Amino Acids Metabolism

Lac.1

By

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- Proteins are the most abundant organic molecules of the living system.
- They occur in the every part of the cell and constitute about 50% of the cellular dry weight.
- Proteins form the fundamental basis of structure and function of life.
- In 1839 Dutch chemist G.J.Mulder while investing the substances such as those found in milk, egg, found that they could be coagulated on heating and were nitrogenous compounds.

- **The term protein is derived from a Greek word *proteios,* meaning first place.**
- ***Berzelius (Swedish chemist)* suggested the name proteins to the group of organic compounds that are utmost important to life.**
- **The proteins are nitrogenous macromolecules composed of many amino acids.**

Biomedical importance of proteins:

- Proteins are the main structural components of the cytoskeleton. They are the sole source to replace nitrogen of the body.
- Bio chemical catalysts known as enzymes are proteins.
- Proteins known as immunoglobulins serve as the first line of defense against bacterial and viral infections.

- Several hormones are protein in nature.
- Structural proteins like actin and myosin are contractile proteins and help in the movement of muscle fibre.

  Some proteins present in cell membrane, cytoplasm and nucleus of the cell act as receptors.
- The transport proteins carry out the function of transporting specific substances either across the membrane or in the body fluids.

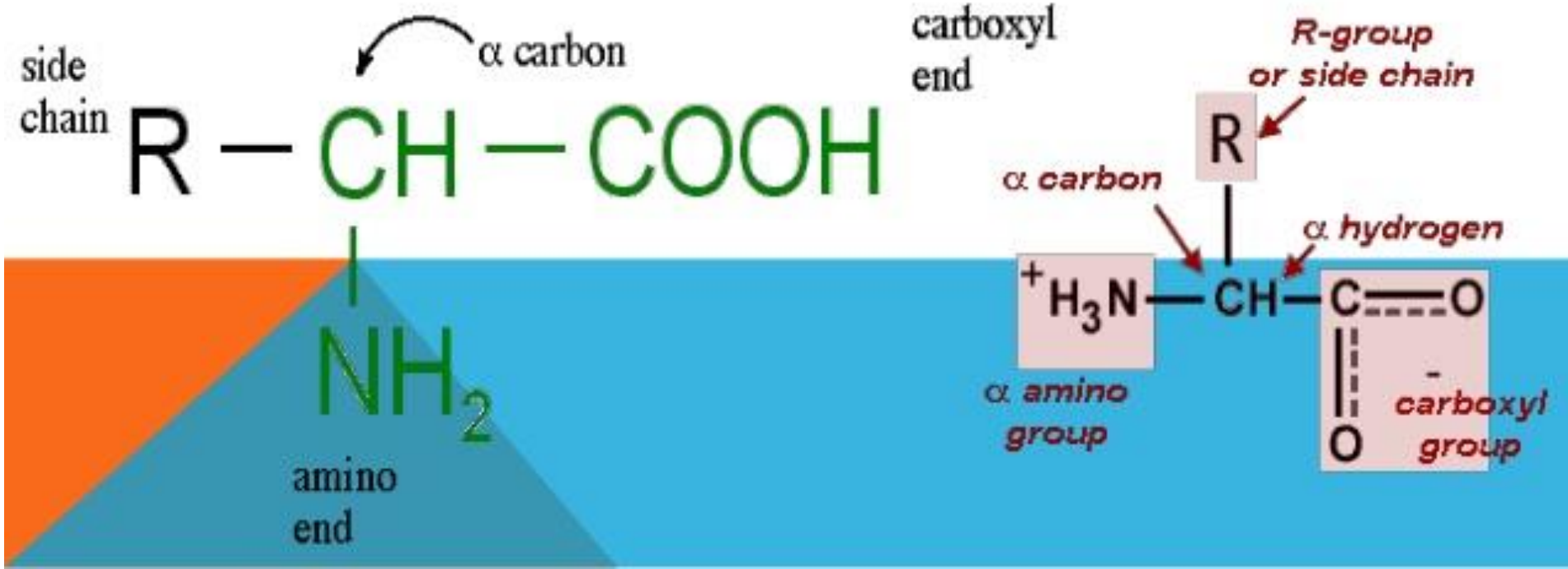
- **Storage proteins bind with specific substances and store them, e.g. iron is stored as ferritin.**
- **Few proteins are constituents of respiratory pigments and occur in electron transport chain, e.g. Cytochromes, hemoglobin, myoglobin**
- **Under certain conditions proteins can be catabolized to supply energy.**
- **Proteins by means of exerting osmotic pressure help in maintenance of electrolyte and water balance in the body.**

OBJECTIVES

- Digestion and absorption of proteins and amino acids
- Introduction to amino acids, structure and types
- Amino acid and nutrition
- General and individual Amino acid metabolism; and inborn errors of metabolism
- Metabolism of ammonia
- Clinical significance of amino acid and ammonia metabolism

WHAT IS AMINO ACID?

Amino acids are derivatives of carboxylic acids formed by substitution of α-hydrogen for amino functional group



WHAT DO AMINO ACIDS DO?

- Amino acids are essential to life, have a role in metabolism, and are important in nutrition.
- They form short polymer chains called peptides, as well as longer chains that are called polypeptides or proteins.
- About 75 percent of the human body is made up of chains of amino acids, which is why they are so vital to how your system functions.
- All the chemical reactions that occur in the body depend on amino acids and the proteins they build.

TYPES OF AMINO ACIDS

Amino acids are classified as

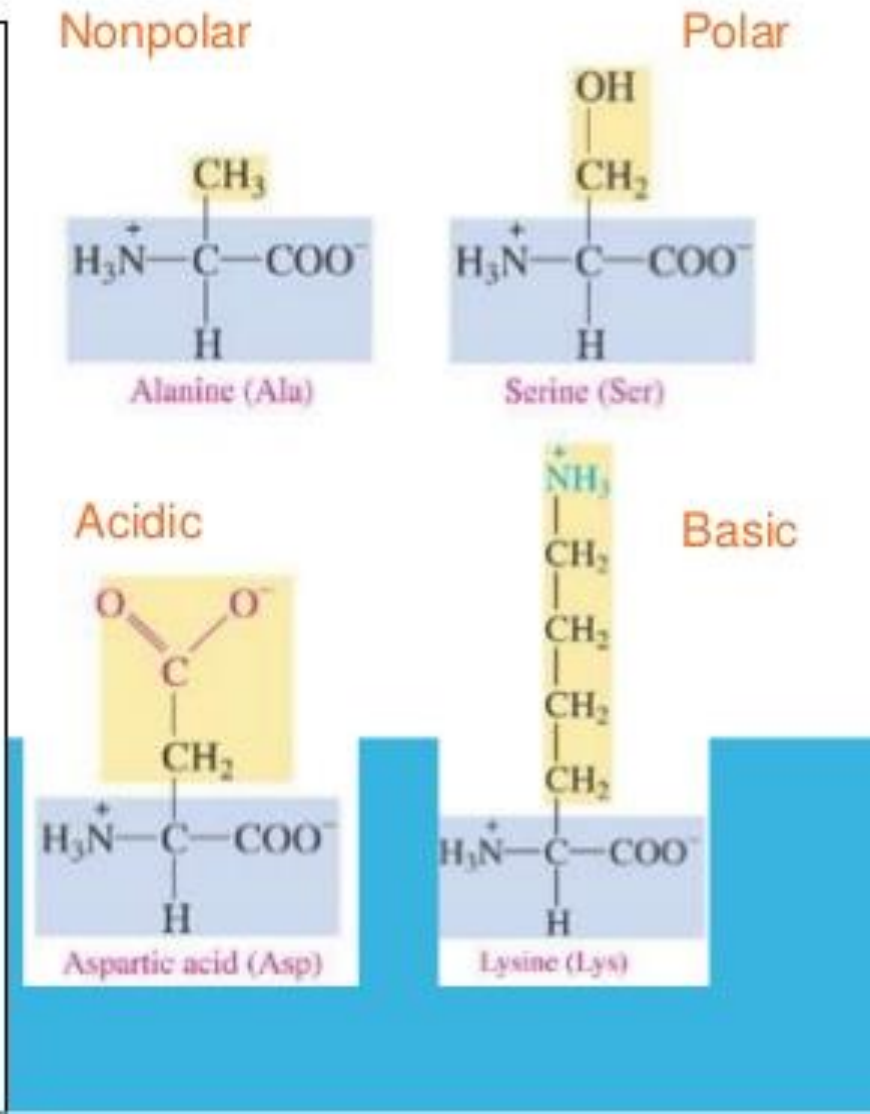
Nonpolar (hydrophobic) with hydrocarbon side chains.

Polar (hydrophilic) with polar or ionic side chains.

Acidic (hydrophilic) with acidic side chains.

Basic (hydrophilic) with

$-NH_2$ side chains.



- non-essential amino acids

  - can be synthesized by an organism

  - usually are prepared from precursors in 1-2 steps

- Essential amino acids

  - cannot be made endogenously

  - must be supplied in diet

    eg. Leu, Phe.....

Nutritionally-Essential amino acids :

Lysine, Leucine, Isoleucine, Valine, Methionine, Phenylalanine,

Threonine, Tryptophan

Nutritionally Nonessential amino acids: Alanine, glycine, aspartate , glutamate, serine, tyrosine, cysteine, proline , glutamine, aspargine

N.B. Histidine & arginine are semi essential. They are essential only for infants growth, but not for old children or adults where in adults histidine requirement is obtained by intestinal flora & arginine by urea cycle

PROTEIN DIGESTION

Digestive Tract of protein

- Proteins are generally too large to be absorbed by the intestine and therefore must be hydrolyzed to the amino acids
- The proteolytic enzymes responsible for hydrolysis are produced by three different organs: the stomach、pancreas and small intestine (the major organ)

Stomach

- HCl (parietal cells) and Pepsinogen (chief cells)
- The pH of gastric juice is around 1.0. Food is retained in the stomach for 2-4 hrs
- HCl kills microorganisms, denatures proteins, and provides an acid environment for the action of pepsin
- **Autocatalysis**: pepsinogen is converted to active pepsin(*Pepsin A)* by HCl

Pancreas and small intestine

- **Endo**peptidase (pancreas)

  Trypsin: carbonyl of arg and lys

  Chymotrypsin: carbonyl of Trp, Tyr, Phe, Met, Leu

  Elastase: carbonyl of Ala, Gly, Ser

  Exopeptidase (pancreas)

  Carboxypeptidase A:amine side of Ala, Ile, Leu, Val

  Carboxypeptidase B: amine side of Arg, lys

- Aminopeptidase (small intestine):

  cleaves N-terminal residue of oligopeptidaes

PROTEIN ABSORPTION

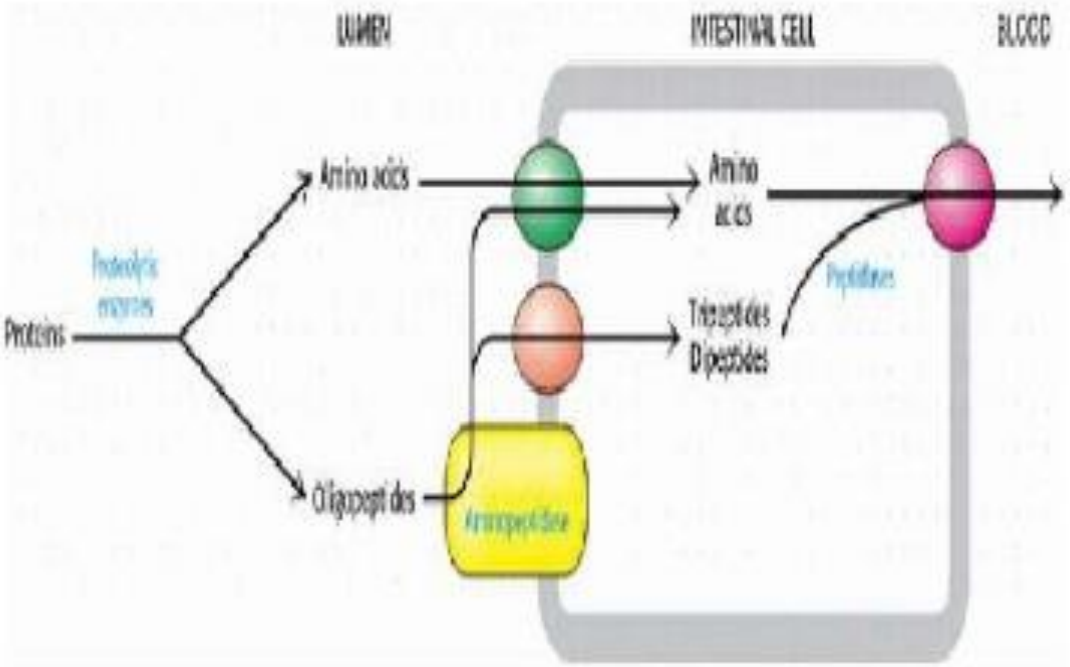
*L-amino acids are actively transported across the intestinal mucosa (need carrier, Na + pump,

Na+ ions, ATP).

Different carrier transport systems are: a) For neut amino acids.
b) For basic amino acid and cysteine.
c) For imino acids and glycine.

d) For acidic amino acids.
e) For B-amino acids (B-alanine & taurine).

*D-isomers transported by simple diffusion.



Nitrogen Balance (NB):

- **Nitrogen balance** is a **comparison between**

  Nitrogen intake (in the form of dietary protein)

  and

  Nitrogen loss (as **undigested protein** in feces ,

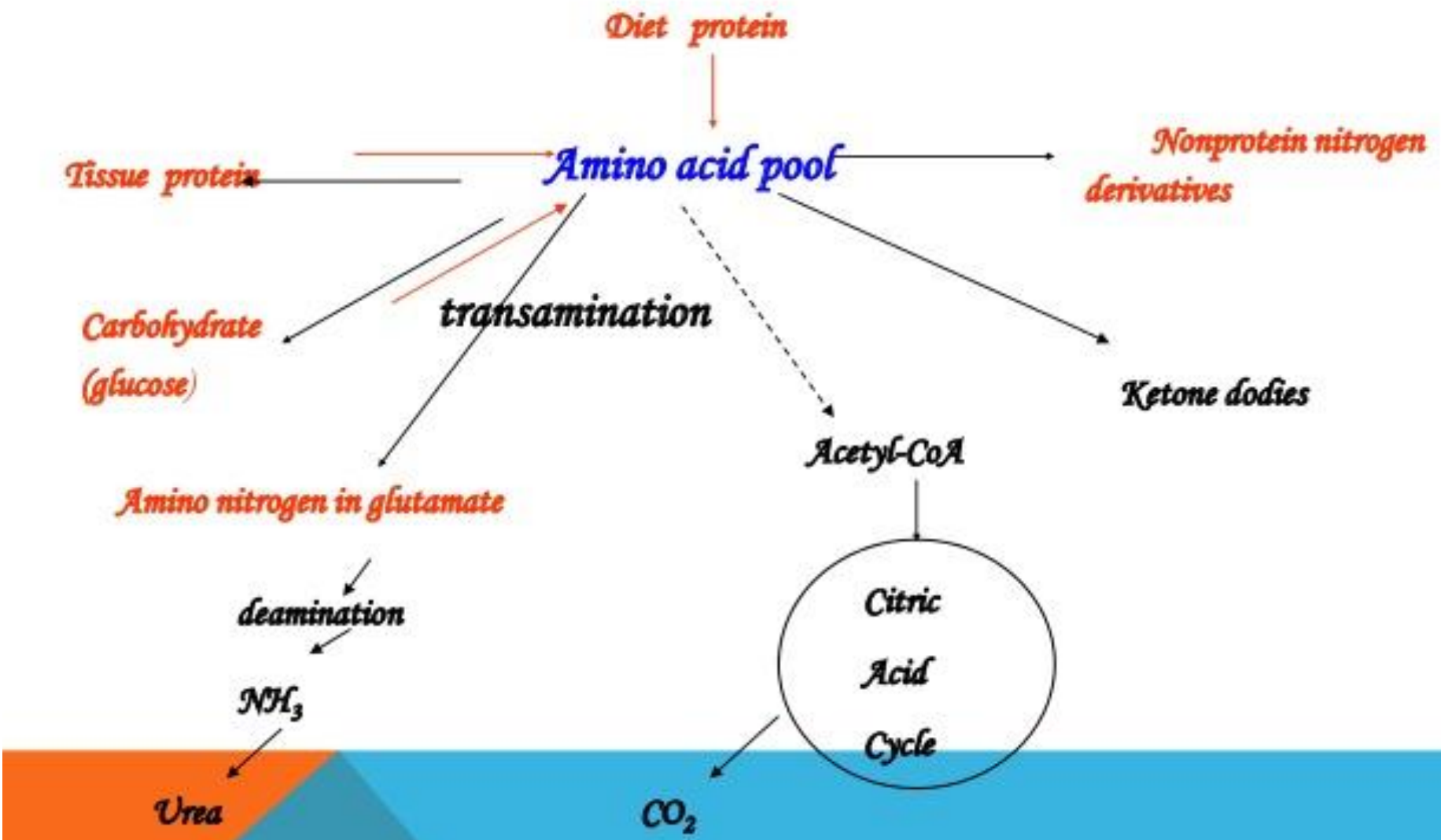
  NPN as urea, ammonia, creatinine & uric acid in urine, sweat & saliva & **losses** by hair, nail, skin).

- NB is important **in** defining

  1.overall protein metabolism of an individual

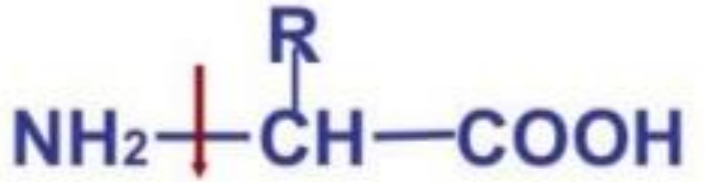
  2.nutritional nitrogen requirement.

AMINO ACID METABOLISM



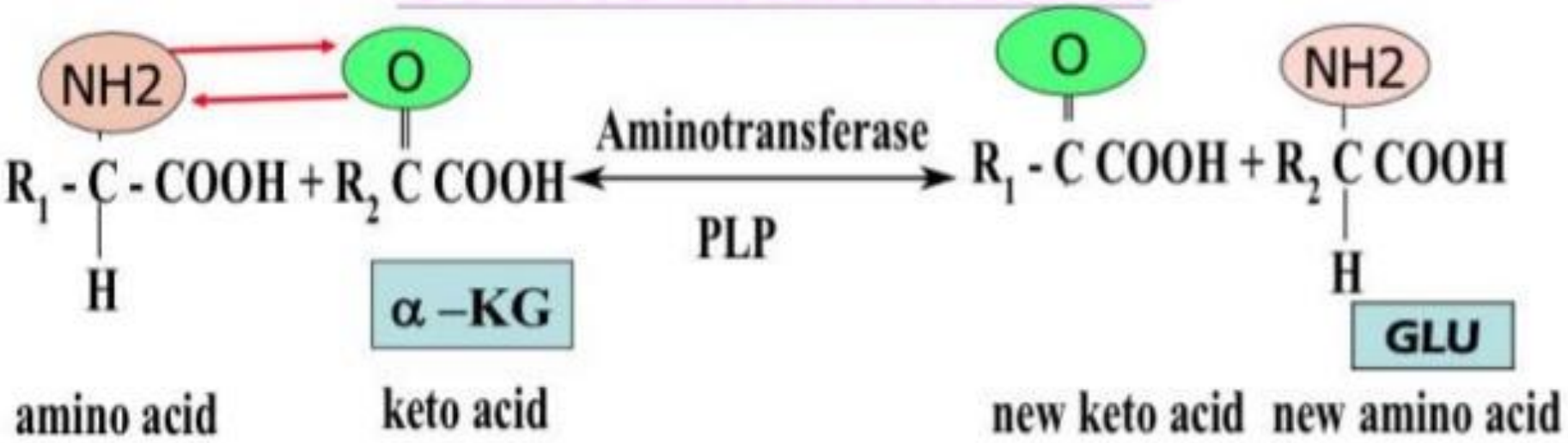
Overview of the protein metabolism

Metabolism OF AMINO ACIDS:



1. **Removal of amonia** by :
   - Deamination → **Oxidative deamination**
     1) glutamate dehydrogenase in mitochondria
     2) amino acid oxidase in peroxisomes
     Direct deamination (nonoxidative)
     1) dea. by dehydration ($-H_2O$)
     2) dea. by desulhydration ($-H_2S$)
   - Transamination (GPT & GOT)
   - and transdeamination.
2. **Fate of carbon-skeletons of amino acids**
3. **Metabolism of ammonia**

Transamination:



Aminotransferases are **active** both in cytoplasm and mitochondria e.g.:

1. **Aspartate aminotransferase (AST)**, Glutamate oxaloacetate transaminase **(GOT),**

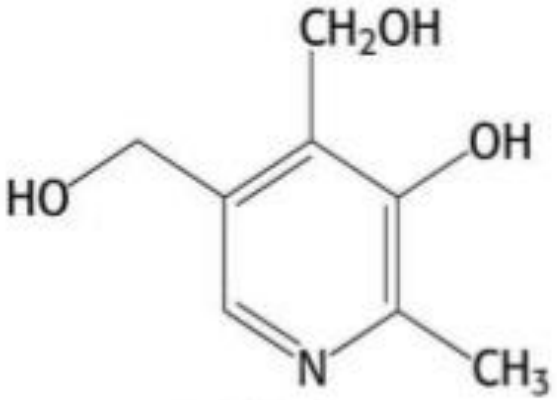
2. **Alanine aminotransferase (ALT)**, Glutamate pyruvate transaminase, **(GPT)**

In all transamination reactions, α-ketoglutarate (α –KG) acts as amino group acceptor.

Most, but not all amino acids undergo transamination reaction with few exceptions **(lysine, threonine and imino acids)**

Mechanism of transamination

All aminotransferases require the prosthetic group ***pyridoxal phosphate (PLP),*** which is derived from ***pyridoxine (vitamin B_6).***

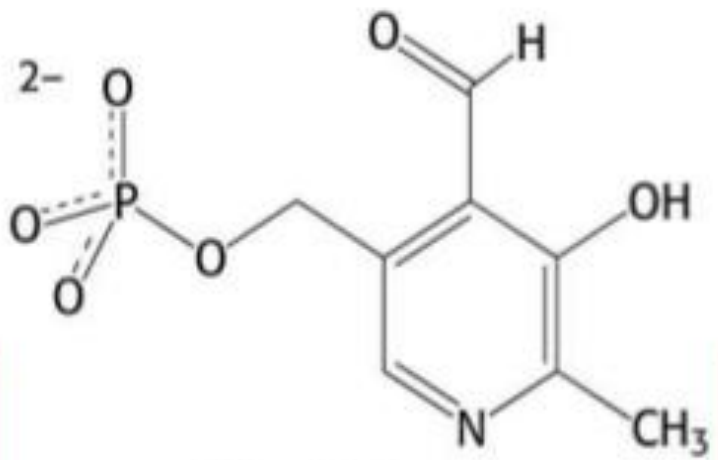


Pyridoxine (Vitamin B_6)

Ping-pong kinetic mechanism

First step: the amino group of amino acid is transferred to pyridoxal phosphate, forming pyridoxamine phosphate and releasing ketoacid.

Second step: α-ketoglutarate reacts with pyridoxamine phosphate forming glutamate



Pyridoxal phosphate (PLP)

B. Oxidative Deamination

- L-glutamate dehydrogenase (in mitochondria)

Glu + NAD^+ (or $NADP^+$) + H_2O ⇆ NH_4^+ + a-ketoglutarate + NAD(P)H +H^+

Requires NAD^+ or $NADP^+$ as a cofactor

Plays a central role in AA metabolism

THE FATE OF CARBON-SKELETONS OF AMINO ACIDS

a) Simple degradation:

(amino acid → Common metabolic intermediate)

Alanine → Pyruvate

Glutamate → α-ketoglutarate

Aspartate → Oxaloacetate

b) Complex degradation:

(amino acid--- Keto acid----- **complex** pathway---- Common metabolic intermediate)

Amino acids whose ketoacids are metabolized via **more complex** pathway **e.g. Tyrosine, Lysine, Tryptophan**

c) Conversion of one amino acid into another amino acid before degradation:

Phenylalanine is converted to **tyrosine** prior to its further degradation.

Metabolism of the Common Intermediates

1. ***Oxidation:*** all amino acids can be oxidized in **TCA** cycle with **energy** production
2. ***Fatty acids synthesis:*** some amino acids provide **acetyl CoA** e.g. leucine and lysine (ketogenic amino acids).
3. ***Gluconeogenesis:*** ketoacids derived from amino acids are used for synthesis of **glucose** (is important in starvation).

Glucogenic	***Ketogenic***	***Glucogenic&Ketogenic***
Ala, Ser, Gly, Cys, Arg, His, Pro, Glu, Gln, Val, Met, Asp, Asn.	Leu , Lys	Phe,Tyr,Trp,Ile,Thr

METABOLISM OF AMMONIA

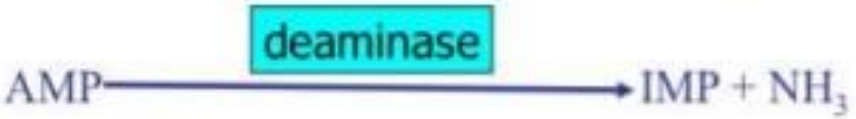
Ammonia is formed in body from:

a) From amino acids: 1.Transdeamination in liver (NOT T.A.)

2.amino acid oxidases and amino acid deaminases in liver and kidney.

b) Deamination of physiological amines: by monoamine oxidase.

c) Deamination of purine nucleotides: especially adenine nucleotides



d) Pyrimidine catabolism.

e) From bacterial action in the intestine **on dietary protein & on urea in the gut.**

NH3 is also produced by glutaminase on glutamine .

TRANSPORT OF AMMONIA TO THE LIVER

Two mechanism are available for the transport of ammonia from peripheral cells to liver for detoxification

The first uses glutamine synthetase to combine glutamate with ammonia

The second , used primarily by muscle, involves transamination of pyruvate to Alanine

GLUTAMATE AND GLUTAMINE RELATIONSHIP

Ammonia Nitrogen can be transported as glutamine.

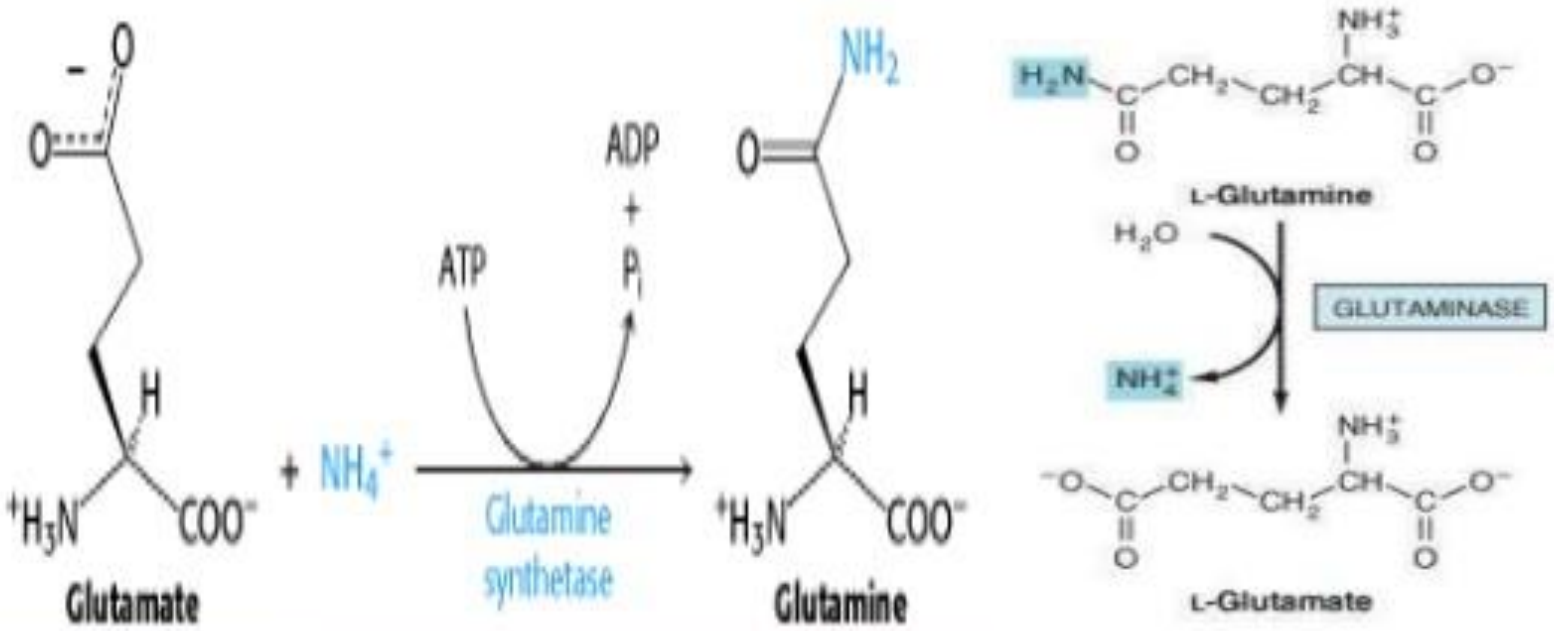
This is the first line of defense in brain cells.

Glutamine synthetase catalyzes the synthesis of glutamine from glutamate and NH_4^+ in an ATP-dependent reaction

The nitrogen of glutamine can be converted to urea in liver by the action of glutaminase in liver

Hydrolytic release of the amide nitrogen of glutamine as ammonia, catalyzed by glutaminase favors glutamate formation.

GLUTAMATE AND GLUTAMINE RELATIONSHIP



The concerted action of glutamine synthase and glutaminase thus catalyzes the interconversion of free ammonium ion and glutamine

GLUCOSE ALANINE CYCLE AND ROLE OF GLUTAMATE

The transport of amino group of amino acids also takes place in the form of Alanine.

Nitrogen is transported from muscle to the liver in two principal transport forms.

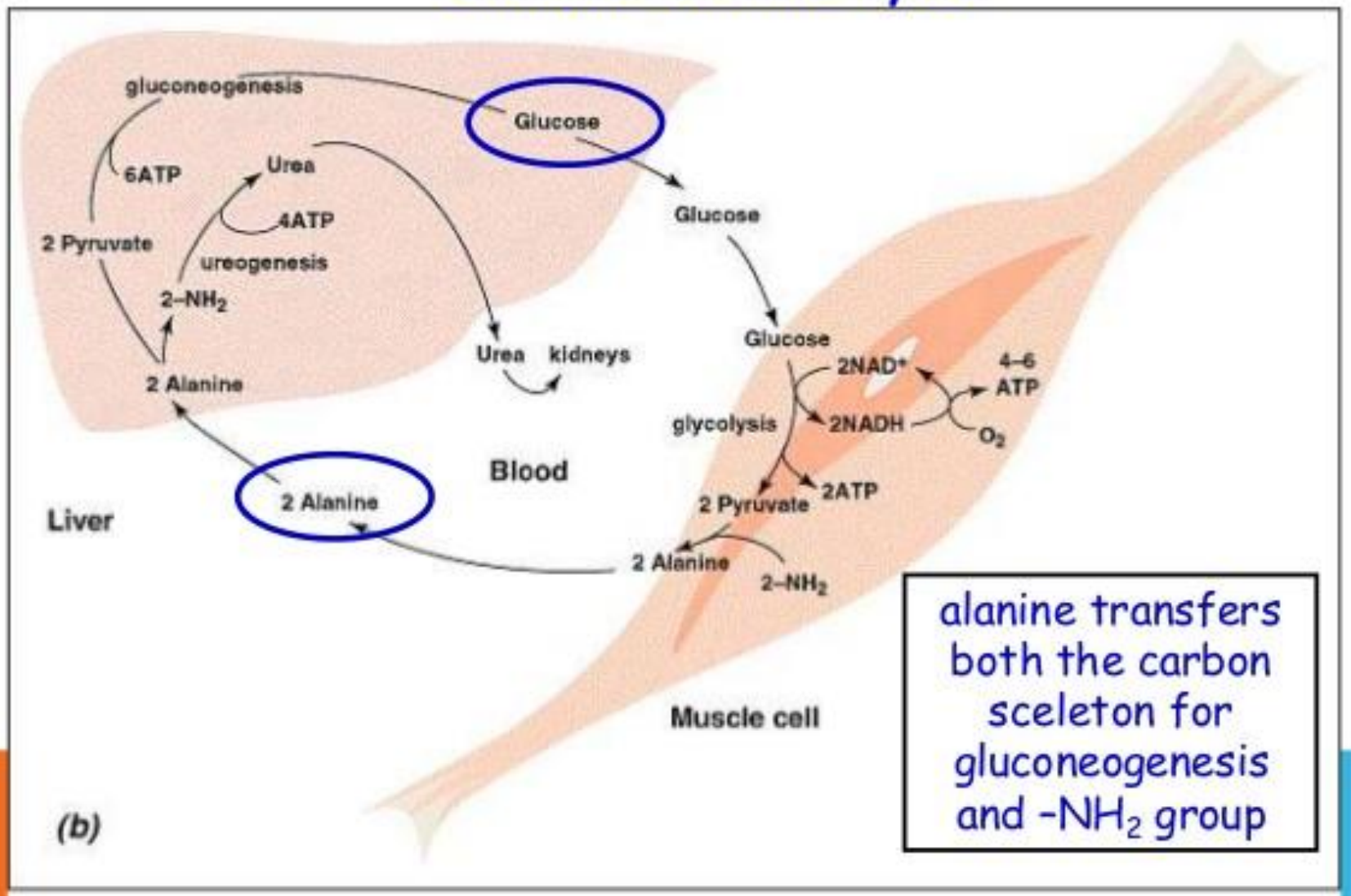
Glutamate is formed by transamination reactions, but the nitrogen is then transferred to pyruvate to form alanine, which is released into the blood.

The liver takes up the alanine and converts it back into pyruvate by transamination.

The pyruvate can be used for gluconeogenesis and the amino group eventually appears as urea.

This transport is referred to as the *alanine cycle.*

Glucose-alanine cycle

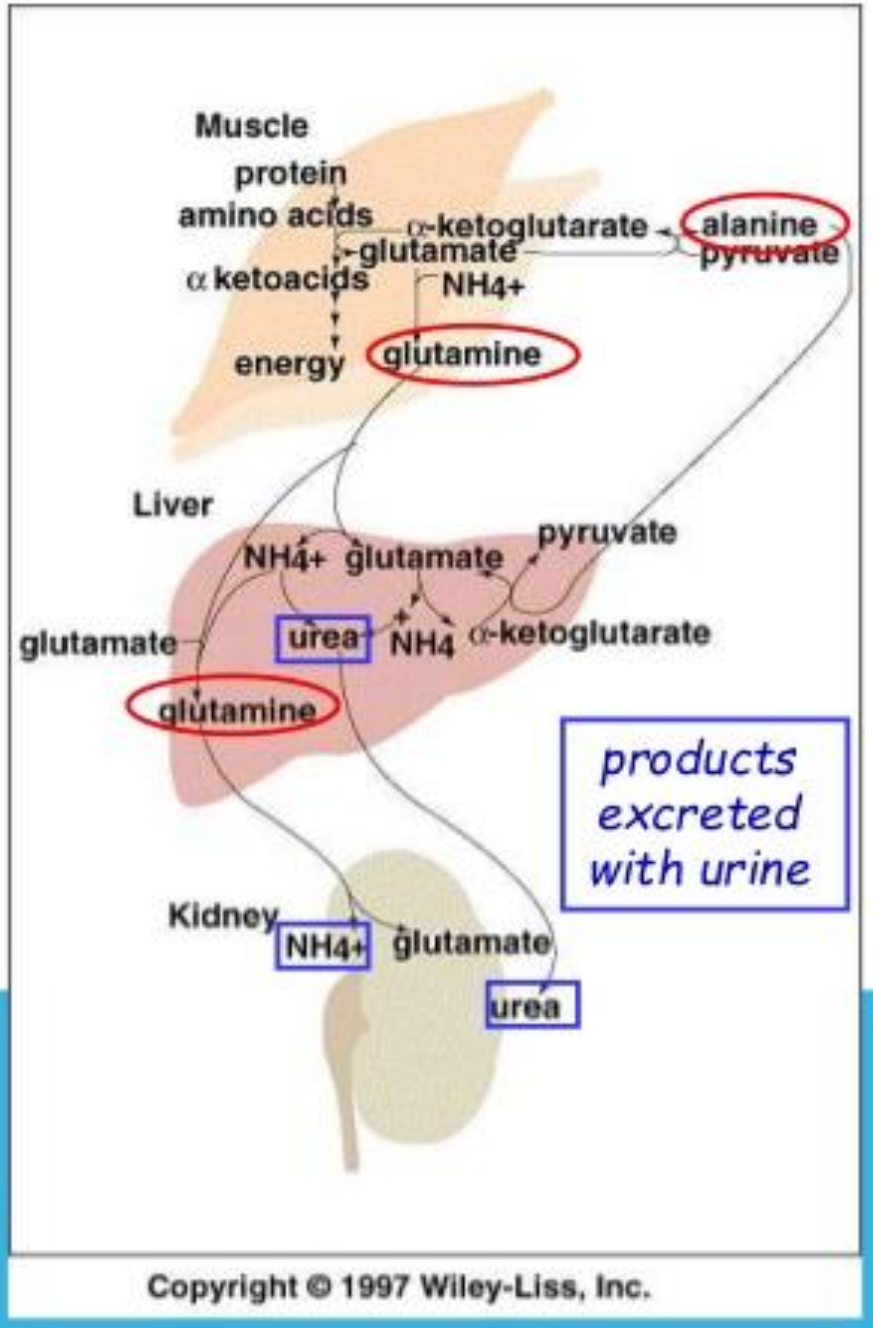


alanine transfers both the carbon sceleton for gluconeogenesis and $-NH_2$ group

The figure was adopted from Devlin, T. M. (editor): Textbook of Biochemistry with Clinical Correlations, 4th ed. Wiley-Liss, Inc., New York, 1997. ISBN 0-471-15451-2

Transport of amino nitrogen

from degraded muscle proteins



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AMMONIA INTOXICATION

The ammonia produced by enteric bacteria and absorbed into portal venous blood and the ammonia produced by tissues are rapidly removed from circulation by the liver and converted to urea.

Thus, only traces (10–20 g/dL) normally are present in peripheral blood.

This is essential, since ammonia is toxic to the central nervous system.

Should portal blood bypass the liver, systemic blood ammonia levels may rise to toxic levels.

This occurs in severely impaired hepatic function or the development of collateral links between the portal and systemic veins in cirrhosis.

AMMONIA INTOXICATION

Excess of ammonia depletes glutamate and hence GABA level in brain

To compensate for glutamate, alpha keto glutarate is used , the decrease concentration of which subsequently depresses TCA and thus deprives brain cells of energy.

Excess Glutamine is exchanged with Tryptophan , a precursor of Serotonin , resulting in hyper excitation.

Symptoms of ammonia intoxication include tremor, slurred speech, blurred vision, coma, and ultimately death.

UREA (ORNITHINE) CYCLE

detoxification pathway (NH_3 is toxic for brain)

proceeds only in the liver

localized in mitochondria /cytoplasm

carbamoyl phosphate synthetase I (= mitoch.)

can acidify an organism (consumes HCO_3^-)

needs energy (3 ATP, but 4 energy rich bonds)

connected with citrate cycle through fumarate

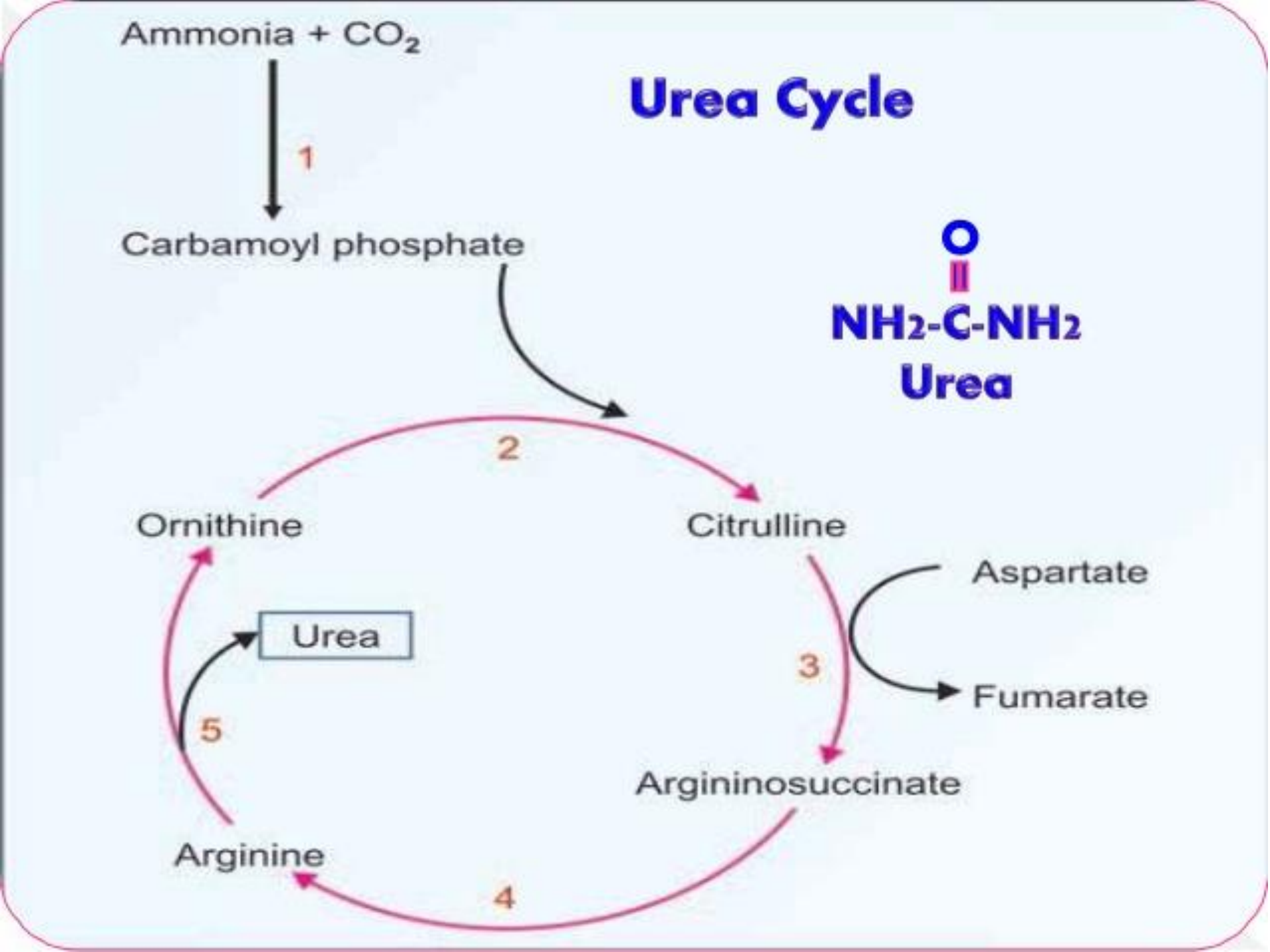
urea is end product of $-NH_2$ metabolism (→ urine)

Urea Cycle

- The urea cycle is the first metabolic pathway to be elucidated.
- The cycle is known as Krebs–Henseleit urea cycle.
- Ornithine is the first member of the reaction, it is also called as Ornithine cycle.
- Urea is synthesized in liver & transported to kidneys for excretion in urine.

- The two nitrogen atoms of urea are derived from two different sources, one from ammonia & the other directly from the a-amino group of aspartic acid.
- Carbon atom is supplied by CO_2
- Urea is the end product of protein metabolism (amino acid metabolism).

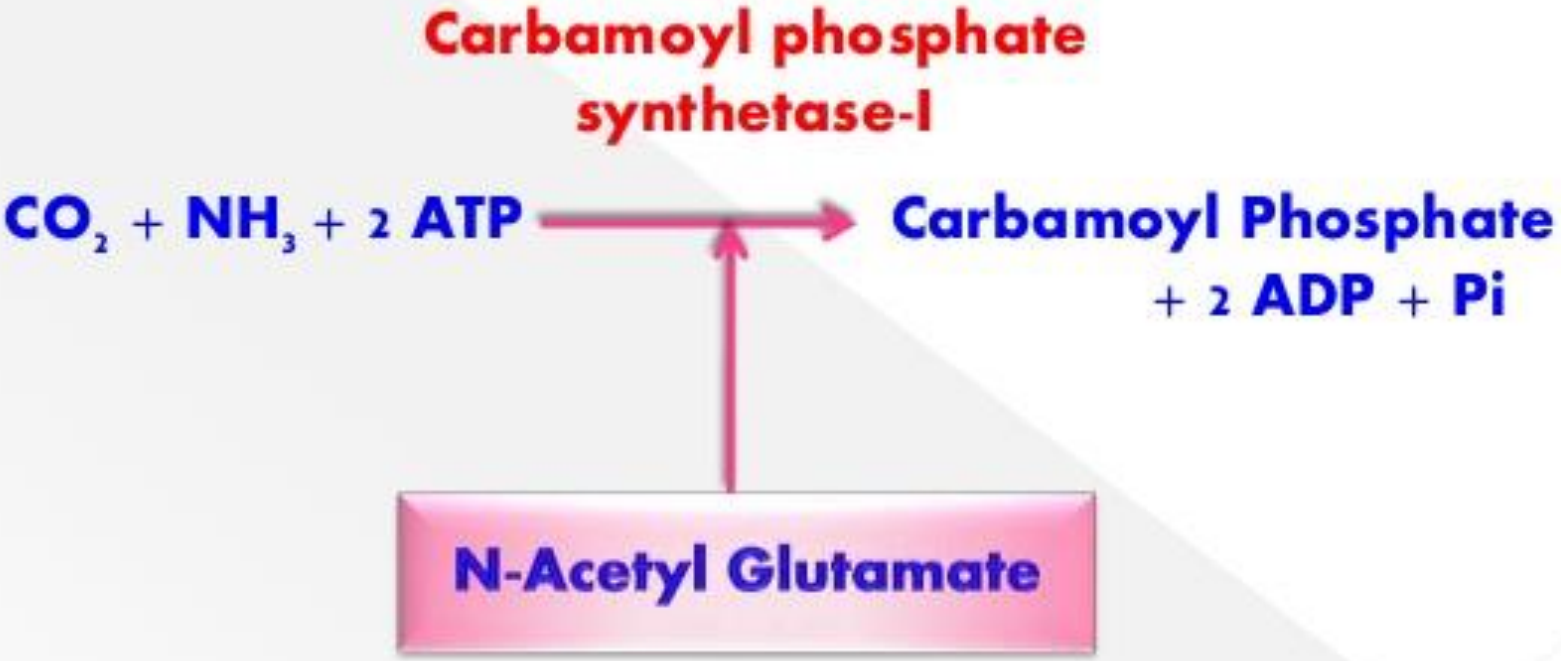
- Urea accounts for 80-90% of the nitrogen containing substances excreted in urine.
- Urea synthesis is a five-step cyclic process, with five distinct enzymes.
- The first two enzymes are present in mitochondria while the rest are localized in cytosol.


Ammonia + CO_2
1
Carbamoyl phosphate
Urea Cycle
NH_2-C-NH_2
Urea
2
Ornithine
Citrulline
Aspartate
Urea
3
Fumarate
5
Argininosuccinate
Arginine
4

Step: 1 Formation of carbamoyl phosphate

- Carbamoyl phosphate synthase I (CPS I) of mitochondria catalyses the condensation of NH_4^+ ions with CO_2 to form carbamoyl phosphate.
- This step consumes two ATP & is irreversible.
- It is a rate-limiting.

Step: 1 Formation of carbamoyl phosphate



Step 2: Formation of Citrulline

- The second reaction is also mitochondrial.
- Citrulline is synthesized from carbamoyl phosphate & ornithine by ornithine transcarbamoylase.
- Ornithine is regenerated & used in urea cycle.

- **Ornithine & citrulline are basic amino acids. (Never found in protein structure due to lack of codons).**
- **Citrulline is transported to cytosol by a transporter system.**
- **Citrulline is neither present in tissue proteins nor in blood; but it is present in milk.**

Step 2: Formation of Citrulline

Step 3: Formation of Arginosuccinate

- Citrulline condenses with aspartate to form arginosuccinate by the enzyme Arginosuccinate synthetase.
- Second amino group of urea is incorporated.
- It requires ATP, it is cleaved to AMP & PPi
- 2 High energy bonds are required.
- Immediately broken down to inorganic phosphate (Pi).

Step:4 Formation of Arginine or cleavage of Arginosuccinate

- The enzyme Argininosuccinase or argininosuccinate lyase cleaves arginosuccinate to arginine & fumarate (an intermediate in TCA cycle)
- Fumarate provides connecting link with TCA cycle or gluconeogenesis.

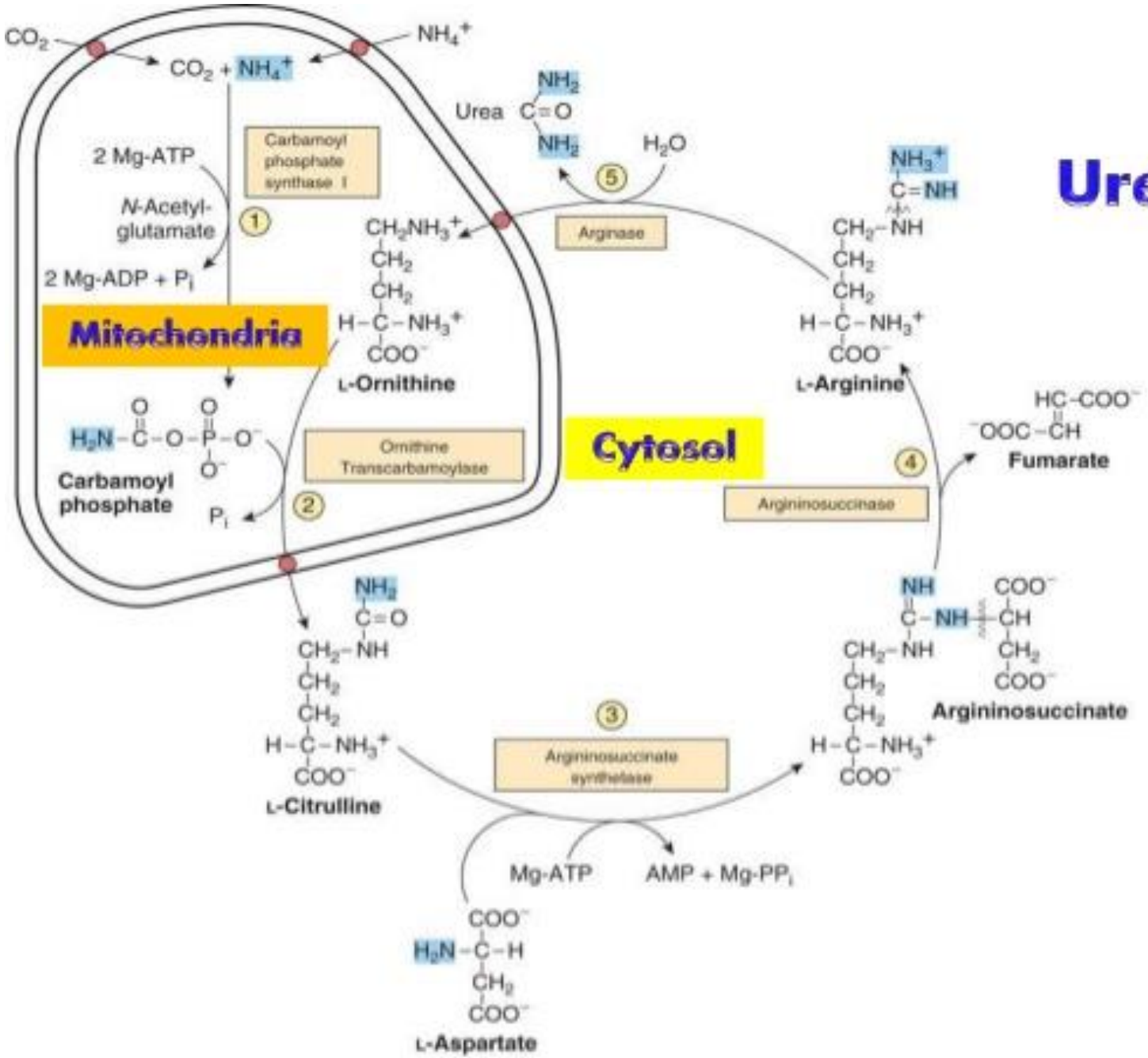
- The fumarate is converted to oxaloacetate via fumarase & MDH & transaminated to aspartate.
- Aspartate is regenerated in this reaction.

Step 5: Formation of Urea

- **Arginase is the 5th and final enzyme that cleaves arginine to yield urea & ornithine.**
- **Ornithine is regenerated, enters mitochondria for its reuse in the urea cycle.**
- **Arginase is activated by Co^{2+} & Mn^{2+}**
- **Ornithine & lysine compete with arginine (competitive inhibition).**

- Arginase is mostly found in the liver, while the rest of the enzymes (four) of urea cycle are also present in other tissues.
- Arginine synthesis may occur to varying degrees in many tissues.
- But only the liver can ultimately produce urea.

Urea Cycle


CO_2
NH_4^+
$CO_2 + NH_4^+$
2 Mg-ATP
Carbamoyl phosphate synthase I
N-Acetyl-glutamate
1
2 Mg-ADP + P_i
Mitochondria
Carbamoyl phosphate
P_i
Ornithine Transcarbamoylase
2
L-Ornithine
Cytosol
Urea
H_2O
5
Arginase
L-Arginine
4
Argininosuccinase
Fumarate
Argininosuccinate
3
Argininosuccinate synthetase
L-Citrulline
Mg-ATP
AMP + Mg-PP_i
L-Aspartate

Energetics of Urea Cycle

- The overall reaction may be summarized as:
- $NH_3 + CO_2 + Aspartate \rightarrow Urea + fumarate$
- 2ATPs are used in the 1st reaction.
- Another ATP is converted to AMP + PPi in the 3rd step, which is equivalent to 2 ATPs.
- The urea cycle consumes 4 high energy phosphate bonds.
- Fumarate formed in the 4th step may be converted to malate.

- Malate when oxidised to oxaloacetate produces 1 NADH equivalent to 2.5 ATP.
- So net energy expenditure is only 1.5 high energy phosphates.
- The urea cycle & TCA cycle are interlinked & it is called as "urea bicycle".

Disposal of urea

- Urea produced in the liver freely diffuses & is transported in blood to kidneys & excreted.
- A small amount of urea enters the intestine where it is broken down to CO_2 & NH_3 by the bacterial enzyme urease.
- This ammonia is either lost in the feces or absorbed into the blood.

Regulation of urea cycle

1. Mitochondrial carbamoyl phosphate synthetase I (CPS I)

CPS I catalyzes the first committed step of the urea cycle

CPS I is also an allosteric enzyme sensitive to activation by N-acetylglutamate（AGA） which is derived from glutamate and acetyl-CoA

Urea Cycle Defects and Hyperammonemia—

(1) Hereditary Hyperammonemia (genetic deficiencies of Urea cycle enzymes)

- Ornithine carbamyl transferase (OTC) deficiency (X linked)
- Carbamyl phosphate synthetase I (CPS I) deficiency
- Citrullinemia (enzyme defect?)
- Arginosuccinic Aciduria (enzyme defect?)
- Argininemia (not severe why?)(enzyme defect?)
- N-acetylGlu synthase deficiency

Urea Cycle Defects and Hyperammonemia

(2) **Acquired Hyperammonemia-------**

a) Liver disease---- (cirrhosis , hepatitis)
b) High protein diet

Clinical significance of blood urea:

- Elevated in renal insufficiency.
- Decreased in hepatic failure.