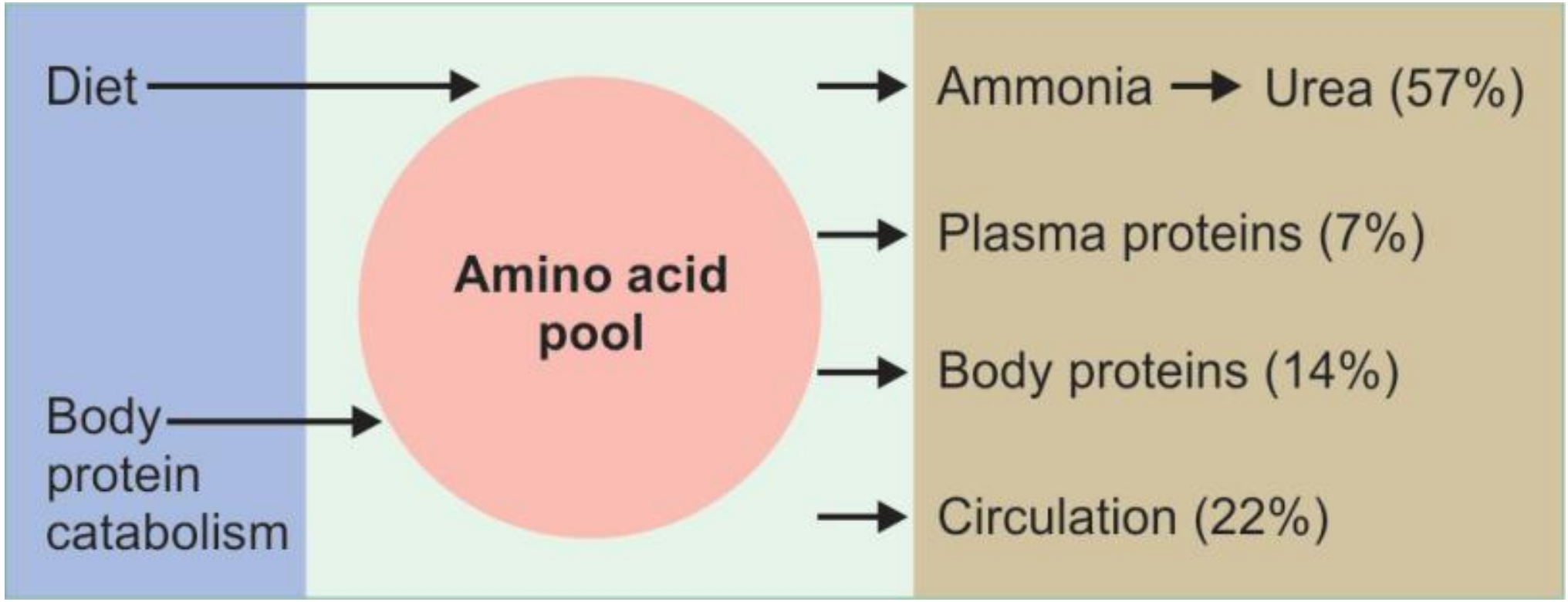
The learner will be able to:

- Mention the significance of transamination and transdeamination.
- Describe the formation of ammonia.
- Outline the urea cycle.
- Enumerate urea cycle disorders.
- Mention the urea level in blood and excretion in urine.
- Explain the genesis of hyperammonemia and the clinical sequelae.



Amino acid pool.

- **Breakdown of muscle protein is the source of amino acids for tissues while liver is the site of disposal.**

In Fasting State

- The muscle releases mainly alanine and glutamine of which **alanine is taken up by liver** and glutamine by kidneys.

- Liver removes the amino group and converts it to urea and the carbon skeleton is used for **gluconeogenesis**.

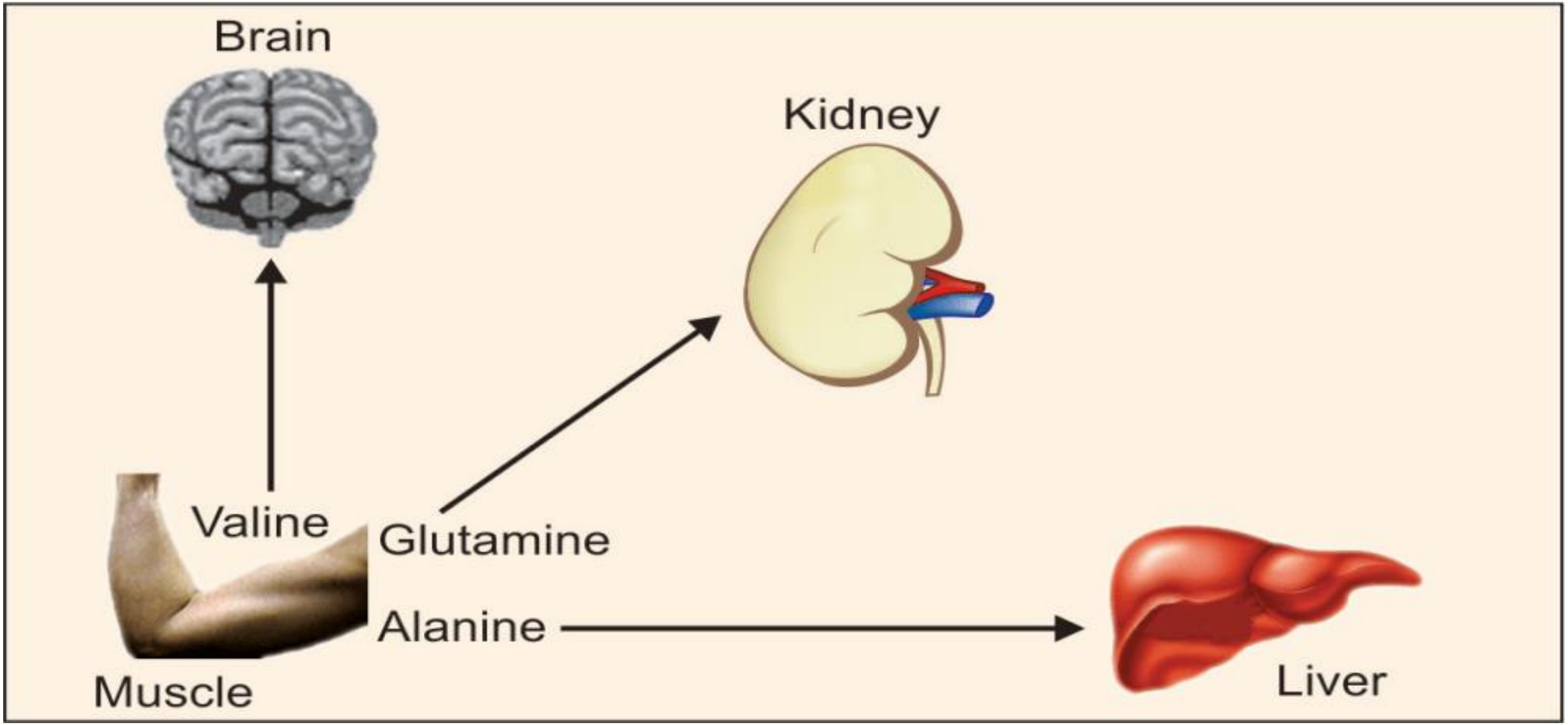
- Glucose-alanine cycle, under gluconeogenesis.
- The brain predominantly takes up branched chain amino acids.

In the Fed State

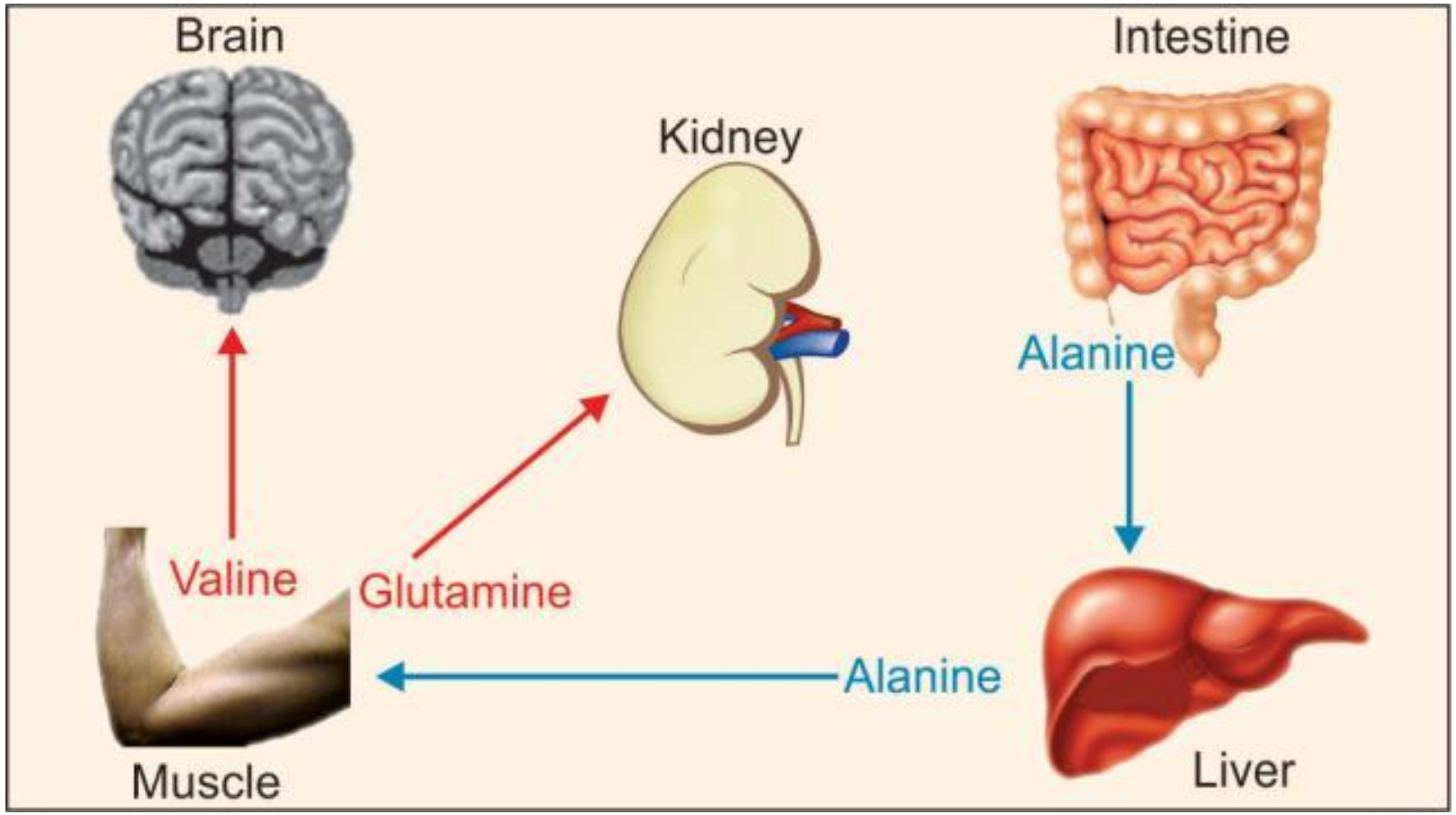
- Amino acids absorbed from the diet are taken up by different tissues.

- Both muscle and brain take up branched chain amino acids, and release glutamine and alanine.

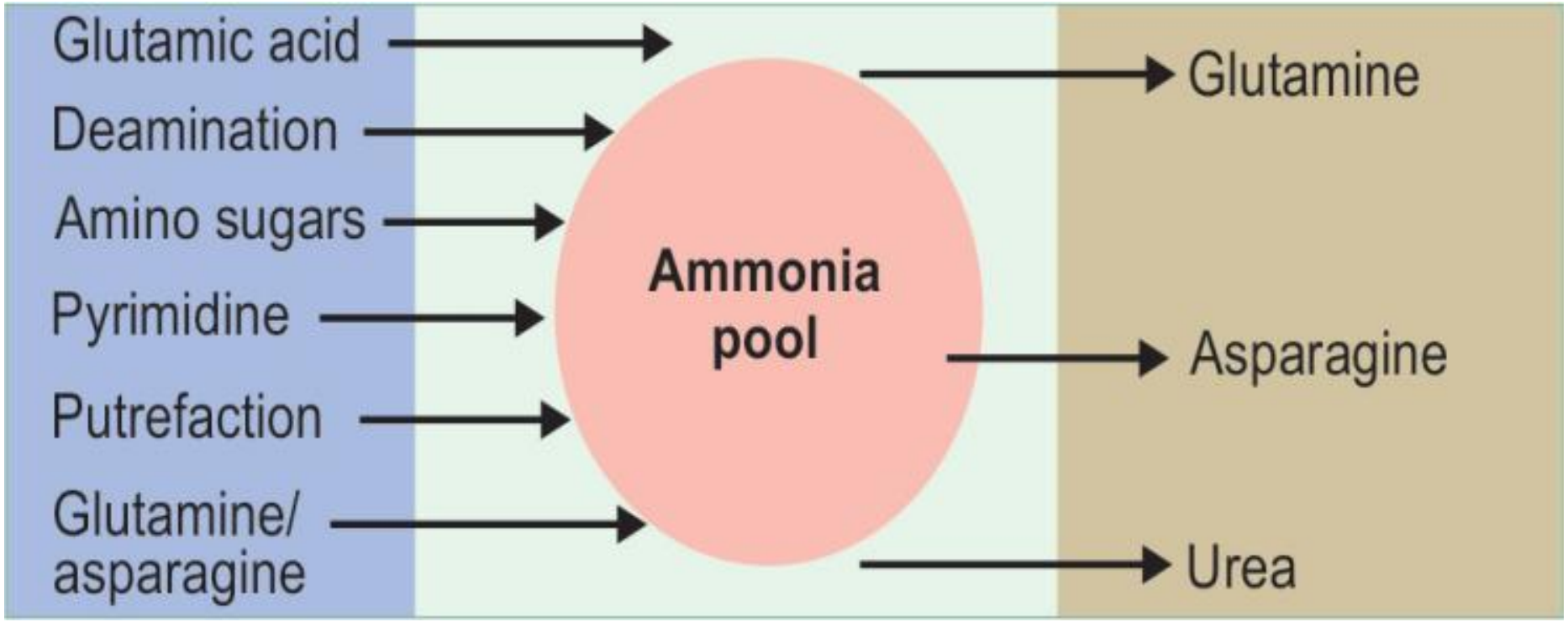
- The glutamine is delivered to kidneys to aid in regulation of acid-base balance, while alanine is taken up by liver.



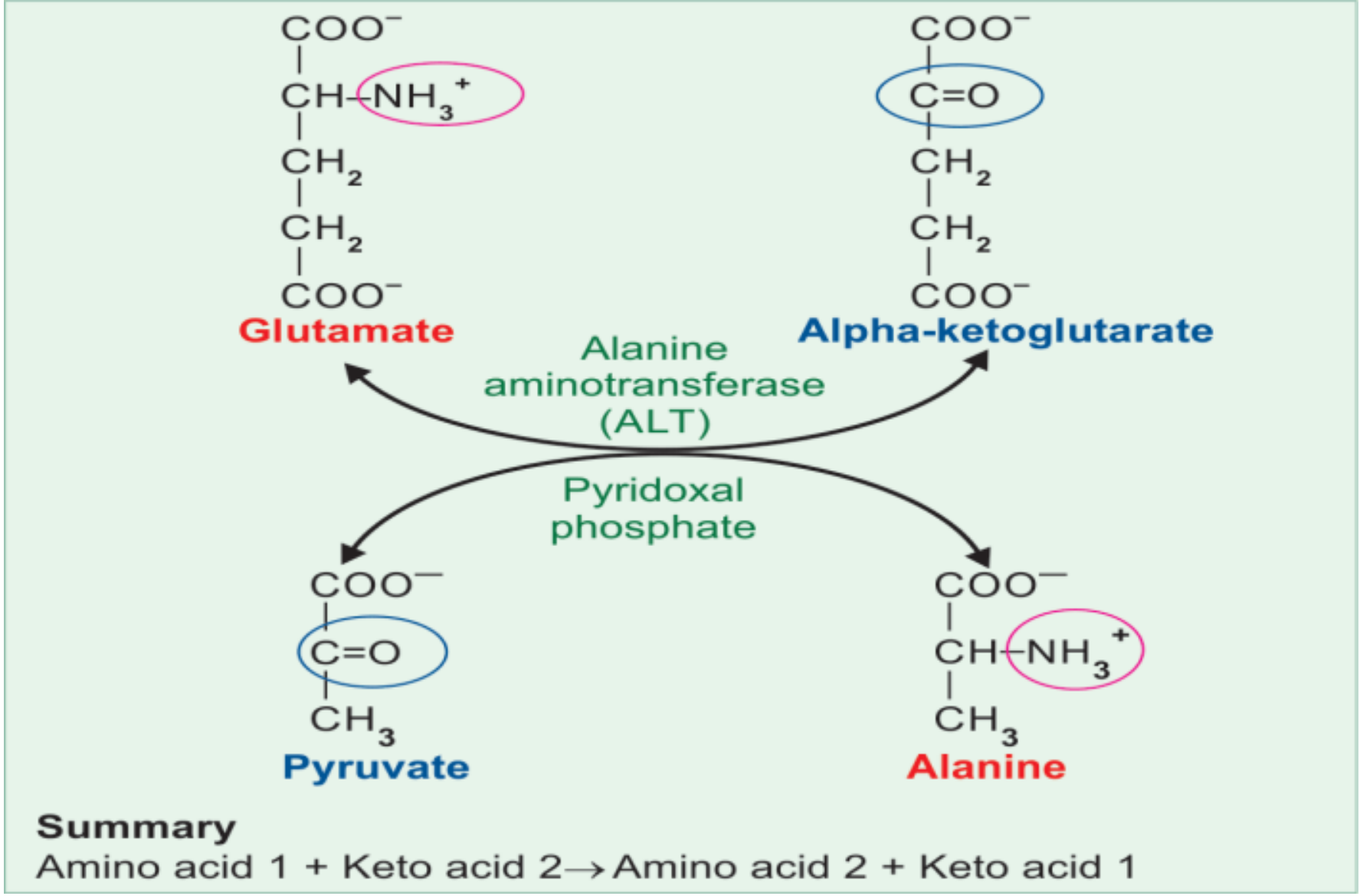
Inter-organ transport of amino acids during fasting conditions



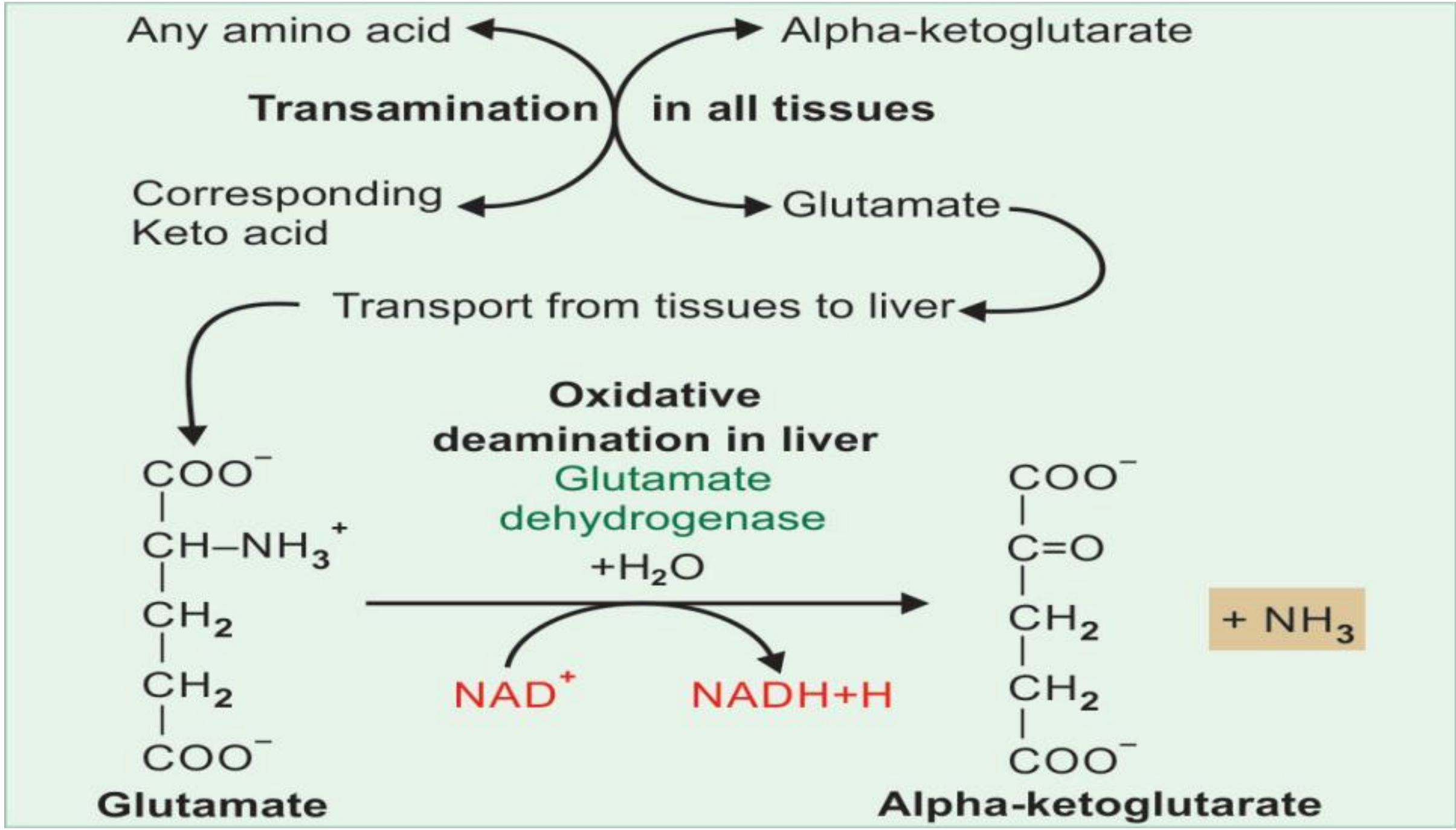
Inter-organ transport of amino acids after taking food (post-prandial condition)



Sources and fate of ammonia



Transamination reaction. In this example, the enzyme is alanine aminotransferase (ALT) and the coenzyme is pyridoxal phosphate. The reaction is readily reversible.


Any amino acid
Alpha-ketoglutarate
Transamination
in all tissues
Corresponding
Keto acid
Glutamate
Transport from tissues to liver
Oxidative
deamination in liver
Glutamate
dehydrogenase
+H2O
NAD+
NADH+H
+ NH3
Glutamate
Alpha-ketoglutarate

Transamination *- First Step of Catabolism*

In this first step, **ammonia** is removed, and the carbon skeleton of the amino acid enters into catabolic pathway.

Transamination + deamination = transdemination

Transamination in all tissues
Deamination only in liver

Clinical Significance of Transamination

- Aspartate amino transferase (**AST**) and Alanine amino transferase (**ALT**) are induced by glucocorticoids, which favour gluconeogenesis.

- AST is increased in **myocardial infarction** and ALT in **liver** diseases.

Biological significance of transamination -

- *Synthesis of Non-essential Amino Acids*
- By means of transamination, all nonessential amino acids can be synthesized by the body from keto acids available from other sources.
- For example, **pyruvate** can be transaminated to synthesize **alanine**.
- Similarly oxaloacetate produces aspartic acid.
- Alpha ketoglutarate is transaminated to form glutamic acid.
- Those amino acids, which cannot be synthesized in this manner, are therefore essential; they should be made available in the food.

- *Interconversion of Amino Acids*

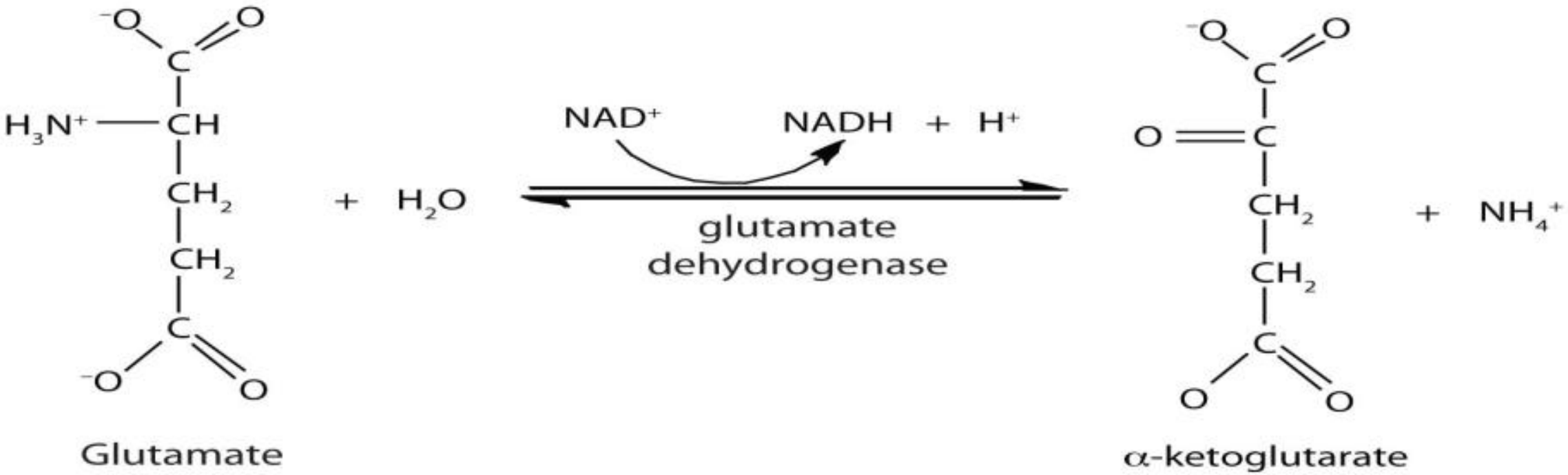
- If amino acid no.1 is high and no. 2 is low; the amino group from no.1 may be transferred to a keto acid to give amino acid no. 2 to equalize the quantity of both.

- This is called **equalization** of quantities of non-essential amino acids.

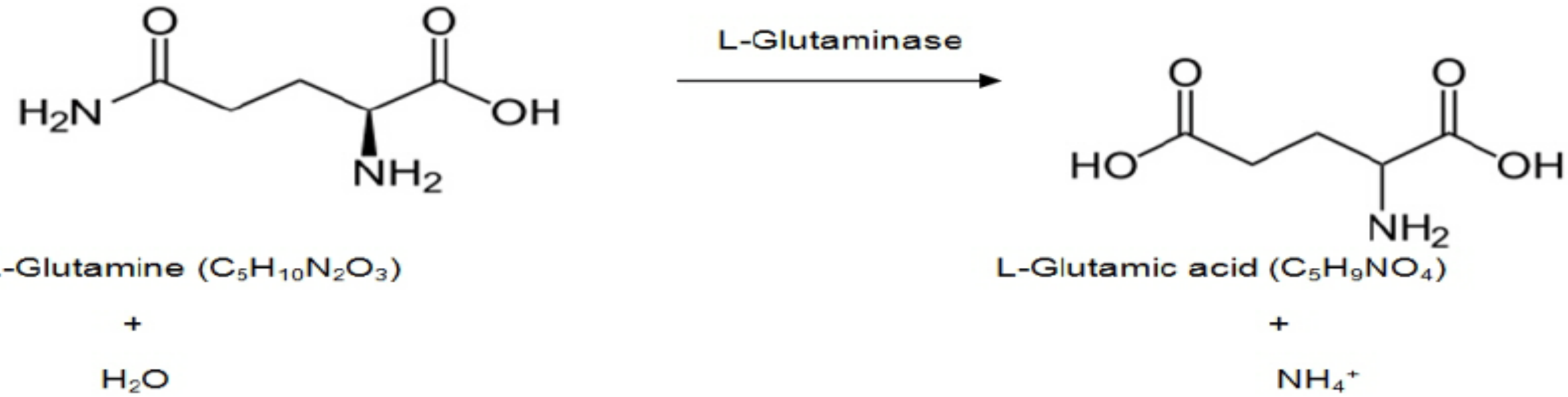
Oxidative deamination of glutamate –

- Only **liver** mitochondria contain **glutamate dehydrogenase (GDH)**
- Amino acids are first transaminated to glutamate, then deaminated at the rate of about 50–70 gram per day.
- During the transamination reaction the amino group of all other amino acids is funneled into glutamate.
- Hence, the glutamate dehydrogenase reaction is the final reaction, which removes the amino group of all amino acids. It needs NAD+ as coenzyme.

It is an allosteric enzyme; it is activated by ADP and inhibited by GTP.



The hydrolysis of glutamine also yields NH3 but this occurs mainly in the kidney where the NH4+ excretion is required for acid-base regulation.



Formation of ammonia -

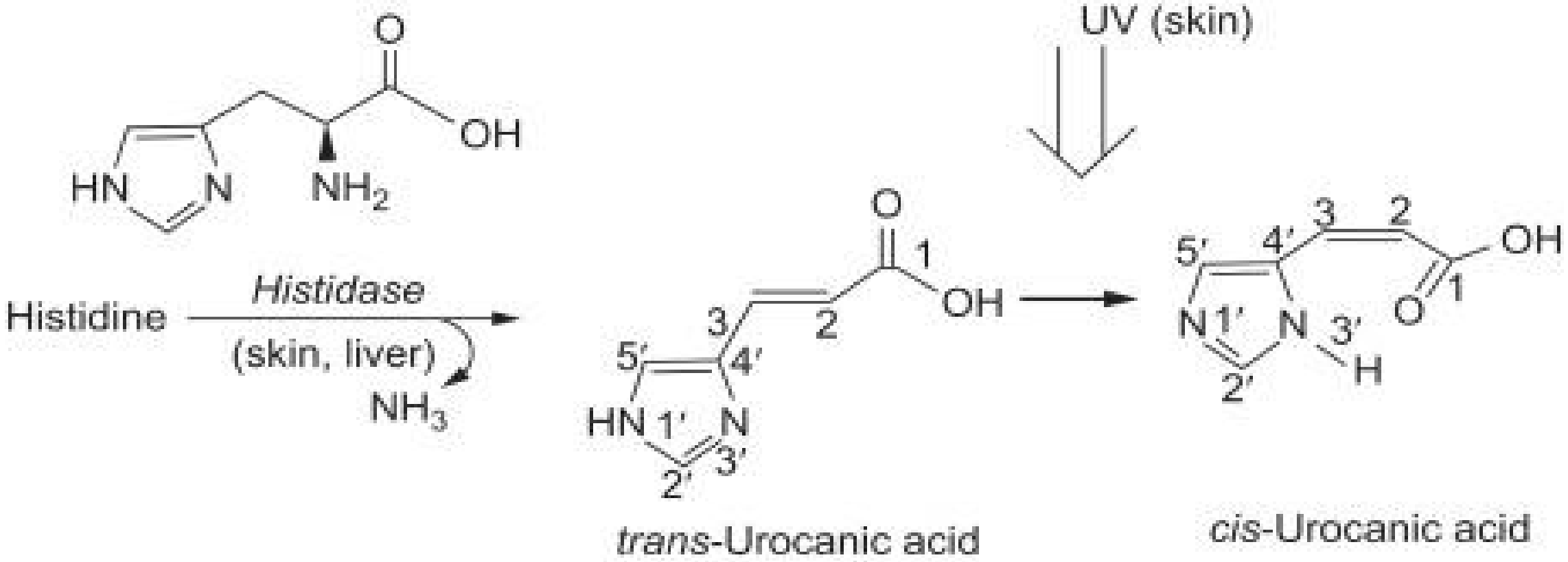
- The first step in the catabolism of amino acids is to remove the amino group as **ammonia**. major source of ammonia.

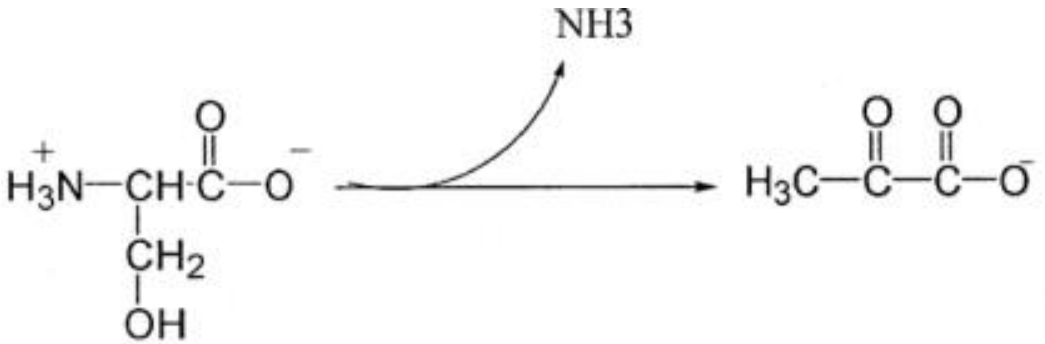
- Ammonia is **highly toxic** especially to the nervous system.

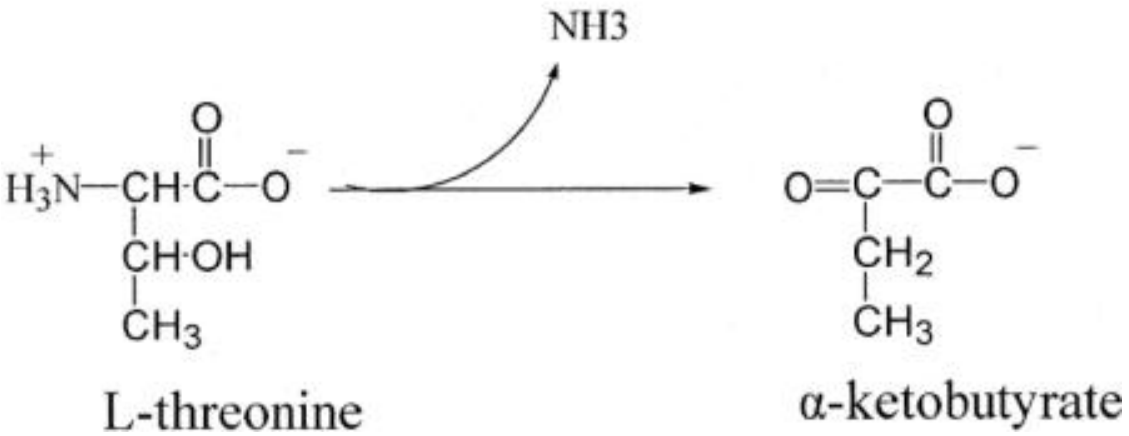
- Detoxification of ammonia is by conversion to urea and excretion through urine.

Minor pathway of Ammonia Production -

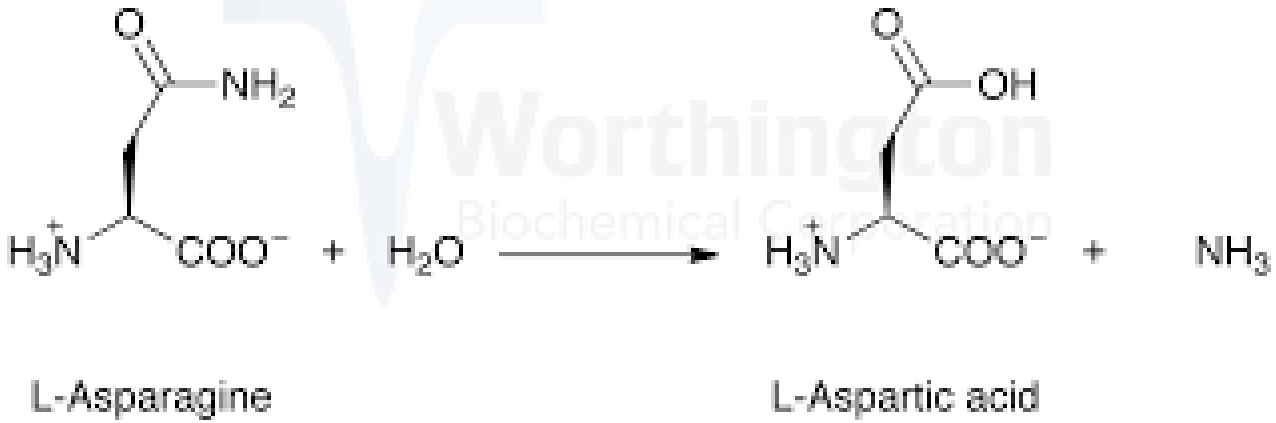
- Histidine to urocanic acid and NH_3 by histidase
- Asparagine to aspartate and NH_3 by asparaginase
- Serine and threonine to pyruvate and alpha ketobutyrate respectively by dehydratases

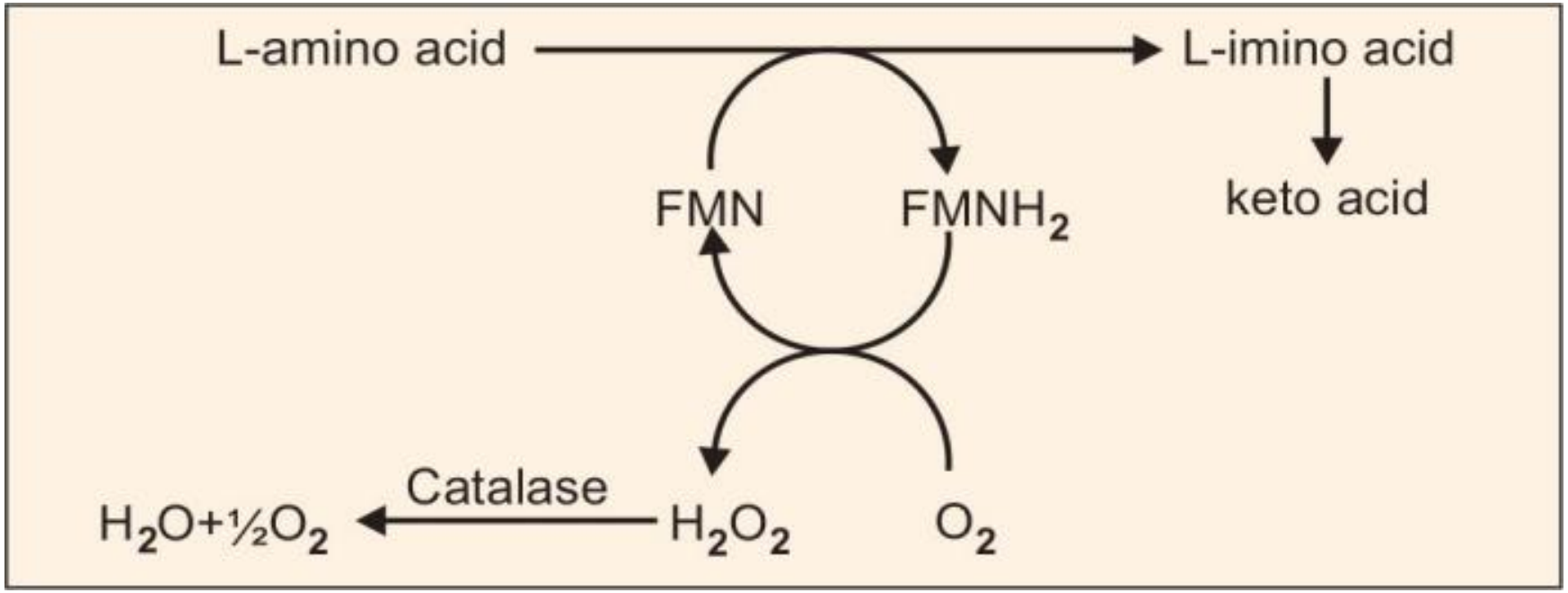






Asparaginase

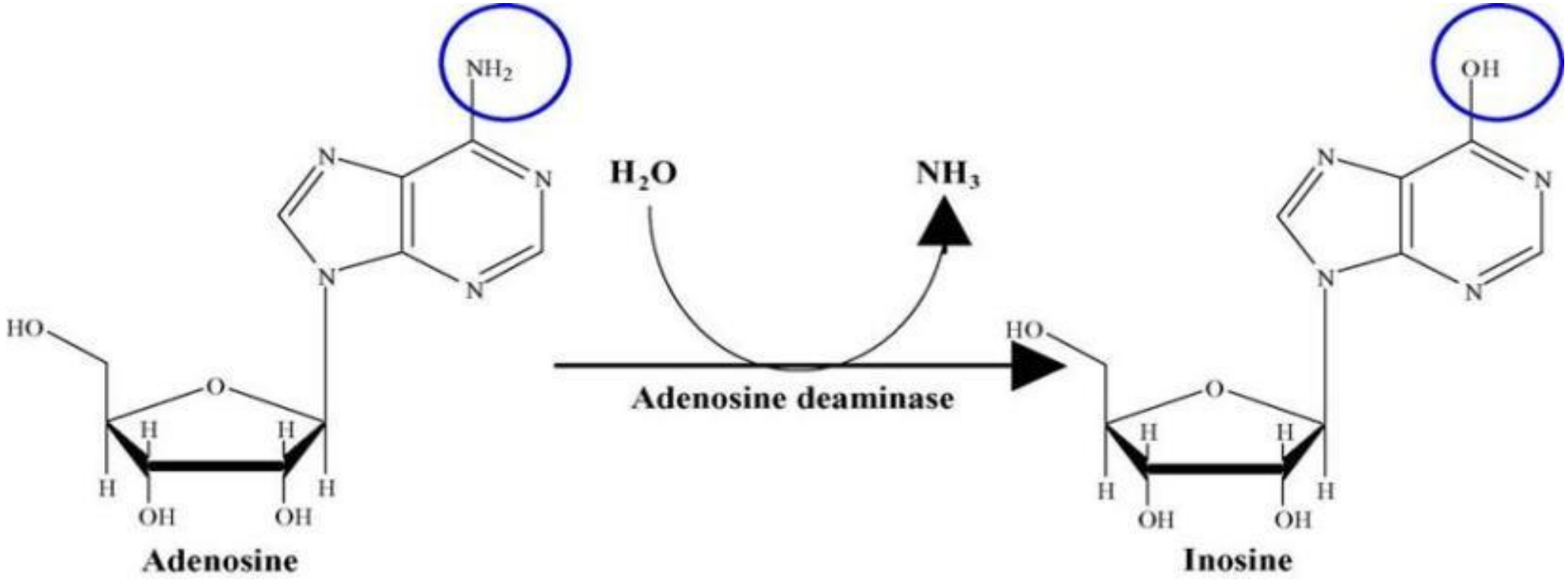




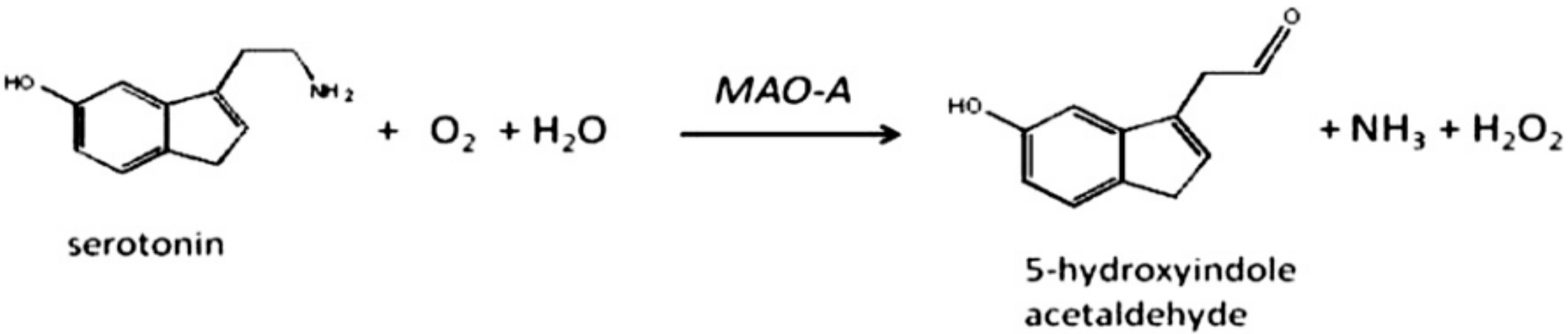
- L amino acid oxidase a flavoprotein also releases ammonia from amino acids.

- Ammonia may also be produced in the gastro-intestinal tract by bacterial putrefaction, bacterial metabolism which reaches the liver through portal vein.

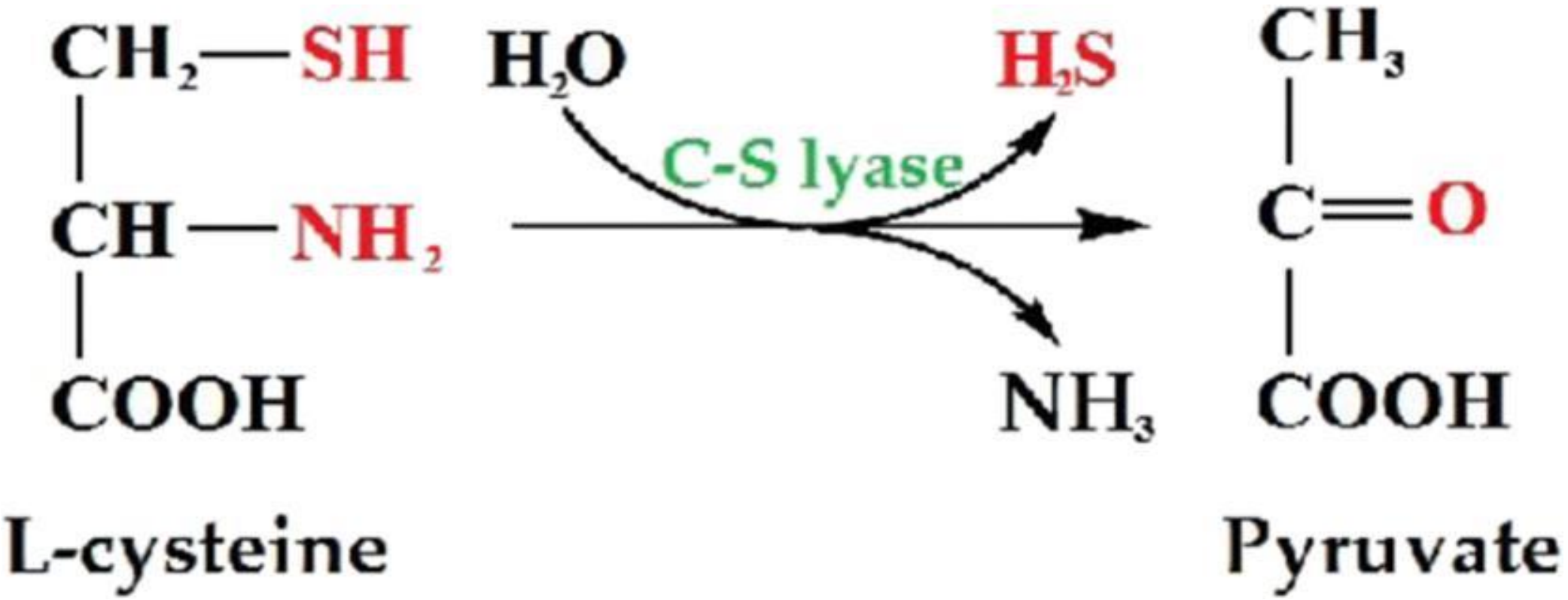
Ammonia is also released during the purine nucleotide cycle (adenylosuccinase), purine catabolism (ADA and guanase) and pyrimidine catabolism.



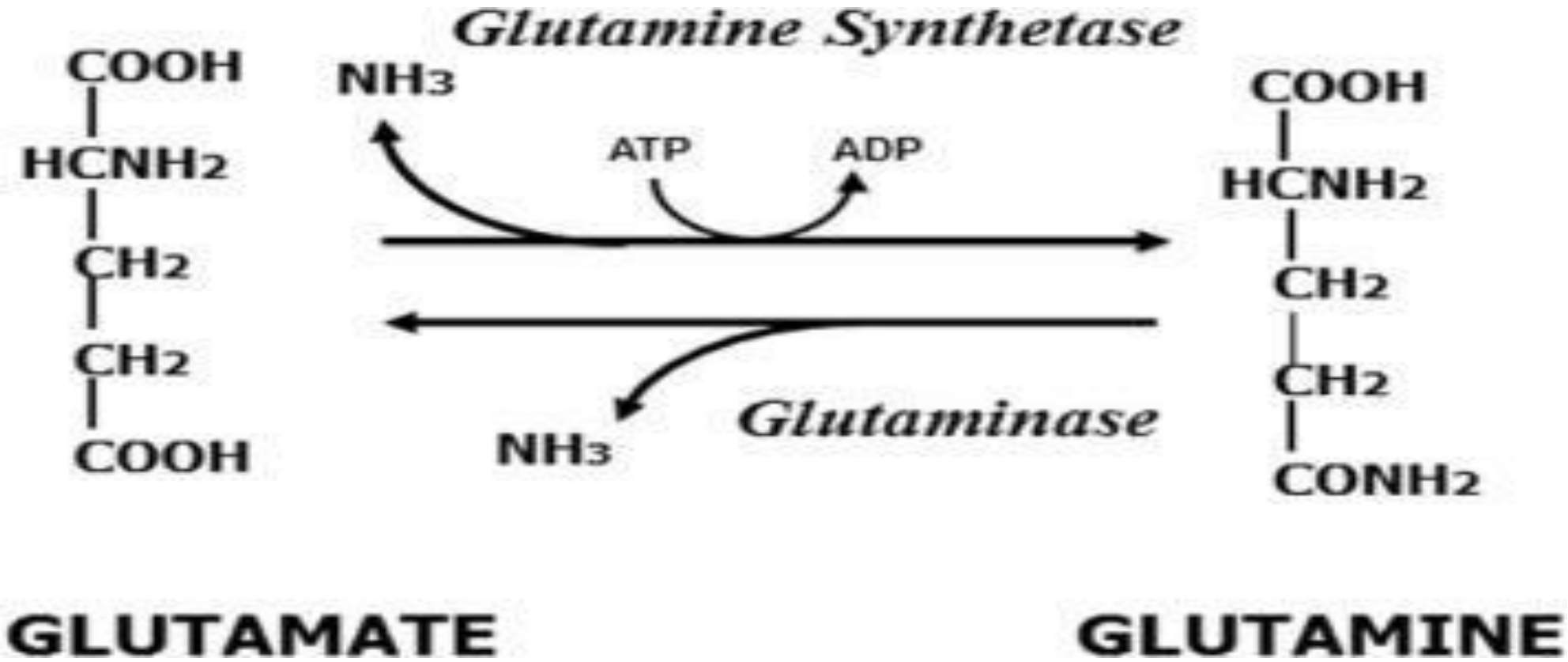
- Ammonia may be formed in the body through minor reactions like oxidation of monoamines by MAO (mono amine oxidase).



- **Cysteine** undergoes deamination and simultaneous trans-sulphuration to form pyruvate by the enzyme desulfhydrase.

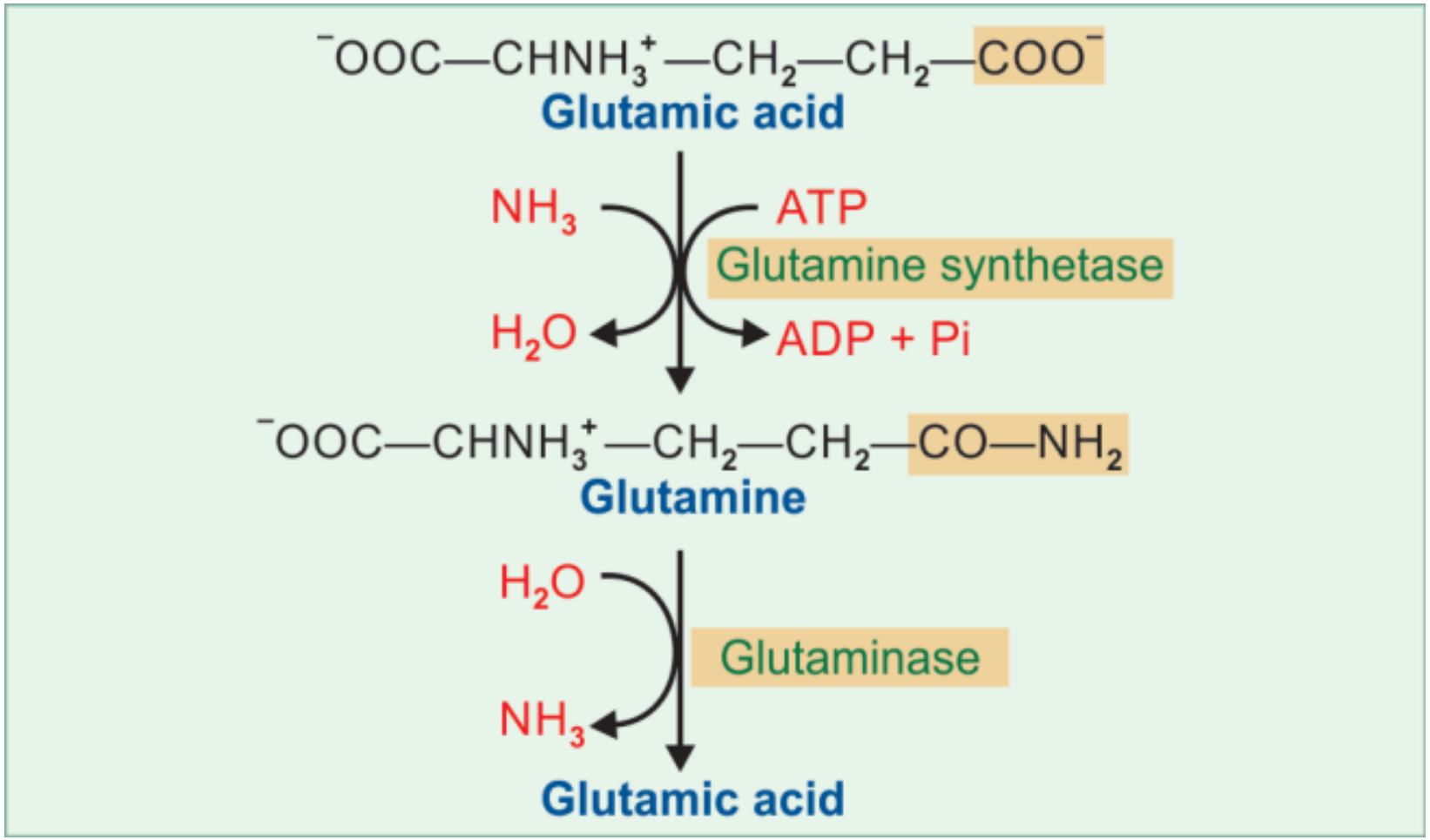


The hydrolysis of glutamine to NH_3 and glutamate in the kidney where the NH_4^+ excretion is required for acid base regulation.



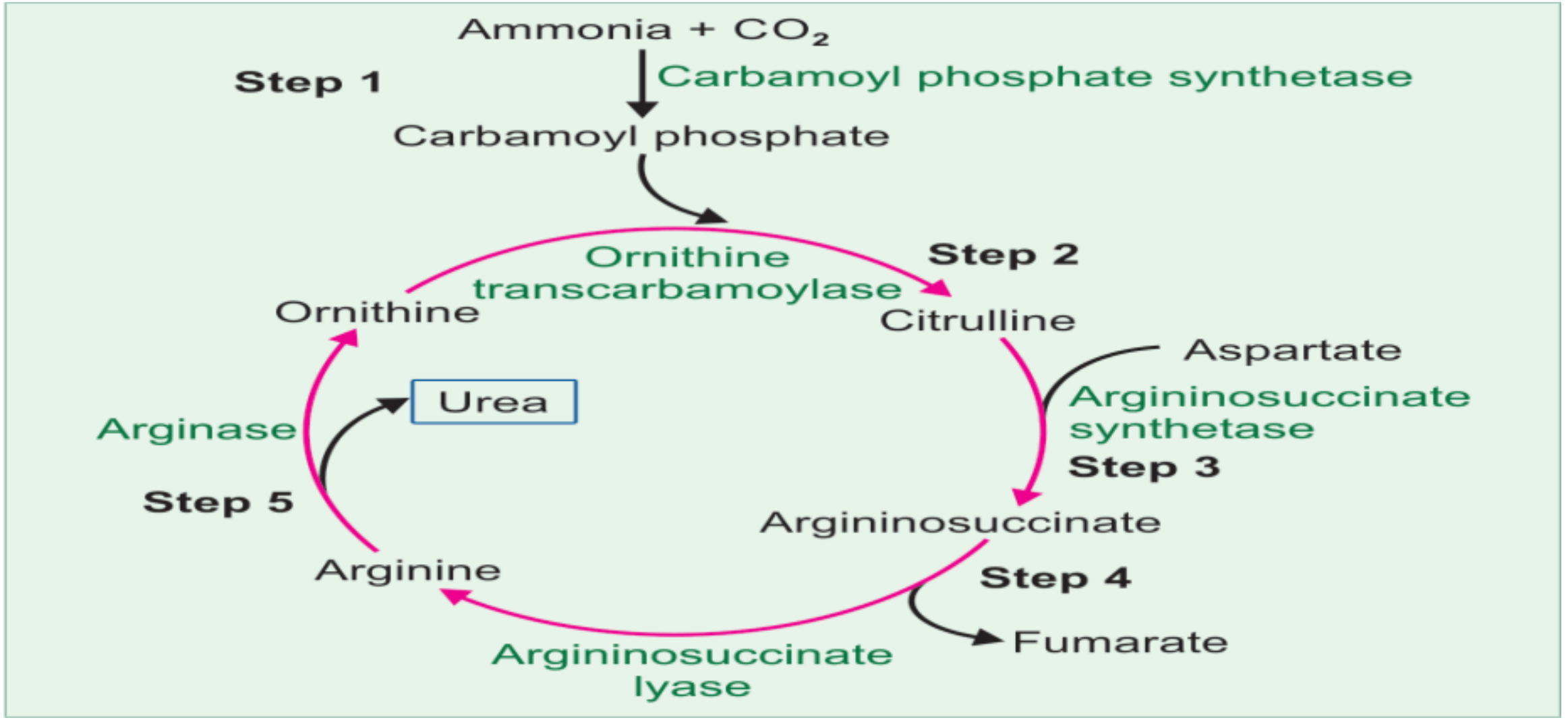
Detoxification of Ammonia

1. Reduced production
2. Ammonia trapping
3. Urea synthesis

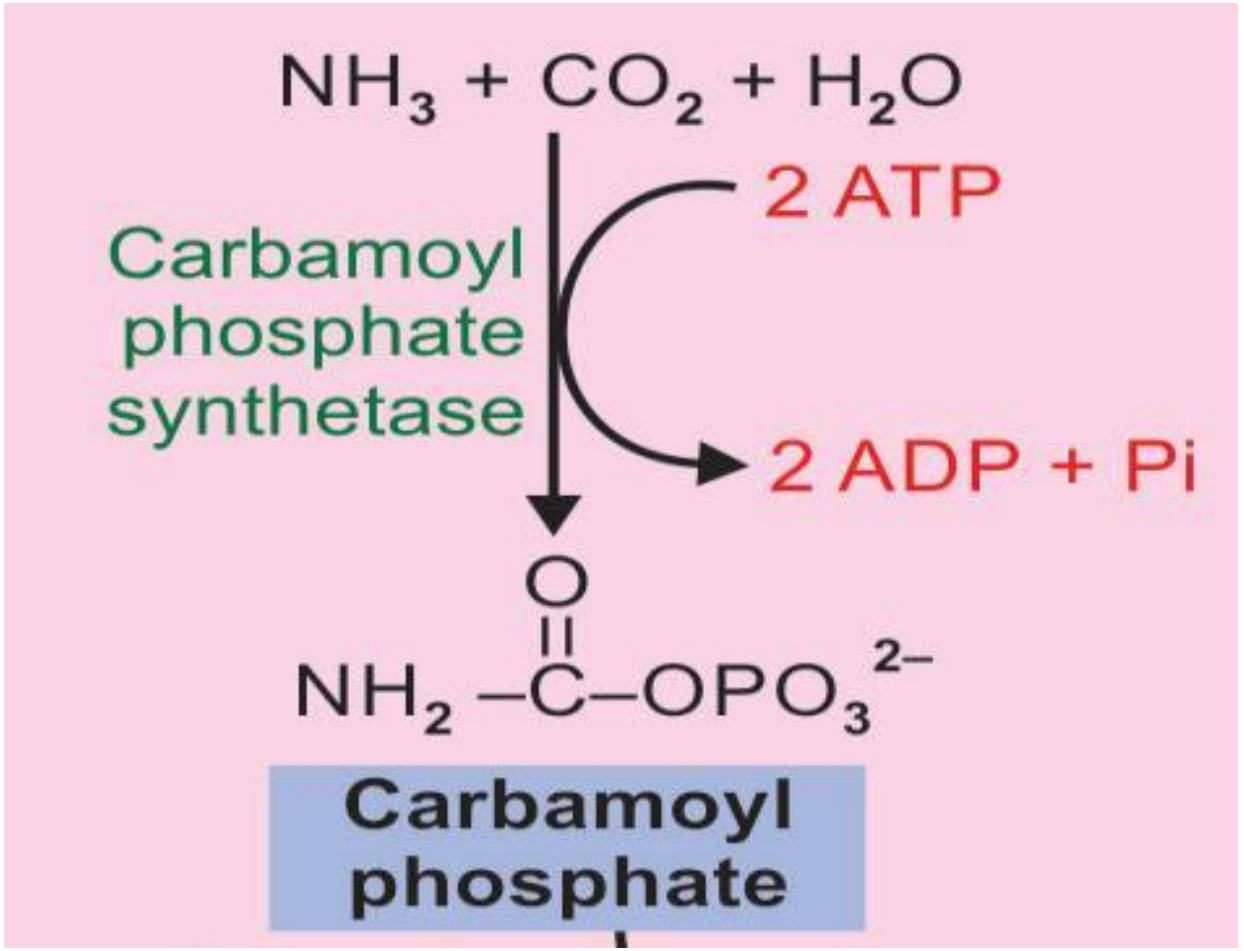


Ammonia trapping as glutamine.

End product of Protein metabolism

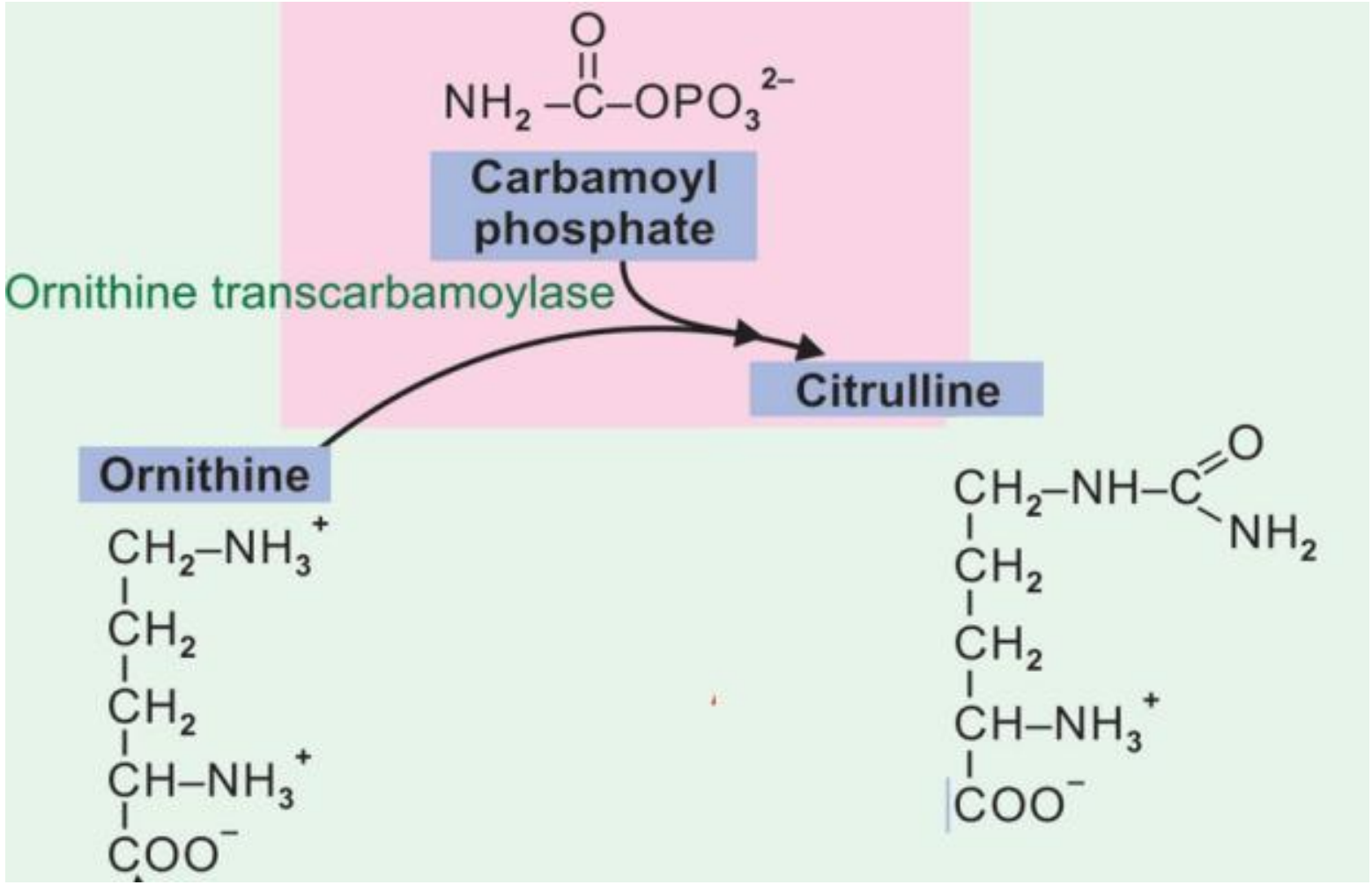


Summary of urea cycle

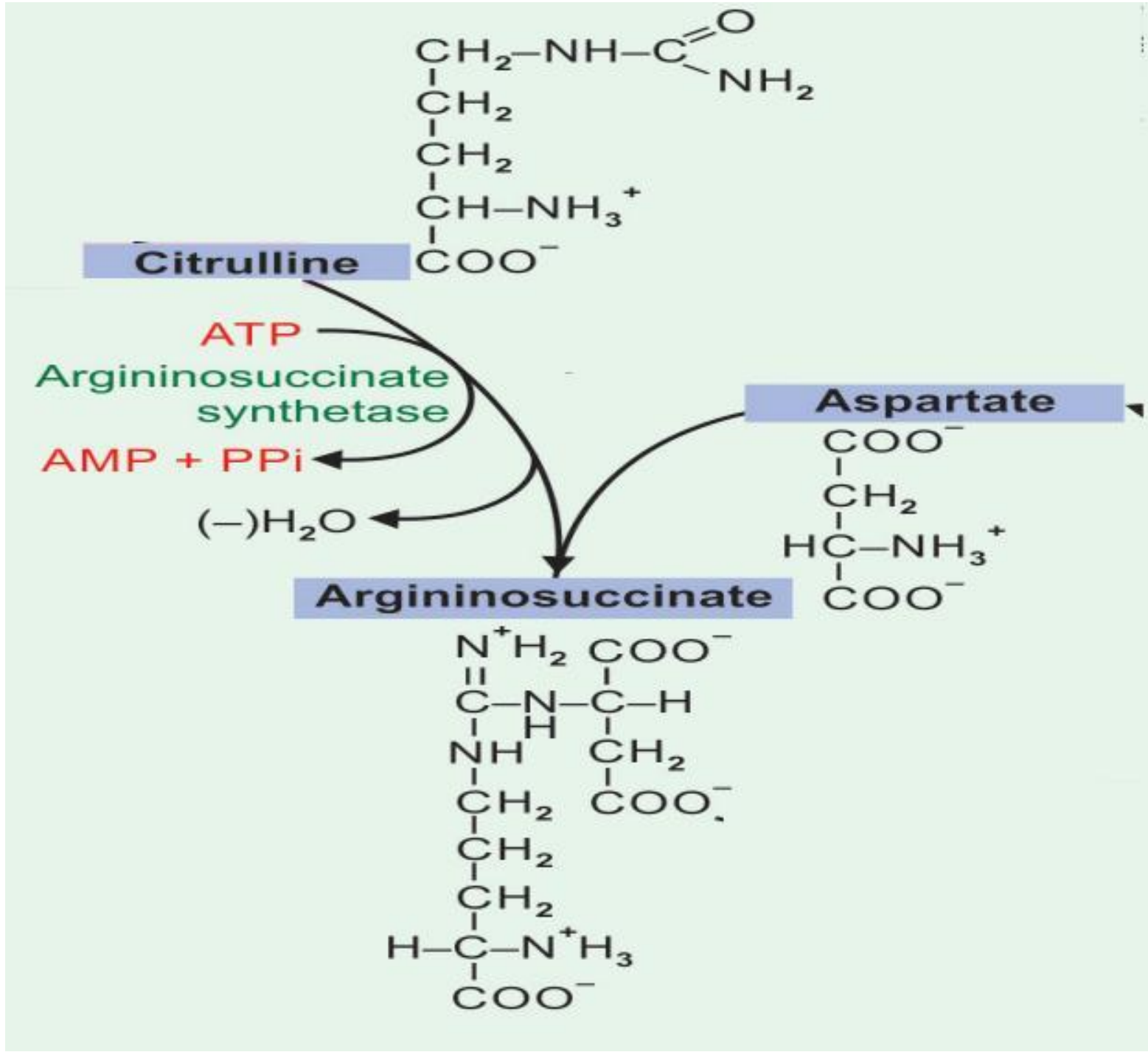


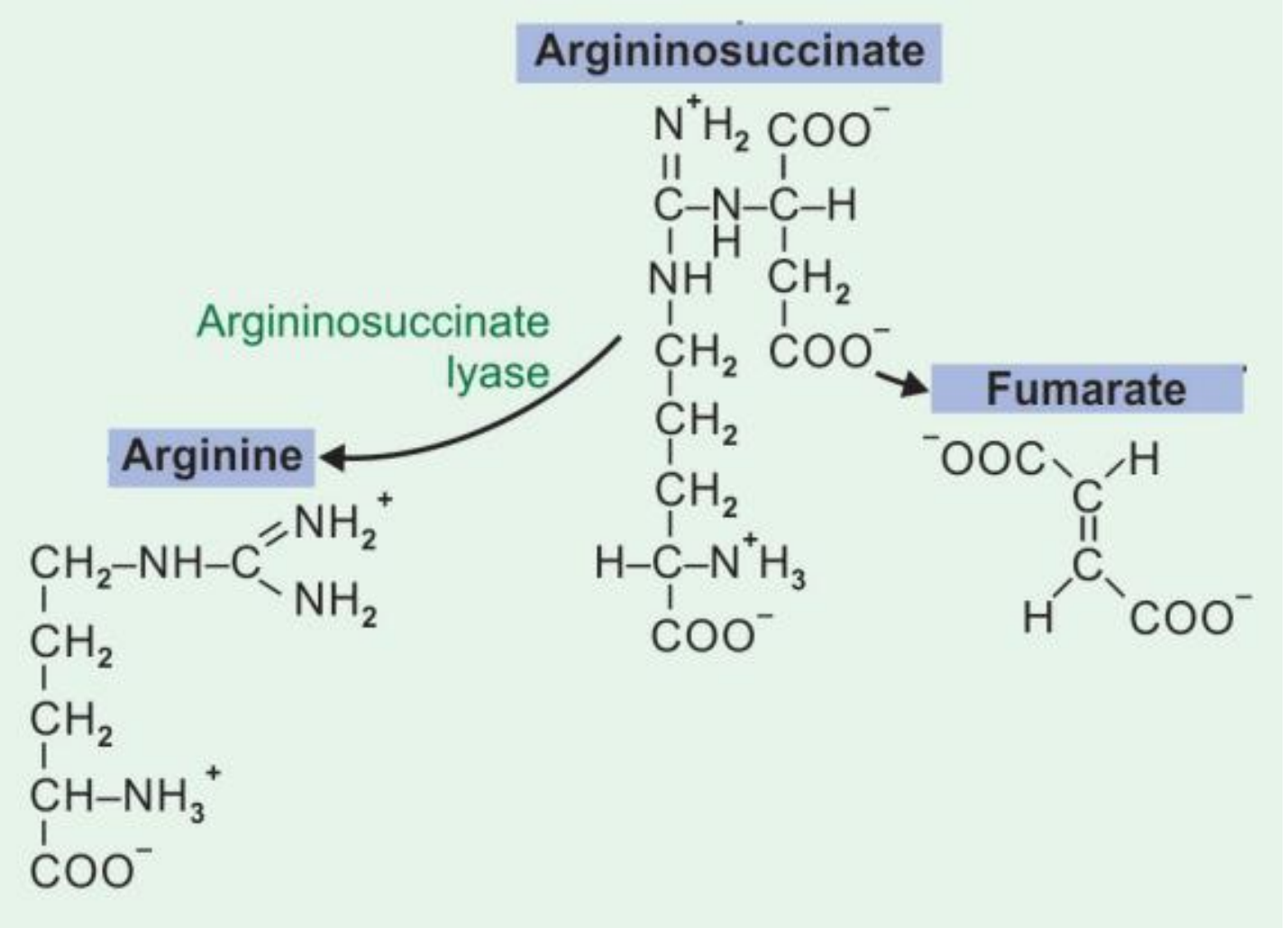
First and Rate limiting step of the urea cycle.

Comparison of CPS I and II enzymes		
	CPS-I	CPS-II
1. Site	Mitochondria	Cytosol
2. Pathway of	Urea	Pyrimidine
3. Positive effector	NAG	Nil
4. Source for N	Ammonia	Glutamine
5. Inhibitor	Nil	CTP

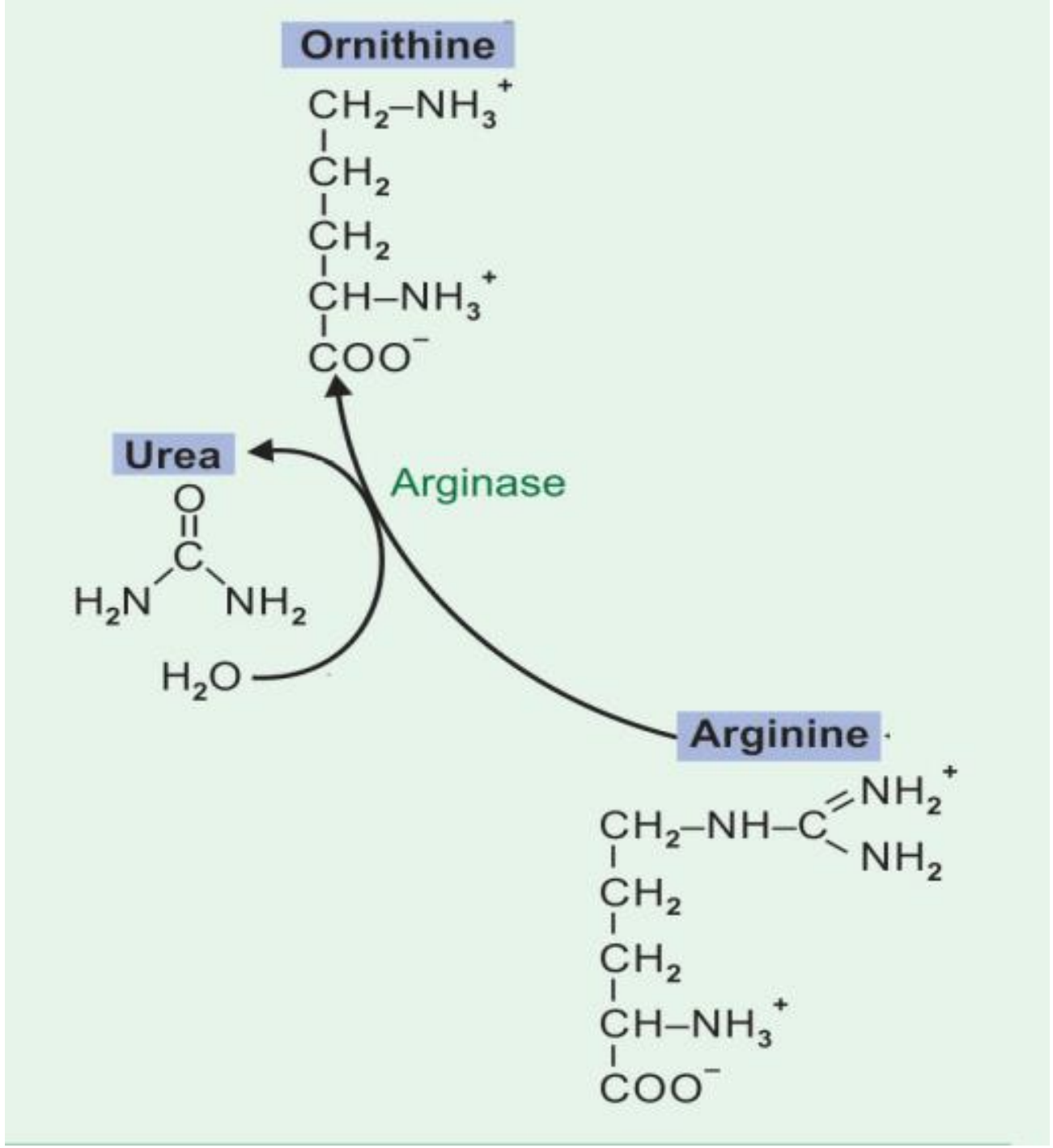

Carbamoyl phosphate
Ornithine transcarbamoylase
Citrulline
Ornithine

Step 2

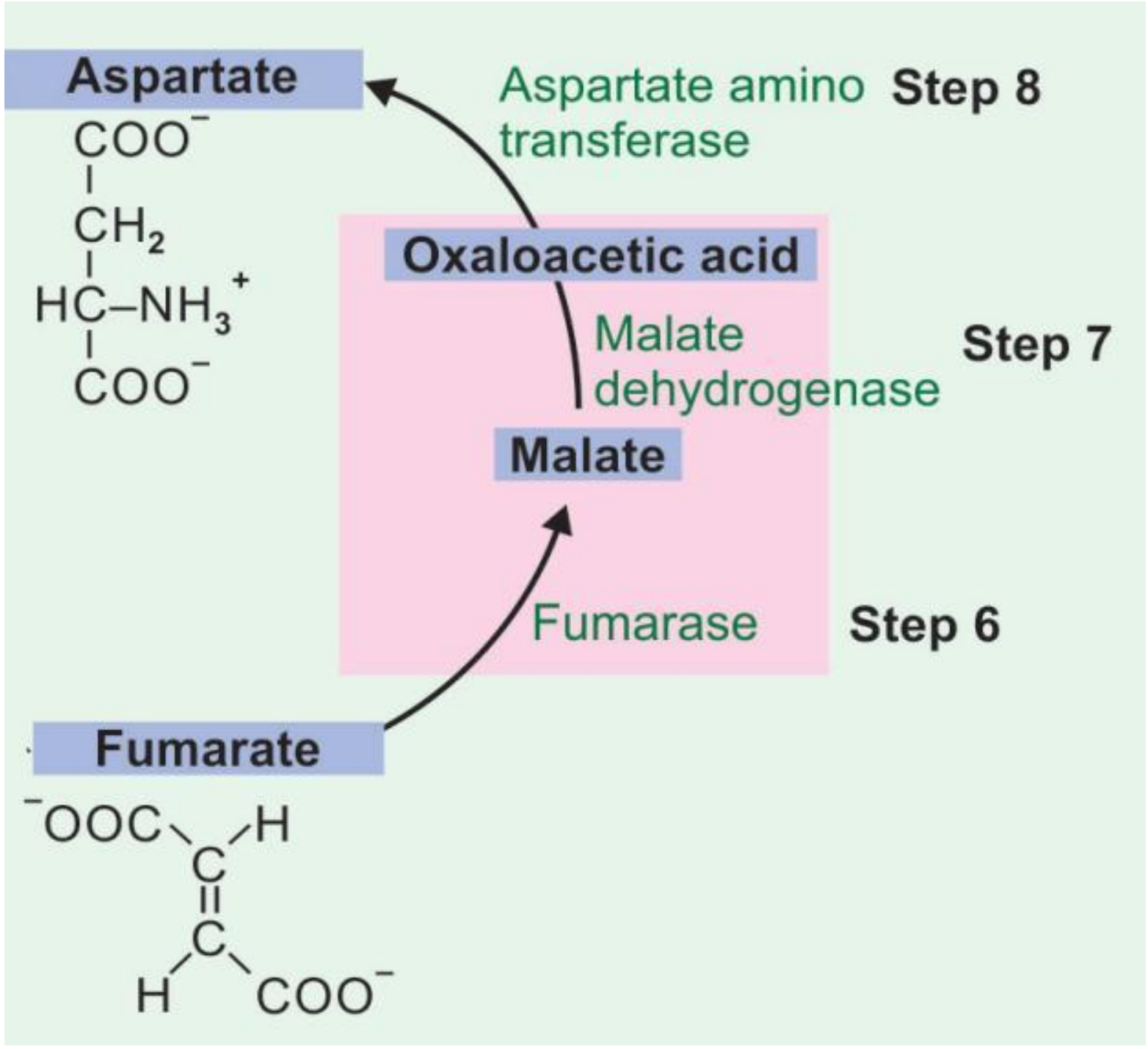

Citrulline
ATP
Argininosuccinate synthetase
AMP + PPi
(–)H2O
Aspartate
Argininosuccinate

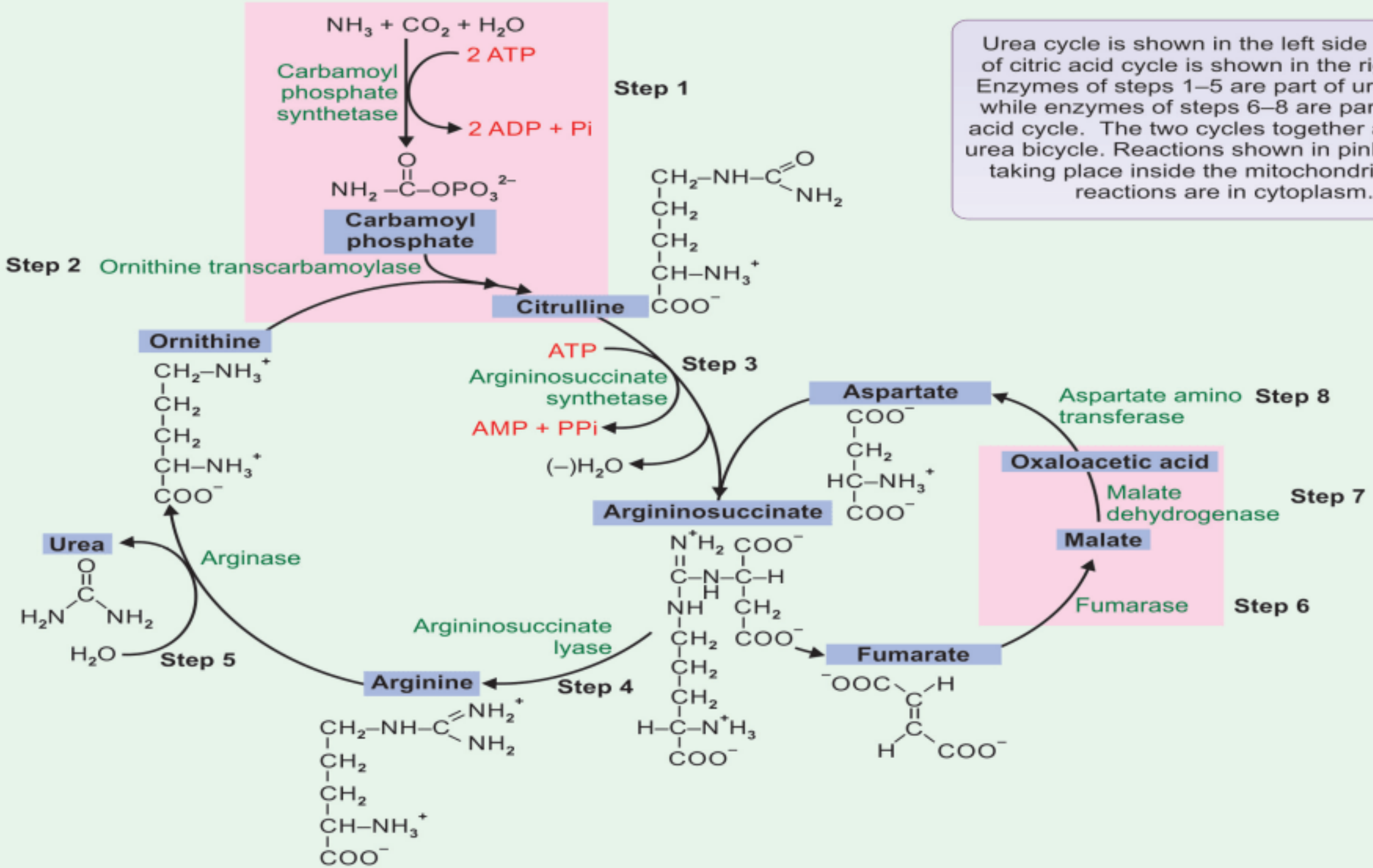

Argininosuccinate
Argininosuccinate lyase
Arginine
Fumarate

Step 4


Ornithine
CH2–NH3+
CH2
CH2
CH–NH3+
COO−
Urea
O
C
H2N
NH2
H2O
Arginase
Arginine
NH2+
CH2–NH–C
NH2
CH2
CH2
CH–NH3+
COO−

Step 5


Aspartate
COO⁻
CH₂
HC–NH₃⁺
COO⁻
Aspartate amino transferase
Step 8
Oxaloacetic acid
Malate dehydrogenase
Step 7
Malate
Fumarase
Step 6
Fumarate
⁻OOC
H
C
C
H
COO⁻


Urea cycle is shown in the left side and part of citric acid cycle is shown in the right side. Enzymes of steps 1–5 are part of urea cycle, while enzymes of steps 6–8 are part of citric acid cycle. The two cycles together are called urea bicycle. Reactions shown in pink area are taking place inside the mitochondria; other reactions are in cytoplasm.
NH3 + CO2 + H2O
2 ATP
Carbamoyl phosphate synthetase
2 ADP + Pi
Step 1
Carbamoyl phosphate
Step 2
Ornithine transcarbamoylase
Citrulline
Ornithine
ATP
Argininosuccinate synthetase
AMP + PPi
(–)H2O
Step 3
Aspartate
Aspartate amino transferase
Step 8
Oxaloacetic acid
Malate dehydrogenase
Step 7
Malate
Fumarase
Step 6
Argininosuccinate
Argininosuccinate lyase
Step 4
Fumarate
Arginine
Arginase
Urea
H2O
Step 5

Energetics of urea cycle

The overall reaction may be summarized as:

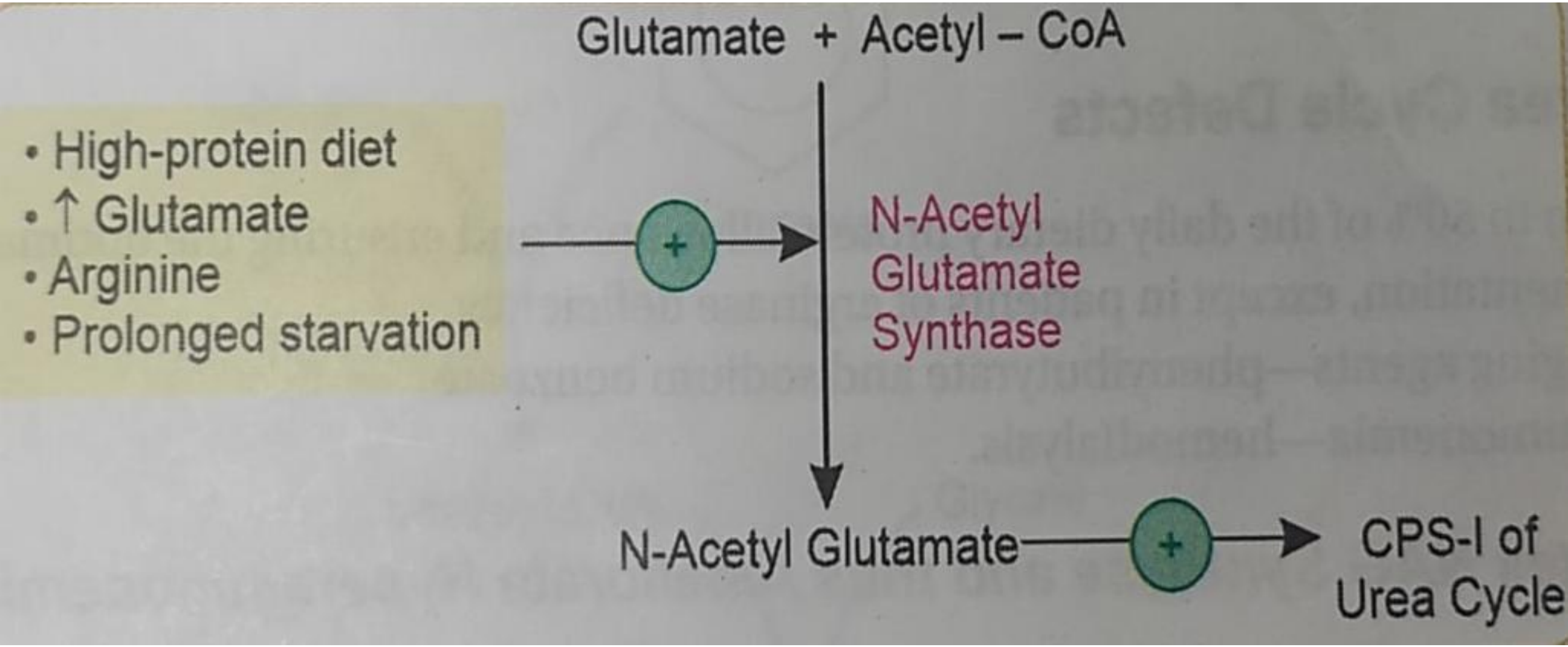
- $NH_3 + CO_2 + Aspartate \rightarrow Urea + Fumarate$
- In the urea cycle 2 ATPs are used in the first reaction.
- Another ATP is converted to AMP and PPi, which is equivalent to 2 ATPs.
- The urea cycle consumes **4 high energy phosphate bonds.**
- However, fumarate formed in the 4th step may be converted to malate.
- Malate when oxidized to oxaloacetate produces 1 NADH equivalent to 2.5 ATP.
- So, **net energy expenditure is only 1.5 high energy phosphates**.
- The urea cycle and TCA cycle are interlinked, and so, it is called as "**urea bicycle**".

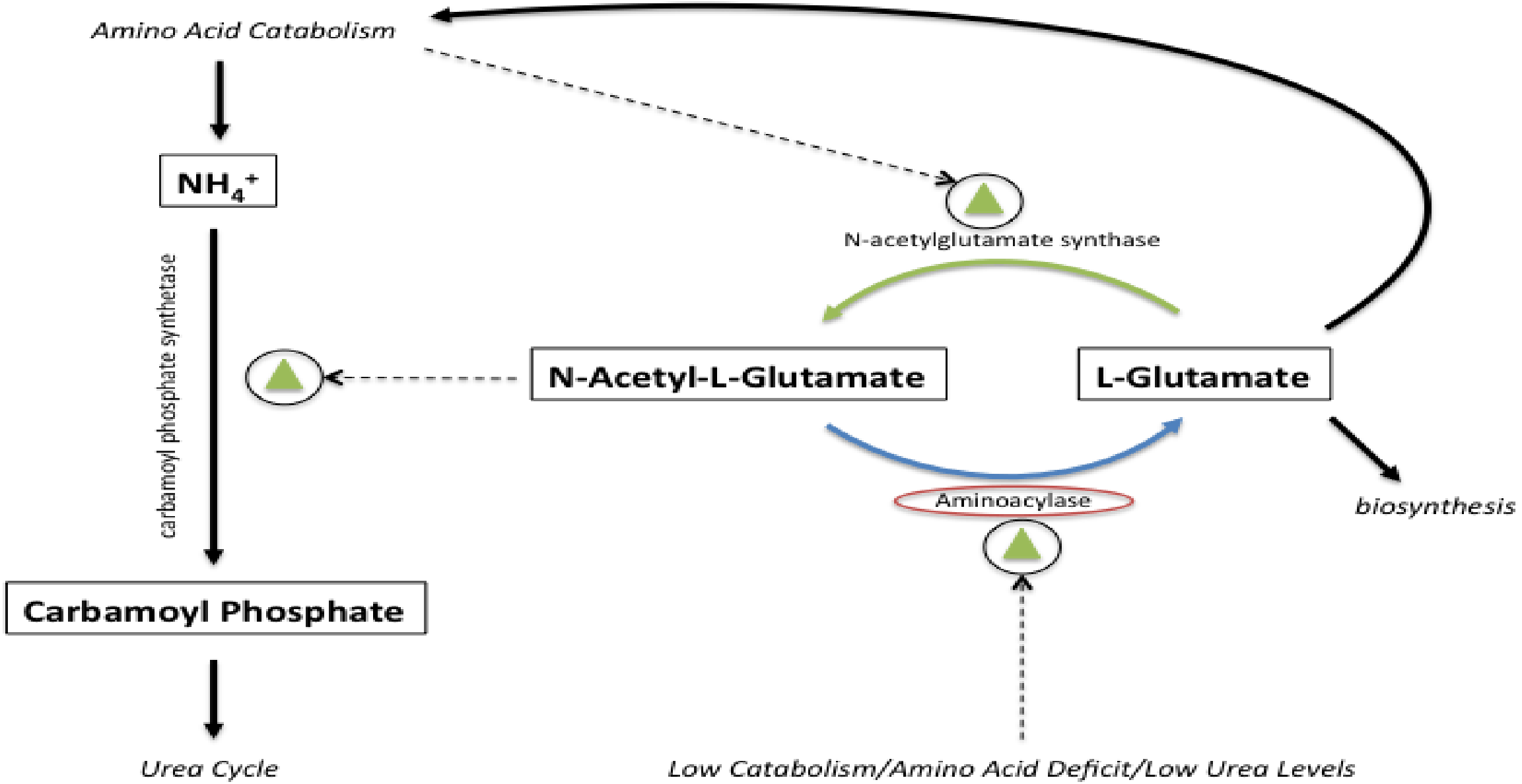
Regulation of urea cycle

- *Coarse Regulation*

During starvation, the activity of urea cycle enzymes is elevated to meet the increased rate of protein catabolism.

- *Fine Regulation*
- The major regulatory enzyme is CPS-I. **N-acetyl glutamate** (NAG) will stimulate this reaction.
- It is formed from glutamate and acetyl CoA.
- Arginine is an activator of NAG synthase.


Glutamate + Acetyl – CoA
• High-protein diet
• ↑ Glutamate
• Arginine
• Prolonged starvation
+
N-Acetyl
Glutamate
Synthase
N-Acetyl Glutamate
+
CPS-I of
Urea Cycle


Amino Acid Catabolism
NH_4^+
carbamoyl phosphate synthetase
Carbamoyl Phosphate
Urea Cycle
N-acetylglutamate synthase
N-Acetyl-L-Glutamate
L-Glutamate
Aminoacylase
biosynthesis
Low Catabolism/Amino Acid Deficit/Low Urea Levels

Free Ammonia is toxic to brain –

- **Depletes α-KG and ATP.**
- **Brain is very sensitive to ammonia. Hepatic Encephalopathy.**
- low protein diet and frequent small feeds are given. (<0.5g/kg/day)
- Lactulose – Osmotic laxative.
- Antibiotics – Rifaximin, Hemodialysis and Liver Transplantation.
- Attempts may be made to eliminate the amino nitrogen in other forms, e.g. as hippuric acid (Benzoyl conjugate of glycine) or phenyl acetyl glutamine.
- Since **Citrulline** is present in significant quantities in milk, breast milk is to be avoided in citrullinemia.

Disorders Of Urea Cycle

- Deficiency of any of the urea cycle enzymes would result in **hyperammonemia**.
- When the block is in one of the earlier steps, the condition is more severe, since ammonia itself accumulates.
- Deficiencies of later enzymes result in the accumulation of other intermediates, which are less toxic and hence symptoms are less.
- As a general description, disorders of urea cycle are characterized by hyperammonemia, encephalopathy and respiratory alkalosis. Clinical symptoms include vomiting, irritability, lethargy and severe mental retardation.
- Infants appear normal at birth, but within days progressive lethargy sets in.

Urea cycle disorders

Diseases	Enzyme deficit	Features
Hyperammonemia type I	CPS-I	Very high NH3 levels in blood. Autosomal recessive. Mental retardation. Incidence is 1 in 100,000.
Hyperammonemia type II	(OTC) Ornithine transcar-bamoy-lase	Ammonia level high in blood. Increased glutamine in blood, CSF and urine. Orotic aciduria due to channelling of carbamoyl phosphate into Pyrimidine synthesis. X-linked.
HyperornithinemiA	Defective ornithine trans-porter protein	Failure to import ornithine from cytoplasm to mitochondria. Defect in ORNT1 gene. Hyperornithinemia, hyperammonemia and homocitrullinuria is seen (HHH syndrome). Decreased urea in blood. Autosomal recessive condition.

Urea cycle disorders

Diseases	Enzyme deficit	Features
Citrullinemia	Argininosuccinate synthe-tase	Autosomal recessive inheritance. High blood levels of ammonia and citrulline. Citrullinuria (1-2 g/day).
Argininosuccinic aciduria	Arginino-succinate lyase	Argininosuccinate in blood and urine. Friable brittle tufted hair (Trichorrhexis nodosa). Incidence 3/200,000
Hyperarginine mia	Arginase	Arginine increased in blood and CSF. Instead of arginine, cysteine and lysine are lost in urine. Incidence 1 in 100,000

Treatment –

- mainly symptomatic.

- Low protein diet with sufficient arginine and energy by frequent feeding can minimize brain damage since ammonia levels do not increase very high.

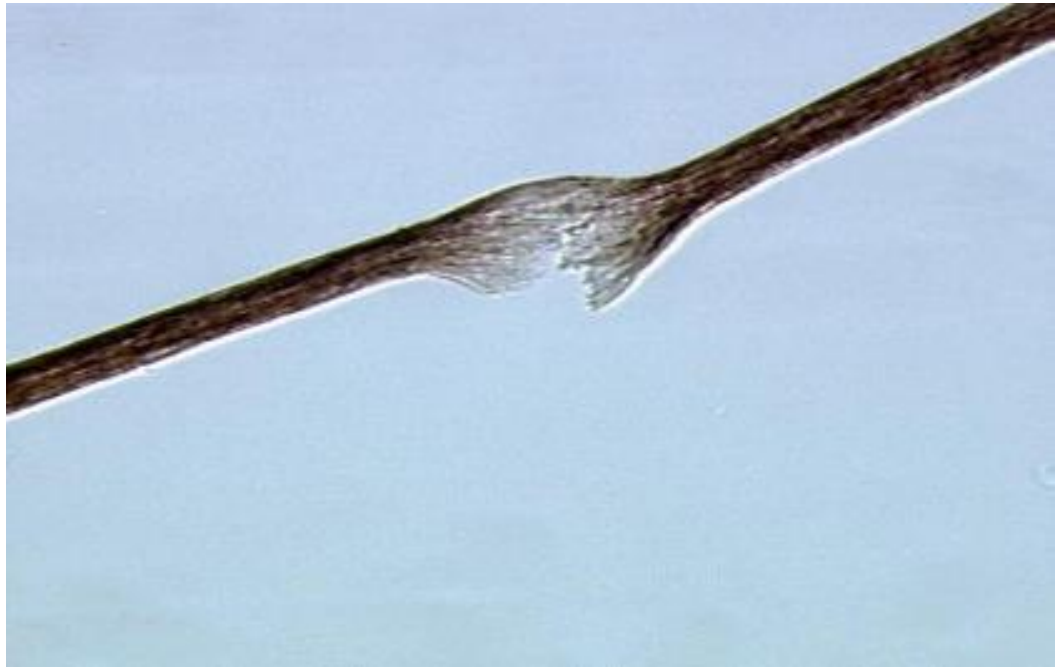
- Ornithine transporter deficiency is characterized by hyperornithinemia, hyperammonemia and homocitrullinuria (HHH syndrome).

- Since ornithine is not available in the mitochondria, lysine is carbamylated to form homocitrulline.

- **Argininosuccinate lyase deficiency** leads to argininosuccinic acidemia and therefore metabolic acidosis.

- Hyperammonemia is less severe and argininosuccinate is elevated in CSF and excreted in urine.

- A typical clinical feature is friable tufted hair (trichorrhexis nodosa).



Phenylbutyrate and benzoate are used to scavenge Ammonia.