UREA CYCLE

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ZOO CC- 410
SEMESTER- IV
UNIT- 4

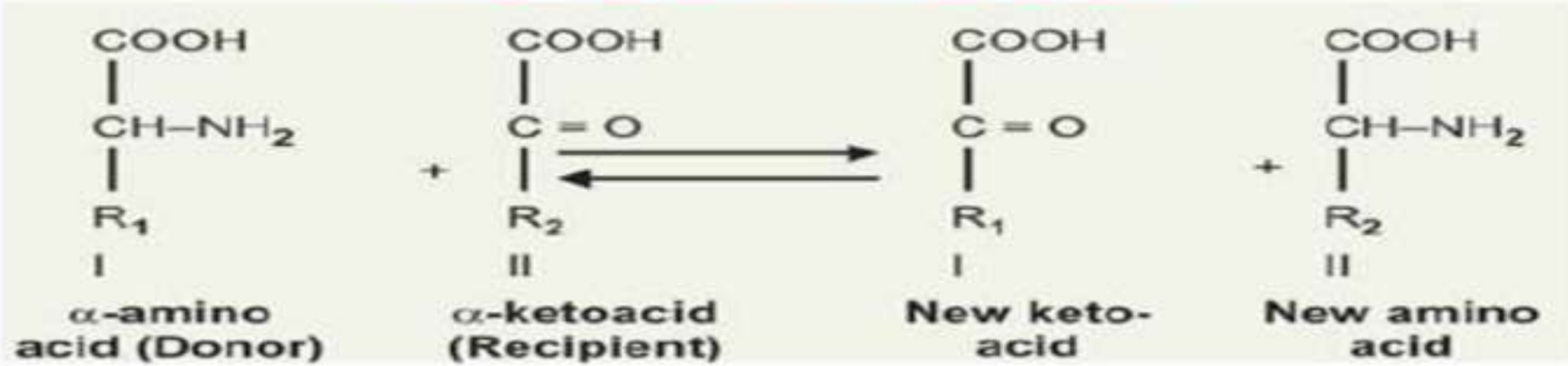
Introduction

- Urea is water soluble compound that could be excreted in urine.
- Urea is the end-product of amino acid metabolism
- Urea is used to get rid of the increased amount of ammonia (NH3) that produces by many processes in human body ...

Formation of NH_3 and urea can be discussed under the following heads:

- Transamination
- Deamination
  - Oxidative deamination
  - Non-oxidative deamination
- Transdeamination
- NH_3 transport
- Formation of urea

Transamination



- **Donor amino acid (I) thus becomes a new ketoacid (I) after losing the α-NH2 group, and the recipient ketoacid (II) becomes a new amino acid (II) after receiving the NH2 group.**
- **The process represents only an intermolecular transfer of NH2 group without the splitting out of NH3. Ammonia formation does not take place by transamination reaction.**

Transamination

- While most amino acids may act as Donor I, the recipient ketoacids may be either- α-keto-(oxo) glutarate, or oxaloacetate or pyruvate.

- It is to be noted that all of these recipient ketoacids are "components of TCA cycle" and hence they are common metabolites of the cell and are easily available.

- The amino acids formed from these recipient ketoacids are respectively glutamic acid, aspartic acid and alanine.

Deamination

- Deamination is the process by which N– of amino acid is removed as NH3.
- It can be of 2 types:

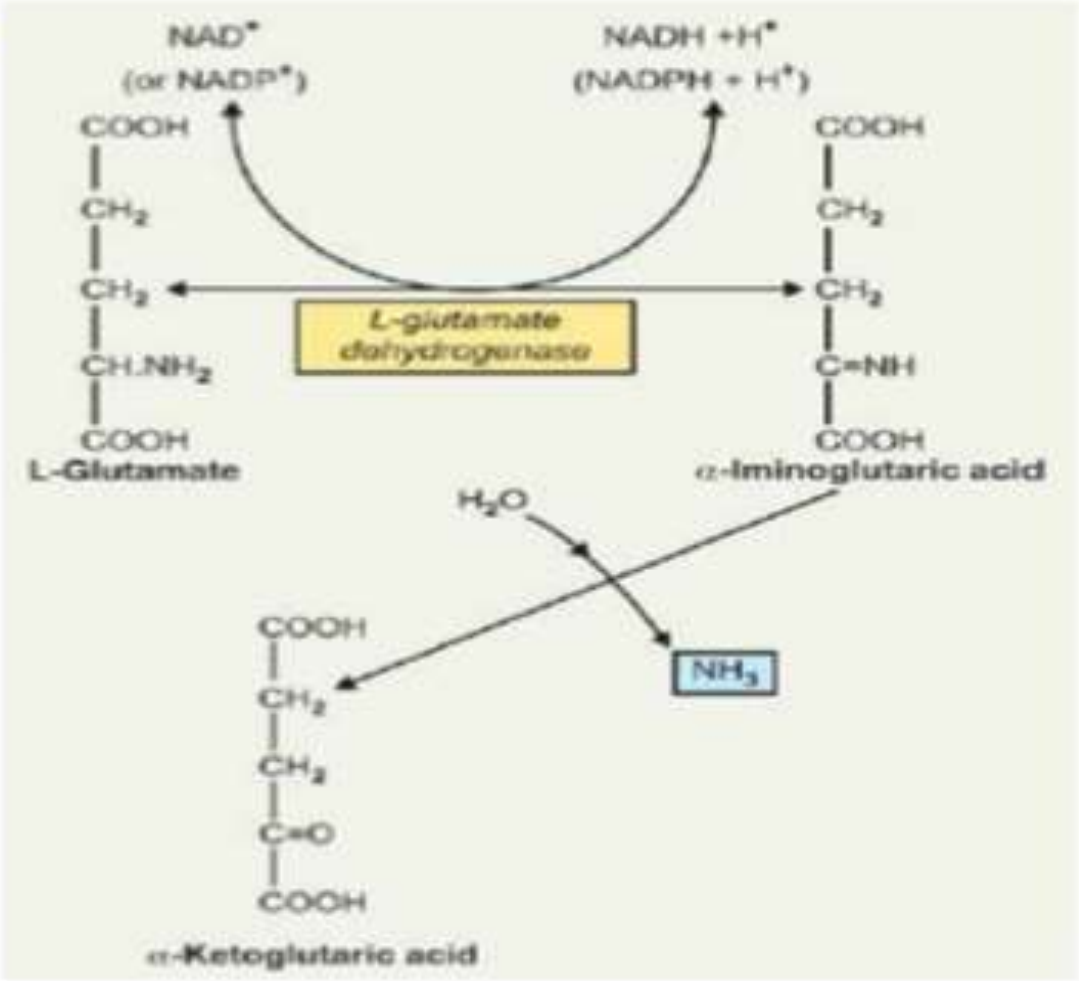
  A. Oxidative deamination

  B. Non-oxidative deamination.

- Oxidative deamination is done by amino acid dehydrogenase enzymes (oxidase enzymes)

Transdeamination

- Specific for Deamination of L-Glutamic Acid.
- This is done by a specific enzyme called L-glutamate dehydrogenase.
- This enzyme is widely distributed in tissues in humans and has high activity, and is specific for L-Glutamate. It requires NAD+ or NADP+ as coenzymes
- It is a regulated enzyme whose activity is affected by allosteric modifiers as ATP, GTP and NADH which inhibit the enzyme, and ADP activates the enzyme



Transdeamination

- The enzyme L-Glutamate dehydrogenase catalyzes the deamination of L-Glutamate to form NH3 and α-Ketoglutarate.

- It is to be noted that the reaction is reversible, and the equilibrium constant favours glutamate formation, but the quick removal of NH3 to form urea in urea cycle and α-Ketoglutarate to TCA cycle favours onward reaction, i.e. NH3 formation

- This mechanism seems to be the major pathway for removal of NH2 group from an L-amino acid and formation of NH3.

Sources of NH3

- Three main sources !!
- 1- transamination, deamination & trandeamination (previously discussed)
- 2- Absorption from gut produced by intestinal bacteria.
- 3- Pyrimidine catabolism. (nitrogenous base of nucleic acids)

Why NH3 is Toxic?

- Only for 3 main reasons …

1. depletion in intermediates of TCA cycle (depletion of energy) since a-keto-acids are major intermediates … (transamination) ← discussed
2. Reduction in synthesis of GABA (an inhibitory brain neurotransmitter) because the reduction of glutamate since it will converted to glutamin. Gamma- Aminobutyric Acid
3. Co-transport in different directions in BBB, glutamine to out and tryptophan to in, tryptophan have a toxic effect on brain tissues.

How brain get rid of excess NH3 ?!!

- No urea cycle occurring in brain tissue, so brain have a mechanism to communicate with urea cycle in liver ... HOW ?!!

Brain → glutamate + NH3 → glutamine

- This reaction is done by glutamine synthetase enzyme.
- Glutamine is soluble in blood and can be transported to liver or Kidney ..
- Other enzyme called glutaminase is found in these organs (liver and kidney)
- In kidney

Glutamine → NH3 + glutamate

- NH3 will dissolve in water and excreted in urine as amonioum ions ..

How brain get rid of excess NH3 ?!!

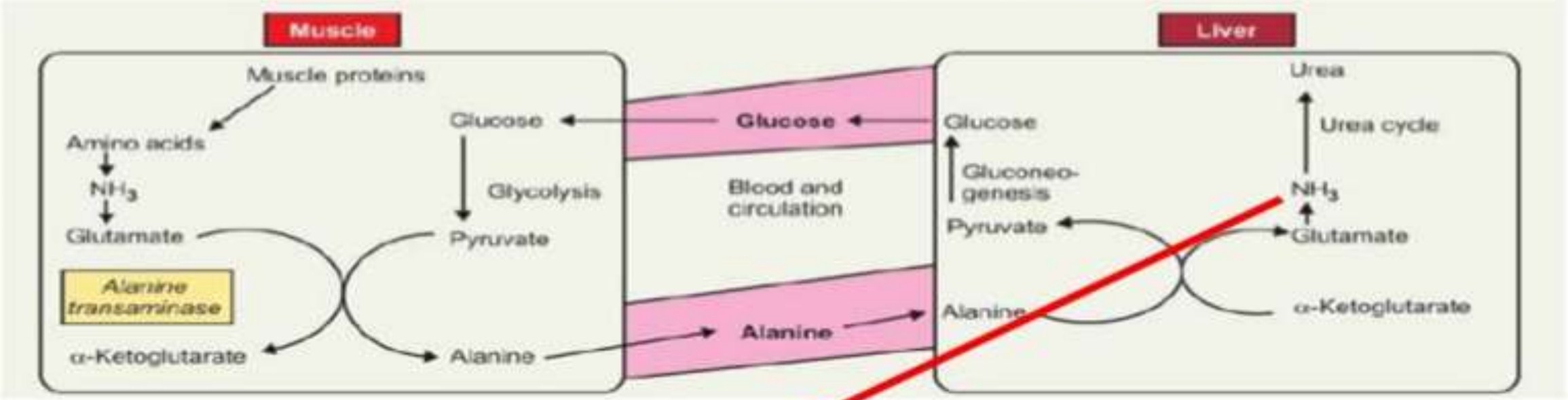
- In liver

Glutamine → glutamate + NH3

- NH3 will enter urea cycle and to lastly converted to urea ..
- Glutamate will converted to aspartate, another substrate for urea cycle (source of the 2nd NH3 of urea)

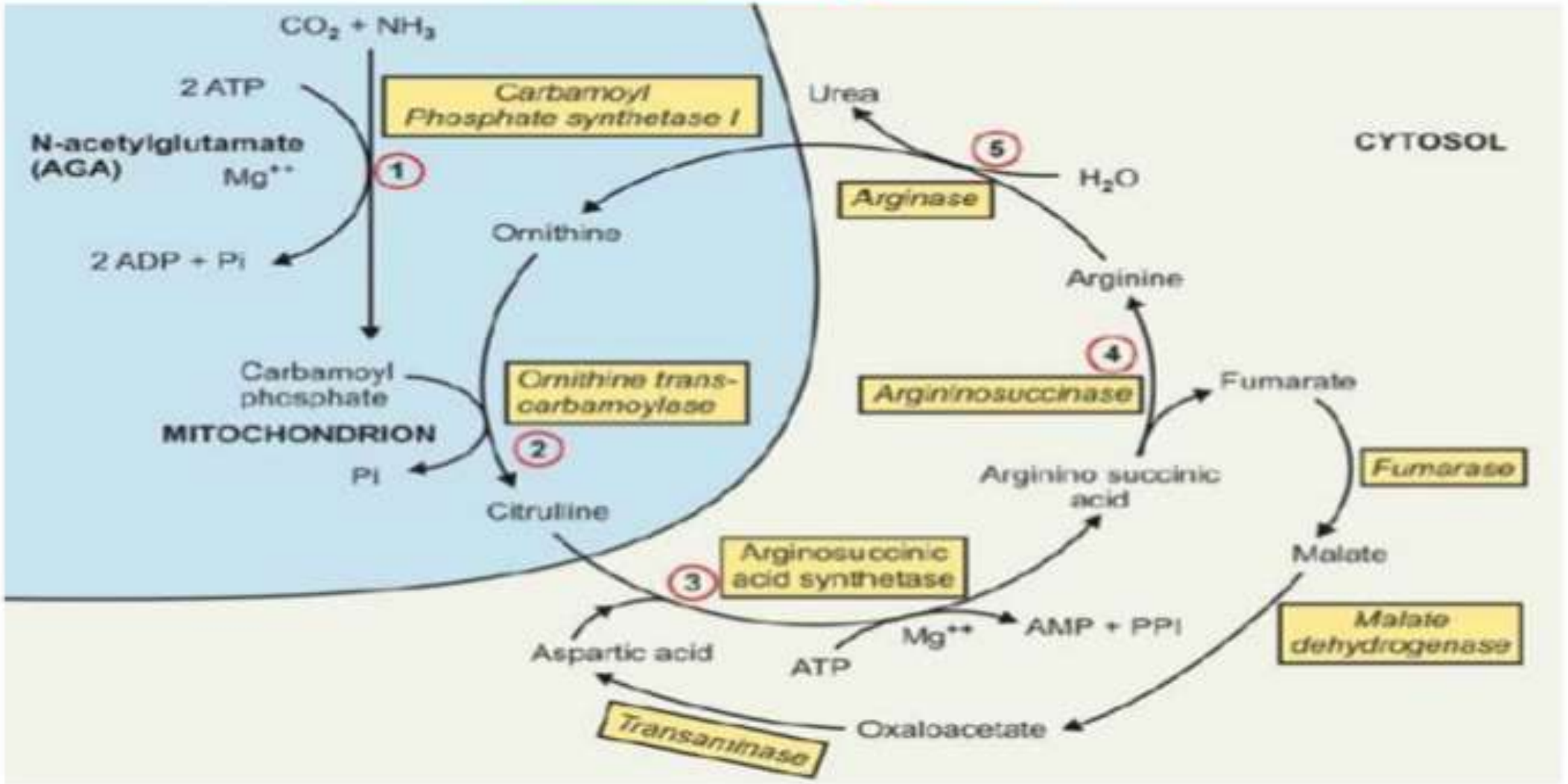
How muscle get rid of excess NH3 ?!

Glucose-alanine cycle



As aspartate since aspartate is a substrate for urea cycle.

Urea cycle


$CO_2 + NH_3$
2 ATP
N-acetylglutamate (AGA)
Mg^{++}
1
Carbamoyl Phosphate synthetase I
2 ADP + Pi
Ornithine
Carbamoyl phosphate
MITOCHONDRION
Pi
2
Ornithine trans-carbamoylase
Citruline
3
Arginosuccinic acid synthetase
Aspartic acid
ATP
Mg^{++}
AMP + PPi
Arginino succinic acid
4
Arginino succinase
Fumarate
Fumarase
Malate
Malate dehydrogenase
Oxaloacetate
Transaminase
Arginine
5
Arginase
H_2O
Urea
CYTOSOL

INTRODUCTION:

- Urea cycle is the first metabolic cycle 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 the major end product of protein metabolism (amino acid metabolism) in humans and mammals.
- Urea has two amino ($-NH_2$) groups, one derived from NH_3 and the other from aspartate.
- Urea is synthesized in the liver.
- Than secreted into blood stream.
- And taken up by the kidneys for excretion in the 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.

CHARACTERISTICS:

- Urea is the major disposal form of amino groups.
- It accounts for 90% of the nitrogen containing components of urine.
- The urea cycle is the sole source of endogenous production of arginine.
- Urea formation takes place in liver.
- Urea excretion occurs through kidney.

Steps in the urea cycle are

Step 1: Formation of carbamoyl phosphate

Step 2: Formation of citrulline

Mitochondria

Step 3: Synthesis of Argininosuccinate

Step 4: Synthesis of Arginine

Step 5: Release of urea and Ornithine

Cytosol

Step 1 : Synthesis of carbamoyl phosphate

Step 1: Takes place in mitochondria

From HCO_3

From deamination Of amino acids

CO_2 + NH_3

2ATP

Mg+2

2ADP + 2Pi

Carbamoyl phosphate synthetase - I

N - Acetyl Glutamate

Carbamoyl phosphate

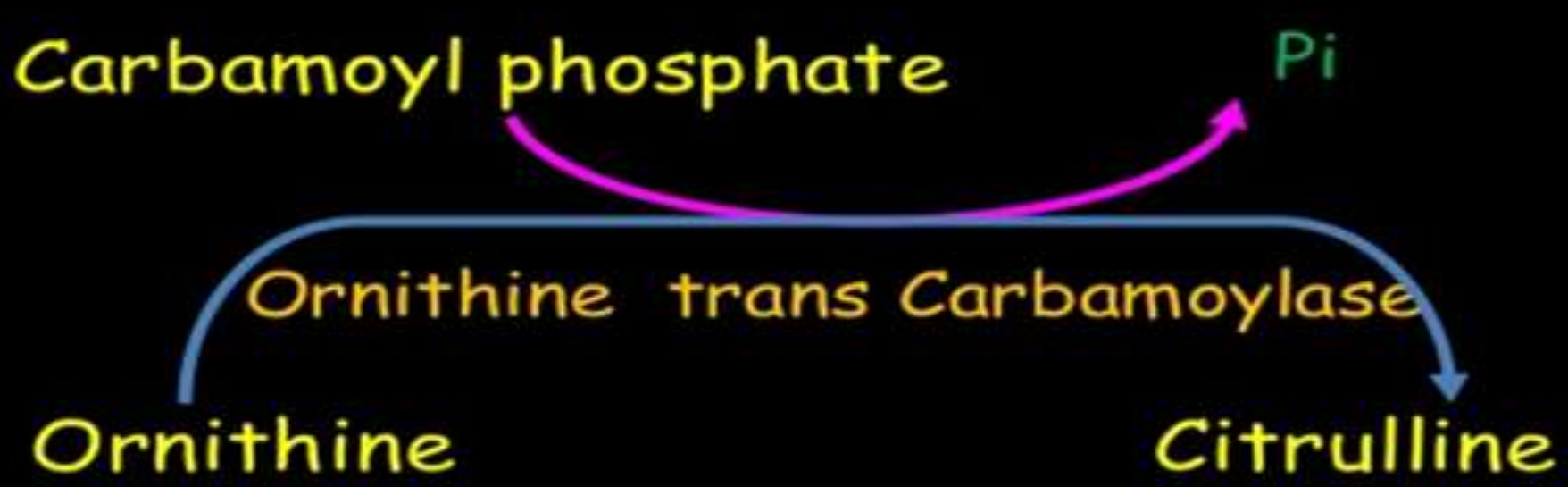
- In liver mitochondria
- NAG allosterically activates CPS-I
- Two high energy phosphate bonds are utilized for the synthesis of carbamoyl Phosphate

Isomers of carbamoyl phosphate synthetase

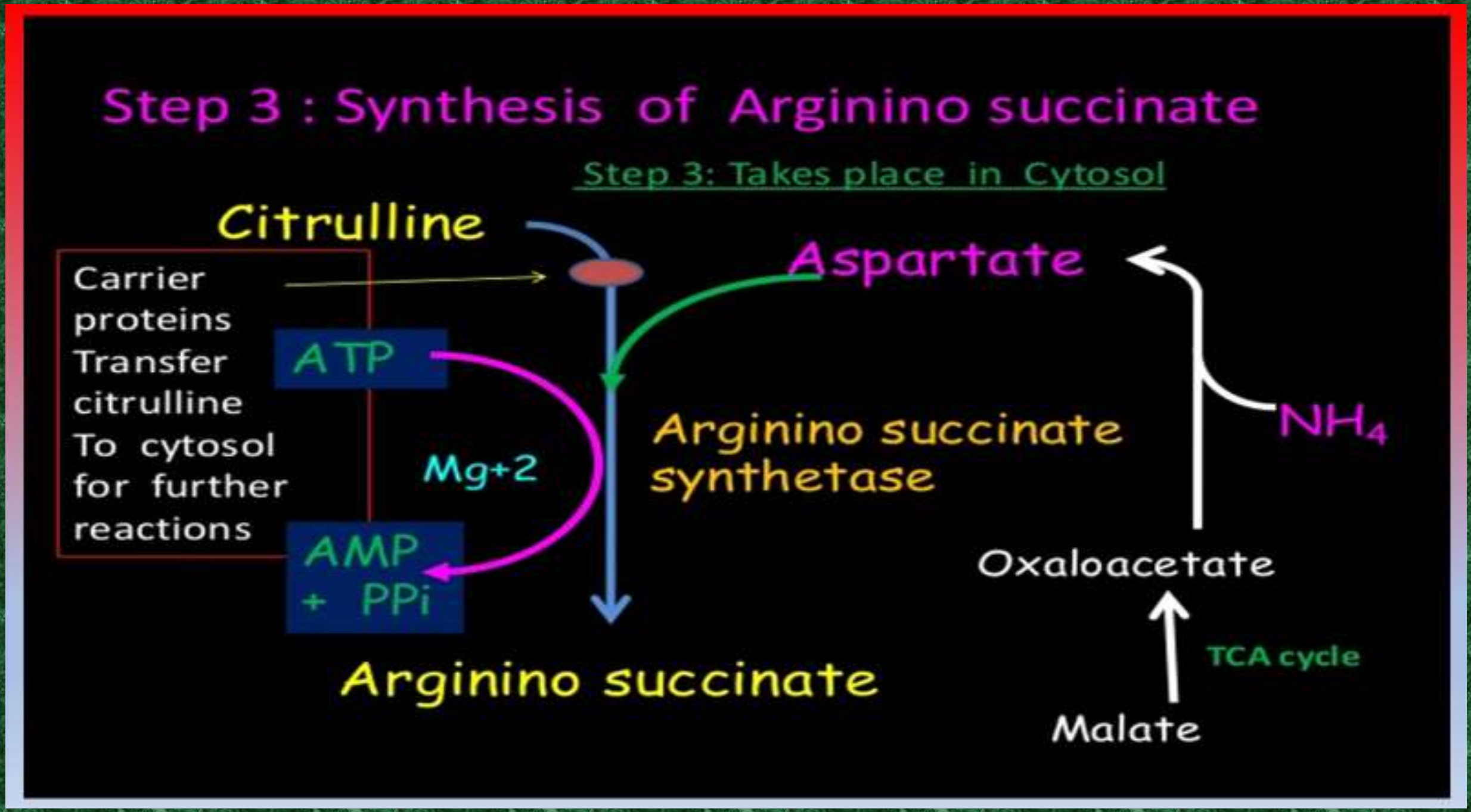
Carbamoyl Phosphate Synthetase I	Carbamoyl Phosphate Synthetase II
1. Found in mitochondria	Cytosol
2. NH_3 is the substrate	Amide of glutamine
3. Urea synthesis	Pyrimidine synthesis
4. N-acetyl glutamate is a positive effector	N-acetyl glutamate has no effect

Step 2 : Synthesis of Citrulline

Step 2 : Takes place in mitochondria



Carrier proteins transfer Ornithine from cytosol to mitochondria matrix


Step 3 : Synthesis of Arginino succinate
Step 3: Takes place in Cytosol
Citrulline
Carrier proteins Transfer citrulline To cytosol for further reactions
ATP
Mg+2
AMP + PPi
Aspartate
Arginino succinate synthetase
NH4
Oxaloacetate
TCA cycle
Malate
Arginino succinate

Step 4 : Synthesis of Arginine

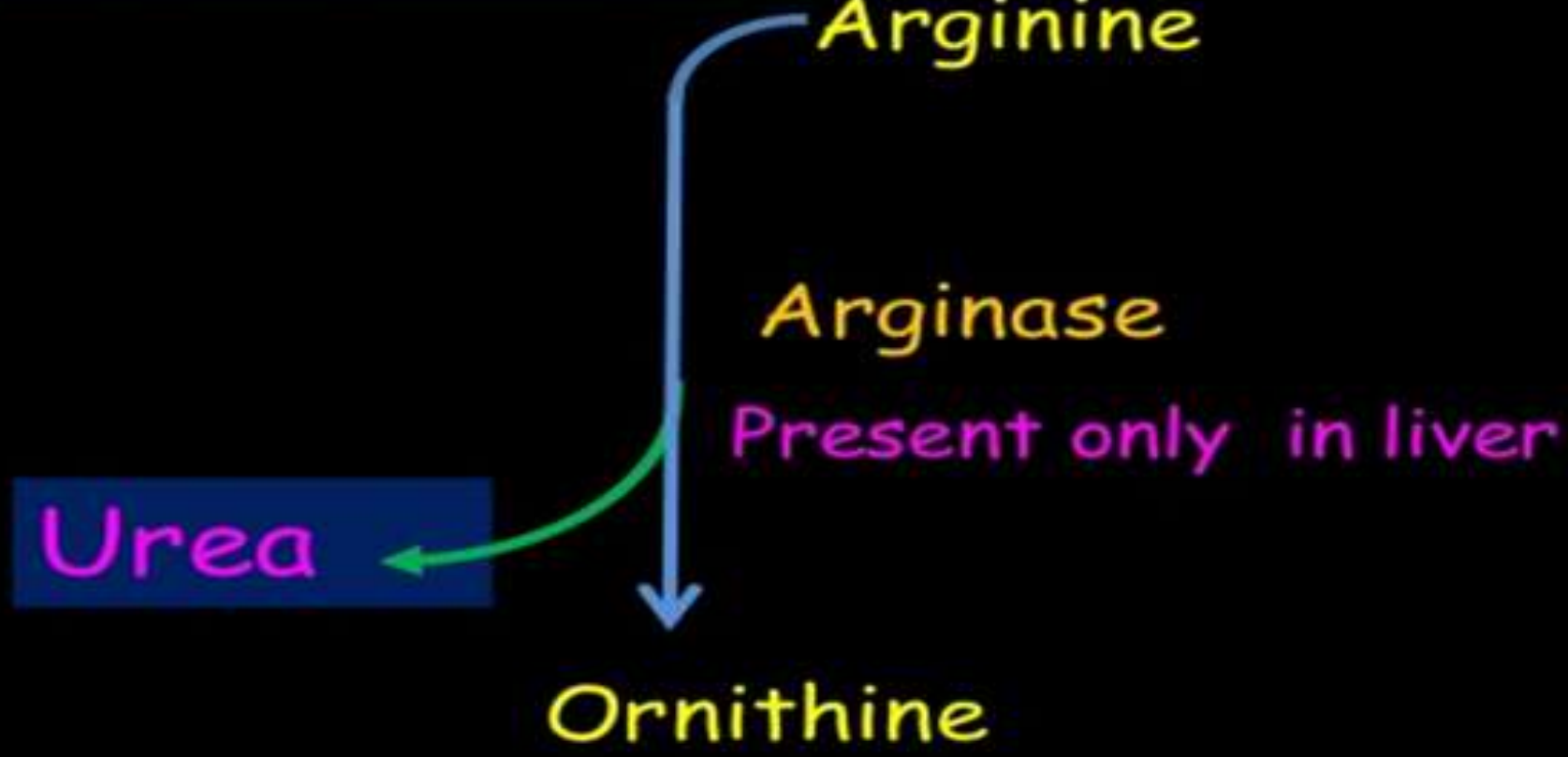
Step 4: Takes place in Cytosol

Arginine - Essential amino acid synthesized in adult

- Arginino succinate is cleaved to arginine and fumarate
- Enzyme is present in cytoplasm of liver and kidney tissues
- Link between Urea cycle and TCA cycle
- Fumarate can be converted to malate and then to oxaloacetate by the intervention of certain TCA cycle enzyme
- Aspartate may be regenerated by transamination

Step 5 : Release of Ornithine and Urea

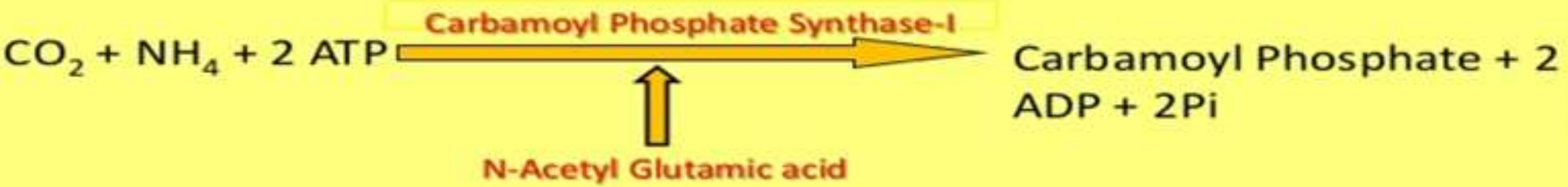
Step 5: Takes place in Cytosol



- Release of urea and ornithine
  - The guanido group of arginine Hydrolytically cleaved by arginase
  - The urea diffuses into blood from where it is cleared by the kidneys
  - Ornithine will enter mitochondria and become substrate for reaction 2

SYNTHESIS:

- STEP I: - Formation of carbamoyl phosphate



- STEP II: - Formation of citrulline

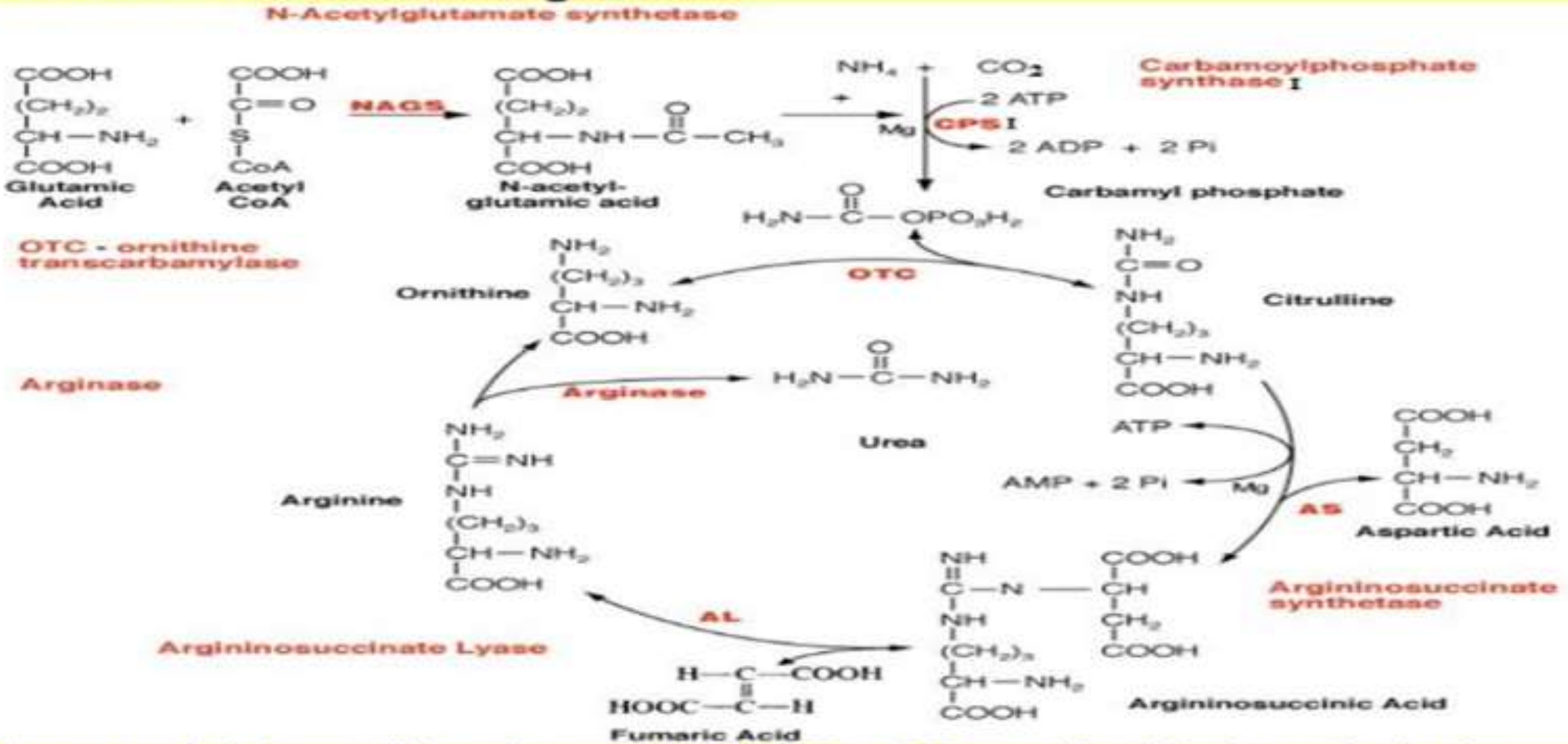
- STEP III: - Formation of Arginosuccinate

❑STEP IV: - Formation of Arginine

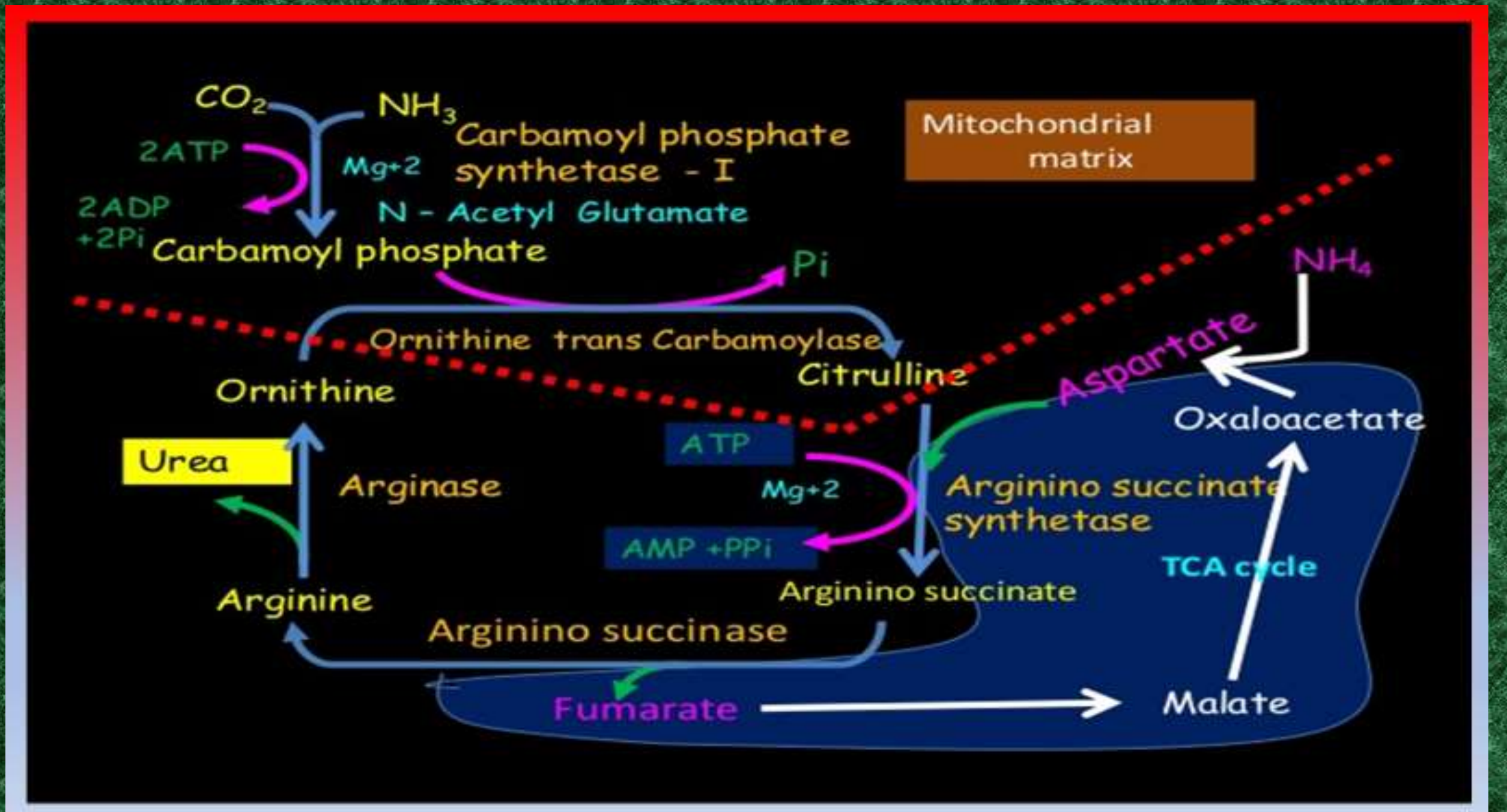
❑STEP V: - Formation of Urea

This Ornithine again bind with Carbamoyl Phosphate to form Citrulline. That's why it is a cyclic process.

Overall reaction and energetic:



The urea cycle is irreversible and 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.


CO_2
NH_3
Carbamoyl phosphate synthetase - I
2ATP
Mg+2
N - Acetyl Glutamate
2ADP +2Pi
Carbamoyl phosphate
Mitochondrial matrix
Pi
NH_4
Ornithine trans Carbamoylase
Citrulline
Aspartate
Ornithine
Oxaloacetate
ATP
Urea
Arginase
Mg+2
Arginino succinate synthetase
AMP +PPi
TCA cycle
Arginine
Arginino succinate
Arginino succinase
Fumarate
Malate

Blood urea

- Normal level- 15 - 45 mg/dl
- Uremia or azotemia is increased blood urea level due to renal failure
- Normally urea excretion is about 20-30 gm per day
- A high value of blood urea indicates significant reduction in the GFR and kidney disease
- Causes for reduction in GFR--- Pre renal, Renal and Post-renal

- Blood Urea estimation is the screening test for the evaluation of Kidney (renal) function.

Blood Urea Significance (continued): -

Pre-renal:

- This is associated with increased protein breakdown, leading to a negative nitrogen balance.
- Observed after major surgery, prolonged fever, diabetic coma, thyrotoxicosis etc.
- In leukemia & bleeding disorders also, blood Urea is elevated.

Renal:

- In renal disorders like acute glomerulonephritis, chronic nephritis, nephrosclerosis, polycystic kidey, blood Urea is increased.

Post-renal:

- Due to obstruction in the Urinary tract (e.g. tumors, stones, enlargement of prostate gland etc.) blood Urea is elevated.
- This is due to increased reabsorption of Urea from the tubules.

Common symptoms of urea cycle disorders includes

- Elevated blood NH_3 level
- Aversion to protein intake
- Tendency for vomiting
- Mental retardation
- Coma, convulsions and death

- Lowering the dietary protein intake has been suggested as a way of treatment

Thank you!