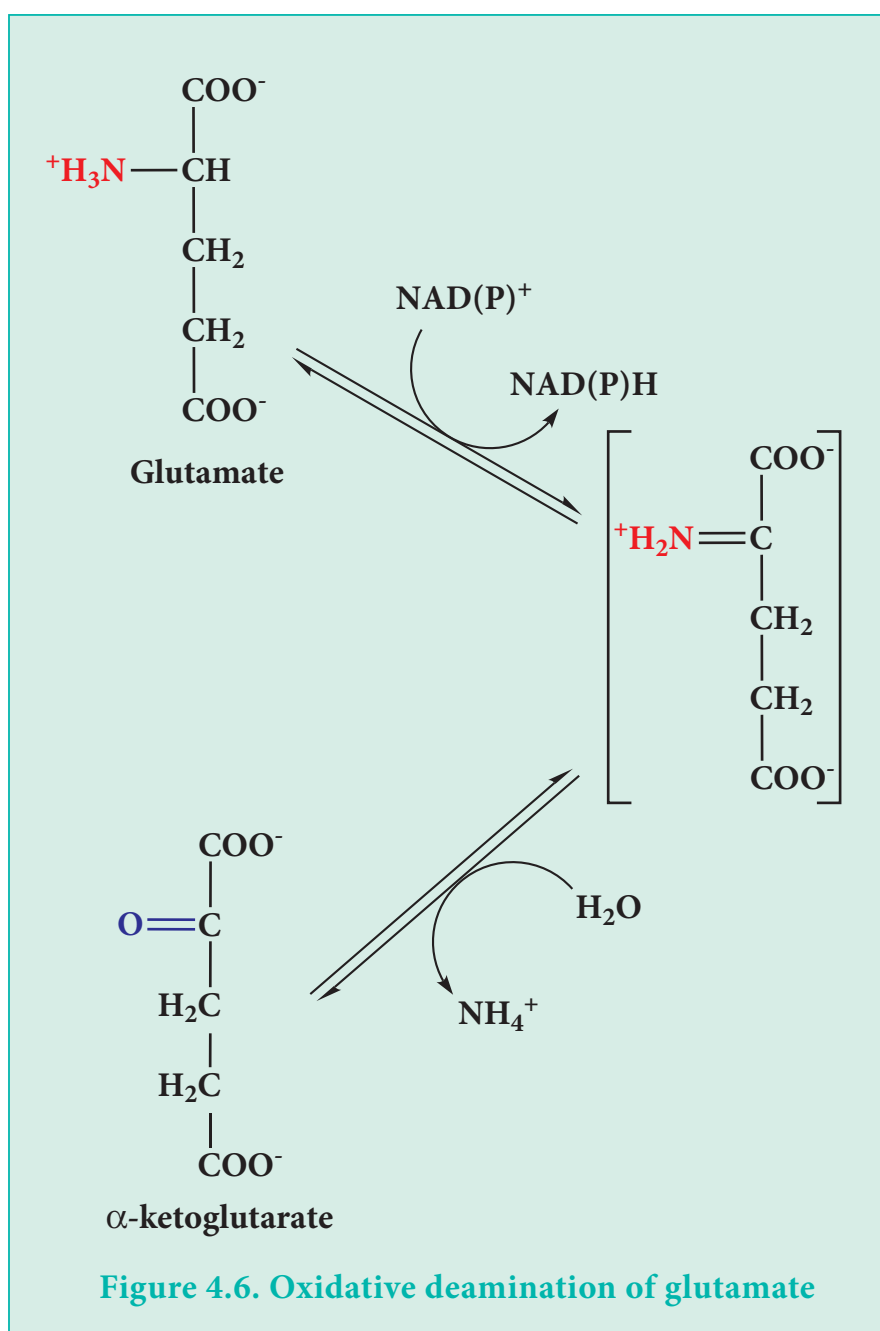
Oxidative Deamination

Oxidative Deamination is a process in which the alpha amino group of the amino acid is removed as ammonia, coupled with oxidation. Most of the amino acids dump their amino groups to alpha ketoglutarate and pyruvate forming glutamate and alanine, respectively.

Oxidative deamination involving the removal of amino group of glutamate as ammonia is the key reaction involved in delivery of amino groups to the liver for urea synthesis. The reaction is catalysed by glutamate dehydrogenase (GDH) with $\text{NAD}^+/\text{NADP}^+$ as the co-enzyme and this reaction is freely reversible. The enzyme is present in liver and kidneys and it plays a central role in nitrogen metabolism. Liver GDH activity is allosterically inhibited by ATP and GTP (Fig 4.6).

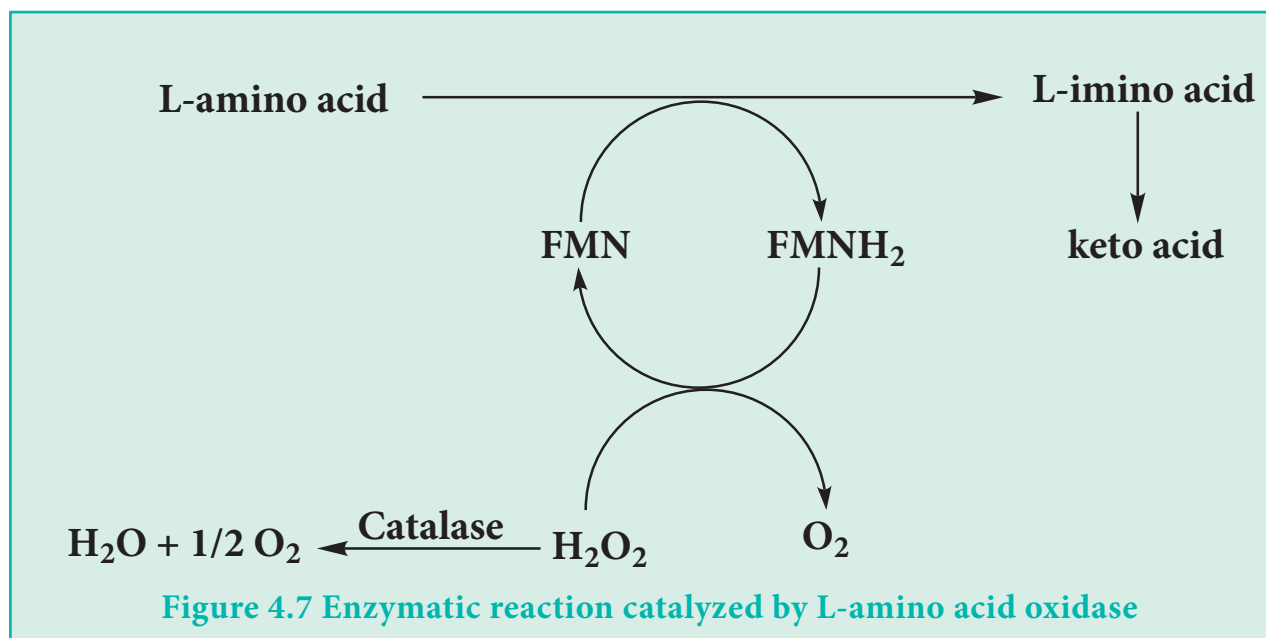
As glutamate acts as a collection centre for all alpha amino groups by transamination and subsequently glutamate liberates ammonia, the process is collectively called as trans-deamination.

L-amino acid oxidases and D-amino acid oxidases are also involved in oxidative deamination.



Steps involved in oxidative deamination:

1. In the first step, L-Amino acid oxidases convert the L-amino acid into L-imino acid, which involves FMN as the coenzyme. The resulting peroxide that is formed is detoxified by catalase (Figure 4.7).



2. In the second step, the imino acid undergoes hydrolytic cleavage to keto acid and ammonia.

All L-amino acids except hydroxyl amino acids and dicarboxy amino acids can be acted upon by L-amino acid oxidases.

D-amino acid oxidases utilize FAD as the coenzyme.

Non-oxidative deamination

Dehydratases act on hydroxyl amino acids like serine and threonine to form pyruvate and alpha keto butyric acid, respectively.

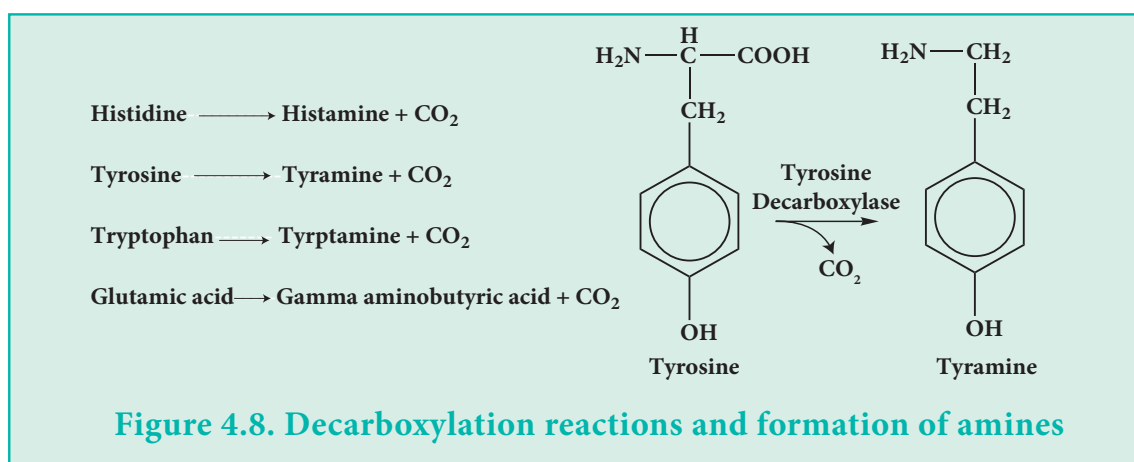
Cysteine desulfurase removes the sulfhydryl group of cysteine to form pyruvate.

The amide groups of glutamine and asparagine are removed by glutaminase and asparaginase, respectively.

4.1.4 Decarboxylation

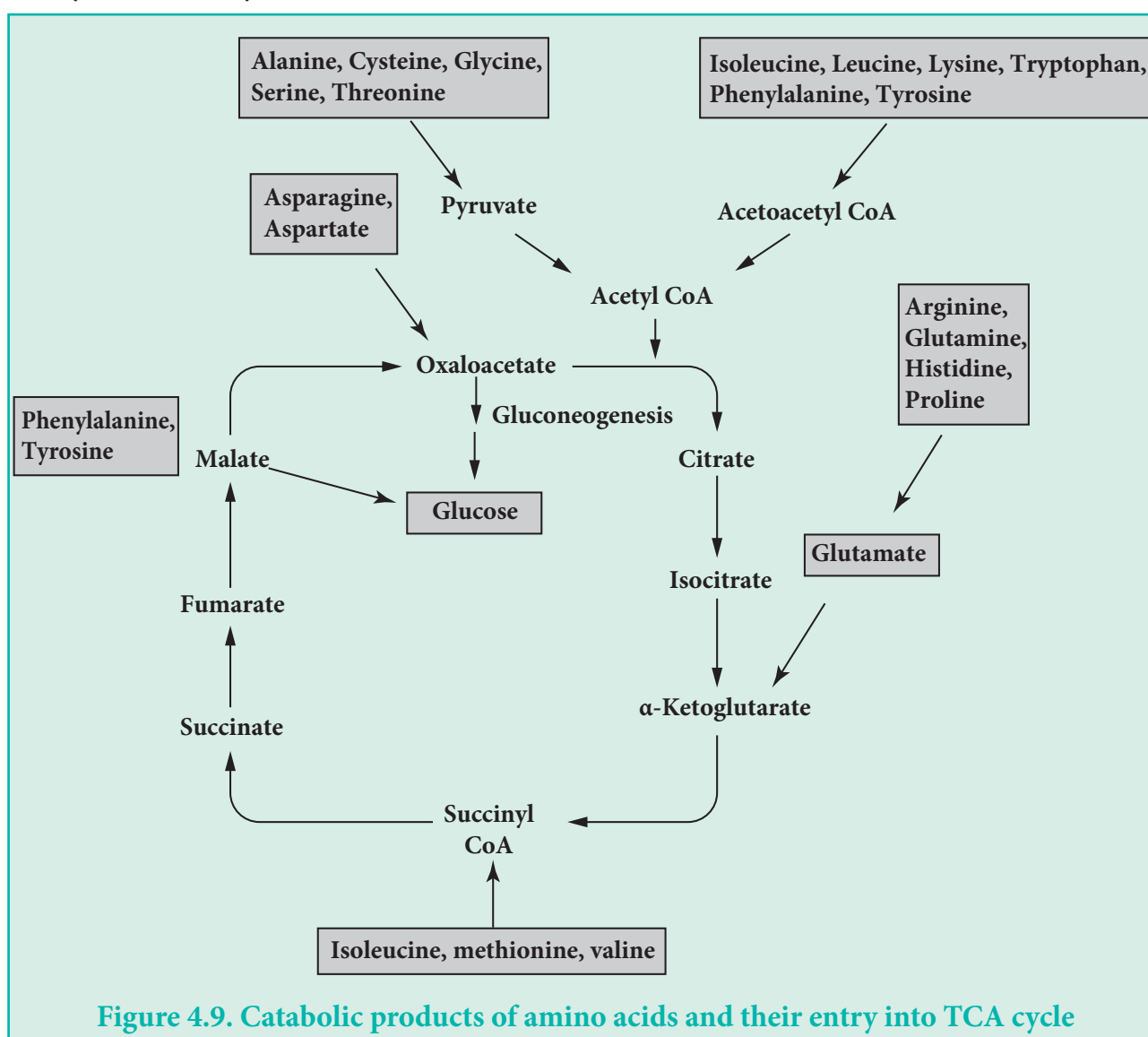
The removal of carboxyl group from the amino acid in the form of CO₂ is called as decarboxylation. Such decarboxylation reactions are involved in the formation of biologically important amines (Figure 4.8). Several amino acid decarboxylases are present in tissues like liver, kidney, lungs and brain that decarboxylate the respective amino acids to form amines.

For example,



4.1.5 Fate of carbon skeleton of amino acids

Entry into TCA cycle



After the amino group is lost, the keto acids formed from the amino acids like pyruvate, alpha ketoglutarate will enter into the TCA cycle and are utilized for energy purposes.

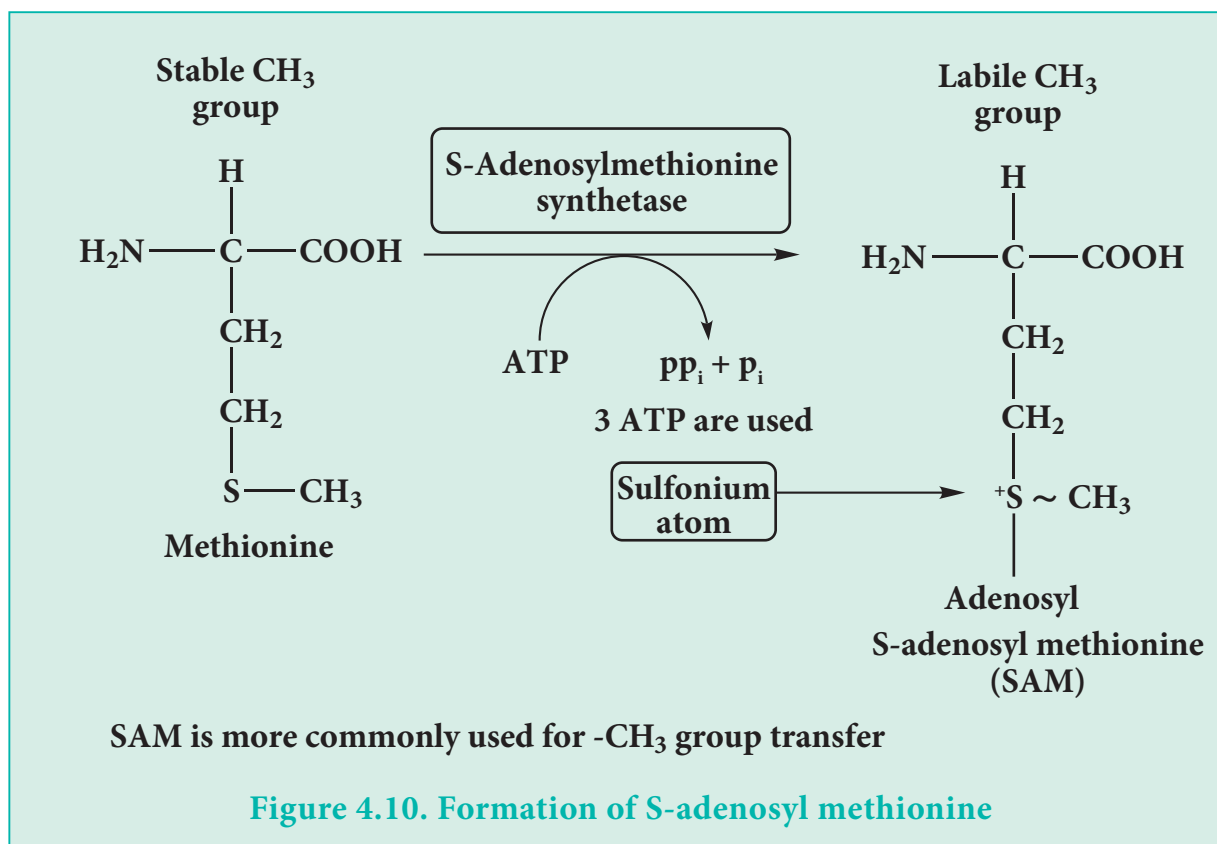
Catabolism of phenylalanine and tyrosine yields fumarate that also enters TCA cycle for further catabolism. These intermediates that enter into TCA cycle can act as precursors of gluconeogenesis (Figure 4.9). Hence, the amino acids that can contribute their carbon skeletons for the synthesis of glucose are called as glucogenic amino acids. eg. glycine, alanine, aspartate, glutamate, etc. However, certain amino acids yield acetyl CoA and acetoacetyl CoA that can act as substrates of fatty acid synthesis or give rise to ketone bodies. These amino acids are called as ketogenic amino acids. Eg. Leucine and Lysine. Certain amino acids like phenylalanine, isoleucine and tyrosine are both glucogenic and ketogenic.

Regeneration of amino acids

By reductive amination or by reversal of trans-deamination, the amino acids can be re-synthesized from their respective keto-acids.

4.1.6 Transmethylation

One carbon metabolism



One carbon metabolism plays an important role in the synthesis of many compounds like purines and pyrimidines. One carbon pool includes formyl, methyl, methylene, methenyl, formimino and hydroxymethyl groups. Except methyl group, all the other one carbon transfers are mediated by Tetrahydrofolate that bears one carbon moieties at N5 and N10 positions. Formate, tryptophan, glycine, serine and choline are the methyl group donors to tetrahydrofolate. Transfer of methyl group involves S-adenosyl methionine (Figure 4.10), which is formed from methionine

S-Adenosyl methionine thus formed acts as a methyl group donor and is involved in the synthesis of epinephrine, creatine and thymine.

4.2. UREA CYCLE

Toxicity of Ammonia

The ammonia formed by trans-deamination and from other sources like bacteria in the gut are transported to the liver and converted to urea. Under physiological conditions, the level of ammonia in the blood is kept under control stringently as the central nervous system is highly susceptible to ammonia intoxication. The major symptoms of ammonia intoxication include slurred speech, tremor, blurred vision, coma and finally death. Ammonia toxicity is because it reacts with α -ketoglutarate to form glutamate, thus depleting the levels of α -ketoglutarate and impairing the function of TCA cycle in neurons.

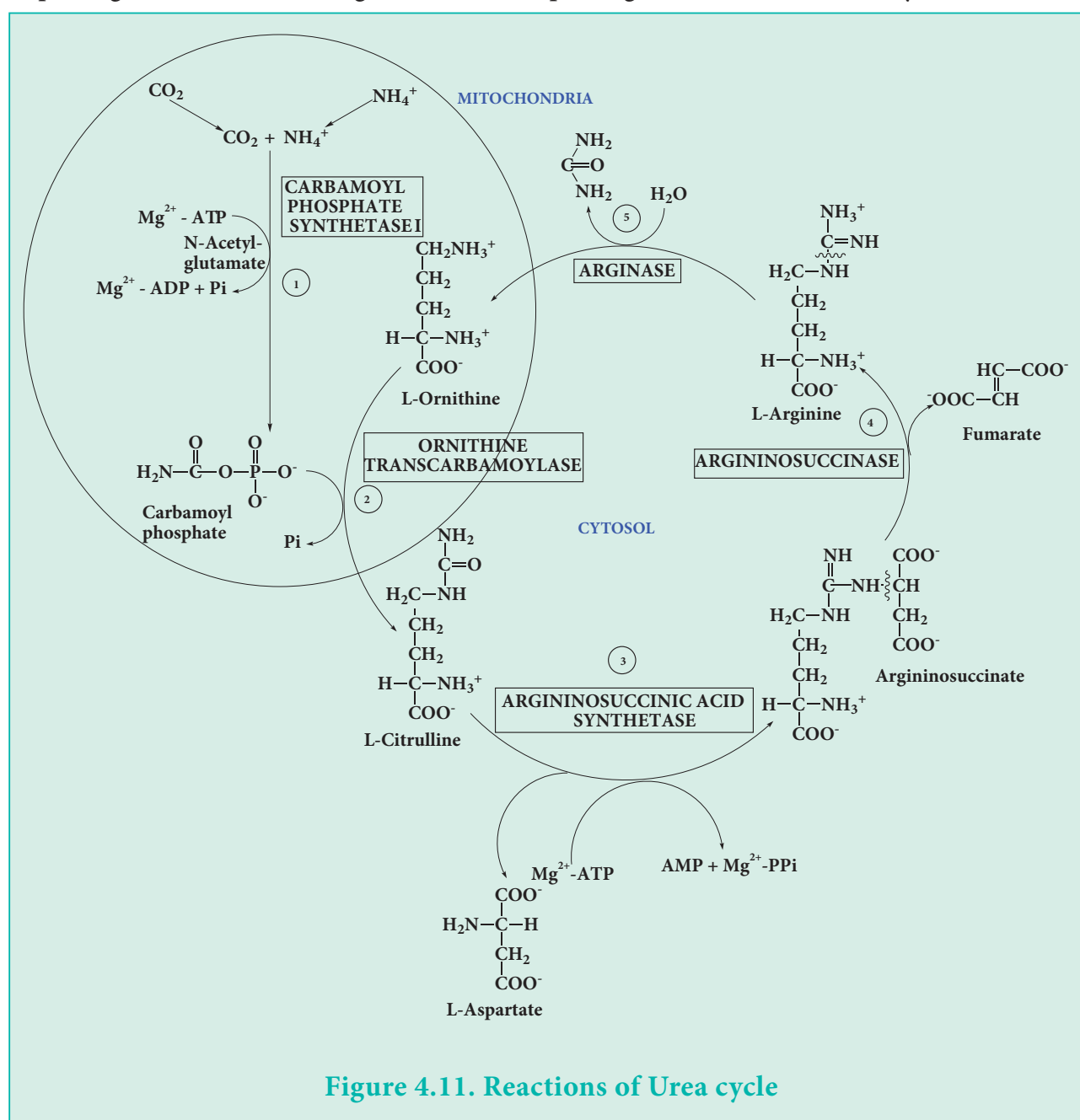


Figure 4.11. Reactions of Urea cycle



The amino group of proteins can be excreted in three forms in living organisms – ammonia, urea or uric acid. Based on the product of elimination, the living organisms can be classified as ammonotelic, ureotelic and uricotelic respectively. Many terrestrial vertebrates excrete the amino nitrogen as urea, while birds and terrestrial reptiles excrete it in the form of uric acid.

In ureotelic animals, the ammonia that is released in the liver is converted to urea and excreted by the kidneys. The pathway of conversion of ammonia to urea involves ornithine in a cyclic fashion. Hence, the pathway is called as urea cycle or Ornithine cycle or Krebs Henseleit cycle after its discoverers Hans Krebs and a medical student, Kurt Henseleit in 1932.

Urea is produced from ammonia with the help of five enzymes and two of these enzymes are present in the mitochondria, while, the rest are present in the cytosol. Out of the two amino groups of urea, one amino group enters the urea cycle as carbamoyl phosphate, and the other enters as aspartate (Figure 4.11).

The five steps of Urea cycle:

- 1. Formation of carbamoyl phosphate:** Carbamoyl phosphate synthetase I present in the mitochondria facilitates the synthesis of carbamoyl phosphate from carbon dioxide, ammonia and ATP. This reaction consumes two ATPs and is irreversible. It is different from the Carbamoyl phosphate synthetase II present in the cytosol, which is involved in pyrimidine biosynthesis, and uses glutamine as the substrate.
- 2. Formation of Citrulline:** Carbamoyl phosphate reacts with ornithine to form citrulline and the reaction is catalysed by Ornithine transcarbamoylase. The other three enzymes of urea cycle are present in the cytosol. Hence, Citrulline is transported out of the mitochondria by a transporter.
- 3. Formation of argininosuccinate:** Citrulline and aspartate condense together to form argininosuccinate in the presence of the enzyme Argininosuccinate synthetase. This reaction requires ATP and is catalyzed by a citrullyl-AMP intermediate (the amino group of Aspartate provides the second nitrogen atom for Urea synthesis).
- 4. Formation of arginine from argininosuccinate:** This reaction catalysed by argininosuccinase releases arginine and fumarate (which enters the citric acid cycle).
- 5. Formation of urea and regeneration of ornithine:** The final step in urea synthesis is the release of urea from arginine by the enzyme arginase and this step regenerates ornithine. The ornithine thus formed enters into the mitochondria with the help of a transporter.

One molecule of Urea is synthesized from one molecule of carbondioxide, one molecule of ammonia and the amino group of aspartate. The reactions consume four high energy bonds equivalent to four ATPs.

4.3 Formation of Niacin

Niacin (pyridine-3-carboxylic acid) is formed from the amino acid tryptophan. Niacin is the precursor for the synthesis of nicotinamide coenzymes. Only 3% of the tryptophan is converted to niacin in the liver. Maize eating population demonstrate niacin deficiency.

The steps involved in the synthesis of niacin (Figure 4.12) from tryptophan are:

1. Tryptophan is oxidized to N-formyl kynurenine.
2. N-formyl kynurenine transfers its formyl group to Tetrahydrofolate and results in the formation of Kynurenine
3. Kynurenine is hydroxylated at the 3rd position to form 3-hydroxy kynurenine.
4. Alanine is removed from 3-hydroxy kynurenine in the presence of pyridoxal phosphate to form 3-hydroxy anthranilic acid.
5. 3-hydroxy anthranilic acid is converted to quinolinic acid
6. Quinolinic acid on decarboxylation yields niacin.

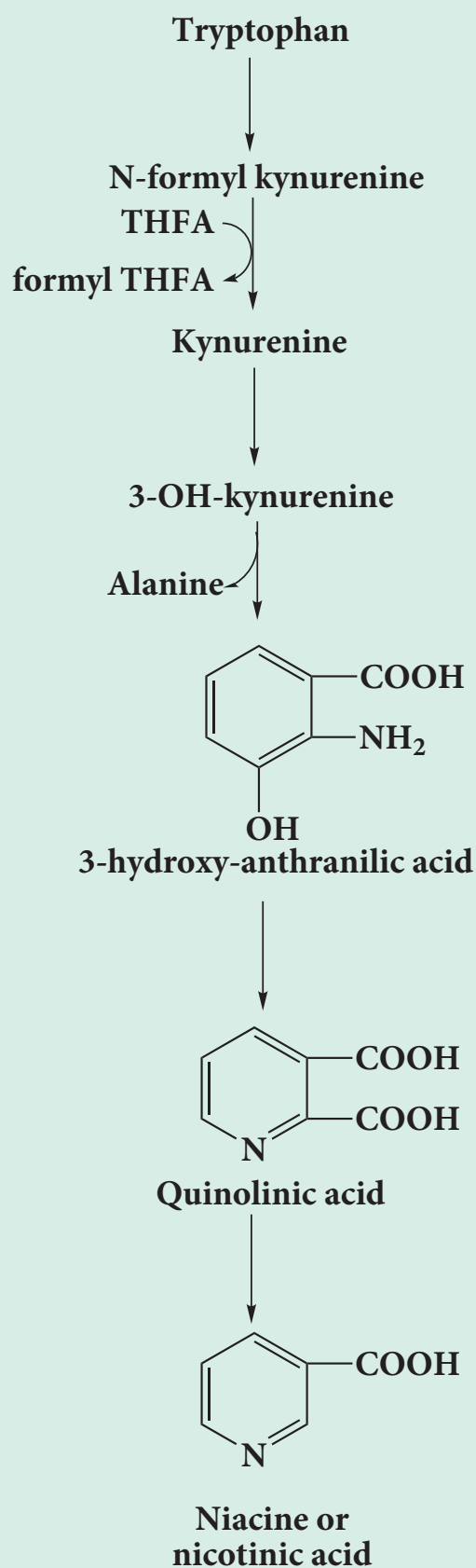
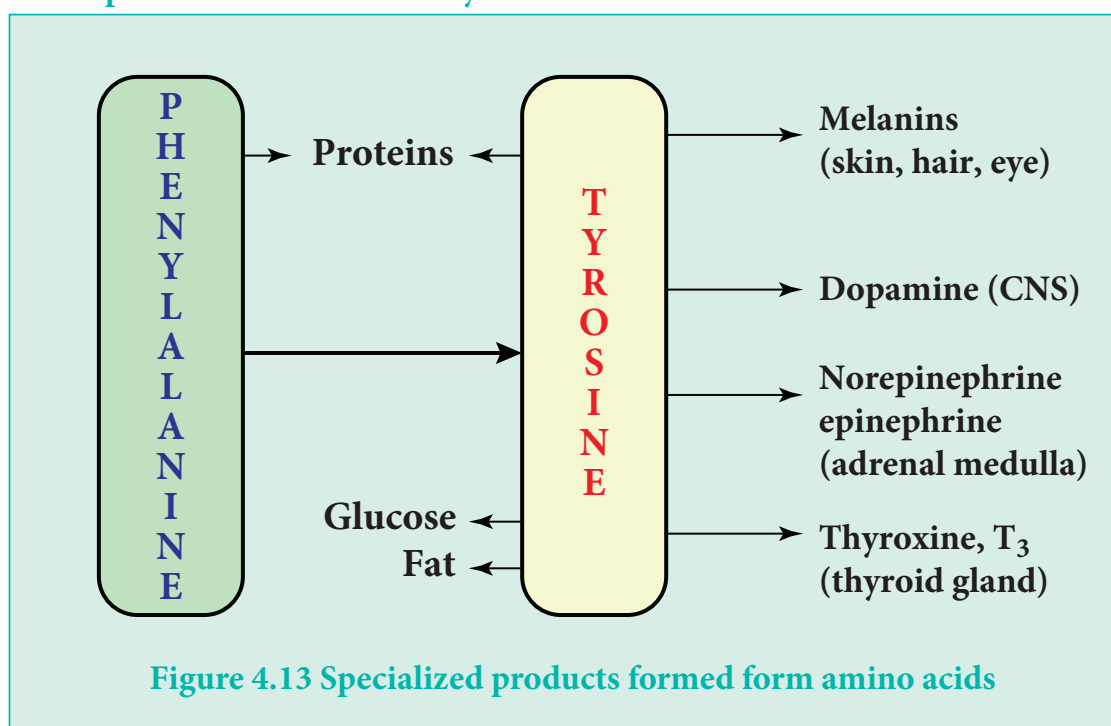


Figure 4.12. Biosynthesis of Niacin

Specialized products formed from tyrosine



Many specialized products are formed from tyrosine in different tissues (Figure 4.13). In the nervous system, tyrosine serves as the precursor for dopamine. In the adrenal glands, it is converted to stress hormones namely, epinephrine and nor-epinephrine. In the thyroid gland, tyrosine is iodinated and converted to thyroid hormones namely tri-iodo thyronine (T₃) and tetraiodothyronine (T₄-thyroxine)

4.4 Formation of Melanin

Melan means black. Melanin is the pigment that is responsible for the colour of the skin, hair and eyes. Melanin is a group of random polymers of indole quinone formed from tyrosine in the melanocytes. The major enzyme involved in its synthesis is Tyrosinase, which is a copper dependent enzyme (Figure 4.14).

1. Tyrosine is hydroxylated to form dihydroxyphenyl alanine (DOPA)
2. DOPA is decarboxylated to DOPA quinone.
3. DOPA quinone is converted to indole quinone by a series of reactions.
4. Indole quinone polymerizes to melanin.

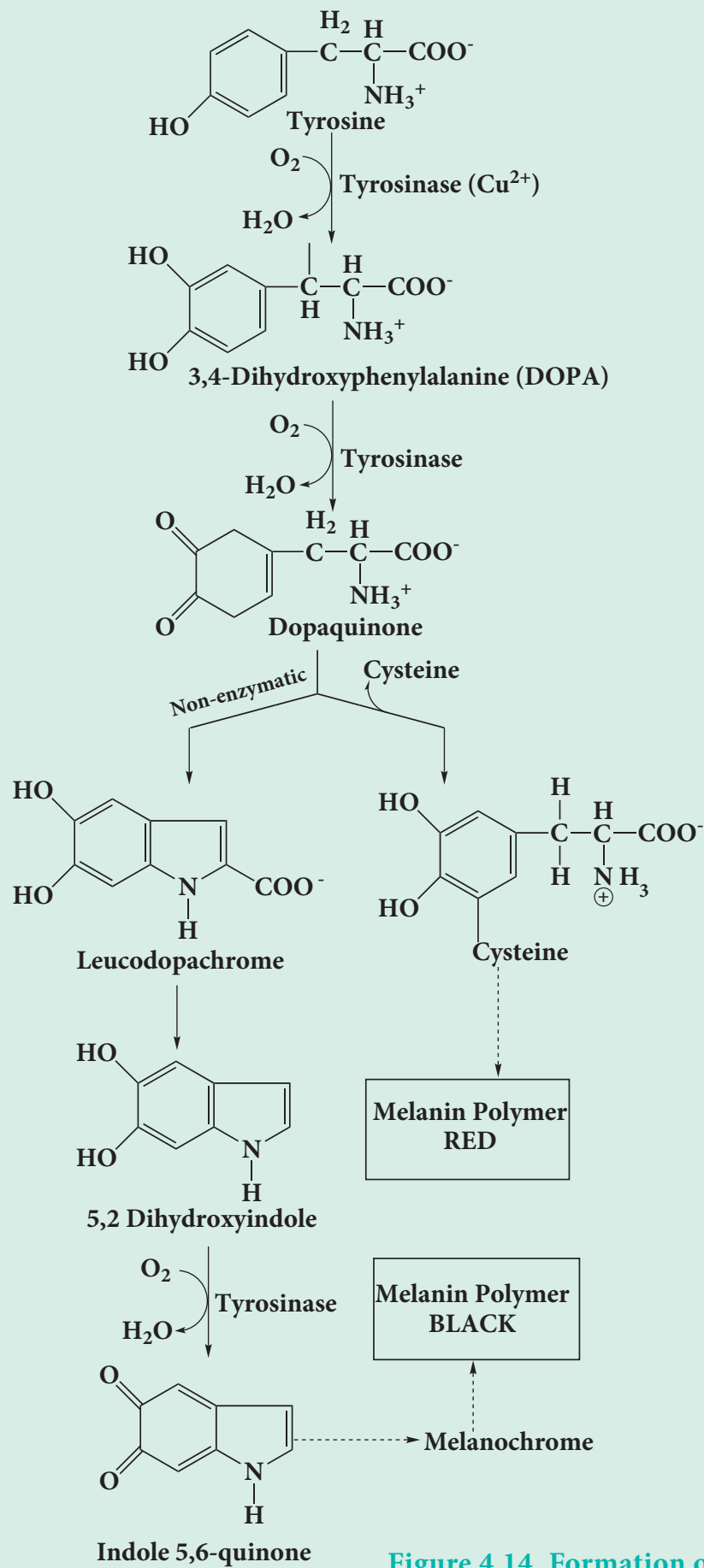


Figure 4.14. Formation of melanin



4.5 Formation of thyroid hormones

Thyroid gland, a bilobed organ weighing about 20 – 25 grams, is responsible for the synthesis of thyroid hormones. Thyroid hormones are responsible for maintenance of basal metabolic rate. Tri-iodothyronine and tetraiodothyronine are the two important thyroid hormones (Fig 4.15).

The steps involved in the synthesis of thyroid hormones are:

1. Conversion of inorganic iodine to iodide (activation of iodine).
2. Iodination of tyrosine rings in the protein thyroglobulin to form di-iodotyrosine
3. Formation of di-iodothyronine and tetraiodothyronine by coupling and removal of alanine. Thyroglobulin bearing iodinated residues is stored in the thyroid gland.
4. Depending upon the needs of the body, TSH signals the thyroid gland resulting in the proteolysis of thyroglobulin and release of T₃ and T₄.

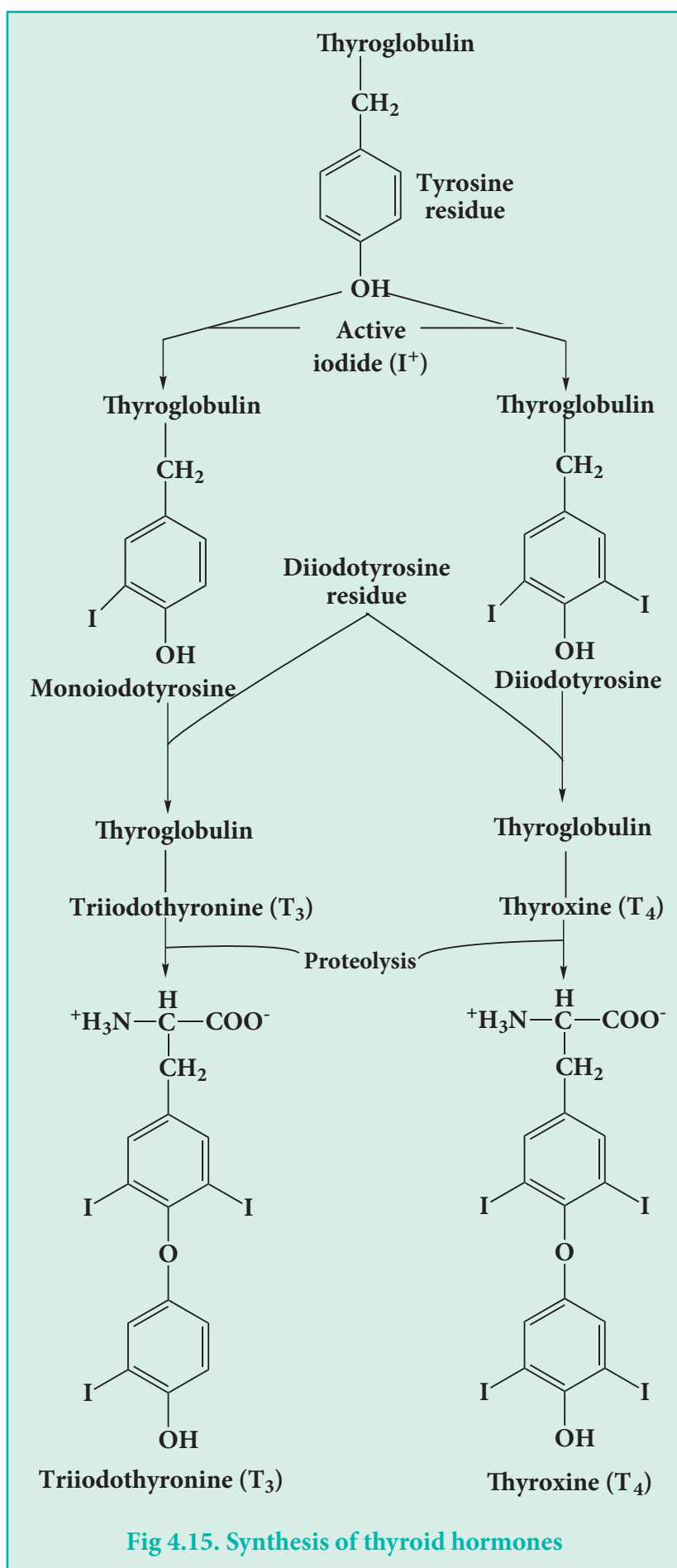


Fig 4.15. Synthesis of thyroid hormones

4.6 Formation of Catecholamines

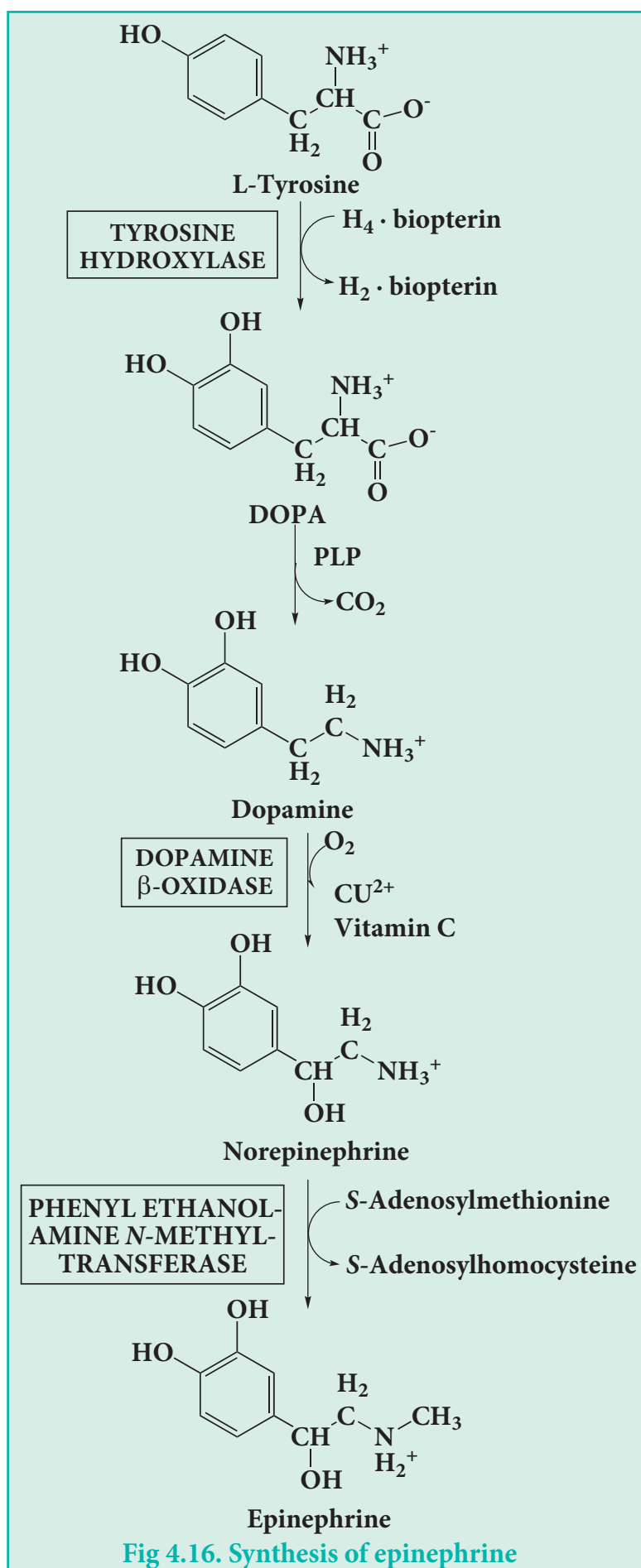
Adrenal glands present in the top of the kidneys are responsible for the secretion of hormones that regulate mineral metabolism (secreted from the adrenal cortex) along with hormones that are secreted in response to stress (secreted by the adrenal medulla), such as epinephrine and nor-epinephrine, that are specialized products of tyrosine (Fig 4.16).

The functions of catecholamines (epinephrine and nor-epinephrine) are:

1. They influence the metabolism of glucose and have an anti-insulin action, i.e. they raise the blood glucose levels.
2. They are responsible for the flight and fight response.
3. These hormones tend to increase the blood pressure by constricting the blood vessels and increasing the force by which heart muscles contract.

The steps involved in the synthesis of catecholamines are:

1. Tyrosine is acted upon by the enzyme tyrosine hydroxylase in the adrenal medulla to form dihydroxy phenyl alanine (DOPA).
2. DOPA is decarboxylated to Dopamine by DOPA decarboxylase.





3. Dopamine is converted to nor-epinephrine (Nor means No 'R' group, ie. Methyl group) by a reaction catalyzed by DOPA hydroxylase.
4. Nor-epinephrine is methylated to Epinephrine. The methyl group donor is S-adenosyl Methionine and the enzyme is N-methyl transferase.

Activity



1. Prepare a chart to explore the link between amino acid metabolism and TCA cycle
2. Prepare a chart to explain urea cycle.
3. With the help of e-books, try to prepare a list of syndromes associated with urea biosynthesis.



- Transaminases AST and ALT are important markers to assess liver damage. Many occupational hazards occur on exposure to solvents like CCl_4 and CHCl_3 . **Example:** Liver damage.
- Insulin, Insulin like Growth factor-1 and Growth hormone have anabolic effects on protein metabolism.
- Ingestion of proteins or infusion with amino acids also have an anabolic effect on protein metabolism.

Summary

- Excess intake of amino acids are used for energy purposes, gluconeogenesis or ketogenesis, while their amino group is converted to urea.
- The carbon skeletons of amino acids are degraded on entry into TCA cycle.
- The first step in the catabolism of amino acids is the removal of its amino group. This removal can happen in two different ways - Transamination and Deamination.
- Transamination is a process, in which transfer of amino groups occur between a keto acid and an amino acid. Alanine transaminase (ALT) and Aspartate transaminase (AST) are the important transaminases.
- Aspartate transaminase catalyses the inter-conversion of aspartate and alpha ketoglutarate to oxaloacetate and glutamate, while alanine transaminase catalyses the conversion of alanine and alpha ketoglutarate to pyruvate and glutamate.



- Serum transaminases are significant markers for diagnosis of liver and heart diseases.
- Many non-essential amino acids are synthesized using transamination reactions.
- Deamination is classified into oxidative deamination and non-oxidative deamination.
- Oxidative Deamination is a process in which the alpha amino group of the amino acid is removed as ammonia, coupled with oxidation.
- Oxidative deamination involving the removal of amino group of glutamate as ammonia is the key reaction involved in delivery of amino groups to the liver for urea synthesis.
- L-amino acid oxidases and D-amino acid oxidases are also involved in oxidative deamination.
- Glutaminase and asparaginase remove the amino groups of glutamine and asparagine.
- Decarboxylation reactions are involved in the formation of biologically important amines.
- After the amino group is lost, the keto acids formed from the amino acids like pyruvate, alpha keto glutarate will enter into the TCA cycle and are utilized for energy purposes.
- Amino acids can be glucogenic and /or ketogenic.
- Urea is produced from ammonia with the help of five enzymes and two of these enzymes are present in the mitochondria, while, the rest are in the cytosol. Out of the two amino groups of the urea, one amino group enters the urea cycle as carbamoyl phosphate, and the other enters as aspartate.
- Niacin (pyridine 3 carboxylic acid) is formed from the amino acid tryptophan.
- Many specialized products are formed from tyrosine in different tissues. In the nervous system, tyrosine serves as the precursor for dopamine. In the adrenal glands, it is converted to stress hormones namely, epinephrine and nor-epinephrine. In the thyroid gland, tyrosine is iodinated and converted to Thyroid hormones namely tri-iodothyronine and tetraiodothyronine.



EVALUATION



I Multiple choice questions

1. Generally, Non-essential amino acids are synthesized by _____ reactions.
 - a) Oxidative deamination
 - b) Oxidative decarboxylation
 - c) **Transamination**
 - d) Non-oxidative deamination
2. Essential Aminoacids
 - a) are synthesized in the liver
 - b) are only used in the synthesis of proteins
 - c) **cannot be synthesized in the body**
 - d) are only catabolized to urea
3. Transamination involves
 - a) **exchange of amino groups between a ketoacid and an aminoacid**
 - b) addition of keto groups from a ketoacid to an aminoacid
 - c) transfer of amino groups from a ketoacid to an aminoacid
 - d) all the above
4. The coenzyme involved in transamination is
 - a) **Pyridoxal phosphate**
 - b) Coenzyme Q
 - c) Thiamine
 - d) Pyridoxamine Phosphate
5. Serum Transaminases are significant markers for
 - a) Liver diseases
 - b) Heart Diseases
 - c) **Both a and b**
 - d) None of the above
6. In ureotelic animals, the amino group is excreted as
 - a) Ammonia
 - b) Urea and Uric Acid



- c) **Urea**
d) Uric acid
7. Carbamoyl Phosphate Synthetase –II is present in the
a) Mitochondria
b) **Cytosol**
c) Partly mitochondria and partly in the cytosol
d) Nucleus
8. DOPA is
a) Dehydroxyphenylalanine
b) Dihydroxyphenylacetate
c) **Dihydroxyphenylalanine**
d) Dehydrophenylalanine
9. The first step in the catabolism of amino acids is
a) **Removal of its amino group**
b) Removal of the carboxyl group
c) Removal of the carbon skeleton
d) Removal of methyl group
10. _____ serves as a precursor for dopamine
a) Tryptophan
b) Hydroxyproline
c) **Tyrosine**
d) Proline
11. The _____ reaction is catalyzed by the glutamate dehydrogenase with _____ as the co-enzyme.
a) Irreversible and $\text{NADH}^+/\text{NADPH}^+$
b) Irreversible and $\text{NAD}^+/\text{NADP}^+$
c) **Reversible and $\text{NAD}^+/\text{NADP}^+$**
d) Reversible and $\text{NADH}^+/\text{NADPH}^+$
12. _____ amino acids are both glucogenic and ketogenic in nature.
a) **Isoleucine and tyrosine**
b) Glycine and alanine
c) Leucine and lysine



- d) Aspartate and glutamate
13. _____ acts as a methyl donor for the synthesis of epinephrine, creatine and thymine.
- a) Methotrexate
 - b) **S- adenosyl methionine**
 - c) Tetrahydrofolate
 - d) Biotin
14. Catabolism of phenylalanine and tyrosine yield ____.
- a) Succinate
 - b) α -ketoglutarate
 - c) Malate
 - d) **Fumarate**
15. Co-enzyme required for the conversion of L-Amino acid into L-Imino acid.
- a) FAD^+
 - b) **FMN**
 - c) NAD^+
 - d) NADP^+

II Give short answer for the following

1. What is the fate of excess dietary amino acids?
2. What are non-essential amino acids? How are they synthesized?
3. Give the reaction catalyzed by AST.
4. What are glucogenic amino acids and ketogenic amino acids? Give examples.
5. What is decarboxylation? Give example.
6. Name the enzymes involved in urea cycle.
7. Mention any four amino acids whose carbon skeletons enter into TCA cycle.
8. How is citrulline formed in the mitochondria converted to argininosuccinate in the cytosol?
9. How is tyrosine converted to dopaquinone?
10. What is the role of S-adenosyl methionine in conversion of norepinephrine to epinephrine?

III Give short answer for the following

1. What is transamination? Add a note on its features.
2. Explain the different types of deamination reactions with examples.
3. Write a note on the formation of amines from amino acids.

- Describe one carbon metabolism and write its significance.
- What is ammonia intoxication? Give its symptoms.
- How is tryptophan converted to niacin?

III Answer the following

- Write in detail about oxidative and Non-oxidative deamination.
- Elaborate on Urea Cycle and its significance.
- Write a note on specialized products formed from tyrosine.
- Briefly discuss on the synthesis and functions of thyroid hormones.
- Enumerate the steps involved in the formation of catecholamines.

Analyze the table and match the following:

Amino acids	Entry into TCA cycle	Glucogenic or Ketogenic
Glutamate	Succinyl Co-A	Ketogenic
Aspartate	Oxaloacetate	Both glucogenic and ketogenic
Tyrosine	Acetyl Co-A	Glucogenic
Isoleucine	Alpha ketoglutarate	Both glucogenic and ketogenic
Leucine	Fumarate	Glucogenic

Answer Table:

Amino acids	Entry into TCA cycle	Glucogenic or Ketogenic
Glutamate	Alpha ketoglutarate	Glucogenic
Aspartate	Oxaloacetate	Glucogenic
Tyrosine	Fumarate	Both glucogenic and ketogenic
Isoleucine	Succinyl Co-A	Both glucogenic and ketogenic
Leucine	Acetyl Co-A	Ketogenic

Assertion and Reason:

Direction: In each of the following questions a statement of assertion (A) is given and a corresponding statement of reason (R) is given just below it. Mark the correct statement as.

- If both A and R are true and R is correct explanation of A
- If both A and R are true but R is not the correct explanation of A
- If R is true but A is false
- If both A and R are false.



1. Assertion: GDH plays a major role in nitrogen metabolism.

Reason : Glutamate acts as a sink for amino groups of amino acid.

2. Assertion: Defects in urea cycle will affect brain.

Reason : Depletion of α -ketoglutarate occurs due to its conversion to glutamate.

3. Assertion: α -ketobutyric acid is formed by transamination reaction.

Reason : Transamination reaction catalyses the inter-conversion of ketoacid and amino acid.

4. Assertion: Essential amino acid are synthesized from α -keto acid by the transfer of amino group with the help of transaminases.

Reason : Transfer of amino group occurs during synthesis and degradation of amino acid.

5. Assertion: Phenyl alanine is a ketogenic amino acid.

Reason : Catabolism of phenylalanine yields fumarate that enters into TCA cycle.

Answer:

1. a) Both A and R are true and R is correct explanation of A

2. a) Both A and R are true and R is correct explanation of A

3. c) R is true but A is false

4. c) R is true but A is false

5. b) Both A and R are true but R is not the correct explanation of A



Concept Map

