THOMAS ADEWUMI UNIVERSITY,
OKO, KWARA STATE
Science | Technology | Medicine

LECTURE NOTE

ON

BCM 226

PROTEIN AND AMINO ACID METABOLISM

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COURSE OUTLINE

- Amino acids
- Proteinogenic & non-proteinogenic amino acids
- Biological functions and applications of amino acids
- Amino acids reactions
- Digestion of proteins and absorption of amino acids
- Amino acids catabolism (breakdown)
- Urea cycle
- Amino acids anabolism (synthesis)
- Diseases of amino acids metabolism
- Protein/peptide formation
- The genetic code (codons)
- Translation and post-translation

FOCUS…

- ✓ Amino acids
- ✓ Amino acids reactions
- ✓ Amino acids catabolism (breakdown)
- ✓ Amino acids anabolism (synthesis)
- ✓ Diseases of amino acids metabolism
- ✓ The genetic code (codons)

DID YOU KNOW?

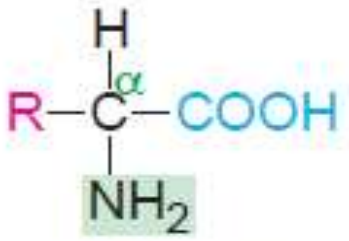
- About 300–400g of protein is constantly degraded and synthesized in the human body every day.
- Ammonia accumulation in the blood is toxic to the brain, and can cause slurring of speech, blurring of vision, tremors and even death.
- Melanin, which is the pigment of skin, hair and eyes, is produced from an amino acid named tyrosine.
- Lack of melanin synthesis (mostly due to a deficiency of the enzyme *tyrosinase*) causes albinism.

AMINO ACIDS

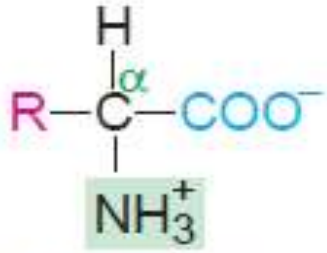
- Amino acids are a group of organic compounds containing the amino and carboxyl functional groups.
- The amino group (—NH2) is basic, while the carboxyl group (—COOH) is acidic in nature.
- Amino acids are the monomeric units of proteins.
- As many as 300 amino acids occur in nature, but only 20 are repeatedly found in the structure of proteins isolated from living organisms.
- These 20 amino acids are known as **standard amino acids**.

STRUCTURE OF AMINO ACIDS

- Amino acids are termed as α-amino acids if both the carboxyl and amino groups are attached to the same carbon atom.
- The α-carbon atom binds to a side chain represented by R, which is different for each of the 20 amino acids found in proteins.
- Amino acids mostly exist in the ionized form in the biological system.

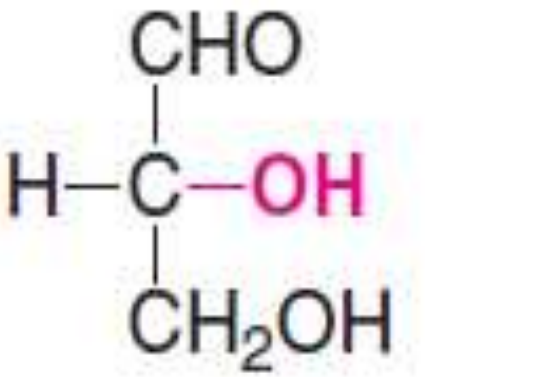


General structure

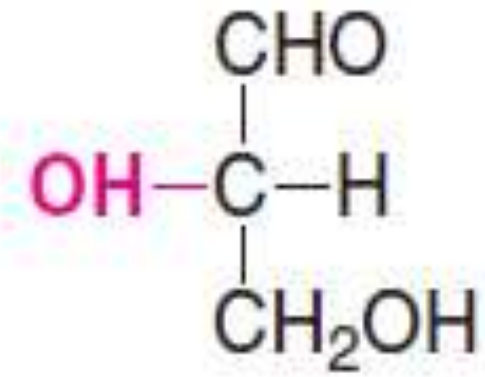


Exists as ion

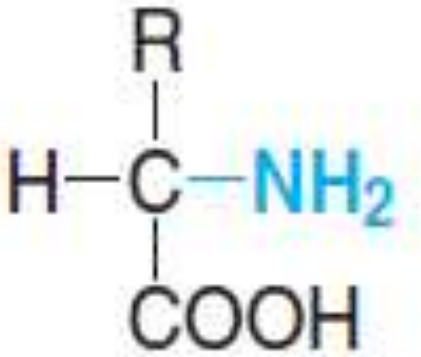
- Due to the presence of asymmetric carbon in their structure, amino acids exhibit optical isomerism.
- An asymmetric carbon is a carbon atom attached to four different groups.
- All amino acids (except glycine) possess four distinct groups (R, H, COO^-, NH_3^+) held by α-carbon.
- Hence, all amino acids (except glycine, where R = H) have optical isomers.
- The structures of L- and D-amino acids are written based on the configuration of L- and D-glyceraldehyde.
- Proteins are composed of L-α-amino acids.



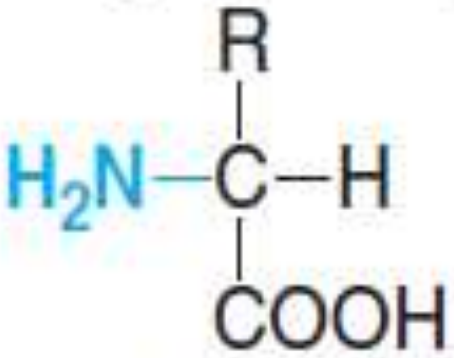
D-Glyceraldehyde



L-Glyceraldehyde



D-Amino acid



L-Amino acid

D- and L-forms of amino acid based on the structure of glyceraldehyde

CLASSIFICATION OF AMINO ACIDS

- There are different ways of classifying amino acids, such as based on their structure and chemical nature; polarity; nutritional requirement; and metabolic fate.

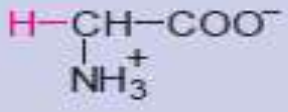
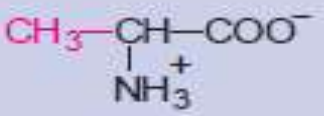
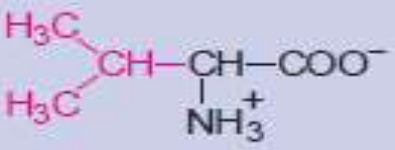
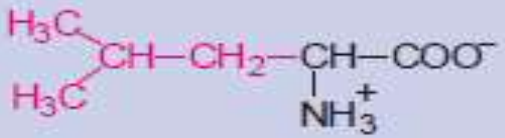
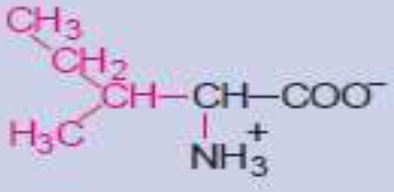
Classification of amino acids based on structure

- A comprehensive classification of amino acids is based on their structure and chemical nature.

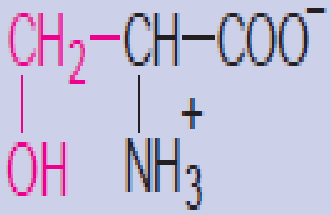
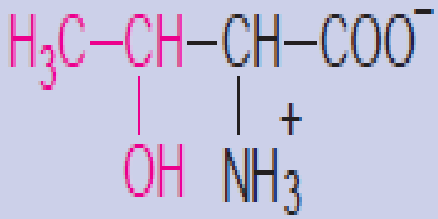
- Each amino acid is assigned a 3 letter or 1 letter symbol.

- These symbols are commonly used to represent amino acids in protein structure.

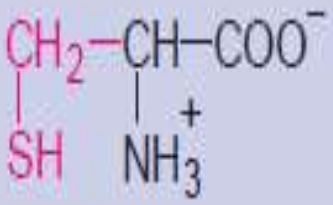
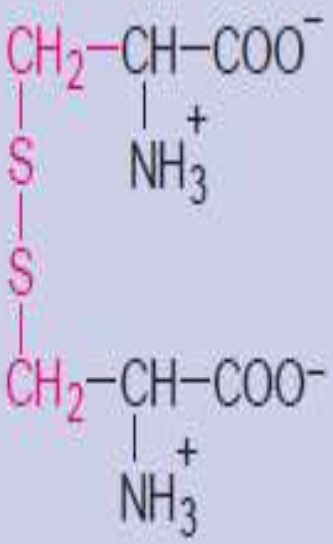
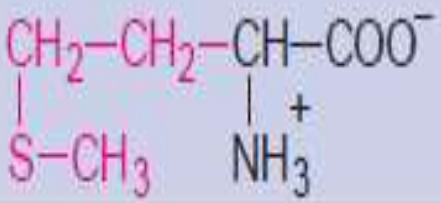
- The 20 amino acids found in proteins are divided into seven distinct groups.

Name	Symbol		Structure	Special group present
	3 letters	1 letter		
I. Amino acids with aliphatic side chains				
1. Glycine	Gly	G		
2. Alanine	Ala	A		
3. Valine	Val	V		Branched chain
4. Leucine	Leu	L		Branched chain
5. Isoleucine	Ile	I		Branched chain

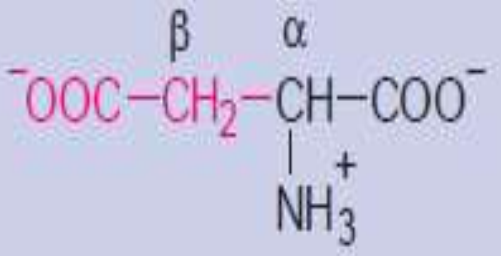
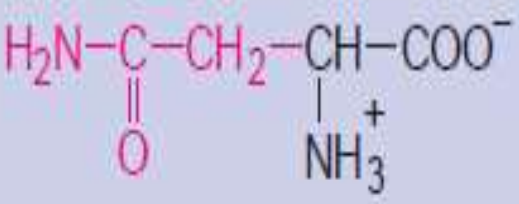
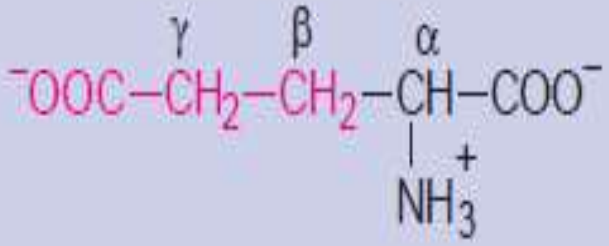
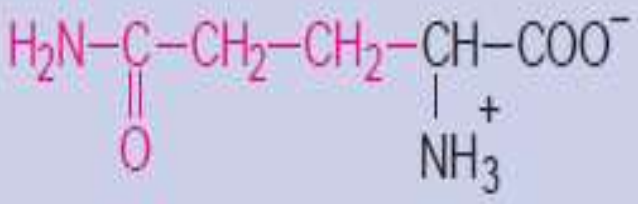
II. Amino acids containing hydroxyl (—OH) groups

6. Serine	Ser	S		Hydroxyl
7. Threonine	Thr	T		Hydroxyl
Tyrosine	Tyr	Y	See under aromatic	Hydroxyl

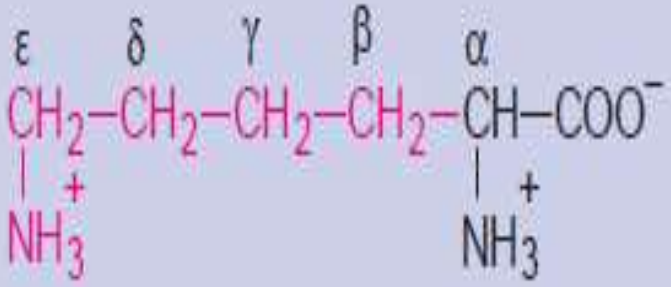
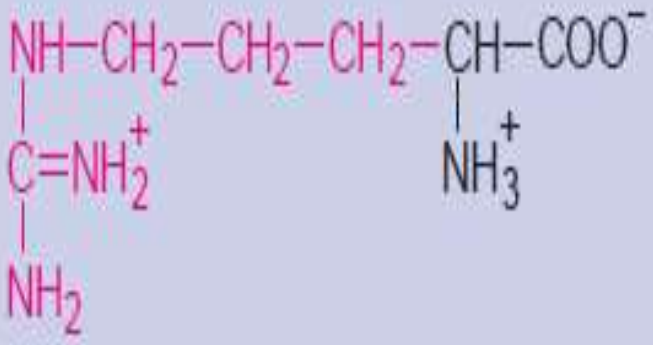
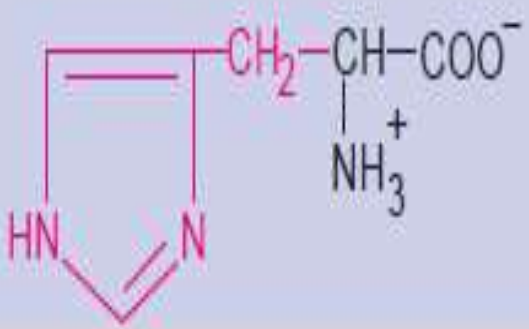
III. Sulfur containing amino acids

8. Cysteine	Cys	C		Sulfhydryl
Cystine	—	—		Disulfide
9. Methionine	Met	M		Thioether

IV. Acidic amino acids and their amides

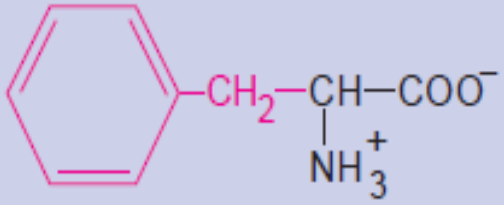
10.	Aspartic acid	Asp	D	 β-Carboxyl
11.	Asparagine	Asn	N	 Amide
12.	Glutamic acid	Glu	E	 γ-Carboxyl
13.	Glutamine	Gln	Q	 Amide

V. Basic amino acids

14. Lysine	Lys	K		ε-Amino
15. Arginine	Arg	R		Guanidino
16. Histidine	His	H		Imidazole

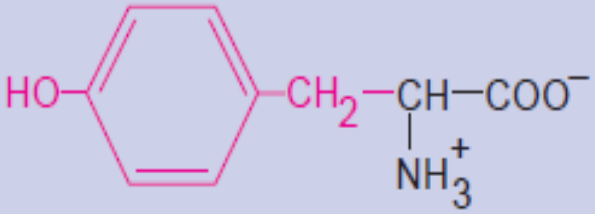
VI. Aromatic amino acids

17. Phenylalanine Phe F



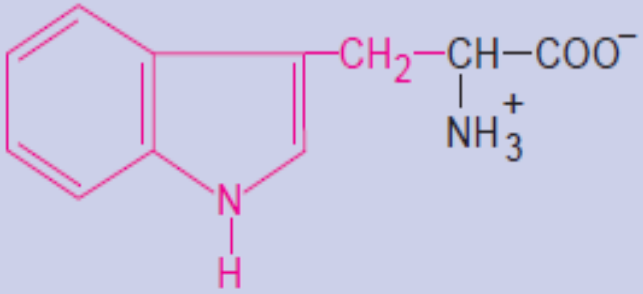
Benzene or phenyl

18. Tyrosine Tyr Y



Phenol

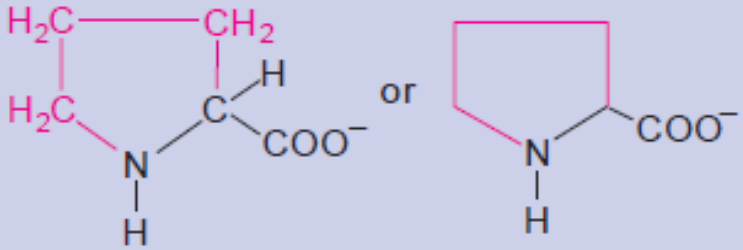
19. Tryptophan Trp W



Indole

VII. Imino acid

20. Proline Pro P



Pyrrolidine

CLASSIFICATION OF AMINO ACIDS BASED ON POLARITY

Amino acids are classified into 4 groups based on their polarity.

1. **Non-polar amino acids**

   These amino acids are also called hydrophobic amino acids.

   They have no charge on their ‘R’ group.

   They include alanine, leucine, isoleucine, valine, methionine, phenylalanine, tryptophan and proline.

2. Polar amino acids with no charge on 'R' group

These amino acids possess groups such as hydroxyl, sulfhydryl and amide.

They participate in hydrogen bonding of protein structure.

The simple amino acid glycine (where R = H) is also considered as part of this category.

The amino acids in this group are glycine, serine, threonine, cysteine, glutamine, asparagine and tyrosine.

3. **Polar amino acids with positive 'R' group:** The three amino acids lysine, arginine and histidine are included in this group.

4. **Polar amino acids with negative 'R' group:** The dicarboxylic monoamino acids: aspartic acid and glutamic acid are considered in this group.

CLASSIFICATION OF AMINO ACIDS BASED ON NUTRITIONAL REQUIREMENT

- The 20 standard amino acids are required for the synthesis of proteins, besides other biological functions.
- However, all these 20 amino acids need not be taken in the diet.
- Based on the nutritional requirements, amino acids are grouped into two classes: essential and non-essential.

ESSENTIAL AMINO ACIDS

- The essential amino acids, also called indispensable amino acids, are the amino acids that cannot be synthesized by the body, and therefore, need to be supplied through diet.
- They are required for proper growth and maintenance of an individual.
- They include arginine, valine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan.
- Arginine and histidine can be synthesized by adults, but not by growing children.
- Hence, they are considered as semi–essential amino acids.
- So, 8 amino acids are absolutely essential, while 2 are semi-essential.

NON-ESSENTIAL AMINO ACIDS

- The non-essential amino acids, also called dispensable amino acids, are the amino acids that can be synthesized by the body, hence, need not be consumed in the diet.

- They are glycine, alanine, serine, cysteine, aspartate, asparagine, glutamate, glutamine, tyrosine and proline.

CLASSIFICATION OF AMINO ACIDS BASED ON THEIR METABOLIC FATE

The carbon skeletons of amino acids can serve as a precursors for the synthesis of glucose (glycogenic) or fat (ketogenic) or both.

Based on this, amino acids are divided into three groups.

1. **Glycogenic (or glucogenic) amino acids:** These amino acids can serve as precursors for the formation of glucose or glycogen. e.g. alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, methionine, proline, serine and valine.

2. **Ketogenic amino acids:** Fat can be synthesized from these amino acids. Two amino acids: leucine and lysine are exclusively ketogenic.

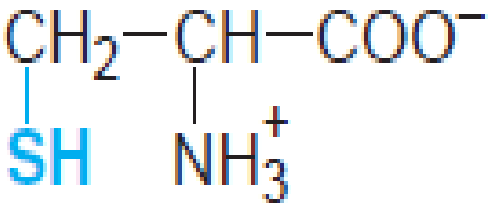
3. **Glycogenic and ketogenic amino acids:** Also known as amphibolic amino acids, they are precursors for synthesis of both glucose and fat. Phenylalanine, isoleucine, tryptophan, tyrosine and threonine belong to this group.

ADDITIONAL AMINO ACIDS

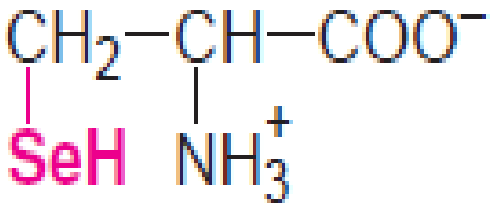
- In recent years, a 21st amino acid named **selenocysteine** has been added to the list of standard amino acids.

- It is found at the active sites of certain enzymes/proteins (selenoproteins). e.g. glutathione peroxidase, glycine reductase, 5´-deiodinase, thioredoxin reductase.

- Selenocysteine is an unusual amino acid containing the trace element selenium in place of the sulfur atom of cysteine.



Cystelne



Selenocystelne

- Incorporation of selenocysteine into proteins during translation is carried out by the codon UGA.

- Interestingly, UGA is normally a stop codon that terminates protein biosynthesis.

- Another unique feature is that selenocysteine is enzymatically generated from serine directly on the tRNA and then incorporated into proteins.

- **Pyrrolysine:** In the year 2002, some researchers described yet another amino acid namely pyrrolysine as the 22nd amino acid present in protein.

- The stop codon UAG can code for pyrrolysine.

NON-STANDARD AMINO ACIDS

- Aside the 20 standard amino acids present in protein structures, there are several other amino acids, which are biologically important.

- They include the amino acid derivatives found in proteins, non-protein amino acids performing specialized functions and the D-amino acids.

- **Amino acid derivatives in proteins (derived amino acids):** Some of the standard amino acids undergo specific modifications after the occurrence of protein synthesis.

- These amino acid derivatives are very important for protein structure and functions.

EXAMPLES OF DERIVED AMINO ACIDS

- **4-hydroxyproline and 5-hydroxylysine:** Collagen, which is the most abundant protein in mammals, contains 4-hydroxyproline and 5-hydroxylysine.

- **γ-Carboxyglutamic acid:** It is found in certain plasma proteins involved in blood clotting.

- **Cystine:** It is formed by combination of two cysteines.

- **Methylated, phosphorylated or acetylated amino acids:** Histones, which are the proteins found in association with DNA, contain many methylated, phosphorylated or acetylated amino acids.

D-AMINO ACIDS

- The vast majority of amino acids isolated from animals and plants are of L-category.
- However, certain D-amino acids are also found in antibiotics (actinomycin-D, valinomycin, gramicidin-S).
- D-serine and D-aspartate are found in brain tissue.
- D-Glutamic acid and D-alanine are present in bacterial cell walls.

NON-PROTEIN AMINO ACIDS

- Although never found in proteins, non-protein amino acids perform several biologically important functions.
- They may be either α- or non-α-amino acids.
- A selected list of these amino acids along with their functions is given in the table below.

A selected list of important non-protein amino acids along with their functions

Amino acids	Function(s)
I. α-Amino acids	
Ornithine, Citrulline, Arginosuccinic acid	Intermediates in the biosynthesis of urea.
Thyroxine, Triiodothyronine	Thyroid hormones derived from tyrosine.
S-Adenosylmethionine	Methyl donor in biological system.
Homocysteine	Intermediate in methionine metabolism. A risk factor for coronary heart diseases
Homoserine	Intermediate in threonine, aspartate and methionine metabolisms.
3, 4-Dihydroxy phenylalanine (DOPA)	A neurotransmitter, serves as a precursor for melanin pigment.
Creatinine	Derived from muscle and excreted in urine
Ovothiol	Sulfur containing amino acid found in fertilized eggs, and acts as an antioxidant
Azaserine	Anticancer drug
Cycloserine	Antituberculosis drug
II. Non-α-amino acids	
β-Alanine	Component of vitamin pantothenic acid and coenzyme A
β-Aminoisobutyric acid	End product of pyrimidine metabolism.
γ-Aminobutyric acid (GABA)	A neurotransmitter produced from glutamic acid
δ-Aminolevulinic acid (ALA)	Intermediate in the synthesis of porphyrin (finally heme)
Taurine	Found in association with bile acids.

Source: Satyanarayana & Chakrapani, 2013

AMINO ACIDS USEFUL AS DRUGS

- There a certain non-standard amino acids that are used as drugs.

- **D-Penicillamine (D-dimethylglycine):** It is a metabolite of penicillin, employed in the chelation therapy of Wilson's disease.

- This is possible since D-penicillamine can effectively chelate copper.

- **N-Acetylcysteine:** It is used in cystic fibrosis, and chronic renal insufficiency, as it can function as an antioxidant.

- **Gabapentin (γ-aminobutyrate linked to cyclohexane):** It is used as an anticonvulsant.

AMINO ACIDS AS NEUROTRANSMITTERS

- A neurotransmitter is an extracellular messenger that can transmit an extracellular message from a neuron to cells.

- Certain amino acids and or their derivatives can serve as neurotransmitters e.g. glycine, glutamate, serotonin, GABA.

- The table below shows a list of important amino acids and amino acid derivatives that serve as neurotransmitters.

Amino acids and their derivatives as neurotransmitters

Amino acid/derivative	*Major function(s)*
Glycine	Inhibitory neurotransmitter in spinal cord
Glutamate	Excitatory neurotransmitter
Dopamine	Increases blood pressure
Norepinephrine and epinephrine	Hormonal neurotransmitters, increase cardaic output and blood pressure
Serotonin	Regulates cerebral activity and behaviour
γ-Aminobutyric acid (GABA)	Inhibitory neurotransmitter in brain

Source: Satyanarayana & Chakrapani, 2013

PHYSICAL PROPERTIES OF AMINO ACIDS

- Amino acids differ in their physico-chemical properties, which ultimately determine the characteristics of proteins.
- **Solubility:** Most of the amino acids are usually soluble in water and insoluble in organic solvents.
- **Melting points:** Amino acids generally melt at higher temperatures, often above 200°C.
- **Taste:** Amino acids may be sweet (Gly, Ala, Val), tasteless (Leu) or bitter (Arg, Ile).
- Monosodium glutamate (MSG; ajinomoto) is used as a flavoring agent in food industry and Chinese foods to increase taste and flavour.
- In some individuals intolerant to MSG, Chinese restaurant syndrome (brief and reversible flu-like symptoms) is observed.

- **Optical properties:** All the amino acids except glycine possess optical isomers due to the presence of asymmetric carbon atom.

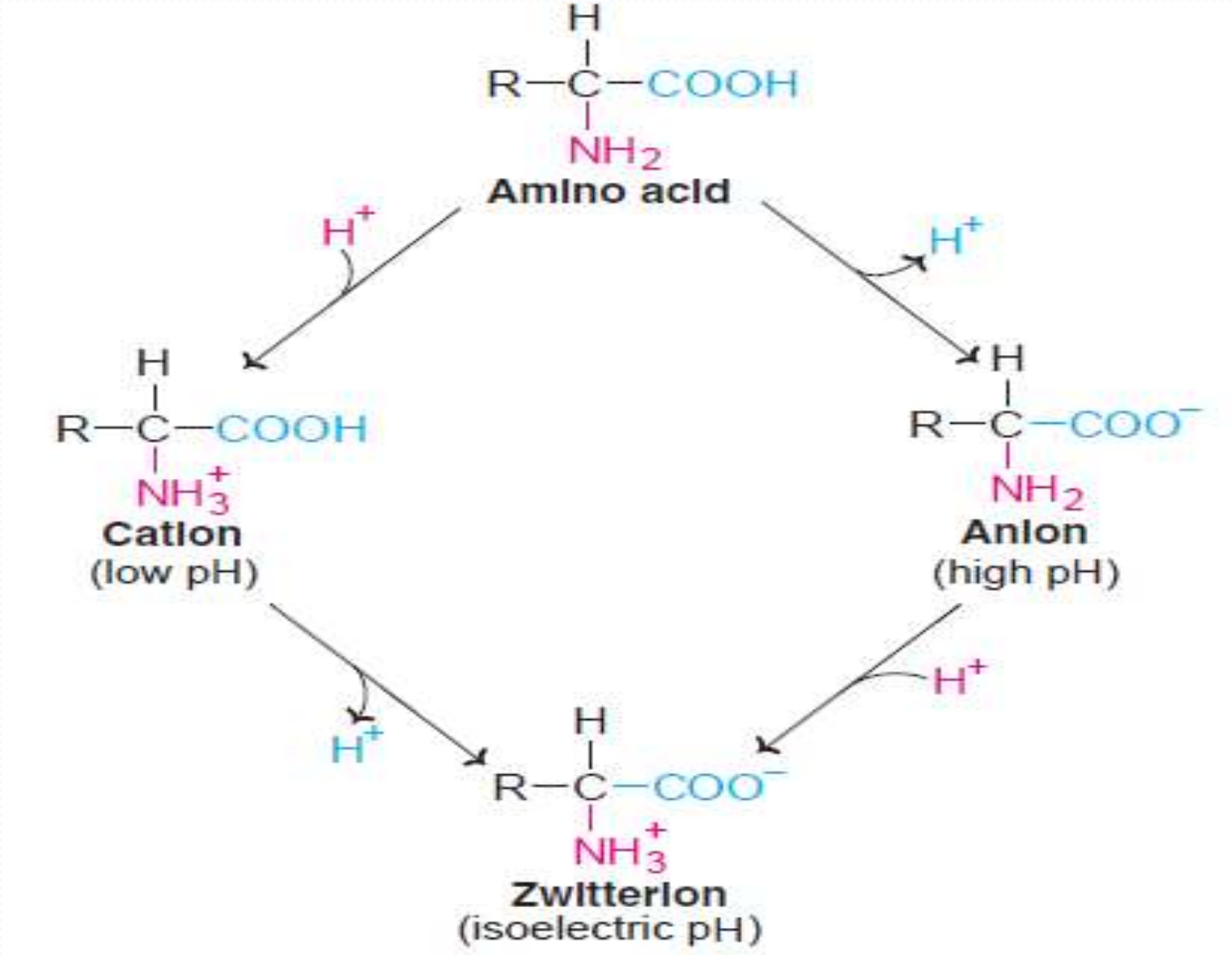
- Some amino acids also have a second asymmetric carbon e.g. isoleucine, threonine.

- **Amino acids as ampholytes:** Amino acids contain both acidic (-COOH) and basic (-NH_2) groups.

- They can donate a proton or accept a proton, hence amino acids are regarded as ampholytes.

ZWITTERION

- The name zwitter is derived from the German word which means hybrid.
- Zwitter ion (or dipolar ion) is a hybrid molecule containing positive and negative ionic groups.
- Amino acids rarely exist in a neutral form, with free carboxylic (-COOH) and free amino (-NH2) groups.
- In strongly acidic pH (low pH), amino acid is positively charged (cation), while in strongly alkaline pH (high pH), it is negatively charged (anion).
- Each amino acid has a characteristic pH (e.g. leucine, pH 6.0) at which it carries both positive and negative charges and exists as zwitterion.



An amino acid as cation, anion and zwitterion

Isoelectric pH

- Isoelectric pH (symbol pI) is defined as the pH at which a molecule exists as a zwitterion or dipolar ion and carries no net charge.
- Hence, the molecule is electrically neutral.
- The pI value can be calculated by taking the average pKa values corresponding to the ionizable groups.
- For example, leucine has two ionizable groups, and its pI can be calculated as follows.

$$pH = \frac{pK_1(COO^-) + pK_2(NH_3^+)}{2}$$

$$pI = \frac{2.4 + 9.6}{2} = 6.0$$

- Leucine exists as cation at pH below 6 and anion at pH above 6.

- At the isoelectric pH (pI = 6.0), leucine is found as zwitterion.

- Thus, the pH of the medium determines the ionic nature of amino acids.

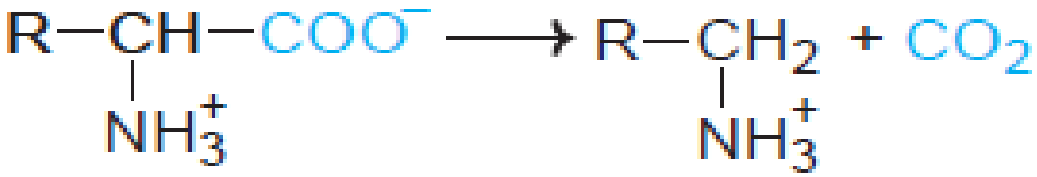
- For the calculation of pI of amino acids with more than two ionizable groups, the pKa for all the groups have to be taken into account.

REACTIONS OF AMINO ACIDS

The general reactions of amino acids are mostly due to the presence of carboxyl (-COOH) and amino (-NH_2) functional groups.

1. **Salt formation:** Amino acids form salts (-COONa) with bases and esters (-COOR´) with alcohols.

2. **Decarboxylation:** Amino acids undergo decarboxylation to produce corresponding amines.



This reaction is significant in living cells due to the formation of many biologically important amines, such as histamine, tyramine and γ-amino butyric acid (GABA) from the amino acids histidine, tyrosine and glutamate, respectively.

3. **Reaction with ammonia:** The carboxyl group of dicarboxylic amino acids reacts with NH_3 to form amide.

4. **Reaction with acids:** The amino groups behave as bases and combine with acids (e.g. HCl) to form salts ($-NH_3^+Cl^-$).

5. **Reaction with ninhydrin:** The α-amino acids react with ninhydrin to form a purple, blue or pink colour complex (Ruhemann's purple).

Ninhydrin reaction is used for the quantitative determination of amino acids and proteins. (Note: Proline and hydroxyproline give yellow colour with ninhydrin).

6. **Transamination:** The transfer of an amino group from an amino acid to a keto acid to form a new amino acid.

It is a very important reaction in the metabolism of amino acids.

7. **Deamination:** Amino acids undergo deamination to liberate free ammonia.

8. **Colour reactions of amino acids:** Amino acids can be identified by specific colour reactions as shown in the table below.

Colour reactions of proteins/amino acids

Reaction	Specific group or amino acid
1. Biuret reaction	Two peptide linkages
2. Ninhydrin reaction	α-Amino acids
3. Xanthoproteic reaction	Benzene ring of aromatic amino acids (Phe, Tyr, Trp)
4. Milllons reaction	Phenolic group (Tyr)
5. Hopkins-Cole reaction	Indole ring (Trp)
6. Sakaguchi reaction	Guanidino group (Arg)
7. Nitroprusside reaction	Sulfhydryl groups (Cys)
8. Sulfur test	Sulfhydryl groups (Cys)
9. Pauly's test	Imidazole ring (His)
10. Folin–Coicalteau's test	Phenolic groups (Tyr)

Source: Satyanarayana & Chakrapani, 2013

AMINO ACIDS CATABOLISM (BREAKDOWN)

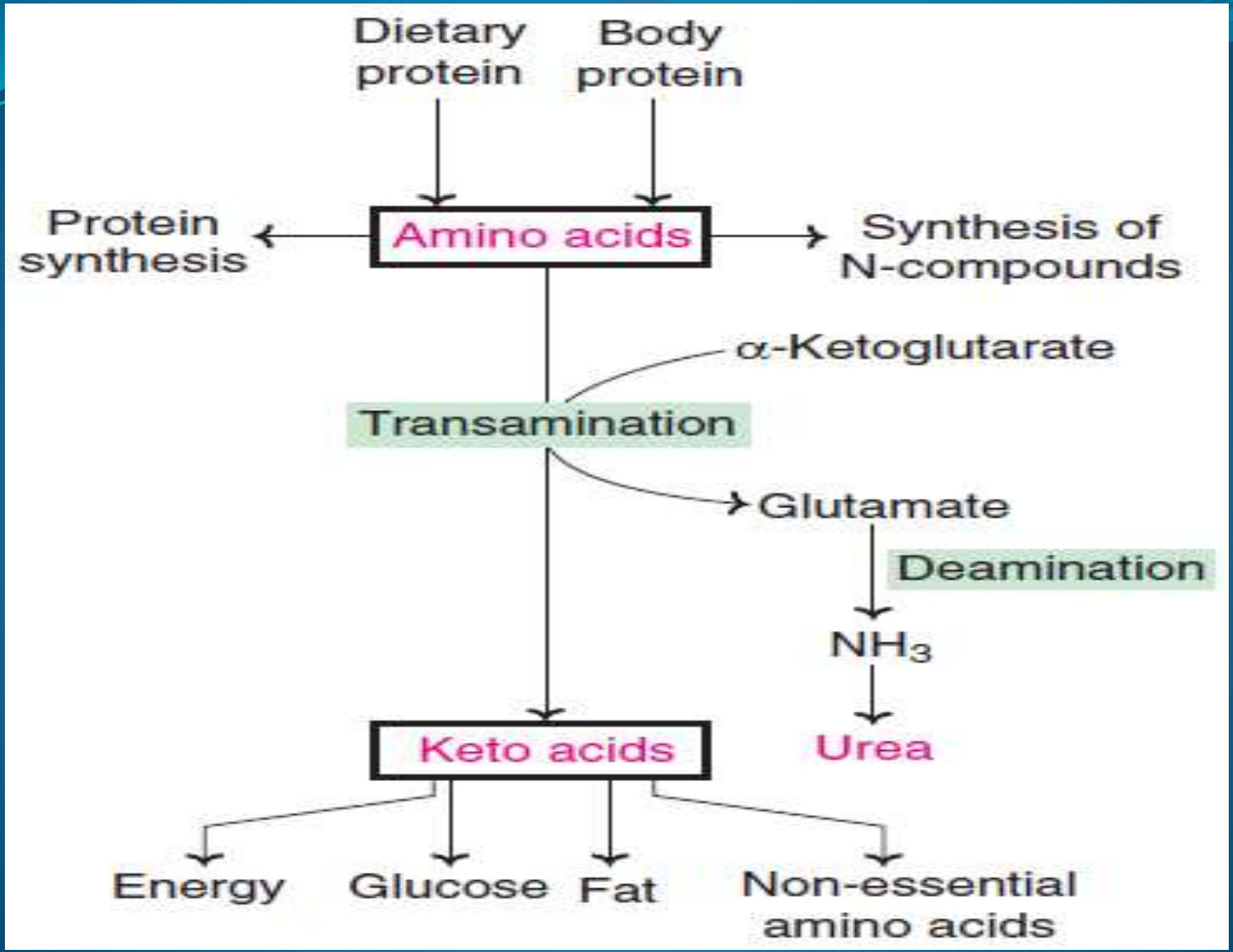
CATABOLISM OF AMINO ACIDS

- Amino acids undergo certain common catabolic reactions, namely *transamination* followed by *deamination* for the liberation of ammonia.

- Ammonia is then converted to urea, which is an excretory end product of protein metabolism.

- The carbon skeleton of amino acids, which is converted to keto acids (by transamination) can be used for the generation of energy and synthesis of important molecules as shown in the chart below.

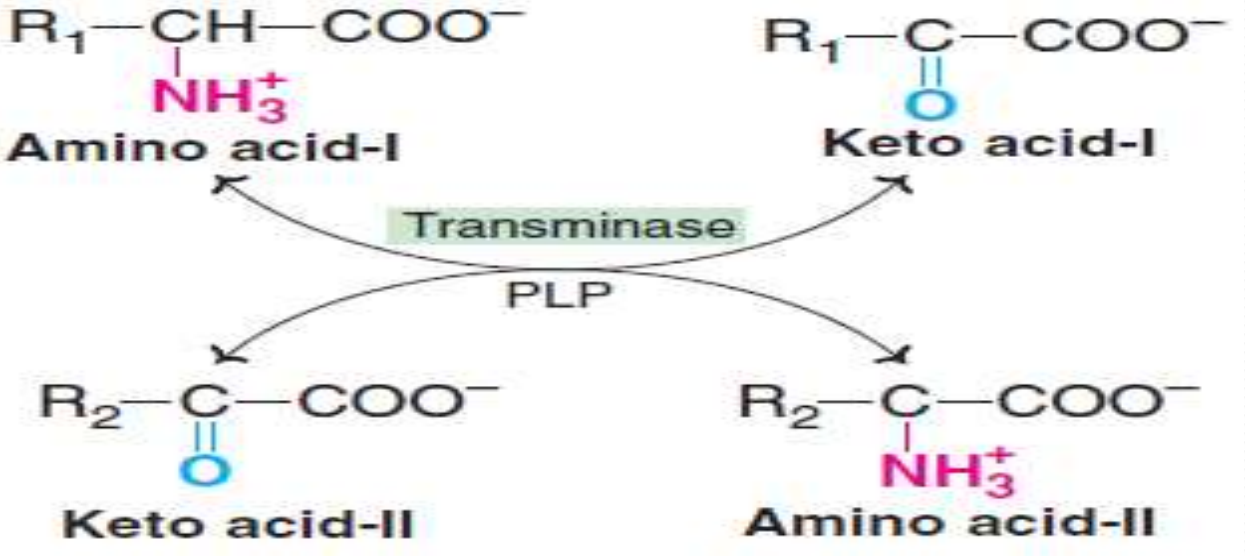
- Amino acids are also used for the synthesis of proteins and important nitrogenous compounds such as porphyrins, purines, pyrimidines, etc.



Source: Satyanarayana & Chakrapani, 2013

TRANSAMINATION

- Transamination is the transfer of an amino group (-NH_2) from an amino acid to a keto acid.
- It involves the interconversion of a pair of amino acids and a pair of keto acids.
- Transamination is catalyzed by a group of enzymes called transaminases (also called aminotransferases).



SALIENT FEATURES OF TRANSAMINATION

- All transaminases require pyridoxal phosphate (PLP), which is a coenzyme derived from vitamin B_6.
- Specific transaminases exist for each pair of amino and keto acids, but only two (aspartate transaminase and alanine transaminase) make a significant contribution for transamination.
- Only the transfer of amino group occurs, so no free NH_3 is liberated.
- Transamination is reversible.
- It involves both catabolism (degradation) and anabolism (synthesis) of amino acids.

- It is vital for the redistribution of amino groups and production of non-essential amino acids based on the cell requirement.
- It channels the excess amino acids towards energy generation.
- Amino acids undergo transamination to finally concentrate nitrogen in glutamate, which undergoes deamination to liberate free NH_3 for urea synthesis.
- With the exception of lysine, threonine, proline and hydroxyproline, all amino acids undergo transamination.
- Transamination is not restricted to α-amino groups only, because for instance, the δ-amino group of ornithine also undergo transamination.
- Serum transaminases are important for diagnostic and prognostic purposes.

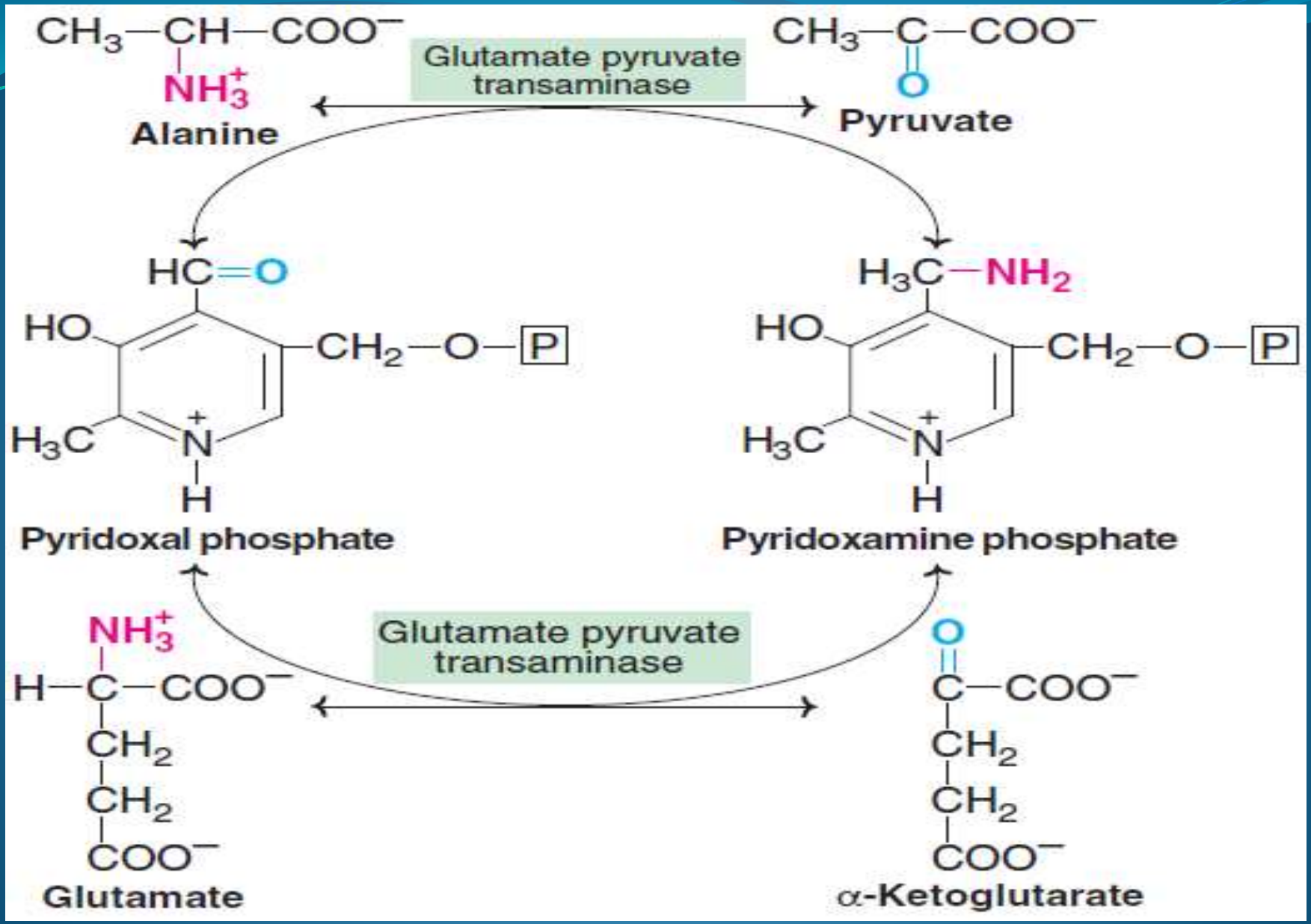
TRANSAMINASES IN DIAGNOSIS

- Analyses of certain enzyme activities in blood serum give valuable diagnostic information for a number of disease conditions.
- **Alanine transaminase** (ALT; also called glutamate-pyruvate transaminase, GPT) and **aspartate transaminase** (AST; also called glutamateoxaloacetate transaminase, GOT) are important in the diagnosis of heart and liver damage.
- This damage could be caused by heart attack, drug toxicity, or infection.
- After a heart attack, a variety of enzymes, including these transaminases, leak from the injured heart cells into the bloodstream.
- Measurements of the blood serum concentrations of the two transaminases by the SGPT and SGOT tests (S for serum)—and of another enzyme, creatine kinase, by the SCK test—can provide information about the severity of the damage.

- Creatine kinase is the first heart enzyme to appear in the blood after a heart attack, and it also disappears quickly from the blood.
- GOT is the next to appear, and GPT follows later.
- Lactate dehydrogenase also leaks from injured or anaerobic heart muscle.
- The SGOT and SGPT tests are also important in occupational medicine, to determine whether people exposed to carbon tetrachloride, chloroform, or other industrial solvents have suffered liver damage.
- Liver degeneration caused by these solvents is accompanied by leakage of various enzymes from injured hepatocytes into the blood.
- Transaminases are most useful in the monitoring of people exposed to these chemicals, because these enzyme activities are high in liver and can be detected in very small amounts.

MECHANISM OF TRANSAMINATION

- Transamination occurs in two stages.
- Firstly, the amino group of an amino acid is transferred to the coenzyme PLP (bound to the transaminase enzyme) to form pyridoxamine phosphate.
- The amino group of pyridoxamine phosphate is then transferred to a keto acid to produce a new amino acid and the enzyme with PLP is regenerated.
- Aminotransferases are classic examples of enzymes catalyzing bimolecular Ping-Pong reactions in which the first substrate reacts and the product must leave the active site of the enzyme before the second substrate can bind.



Mechanism of Transamination

Source: Satyanarayana & Chakrapani, 2013

DEAMINATION

- Deamination is the removal of amino group from amino acids as NH_3.
- Unlike transamination, which involves the transfer of amino group, deamination results in the liberation of ammonia and formation of a keto acid.
- Interestingly, transamination and deamination occur simultaneously, often involving glutamate as the central molecule.
- The term **transdeamination** is used while describing the reactions of transamination and deamination, particularly those involving glutamate.
- Deamination may be either oxidative or non-oxidative.

OXIDATIVE DEAMINATION

- Oxidative deamination is the liberation of free ammonia from the amino group of amino acids, coupled with oxidation.
- This takes place mostly in liver and kidney.
- The purpose of oxidative deamination is to provide NH_3 for urea synthesis and α-keto acids for a variety of reactions, including energy generation.
- The transamination of most amino acids occur with α-ketoglutarate to produce glutamate.

- Therefore, glutamate serves as a ‘collection centre’ for amino groups in the biological system.

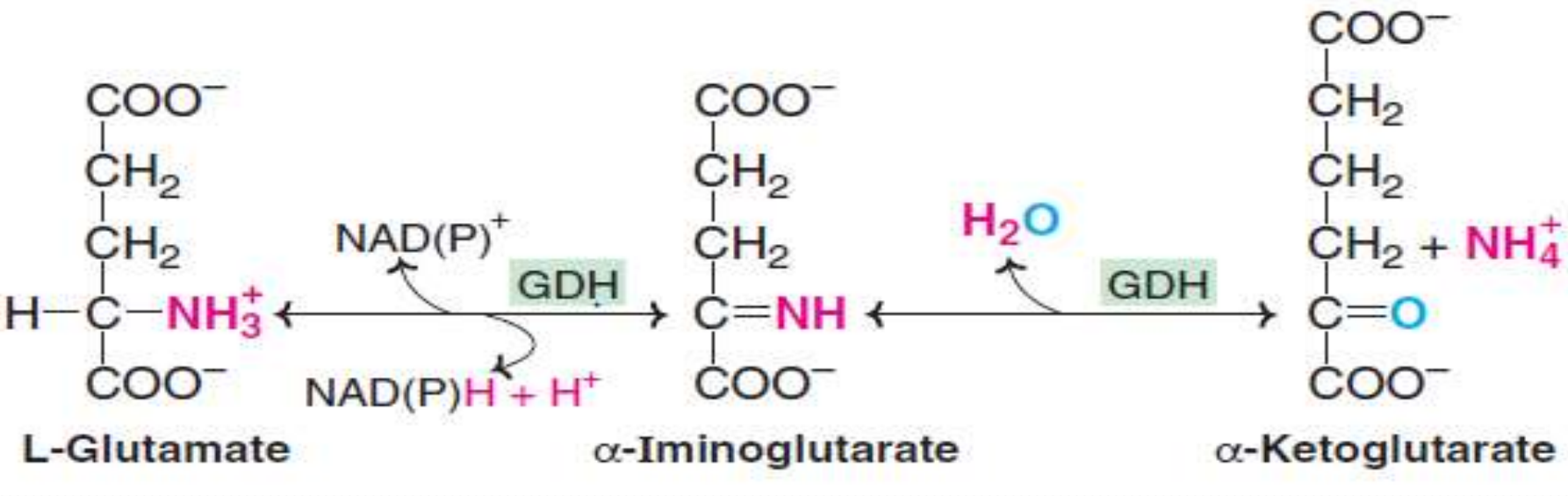
- Glutamate rapidly undergoes oxidative deamination, catalyzed by **glutamate dehydrogenase** (GDH) to liberate ammonia.

- GDH utilizes either NAD^+ or $NADP^+$ as a coenzyme, and is involved in both catabolic and anabolic reactions.

- The conversion of glutamate to α-ketoglutarate occurs through the formation of an intermediate, α-iminoglutarate.

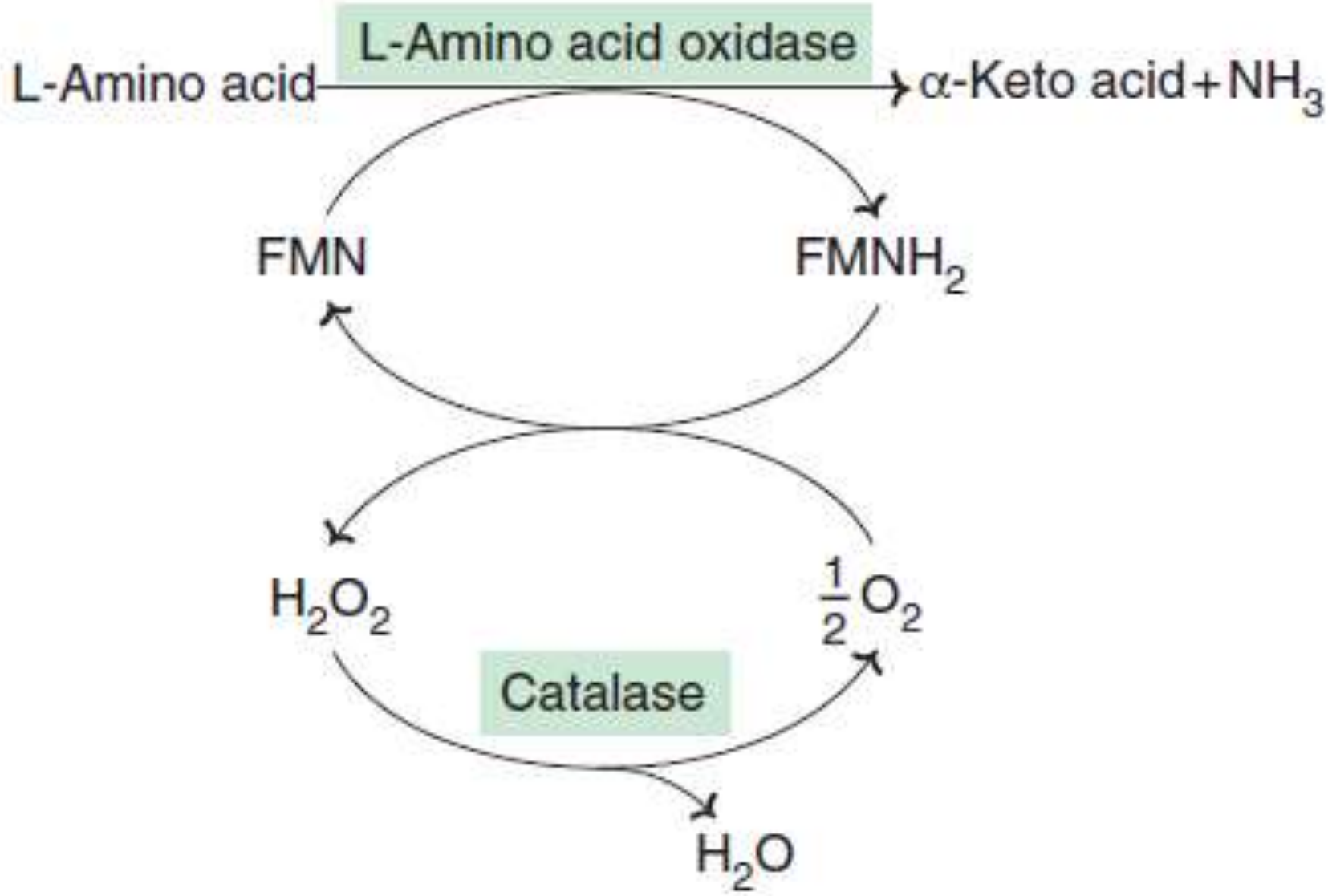
- The GDH reaction reversibly links up glutamate metabolism with TCA cycle through α-ketoglutarate.

- Ingestion of a protein-rich meal raises the level of liver glutamate, which is converted to α-ketoglutarate with liberation of NH_3.
- Also, when the cellular energy levels are low, the degradation of glutamate is increased to provide α-ketoglutarate, which enters TCA cycle to liberate energy.
- GDH is a complex mitochondrial enzyme controlled allosterically, and is inhibited by GTP and ATP, while GDP and ADP activate it.
- Steroid and thyroid hormones also inhibit GDH.



OXIDATIVE DEAMINATION BY AMINO ACID OXIDASES

- L-Amino acid oxidase and D-amino acid oxidase are flavoproteins, possessing FMN and FAD, respectively.
- They act on the corresponding amino acids (L or D) to produce α-keto acids and NH_3.
- In this reaction, oxygen is reduced to H_2O_2, which is later decomposed by catalase.
- The activity of L-amino acid oxidase is much lower, while that of D-amino acid oxidase is high in tissues (mostly liver and kidney).
- L-Amino acid oxidase does not act on glycine and dicarboxylic acids.
- Due to its very low activity, this enzyme does not appear to play any significant role in amino acid metabolism.

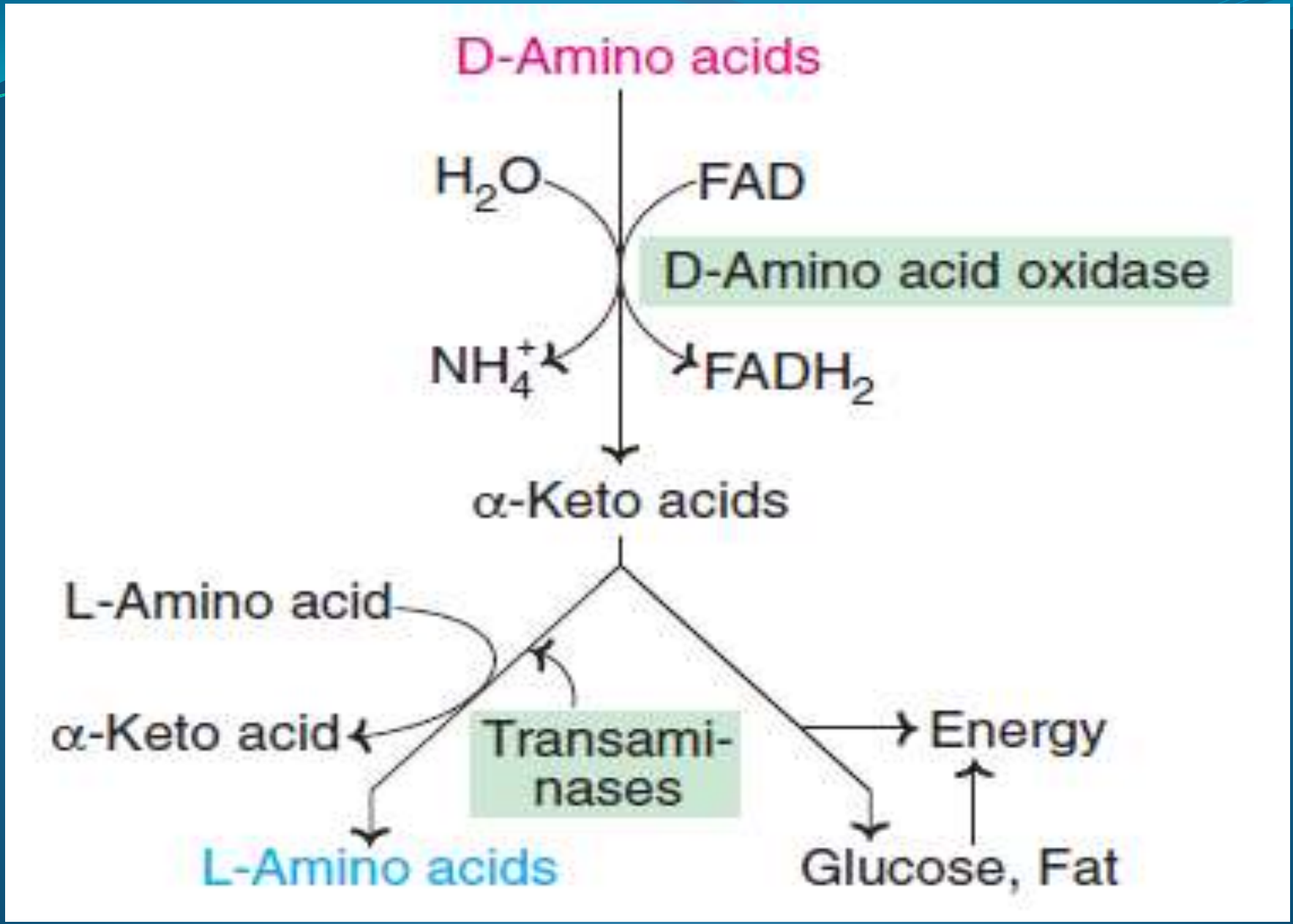


Oxidative Deamination of Amino Acids by L-Amino Acid Oxidase

Source: Satyanarayana & Chakrapani, 2013

OXIDATIVE DEAMINATION OF D-AMINO ACIDS

- D-amino acids are not present in mammalian proteins but found in plants and microorganisms.
- However, they are regularly taken in the diet and metabolized by the body.
- D-amino acid oxidase converts them to the respective α-keto acids by oxidative deamination.
- The α-keto acids produced undergo transamination to be converted to L-amino acids, which participate in various metabolisms.
- Keto acids may also be oxidized to generate energy or serve as precursors for glucose and fat synthesis.
- Thus, D-amino acid oxidase is important as it initiates the first step for the conversion of unnatural D-amino acids to L-amino acids in the body.

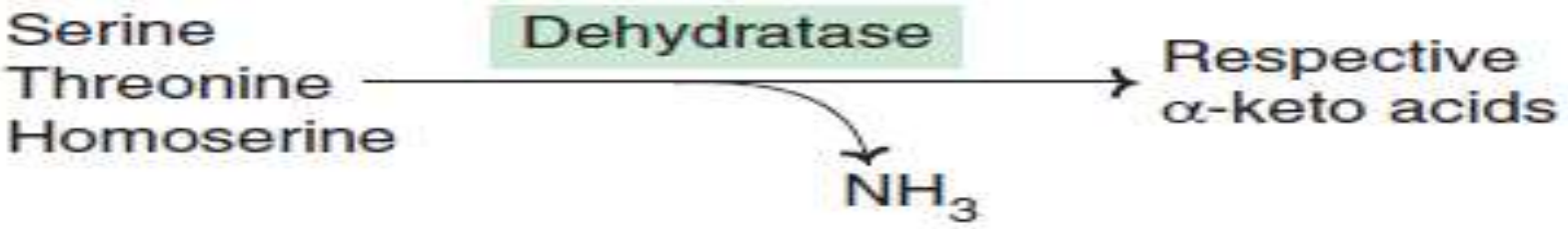


Source: Satyanarayana & Chakrapani, 2013

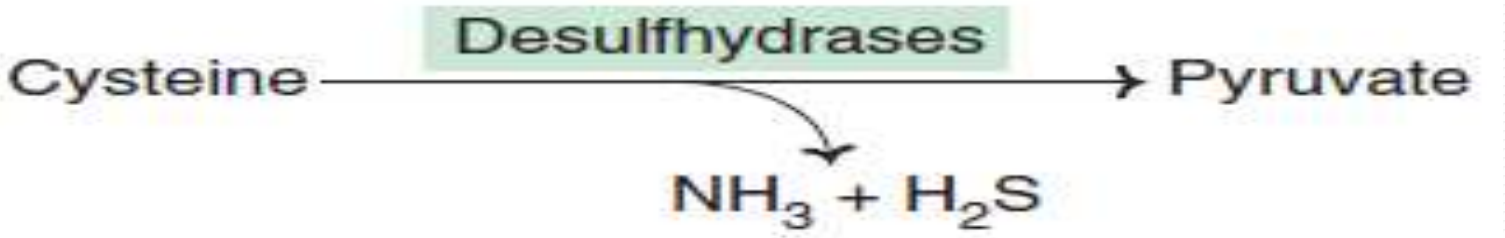
Metabolic Fate of D-Amino Acids

NON-OXIDATIVE DEAMINATION

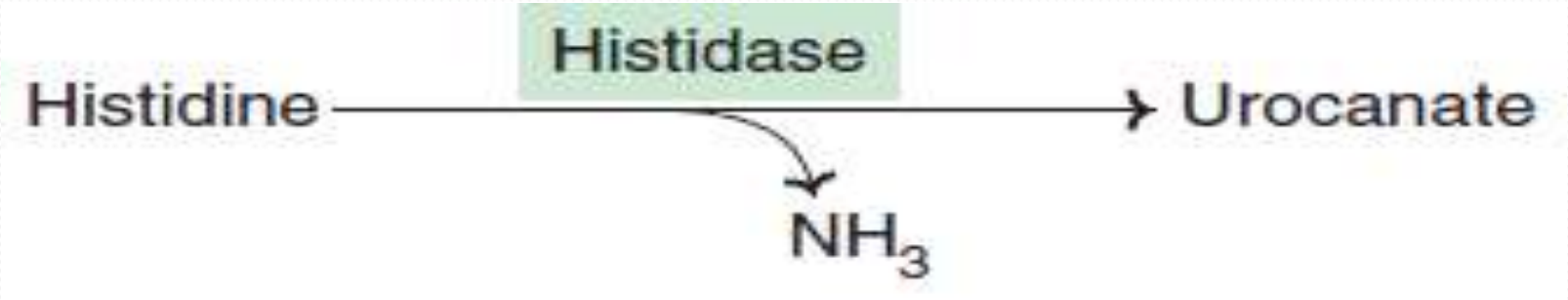
- Some amino acids can be deaminated to liberate NH_3 without undergoing oxidation.
- Serine, threonine and homoserine are the hydroxy amino acids, and they undergo non-oxidative deamination catalysed by PLP-dependent dehydrases (dehydratases).



- The sulfur amino acids, namely cysteine and homocysteine, undergo deamination coupled with desulfhydration to give keto acids.



- The enzyme **histidase** acts on histidine to liberate NH_3 by a non-oxidative deamination process.



AMMONIA METABOLISM

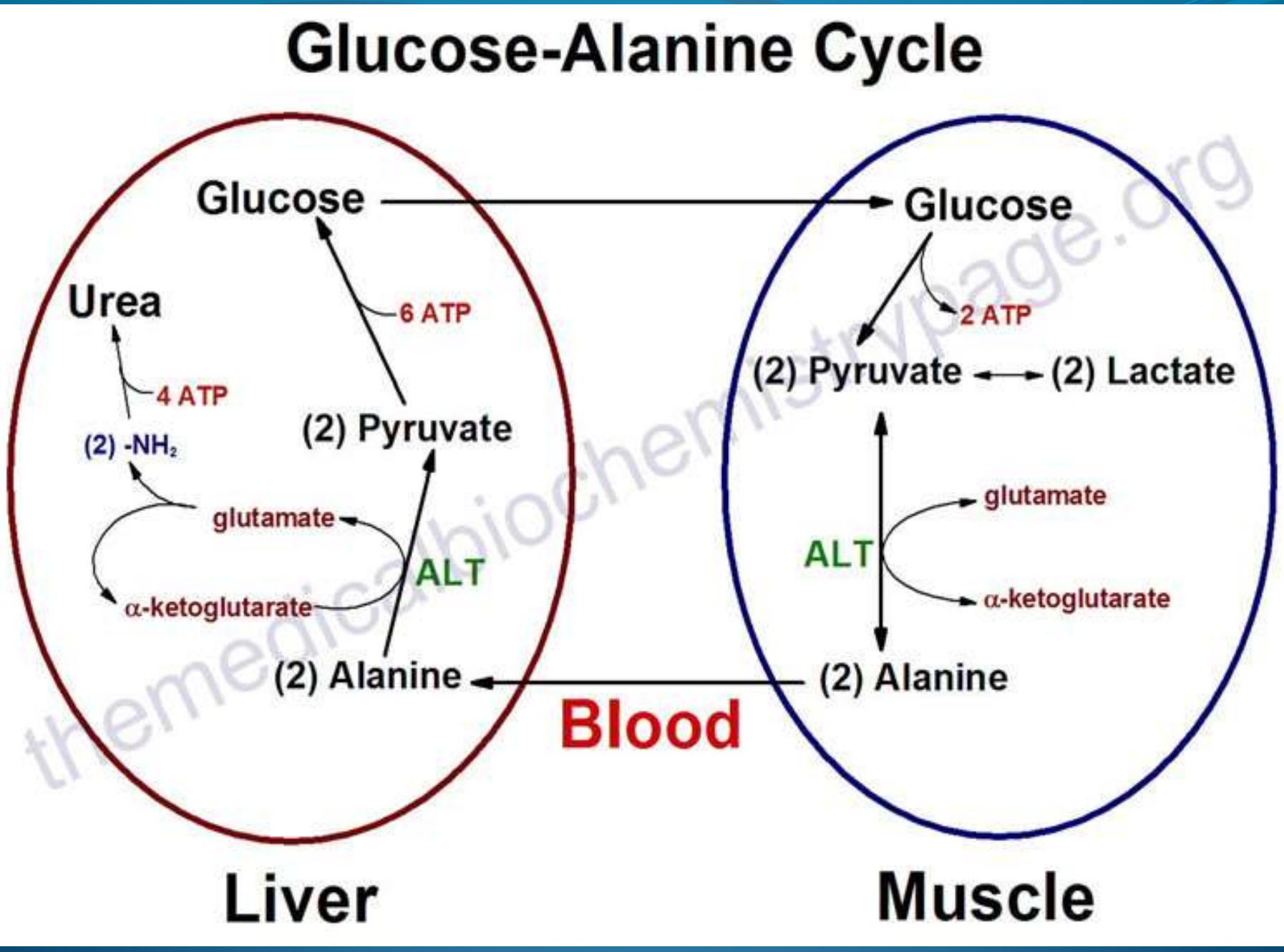
- The formation of NH_3 occurs from the breakdown of amino acids (transamination and deamination), biogenic amines, amino group of purines and pyrimidines and by the action of intestinal bacteria (urease) on urea.

- At physiological pH, ammonia exists as ammonium (NH_4^+) ion.

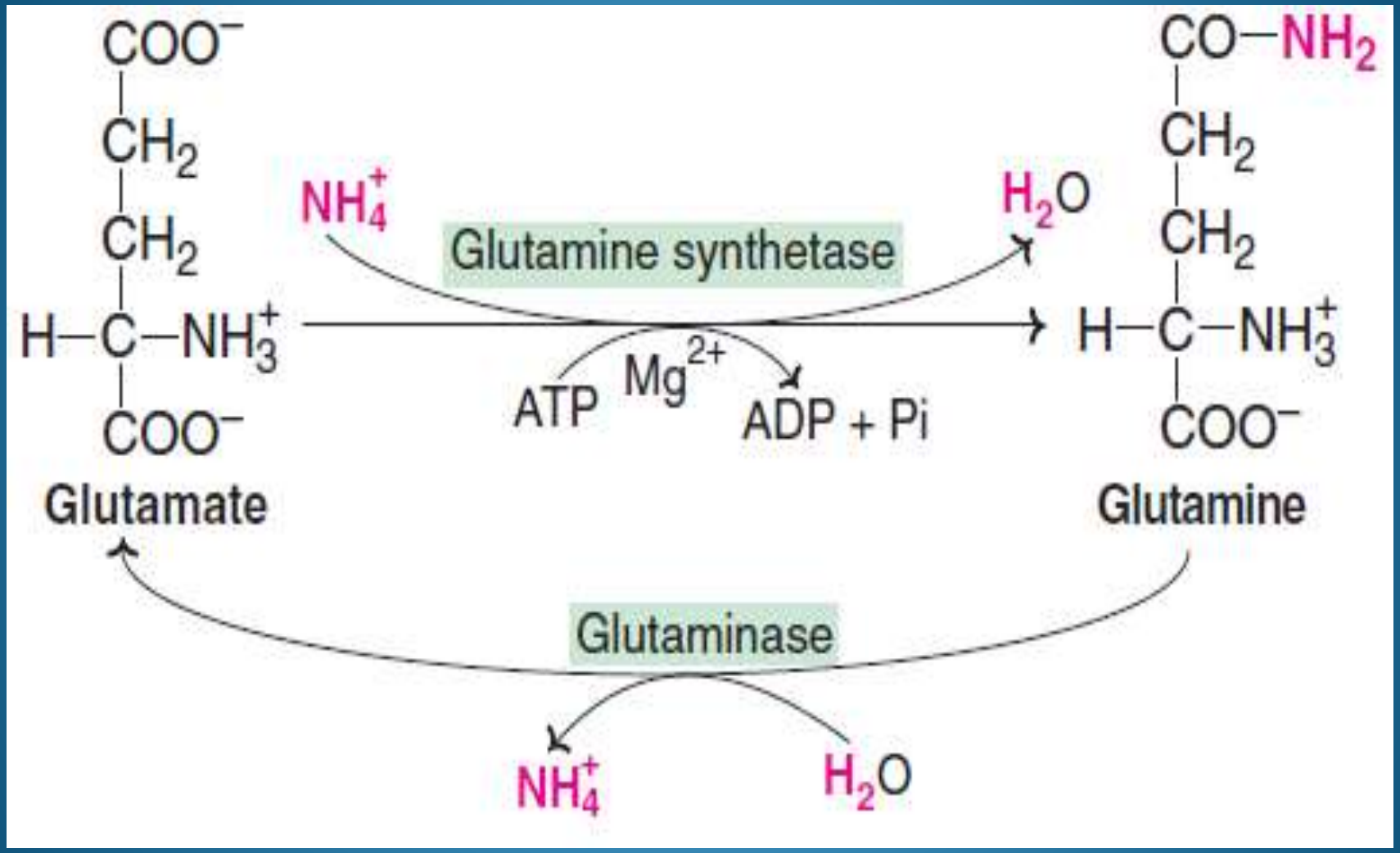
- The body's efficient transport mechanism for NH_3 and its immediate utilization for urea synthesis keep the concentration of NH_3 in the circulation low (normal plasma 10-20 mg/dl).

- Ammonia is mostly transported between various tissues and the liver in the form of glutamine or alanine, not as free ammonia.

- Alanine plays an important role in the glucose-alanine cycle, which facilitates NH_3 transport from muscle to the liver.


Glucose-Alanine Cycle
Glucose
Glucose
Urea
6 ATP
2 ATP
4 ATP
(2) Pyruvate
(2) Lactate
(2) -NH2
(2) Pyruvate
glutamate
glutamate
ALT
ALT
α-ketoglutarate
α-ketoglutarate
(2) Alanine
(2) Alanine
Blood
Liver
Muscle
themedicalbiochemistrypage.org

- Glutamine serves as a storage and transport form of NH_3.
- It is the most abundant amino acid in the human body (8 mg/dl in adults), and regarded as a storehouse of NH_3.
- Ammonia synthesis mostly occurs in liver, brain and muscle.
- It is removed from the brain predominantly as glutamine, which is freely diffusible in tissues, hence, easily transported.
- A mitochondrial enzyme called **glutamine synthetase** is responsible for the synthesis of glutamine from glutamate and ammonia.
- Glutamine can be deaminated by hydrolysis to release ammonia by **glutaminase**, an enzyme mostly found in kidney and intestinal cells.



Synthesis of glutamine and its conversion to glutamate

Source: Satyanarayana & Chakrapani, 2013

FUNCTIONS OF AMMONIA

- Ammonia is not just a waste product of nitrogen metabolism.
- It is involved (directly or via glutamine) for the synthesis of many compounds in the body.
- These include non-essential amino acids, purines, pyrimidines, amino sugars etc.
- Ammonium ions (NH_4^+) are also very important for maintaining the acid-base balance of the body.

AMMONIA EXCRETION

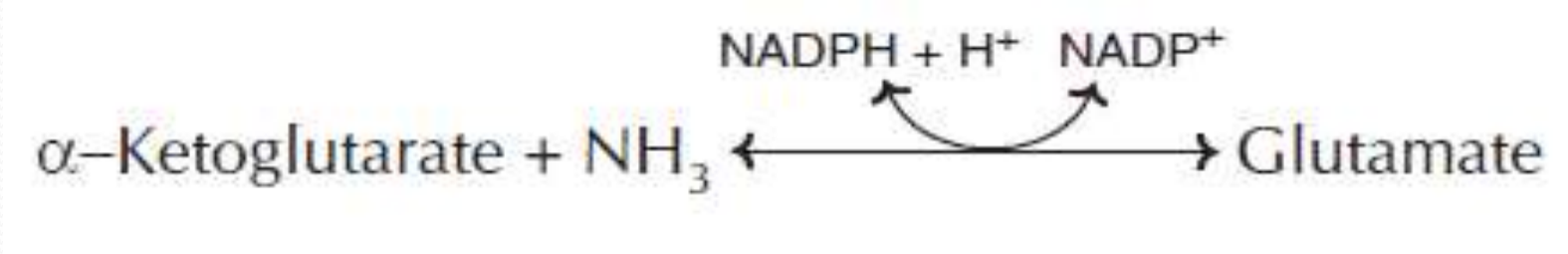
- Organisms possess different mechanisms for the disposal of ammonia from the body.
- In this regard, animals are of three types:
- **Ammoniotelic:** Aquatic animals dispose NH_3 into the surrounding water.
- **Uricotelic:** Reptiles and birds convert ammonia mostly to uric acid.
- **Ureotelic:** Mammals, including man, convert NH_3 to urea.

  Urea is a non-toxic and soluble compound, hence easily excreted.

AMMONIA TOXICITY

- The brain is harmed by even a slight elevation in blood ammonia concentration.
- Ammonia accumulation in the body results in slurring of speech, blurring of vision, tremors, and if not corrected, may lead to coma and finally, death.
- The elevation in blood NH_3 level may be genetic or acquired.
- Impairment in urea synthesis due to a defect in any one of the five enzymes involved in urea synthesis can also cause a rise in blood NH_3 level .
- The acquired hyperammonemia may be due to hepatitis, alcoholism etc. where the urea synthesis becomes defective, hence NH_3 accumulates.
- All these disorders lead to **hyperammonemia** and cause hepatic coma and mental retardation.

- The **glutamate dehydrogenase** reaction probably explains the toxic effects of NH_3 on the brain.



- The accumulation of NH_3 shifts the equilibrium to the right with more glutamate formation, hence more utilization of α-ketoglutarate.

- α-Ketoglutarate is a key intermediate in TCA cycle and its depleted levels impair the TCA cycle.

- The net result is that production of energy (ATP) by the brain is reduced.

- Hence, the toxic effects of NH_3 on the brain are due to impairment in ATP formation.

- When the plasma level of ammonia is highly elevated, intravenous administration of sodium benzoate and phenyllactate is done.

- These compounds can respectively condense with glycine and glutamate to form water-soluble products that can be easily excreted.

- By this way, ammonia can be trapped and removed from the body.

- In some instances of toxic hyperammonemia, hemodialysis may become necessary.

ONE-CARBON METABOLISM

- Amino acid metabolism is particularly important for the transfer or exchange of one-carbon units.

- The following one-carbon fragments are encountered in the biological reactions, which constitute one-carbon pool:

Methyl (-CH_3)

Hydroxymethyl (-CH_2OH)

Methylene (=CH_2)

Methenyl (-CH=)

Formyl (-CH=O)

Formimino (-CH=NH)

- Note that CO_2 is also a one-carbon unit, and it is involved in many biochemical reactions (carboxylation), which are dependent on biotin.

- For instance, conversion of pyruvate to oxaloacetate in gluconeogenesis.

- However, most authors ignore CO_2 as a one-carbon unit, and do not include it in the list.

- Tetrahydrofolate (THF) is a versatile coenzyme that actively participates in one-carbon metabolism.

- With regard to the transfer of methyl groups from S-adenosylmethionine, vitamin B_{12} is also involved besides THF.

- One-carbon unit covalently binds with THF at position N^5 or N^{10} or on both N^5 and N^{10} of pteroyl structure of folate.

- One-carbon metabolism is rather complex, involving many reactions.

- However, for better understanding, it is divided into *generation* and *utilization* of one-carbon units, and the *role of methionine and vitamin* B_{12}.

GENERATION OF ONE-CARBON UNITS

Many compounds (particularly amino acids) act as donors of one-carbon fragments.

1. The formate released from glycine and tryptophan metabolism combines with THF to form N^{10}-formyl THF.

2. Histidine contributes formimino fragment to produce N^5-formimino THF.

3. When serine is converted to glycine, N^5, N^{10}-methylene THF is formed.

   This is the most predominant entry of one carbon units into one-carbon pool.

4. Choline and betaine contribute to the formation of N^5-methyl THF.

   The different derivatives of THF carrying one-carbon units are interconvertible, and this is metabolically significant for the continuity of the one-carbon pool.

UTILIZATION OF ONE-CARBON MOIETIES

- One-carbon fragments from THF are used for the synthesis of a wide variety of compounds.

- These include purines, formylmethionine tRNA (required for initiation of protein synthesis), glycine, pyrimidine nucleotide (thymidylate) etc.

ROLE OF METHIONINE AND B_{12} IN ONE-CARBON METABOLISM

- Methyl ($-CH_3$) group is an important one-carbon unit.
- The role of active methionine as methyl donor in transmethylation reactions is important.
- After the release of methyl group, methionine is converted to homocysteine.
- For the regeneration of methionine, free homocysteine and N^5-methyl THF are required and this reaction is dependent on methylcobalamin (vitamin B_{12}).
- The one-carbon pool, under the control of THF, is linked with methionine metabolism (transmethylation) through vitamin B_{12}.
- Hence vitamin B_{12} is also involved in one-carbon metabolism.

CARBON SKELETON OF AMINO ACIDS

- After the removal of amino group from individual amino acids, the carbon skeletons are converted to intermediates of TCA cycle or their precursors.

- The carbon skeleton finally has one or more of the following fates:

1. Oxidation via TCA cycle to produce energy (about 10-15% of body requirement).
2. Synthesis of glucose.
3. Formation of lipids: fatty acids and ketone bodies.
4. Synthesis of non-essential amino acids.

- The carbon skeletons of the 20 standard amino acids are degraded to one of the following seven products: pyruvate, α-ketoglutarate, succinyl CoA, fumarate, oxaloacetate, acetyl CoA and acetoacetate.

- As described above, amino acids are classified into three groups, based on the nature of the metabolic end products of their carbon skeleton (i.e., their metabolic fate).

- **Glycogenic (glucogenic) amino acids:** These are the amino acids whose carbon skeletons are finally degraded to pyruvate or one of the intermediates of TCA cycle (α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate).

- These intermediates serve as good substrates for gluconeogenesis, leading to the formation of glucose or glycogen.

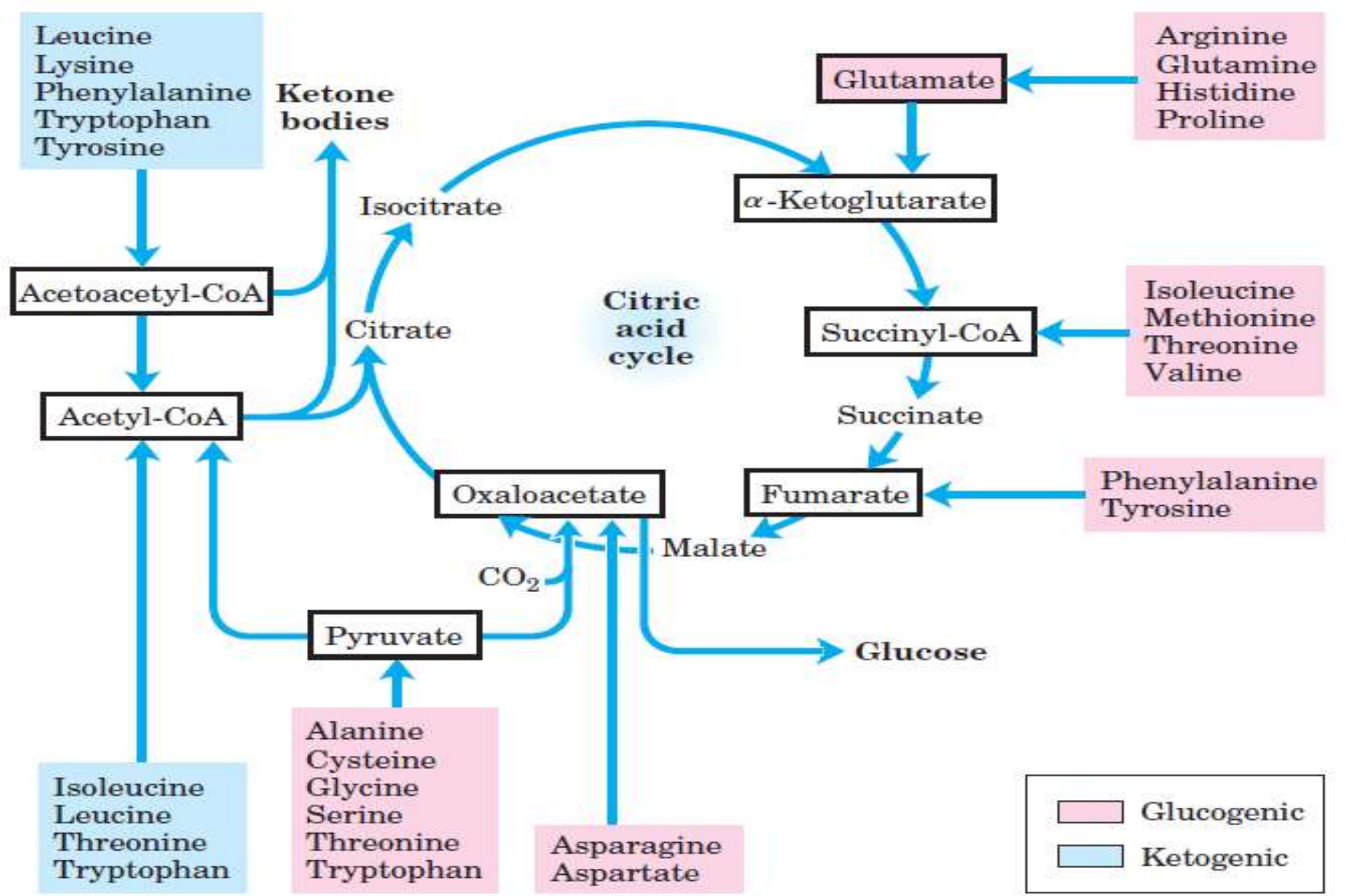
- **Ketogenic amino acids:** The amino acids whose carbon skeletons are metabolized to acetyl CoA or acetoacetate can be converted to fat (i.e., fatty acids or ketone bodies).

- Acetoacetate is a ketone body (besides acetone and β-hydroxybutyrate).

- **Glycogenic (glucogenic) and ketogenic amino acids:** Some of the amino acids are both glycogenic and ketogenic.

- They serve as precursors for glucose as well as fat.

- Hence, they have multiple points of entry into the TCA cycle.



AN OVERVIEW OF THE FATE OF THE CARBON SKELETON OF AMINO ACIDS

Source: Nelson & Cox, 2016

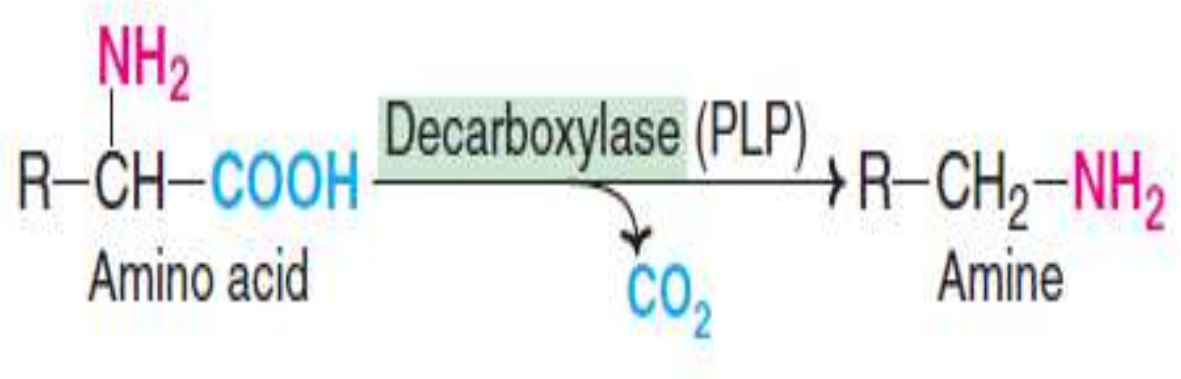
A summary of the specialized products formed/contributed by amino acids

Amino acid	*Specialized product(s)*
Glycine	Creatine, glutathione, heme, purines, conjugated bile acids.
Tyrosine	Thyroxine, triiodothyronine, epinephrine, norepinephrine, dopamine, melanin.
Tryptophan	NAD^+, $NADP^+$ (coenzymes of niacin), serotonin, melatonin.
Methionine	Active methionine, creatine, epinephrine, polyamines.
Cysteine	Glutathione, taurine, coenzyme A, active sulfate.
Histidine	Histamine
Arginine	Creatine, nitric oxide
Lysine	Carnitine
Glutamate	γ-Amino butyric acid, glutathione, γ-carboxyglutamate.
Glutamine	Purines, pyrimidines, amino sugars.
Aspartate	Purines, pyrimidines
Serine	Phosphatidylserine, sphingomyelins, choline.
β-Alanine	Coenzyme A

Source: Satyanarayana & Chakrapani, 2013

BIOGENIC AMINES

- The decarboxylation of amino acids or their derivatives generally results in the formation of amines.



- The table below shows a summary of the biogenic amines derived from different amino acids and their major functions.

summary of the biogenic amines and their functions

Amino acid	*Amine*	*Function(s)*
Serine	Ethanolamine	Forms choline
Glutamate	γ-Aminobutyric acid	Inhibitory neurotransmitter
Histidine	Histamine	Vasodilator, promotes gastric HCl and pepsin synthesis
Phenylalanine	Dopamine	For the synthesis of nore-pinephrine and epinephrine
Tyrosine	Tyramine	Vasoconstrictor (increases blood pressure)
Tryptophan	Tryptamine	Elevates blood pressure
	Serotonin	Stimulates cerebral activity
	Melatonin	Circadian rhythms
Cysteine	Taurine	Constituent of bile acid (taurocholic acid)

Source: Satyanarayana & Chakrapani, 2013

POLYAMINES

- Polyamines (Greek: poly—many) possess multiple amino groups.
- The biologically important polyamines are putrescine, spermine and spermidine.
- Spermine and spermidine were originally detected in human semen (sperms), hence, the name.
- Polyamines are basic in nature and possess multiple positive charges, hence, they are readily associated with nucleic acids (DNA and RNA).

FUNCTIONS OF POLYAMINES

1. They are involved in the synthesis of DNA, RNA and proteins.

2. They are essential for cell growth and proliferation.

3. Some enzymes are inhibited by polyamines, e.g. protein kinase.

4. They are believed to be involved in the stabilization of membrane structure (cell and cellular organelles).

CLINICAL IMPORTANCE OF POLYAMINES

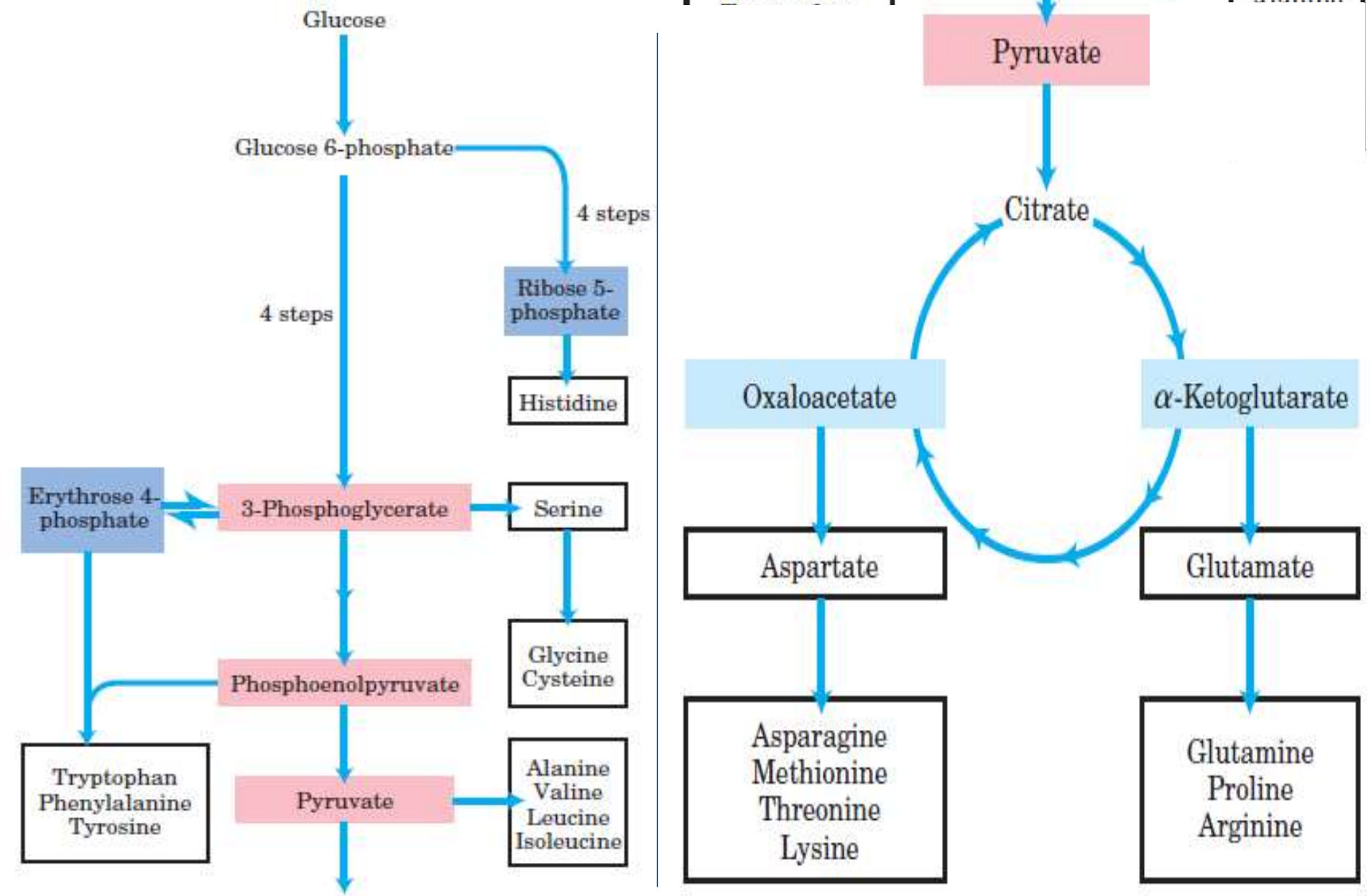
- The excretion of polyamines is found to be elevated in almost all types of cancers, e.g. leukemias; carcinoma of lungs, bladder, kidney etc.

- Putrescine is an ideal diagnostic marker for cell proliferation, whereas spermidine is suitable for the assessment of cell destruction.

AMINO ACIDS ANABOLISM (SYNTHESIS)

BIOSYNTHESIS OF AMINO ACIDS

- All amino acids are derived from intermediates of glycolysis, TCA cycle, or the pentose phosphate pathway.
- Nitrogen enters these pathways through glutamate and glutamine.
- Organisms vary greatly in their ability to synthesize the 20 common amino acids.
- Whereas most bacteria and plants can synthesize all 20, mammals can synthesize only about half of them—generally those with simple pathways.
- These are the non-essential amino acids, not needed in the diet.
- The remainder, the essential amino acids, must be obtained from food.
- The pathways for the 20 common amino acids presented below are those operative in bacteria.



AN OVERVIEW OF AMINO ACIDS BIOSYNTHESIS

Source: Nelson & Cox, 2016

GROUP ASSIGNMENT FOR PRESENTATION

Write on the metabolism (breakdown and synthesis) of the following amino acids:

Group A – Glycine and Serine

Group B – Phenylalanine and Tyrosine

Group C – Tryptophan

Group D – Methionine and Cysteine

Group E – Valine, Leucine and Isoleucine

Group F – Histidine, Proline and Arginine

Group G – Lysine

Group H – Glutamate and Glutamine

Group I – Aspartate and Asparagine

Group J – Threonine

Group K – Alanine

DISEASES OF AMINO ACIDS METABOLISM

DISEASES OF AMINO ACID METABOLISM

- Several diseases are associated with amino acids metabolism.
- The details of these metabolic disorders have been described under the respective amino acids.
- The table below gives a summary of the inborn errors of amino acid metabolism.

Some Human Genetic Disorders Affecting Amino Acid Catabolism

Medical condition	*Approximate incidence (per 100,000 births)*	*Defective process*	*Defective enzyme*	*Symptoms and effects*
Albinism	<3	Melanin synthesis from tyrosine	Tyrosine 3-monooxygenase (tyrosinase)	Lack of pigmentation: white hair, pink skin
Alkaptonuria	<0.4	Tyrosine degradation	Homogentisate 1,2-dioxygenase	Dark pigment in urine; late-developing arthritis
Argininemia	<0.5	Urea synthesis	Arginase	Mental retardation
Argininosuccinic acidemia	<1.5	Urea synthesis	Argininosuccinase	Vomiting; convulsions
Carbamoyl phosphate synthetase I deficiency	<0.5	Urea synthesis	Carbamoyl phosphate synthetase I	Lethargy; convulsions; early death
Homocystinuria	<0.5	Methionine degradation	Cystathionine β-synthase	Faulty bone development; mental retardation
Maple syrup urine disease (branched-chain ketoaciduria)	<0.4	Isoleucine, leucine, and valine degradation	Branched-chain α-keto acid dehydrogenase complex	Vomiting; convulsions; mental retardation; early death
Methylmalonic acidemia	<0.5	Conversion of propionyl-CoA to succinyl-CoA	Methylmalonyl-CoA mutase	Vomiting; convulsions; mental retardation; early death
Phenylketonuria	<8	Conversion of phenylalanine to tyrosine	Phenylalanine hydroxylase	Neonatal vomiting; mental retardation

Source: Nelson & Cox, 2016

THE GENETIC CODE (CODONS)

GENETIC CODE (CODONS)

- The three nucleotide (triplet) base sequences in mRNA that act as code words for amino acids in protein constitute the genetic code or simply **codons**.
- Protein biosynthesis (translation) can occur only when all the amino acids needed for a particular protein are available.
- The genetic code is regarded as a dictionary of nucleotide bases (A, G, C and U) that determines the sequence of amino acids in proteins.
- These four bases produce 64 different combinations (4^3) of three base codons.
- The nucleotide sequence of the codon on mRNA is written from the 5'-end to 3' end.
- The process depends on the formation of peptide linkages between the carboxyl group of one amino acid and the amino group of another.

- Sixty one codons code for the 20 amino acids found in protein.

- The three codons UAA, UAG and UGA do not code for amino acids, but they act as **stop signals** in protein synthesis.

- These three codons are collectively known as **stop codons**, **termination codons** or **non-sense codons**.

- The codons UAG, UAA and UGA are often referred to as amber, ochre and opal codons respectively.

- The codons AUG—and, sometimes, GUG—are the chain **initiating codons** or simply **start codons**.

CHARACTERISTICS OF THE GENETIC CODE

- **Universality**: The same codons are used to code for the same amino acids in all the living organisms, except for a few exceptions like AUA, which is the codon for methionine in mitochondria and codes for isoleucine in cytoplasm.

- **Specificity**: A particular codon always codes for the same amino acid, hence the genetic code is highly specific or unambiguous.

- **Non-overlapping**: The genetic code is read from a fixed point as a continuous base sequence that is non-overlapping and without any punctuations, e.g., UUUCUUAGAGGG is read as UUU/CUU/AGA/GGG.

- **Degenerate**: The codon is degenerate or redundant, since there are 61 codons available to code for only 20 amino acids, so most of the amino acids have more than one codon.

The genetic code along with respective amino acids

First base 5′end	Second base (middle one) U	C	A	G	Third base 3′end
U	UUU Phe	UCU Ser	UAU Try	UGU Cys	U
	UUC Phe	UCC Ser	UAC Try	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG* Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

*AUG serves as initiating codon, besides coding for methionine residue in protein synthesis; UAA, UAG and UGA called as nonsense codons, are responsible for termination of protein synthesis.

Source: Satyanarayana & Chakrapani, 2013

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