VIVEKANANDA COLLEGE
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NAAC ACCREDITED 'A' GRADE

Topic: Protein Metabolism
Course Title: Biochemistry and Genetic Engineering
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Unit:
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Name of the Teacher: Dr. Samita Kundu
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Protein metabolism

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Protein degradation

- Two major enzyme systems responsible for degrading proteins: the ATP-dependent ubiquitin proteasome system of the cytosol, and the ATP-independent degradative enzyme system of the lysosomes.
- Proteasomes selectively degrade damaged or short-lived proteins.
- Lysosomes use acid hydrolases to nonselectively degrade intracellular proteins (“autophagy”) and extracellular proteins (“heterophagy”), such as plasma proteins, that are taken into the cell by endocytosis

- The relative susceptibility of a protein to degradation is expressed as its **half-life (*t*½)**, the time required to lower its concentration to half of its initial value.
- Intracellular proteases hydrolyze internal peptide bonds.
- The resulting peptides are then degraded to amino acids by endopeptidases that hydrolyze internal peptide bonds, and by aminopeptidases and carboxypeptidases that remove amino acids sequentially from the amino- and carboxyl-termini, respectively

Steps of amino acid metabolism

- ✓ **First phase:** Removal of α-amino groups (by transamination and oxidative deamination), forming ammonia and the corresponding α-keto acids (the carbon-skeletons of amino acids)
- ✓ **Second phase:** The carbon skeletons of the α-keto acids are converted to common intermediates of energy producing metabolic pathways. These compounds are ultimately metabolized to CO_2 and water, glucose, fatty acids or ketone bodies.

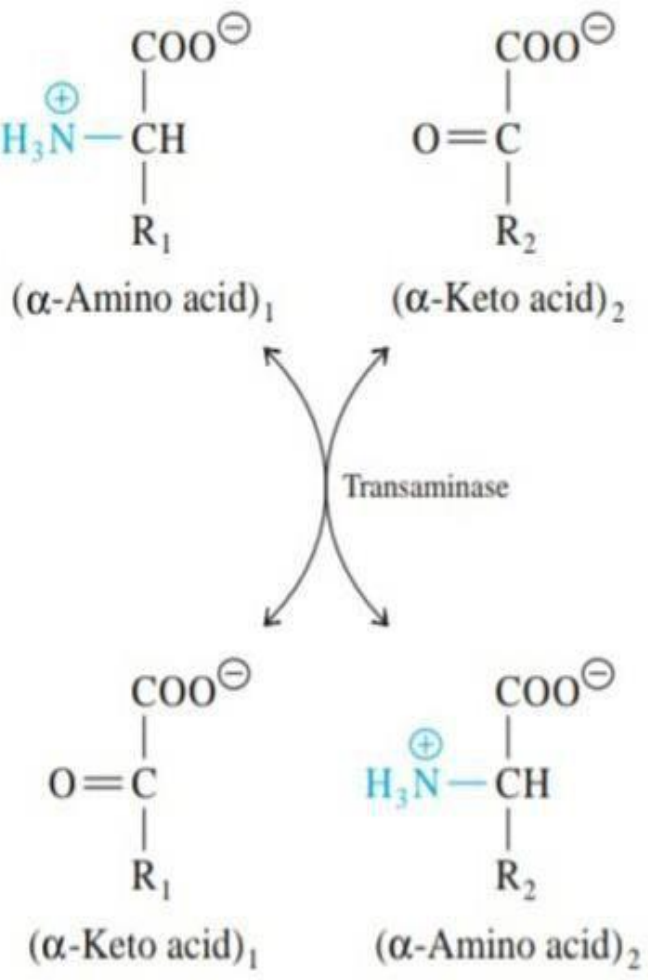
Need for removal of α-amino group

- The first part of amino acid metabolism is basically the metabolism (catabolism) of ***Nitrogen***- containing molecules (here, removal of the α-amino group, -NH_2)
- The presence of the α-amino group prevents amino acids from oxidative breakdown. So removal of the α-NH_2 group is an obligatory step and is essential for producing energy.
- N-catabolism consists of removal of α -NH_2 group as NH_3 and conversion of this NH_3 to excretory end products like urea and uric acid.
- Removal of this α -NH_2 group is accomplished by basically 2
- processes –
  - *Trans*amination (*transfer* of -NH_2 group)
  - *De*amination (*removal* of -NH_2 group)

Pathways of Nitrogen catabolism

- **Transamination**

Transfer of the *amino* group of an Amino acid (α-AA_1) to a keto acid (α-KA_2), changing that keto acid (α-KA_2) into a new amino acid (α-AA_2) and the original Amino acid (α-AA_1) into a new keto acid (α-KA_1)



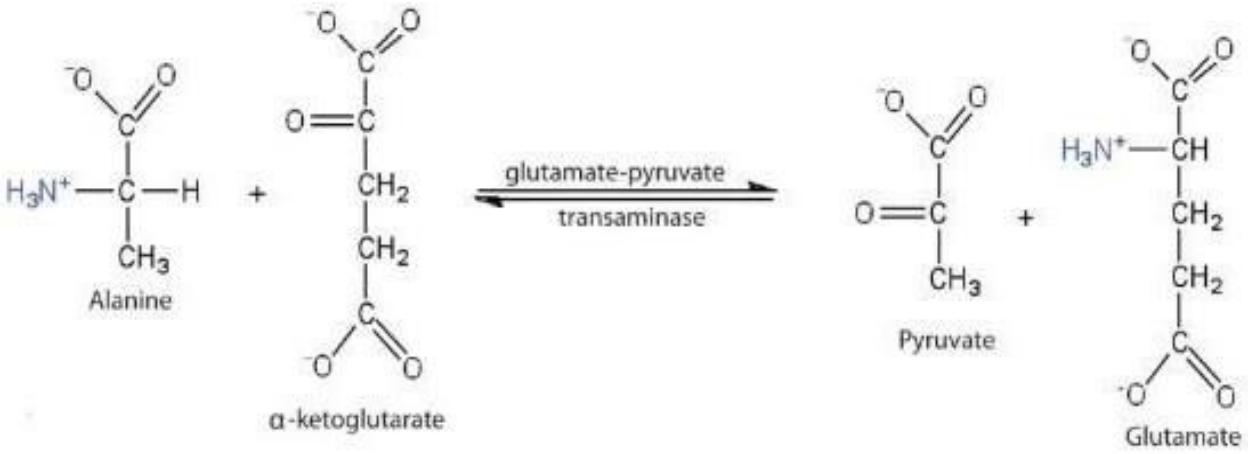
- The enzymes are known as *transaminases* (or *aminotransferases)*

- Occurs in the mitochondria and cytoplasm of liver, kidney, heart, testes and brain.

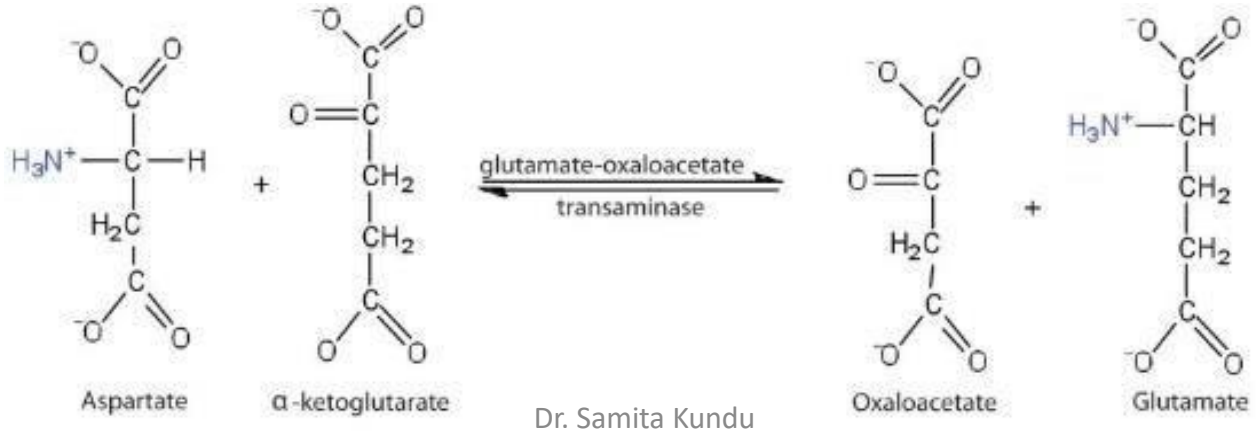
- Reversible reactions that can serve in both formation of amino acid from keto acid and catabolism of former to the latter.

❑ *Examples:*

- Alanine transaminase (ALT/GPT) catalyzes the transamination of alanine to pyruvate.



- Aspartate transaminase (AST/GOT) catalyzes the transamination of aspartate to oxaloacetate.

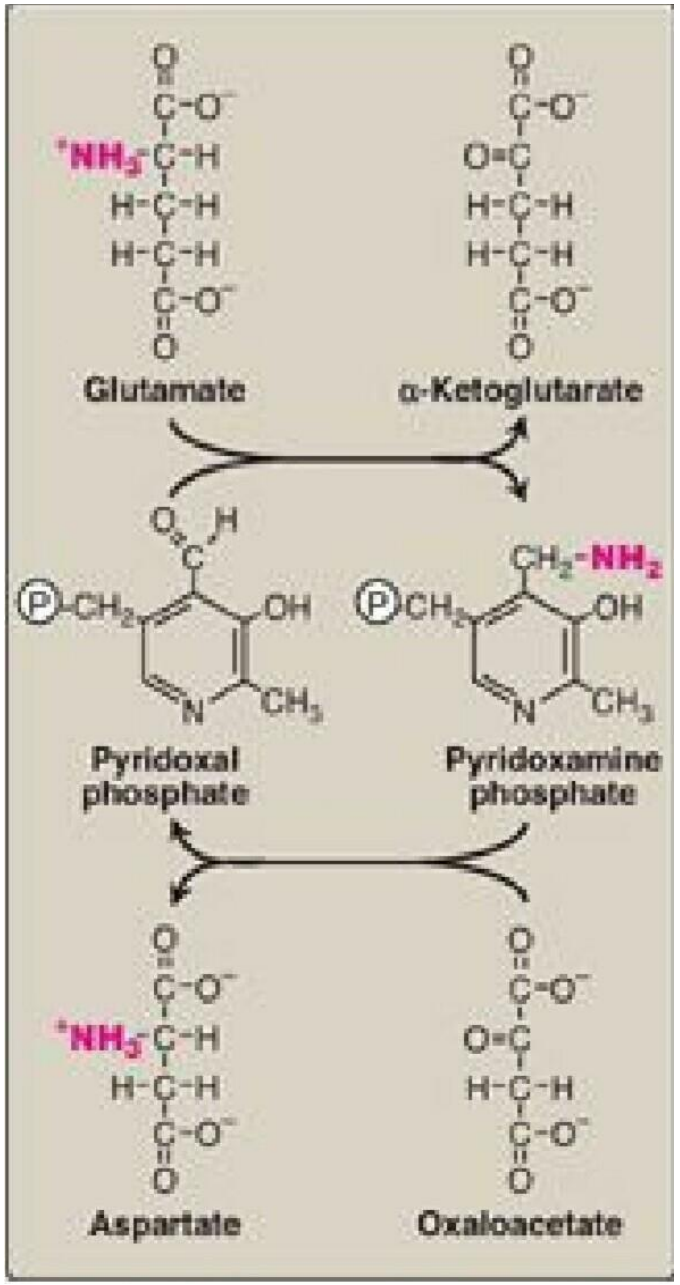


❑ *Mechanism:*

- ✓ Transaminases require pyridoxal phosphate (PLP) as the prosthetic group.
- ✓ Double displacement (ping-pong) type of bisubstrate reaction where 2 substrates, amino acid and α-keto acid bind separately and successively with the prosthetic group of the enzyme.
- ✓ Transfers $-NH_2$ group to pyridoxal part of PLP to generate pyridoxamine phosphate (PMP), which then reacts with an KA to form AA and regenerating the original aldehyde (PLP) form of the coenzyme.

(i) $\alpha\text{-}AA_1 + \text{enzyme-PLP} \leftrightarrow \alpha\text{-}KA_1 + \text{enzyme-PMP}$

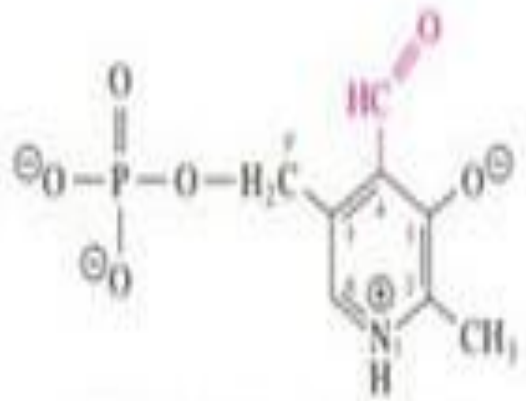
(ii) $\alpha\text{-}KA_2 + \text{enzyme-PMP} \leftrightarrow \alpha\text{-}AA_2 + \text{enzyme-PLP}$



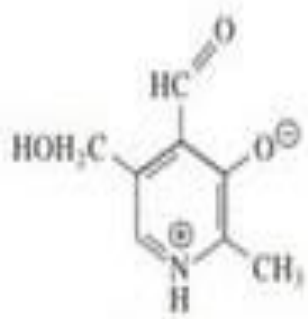
❖ *Pyridoxal phosphate (PLP)*

- PLP is a derivative of Vitamin B_6 (Pyridoxine).
- PLP contains a hydroxymethyl (-CH_2OH) group at position 4 of the pyridine ring.
- In PLP this group has been oxidized to an aldehyde.
- The -CH_2OH group at position 5 is phosphorylated.
- PLP functions as an intermediate carrier of –NH_2 groups at the active site of transaminases.
- It undergoes reversible transformation between its aldehyde form PLP, which can accept a –NH_2 group from an amino acid, and its aminated form PMP, which can donate its –NH_2 group to a keto acid.

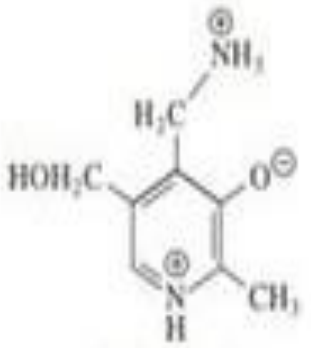
Pyridoxine



Pyridoxal 5'-phosphate (PLP)



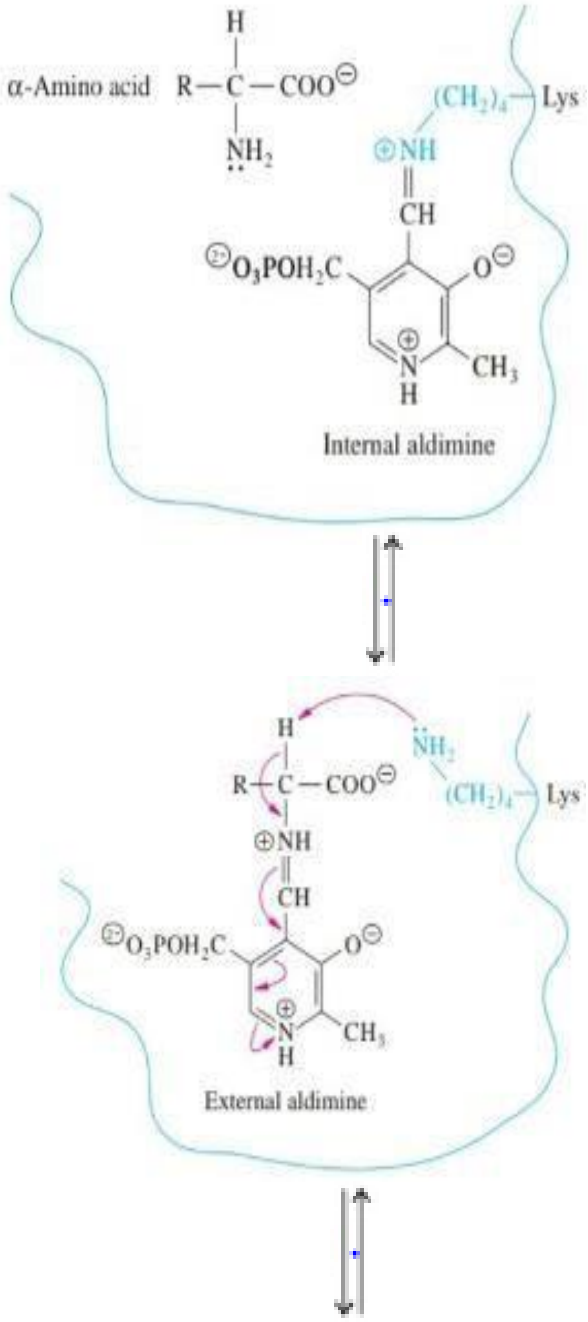
Pyridoxal



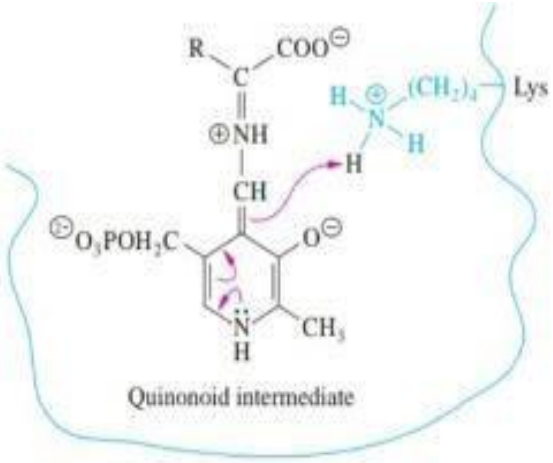
Pyridoxamine

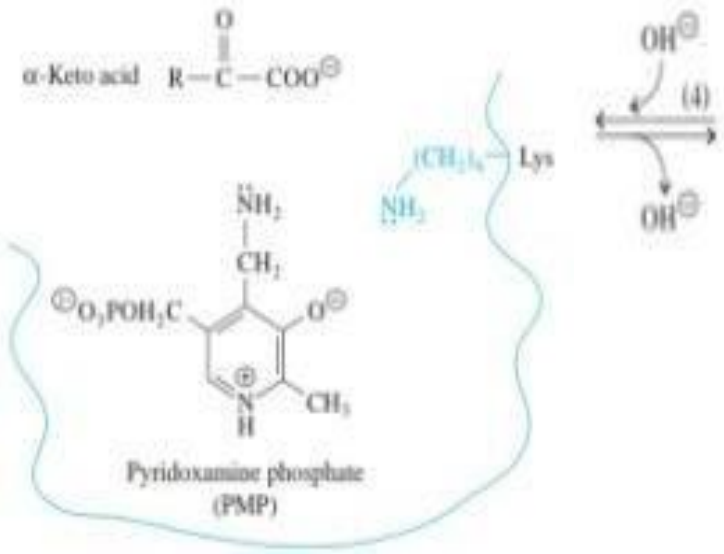
❖ *Steps of transamination*

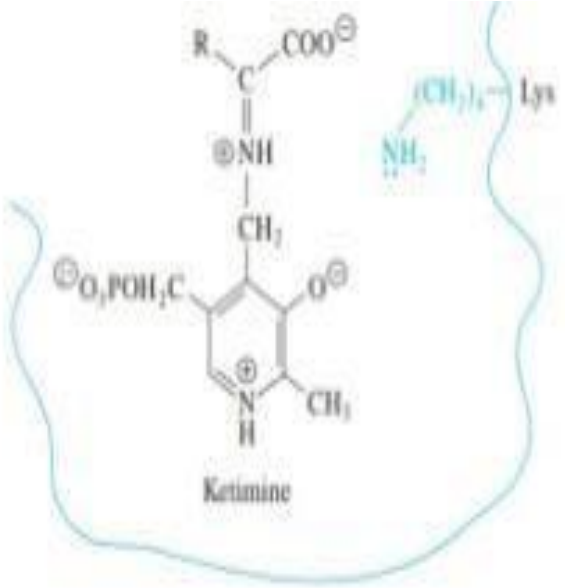
- ✓ PLP is generally covalently bound to the active site of transaminase through a Schiff base (aldimine, *with a double bond between -N and carbonyl-C of PLP*) linkage to the €-amino group of a Lysine residue of the enzyme.
- ✓ A new Schiff base linkage is formed on addition of an amino acid substrate. The α-NH_2 group of the amino acid substrate displaces the €-NH_2 group of the active site Lys.
- ✓ Thus, *an internal aldimine becomes an external aldimine*, with release of the Lys amino group of the enzyme.



- ✓ A bond to the α-C is broken, removing a proton and leaving behind a free electron pair on it (unstable carbanion intermediate).
- ✓ The electrophilic N of the pyridine ring of PLP acts as an electron sink, drawing electrons away from the AA and providing a resonance-stabilized carbanion with a quinonoid structure.
- ✓ Reprotonation yields a ketimine, (*with a double bond between -N and -C_α of the substrate*).







- ✓ The α-keto acid-PMP Schiff base (ketimine) is hydrolyzed to PMP and an α-keto acid.
- ✓ The second half occurs by a reversal of the above steps to regain enzyme-PLP.

- **Deamination**

✓ *Removal* of the *amino* (-NH_2) group of an amino acid as free ammonia (NH_3)

✓ It is of 2 types:

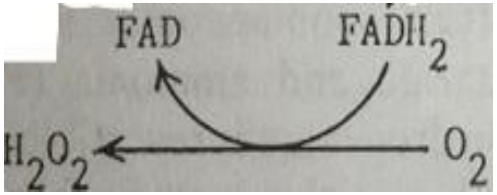
A. Oxidative deamination

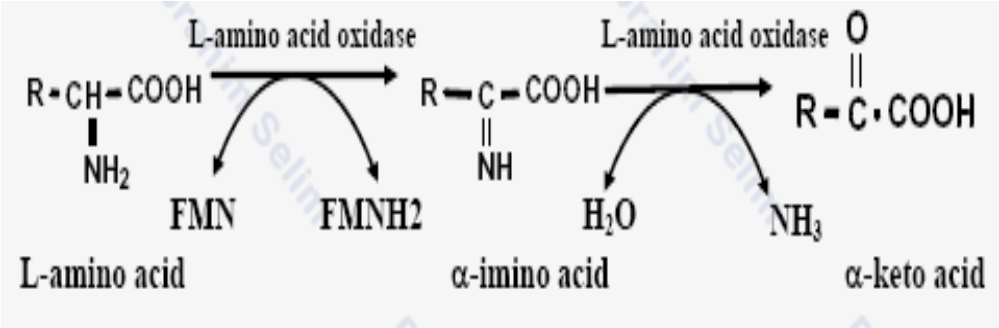
- Liberates the amino (-NH_2) group as free NH_3 along with simultaneous oxidation of the carbon skeleton.
- Occurs primarily in the liver and kidney mitochondria.
- Catalyzed mainly by two non-specific flavoprotein enzymes called amino acid oxidases (glycine oxidase for glycine) and glutamate dehydrogenase.
  - D-amino acid oxidase is specific for D-amino acids and carries FAD (*F*lavin *A*denine *D*inucleotide), while L-amino acid oxidase is for L-amino acids and requires FMN (*F*lavin *M*ono *N*ucleotide).

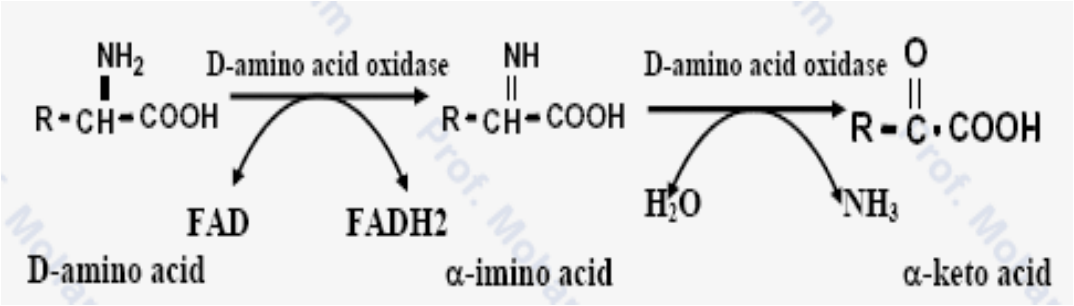
D(/L)-Amino acid + FAD(/FMN) + H_2O→α-Keto acid + NH_3 + $FADH_2$(/$FMNH_2$)

Mechanism of action:

- Amino acid oxidases oxidize an amino acid to the corresponding imino acid by transferring reducing equivalents (H^+ and *e*) from the amino acid to the flavin nucleotide.
- The imino acid reacts spontaneously with water to give an α-keto acid and NH_3.
- $FADH_2/FMNH_2$ is reoxidized directly by molecular O_2, producing H_2O_2.



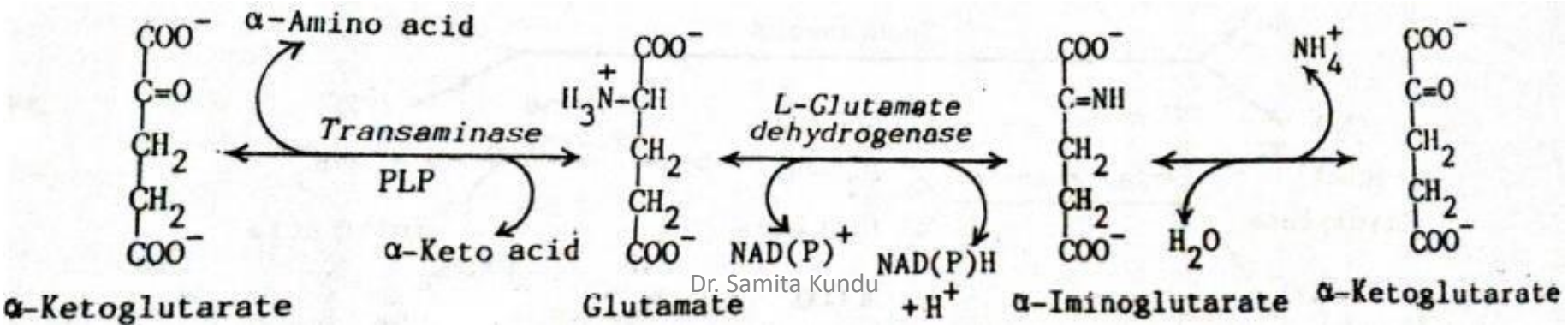




- **Transdeamination:**
- (Transamination followed by oxidative deamination)
- Occurs in the liver cytoplasm and mitochondria.
- ***Mechanism***:

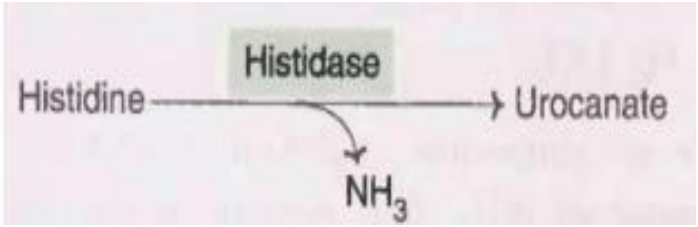
1. *Transamination*: A transaminase transfers the $-NH_2$ group of an amino acid to α-ketoglutarate, producing a new α-keto acid and glutamate.
2. *Oxidative deamination*: (i) L-Glutamate dehydrogenase first uses NAD^+ in mitochondria (or $NADP^+$ in cytosol) as the electron acceptor to oxidize glutamate to α-iminoglutarate.

   (ii) α-iminoglutarate is then spontaneously hydrolyzed into α-ketoglutarate and ammonia.

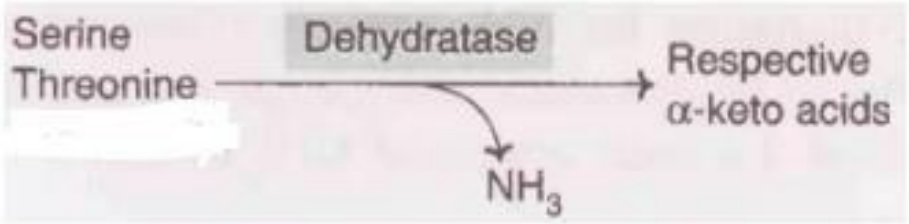


B. Non-oxidative deamination

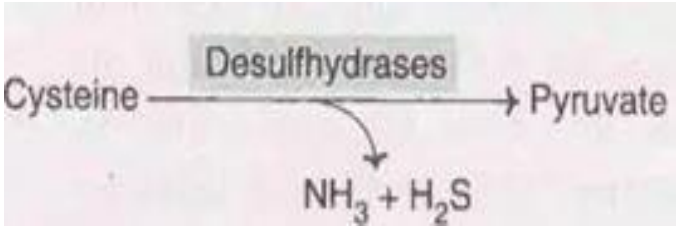
- Deamination of amino acids i.e removal of the amino group of amino acids as ammonia, but involving no oxidation of the carbon skeleton.
- **Amino acid lyases**: these are C-N lyases that deaminate significant amounts of histidine (histidase) and aspartic acid (aspartate ammonia lyase)



- **Amino acid dehydratases**: PLP containing enzymes that catalyze the dehydration followed by deamination of hydroxy amino acids like serine (by serine dehydratase) and threonine (by threonine dehydratase).



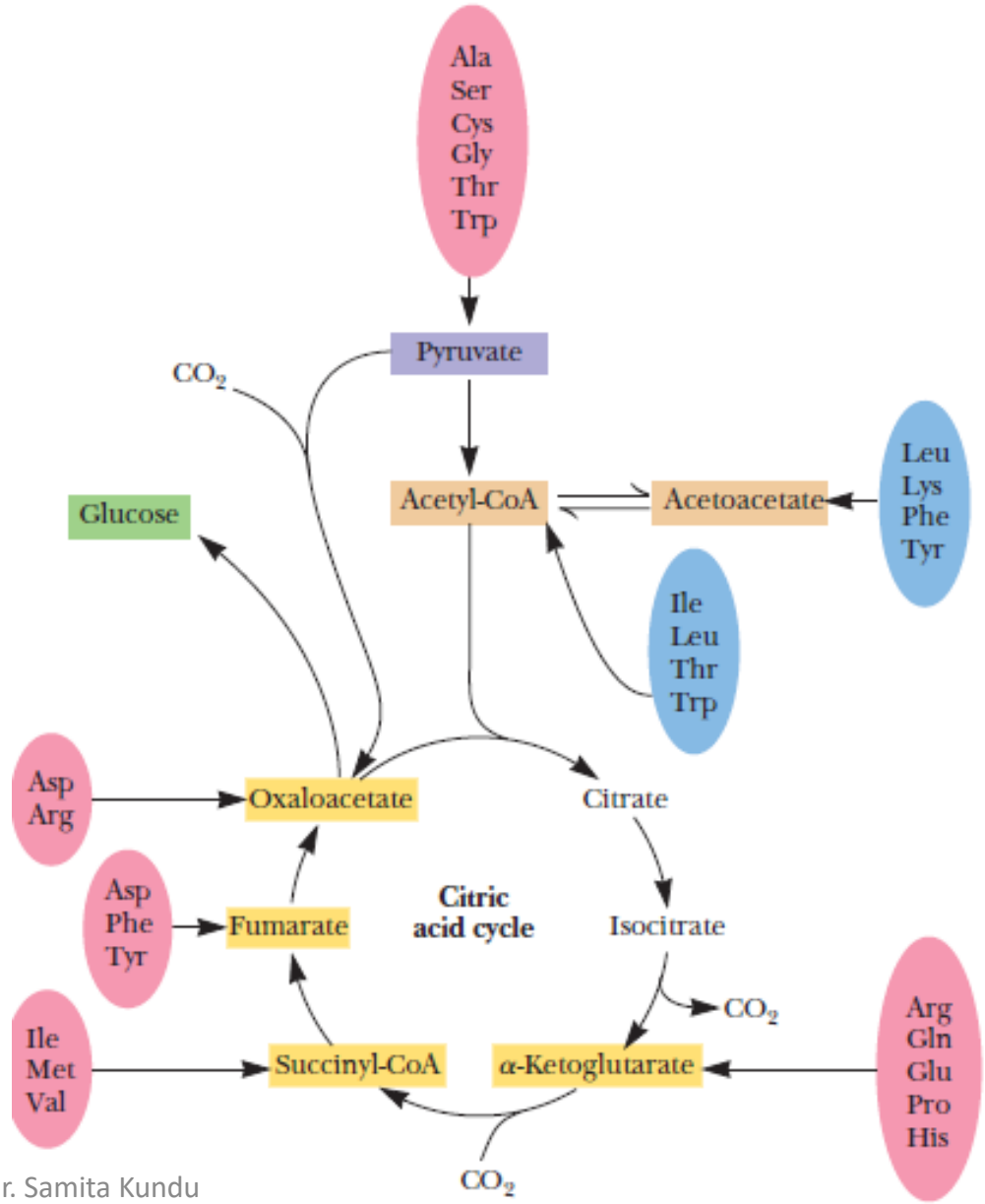
- **Amino acid desulfhydrases**: PLP containing enzymes that catalyzes desulfhydration and then deamination of sulfur containing amino acid like cysteine (by cysteine desulfhydrase)



- **Amino acid amide hydrolases:** Catalyze hydrolytic deamination of amino acid amides like glutamine and asparagine, releasing their amide groups as ammonia and changing them respectively to glutamate and aspartate.

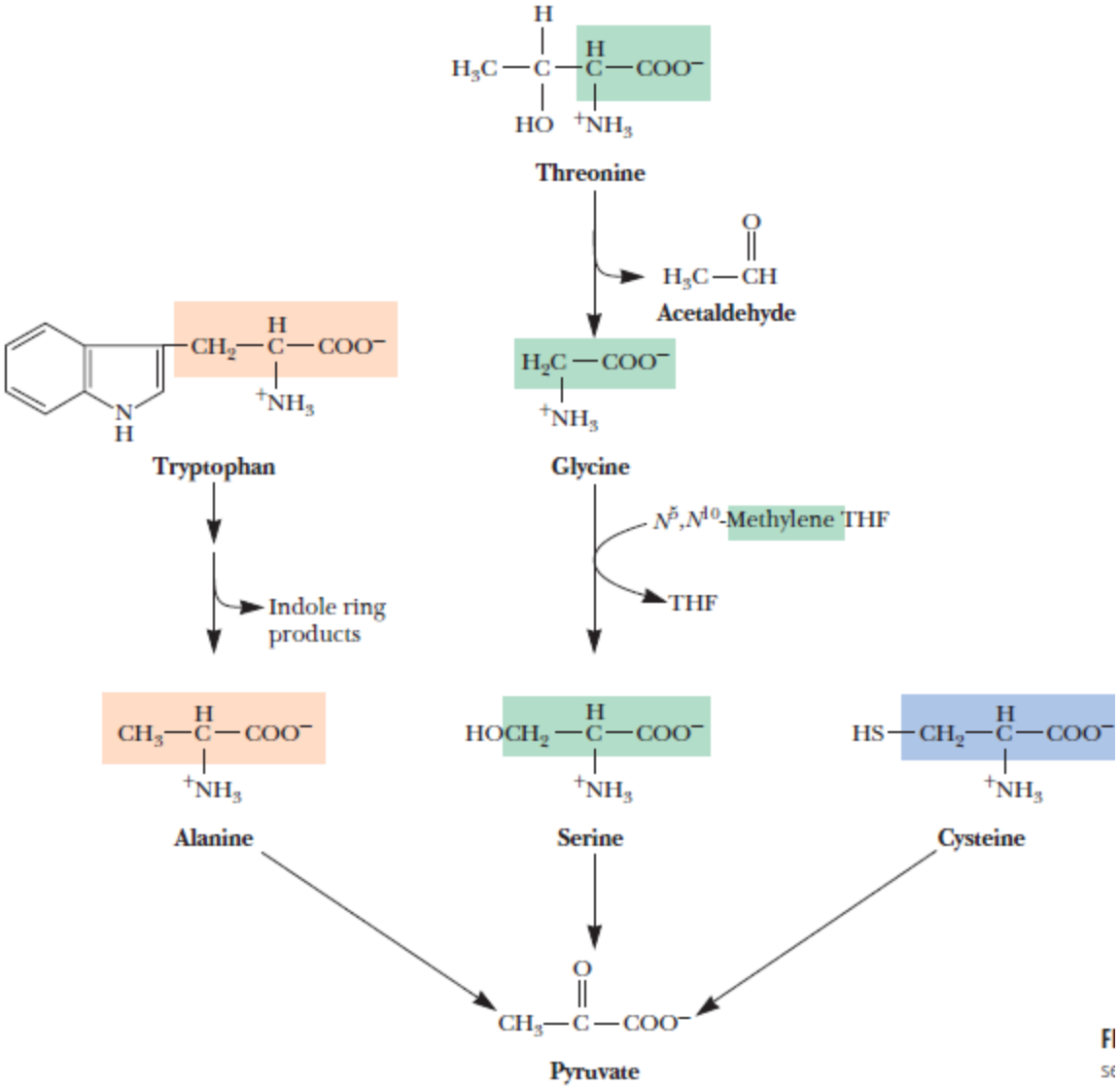
Degradation of Amino acids

- Degradation of the carbon skeletons of the 20 common amino acids converges to just 7 metabolic intermediates: *acetyl-CoA, succinyl-CoA, pyruvate, α-ketoglutarate, fumarate, oxaloacetate,* and *acetoacetate.*



Alanine, Serine, Cysteine, Glycine, Threonine, Tryptophan

- C skeletons of converge to *pyruvate*



Aspartate and Asparagine

- Transamination of aspartate gives *oxaloacetate*
- Hydrolysis of asparagine by asparaginase yields aspartate and NH_4
- Aspartate degradation via the urea cycle leads to a different citric acid cycle intermediate, namely *fumarate*

Glutamate, Glutamine, Proline, Arginine, and Histidine

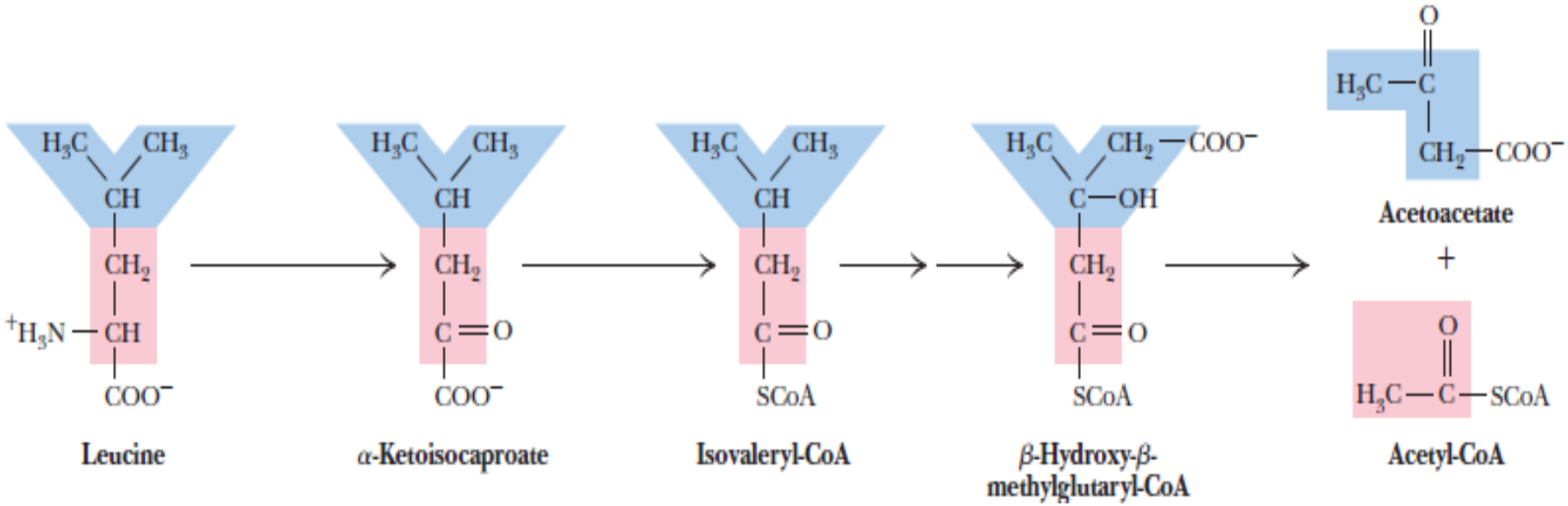
- Glutamate *and* any amino acid convertible to glutamate are classified within the C-5 family
- The carbon skeletons enter the citric acid cycle as α-ketoglutarate

Valine, Isoleucine, and Methionine

- Degradation Leads to Succinyl-CoA
- Breakdown of valine, isoleucine, and methionine converges at *propionyl-CoA*
- Propionyl-CoA is subsequently converted to methylmalonyl-CoA and thence to *succinyl-CoA*

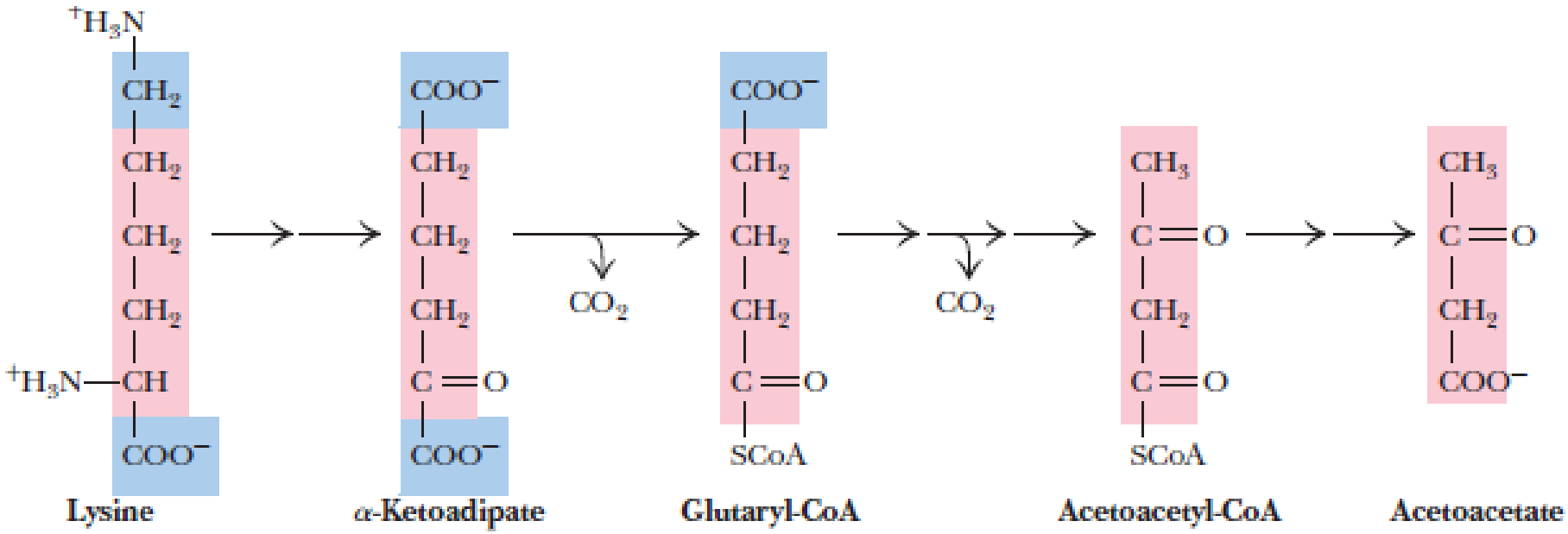
Leucine

- Degraded to Acetyl-CoA and Acetoacetate



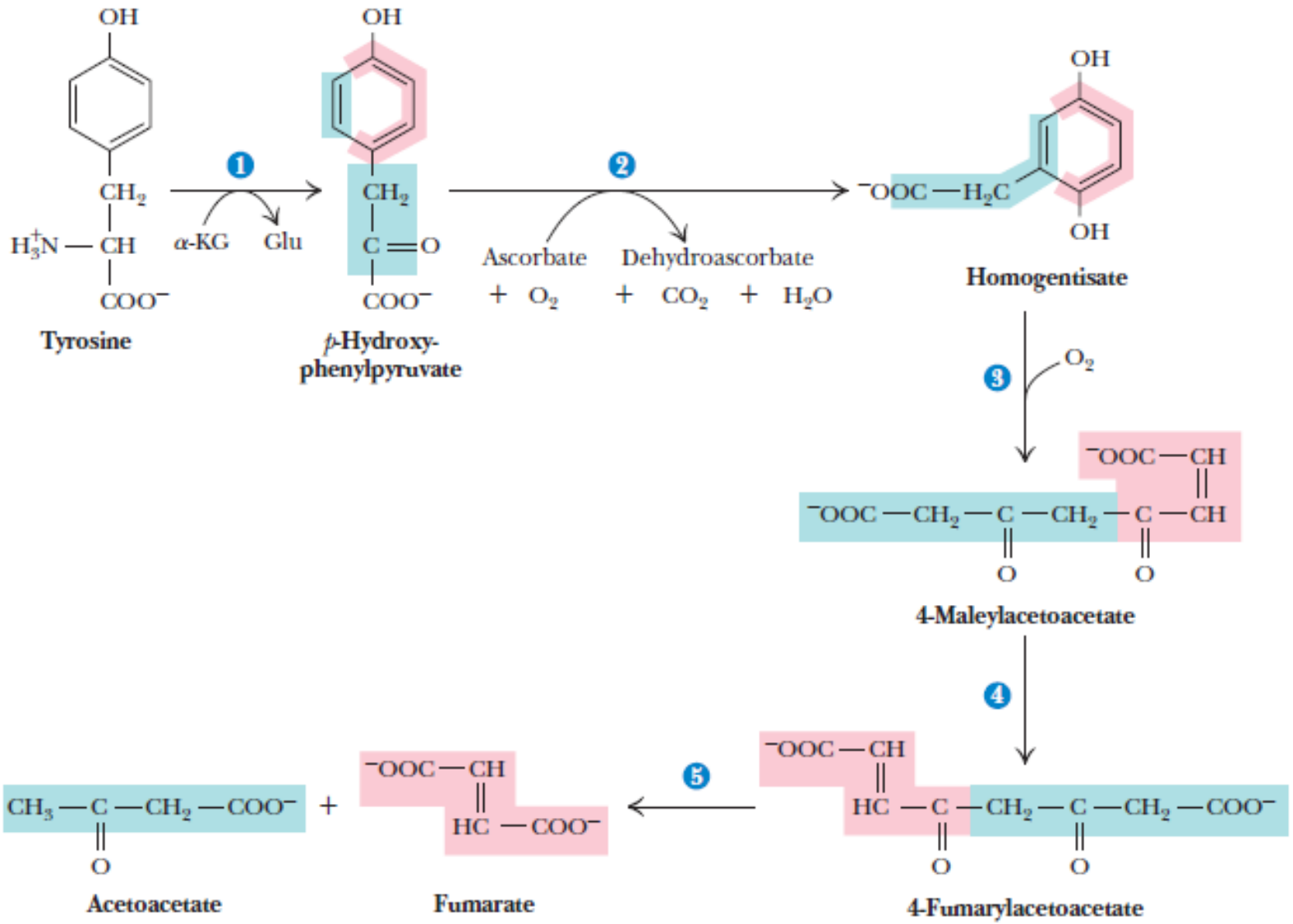
Lysine

- Transformed into *acetoacetyl-CoA* and ultimately into the ketone body, *acetoacetate*.

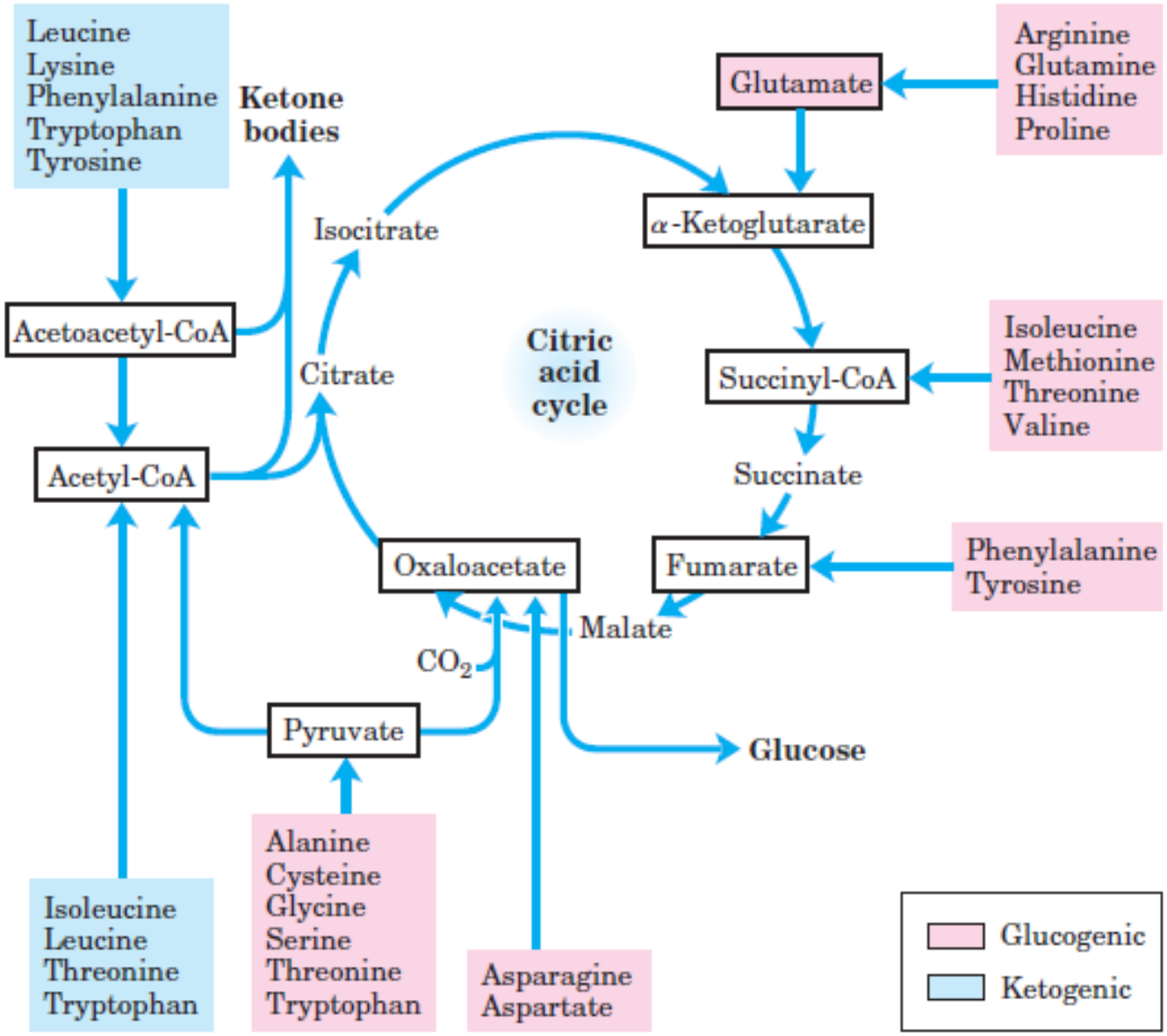


Phenylalanine and Tyrosine

- Degraded to Acetoacetate and Fumarate



Summary of amino acid catabolism

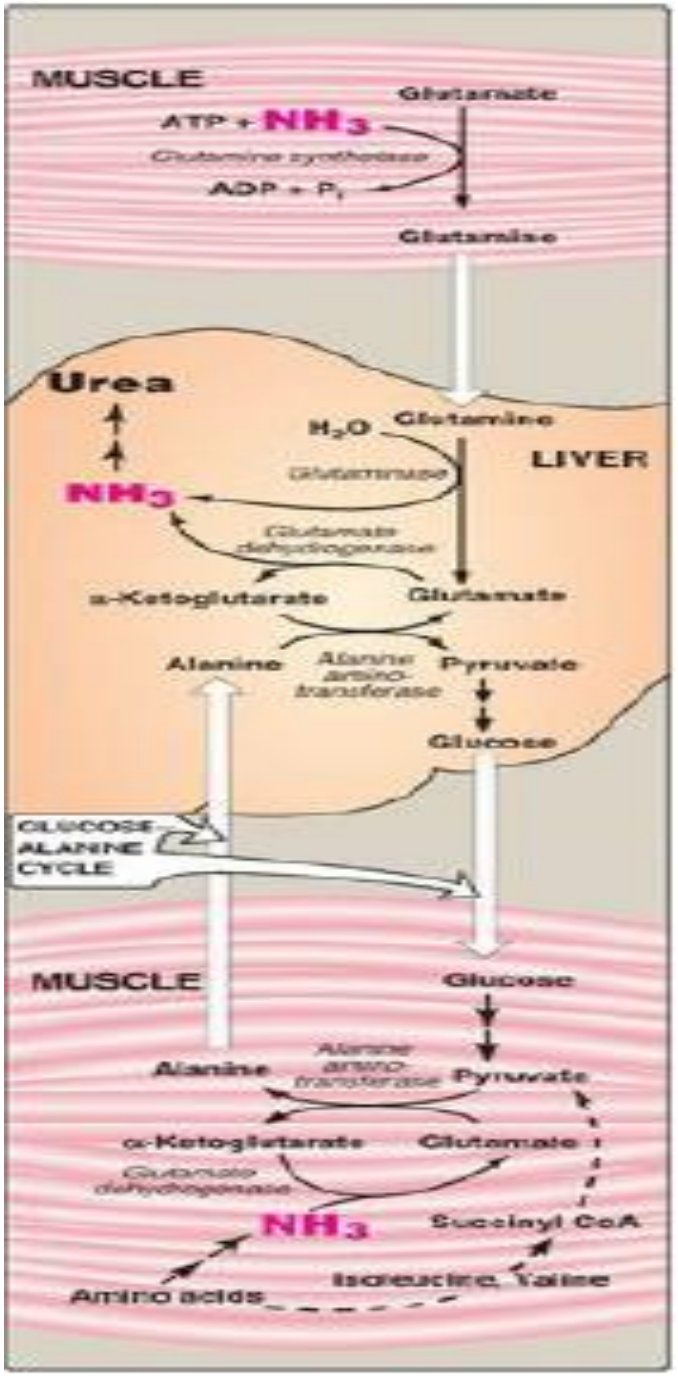


Fate of ammonia

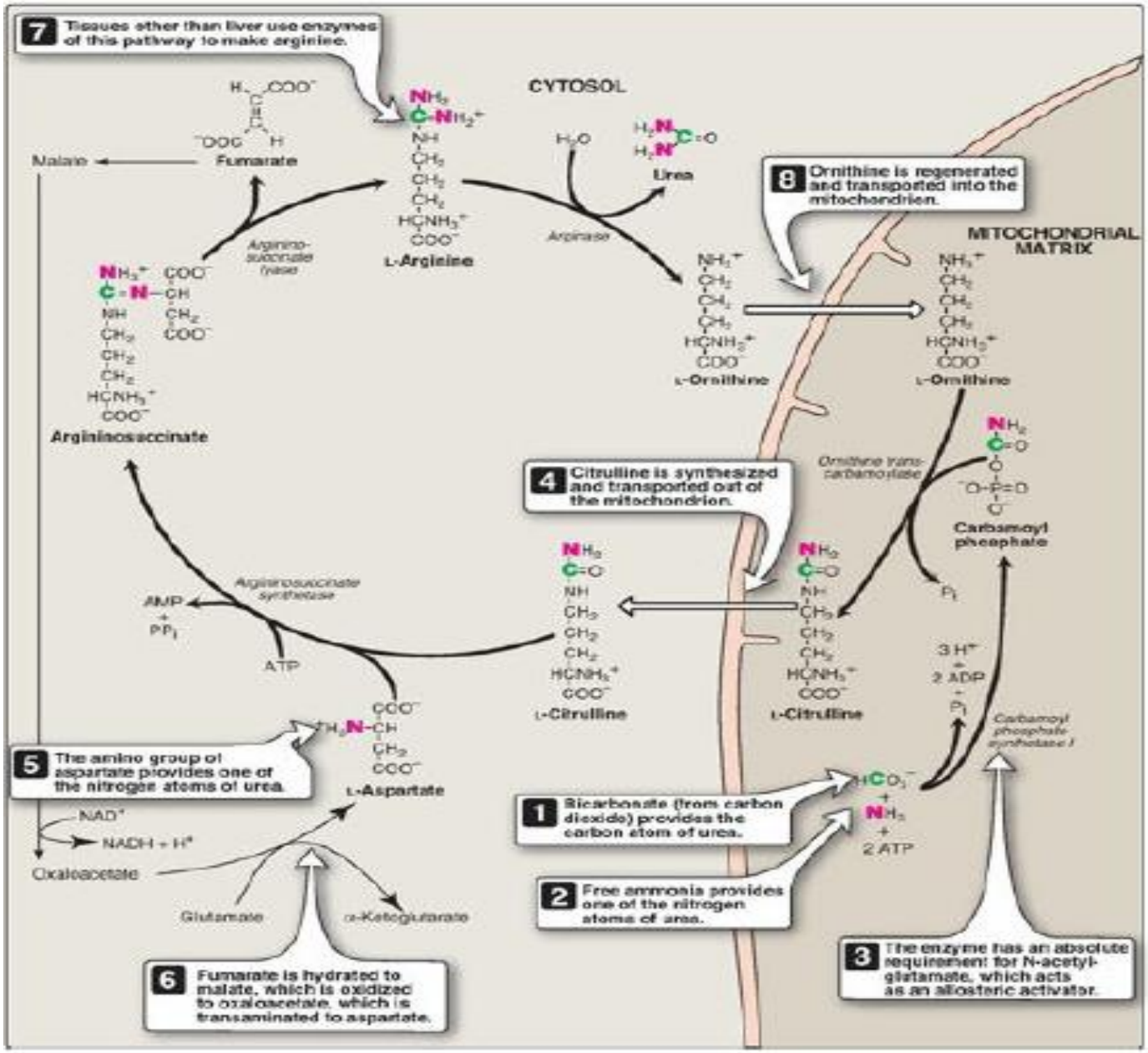
- ✓ Ammonia is highly toxic, so needs to be rapidly eliminated from the body.

- **Metabolic fate**

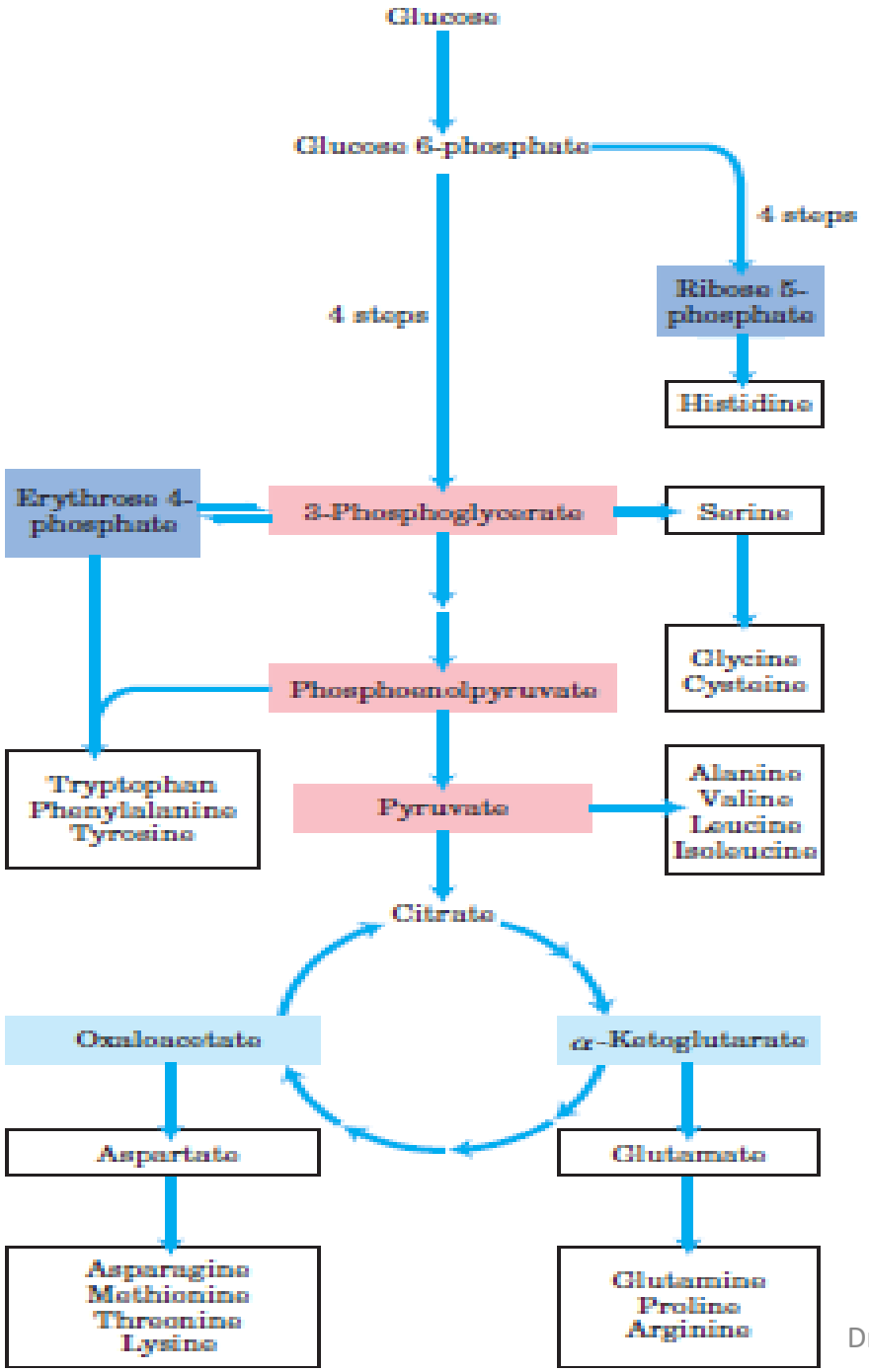
1. Mainly converted to urea in the liver by urea cycle.
2. Formation of glutamine (a non-toxic form of ammonia that can be transported to liver, kidneys and intestine via blood)
3. Amination of α- keto acid to form α-amino acid.



UREA CYCLE


7 Tissues other than liver use enzymes of this pathway to make arginine.
CYTOSOL
Fumarate
Malate
L-Arginine
Urea
Arginino-succinate lyase
Arginase
8 Ornithine is regenerated and transported into the mitochondrion.
MITOCHONDRIAL MATRIX
L-Ornithine
L-Ornithine
Argininosuccinate
4 Citrulline is synthesized and transported out of the mitochondrion.
Ornithine trans-carbamoylase
Carbamoyl phosphate
Argininosuccinate synthetase
AMP + PPi
ATP
L-Citrulline
L-Citrulline
Pi
3 H+
2 ADP + Pi
Carbamoyl phosphate synthetase I
5 The amino group of aspartate provides one of the nitrogen atoms of urea.
L-Aspartate
NAD+
NADH + H+
Oxaloacetate
Glutamate
α-Ketoglutarate
1 Bicarbonate (from carbon dioxide) provides the carbon atom of urea.
2 ATP
2 Free ammonia provides one of the nitrogen atoms of urea.
3 The enzyme has an absolute requirement for N-acetyl-glutamate, which acts as an allosteric activator.
6 Fumarate is hydrated to malate, which is oxidized to oxaloacetate, which is transaminated to aspartate.

Biosynthesis of Amino Acids



- All amino acids are derived from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway
- Nitrogen enters these pathways by way of glutamate and glutamine.

TABLE 22–1 Amino Acid Biosynthetic Families, Grouped by Metabolic Precursor

α-Ketoglutarate
Glutamate
Glutamine
Proline
Arginine

3-Phosphoglycerate
Serine
Glycine
Cysteine

Oxaloacetate
Aspartate
Asparagine
Methionine*
Threonine*
Lysine*

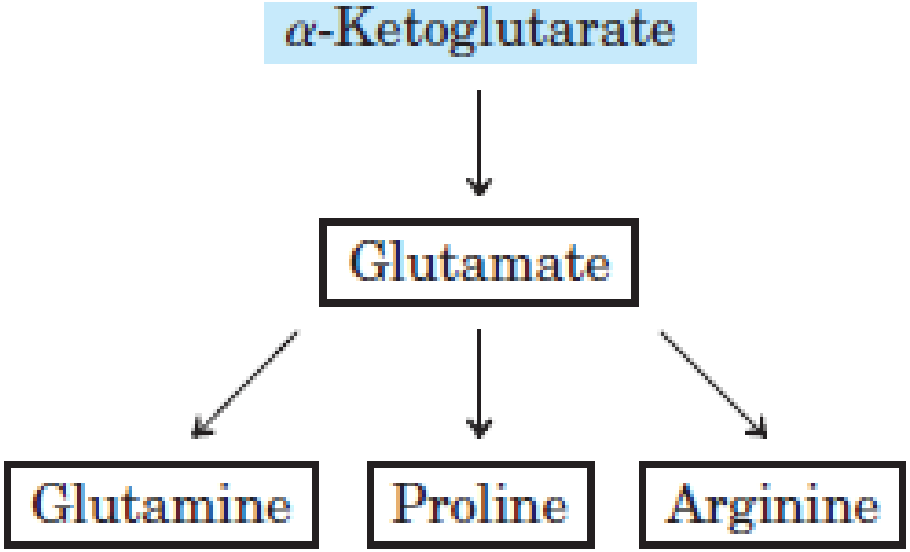
Pyruvate
Alanine
Valine*
Leucine*
Isoleucine*

Phosphoenolpyruvate and erythrose 4-phosphate
Tryptophan*
Phenylalanine*
Tyrosine†

Ribose 5-phosphate
Histidine*

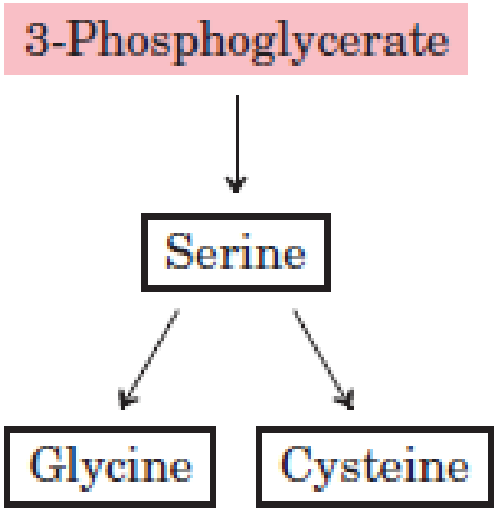
Glutamate, Glutamine, Proline and Arginine

- L-glutamate dehydrogenase catalyzes the reaction of α-ketoglutarate and NH_4 to form glutamate (reductive amination)
- Glutamine synthetase catalyzes the reaction of glutamate and NH_4 to yield glutamine.
- Proline is a cyclized derivative of glutamate
- Arginine is synthesized from glutamate via ornithine and the urea cycle in animals



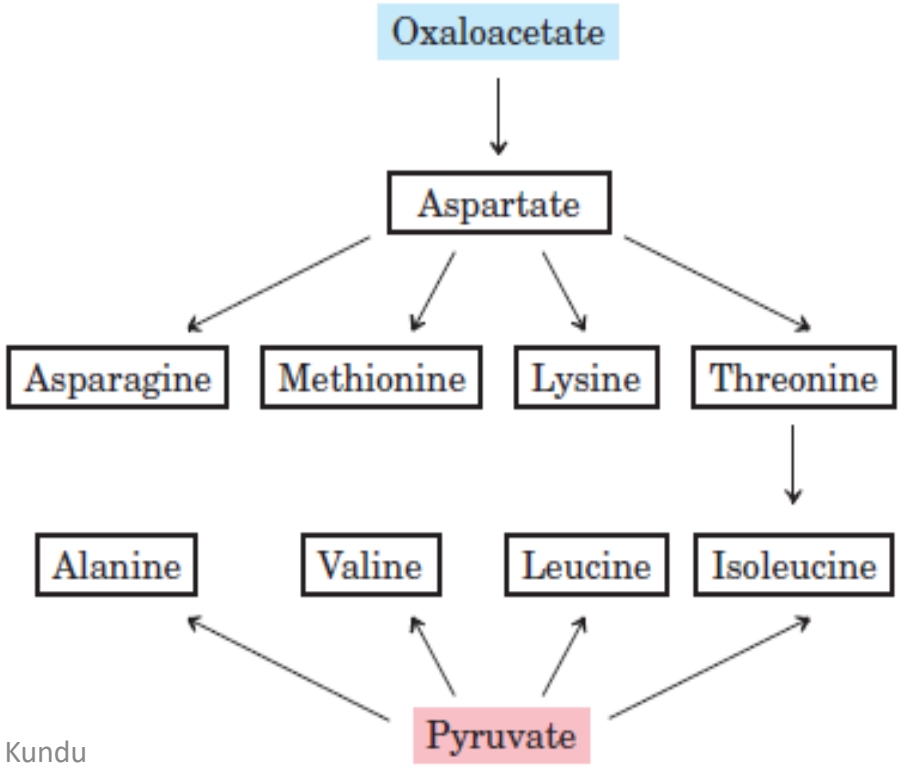
Serine, Glycine and Cysteine

- The hydroxyl group of 3-phosphoglycerate is oxidized (using NAD) to 3-phosphohydroxypyruvate. Transamination from glutamate yields 3-phosphoserine, which is hydrolyzed to free serine by phosphoserine phosphatase.
- Serine is the precursor of glycine through removal of a carbon atom by serine hydroxymethyltransferase
- Environmental sulfate is activated in two steps to produce 3-phosphoadenosine 5-phosphosulfate (PAPS), which undergoes reduction to sulfide. The sulfide is then used in the formation of Cys from Ser in a two steps. Mammals synthesize Cys from two amino acids: Met furnishes the sulfur atom, and Ser furnishes the carbon skeleton.



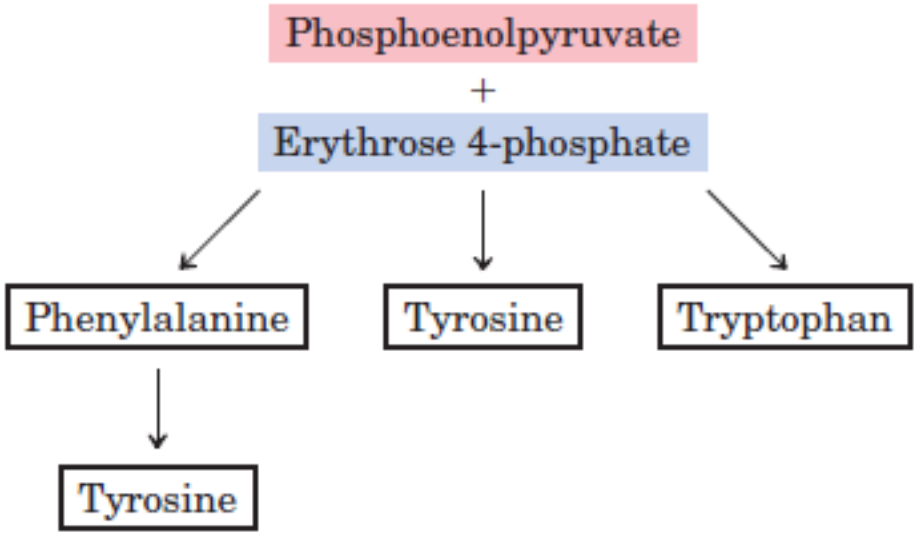
Aspartate, Asparagine, Methionine, Lysine, Threonine, Alalnine, Valine, Leucine, Isoleucine

- Ala and Asp are synthesized from pyruvate and oxaloacetate, respectively, by transamination from Glu.
- Asn is synthesized by amidation of Asp with Gln.
- Asp gives rise to Met, Thr and Lys.
- Thr is one of the precursors of Ile.
- Pyruvate gives rise to Val and Ile in pathways that begin with condensation of two carbons of pyruvate (hydroxyethyl thiamine pyrophosphate) with another molecule of pyruvate (Val) or α-ketobutyrate (Ile).
- An intermediate in the Val pathway, α-ketoisovalerate, is the starting point for Leu



Phenylalanine, Tyrosine, Tryptophan

- The first four steps produce shikimate, a 7C molecule derived from erythrose 4-phosphate and phosphoenolpyruvate. Shikimate is converted to chorismate in three steps that include the addition of 3 more carbons from another molecule of phosphoenolpyruvate.
- Chorismate is the first branch point of the pathway, with one branch leading to Trp, the other to Phe and Tyr.



Histidine

- 5-phosphoribosyl- 1-pyrophosphate (PRPP) is synthesized from ribose 5-phosphate derived from the pentose phosphate pathway, in a reaction catalyzed by ribose phosphate pyrophosphokinase
- Five of histidine's six C atoms are derived from PRPP.
- The sixth carbon and a nitrogen originates from purine ring of ATP.
- Glutamine supplies the second ring nitrogen