

# Protein Metabolism

# Learning Objectives

- Describe the digestion, absorption and transport of dietary proteins with associated disorders
- Explain amino acid pool
- Enumerate the stages of catabolism of amino acids
- Describe formation and transport of ammonia and explain the role of transamination, deamination reactions in the formation of ammonia
- Discuss disposal of ammonia in the form of urea (urea cycle), its significance, regulation and metabolic inborn disorders of urea cycle

- Describe the metabolic pathways for glycine and branched chain amino acids valine, isoleucine and leucine and inborn disorders associated with metabolism of each amino acid
- Describe the metabolic pathways for aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and inborn disorders associated with metabolism of each amino acid
- Describe the metabolic pathways for sulfur-containing amino acids (methionine, cysteine and cystine) and inborn disorders associated with metabolism of each amino acid

# Digestion And Absorption Of Proteins

- Proteolytic enzymes break down dietary proteins into their constituent **amino acids**.
- These enzymes are produced by:
  - stomach*
  - pancreas*
  - small intestine.*
- There is no digestion of protein in mouth.

## *Digestion in Stomach*

When protein enters the stomach, *gastrin* stimulates the release of :

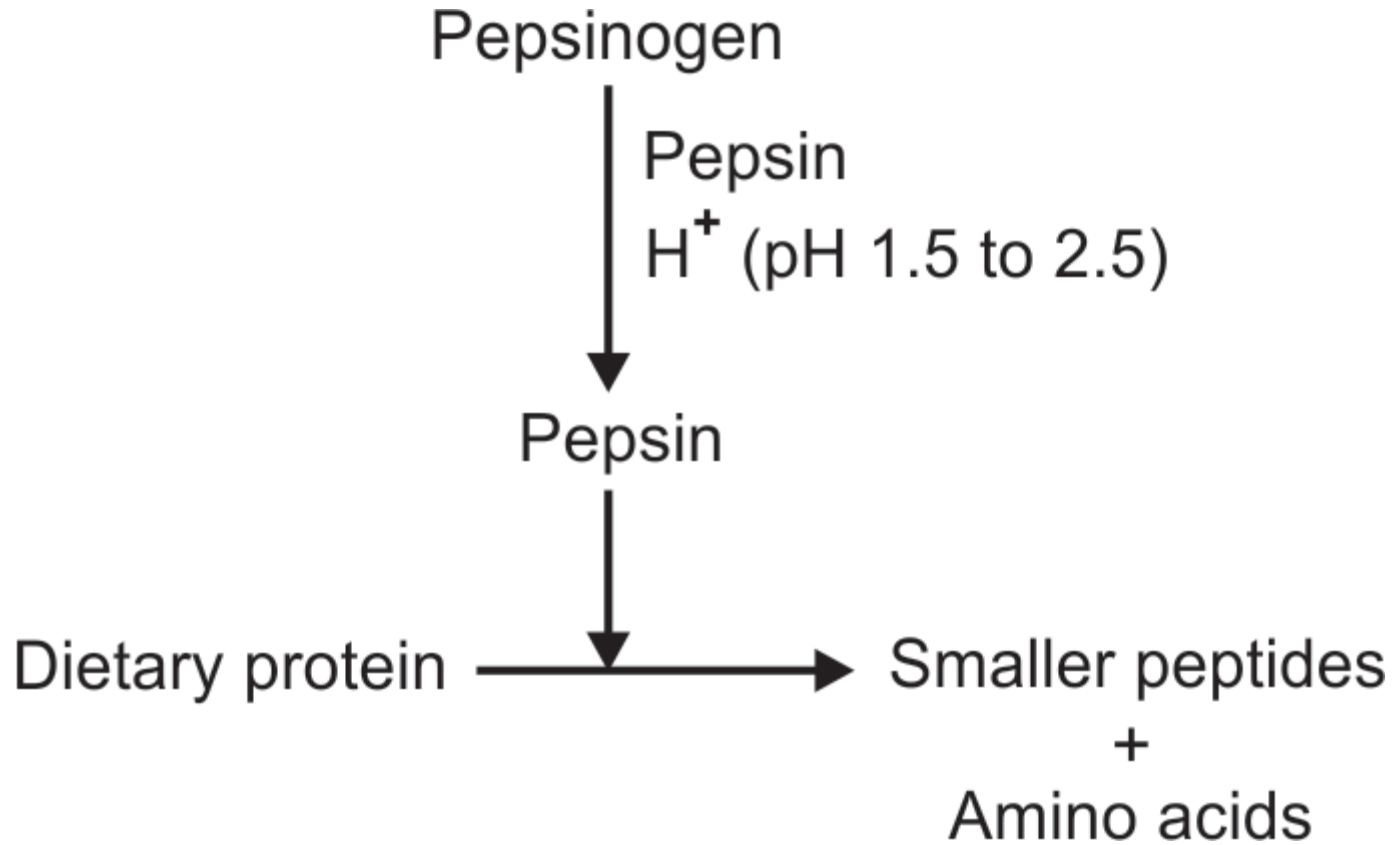
- Hydrochloric acid
- Pepsinogen
- Rennin in infants.

# Hydrochloric acid

- **Denatures** proteins making their internal peptide bonds more accessible to subsequent **hydrolysis** by proteases
- Provides an **acid environment** for the action of **pepsin**.

# Pepsin

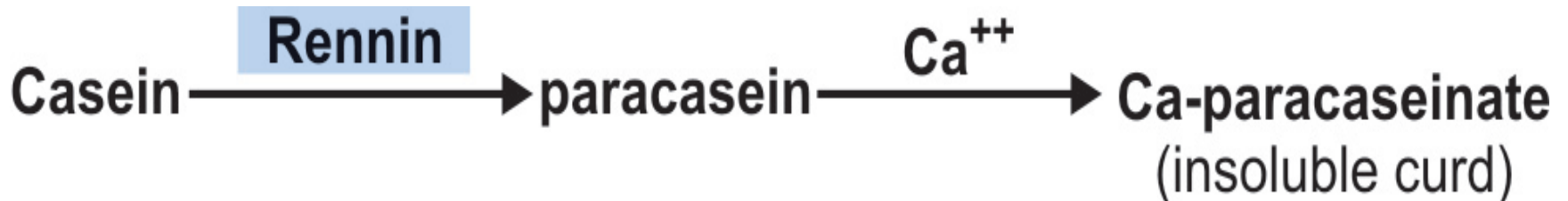
- It is secreted as the *pepsinogen* an inactive form.
- Pepsin cleaves those peptide bonds of protein involving :
  - *Aromatic amino acids* (phenylalanine, tyrosine and tryptophan)
  - *Acidic amino acids* (aspartic acid and glutamic acid).
- Pepsin cleaves long polypeptide chains into a mixture of *smaller peptides* and some *free amino acids*.



**Activation of pepsinogen and action of pepsin**

# Rennin

- Absent in adults.
- Also called **chymosin** or **rennet**.
- Action of rennin is to **clot milk**. The purpose of this reaction is to convert milk into a more solid form **to prevent the rapid passage** of milk from the stomach of infants.



**Rennin** and **renin** are different. Renin is secreted by kidney and is involved in regulation of water and electrolyte balance and blood pressure.

# Digestion in Intestine by Pancreatic Enzymes

There are two types of peptidase enzymes secreted by pancreas.

- 1 Endopeptidase
- 2 Exopeptidase

- Endopeptidases cleave **internal peptide bonds**.
  
- Exopeptidase hydrolyse the peptide bonds of **terminal amino acids**.
  
- Endopeptidases secreted by pancreas are:
  - *Trypsin*
  - *Chymotrypsin*
  - *Elastase*.

➤ Endopeptidase are secreted in **proenzyme** (inactive) forms,

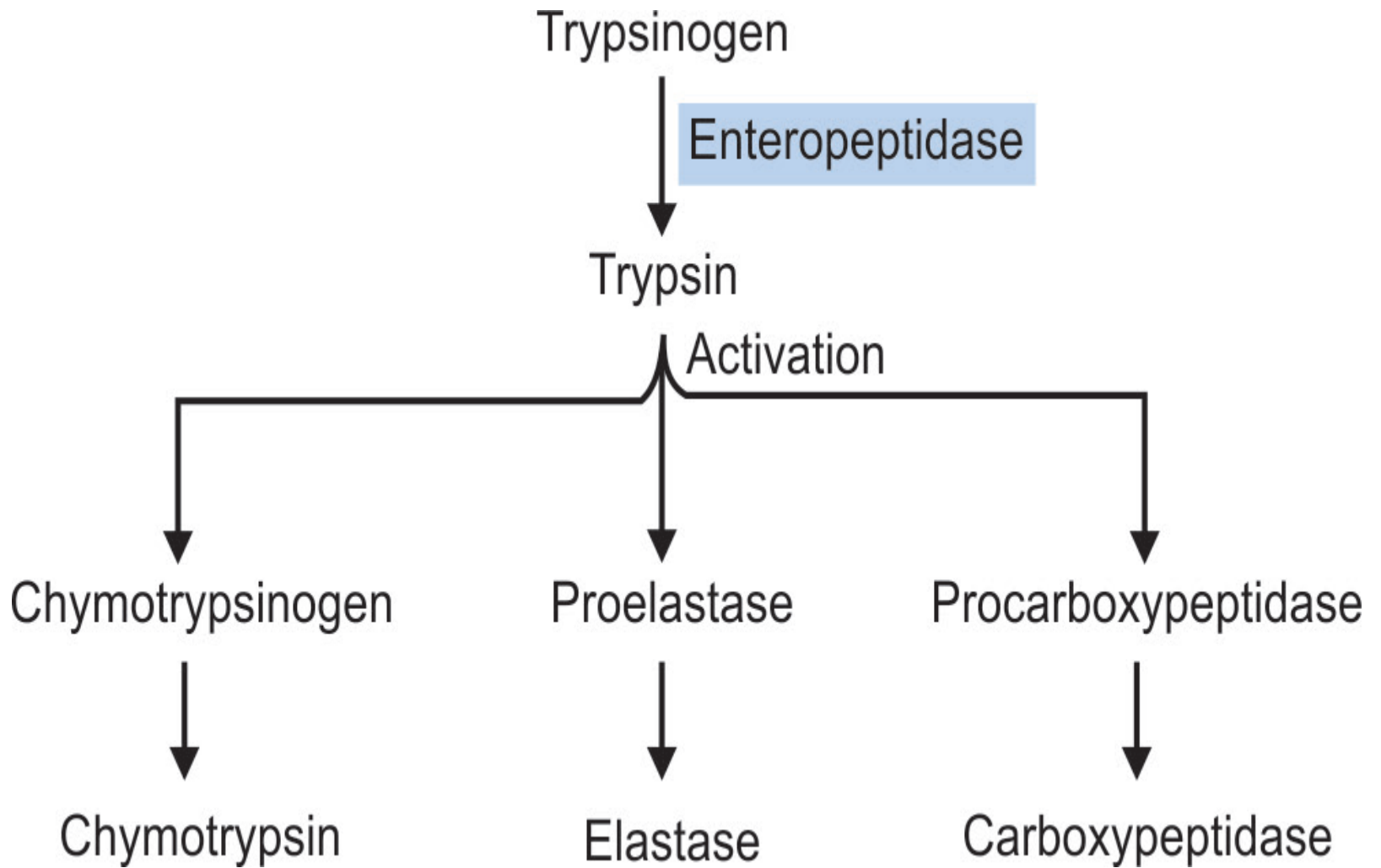
Trypsinogen

Chymotrypsinogen

Proelastase.

➤ Exopeptidase are of two types:

- **Carboxypeptidase** secreted by pancreas act on C-terminal amino acid
- **Aminopeptidases** secreted by mucosal cell act on N-terminal amino acid.



**Figure 12.1:** Activation of pancreatic proenzymes.

**TABLE 12.1: Specificity of proteolytic enzymes.**

<i>Enzymes</i>	<i>Site of action</i>	<i>Cleavage points of peptide bond</i>
Pepsin	Stomach	Phe, Trp, Tyr, and Leu (N)
Trypsin	Intestine	Lys, Arg (C)
Chymotrypsin	Intestine	Phe, Trp, and Tyr (C)
Elastase	Intestine	Small nonpolar amino acids like alanine, serine, and glycine
Carboxypeptidase	Intestine	Successive C-terminal amino acids
Aminopeptidase	Intestinal mucosa	Successive N-terminal amino acids

Peptide bond cleavage occurs on either the carbonyl (C) or the amino (N) side of the indicated amino acid residues.

## *Digestion in Intestine by Intestinal Proteases*

- Enzymes secreted by the mucosa of the small intestine:

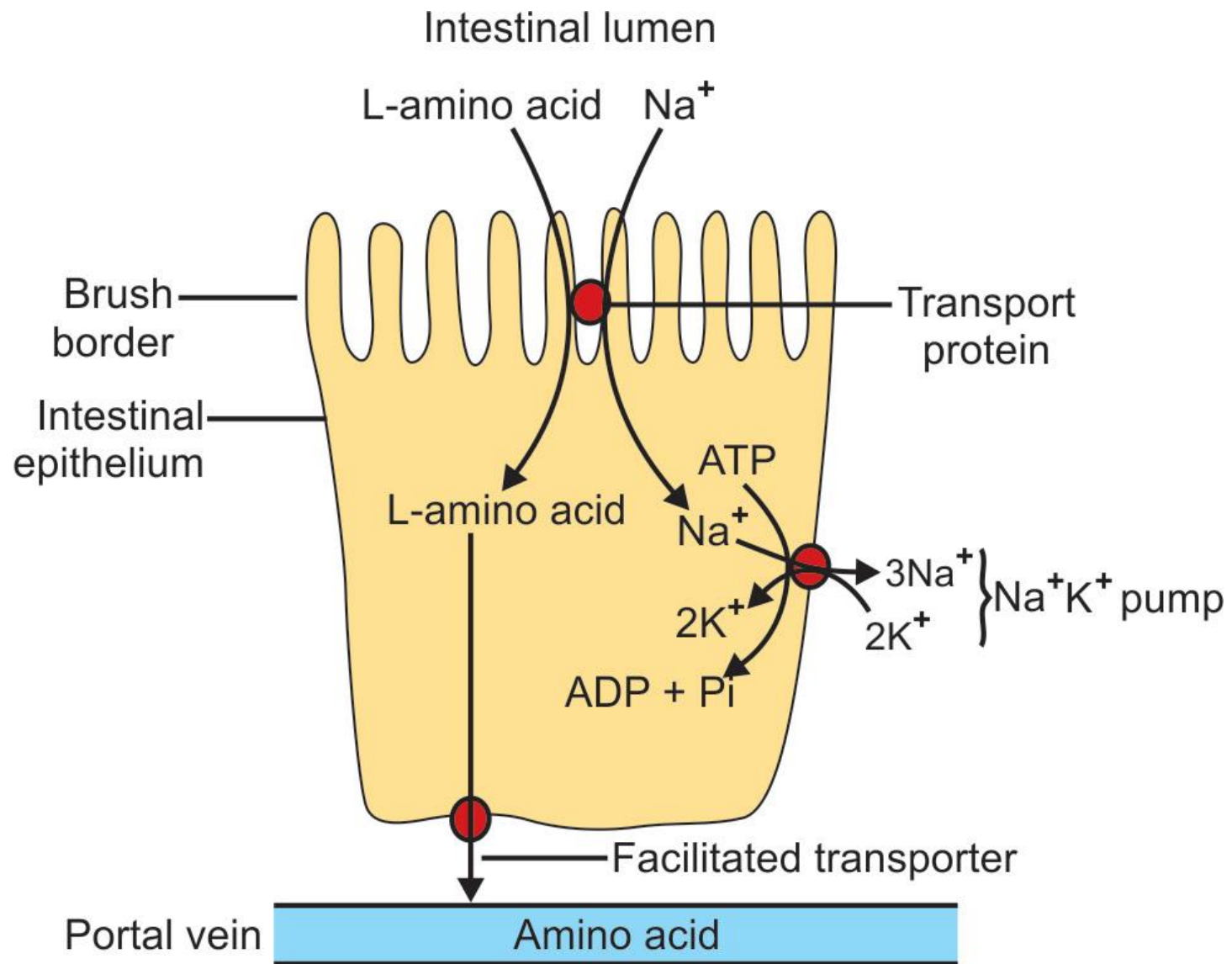
Aminopeptidases

Dipeptidases.

- **Aminopeptidase** is an exopeptidase, hydrolyze peptide bonds next to N-terminal amino acids of the short peptides.
- The **dipeptidases** complete digestion of dipeptides to free amino acids.

# Absorption of Amino Acid

- The absorption of most amino acids involves an *active transport mechanism*, requiring **ATP** and specific **transport proteins**
- Many transporters have **Na<sup>+</sup> dependent** mechanisms, coupled with **Na<sup>+</sup> K<sup>+</sup> pump** .
- All are specific for only **L-amino acid**.
- **D-amino acids** are transported by **passive diffusion**.



**Figure 12.2:** Stereospecific transport of L-amino acid across the intestinal epithelium.

Alton Meister proposed that **glutathione** ( $\gamma$ -glutamyl cysteinylglycine) participates in absorption of amino acids in intestine, kidneys and brain and the cycle is called **gammaglutamyl cycle** or **Meister cycle**.

## Absorption of Intact Protein

- Small intestinal cells of fetal and new-born infants are able to absorb intact proteins, e.g. **immunoglobulin**
- **IgA** from colostrum of maternal milk are absorbed intact without loss of biologic activity, so that they provide passive immunity to the infant.
- The intact proteins are **not absorbed** by **adult** intestine

## Disorders Associated with Amino Acid Transport

Genetic diseases arising from defects in amino acid transporters have been reported

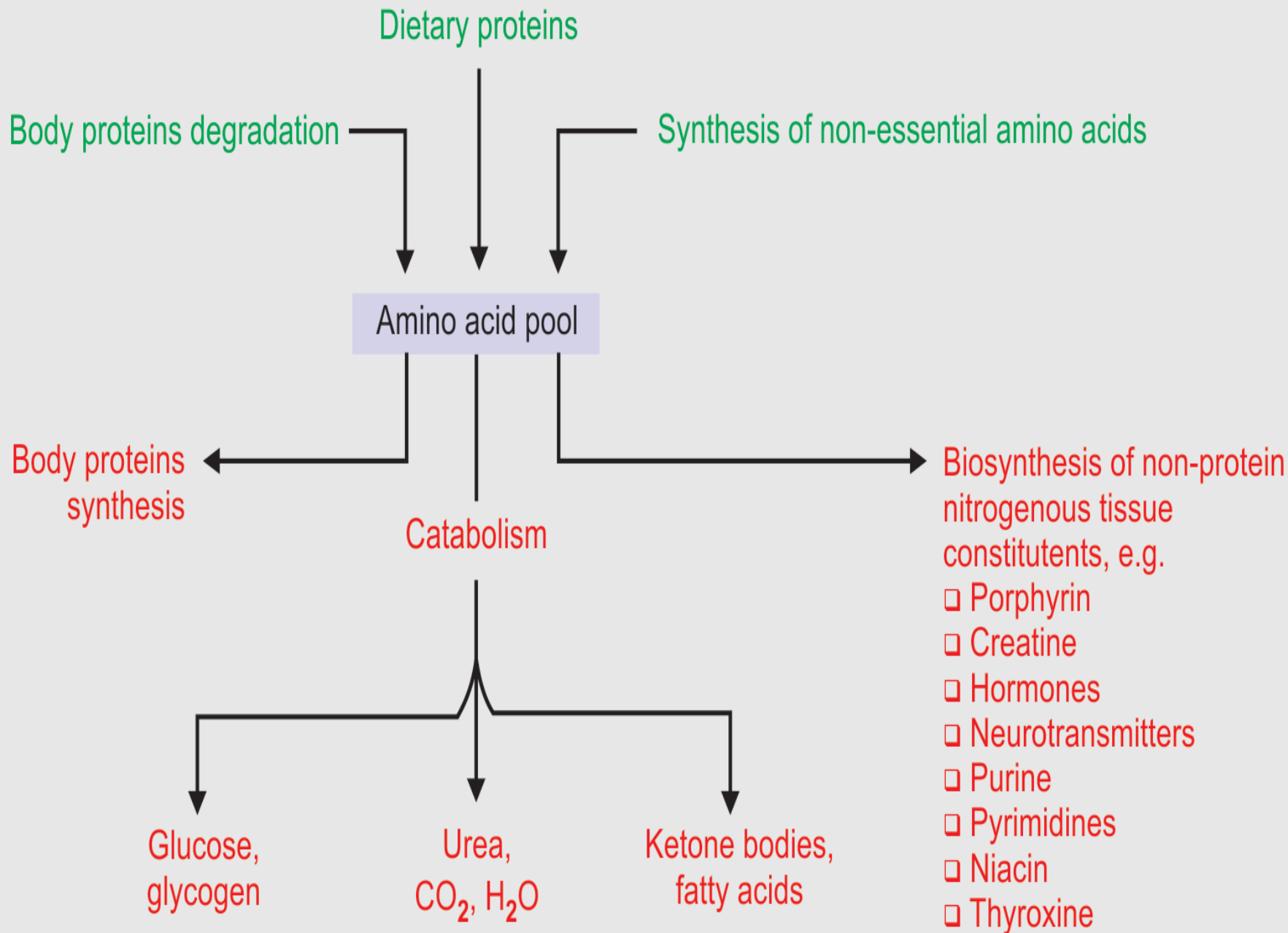
**TABLE 12.2: Amino acid transport systems and disorders.**

<i>Transport systems</i>	<i>Amino acid transported</i>	<i>Disorders associated with defective transport</i>
Small neutral amino acids	Alanine, serine, and threonine	Hartnup disease
Large neutral amino acids	Isoleucine, leucine, valine, tyrosine, tryptophan, and phenylalanine	
Basic amino acids and cystine	Arginine, lysine, and cystine	Cystinuria
Acidic amino acid	Glutamic acid and aspartic acid	
Imino acid and glycine	Proline, hydroxyproline, and glycine	Glycinuria

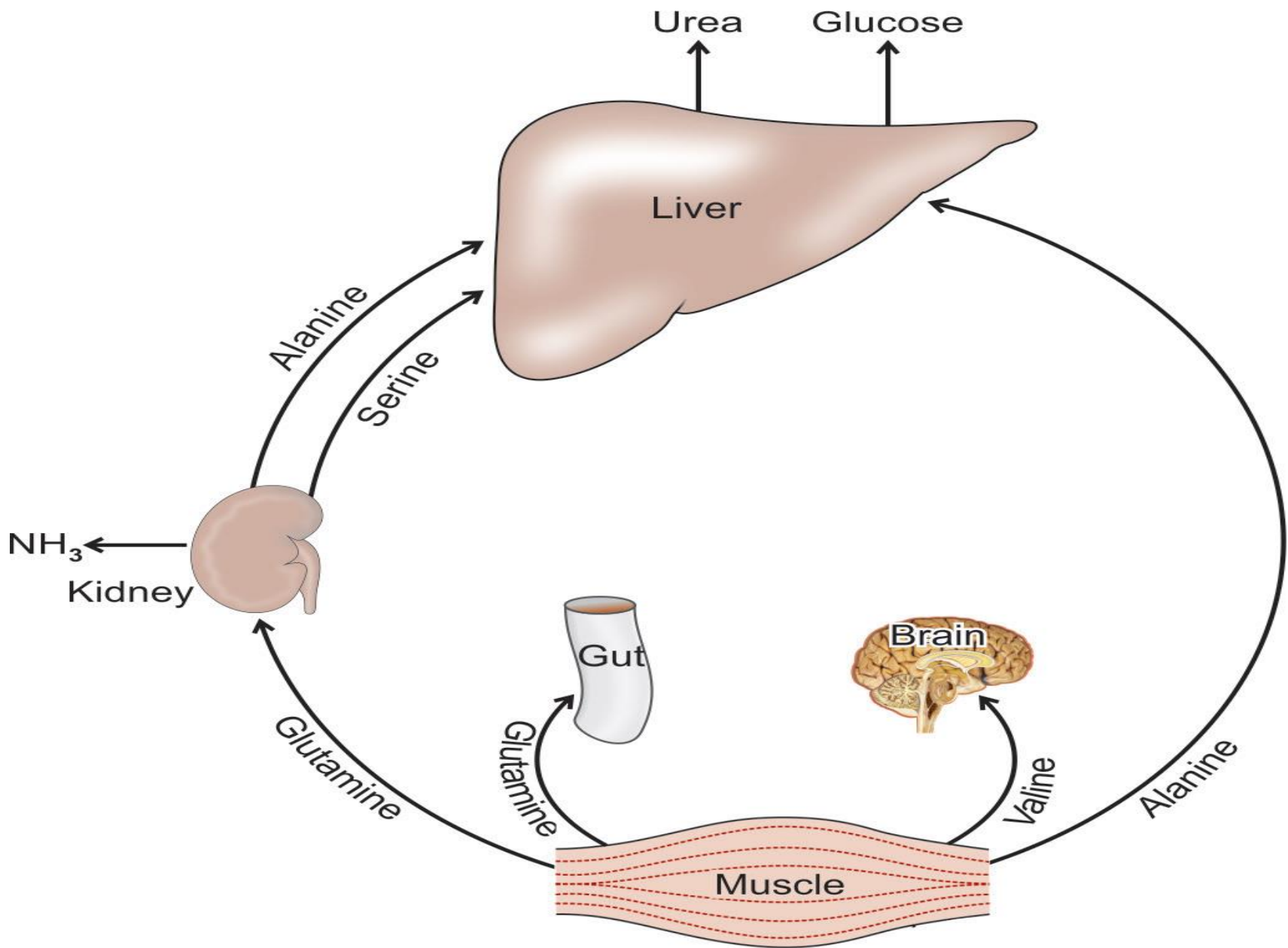
# Amino Acid Pool

Amino acids, released by hydrolysis of dietary protein, and tissue proteins together constitute the *amino acid pool*.

- The primary role of amino acids is to serve as building blocks of synthesis of **tissue protein** and other **nitrogen containing compounds**
- Amino acids, in excess of those needed for the synthesis of proteins and other biomolecules **cannot be stored** nor are they excreted. Surplus amino acids are oxidized for energy.



**Figure 12.3: Amino acid pool**



**Figure 12.4:** Amino acid exchanges between organs and key role of alanine.

# STAGES OF CATABOLISM OF AMINO ACIDS

- In the catabolism, amino acids lose their **amino groups** in the form of **ammonia**, which is excreted in the form of **urea** in the liver by reactions of the **urea cycle**.
- The remaining **carbon skeleton** of amino acids, the  $\alpha$ -keto acids undergo oxidation to carbon dioxide and water by reactions of **citric acid cycle**.

The complete catabolism of amino acids includes:

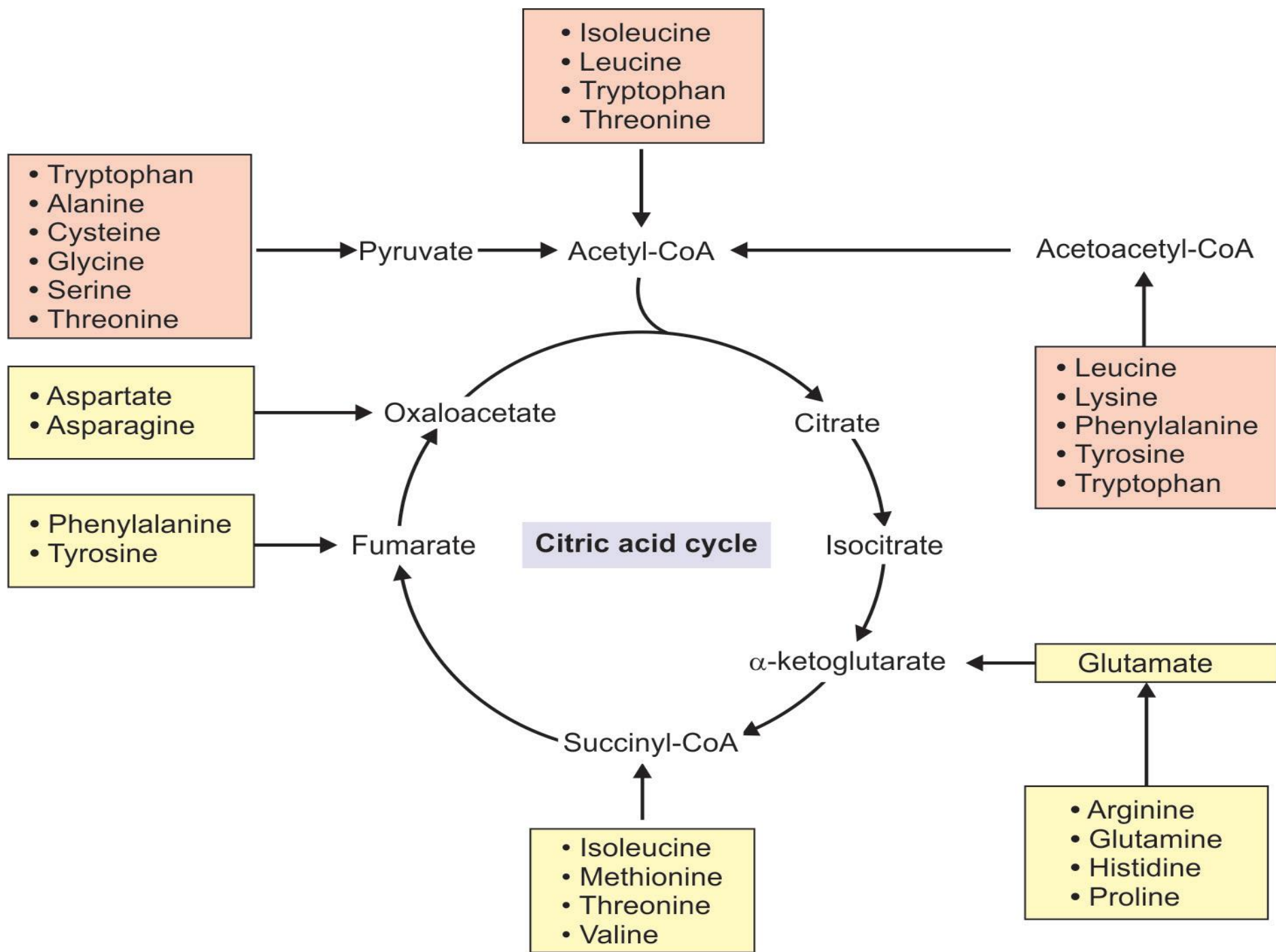
1. The removal of  $\alpha$ -amino group in the form of ammonia by following reactions:

- Transamination by the enzyme aminotransferase, also called transaminase.
- Deamination may be oxidative or non-oxidative.
  - a. Oxidative deamination is by glutamate dehydrogenase or amino acid oxidase.
  - b. Non-oxidative deamination by amino acid dehydratase.

2. Transport of ammonia.
3. Disposal of ammonia in the form of urea in the liver by reactions of the urea cycle.
4. Disposal (catabolism) of carbon skeleton of amino acids after the removal of  $\alpha$ -amino group. The carbon skeletons of 20 amino acids converged to form only seven major products. These are:

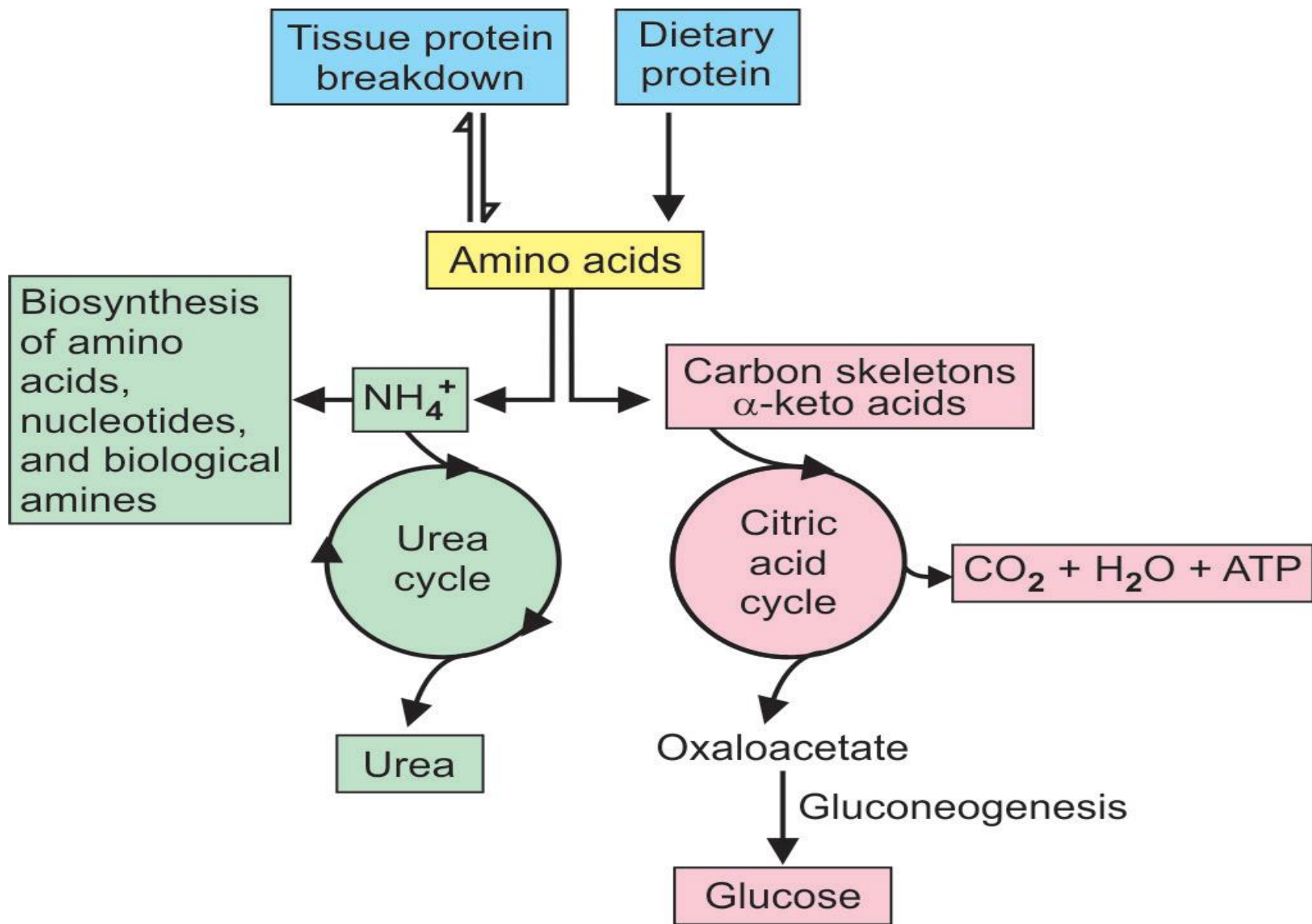
1. Pyruvate
2. Acetyl-CoA
3. Acetoacetyl-CoA
4.  $\alpha$ -ketoglutarate
5. Succinyl-CoA
6. Fumarate
7. Oxaloacetate

- All these enter the citric acid cycle (**Figure 12.5**).
- From here the carbon skeletons are diverted to gluconeogenesis or ketogenesis or are completely oxidized to CO<sub>2</sub> and H<sub>2</sub>O.



## Figure 12.5: Metabolic fate of carbon skeleton of amino acids.

- Yellow shade: Glucogenic amino acids;
- Red shade: Ketogenic amino acid.
- Several amino acids are both glucogenic and ketogenic.
- Only two amino acids, leucine and lysine, are exclusively ketogenic.

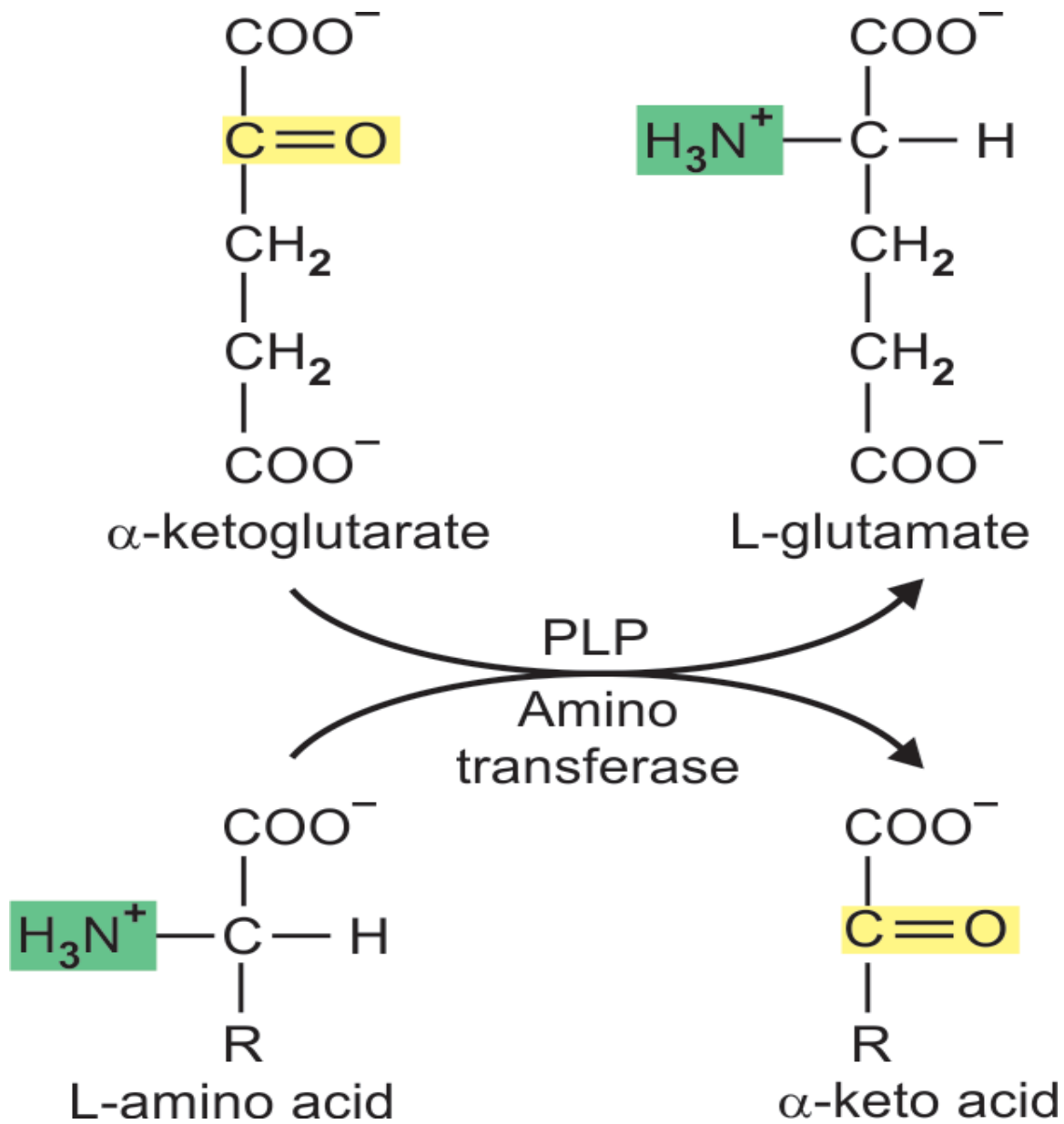


**Figure 12.6:** Amino acid catabolism overview.

1. The removal of  $\alpha$ -amino group in the form of ammonia

# *Transamination*

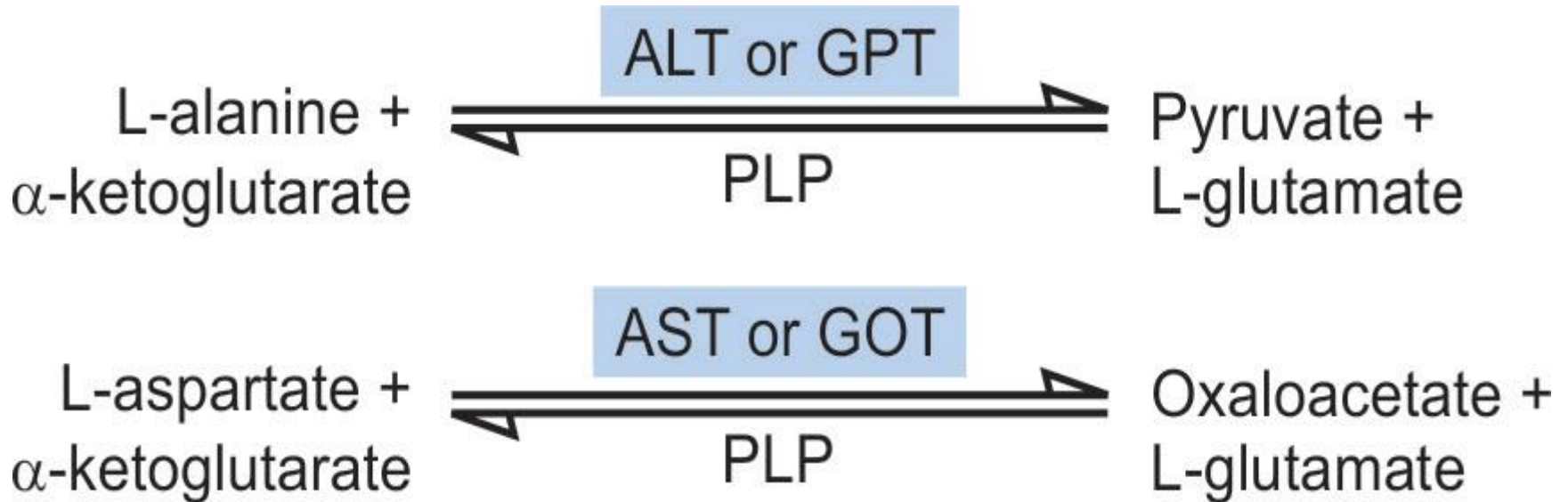
- Transamination involves the **transfer of  $\alpha$ -amino group** of  $\alpha$ -amino acid to an  **$\alpha$ -keto acid** to form new amino acid and a new keto acid.
- The enzymes that catalyze these reactions are called *aminotransferases* or *transaminases*
- All transaminases require **pyridoxal phosphate (PLP)** as a coenzyme.



**Figure 12.7:** Transamination reaction.

➤ Some of the most important transaminases are :

- Alanine transaminase (ALT) or  
Glutamate pyruvate transaminase (GPT)
- Aspartate transaminase (AST) or  
Glutamate oxaloacetate transaminase (GOT)



**Figure 12.8:** Reactions catalyzed by alanine transaminase and aspartate transaminase.

- Except *lysine*, *threonine*, *proline* and *hydroxyproline* all amino acids undergo transamination reaction
- There is **no net loss of amino groups** in transamination reactions.

# Metabolic significance of transamination reactions

- This reaction provides a mechanism for collecting the amino groups from many different amino acids into one common product **L-glutamate**.
- L-glutamate, produced by transamination, can be used as an amino group donor in **the synthesis of non-essential amino acids**.

# Clinical significance of transaminase enzyme

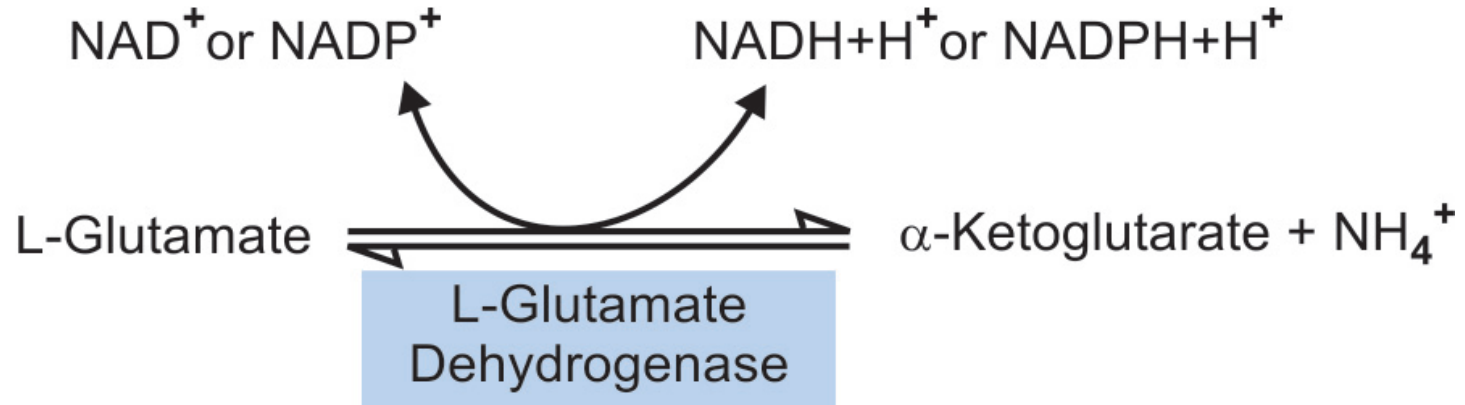
**ALT** and **AST** are important in the diagnosis of **liver** and **heart** damage

# Deamination

Deamination may be **oxidative** or **non-oxidative**

- Oxidative deamination is by **glutamate dehydrogenase** or **amino acid oxidase**.
- Nonoxidative deamination is by **amino acid dehydratase**.

## Oxidative deamination by glutamate dehydrogenase



The net removal of  $\alpha$ -amino groups to ammonia, requires the combined action of glutamate transaminase and glutamate dehydrogenase.

## *Metabolic significance*

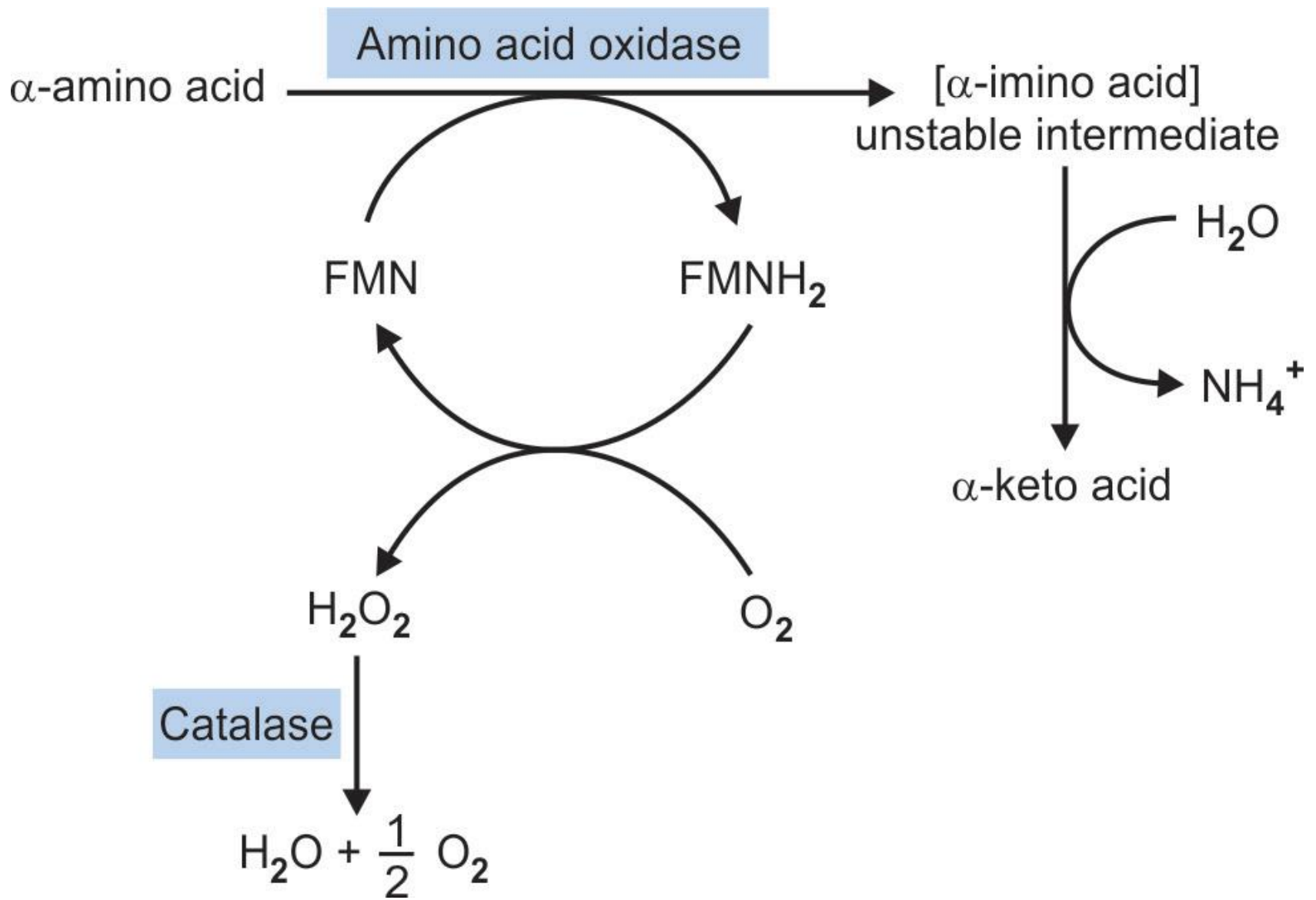
- This reaction functions both in amino acid **catabolism** and **biosynthesis**.
- **Catabolically** it channels nitrogen from **glutamate** to **ammonia**
- **Anabolically** it catalyzes amination of  **$\alpha$ -ketoglutarate** to **glutamate**.

*Clinical significance of glutamate dehydrogenase  
(GLD)*

GLD is present in normal serum in trace amount only, but increased activities are observed in cases of liver disease.

## *Oxidative deamination by amino acid oxidases*

- Both **L-** and **D-amino acid oxidases** occur in the kidneys and the liver.
- Amino acid oxidases use **auto-oxidizable FMN or FAD** as coenzyme

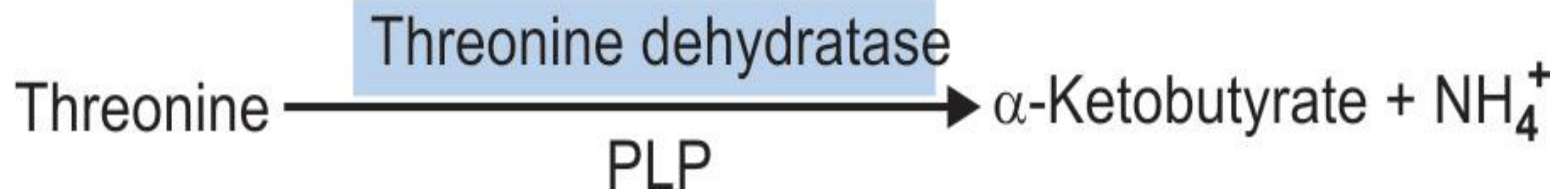
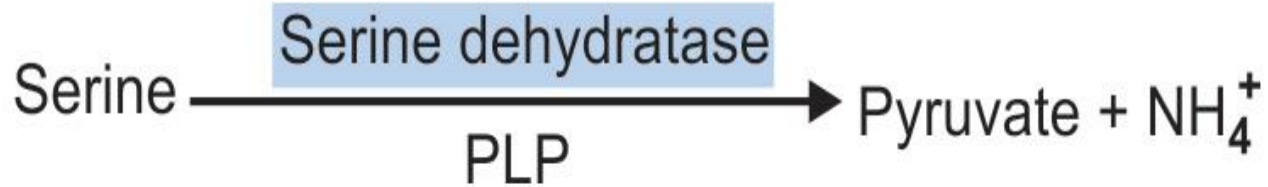


**Figure 12.10:** Oxidative deamination by amino acid oxidase.

## *Metabolic significance*

D-amino acids present in the diet are metabolized by D-amino acid oxidase in the liver

# Nonoxidative deamination by amino acid dehydratase



## 2. Transport of ammonia.

Disposal Of Ammonia Or Metabolic Fate Of Ammonia

## Transport Ammonia from Peripheral Tissues to the Liver

- In many tissues, including the brain, some processes such as nucleotide degradation generate free ammonia.
- Since ammonia is extremely toxic, much of the free ammonia is immediately converted to nontoxic metabolites before export from the extrahepatic tissues into the blood and transport to the liver or kidneys.

- From extrahepatic tissues and muscles, free ammonia is transported to the liver in two principal transport forms:

- 1. Glutamine, and**

- 2. Alanine.**

## *Transport of Ammonia in the Form of Glutamine*

- In many tissues (liver, kidney, and brain), free ammonia is combined with **glutamate** to yield **glutamine** by the action of **glutamine synthetase**.
- The glutamine, so formed, is a neutral nontoxic compound, which can readily pass through cell membrane, whereas **glutamate cannot**.

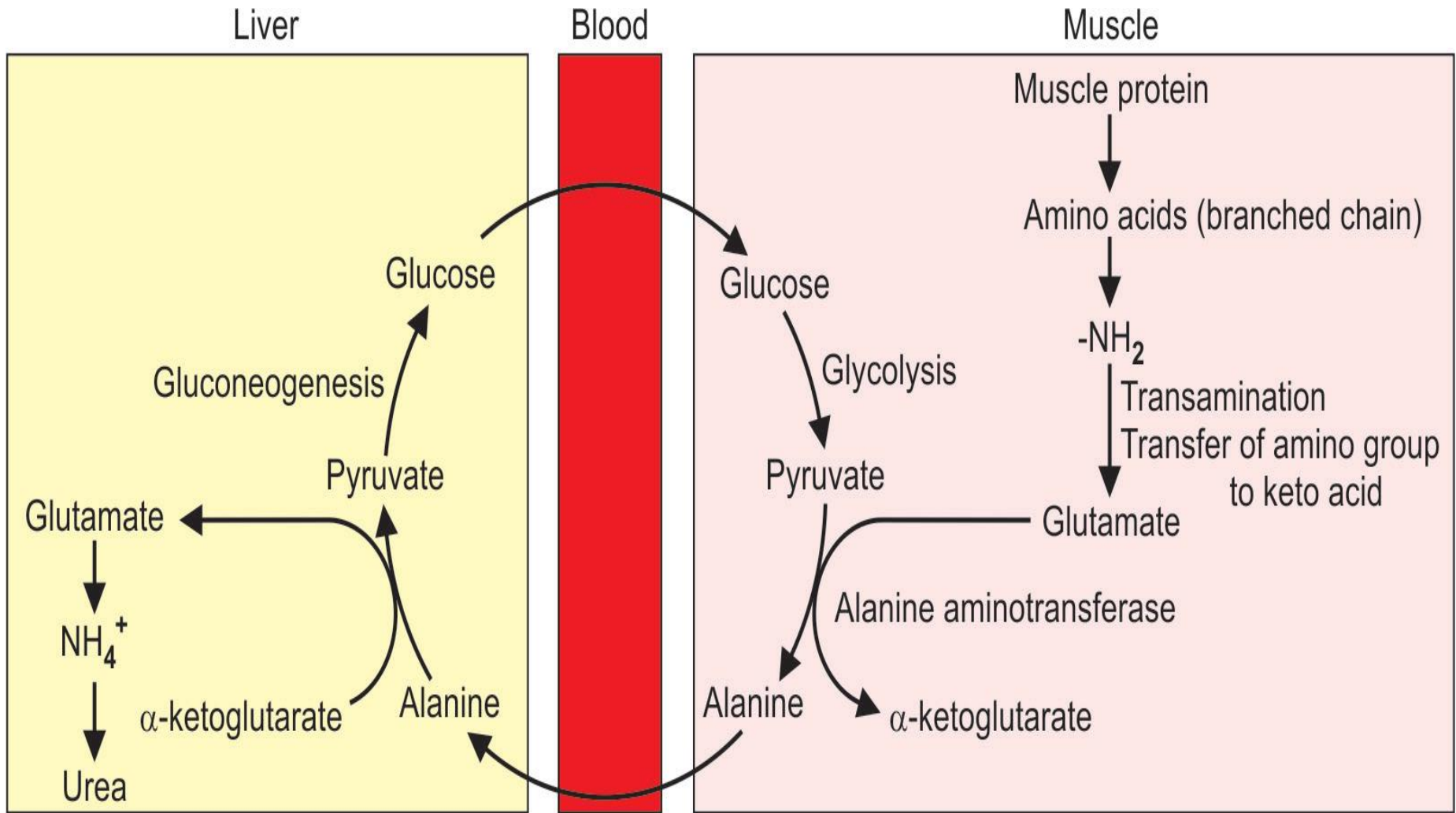
- Glutamine is normally present in blood in much higher concentrations than other amino acids.
- Glutamine also serves as a source of amino groups in a variety of biosynthetic reactions.
- The glutamine is carried via blood to the liver, where it can be acted upon by glutaminase to yield glutamate and ammonia



Glutamine is a major transport and temporary storage form of ammonia in the body.

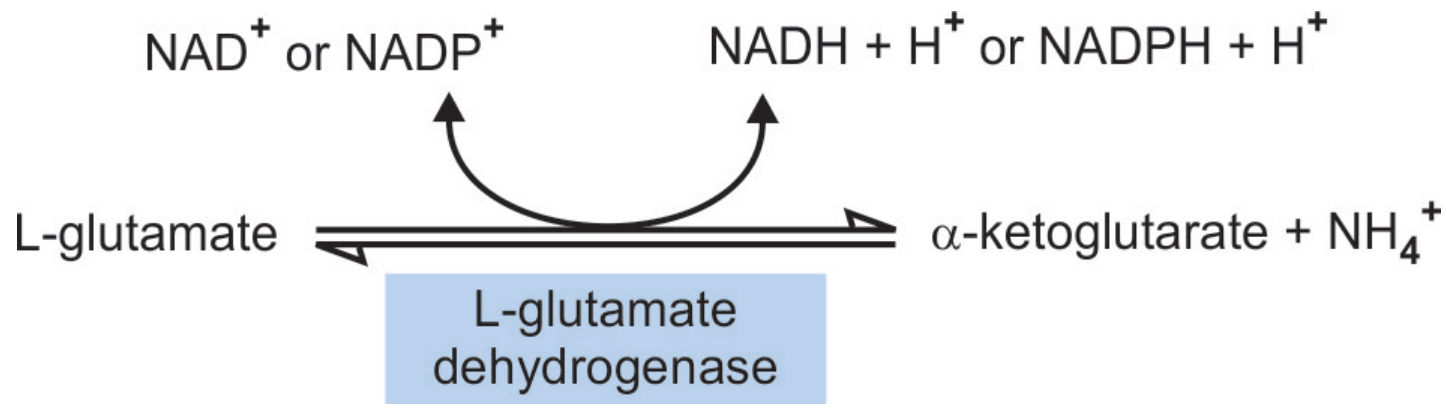
## *Transport of Ammonia in the Form of Alanine*

- Alanine transports ammonia to the liver in nontoxic form via a pathway called the **glucose alanine cycle**.
- In muscle, amino groups from amino acids are collected in the form of **glutamate** by transamination.
- Glutamate transfer its  $\alpha$ -amino group to **pyruvate** by the action of **alanine aminotransferase (ALT)** to form **alanine**. Alanine so formed passes into the blood and travels to the liver.



**Figure 12.13:** Transport of ammonia from muscle to liver by glucose-alanine cycle.

- In the liver by reverse reaction, ALT transfers the amino group from alanine to  $\alpha$ -ketoglutarate, forming pyruvate and glutamate.
- Glutamate can then enter mitochondria where **glutamate dehydrogenase** reaction release ammonia.



# The overview of catabolism of amino groups

# Liver

Cellular proteins



Amino acids

Dietary amino acids

Transamination

$\alpha$ -keto acid

Pyruvate

1

$\alpha$ -ketoglutarate

Glutamate

Alanine

Alanine from muscle

3

Transamination

Transport

2

Deamination

Glutaminase

Glutamine

Glutamine from muscle and other tissues

3

Transport

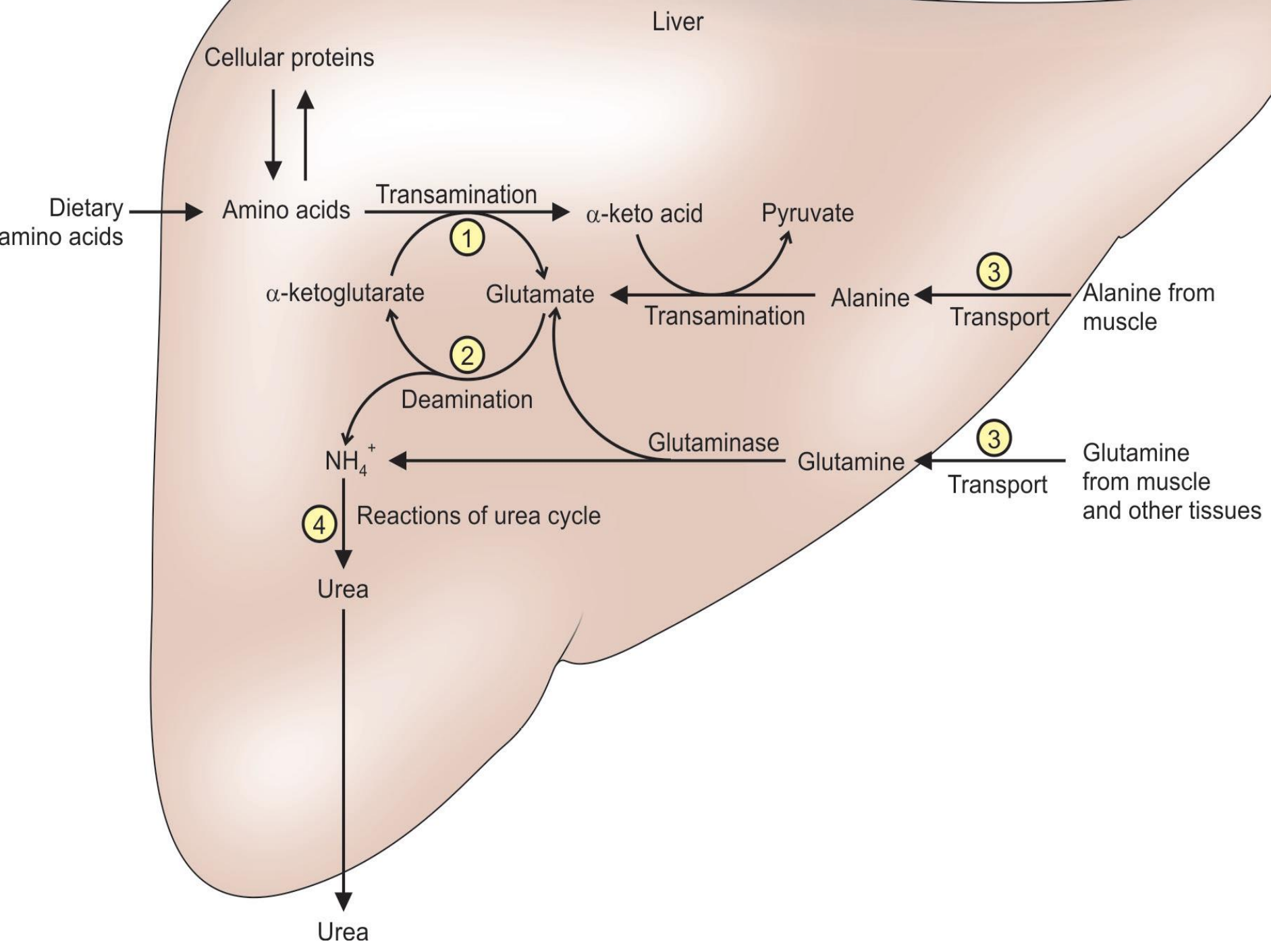
$\text{NH}_4^+$

Reactions of urea cycle

4

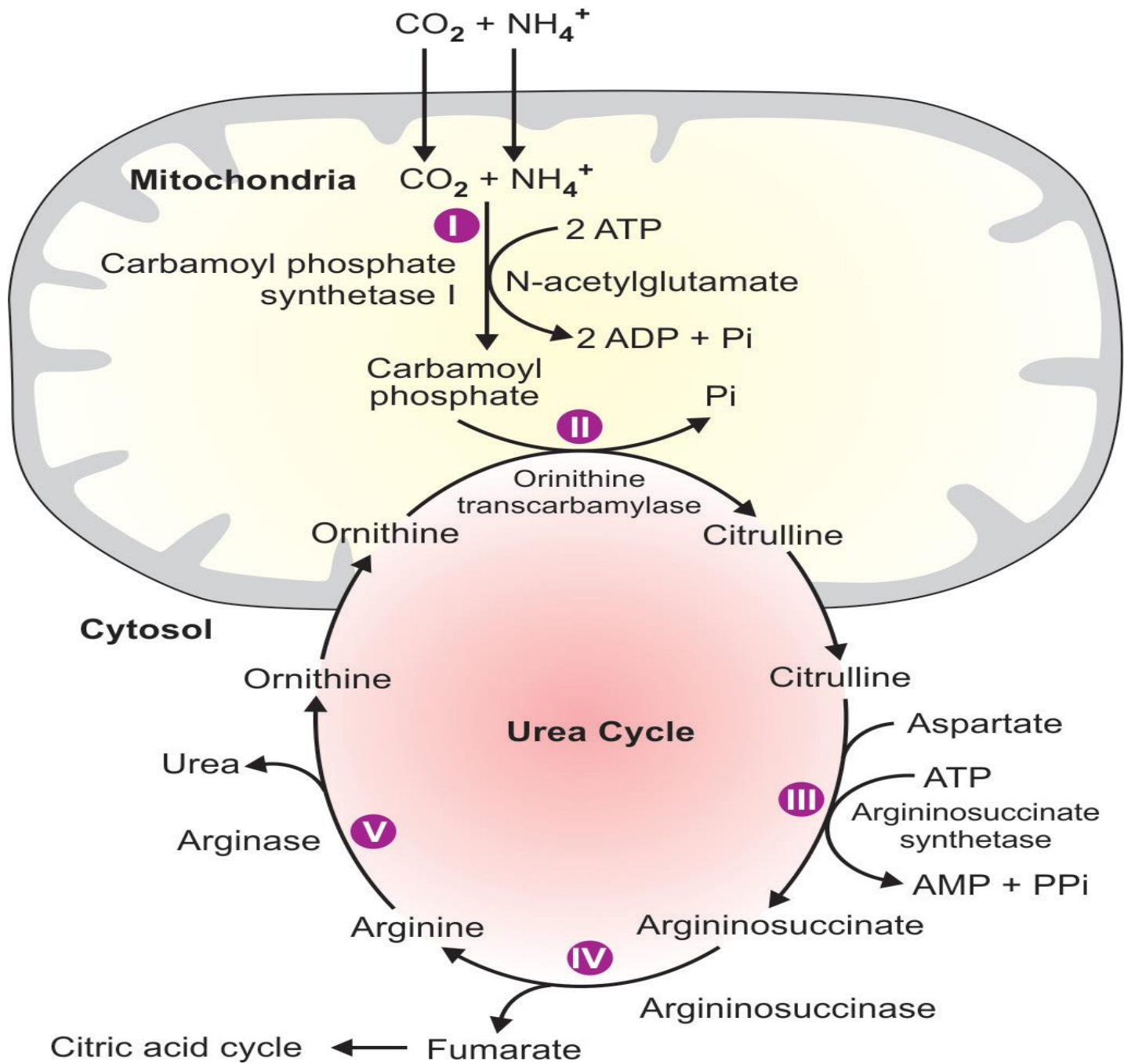
Urea

Urea



3. Disposal of ammonia in the form of urea in the liver by reactions of the urea cycle.

- Ammonia formed in the breakdown of amino acids if not reused for the synthesis of new amino acids or other nitrogenous compounds, the excess ammonia is converted into urea in the urea cycle and excreted into the urine.
- Formation of urea by "*Kreb's Henseleit urea cycle*" is an ultimate route for the metabolic disposal of ammonia.
- Urea is produced **exclusively by the liver**.
- Enzymes catalyzing the urea cycle reactions are distributed between the *mitochondria* and the *cytosol* of the liver



- *Carbamoyl phosphate synthase-I* is a hepatic mitochondrial enzyme functions in **urea synthesis**.
- *Carbamoyl phosphate synthase-II* a cytosolic enzyme that uses glutamine rather than ammonia as a nitrogen source and functions in **pyrimidine nucleotide biosynthesis**

## The Energy Cost of Urea Cycle

**Four ATPs** are consumed in the synthesis of each molecule of urea as follows:

- **Two ATP** are needed to make **carbamoyl phosphate**.
  - One ATP serves as a **source of phosphate**
  - Second ATP is converted to **AMP + PPi**.
- **One ATP** is required to make **arginosuccinate**.
- **One ATP** is required to **restore AMP to ATP**.

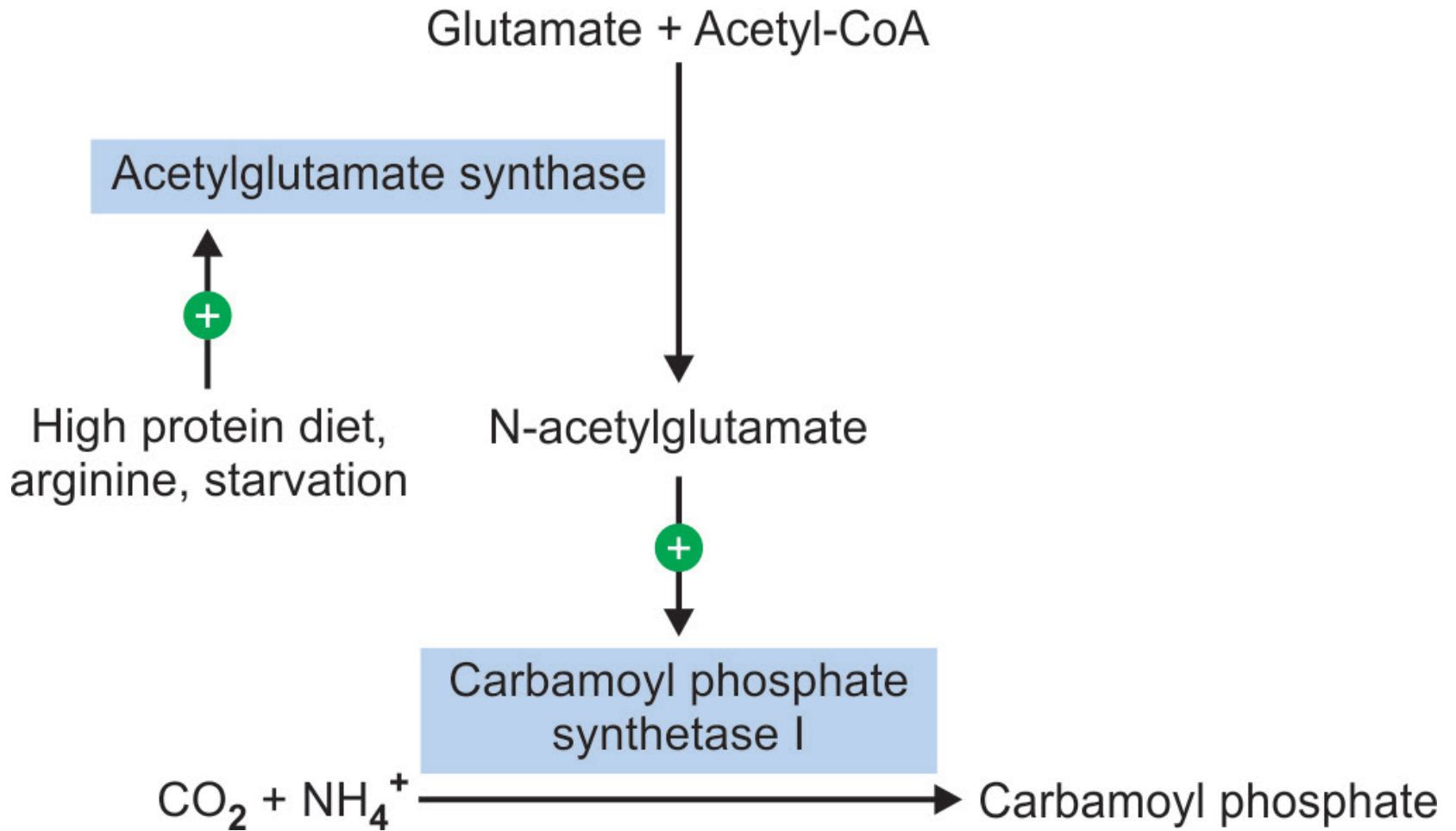
# Significance of Urea Cycle

- The toxic ammonia is converted into the harmless nontoxic urea.
- It disposes off two waste products, **ammonia and carbon dioxide**.
- It participates in the regulation of blood pH, which depends upon the ratio of dissolved  $\text{CO}_2$ , i.e.  $\text{H}_2\text{CO}_3$  to  $\text{HCO}_3^-$ .

- It forms semi-essential amino acid, arginine. Arginine also serves as the precursor of the potent muscle relaxants nitric oxide (NO).
- Ornithine, which is formed in urea cycle, is a precursor for the formation of polyamines like putrescine, spermidine, and spermine and can also be involved in formation of nonessential amino acid **proline**

# Regulation of Urea Cycle

*Carbamoyl phosphate synthetase-I* is an allosteric regulatory enzyme of urea cycle, which is allosterically activated by *N-acetylglutamate (NAG)*.



**Figure 12.18:** Activation of N-acetylglutamate.

# Metabolic Inborn Errors of Urea Cycle

- **Five disorders** associated with each of the **five enzymes** of urea cycle have been reported.
- Since urea synthesis converts toxic ammonia to non-toxic urea, all defects in urea synthesis result in *hyperammonemia* and *ammonia intoxication*.

- This intoxication is more severe when the metabolic block occurs at **reaction I** or **II**, since it accumulates **ammonia** itself.
- Deficiency of later enzymes result in the accumulation of other **intermediates of the urea cycle**, which are **less toxic** and therefore severity of symptoms is less.

## *Symptoms*

Clinical symptoms, common to all urea cycle, disorders include:

- Ammonia intoxication
- Protein-induced vomiting
- Intermittent ataxia
- Irritability
- Lethargy
- Mental retardation.

**TABLE 12.3: Disorders caused by genetic defects of urea cycle enzymes.**

<i>Disorders</i>	<i>Defective enzymes</i>	<i>Products accumulated</i>
Hyperammonemia type-I	Carbamoyl phosphate synthetase-I	Ammonia
Hyperammonemia type-II	Ornithine transcarbamylase	Ammonia
Citrullinemia	Argininosuccinate synthetase	Citrulline
Argininosuccinic aciduria	Argininosuccinase	Argininosuccinate
Argininemia	Arginase	Arginine

## *Treatment*

Low protein diet, food intake should be in frequent small meals to avoid sudden increase in blood ammonia levels.

The Metabolic Pathways For Aliphatic Side Chain  
Containing Amino Acids (Glycine, Alanine, Valine,  
Isoleucine And Leucine)

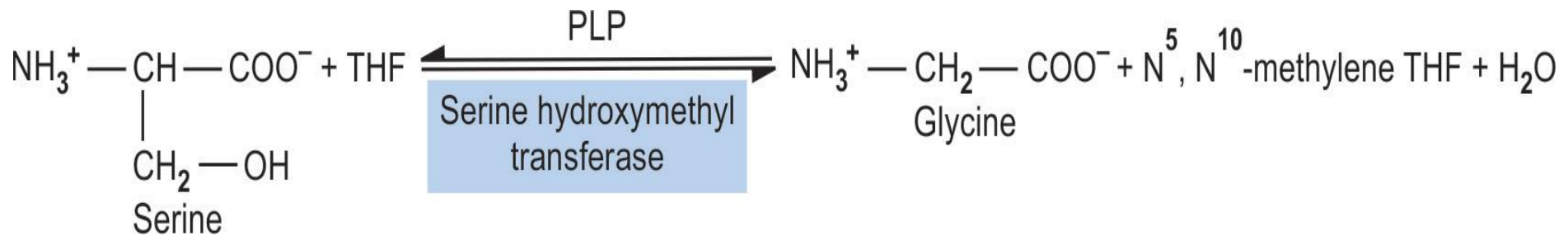
## Metabolism of Glycine

- Glycine is a non-essential or dispensable amino acid.

### *Synthesis of Glycine*

- It is synthesized from **serine** by removal of **hydroxymethyl** group from the side chain of serine.

The methylene carbon

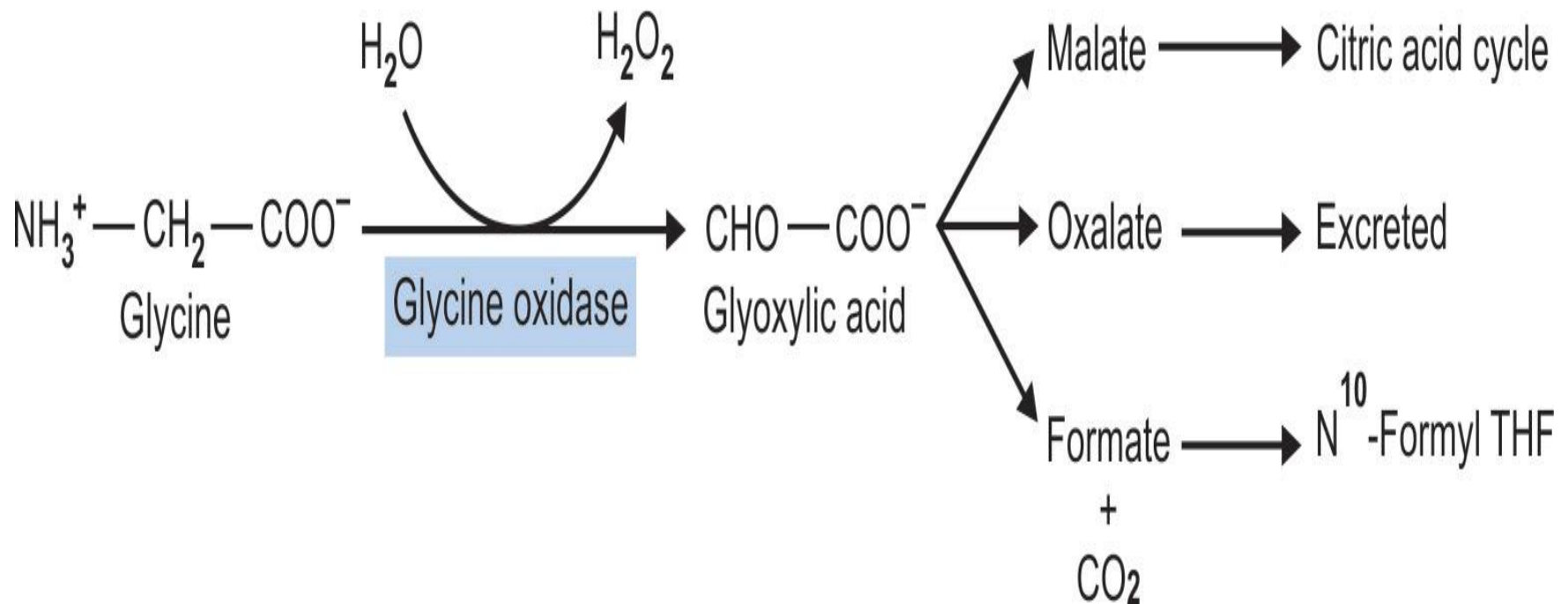
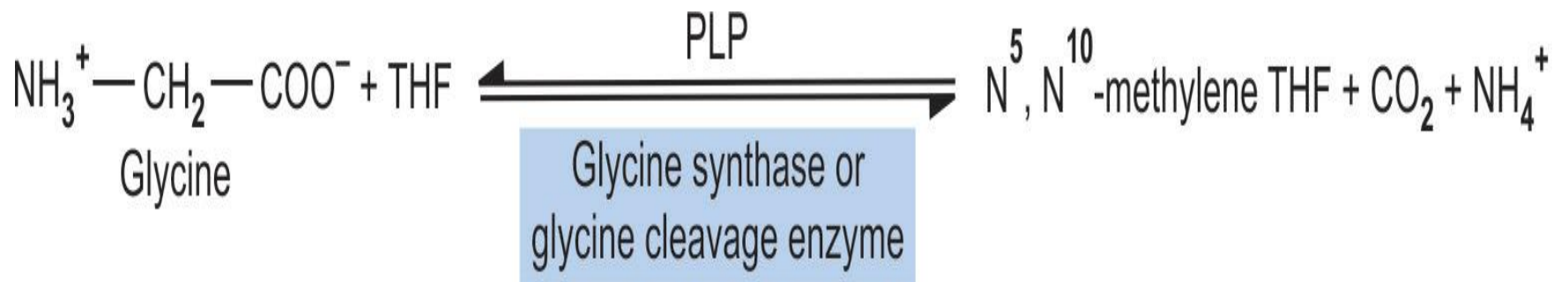


**Figure 12.19:** Synthesis of glycine from serine.  
(THF: tetrahydrofolate)

# *Catabolism of Glycine*

Glycine is catabolized by following **three** ways:

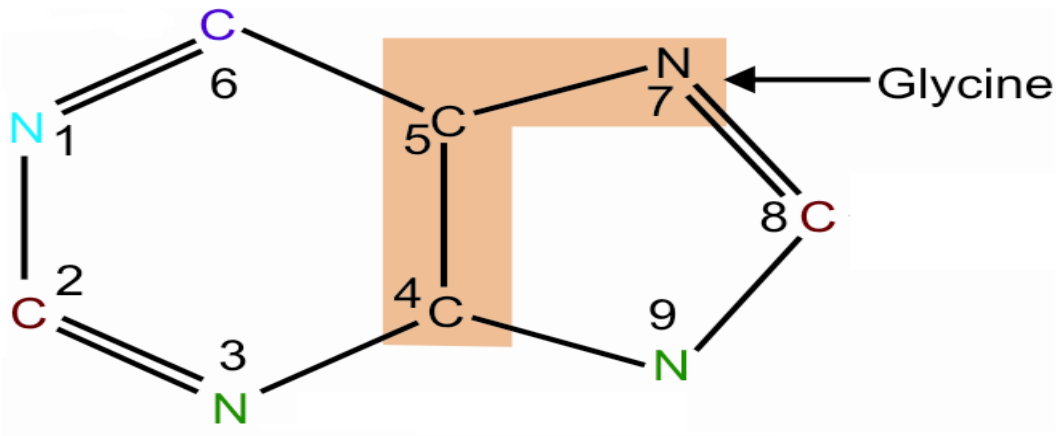
1. Conversion into **serine**
2. Oxidative cleavage into **CO<sub>2</sub>** and **NH<sub>4</sub><sup>+</sup>**.
3. Oxidatively deamination to **glyoxylic acid** which:
  - decarboxylated to **formaldehyde** and **CO<sub>2</sub>**
  - converted to **malate**
  - oxidized to **oxalate** and excreted.



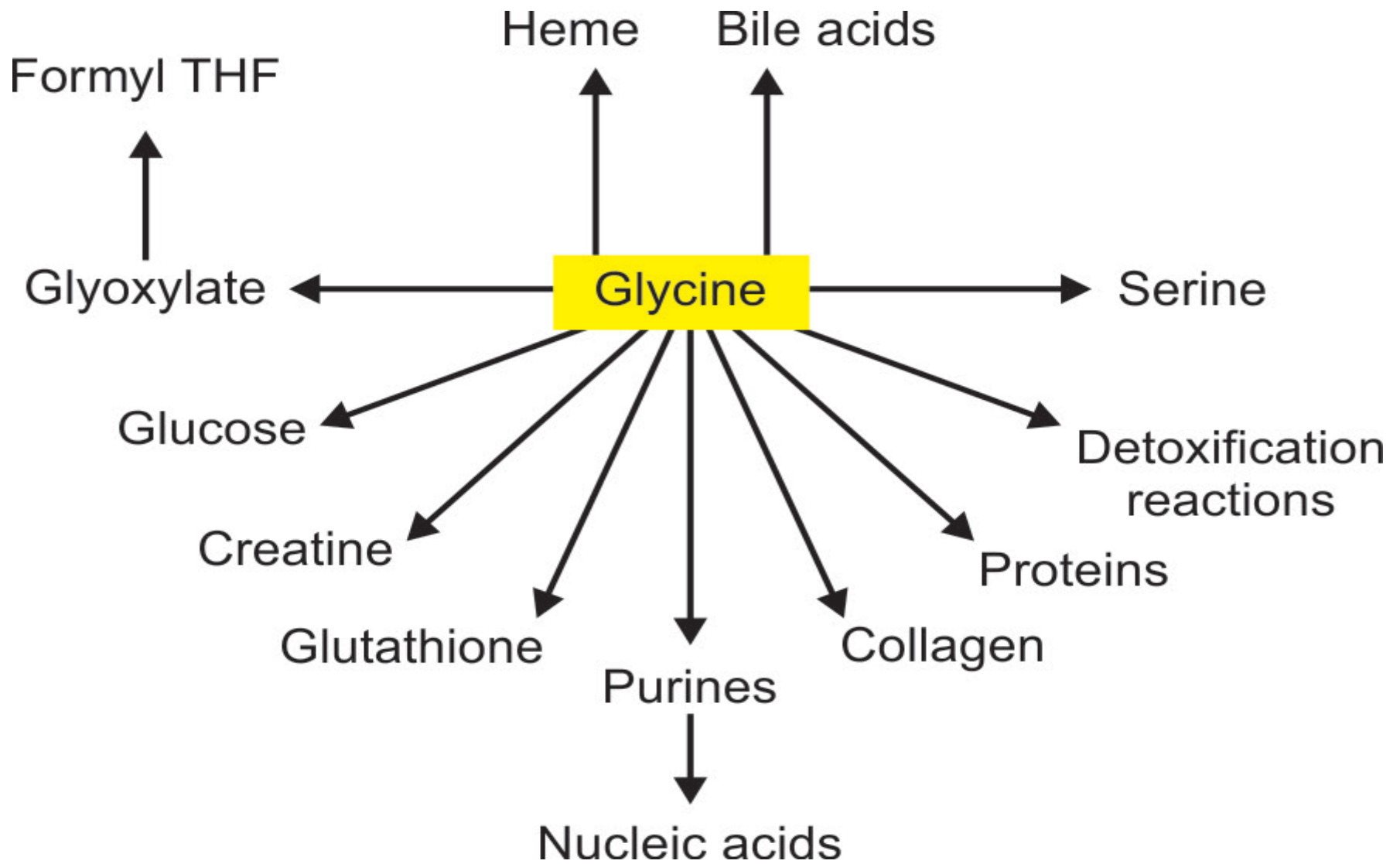
**Figure 12.20:** Catabolic pathways of glycine.

# Metabolic Importance of Glycine

- Synthesis of **heme**
- Synthesis of **glutathione** ( $\gamma$ -glutamyl-cysteinyl-glycine).
- Formation of **purine ring**



- Formation of **bile acids**: glycocholic & glycochenodeoxycholic acid.
- Glycine is involved in **detoxification reactions** :e.g. benzoic acid is detoxicated by conjugating with glycine to hippuric acid.
- Synthesis of **creatin**: Glycine +Arginine +Methionine
- **Collagen** formation
- Synthesis of **glucose**.
- Constituent of various tissue **proteins, hormones ,enzymes**.



**Figure 12.21:** Metabolic importance of glycine.

## *Metabolic Disorders of Glycine*

### *Primary hyperoxaluria*

- Inborn error, characterized by high urinary excretion of oxalate.
- Failure to catabolize glyoxylate, which gets oxidized to oxalate.

- Increased level of oxalate results in **urolithiasis** (stone in urinary tract), **nephrocalcinosis** (presence of Ca deposits in kidneys) and recurrent **infections of urinary tract**.
- **Death** occurs in childhood or early adult life from renal failure

## *Glycinuria*

- It is an **inborn error** characterized by increased **excretion of glycine through urine**, despite plasma concentration of glycine is normal.
- Since plasma levels are normal, glycinuria occurs probably due to a *defect in renal tubular reabsorption of glycine*.
- Glycinuria is characterized by increased tendency for the formation of **oxalate renal stones**.

## *Non- ketotic Hyperglycinemia*

- Non- ketotic hyperglycinemia is due to defects in glycine cleavage enzyme, **glycine synthase** of catabolic pathway of glycine.
- Non-ketotic hyperglycinemia is characterized by elevated serum levels of glycine leading to severe mental deficiencies and death in very early childhood.
- At high levels, glycine is an inhibitory neurotransmitters leading to neurological defects.

# Metabolism Of Branched Chain Amino Acids

**Valine**, **Isoleucine** and **leucine** are branched essential amino acids.

- Valine: Glucogenic
- Isoleucine : Both glucogenic and ketogenic
- Leucine: Ketogenic

First three catabolic reactions for branched chain amino acids are similar and are catalyzed by common enzymes

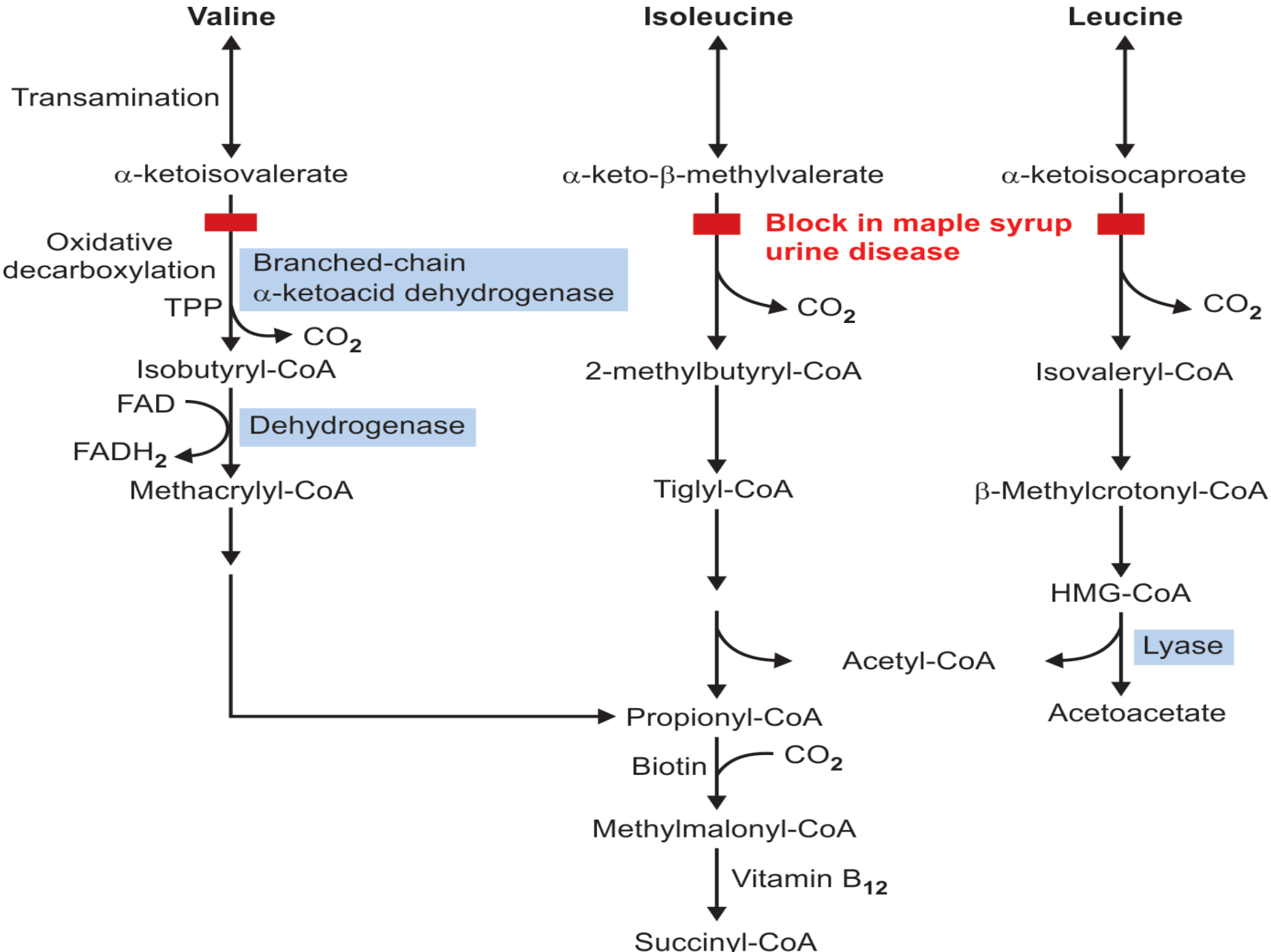
1. **Transamination** : to yield corresponding  $\alpha$ -keto acids.
2. **Oxidative decarboxylation** :In the second step, the  $\alpha$  -keto acids are oxidatively decarboxylated to the corresponding acyl-CoA thioester by branched-chain  $\alpha$  -keto acid dehydrogenase complex. TPP, FAD, Lipoic acid, NAD<sup>+</sup> and CoA are required as coenzymes in this reaction.

3. **Dehydrogenation** :The third reaction is a FAD dependent dehydrogenation

4. The subsequent reactions of all the three branched chain amino acids differs and is catabolised as follows:

- Valine is converted to succinyl-CoA accounting for the glucogenic nature of the valine.
- Isoleucine is converted to succinyl-CoA and acetyl- CoA, accounting for the ketogenic and glucogenic nature of the isoleucine.

- Leucine forms acetoacetate and acetyl-CoA but does not produce succinyl-CoA, which accounts for exclusively the *ketogenic nature of the leucine*.
- Leucine produces hydroxymethyl glutaryl-CoA (HMG-CoA) intermediate product which is a precursor of cholesterol biosynthesis and ketone body formation.



*Metabolic Disorder of Branched Chain Amino Acids*

*Maple syrup urine disease (MSUD) or branched chain  
keto aciduria*

- Inherited defect in the branched chain  $\alpha$ -keto acid dehydrogenase.
- Branched chain amino acids, leucine, isoleucine and valine, and their  $\alpha$ -keto acids accumulate in blood, urine and CSF.
- $\alpha$ -keto acids impart a characteristic sweet odour to the urine of the affected individuals which resembles with maple syrup or burnt sugar hence the name.

## *Symptoms*

- Vomiting
- dehydration
- metabolic acidosis
- maple syrup odour to the urine.
- If untreated, it leads to mental retardation, coma and even death within one year after birth.

## Diagnosis

- Many infants with MSUD are identified through newborn screening programs.
- **Tandem mass spectrometry**, an advanced newborn screening test that screens for more than 30 different disorders through one blood sample, has aided in the diagnosis of MSUD.

## *Treatment*

- Treatment involves replacing dietary protein by mixture of amino acids that contain low or no leucine, isoleucine, and valine.
- To monitor the effectiveness of the dietary treatment, plasma and urinary levels of branched-chain amino acids with dinitrophenylhydrazine (DNPH) should be measured constantly.

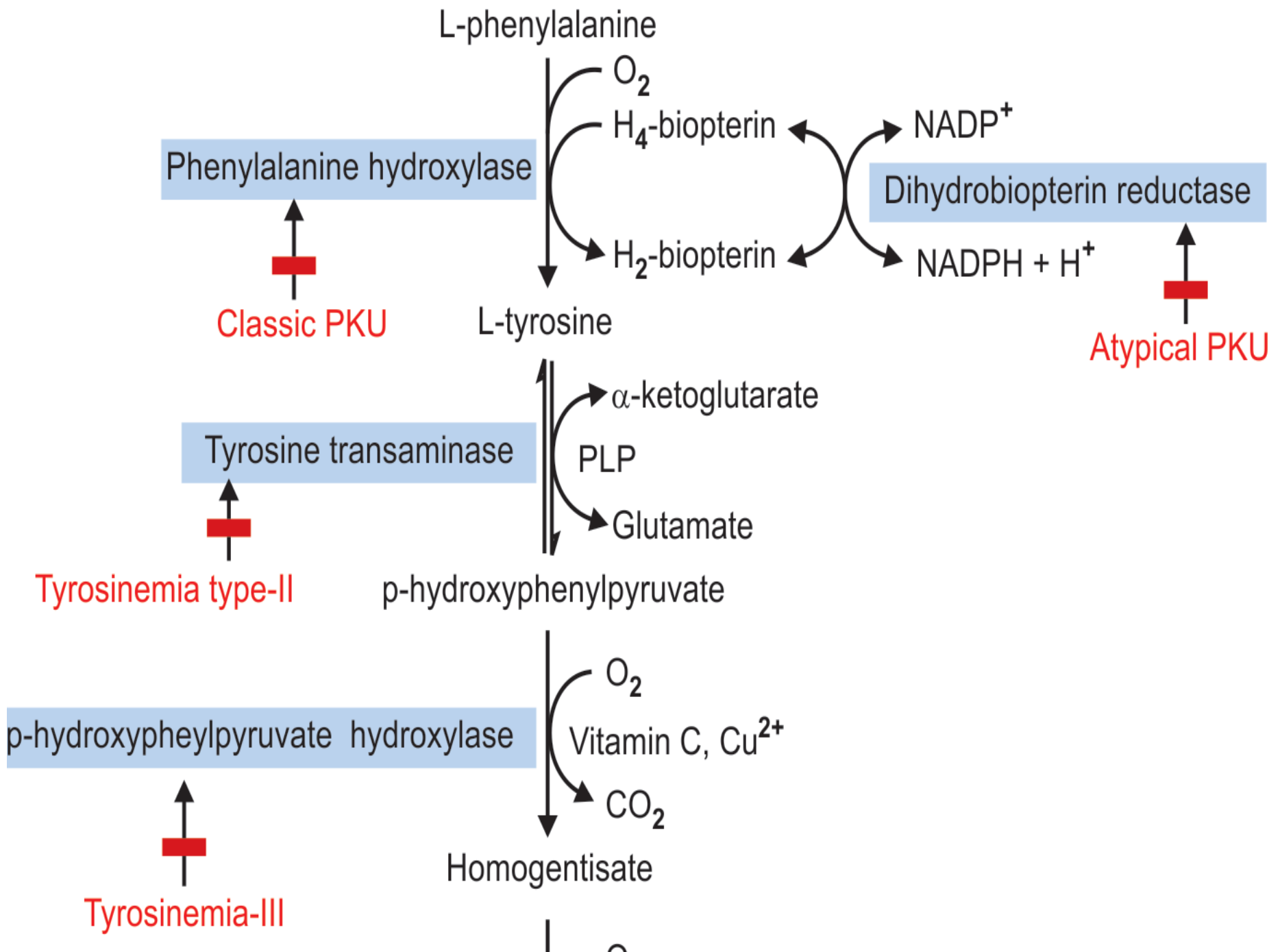
# ***Metabolism of Phenylalanine & Tyrosine***

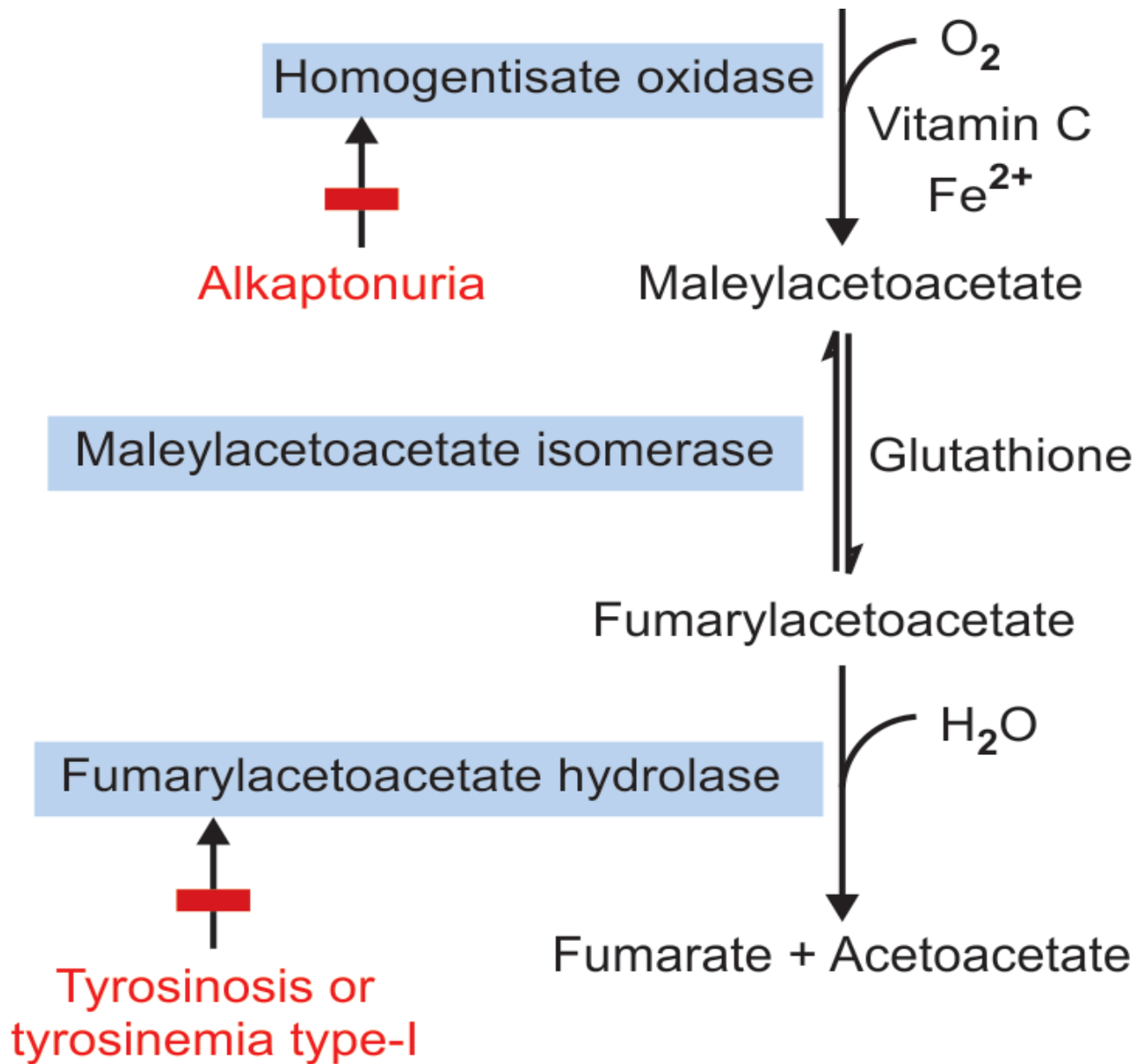
- Phenylalanine and tyrosine are the **aromatic amino acids**.
- **Phenylalanine** is nutritionally **essential** amino acids but **tyrosine is not** as it can be synthesized from phenylalanine.

## *Catabolism of Phenylalanine and Tyrosine*

- Phenylalanine metabolism is initiated by its **oxidation to tyrosine** which then undergoes oxidative degradation.
- Thus, catabolic pathway for phenylalanine and tyrosine is same.

**Figure 12.24:** Catabolic pathway for phenylalanine and tyrosine showing metabolic disorders with respect to genetically defective enzymes.

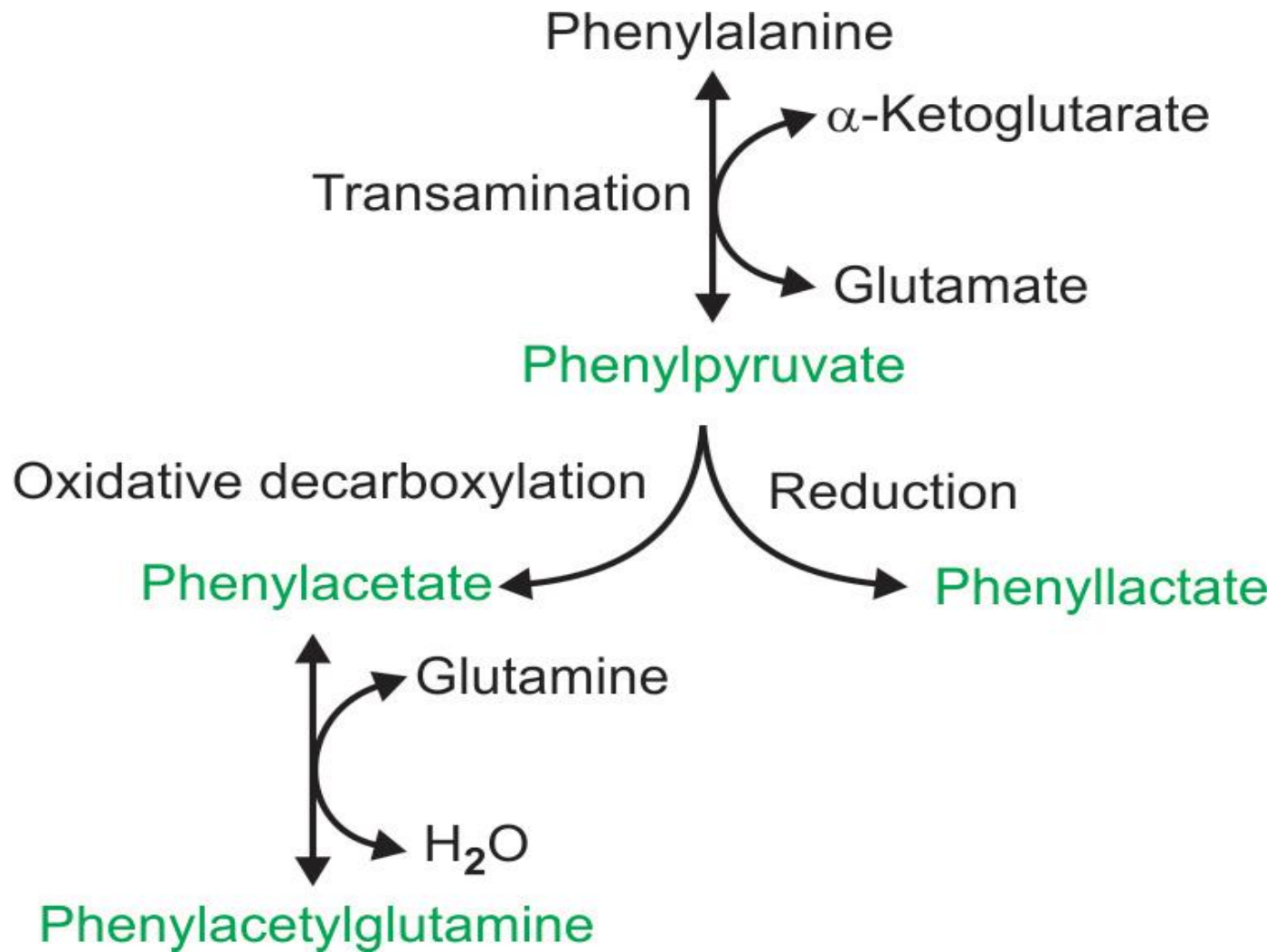




# Metabolic Disorders of Phenylalanine and Tyrosine

## *Phenylketonuria (PKU)*

- Inborn error of phenylalanine metabolism, associated with the inability to convert **phenylalanine** to **tyrosine**.
- Result in accumulation of phenylalanine in tissues and blood and its increased excretion in urine.
- Accumulation of toxic metabolites of phenylalanine such as, *phenylpyruvate*, *phenylacetate*, *phenyllactate* and *phenylacetyl glutamine* occurs.



**Figure 12.25:** Alternative pathways of phenylalanine catabolism in phenylketonurics.

## *Classification of PKU*

- PKU may be classified into three broad groups
- PKU, caused by deficiency of phenylalanine hydroxylase, is the most commonly encountered error.

# *Classification of PKU*

## *Types of phenylketonuria with their defects*

### *Type of PKU*

### *Defect*

Classic phenylketonuria or  
Hyperphenylalaninemia type-I

Phenylalanine  
hydroxylase

Atypical phenylketonuria or  
Hyperphenylalaninemia type-II  
and III

Dihydrobiopterin  
reductase

Hyperphenylalaninemia type-IV  
and V

Dihydrobiopterin  
synthesis

## *Characteristics of PKU*

- In tissues, plasma and urine increased level of:
  - Phenylalanine,
  - phenylacetate,
  - phenyl-lactate,
  - phenylpyruvate
  - phenylacetylglutamine,.
- Phenylacetate gives the urine a **mousy odour**.

- **Neurological symptoms:** Mental retardation, failure to walk, to talk, seizures, psychoses, tremor and failure to grow.
- **Hypopigmentation:** Phenylketonurics have a lighter skin colour, fair hair and blue eyes due to deficiency of pigment **melanin**.
- The hydroxylation of tyrosine by *tyrosinase* is the first step in the formation of the pigment **melanin** is competitively inhibited by the high levels of phenylalanine in PKU

## *Treatment of PKU*

### Treatment due to Defect in Phenylalanine Hydroxylase

- Low phenylalanine diet supplemented with tyrosine, because tyrosine is normally synthesized from phenylalanine.
- The aim is to provide just enough phenylalanine to meet the needs for growth and replacement.

- Proteins that have a low content of phenylalanine, such as casein from milk are hydrolyzed and phenylalanine is removed by adsorption.
- A low phenylalanine diet must be started very soon after birth to prevent irreversible brain damage.

## *Treatment due to Defect in Dihydrobiopterin Reductase*

- The treatment in this case is more complex than restricting the intake of phenylalanine and tyrosine.
- Tetrahydrobiopterin is also required for the formation of **L-3,4-dihydroxyphenylalanine (L-Dopa)** and **5-hydroxytryptophan**, precursors of the neurotransmitters, norepinephrine and serotonin, respectively.
- In phenylketonuria of this type, these precursors must be supplied in the diet, along with tetrahydrobiopterin.

## *Diagnostic Tests for PKU*

- In the past years, the urine of newborns was assayed by the addition of  $\text{FeCl}_3$  which gives an olive color in the presence of phenylpyruvate.
- The phenylalanine level in blood is detected by screening by using Guthrie test; a bacterial assay for phenylalanine is more reliable diagnostic test.
- The gene for human phenylalanine hydroxylase has been cloned, so that prenatal diagnosis of PKU is now possible with DNA probes.

# *Tyrosinemia*

There are three types of tyrosinemia:

1. Tyrosinemia type-I (tyrosinosis/hepatorenal tyrosinemia)
2. Tyrosinemia type-II (Richner–Hanhart syndrome)
3. Tyrosinemia type-III (neonatal tyrosinemia).

# *Tyrosinemia type-I*

- Tyrosinemia type-I, also called **tyrosinosis**
- Caused by a genetic deficiency of **fumarylacetoacetate hydroxylase**.

➤ Results in accumulation and excretion of tyrosine and its metabolites:

P- hydroxyphenyl-pyruvate,

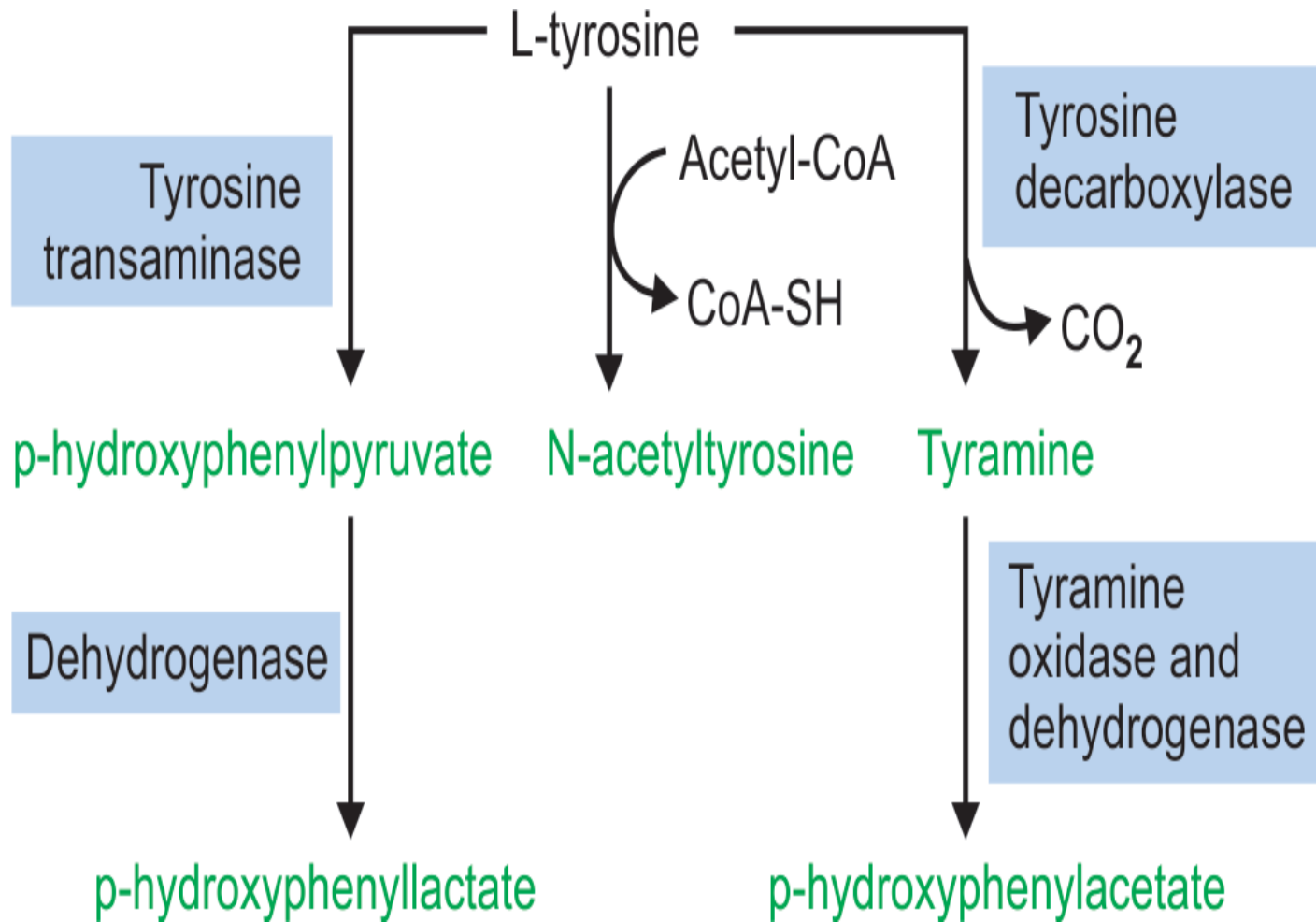
P- hydroxyphenyl-lactate

P-hydroxyphenyl-acetate,

N- acetyltyrosin

Tyramine.

➤ The deficiency of enzyme fumarylacetoacetate hydroxylase causes liver failure, kidney dysfunction, polyneuropathy, and vitamin D-resistant rickets.



**Figure 12.26:** Alternative catabolic pathway for tyrosine in tyrosinemia.

## Clinical Features

- In acute tyrosinosis, the infant exhibits diarrhea, vomiting, and cabbage-like odor to skin and urine due to **succinylacetone** an abnormal metabolite, derived from fumarylacetoacetate.
- Death may occur in infancy due to acute liver failure (within first year of life).
- Whereas in chronic tyrosinosis, in later life, it develops liver cirrhosis and death that occurs by age 10 years

## Diagnosis

- A diagnosis of tyrosinemia type I may be suspected in infants who display failure to thrive and an enlarged liver (hepatomegaly) during the first three months of life.
- The diagnosis is expected when tyrosine metabolites and succinylacetone are detected in the urine.
- It is also possible to make the diagnosis based on decreased activity of **fumarylacetoacetate hydroxylase** (FAH) in liver tissue but this test is not readily available.

- Molecular genetic testing for *FAH* gene mutations is available to confirm the diagnosis.
- Tyrosinemia type-I may also be diagnosed through newborn screening programs. Succinylacetone can be measured on the newborn blood spot by tandem mass spectroscopy.
- Carrier testing and prenatal diagnosis by DNA analysis are available if the specific gene-causing mutation has been identified in the family. Prenatal diagnosis is also possible by detection of succinylacetone in amniotic fluid.

## *Treatment*

The patient should be kept on diet low in phenylalanine and tyrosine.

## *Tyrosinemia Type-II (Richner-Hanhart Syndrome)*

### *Cause*

Tyrosinemia type-II is caused by genetic deficiency of hepatic enzyme **tyrosine aminotransferase** (tyrosine transaminase).

### *Clinical features*

- tyrosine and its toxic metabolites accumulates in blood and tissues and appears in urine.
- The accumulation of tyrosine produces **lesions in eye** and **skin** and causes **mental retardation**

## *Treatment*

- Diet low in tyrosine and phenylalanine is recommended.
- Diet with vitamin C may benefit the corneal and skin lesions of tyrosine aminotransferase deficiency, but not the mental retardation.

## Tyrosinemia Type-III (Neonatal Tyrosinemia)

- Caused by absence of the enzyme **P- hydroxyphenyl-pyruvate hydroxylase**
- Serum **tyrosine** levels are **high** in premature infants resulting from an **immature liver** and its limited ability to synthesize the enzyme, p-hydroxyphenyl-pyruvate hydroxylase.

- As the liver matures, the accumulated tyrosine is metabolized and serum levels decrease within 4 to 8 weeks of age.
- It is **benign** condition and responds well to ascorbic acid.

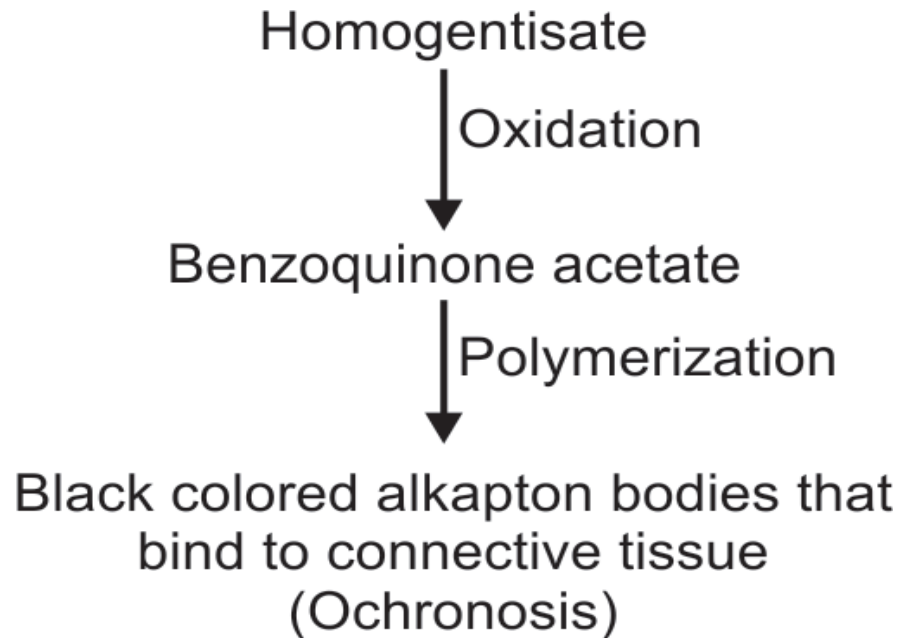
# *Alkaptonuria*

## *Cause*

- Defect in the enzyme *homogentisate oxidase*, that catalyzes oxidation of homogentisate
- Homogentisate accumulates in blood and body tissues and is excreted in large amounts in urine.

## *Clinical features*

The **urine of alkaptonuric** patients becomes **dark** after being exposed to air



**Formation of alkapton bodies**

- The **alkapton** imparts a characteristic black-brown color to urine.
- Alkaptonuria is a **harmless** condition.
- Later in life **deposition** of dark colored **alkapton pigments** in **connective tissues** and **bones** occur.

- This results in black pigmentation of the sclera, ear, nose and cheeks and the clinical condition is known as **ochronosis** (because ochre color of the deposit).
- Ochronosis leads to tissue damage and may develop joint pain, arthritis and backache.

## *Treatment*

Since alkaptonuria is not considered life threatening, this condition is not treated. Later in life, the symptoms of arthritis may be treated but the condition itself is not.

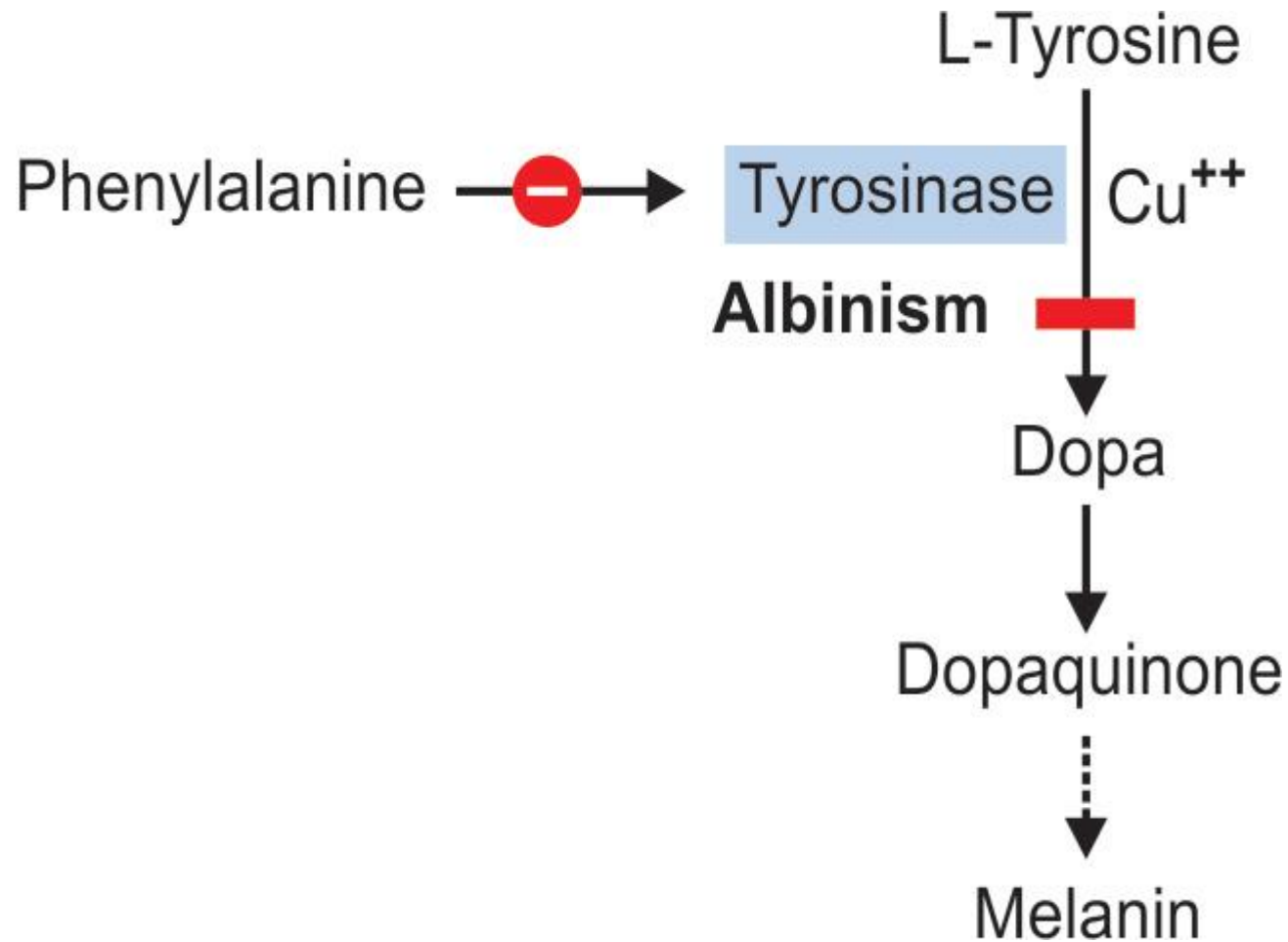
## *Diagnosis*

The urine sample of patients of alkaptonuria **turns dark on standing in air**. The urine gives positive test with **ferric chloride** and **silver nitrate** due to reducing activity of homogentisate.

# *Albinism*

## *Cause*

- Inborn error of **tyrosine catabolism**.
- It is due to inherited deficiency of enzyme **tyrosinase** which impairs the synthesis of **melanin** from tyrosine.
- Melanin protects the body from harmful radiation of sunlight.



**Figure 12.30:** Biosynthesis of melanin.

## *Clinical features*

- Impaired synthesis of melanin results in either **hypopigmentation** or no pigmentation of hair, skin and eyes and leads to white hair and skin.
- Albinos are highly sensitive to sunlight and can lead to **skin cancer**.

- The lack of melanin pigment in eyes is responsible for **photophobia** (intolerance to light) and **nystagmus** (**rapid involuntary oscillation of the eyeballs**)

## *Biologically Important Compounds Derived from Tyrosine*

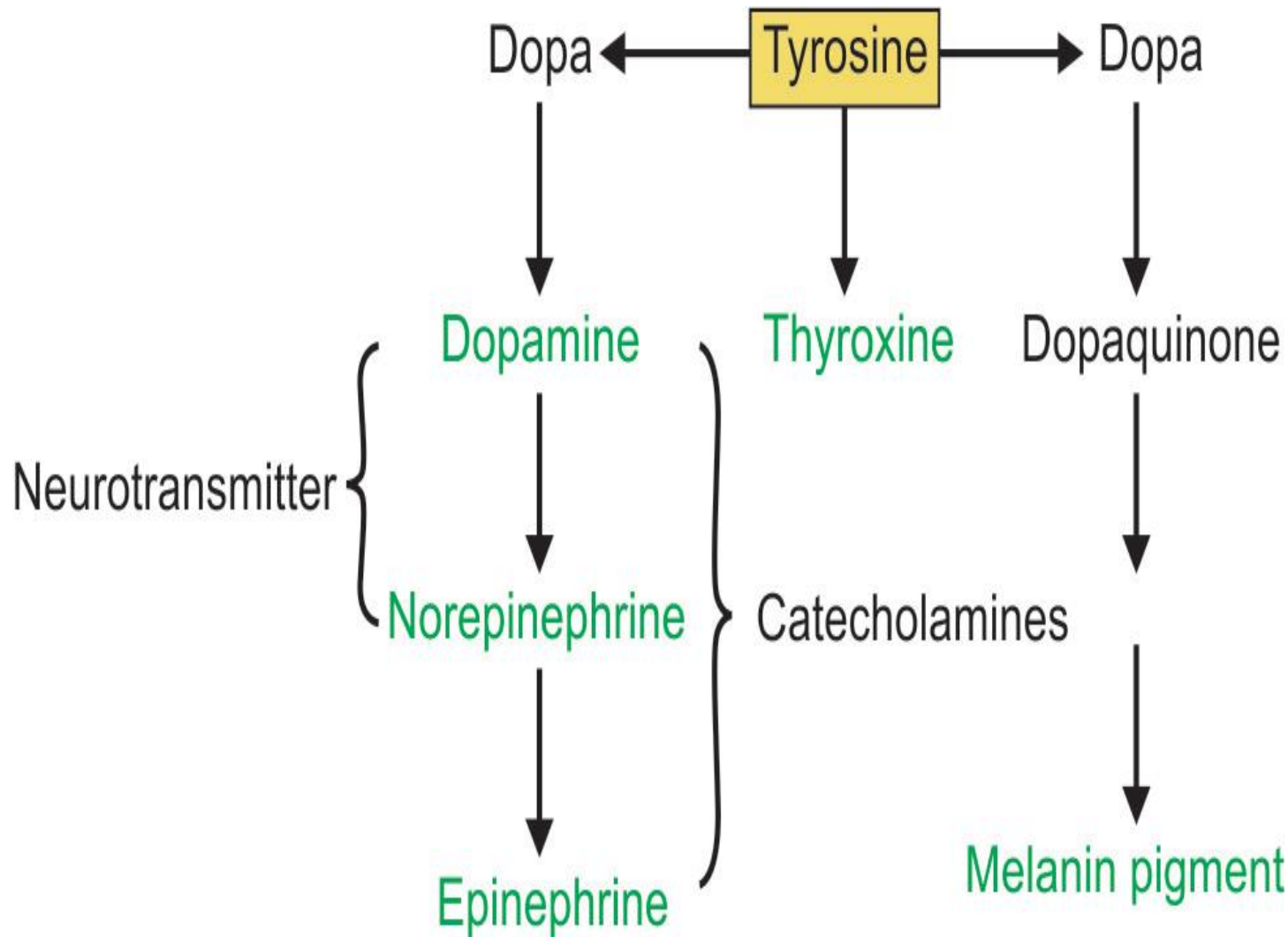
Tyrosine serves as a precursor for following several biologically important compounds :

### 1. Catecholamine

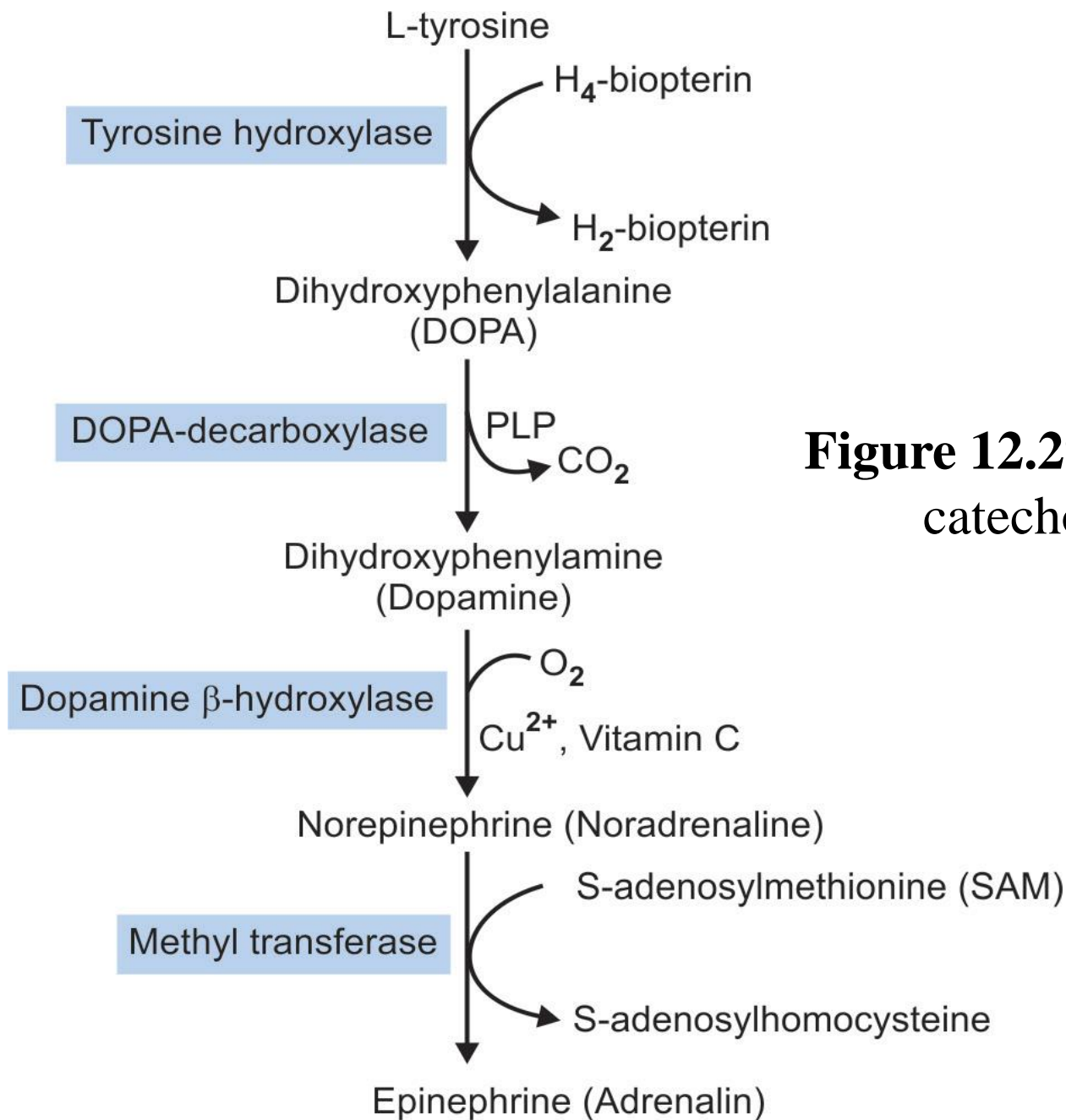
- Dopamine
- Norepinephrine
- Epinephrine

### 2. Melanin pigment

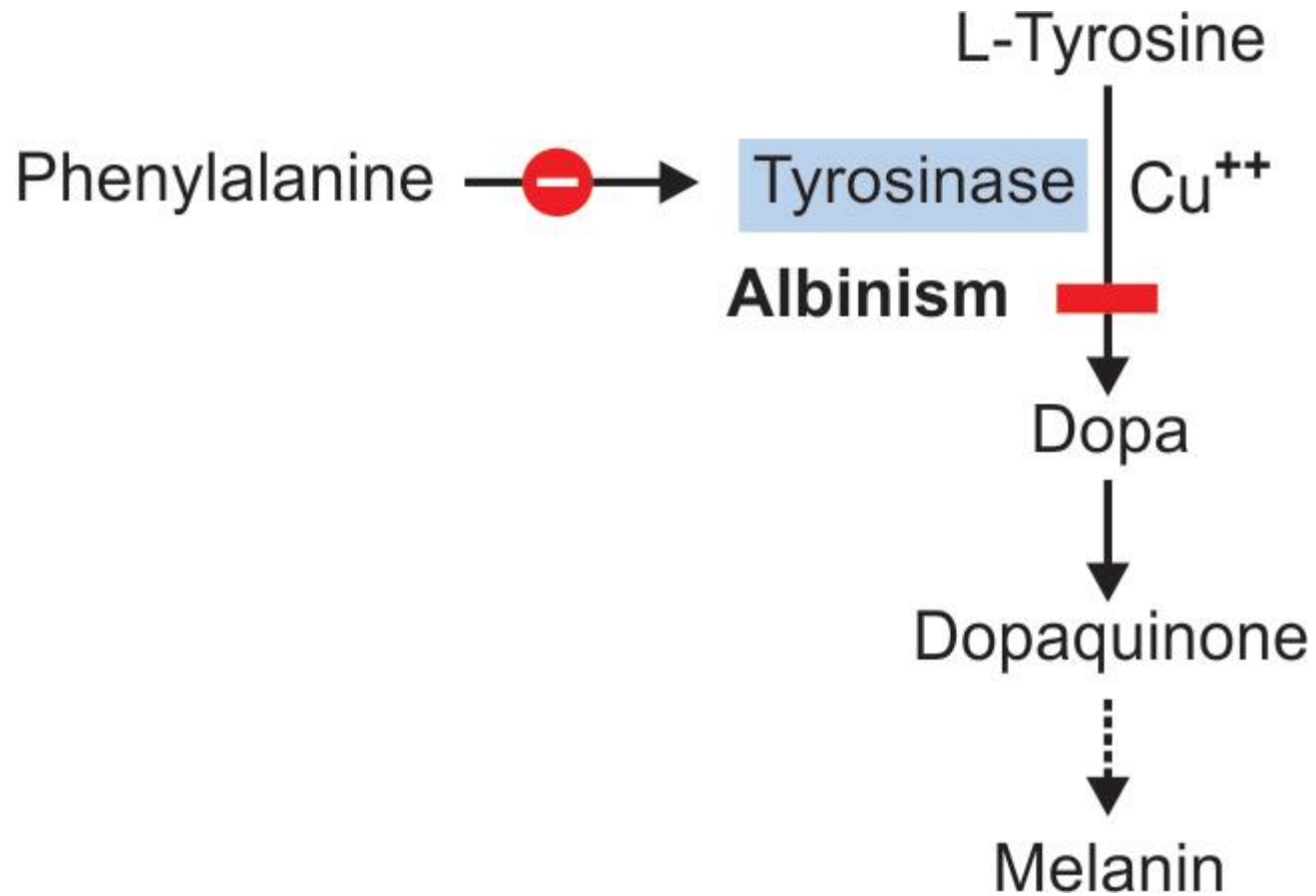
### 3. Thyroxine.



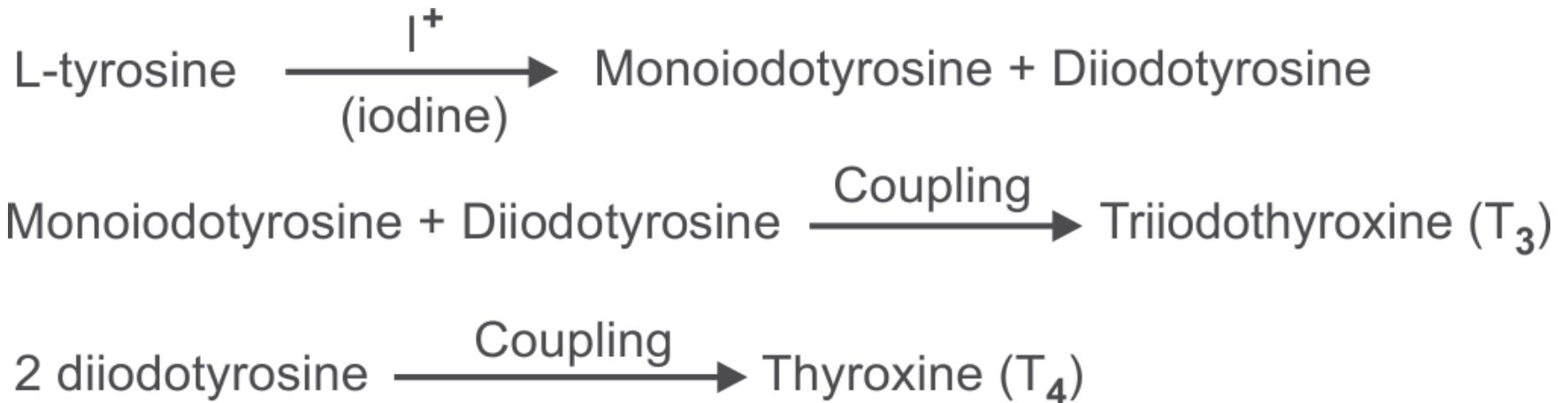
**Figure 12.28:** Biosynthesis of biologically important compounds from tyrosine.



**Figure 12.29: Synthesis of catecholamines.**



## Biosynthesis of melanin



**Figure 12.31:** Biosynthesis of T<sub>3</sub> and T<sub>4</sub>.

# **METABOLISM OF TRYPTOPHAN**

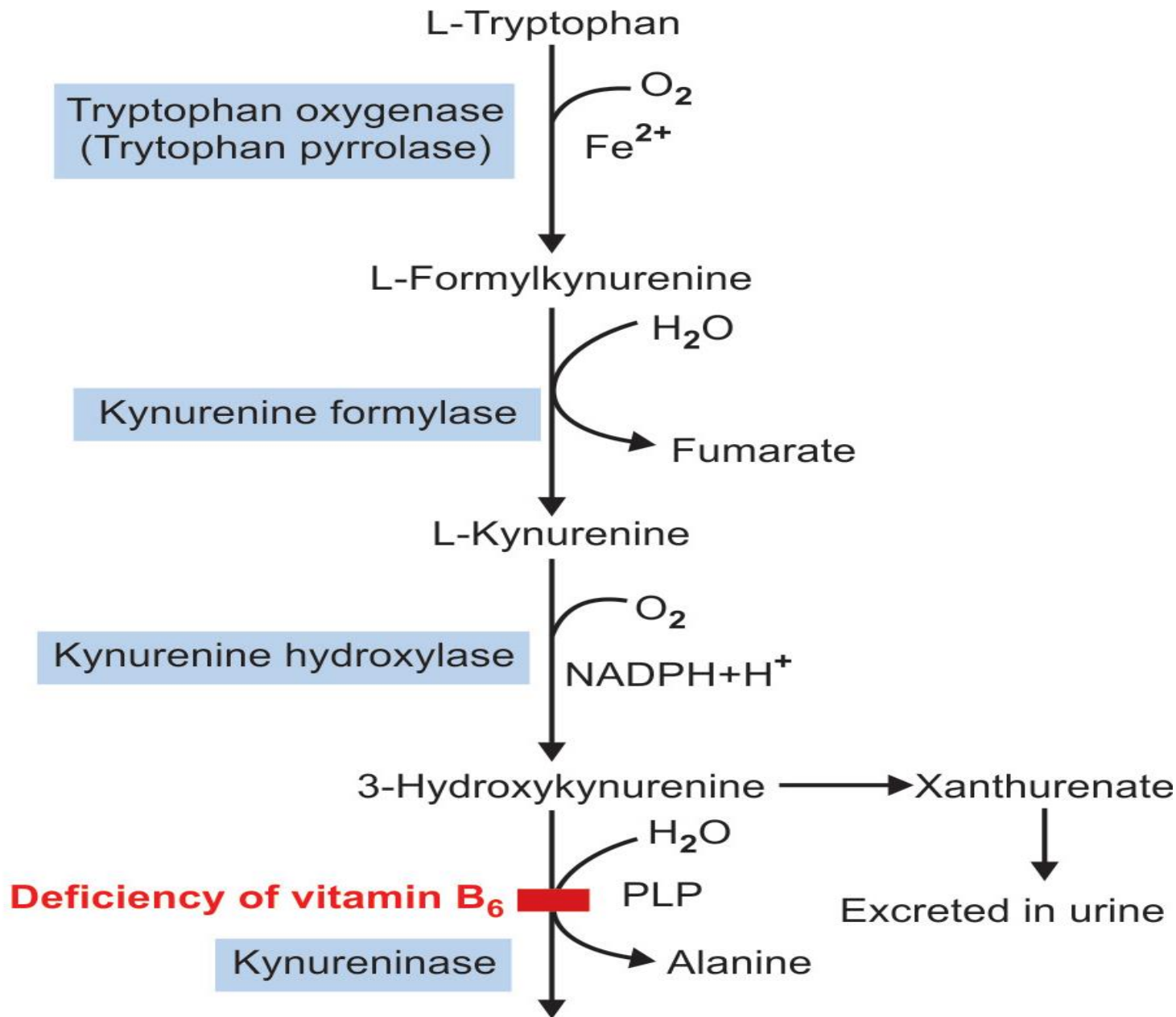
➤ Tryptophan is an **essential amino acid**, containing *indol ring*.

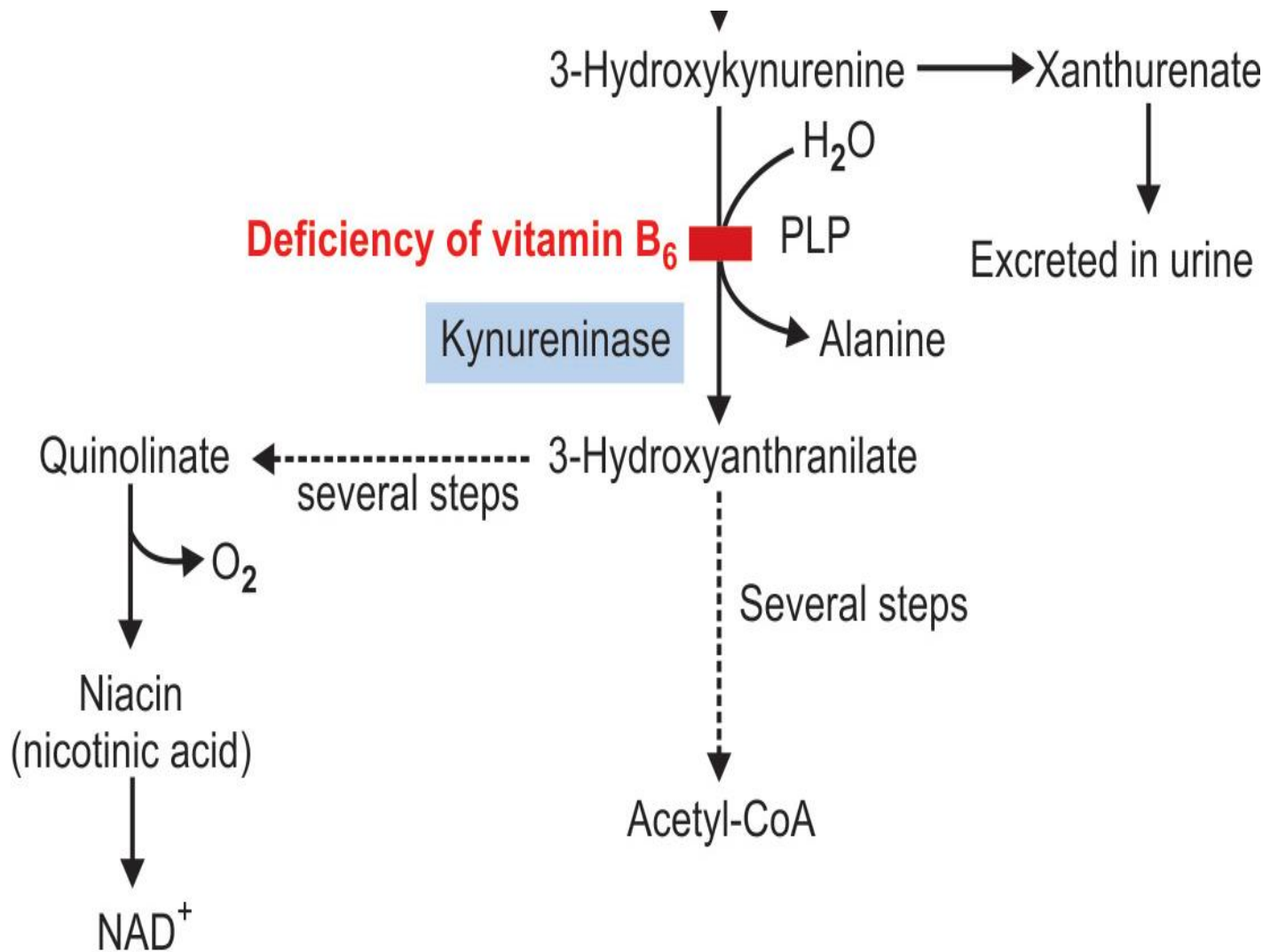
➤ Tryptophan is a precursor for the synthesis of:

- **Vitamin niacin (vitamin B<sub>3</sub>)**
- **Neurotransmitter serotonin**
- **Hormone melatonin.**

Tryptophan is metabolized by two pathways:

- 1 Kynurenine pathway
2. Serotonin pathway.





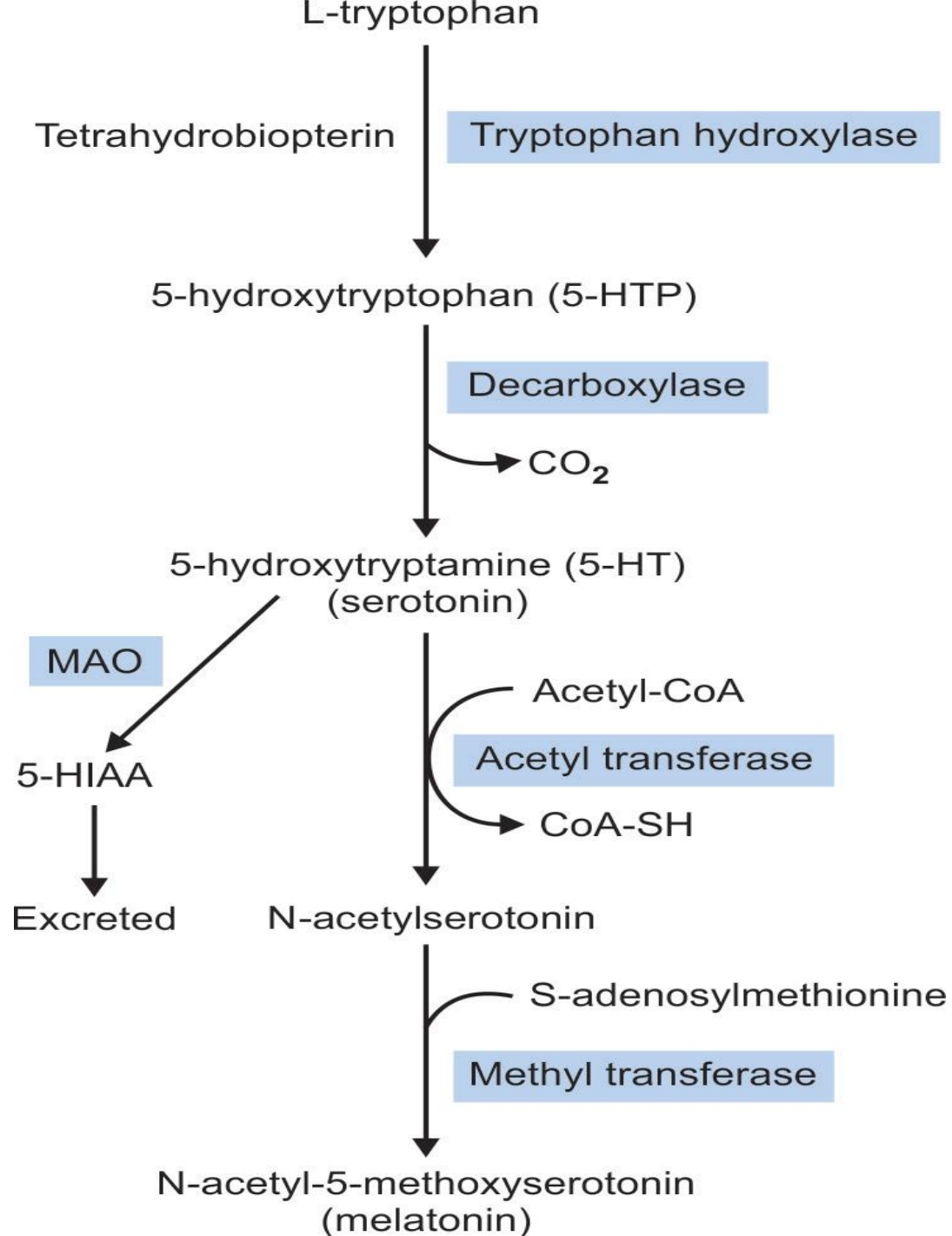
**Figure 12.32:** Kynurenine catabolic pathway of tryptophan and biosynthesis of vitamin niacin.

For every 60 mg of tryptophan, 1 mg equivalent of  
niacin can be generated.

**Figure 12.33:** Serotonin catabolic pathway of tryptophan and formation of melatonin.

MAO: monoamine oxidase;

5-HIAA: 5-hydroxyindoleacetic acid



*Biologically Important Compounds Derived  
from Tryptophan*

Tryptophan is a precursor for the synthesis of:

- Neurotransmitter serotonin
- Hormone melatonin
- Vitamin niacin (vitamin B3).

# *Serotonin*

- Serotonin is synthesized from **tryptophan** by neurons, pineal glands and intestinal argentaffin cells.
- In normal adult, about **1% of tryptophan** is converted to serotonin.

## Clinical Importance

- Clinically, serotonin plays a role in **depression** and **carcinoid tumors**.
- The most important physiological role of serotonin is that of a **transmitter in serotonergic neurons** within the brain.

- In humans, serotonin is involved in a variety of behavioral patterns including:
  - Sleep
  - Perception of pain
  - Social behavior
  - Schizophrenia
  - Mental depression.

## *Functions of serotonin*

- Serotonin is a **neurotransmitter** and stimulates cerebral activity.
- Serotonin deficiency causes a decrease in cerebral (brain) activity, which leads to **depression**.
- Serotonin is involved in a variety of behavioural patterns, including **sleep, body temperature** and **blood pressure**.

- Serotonin produced in intestinal cells stimulates the **release of gastrointestinal peptide hormones.**
- Serotonin serves as **precursor of melatonin** in the pineal gland.
- Serotonin is also a powerful **vasoconstrictor** and **stimulator of smooth muscle contraction.**

## Carcinoid Tumors or Argentaffinomas

- A carcinoid tumor is a serotonin-secreting neoplasm.
- Carcinoid tumors develop from enterochromaffin cells, which are distributed widely throughout the gastrointestinal tract, biliary tract and gallbladder, pancreatic ducts, and bronchial tree.

- Normally, about 1-3 % of tryptophan is converted to serotonin, however, in carcinoid syndrome about 60% of tryptophan is diverted to serotonin formation resulting in decreased synthesis of vitamin niacin. Therefore, niacin deficiency (pellagra) may also be seen in this carcinoid syndrome.

# Clinical symptoms of carcinoid syndrome

- Prominent flushing
- Bronchial constriction
- Diarrhea
- Cardiac lesions and often is associated with heart failure
- Individuals have large increase in urinary excretion of 5-HIAA.

## **Diagnostic test**

Measurement of urinary 5-HIAA concentration.

## *Melatonin*

- Melatonin is a hormone produced from serotonin by the pineal gland
- Synthesis of melatonin is regulated by **light-dark cycle**.
- It is synthesized mostly at night.
- It is an inhibitor of **melanocyte-stimulating hormone (MSH)** and **adrenocorticotrophic hormone (ACTH)**.

- Melatonin is a sleep-inducing substance and is involved in regulation of circadian rhythm of body. It may also be involved in regulating **reproductive functions**.

## *Metabolic Disorder of Tryptophan*

### *Hartnup's disease*

- Inherited disorder of **tryptophan metabolism**.
- This disorder was first of all reported in the family of **Hartnup**
- Defect in the intestinal and renal **transport of tryptophan** and other neutral amino acids and leads to tryptophan deficiency.

- Tryptophan deficiency leads to decreased synthesis of **vitamin niacin** and **serotonin**.
- Decreased synthesis of niacin leads to **pellagra** like symptoms
- and decreased serotonin synthesis is responsible for **neurological symptoms**
- There is **amino aciduria** due to failure of transport of amino acids from kidney.
- This causes increased urinary loss of alanine, serine, threonine, asparagine, glutamine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine, glycine, and citrulline.

➤ Unabsorbed tryptophan remaining in the intestine is metabolized by intestinal bacteria to **indolacetic acid** and **indolpyruvic acid** which are subsequently excreted in urine.

# Clinical Features

Hartnup disease causes:

- An intermittent photosensitive red scaly rash resembling the rash of pellagra
- Severe but reversible cerebellar ataxia
- Headache
- Muscle discomfort and
- Occasionally psychological disturbances
- Generalized neutral aminoaciduria

## Diagnosis

- Due to the variability of symptoms, unambiguous diagnosis can only be made through urine analysis.
- The test is based on the detection of elevated amino acids in the urine by chromatography.
- There is increased urinary excretion of **indolacetic acid** and **indolpyruvic acid** and other indole derivatives.
- Molecular genetic testing can confirm a diagnosis of Hartnup disease in some cases. Molecular genetic testing can detect genetic alterations in the *SLC19A6* gene known to cause the disorder, but usually is not necessary to obtain a diagnosis.

## Therapy

Oral nicotinic acid supplementation will permit adequate synthesis of NAD<sup>+</sup> in patients with Hartnup disease. This corrects pellagra like symptoms of the disorder. The aminoaciduria remains unaltered.

# Metabolism Of Sulfur Containing amino Acids

Three sulphur containing amino acids are:

Cystine

Cysteine

Methionine

- Methionine is an essential whereas cysteine and cystine are nonessential amino acids.
- Cysteine and cystine are synthesized from two amino acids, methionine, and serine
- Cystine and cysteine are readily inter-convertible in the body.

## Metabolism Of Methionine

Methionine is metabolized by:

1. Transfer of methyl group of methionine by

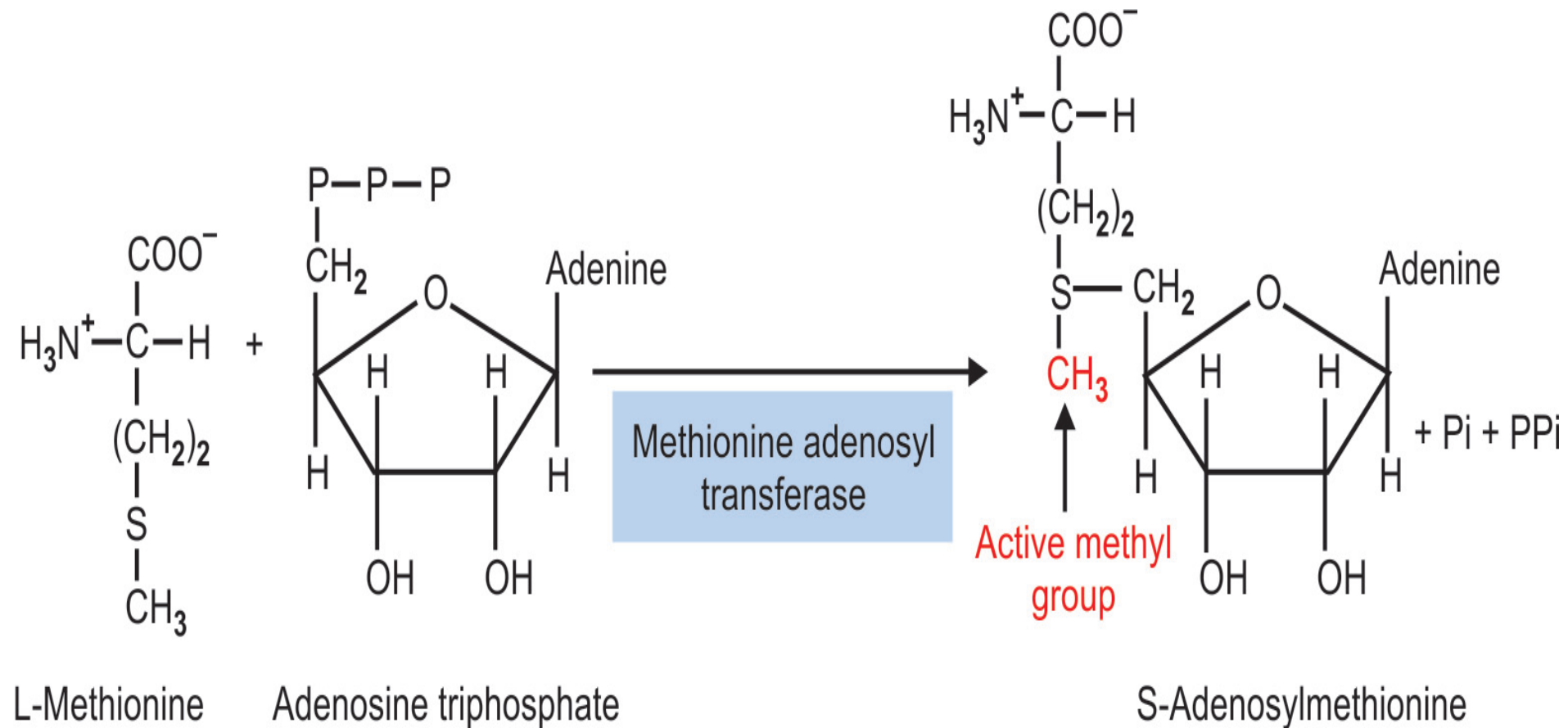
**Transmethylation** reactions

2. Conversion of demethylated portion of the methionine to **cysteine** and **cystine**.

# *Transfer of Methyl Group of Methionine*

## *(Transmethylation reactions)*

- Transfer of methyl group ( $-\text{CH}_3$ ) from methionine to an acceptor molecule is termed as **transmethylation**.
- The methyl group of methionine becomes available for transmethylation only in an active form of methionine, **S-adenosylmethionine (SAM)**.

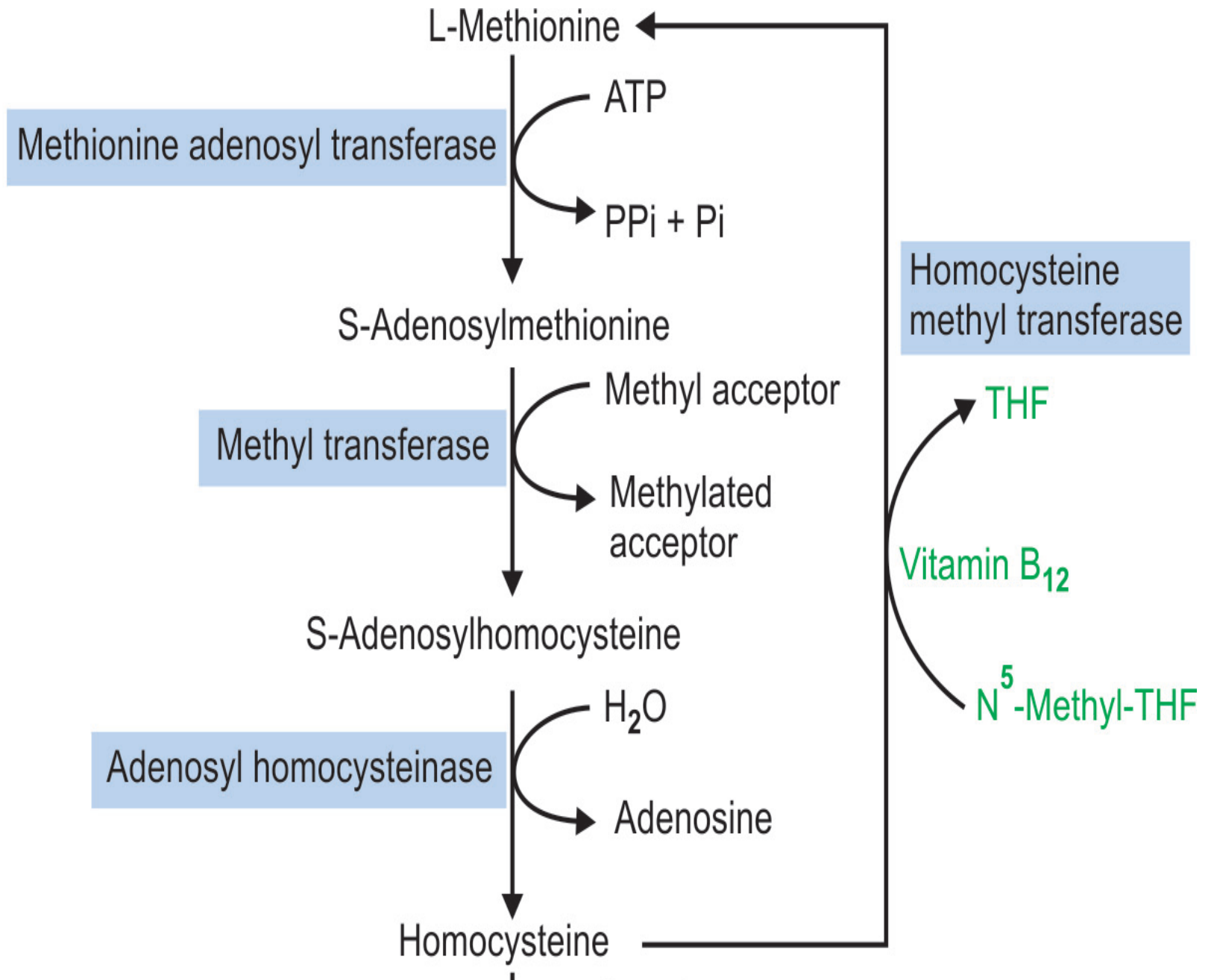


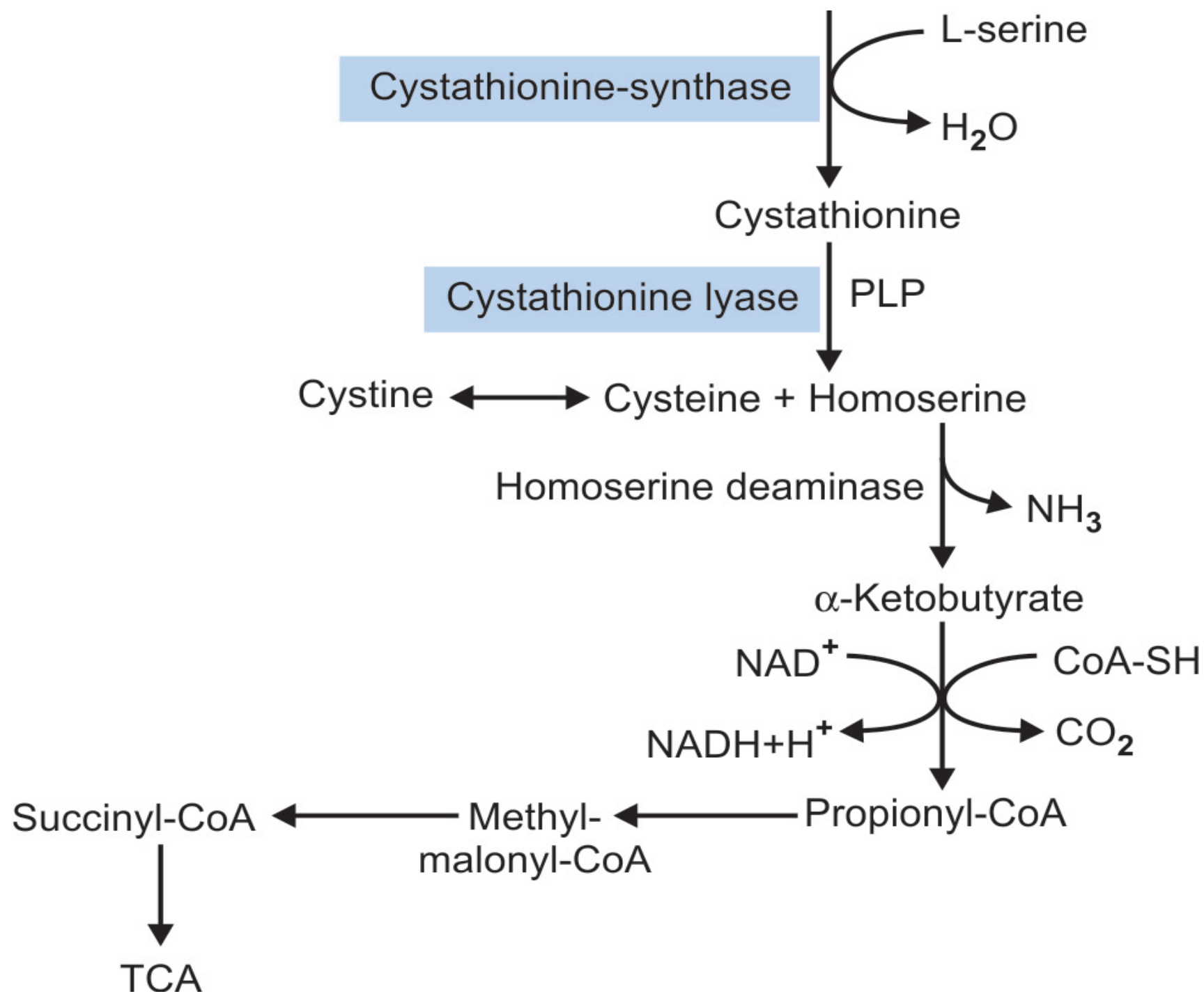
## Formation of active methionine

Some important transmethylation reactions are:

- Norepinephrine to epinephrine
- Phosphatidylethanolamine to phosphatidylcholine
- Guanidoacetoacetate to creatine
- Ethanolamine to choline
- Acetyl serotonin to melatonin
- Nucleotides to methylated nucleotides

Conversion of demethylated portion of the methionine  
to cysteine and cystine





- It should be noted that cysteine is synthesized from two amino acids, **methionine** and **serine**.
- **Methionine** furnishes the **sulphur** atom and **serine** furnishes the **carbon skeleton**.

## *Synthesis of methionine from homocysteine*

- Remethylation of homocysteine requires both **tetrahydrofolate (THF)** and **vitamin B<sub>12</sub>**.
- Methyl group of N<sup>5</sup>-methyl THF is transferred to vitamin B<sub>12</sub> forming **methyl B<sub>12</sub>**. Then methyl B<sub>12</sub> transfers the methyl group to **homocysteine** which is converted to methionine.
- There is no net synthesis of methionine because homocysteine which serves as precursor for methionine has to be derived from methionine.

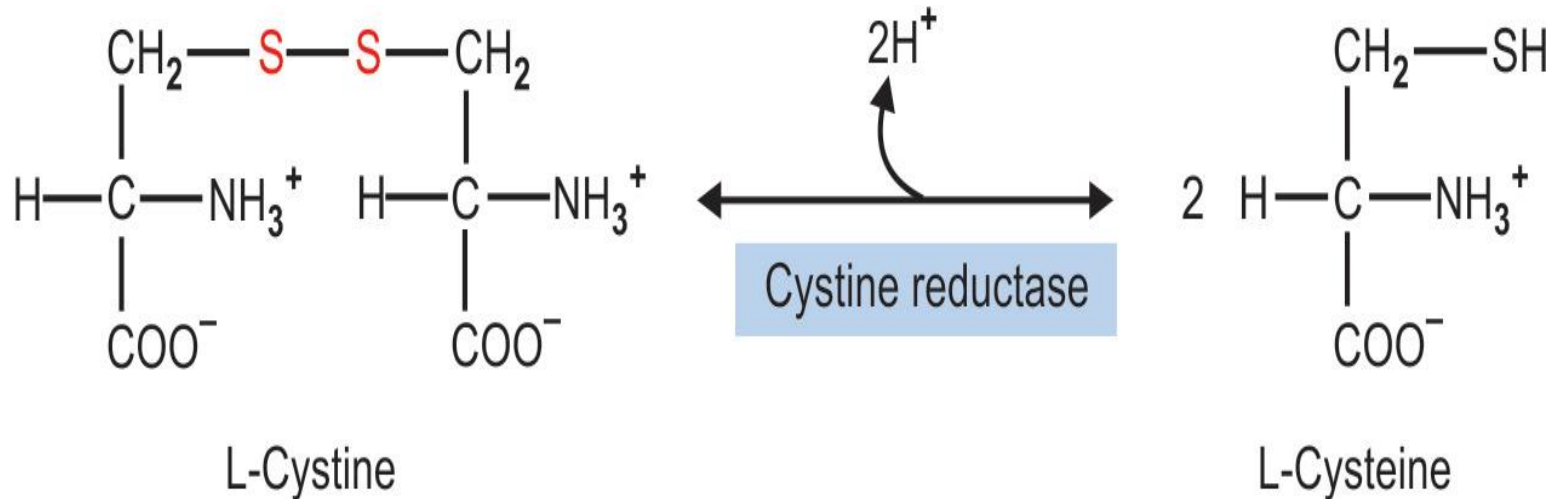
# Metabolism of cysteine and cystine

## *Synthesis of Cysteine and Cystine*

- Cysteine is a nutritionally **non-essential**, **glucogenic** amino acid.
- Synthesized from two amino acids, *methionine*, which is nutritionally essential and *serine*, which is not.
- **Methionine** furnishes **sulphur** atom and **serine** furnishes the **carbon skeleton**
- **Cystine** is formed by oxidation of **cysteine**.

## *Catabolism of Cystine and Cysteine*

Cystine is converted to cysteine. Catabolism of cystine then occurs simultaneously with that of cysteine.

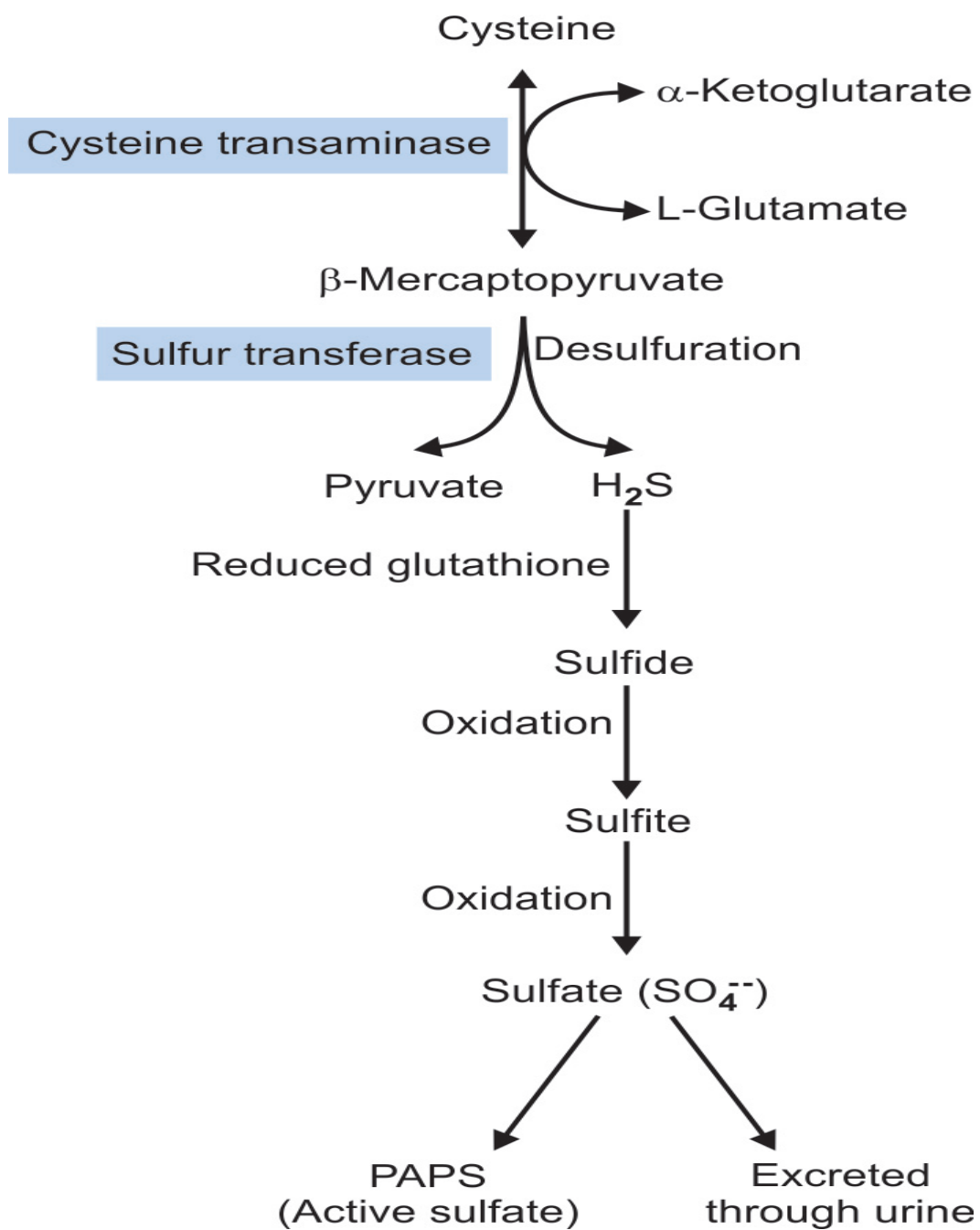


Conversion of cystine to cysteine

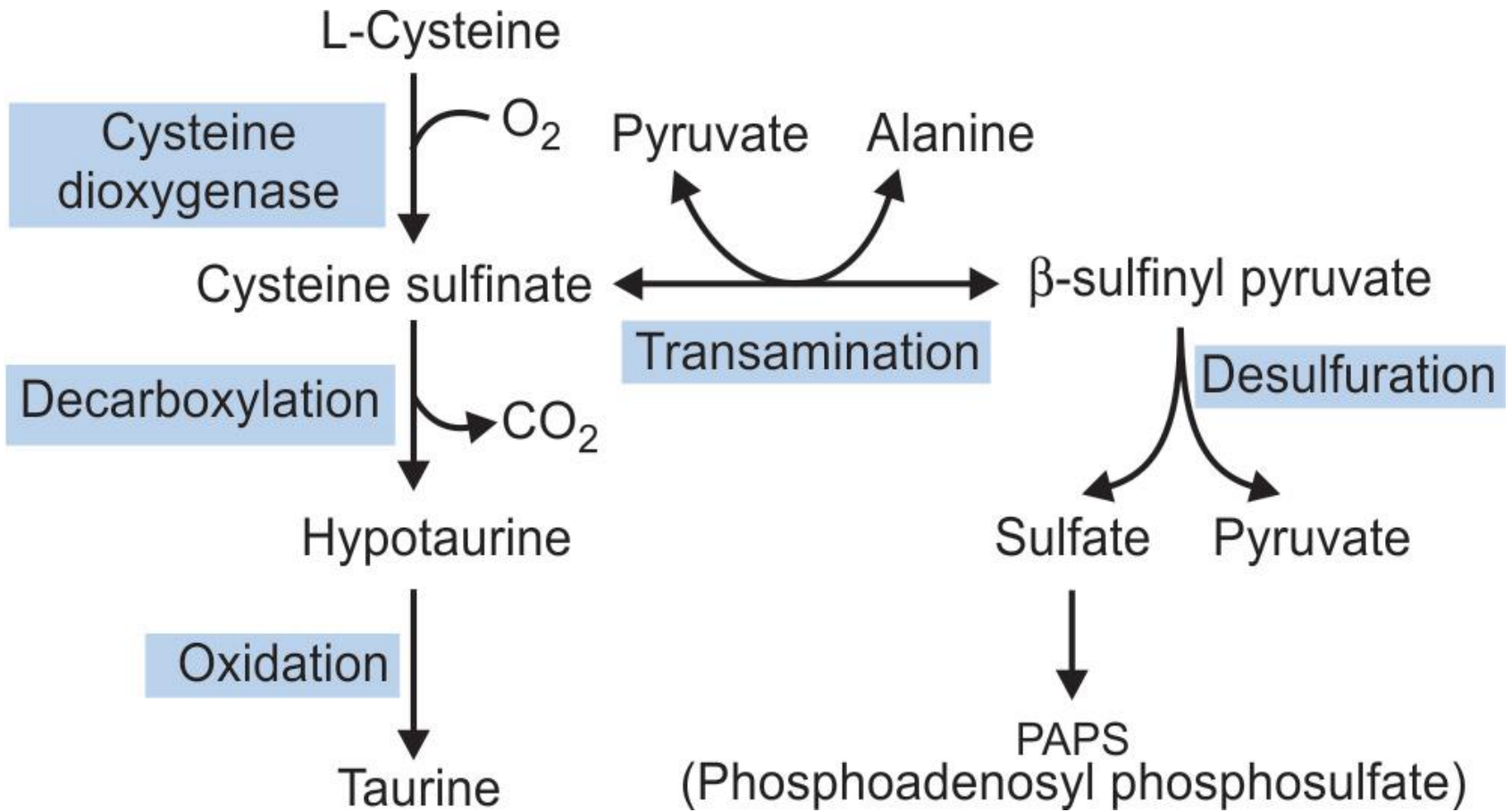
Cysteine can be catabolized by two routes:

1. The transamination pathway

2. Direct oxidative pathway



Transamination pathway



Direct oxidative pathway for catabolism of cysteine

## *Importance of cysteine*

- Glucogenic amino acid involved in formation of **glucose**.
- Cysteine is most important **dietary source of sulphur**.
- Physiologically important sulphur containing compounds derived from cysteine are:
  - **Taurine**
  - *Insulin*
  - *Coenzyme-A*
  - *Glutathione*
  - *Vasopressin*
- It is also involved in **detoxification** mechanisms.

# *Metabolic Disorders of Sulphur Containing Amino Acids*

## *Cystinuria (Cystin-lysinuria)*

- Cystinuria is the most common **inborn error** of amino acid transport.
- Cystinuria is an inherited disorder in which kidney tubules fail to reabsorb the amino acids **cystine, ornithine, arginine** and **lysine** (the mnemonic is **COAL**).
- This is characterized by massive **urinary excretion** of **cystine, ornithine, arginine** and **lysine**.

## *Cause*

- Normally, these amino acids are filtered by the glomerulus and reabsorbed in the proximal renal tubule by specific carrier proteins.
- In cystinuria defect in this carrier system leads to the excretion of all these four amino acids.

## *Clinical features*

- ❖ Since cystine is the **least soluble** its over excretion often leads to precipitation and formation of **cystine calculi (stones)** in the renal tubules and leads to **obstruction, infection** and **renal insufficiency** in cystinuric patient.

## *Treatment*

- ❖ Treatment involves ingestion of **large amounts of water**, which increases cystine solubility through maintenance of alkaline urine

## *Cystinosis (cystine storage disease)*

Cystinosis is a rare but serious **lysosomal disorder**.

### *Cause*

- ❖ Caused by a **defective carrier** that transports cystine across the lysosomal membrane from lysosome to the cytosol.

## *Clinical features*

- ❖ Cystine accumulates in the lysosomes in many tissues and forms crystals impairing their function.
- ❖ Cystinosis is usually accompanied by a generalized aminoaciduria.
- ❖ Patients usually die within 10 years due to acute renal failure

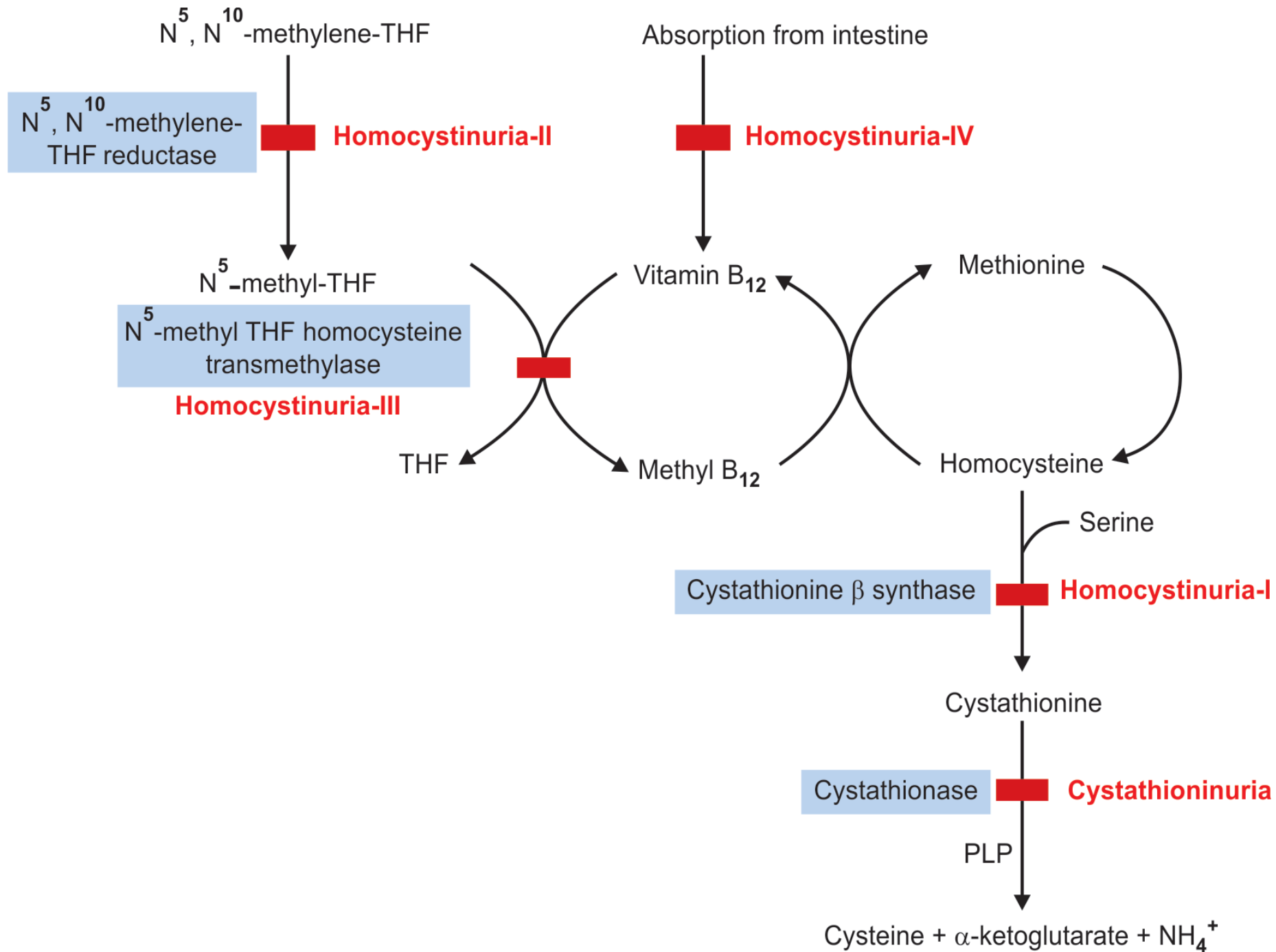
## *Homocystinuria*

- Homocystinurias are a group of disorder of **methionine metabolism**.
- It is characterized by **high blood** and urinary levels of **homocysteine** and **methionine**.
- Four metabolic defects cause **four types of Homocystinuria**.

**TABLE 12.5:** Different types of homocystinuria with their defects.

<i>Types</i>	<i>Defects</i>
Homocystinuria-I	Cystathionine $\beta$ synthase
Homocystinuria-II	Synthesis of N <sup>5</sup> -methyltetrahydrofolate
Homocystinuria-III	Deficiency of methyl B <sub>12</sub>
Homocystinuria-IV	Defective intestinal absorption of vitamin B <sub>12</sub>

**Figure 12.39:** Metabolic blocks in homocystinuria.



- ❖ A deficiency of **cystathionine synthase** is the most **common cause** of **homocystinuria**.
- ❖ Other types of homocystinuria are due to **defects** in the **remethylation of homocysteine** to form methionine
- ❖ The *accumulation of **homocysteine** causes skeletal abnormalities, **ectopia lentis** (dislocation of the lenses in the eyes), **osteoporosis, mental retardation and thrombosis.***
- ❖ *Thrombosis may result in **myocardial infarction, pulmonary embolism or stroke.***

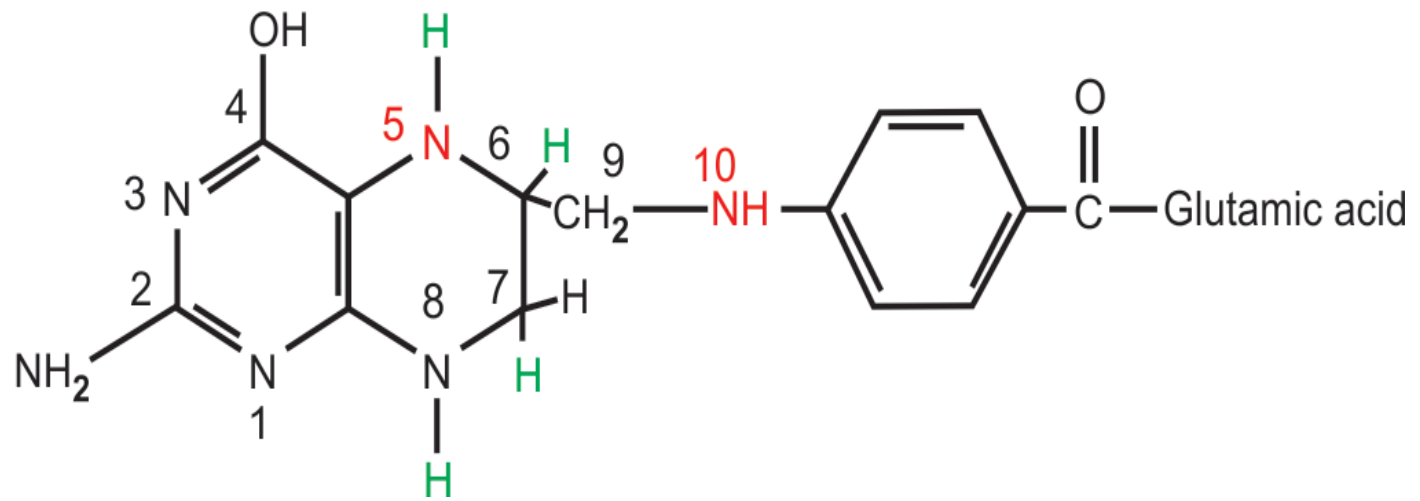
## *Treatment*

- The biochemical defect in cystathionine synthase can be corrected in some cases by **providing pyridoxine (vitamin B<sub>6</sub>)**. Pyridoxine is needed to activate the enzyme cystathionine synthase.
- Those with complete enzyme deficiency should be treated with a **diet low in methionine and supplemented with cysteine**.
- **Vitamin B<sub>12</sub>** may be given in instances of vitamin B<sub>12</sub> deficiency.

# ONE CARBON METABOLISM

- Groups, containing a **single carbon** atoms are called one carbon groups. One carbon groups are formed from several amino acids during their metabolism.
- These include **serine, glycine, histidine** and **tryptophan**.

- One carbon groups formed during metabolism are:
  - Methyl ( $\text{CH}_3$ )
  - Methylene ( $\text{CH}_2$ )
  - Methenyl ( $\text{CH}$ )
  - Formyl ( $\text{CHO}$ )
  - Formimino ( $\text{CH}=\text{NH}$ )
  
- These one carbon groups are transferred by way of tetrahydrofolate (THF).



Structure of tetrahydrofolate

- One carbon groups carried by THF are attached either to nitrogen  $N^5$  or  $N^{10}$  or to both  $N^5$  and  $N^{10}$
- The different one carbon derivatives of THF are
  - $N^5$ -methyl THF
  - $N^5, N^{10}$ -methylene THF
  - $N^5, N^{10}$ -methenyl THF
  - $N^5$ -formyl THF
  - $N^5$ -formimino THF.
- These different derivatives of THF are interconvertible.

Sources of one-carbon group	Carrier derivatives	Utilization of one-carbon group
Glycine } Tryptophan } → Formate	$N^{10}$ -formyl THF	Purine synthesis
Histidine → FIGLU	$N^5, N^{10}$ -methenyl THF	Purine synthesis
Serine ← Glycine	$N^5, N^{10}$ -methenyl THF	Pyrimidine nucleotide (Thymidylate synthesis)
Choline } Betaine }	$N^5$ -methyl THF	Synthesis of methionine from homocysteine
Methionine	SAM	Synthesis of choline, creatine, epinephrine, tRNA, DNA

## *Importance of one carbon group metabolism*

One carbon groups at different levels of oxidation are transferred and made available by way of the tetrahydrofolate and vitamin B<sub>12</sub> coenzymes for use in a wide variety of vital anabolic processes.

## BIOGENIC AMINES

➤ Decarboxylation of amino acids results in the formation of **amines**. These amines are called biogenic amines.

They have diverse biological function.

➤ Decarboxylation reactions are catalyzed by **PLP** dependent decarboxylases.

**TABLE 12.6: Some important biogenic amines and their functions.**

<i>Amines</i>	<i>Amino acid precursors</i>	<i>Functions</i>
Dopamine	Tyrosine	Neurotransmitter
Norepinephrine	Tyrosine	Neurotransmitter
Epinephrine	Tyrosine	Hormone
Tyramine	Tyrosine	Vasoconstrictor
Serotonin	Tryptophan	Vasoconstrictor
Melatonin	Tryptophan	Vasoconstrictor
GABA	Glutamate	Neurotransmitter
Histamine	Histidine	Vasodilator
Taurine	Cysteine	Neurotransmitter
Spermine	Ornithine and methionine	Growth factor, regulator of transcription and translation

## *Polyamines*

- Biological amines made up of multiple amino acids called polyamines, e.g.
  - Putrescine
  - Spermidine
  - Spermine.
- Polyamines are positively charged at physiological pH and associate with negatively charged nuclear DNA.
- These are present in high concentration in semen. The concentration of polyamines in brain is about 2 mM.

- *Putrescine, spermidine* and *spermine* are derived from **ornithine** and **methionine**.
- It is presumed that the **15% of methionine** is used for polyamine synthesis.
- Polyamines are involved in regulation of **transcription** and **translation**.

- They act as a **growth factor** and function in cell proliferation and growth.
- Polyamines are involved in stabilization of intact cells, subcellular organelles and membranes.

## *Catabolism and excretion of polyamines*

- The enzyme **polyamine oxidase** present in liver peroxisomes oxidizes spermine to spermidine and spermidine to putrescine.
- Putrescine is then oxidized by a copper containing **diamine oxidase** to  $\text{CO}_2$  and  $\text{NH}_3$ .
- Major portions of putrescine and spermidine are excreted in urine after conjugation with acetyl-CoA as acetylated derivatives.

## *Clinical significance of polyamines*

- Polyamines and their derivatives have application in **diagnosis** and **treatment of cancer**.
- Their levels have been shown to increase in response to cell growth and differentiation.
- Their concentration is elevated in body fluids of cancer patients.
- Assays of urinary and blood polyamines have been used to detect **cancer** and to determine the success of **therapy** (diagnostic indicator).

**THANK YOU**