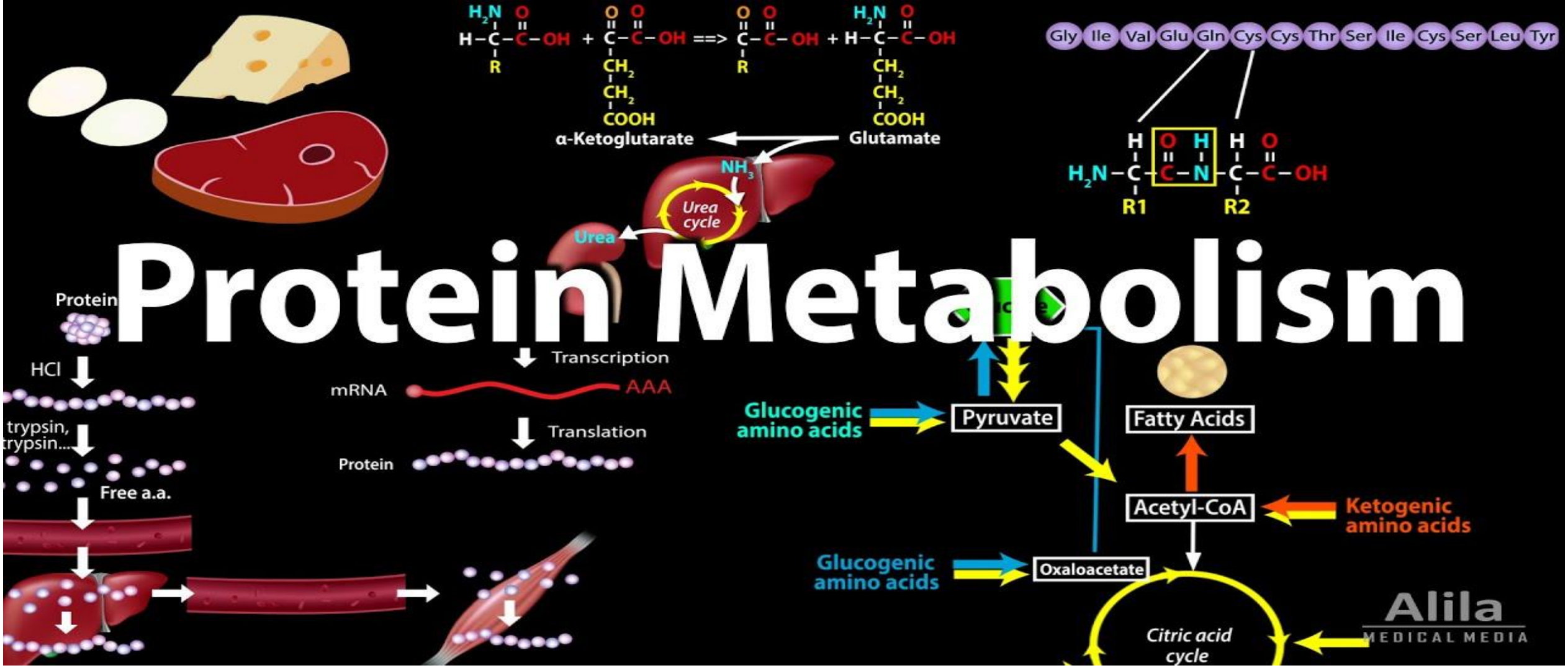


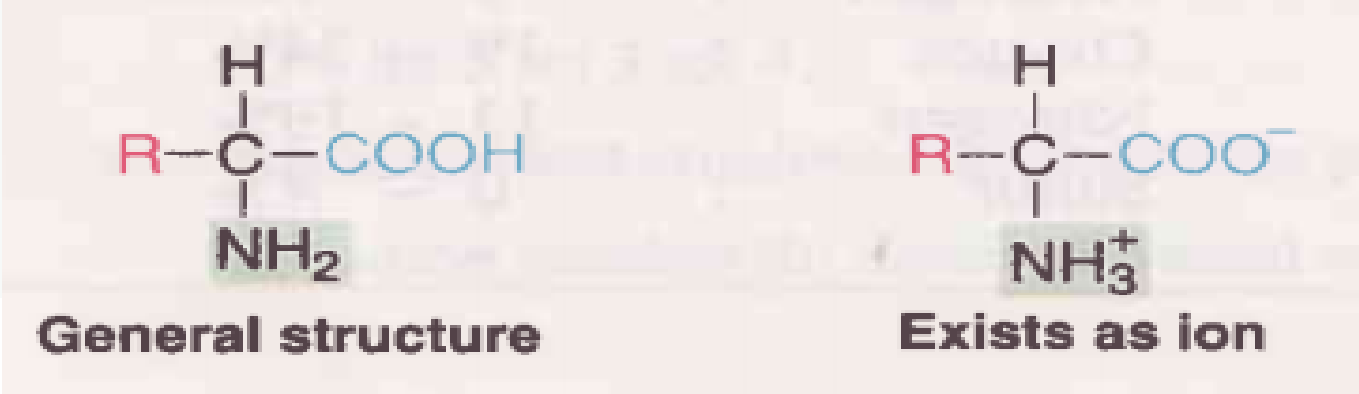
By: Dr. Kalpana Jorasia
Assistant Professor
(VPB)

AMINO ACID AND PROTEIN METABOLISM - AN INTRODUCTION

- The protein digestion begins in the stomach and is completed in the intestine. Proteolytic enzymes like proteases and peptidases degrade dietary proteins into their corresponding amino acids.
- The amino acids are then absorbed from the intestinal lumen through secondary active Na^+ dependent transport, through facilitated diffusion and through transport linked to the gamma - glutamyl cycle.
- Major functions of carbohydrates and triacylglycerols are to provide energy, but the primary role of amino acid is to serve as building blocks in the synthesis of tissue proteins. Protein is used secondarily as fuel.

INTRODUCTION

- Proteins are **nitrogen-containing** macromolecules consisting of L-α-amino acids
- AA catabolism is part of the whole body catabolism
- Nitrogen enters the body in a variety of compounds present in the food, the most important being AAs present in the dietary protein.
- Nitrogen leaves the body as urea, ammonia, and other products derived from AA metabolism.



PROTEIN TURN OVER

The turnover is high in infancy and decreases with age advance.

Degradation

- 300 to 400 gm/day

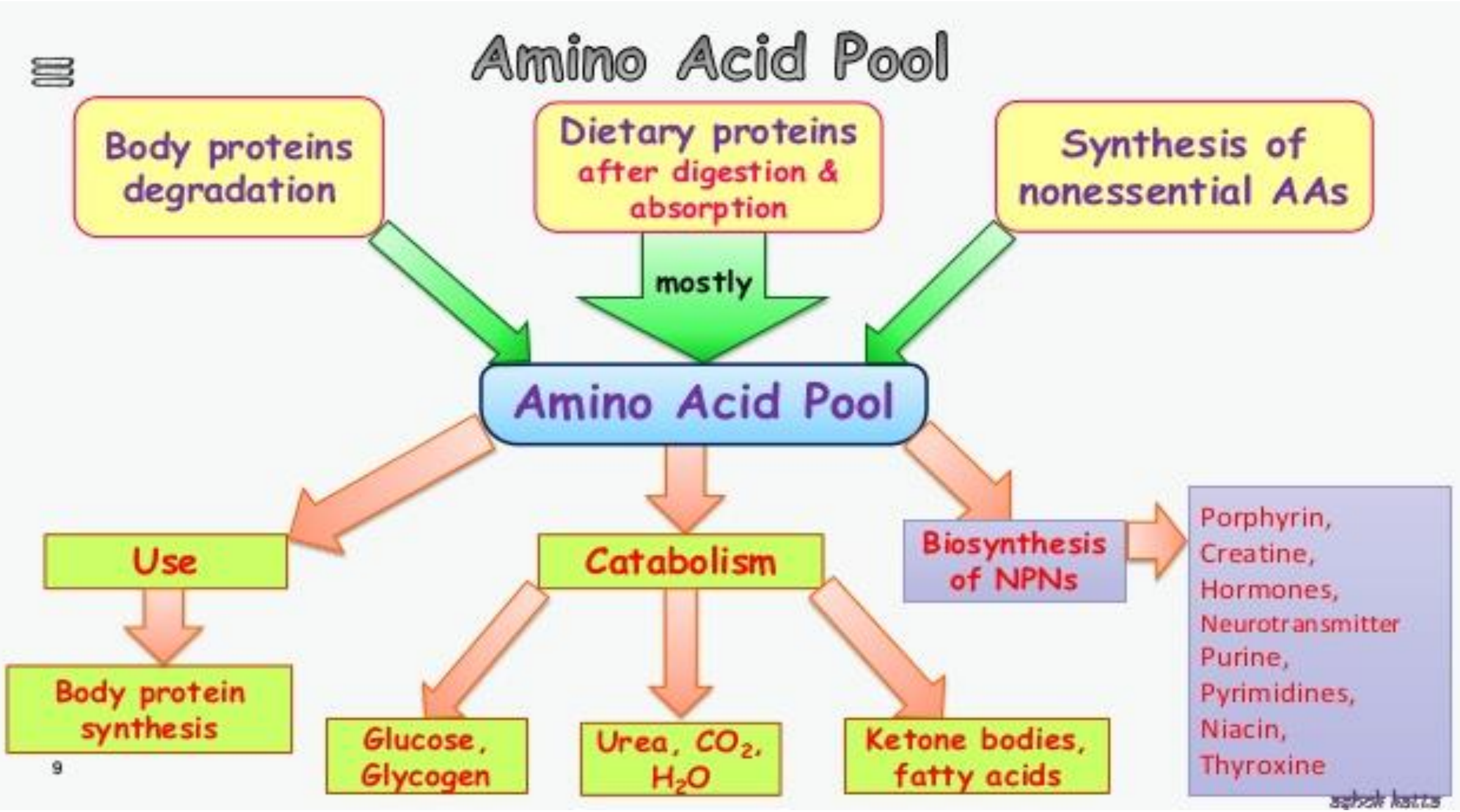
Synthesis

- 300 to 400 gm/day

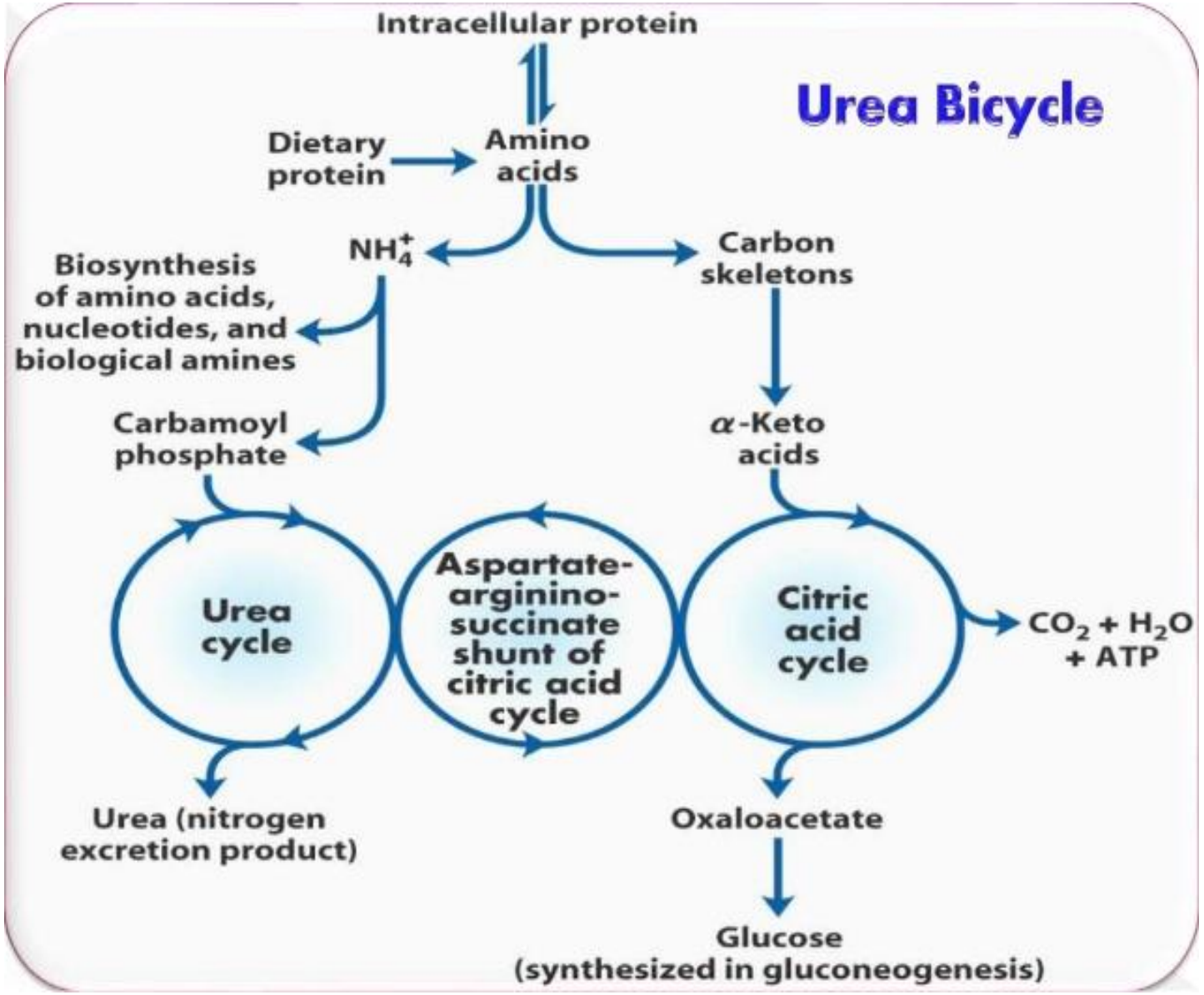
Control of protein turnover

- The turnover of the protein is influenced by many factors.
- A small polypeptide called **ubiquitin** tags with the proteins and facilitates degradation.

There is no storage form of amino acids as is the case for carbohydrates (glycogen) and lipids (triacylglycerols). The excess intake of amino acids are metabolized-oxidized to provide energy/ converted to glucose or fat


Amino Acid Pool
Body proteins degradation
Dietary proteins after digestion & absorption
Synthesis of nonessential AAs
mostly
Amino Acid Pool
Use
Catabolism
Biosynthesis of NPNs
Body protein synthesis
Glucose, Glycogen
Urea, CO_2, H_2O
Ketone bodies, fatty acids
Porphyrin,
Creatine,
Hormones,
Neurotransmitter
Purine,
Pyrimidines,
Niacin,
Thyroxine

Overview of amino acid catabolism in mammals



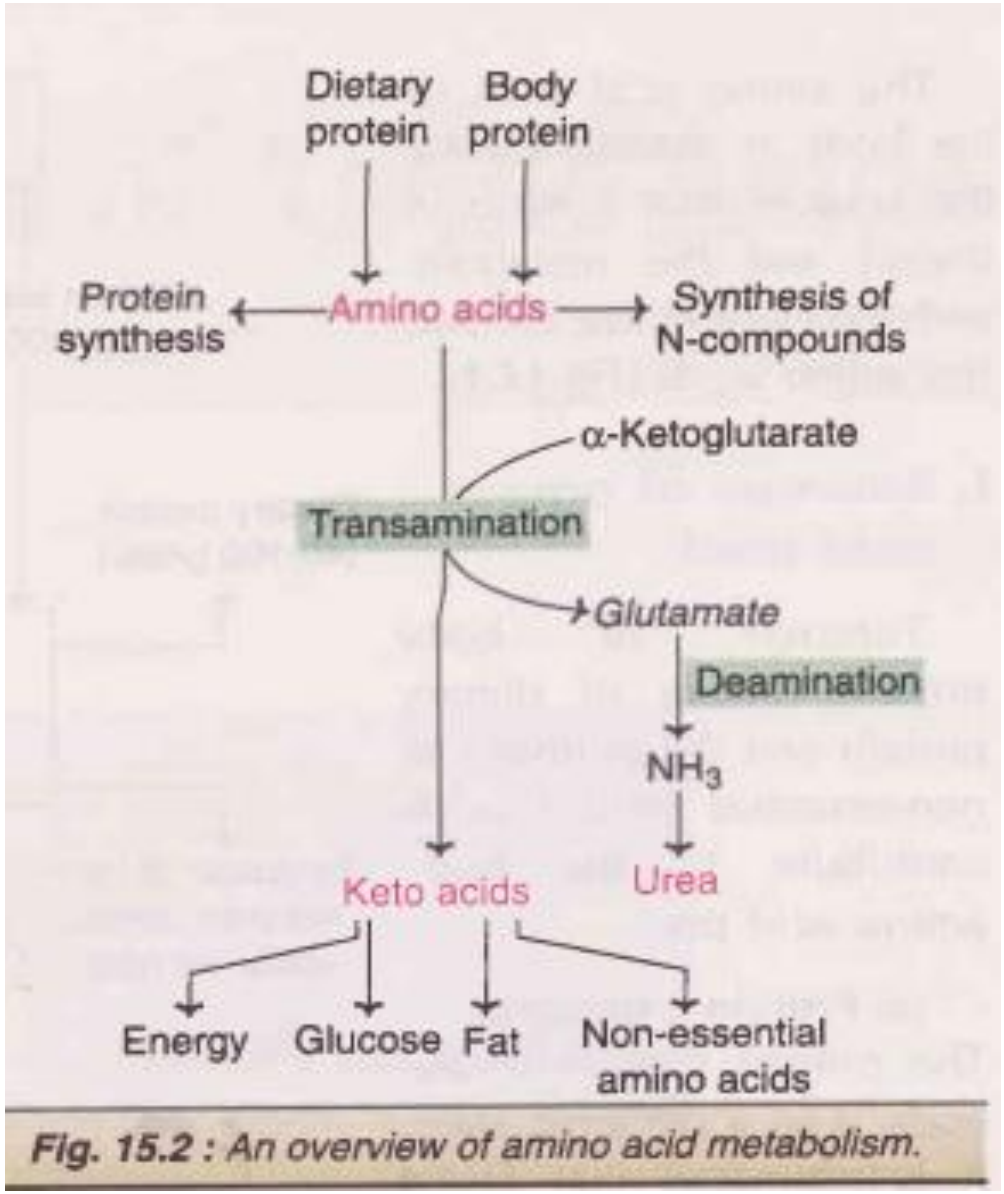
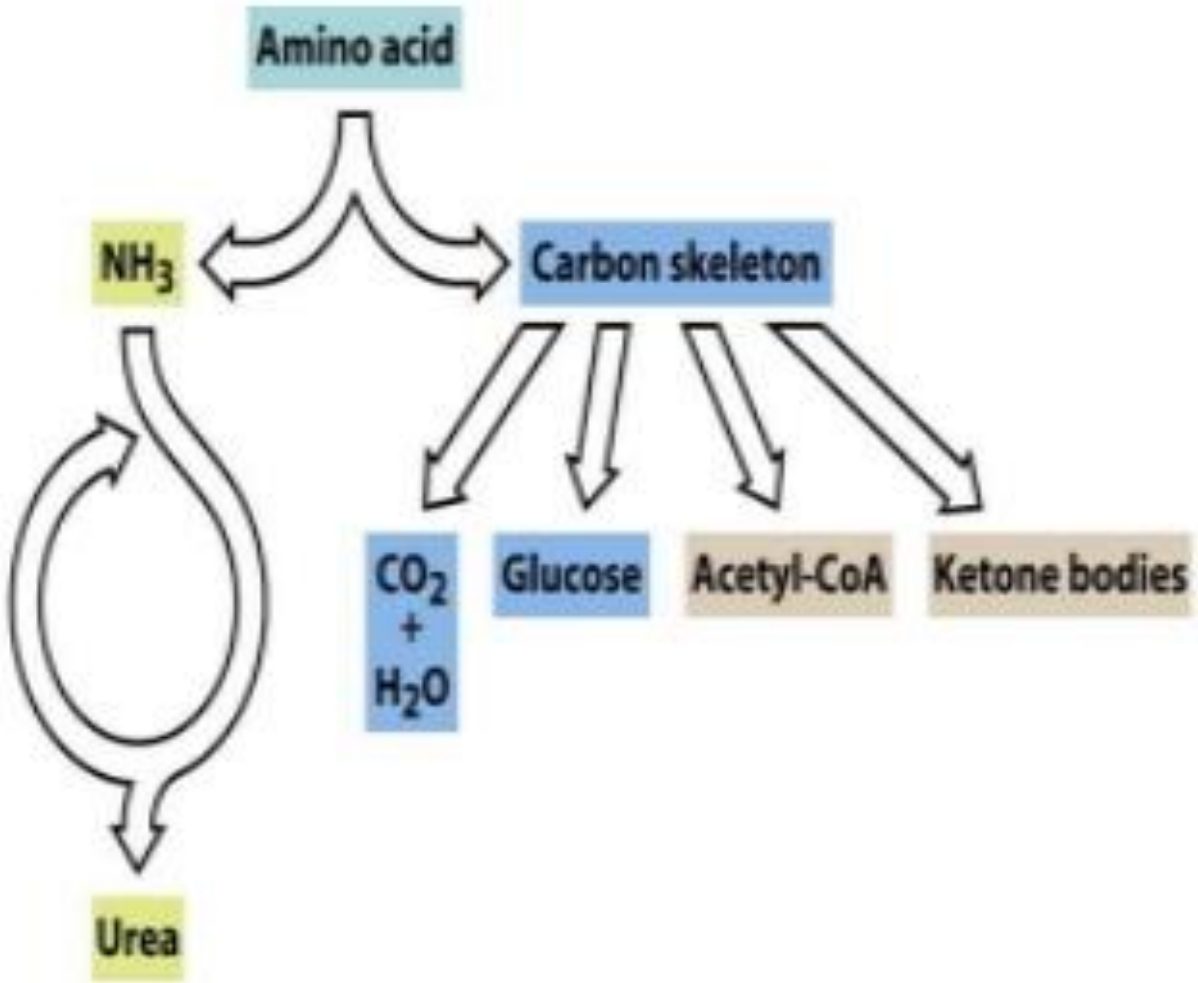
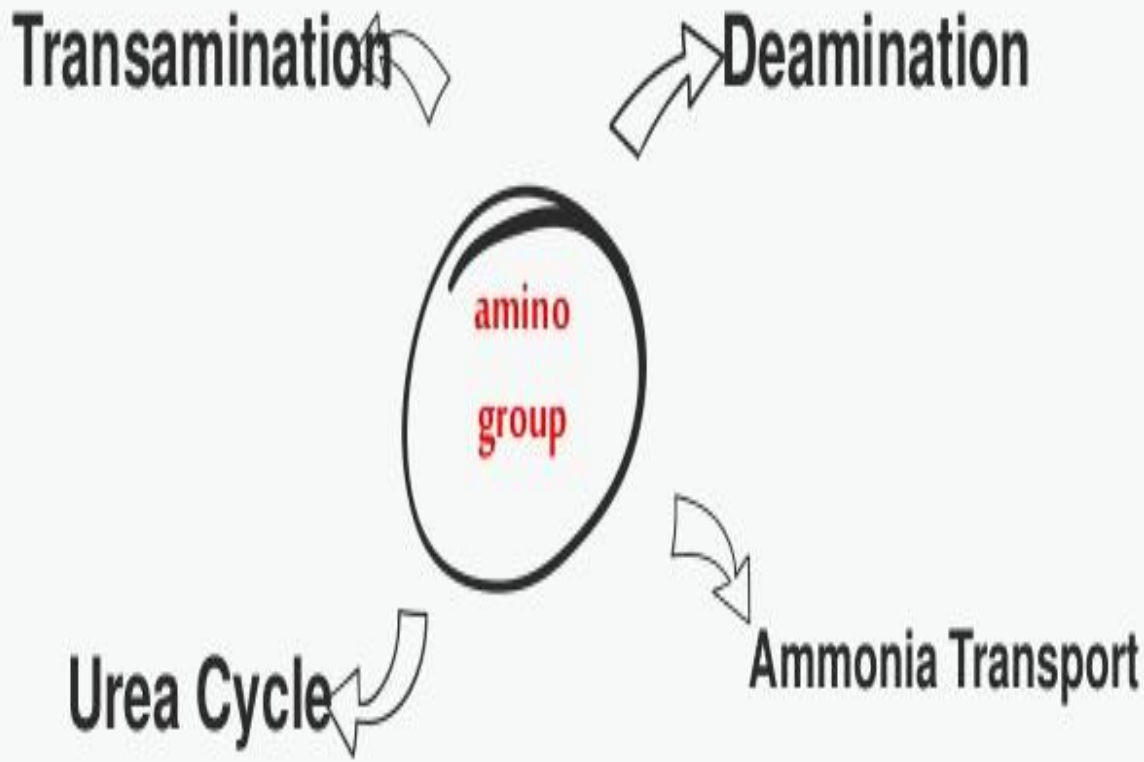


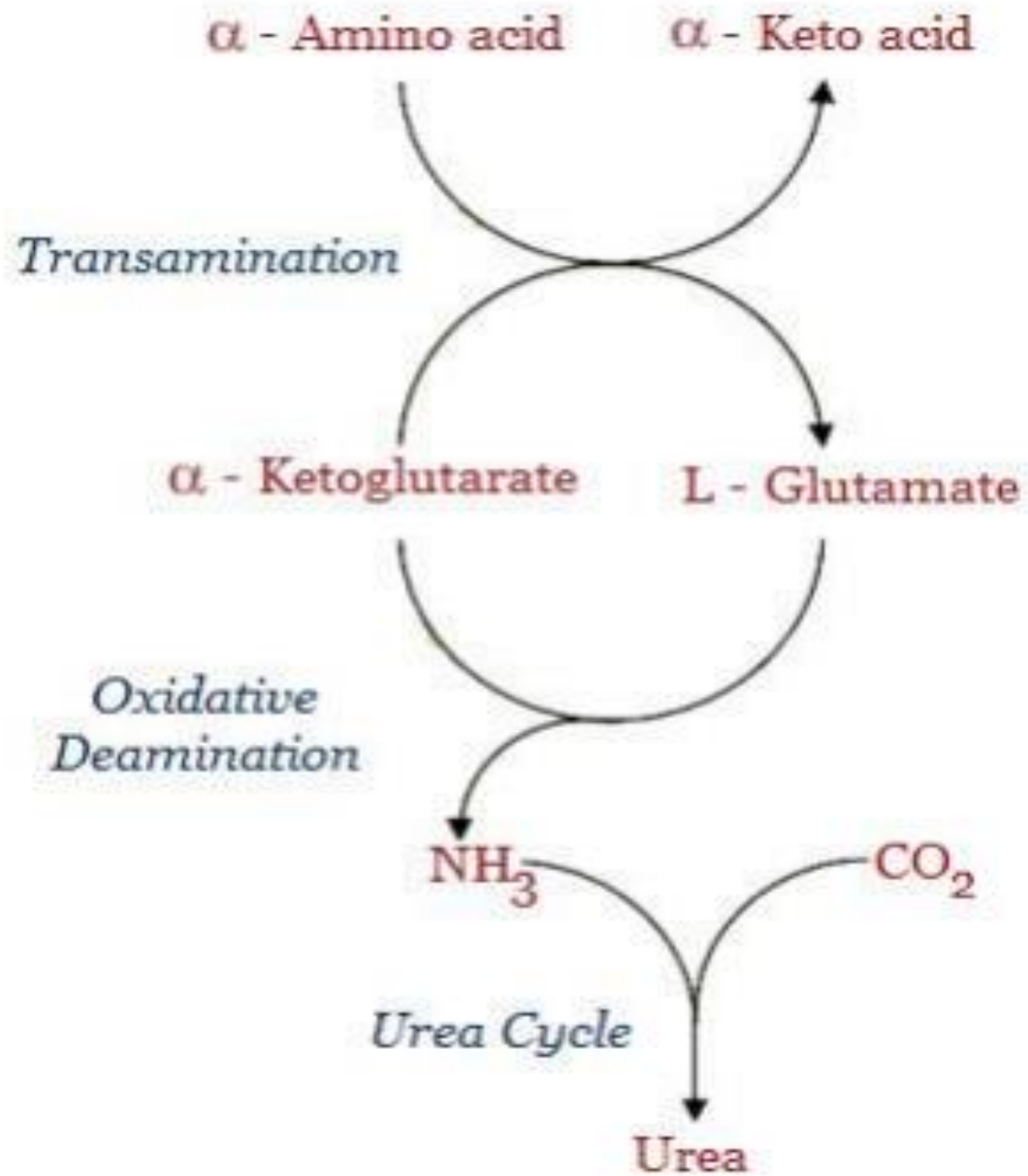
Fig. 15.2 : An overview of amino acid metabolism.



CATABOLISM OF AMINO ACIDS

Catabolism of amino group





TRANSAMINATION

Amphibolic in nature

Transamination

- The transfer of an amino (-NH2) group from an amino acid to a keto acid, with the formation of a new A. A & a new ketoacid.

- Catalysed by a group of enzymes called **transaminases (aminotransferases)**

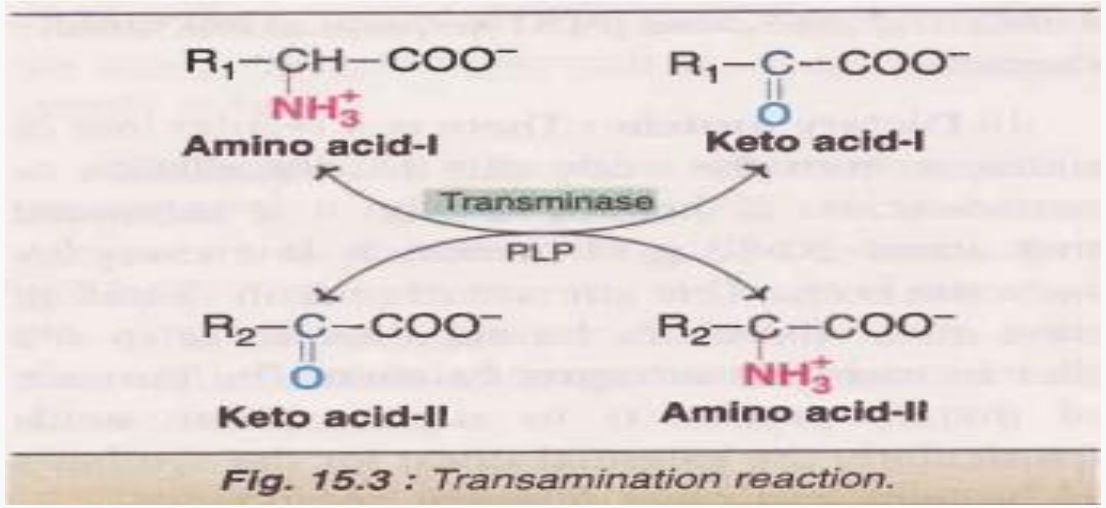


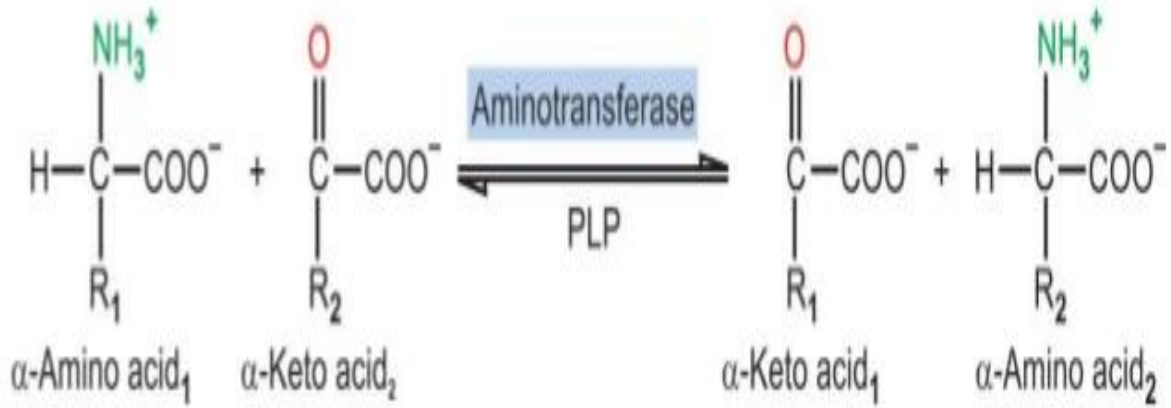
Fig. 15.3 : Transamination reaction.

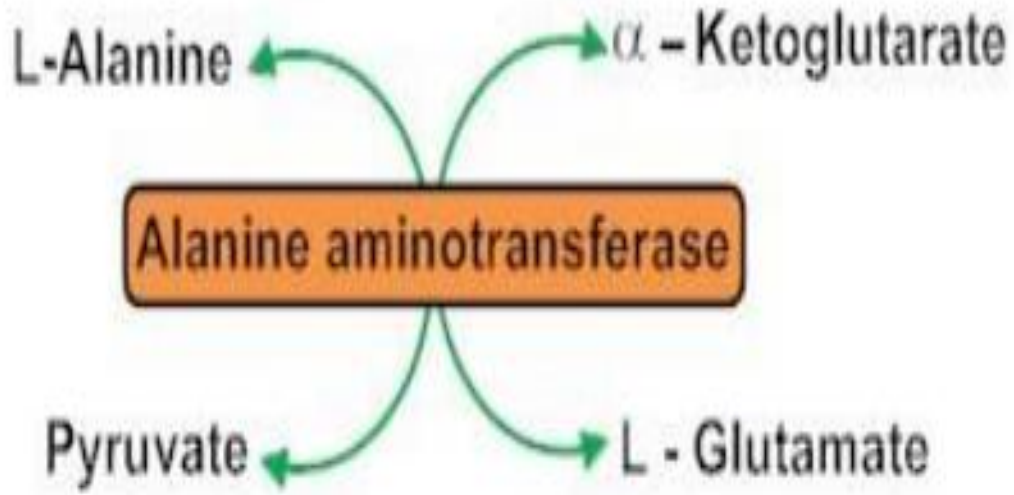
Alanine transferase(ALT)
Aspartate transaminase(AST)

Characteristics of Transamination

- Pyridoxal Phosphate – COFACTOR **PLP(pyridoxal phosphate)- vitamine B_6**
- Transamination is reversible.
- It involves both anabolism and catabolism,
- It diverts the excess AAs towards **energy production**.
- There is no free NH_3 liberated, **only transfer of amino group**.
- Except **lysine, threonine, proline, and hydroxy proline,** all AAs participate in transamination reactions.
- Transamination is not restricted to α -amino groups.
- The δ -amino group of ornithine and the Σ -amino group of lysine—readily undergoes transamination.
- Liver, Kidney, Heart, Brain- adequate amount of these enzymes.

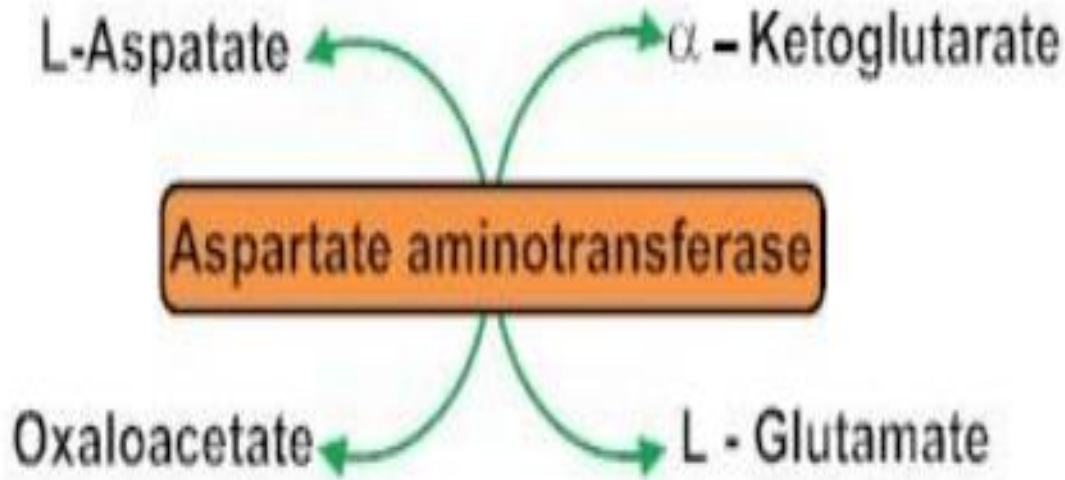
- **Serum transaminases are important for diagnostic and prognostic purpose**



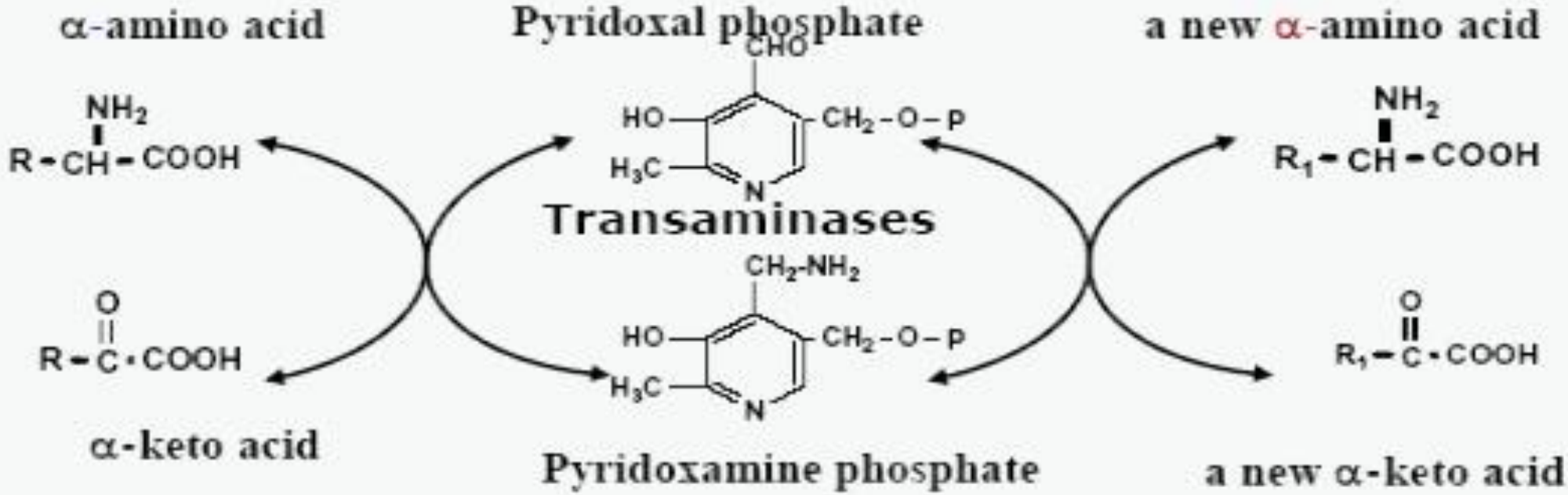


L-Alanine + α-Ketoglutarate ⇌ (ALT or GPT; PLP) Pyruvate + L-Glutamate

L-Aspartate + α-Ketoglutarate ⇌ (AST or GOT; PLP) Oxaloacetate + L-Glutamate



Role of B6 Phosphate in transamination



- The transfer of α-amino group from donor amino acid to **Pyridoxal phosphate** forms Pyridoxamine phosphate, and a keto acid.
- The α-amino group is finally passed on to acceptor α-keto acid to form a new amino acid.

Metabolic significance of Transamination reactions

- This reaction provides a mechanism for **collecting the amino groups** from various AAs into one common product **L-glutamate**.
- This is important because glutamate is the only AA whose α-amino group can be directly removed at a high rate by **oxidative deamination**.
- Functions both in amino acid **catabolism & biosynthesis**.
- The synthesis of **non-essential amino acids**.

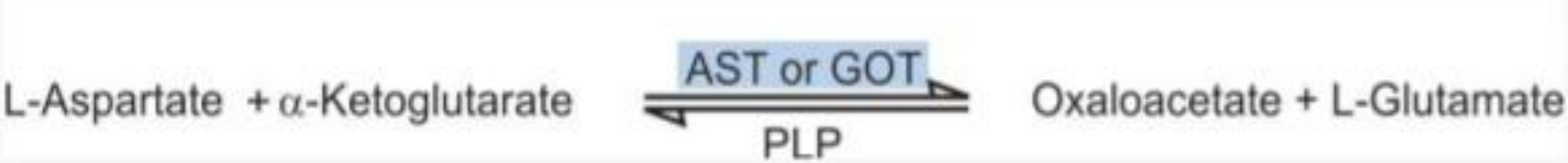
Clinical significance of transaminase enzyme

Serum levels of some transaminases are elevated in some disease state and measurement of these are useful in medical diagnosis, e.g. ALT (SGPT) and AST (SGOT) are important in the diagnosis of liver and heart damage

Aspartate transaminase (AST / SGOT)

Serum glutamate oxalo transaminase

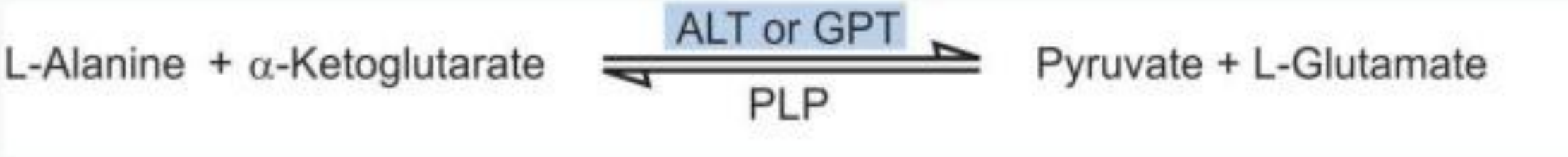
- mitochondrial enzyme.
- heart, liver, muscle, kidney.
- specific to heart damage
- important in liver since half of urea-N is from Aspartate.



Alanine Transaminase (ALT/ SGPT)

Serum glutamate pyruvate transaminase

- cytosolic enzyme,
- specific to liver damage.
- important in muscle where ~25% of AA-N is transported out as alanine
- In liver, reverse reaction moves AA-N back on GLU .



DEAMINATION

Mitochondrial Matrix

Deamination

- The removal of amino group from A.A , as free NH3.
- This occurs both as
  - Oxidative deamination.
  - Non-oxidative deamination.

- **Deamination results in the liberation of ammonia for urea synthesis**

Some authors use the term transdeamination while describing the reactions of transamination and deamination,particularly involving glutamate

Glutamate is the only amino acid that undergoes **oxidative deamination** to a significant extent to **liberate free NH3** for **urea synthesis**

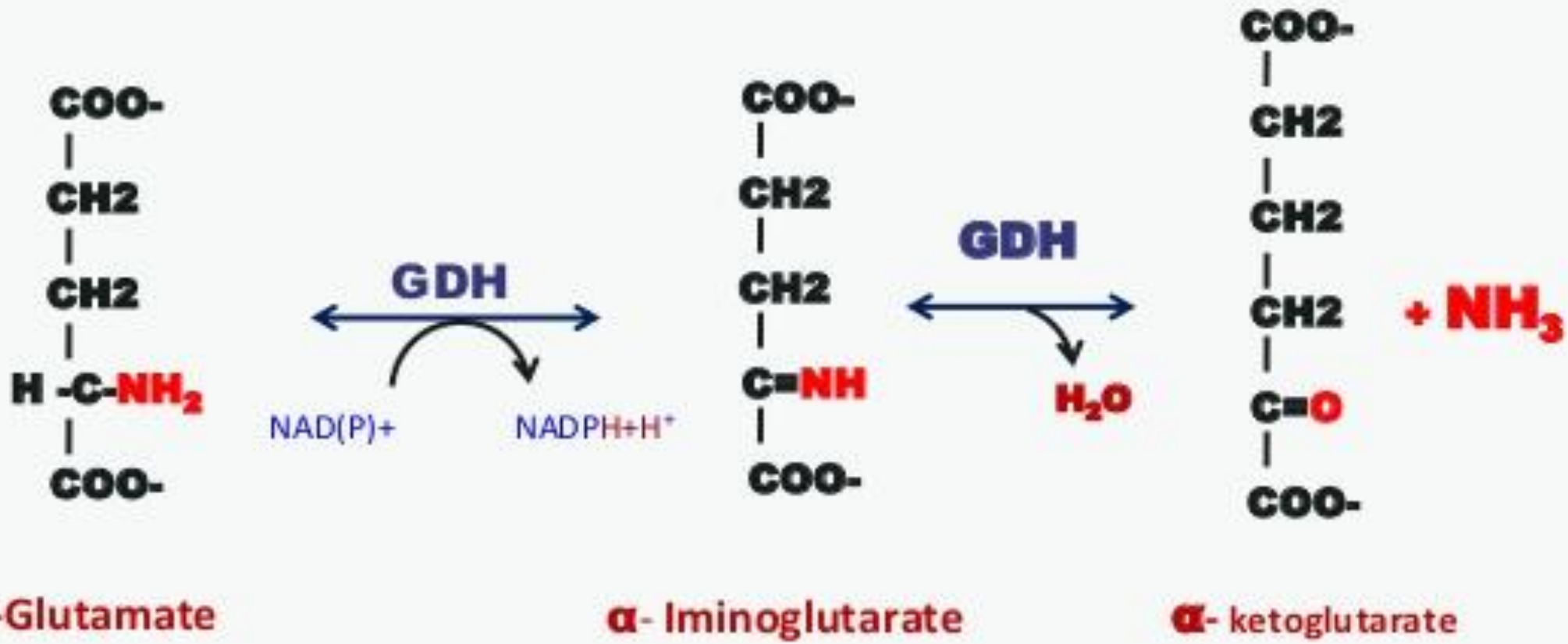
OXIDATIVE DEAMINATION

- ✓ In this **deamination coupled with oxidation**.
- ✓ Removal of NH3 from amino group of Glutamate, coupled with oxidation.
- ✓ Glutamate is the only AA , which undergoes oxidative deamination at a significant rate.
- ✓ Site:
  - ✓ **Liver** and kidney.
  - ✓ Mitochondria.
- ✓ Catalysed by **Glutamate Dehydrogenase** –
- ✓ Co-enzyme – NAD^+ / $NADP^+$.

Glutamate dehydrogenase *(gdh)*

- ✓ Mitochondrial enzyme
- ✓ Metalloenzyme (Zn^{2+}).
- ✓ It is the only enzyme that can accept either NAD^+ or $NADP^+$ as its coenzyme.

Glutamate serves as a 'collection centre' for amino groups in the biological system.


COO-
CH2
CH2
H -C-NH2
COO-
GDH
NAD(P)+
NADPH+H+
COO-
CH2
CH2
C=NH
COO-
GDH
H2O
COO-
CH2
CH2
CH2
C=O
COO-
+ NH3
L-Glutamate
α- Iminoglutarate
α- ketoglutarate

Regulation of GDH activity

- The activity of glutamate dehydrogenase is allosterically regulated.
- Consists of 6 identical subunits .
- Allosteric regulation–
  - GTP and ATP – allosteric inhibitors.
  - GDP and ADP – allosteric activators.
- ↓ Energy – ↑ oxidation of A.A.
- Steroid and thyroid hormones inhibit GDH

Metabolic Significance

- Reversible Reaction
- Both **Anabolic and Catabolic**.
- Catabolic – Channels nitrogen from Glutamate to urea.
- Anabolic – amination of α- KG by NH3 to Glutamate.

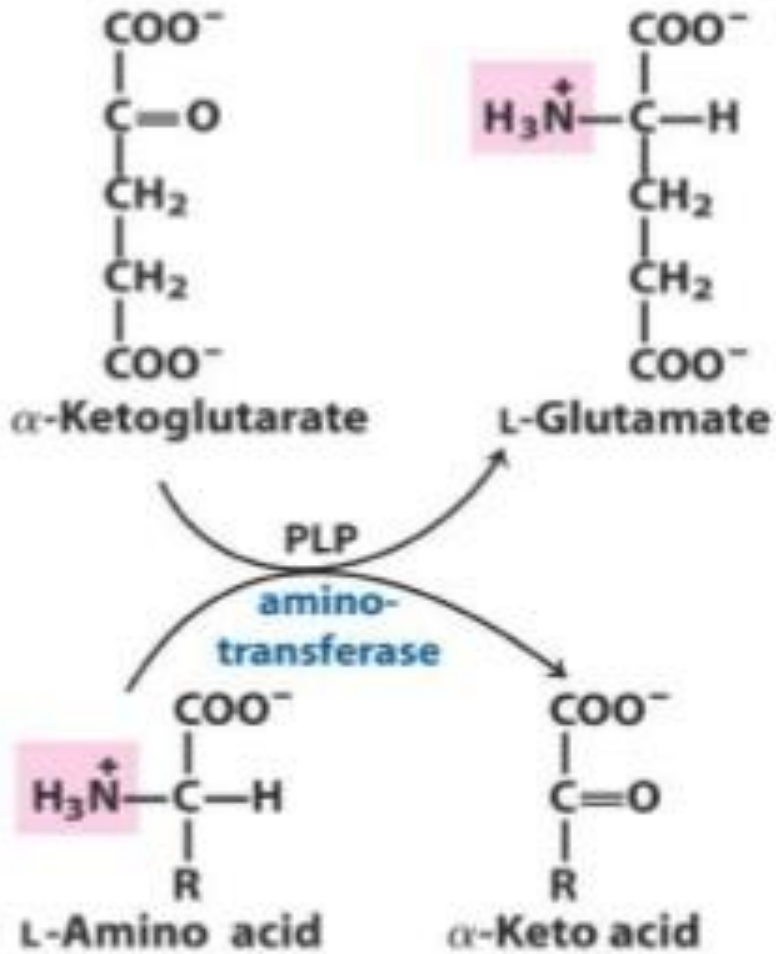
Role of Glutamate

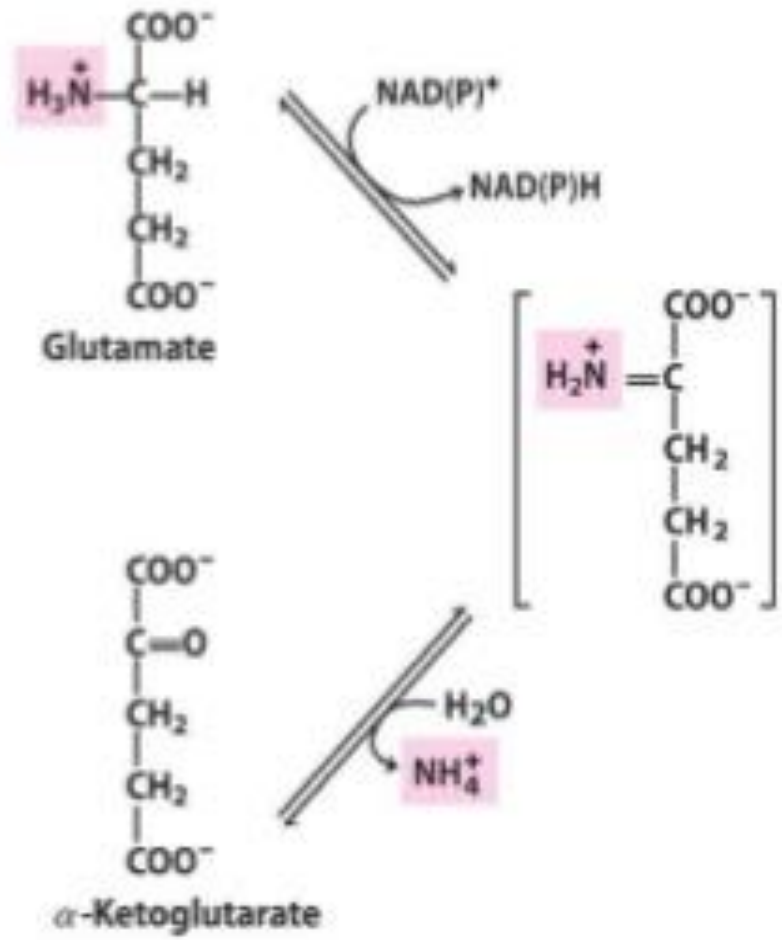
- Glutamate occupies a central place in the amino acid metabolism.
- Basically it acts as **a collector of amino group** of the amino acids.
- All the amino nitrogen from amino acids that undergo transamination can be **concentrated in glutamate**.
- L-glutamate is the only amino acid that undergoes oxidative deamination at an appreciable rate in mammalian tissues.
- The formation of ammonia from α -amino groups thus occurs mainly via the α -amino nitrogen of L-glutamate.

Role of Glutamate & Glutamate dehydrogenase

- Since in majority of the transamination reactions **alpha ketoglutarate is the acceptor** keto acid forming Glutamate, that is **oxidatively deaminated** in the liver by Glutamate dehydrogenase to form alpha ketoglutarate and ammonia.
- Conversion of α-amino nitrogen to ammonia by the concerted action of glutamate aminotransferase and **GDH is often termed "transdeamination."**
- Thus Transamination and deamination are coupled processes though they occur at distant places.

Transdeamination





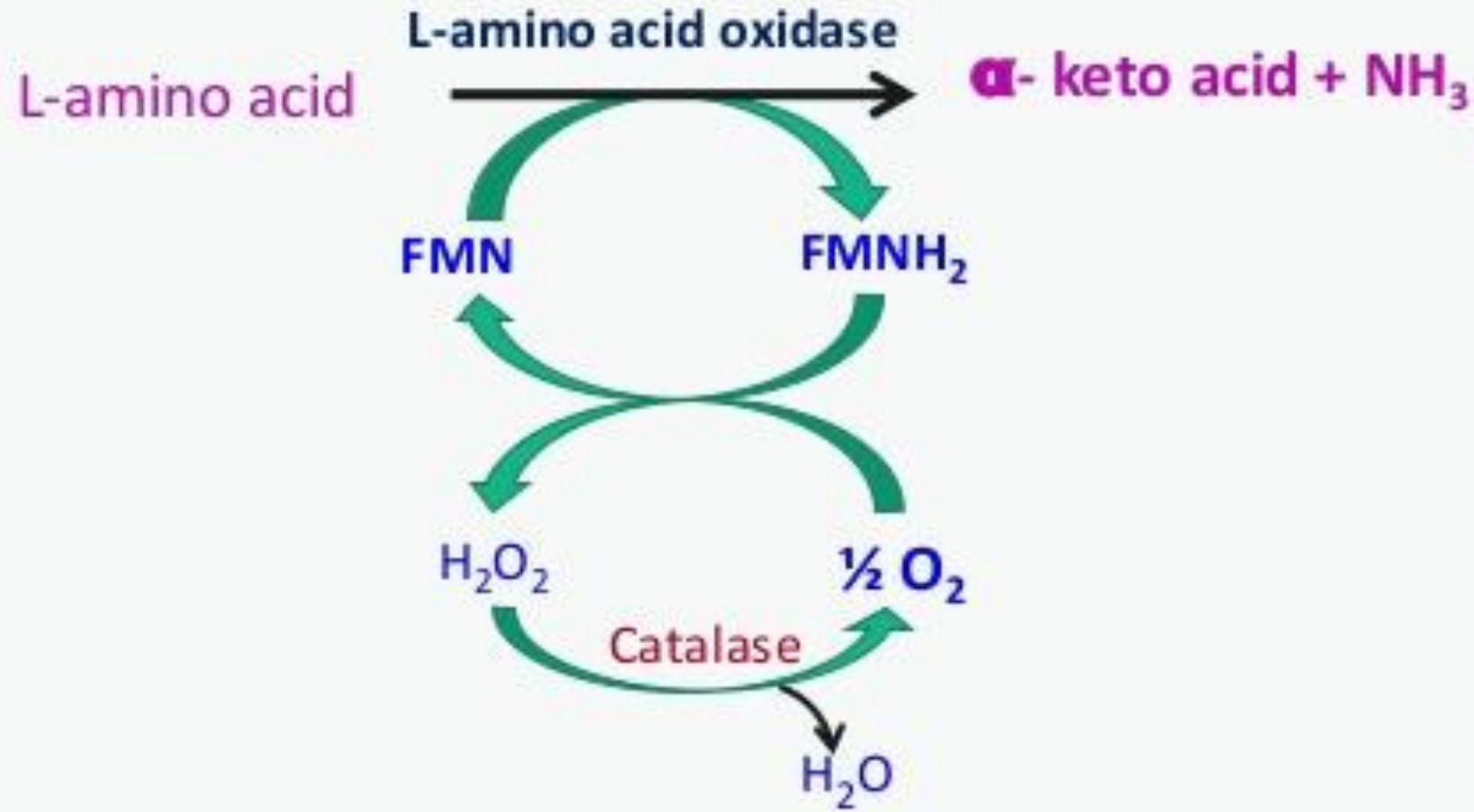
MINOR PATHWAYS OF DEAMINATION

Amino acid Oxidases

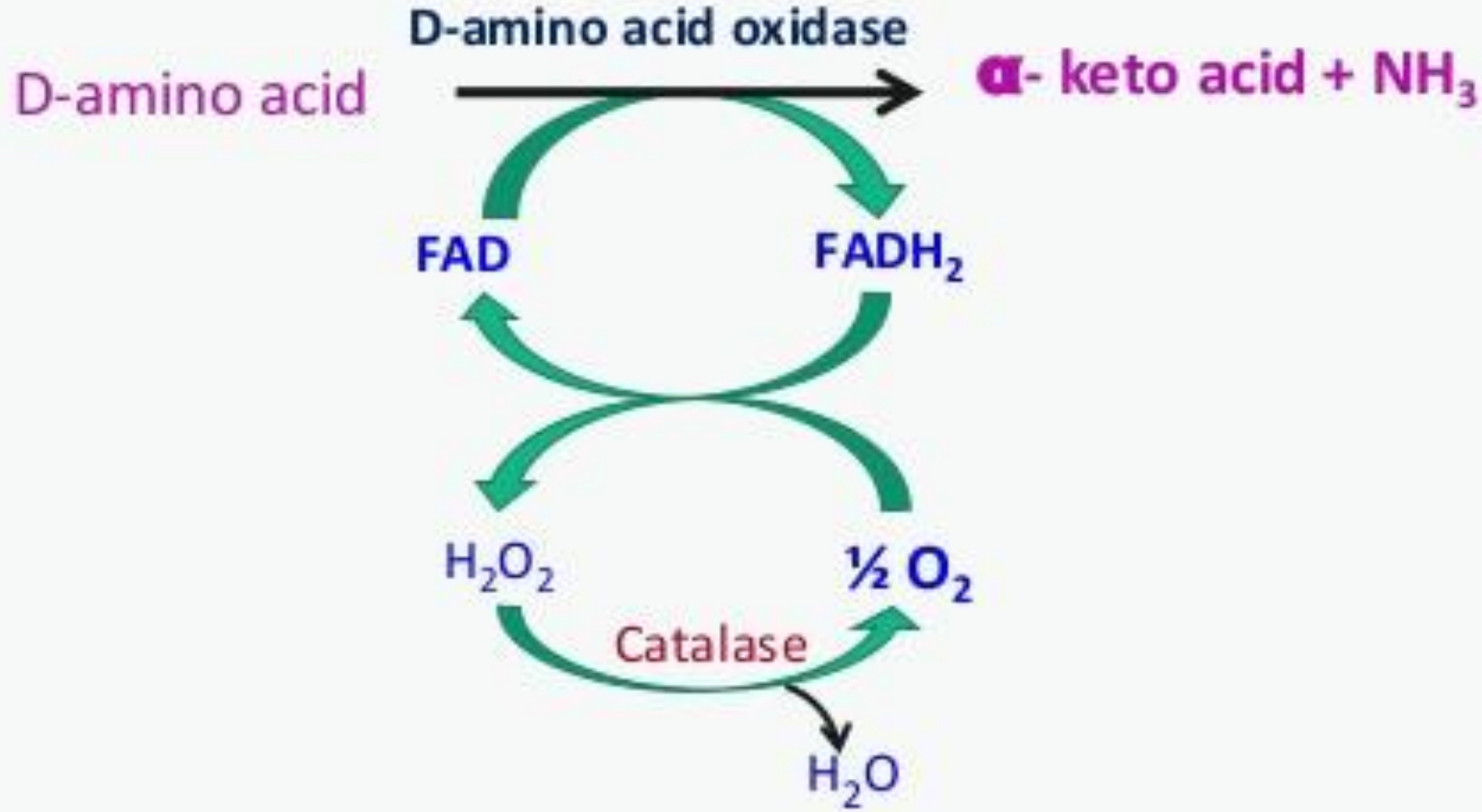
L-amino acid oxidase and D-amino acid oxidase

- Flavoproteins
- Cofactors - FMN and FAD .

- Site - Liver, kidney .
  Peroxisomes .
- Activity of L-AA Oxidase is low.
- Thus , a minor role in AA catabolism.

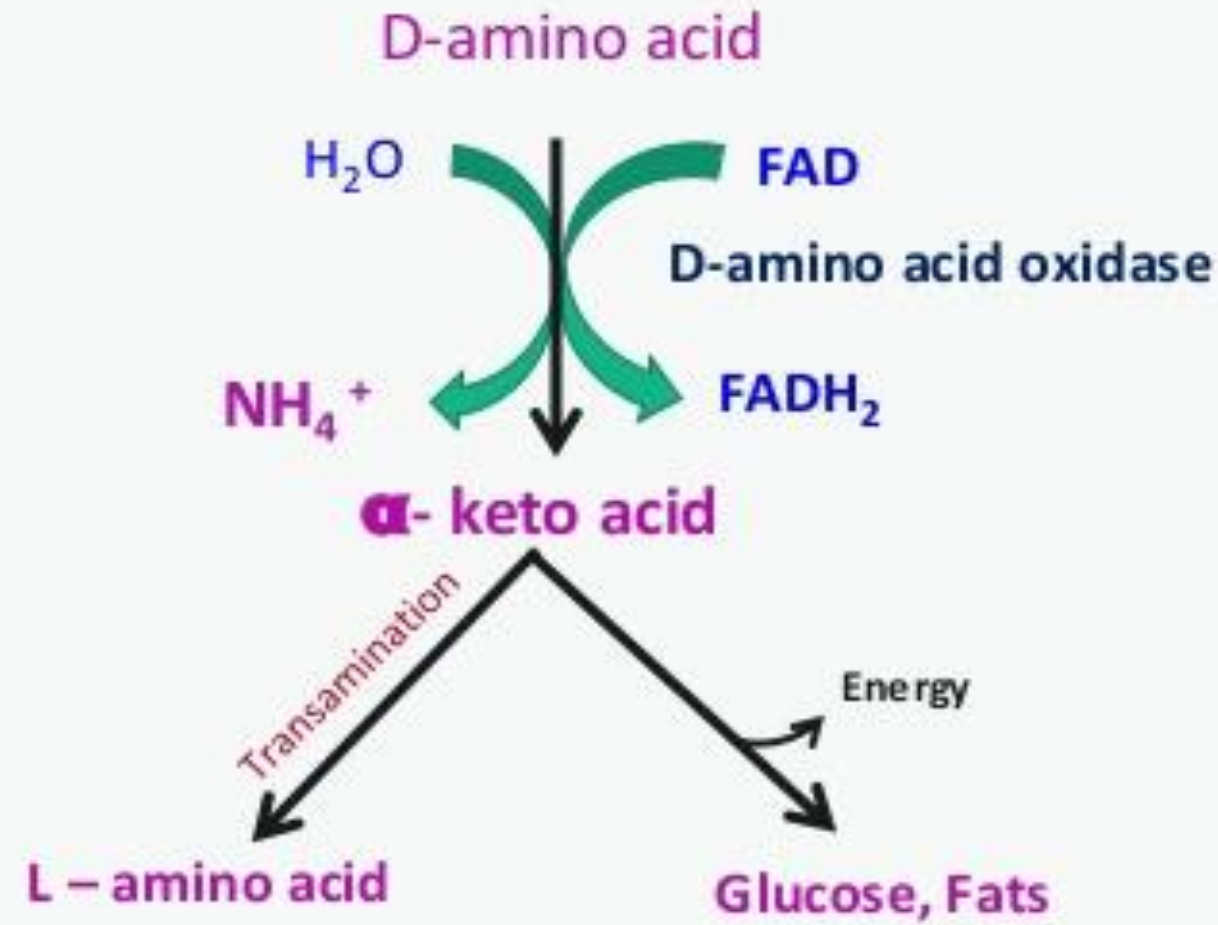


L-AA Oxidase – acts on all A.A, except Hydroxy , dicarboxylic AA .



Activity of D-AA oxidase is high than that of L-AA oxidase.
(Degrades D-AA in bacterial cell wall).

Metabolic fate of D - amino acids



Fate of D-amino acids

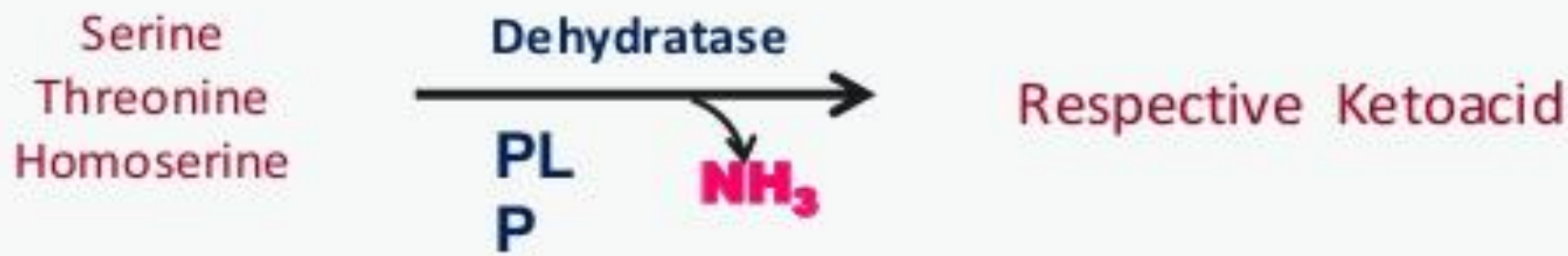
- D-amino acids are found in plants and microorganisms.
- They are not present in mammalian proteins
- D-amino acids are taken in the diet / bacterial cell wall, absorbed from gut - acted on by D- AA oxidase to the respective α-keto acids.
- The α-keto acids undergo transamination to be converted to L-amino acids Which participate in various metabolic pathways.

Non -Oxidative deamination

- Direct deamination, without oxidation.

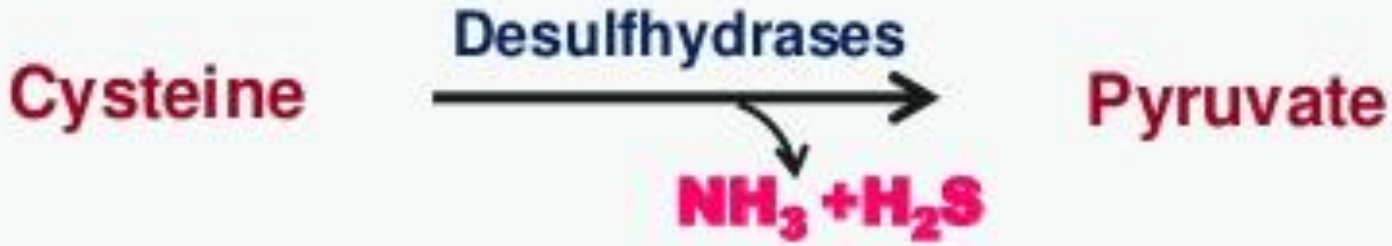
Amino acid Dehydratases :

- Serine, threonine and homoserine are the hydroxy amino acids.
- They undergo non-oxidative deamination catalyzed by PLP-dependent dehydratases



Amino acid desulfhydrases

- Cysteine and homocysteine undergo deamination coupled with desulfhydration to give keto acids.
-



- Deamination of histidine.

METABOLISM OF AMMONIA

metabolism of ammonia

- At the physiological pH, ammonia exists as NH_4^+ ions.

Formation of ammonia:

- Amino acids – by Trans-deamination.
- Biogenic amines
- Pyrimidine catabolism.
- by action of intestinal bacteria on urea.

metabolism of ammonia

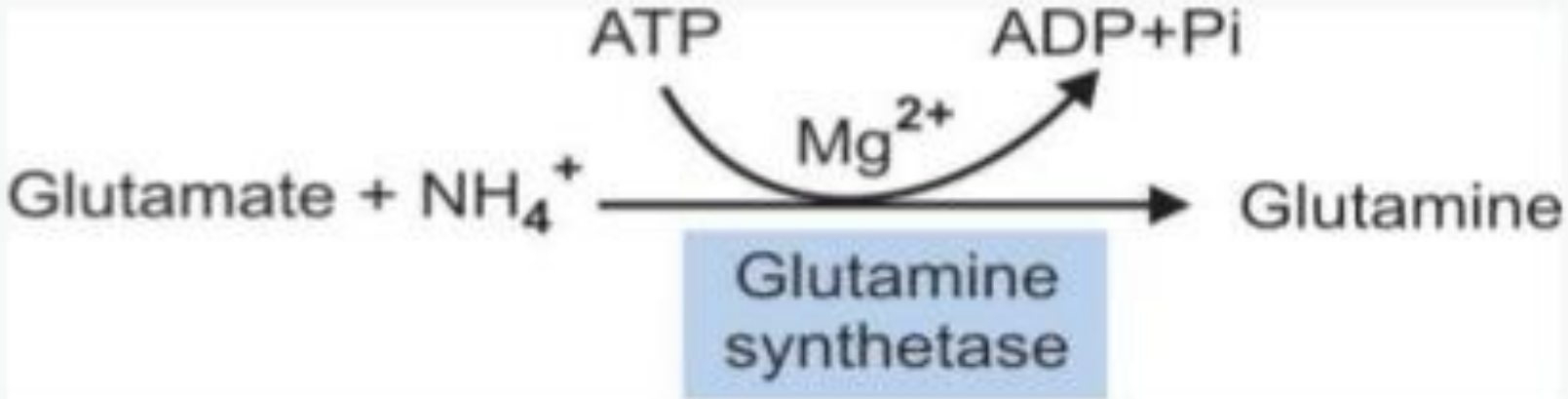
- Ammonia is produced in most tissues.
- Since ammonia is extremely toxic,
- For the ultimate conversion of ammonia to urea, it is transported to the liver.
- It is immediately converted to nontoxic metabolites such as,
  - glutamate (All tissues)
  - glutamine (Brain)
  - alanine (Muscle) &
  - ultimately to urea.

Transport of ammonia

- Since free ammonia is highly toxic, it is never transported in free form in blood.
- Two mechanisms are available in humans for the transport of ammonia from the peripheral tissues to the liver for its ultimate conversion to urea.
  - **Transport of Ammonia in the Form of Glutamine**
  - **Transport of Ammonia in the Form of Alanine**

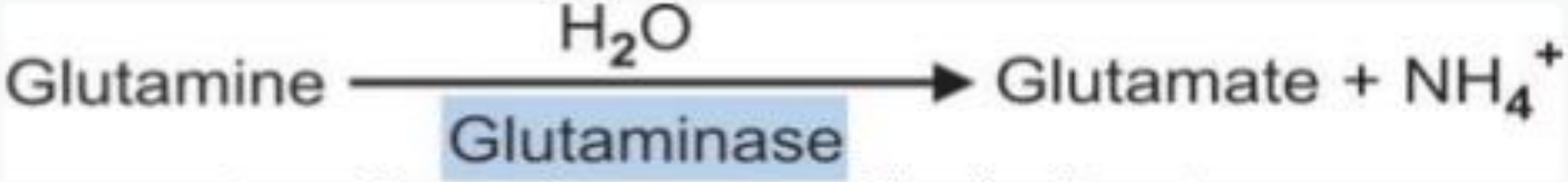
Transport of Ammonia in the Form of Glutamine

- Glutamine is a storehouse of NH3.
- It is present at the highest conc. in blood among the AAs.
- Glutamine serves as a storage and transport form of NH3.
- In many tissues (liver, kidney and brain), ammonia is enzymatically combined with glutamate to yield glutamine by the action of **glutamine synthetase.**



Transport of Ammonia in the Form of Glutamine

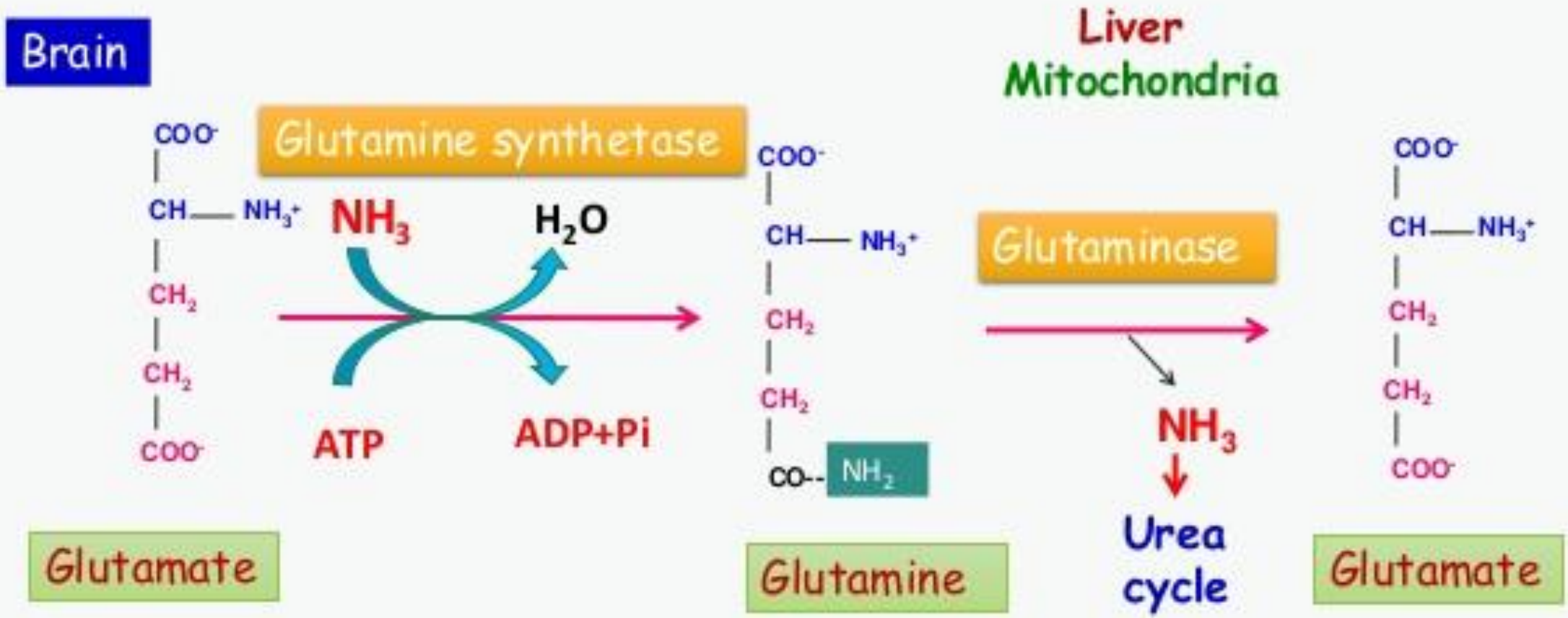
- The glutamine, so formed is a **neutral nontoxic** major transport form of ammonia.
- The glutamine is transported by blood to the liver, where it is cleaved by glutaminase to yield glutamate and free ammonia.



- The ammonia so formed is converted by the liver into urea.

Glutamine is a store house of NH_3(ammonia).

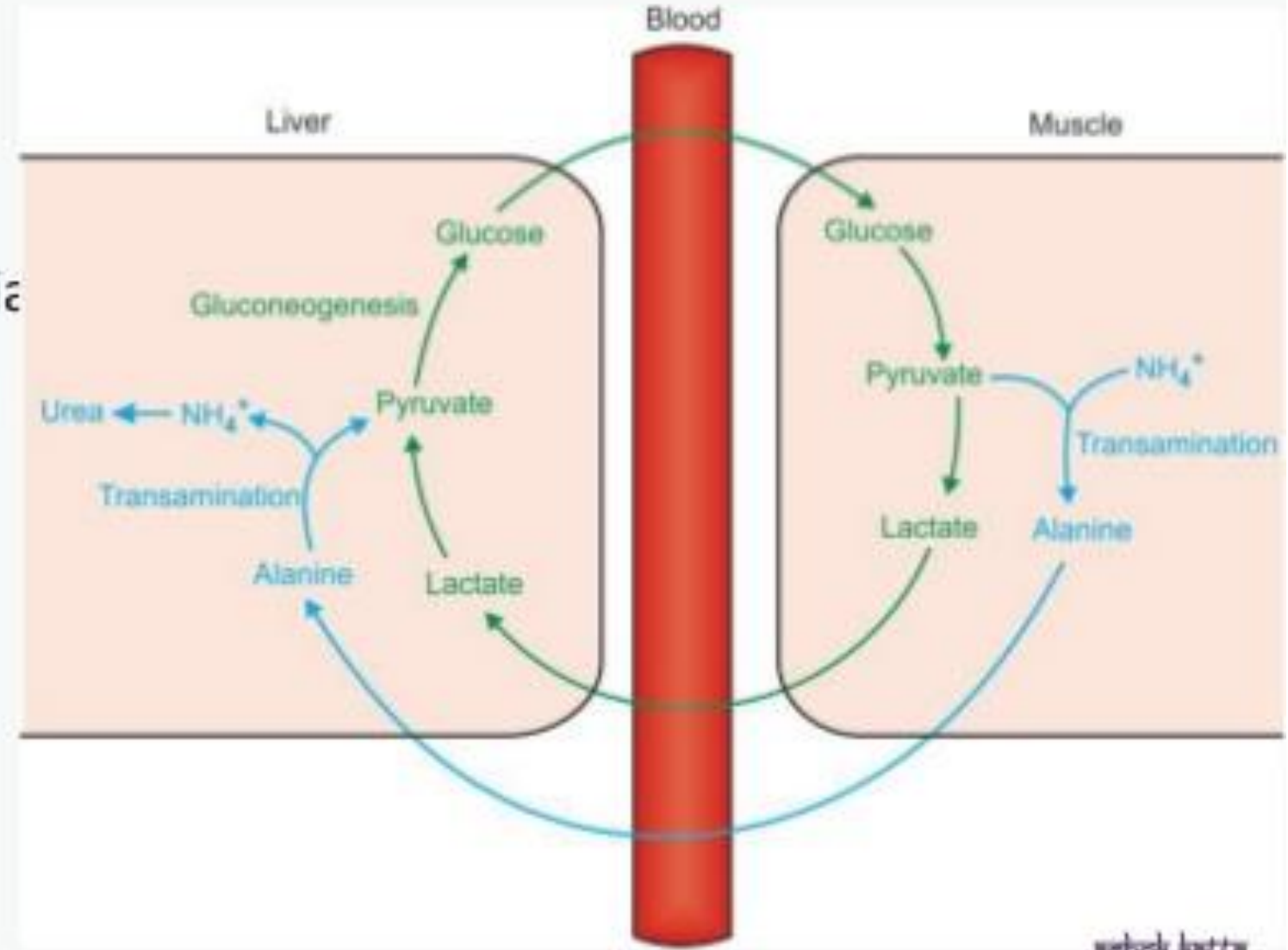
Synthesis Of Glutamine & Its Conversion To Glutamate



Glutamine is a non-toxic carrier of ammonia from Brain . It is released into blood circulation and carried to liver.

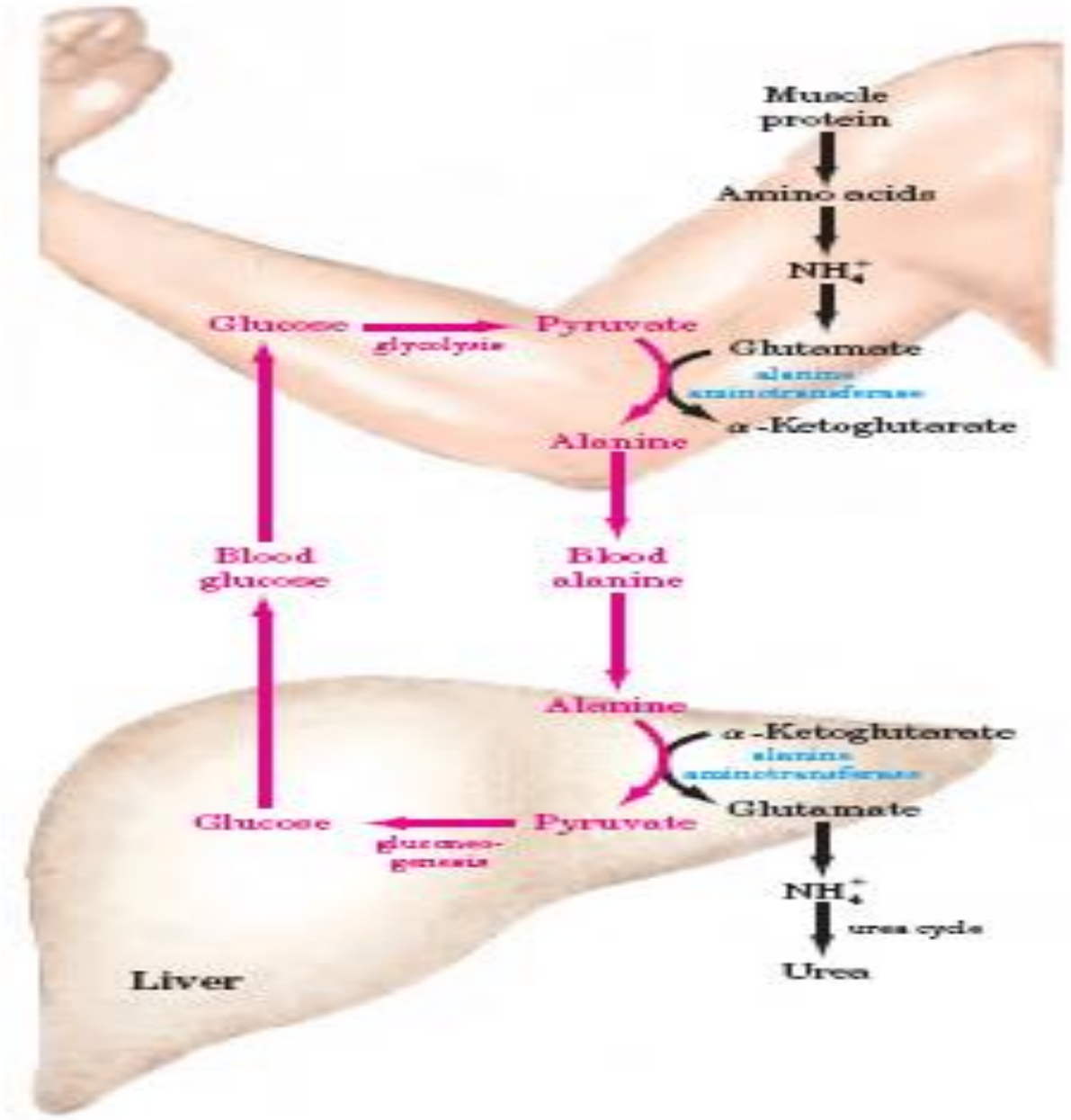
Transport of Ammonia in the Form of alanine

- Alanine transports ammonia from muscles to the liver through **glucose alanine cycle**.



Glucose-alanine cycle

FIGURE 18–9 Glucose-alanine cycle. Alanine serves as a carrier of ammonia and of the carbon skeleton of pyruvate from skeletal muscle to liver. The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.



1.Glutamate –

- ✓formed from cellular transamination.
- ✓major role in inter-organ transport of NH3.
- ✓Conc. of glutamate in blood is 10 times high than other AAs.
- ✓Glutamate from blood reaches Liver.
- ✓Here ,GDH liberates NH3 from Glutamate .

2. Glutamine from Brain .

- ✓Brain is very sensitive to NH_3, so, it has a special mechanism for immediate detoxification.

Glutamate – charged

- cannot pass .

Glutamine – neutral, non-toxic

- can pass through cell membrane.

Disposal of ammonia

(a) **Ammoniotelic** : The aquatic animals dispose off NH_3 into the surrounding water.

(b) **Uricotelic** : Ammonia is converted mostly to uric acid e.g. reptiles and birds.

(c) **Ureotelic** : The mammals including man convert NH_3 to urea. Urea is a non-toxic and soluble compound, hence easily excreted.

AMMONIA TOXICITY

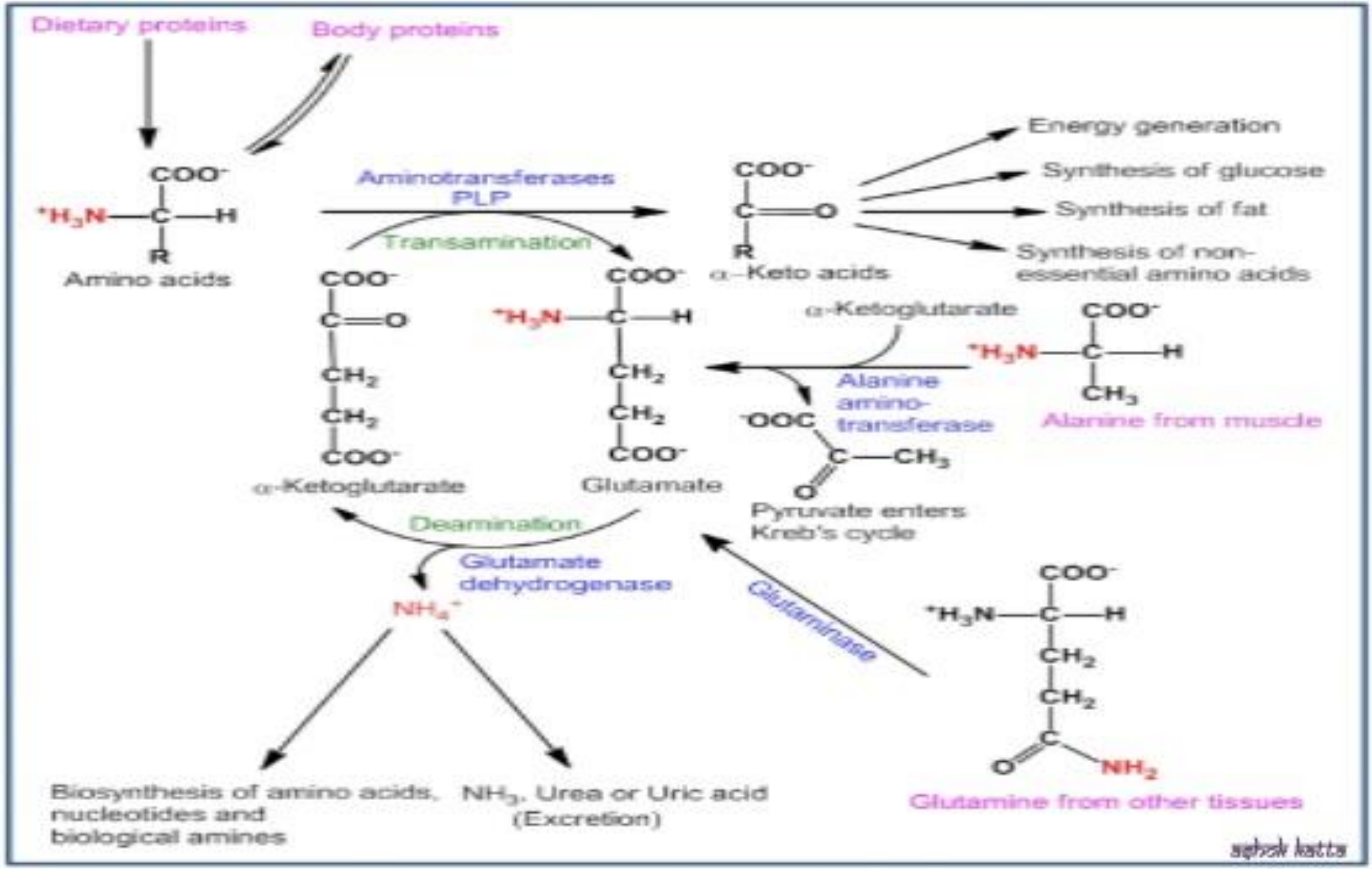
- Even a marginal elevation in the blood ammonia conc. is **harmful to the brain**.
- when ammonia accumulates in the body, results in...
  - slurring of speech,
  - Blurring of the vision, Tremors.
  - May lead to coma and finally,
  - Death, if not corrected.
- Elevation in blood NH_3 level may be genetic or acquired.
- Impairment in urea synthesis leads to hyperammonemia.
- And cause **hepatic coma** and **mental retardation.**
- The acquired hyperammonemia may be due to hepatitis, alcoholism etc.

Functions of Ammonia

- Ammonia is essential for the synthesis of non-essential amino acids, purines, pyrimidines, amino sugars & aspargine.
- Ammonium ions are very important to maintain acid-base balance of the body.

Ammonium ions= NH_4^+

Summary


Dietary proteins
Body proteins
Amino acids
Aminotransferases
PLP
Transamination
α-Keto acids
Energy generation
Synthesis of glucose
Synthesis of fat
Synthesis of non-essential amino acids
α-Ketoglutarate
Glutamate
α-Ketoglutarate
Alanine amino-transferase
Alanine from muscle
Pyruvate enters Kreb's cycle
Deamination
Glutamate dehydrogenase
NH_4^+
Glutaminase
Glutamine from other tissues
Biosynthesis of amino acids, nucleotides and biological amines
NH_3, Urea or Uric acid (Excretion)

Urea Cycle

- Urea is the **end product** of protein metabolism (amino acid metabolism).
- Urea is synthesized in **liver** and transported to kidneys for excretion in urine.
- Urea cycle is the first metabolic cycle that was elucidated by Hans Krebs and Kurt Henseleit (1932), hence it is known as Krebs-Henseleit cycle.
- Urea has **two amino (-NH) groups**, one derived from **NH_3** and the other from **aspartate.**
- Carbon atom is supplied by CO_2.
- The **first two enzymes are present in mitochondria** while the **rest are localized in cytosol.**
- Urea accounts for 80-90% of the nitrogen containing substances excreted in urine.

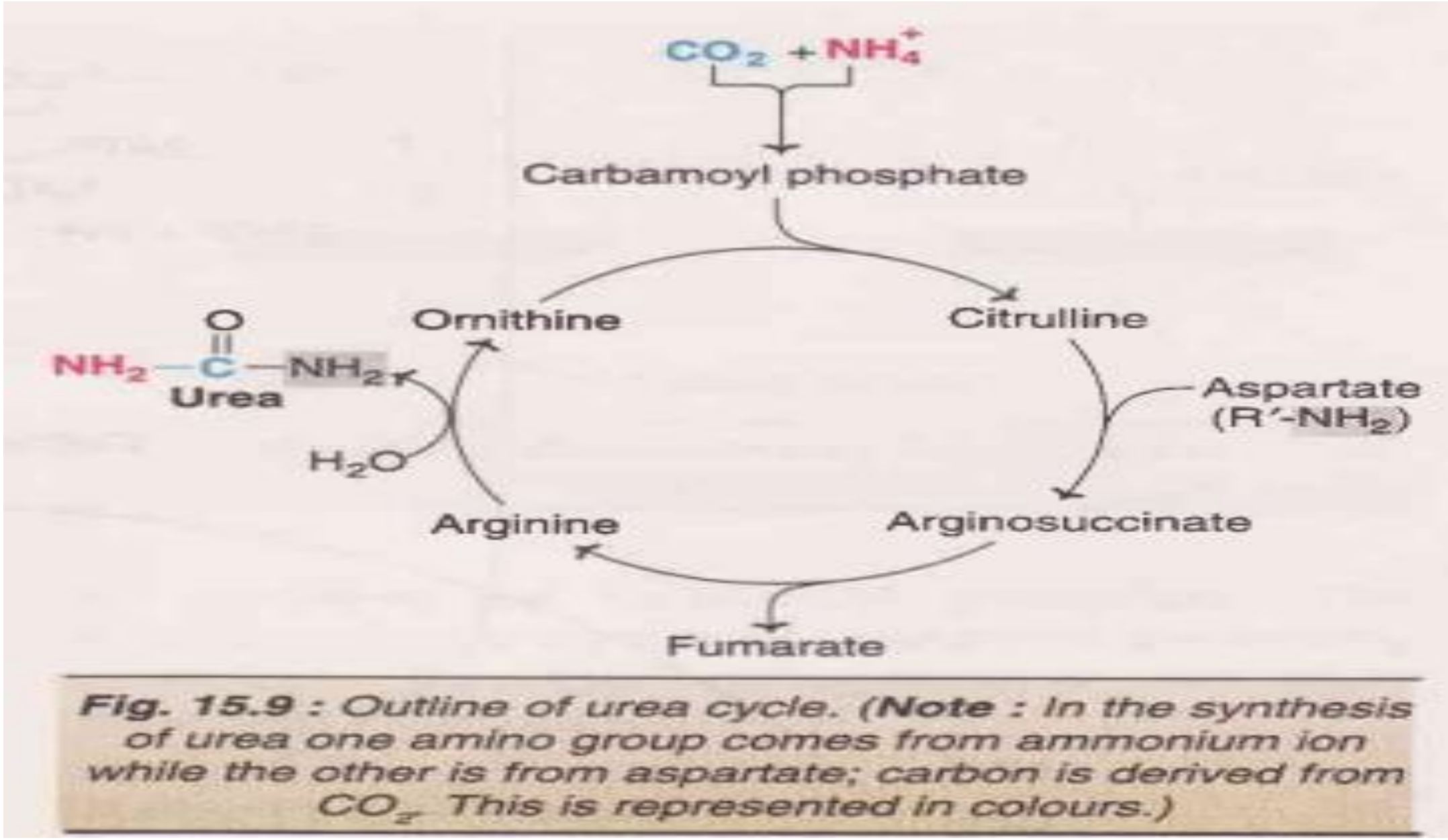


***Fig. 15.9** : Outline of urea cycle. (**Note** : In the synthesis of urea one amino group comes from ammonium ion while the other is from aspartate; carbon is derived from CO_2. This is represented in colours.)*

1. Synthesis of carbamoyl phosphate :

- **Carbamoyl phosphate synthase | (CPS I)** of mitochondria catalyses the condensation of NH_4+ ions with CO_2 to form carbamoyl phosphate. This step consumes **two ATP** and is **irreversible**, and **rate-limiting**.
- CPS I requires **N-acetylglutamate (NAG)** for its activity.

2. **Arginase** is mostly found in the **liver**, while the rest of the enzymes (four)of urea cycle are also present in other tissues.

For this reason, arginine synthesis may occur to varying degrees in many tissues. But only the liver can ultimately produce urea.

Carbamoyl Phosphate Synthetases

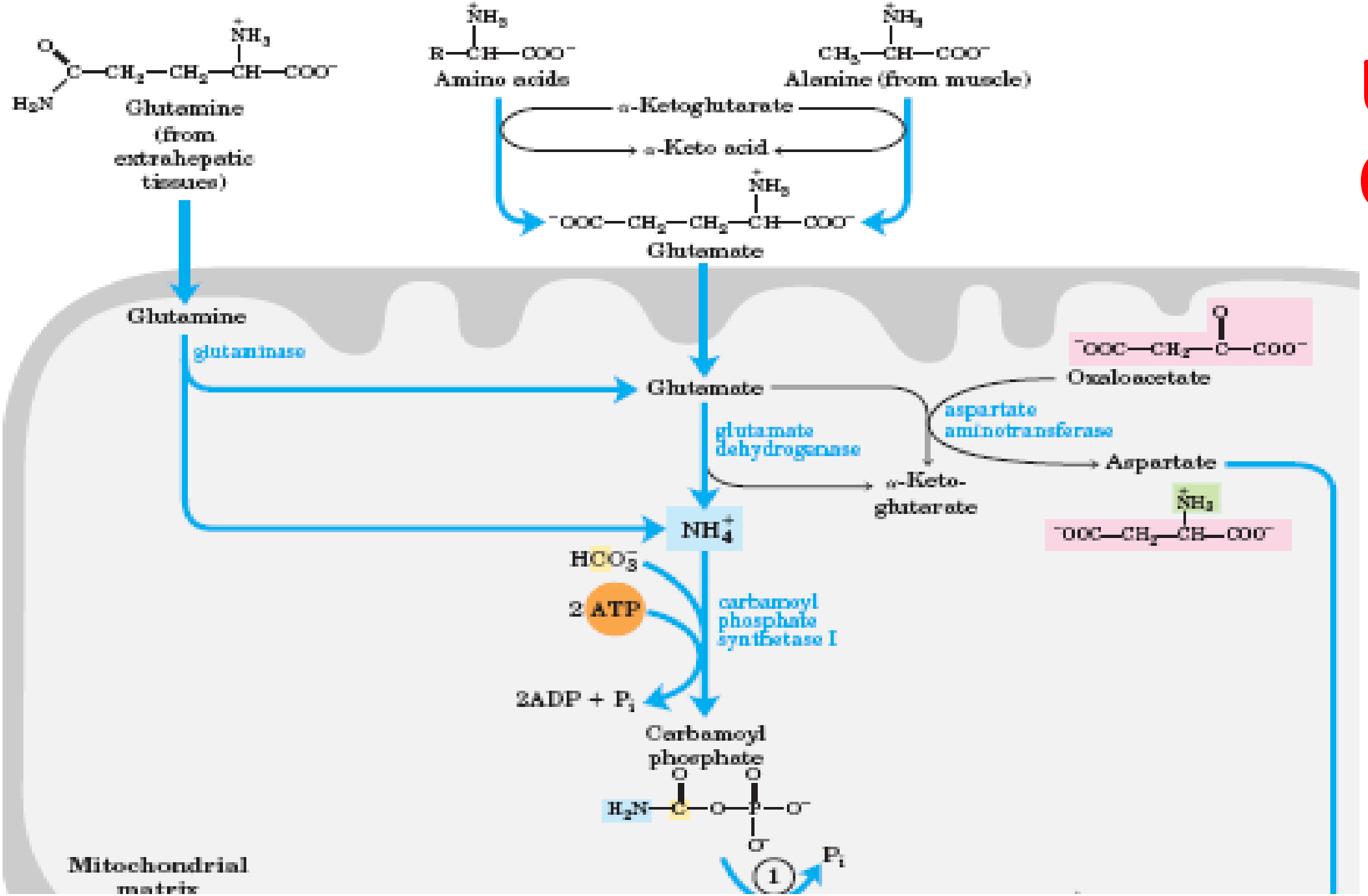
CPS-I

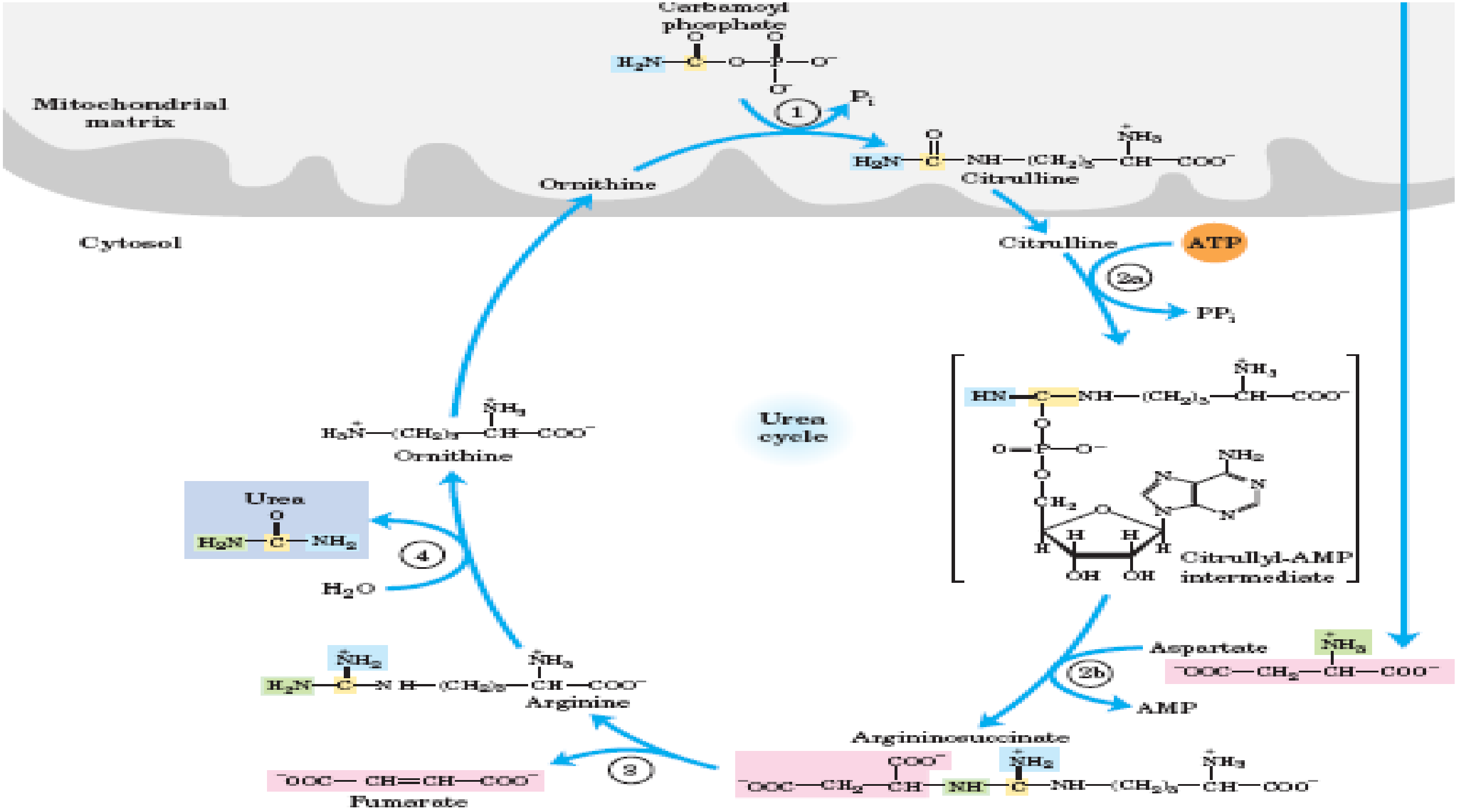
- Mitochondria
- Uses NH_3
- Urea Cycle
- Activated – NAG

CPS-II

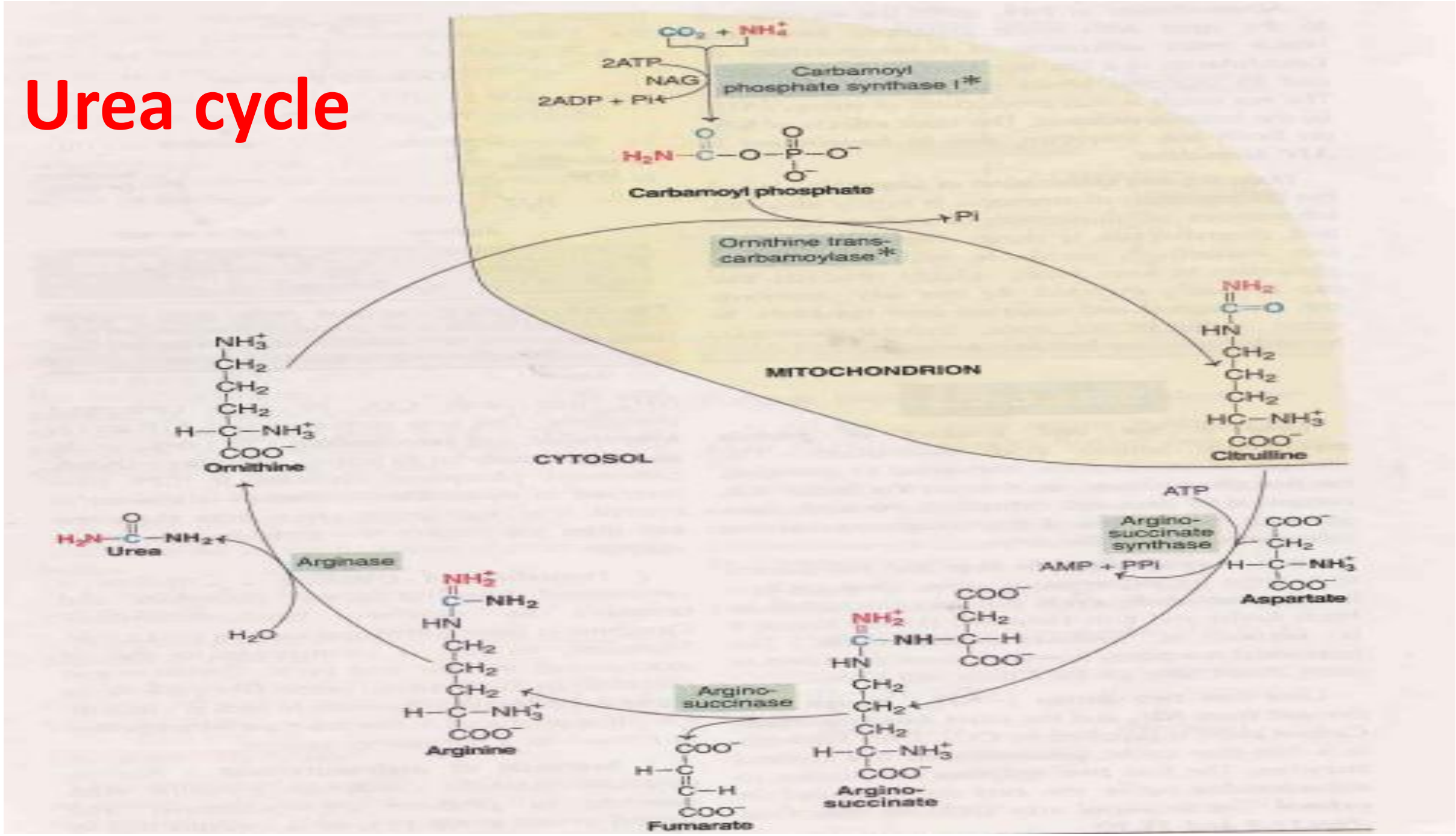
- Cytosol
- Uses Glutamine
- Pyrimidine biosynthesis
- Inhibited - CTP

Urea Cycle


Glutamine
(from extrahepatic tissues)
Amino acids
Alanine (from muscle)
α-Ketoglutarate
α-Keto acid
Glutamate
Glutamine
glutaminase
Glutamate
glutamate dehydrogenase
α-Keto-glutarate
Oxaloacetate
aspartate aminotransferase
Aspartate
NH_4^+
HCO_3^-
2 ATP
carbamoyl phosphate synthetase I
2ADP + P_i
Carbamoyl phosphate
P_i
1
Mitochondrial matrix


Carbamoyl phosphate
Mitochondrial matrix
Cytosol
Ornithine
Citrulline
ATP
PPi
Urea cycle
Citrullyl-AMP intermediate
Aspartate
AMP
Argininosuccinate
Fumarate
Arginine
Urea
H2O
Ornithine

Urea cycle


CO_2 + NH_4^+
2ATP
NAG
2ADP + Pi
Carbamoyl phosphate synthase I*
Carbamoyl phosphate
Pi
Ornithine trans-carbamoylase*
MITOCHONDRION
CYTOSOL
Ornithine
Citrulline
ATP
Argino-succinate synthase
AMP + PPi
Aspartate
Urea
Arginase
H_2O
Arginine
Argino-succinase
Fumarate
Argino-succinate

Regulation of urea cycle

- The first reaction catalysed by carbamoyl phosphate synthase I (CPS l) is rate Jimiting reaction or committed step in urea synthesis.
- CPS I is allosterically **activated by N-acetylglutamate (NAG).**
- It is synthesized from glutamate and acetyl CoA by synthase and degraded by a hydrolase.

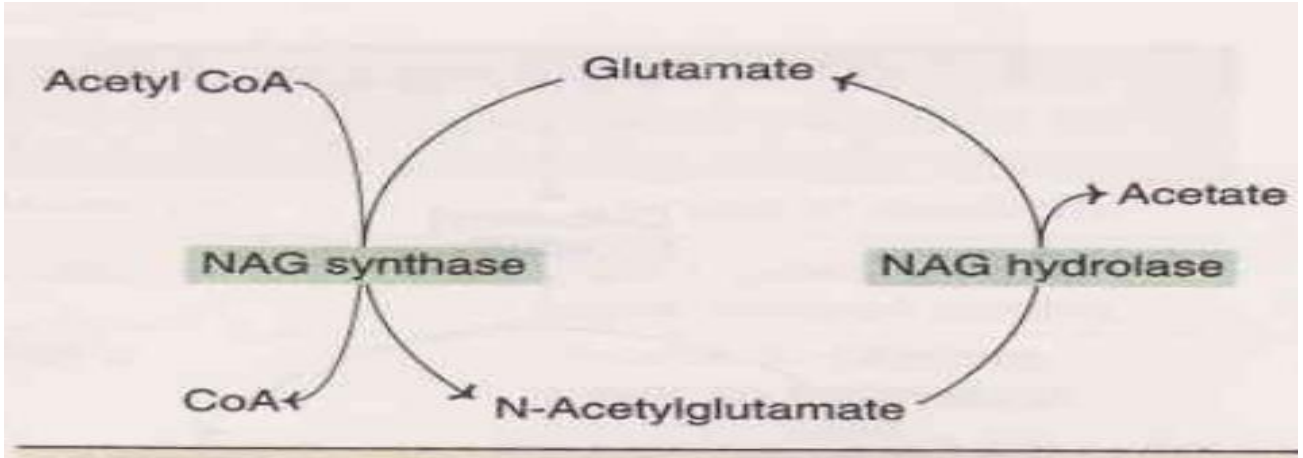


Fig. 15.11 : Formation and degradation of N-acetylglutamate.

Bioenergetics of urea cycle

- The urea cycle is **irreversible**
- consumes 4 ATP.
- Two ATP are utilized for the synthesis of carbamoyl phosphate.
- One ATP is converted to AMP and PPi to produce arginosuccinate which equals to 2 ATP.
- Hence **4 ATP are actually consumed.**

Inherited disorders of urea cycle

Disoeders	Defective Enzyme	Products accumulated
Hyperammonaemia-1	Carbamoyl Phosphate Syntethase -1	Ammonia
Hyperammonaemia-2	Ornithine transcarbomylase (orotic aciduria-most common)	Ammonia
Citrullinemia	Argininino succinate Syntheatse	Citrulline
Arginosuccinic aciduria	Argininosuccinate lyase	Arginosuccinate
Argininemia	Arginase	Arginine

Integration by urea cycle and TCA cycle

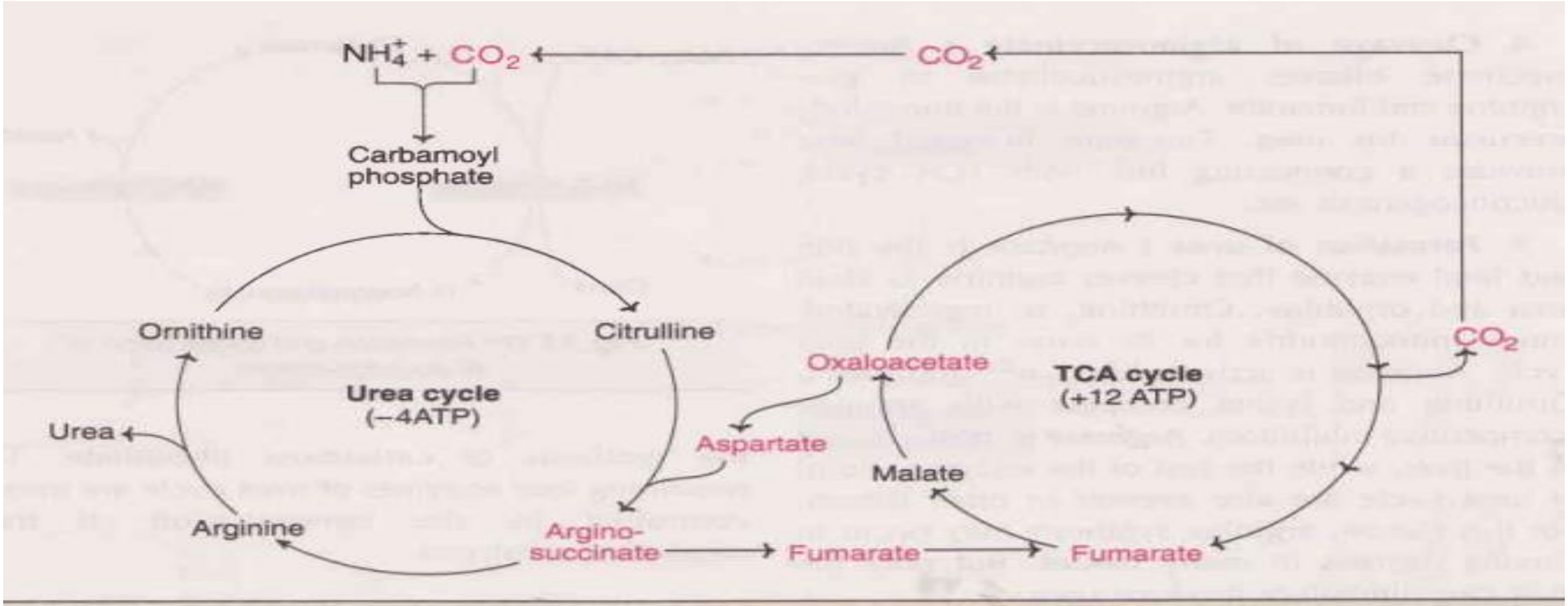


Fig. 15.12 : Interrelation between urea and tricarboxylic acid (TCA) cycle (Depicted in blue colour).

- ATP (12) are generated in the TCA cycle
- while ATP (4) are utilized for urea synthesis

- The term '**uremia**' is used to indicate increased blood urea levels due to renal failure.
- **Azotemia** reflects a condition with elevation in blood urea,/or other nitrogen metabolites which may or may not be associated with renal diseases.

Non protein nitrogen (NPN)

- NPN refers to all the nitrogen-containing substances other than proteins. These include urea (most abundant), creatinine, creatine, uric acid, peptides, amino acids etc.
- The molecular weight of urea is 60 and about half of it (28) is contributed by the two nitrogen atoms. Thus, if blood urea concentration is 60 mg, then about half of it-28 mg-is blood urea nitrogen (BU N). Therefore,
- BUN = 1/2 NPN
- NPN = 2 BUN

Summary of amino acid metabolism

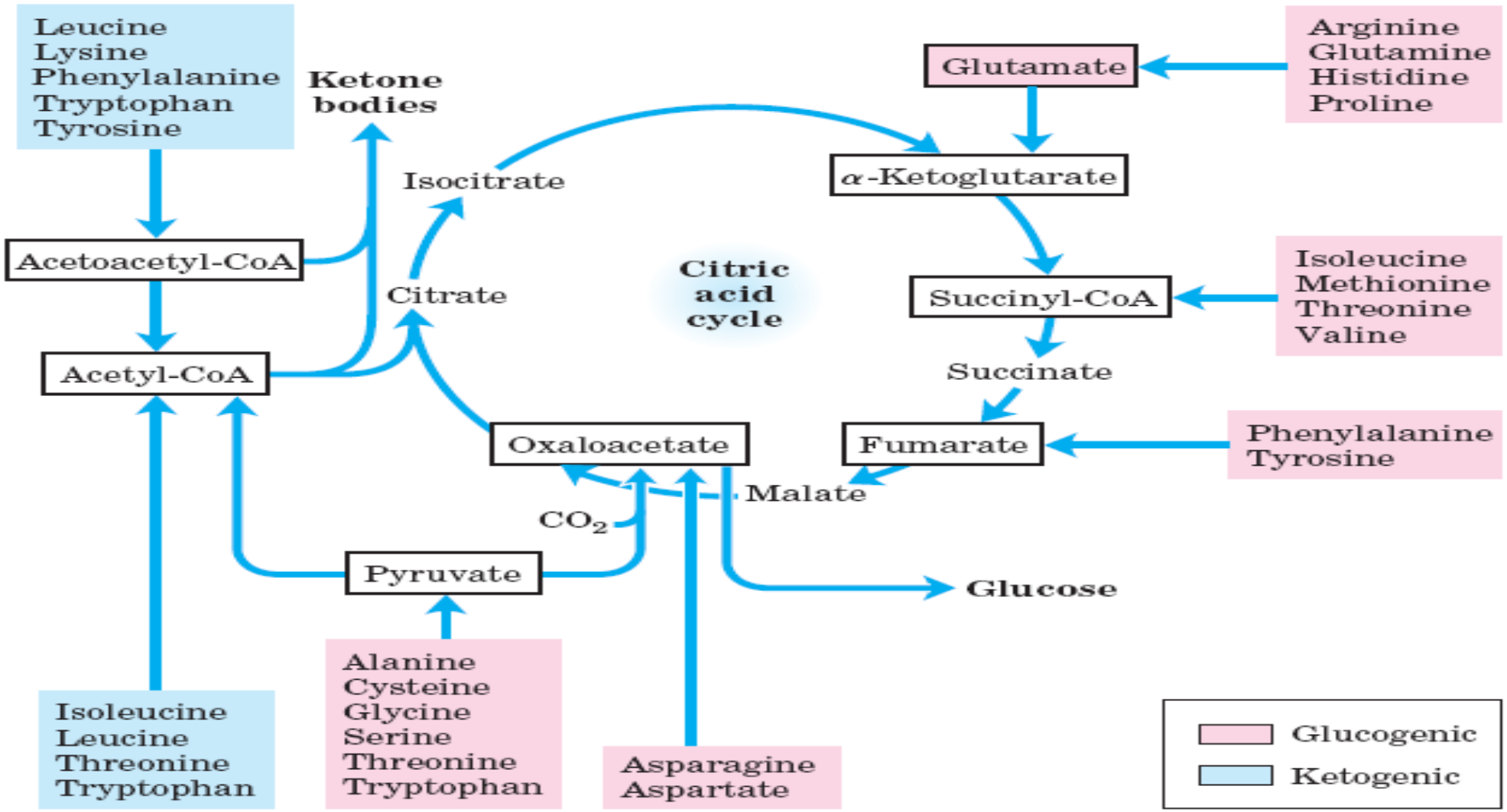


Table 15.4 Classification of amino acids based on the fate of carbon skeleton

Glycogenic (glucogenic)	***Glycogenic and ketogenic***	*Ketogenic*
Alanine	Phenylalanine*	Leucine*
Arginine*	Isoleucine*	Lysine*
Aspartate	Tyrosine	
Cysteine	Tryptophan*	
Glutamine		
Glutamate		
Glycine		
Histidine*		
Hydroxyproline		
Methionine*		
Proline		
Serine		
Threonine*		
Valine*		

** Essential amino acids; (Helpful tips to recall—ketogenic amino acids start with letter 'L'; PITT for glyco- and ketogenic amino acids; rest of the 20 amino acids are only glycogenic).*

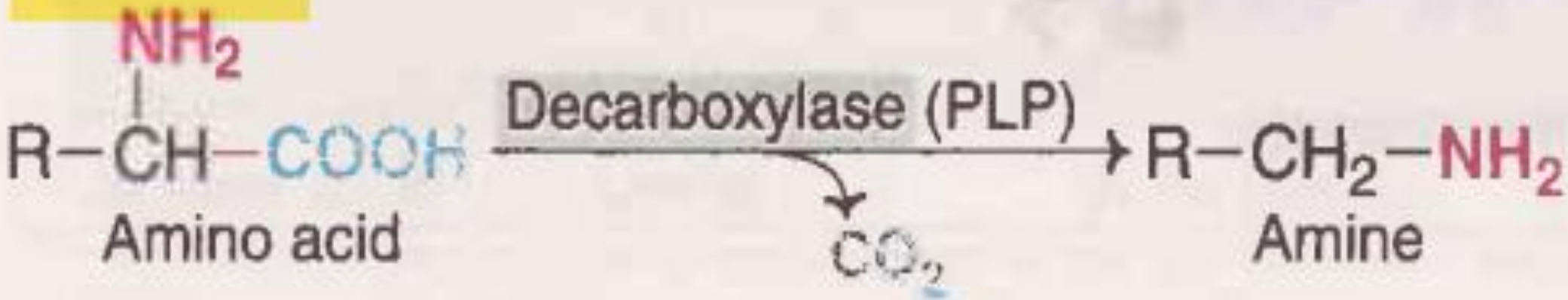
DECARBOXYLATION

Decarboxylation reaction of Amino acids

- Decarboxylation is a reaction in which carboxyl group is removed as CO_2.
- The enzymes that catalyze the reactions are generally called as decarboxylases. Pyridoxal phosphate is required as coenzyme for all decarboxylases except Histidine decarboxylase.
- Following are some of the decarboxylation reactions, resulting in the production of useful substances.
    - Tyrosine → Catecholamine (hormones)
    - Glutamate → γ-amino butyrate (Inhibitory neurotransmitter)
    - Tryptophan → Serotonin (neurotransmitter /vasodilator)
    - Histidine → Histamine (Vasodialator)
    - Methionine → Spermine (Involved in DNA packaging)
    - Ornithine → Spermidine (Involved in DNA packaging)

BIOGENIC AMINES

In general, the decarboxylation of amino acids or their derivatives results in the formation of amines.




thank you