Nitrogen Excretion and the Urea Cycle

Nebras Melhem

Sources of ammonia in the body

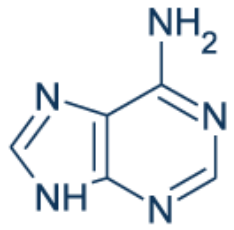
- The deamination of amino acids is the major source.
- Catabolism of purines & pyrimidines.
- Dietary glutamine by intestinal **glutaminase** increases ammonia absorbed from intestine.
- In addition, bacterial putrefaction in the intestines produces small amounts of ammonia.
- Urea excreted with bile is hydrolysed by bacterial enzyme (**Urease**) and increase ammonia absorbed from intestine.

FATE OF PRODUCTS OF DEAMINATION

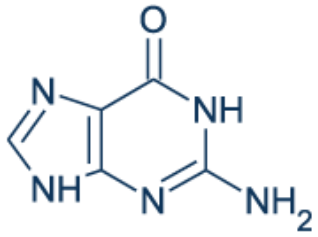
- The ammonia removed by deamination may be used for the **synthesis of some compounds**, e.g., the nonessential amino acids, purines, pyrimidines, and amino sugars.

- Actually, **nearly all the nitrogenous compounds** in the body are **derived** from amino acids.

Purines

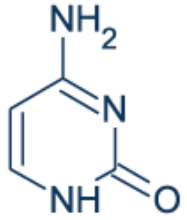


Adenine

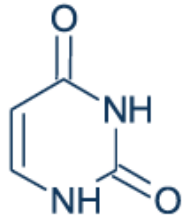


Guanine

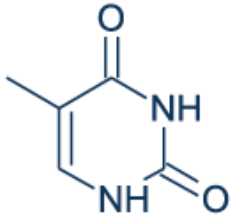
Pyrimidines



Cytosine



Uracil



Thymine

In The Liver

- The liver is the main site of deamination of amino acids.
- Most of the ammonia released (from deamination of amino acids or from glutamine by **glutaminase**) is converted to urea (about 90%).
- The urea formed goes via the blood to the kidneys to be excreted in urine.
- Some of the ammonia is also converted to **glutamine**.

In The Kidneys

- The ammonia resulting from the deamination of amino acids in the kidneys is directly excreted in the urine.

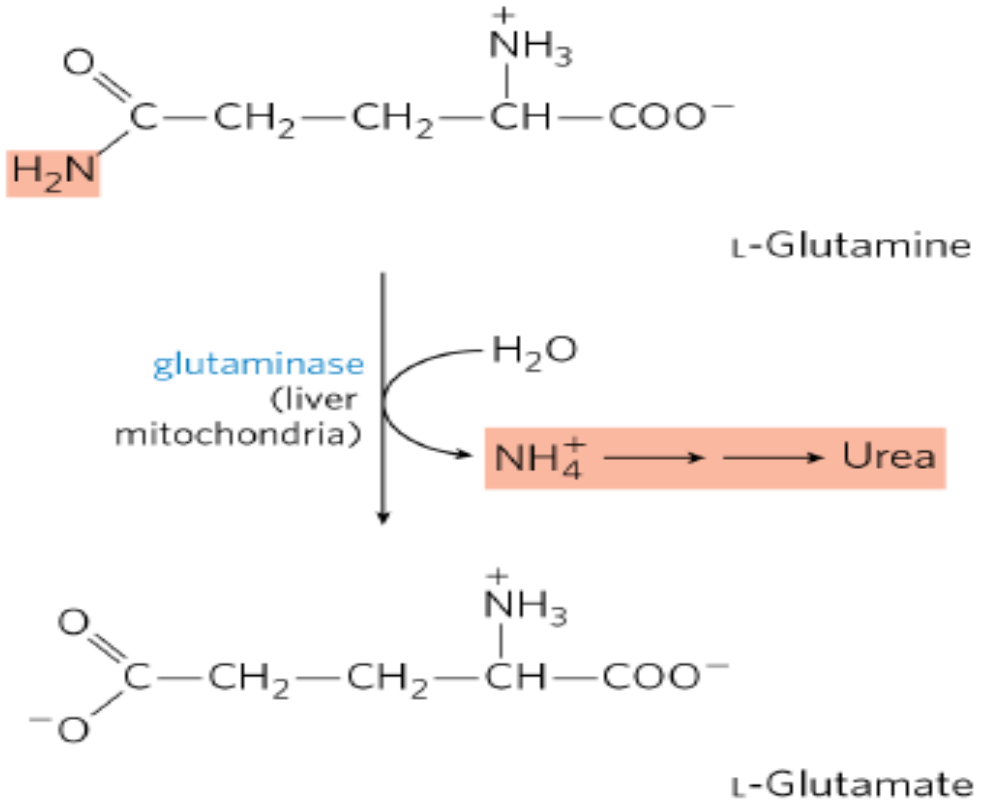
- This accounts for about 40% of the urinary ammonia.

In Extra Renal Tissues

- The ammonia resulting from the deamination of amino acids in extra renal tissues, particularly the brain is converted to glutamine.

- Glutamine goes, via the blood, to the kidneys where it becomes hydrolyzed by **glutaminase** into **glutamate and ammonia.**

- The ammonia is excreted in the urine, accounting for about 60% of urinary ammonia.


NH3+
O
C—CH2—CH2—CH—COO−
H2N
L-Glutamine
glutaminase
(liver
mitochondria)
H2O
NH4+
Urea
NH3+
O
C—CH2—CH2—CH—COO−
−O
L-Glutamate

Transport of ammonia in the circulation

- Although ammonia is constantly produced in the tissues, it is present at very low levels in blood (0.1 mg/dL).

- This is due to both the rapid removal of ammonia from the blood by the **liver** and the fact that many tissues, particularly **muscle**, release amino acid nitrogen in the form of glutamine or alanine, rather than as free ammonia.

Urea Cycle

- This pathway was discovered in 1932 by Hans Krebs-Kurt Henseleit who later also discovered the citric acid cycle.
- Urea production occurs almost exclusively in the liver and is the fate of most of the ammonia channeled there.
- The urea passes into the bloodstream and thus to the kidneys and is excreted into the urine.

Urea Cycle

- This is the **principal pathway of disposal of ammonia** resulting from the deamination of amino acids.

- It allows the body to get rid of about 80-90% of the amino groups of amino acids in a neutral nontoxic form.

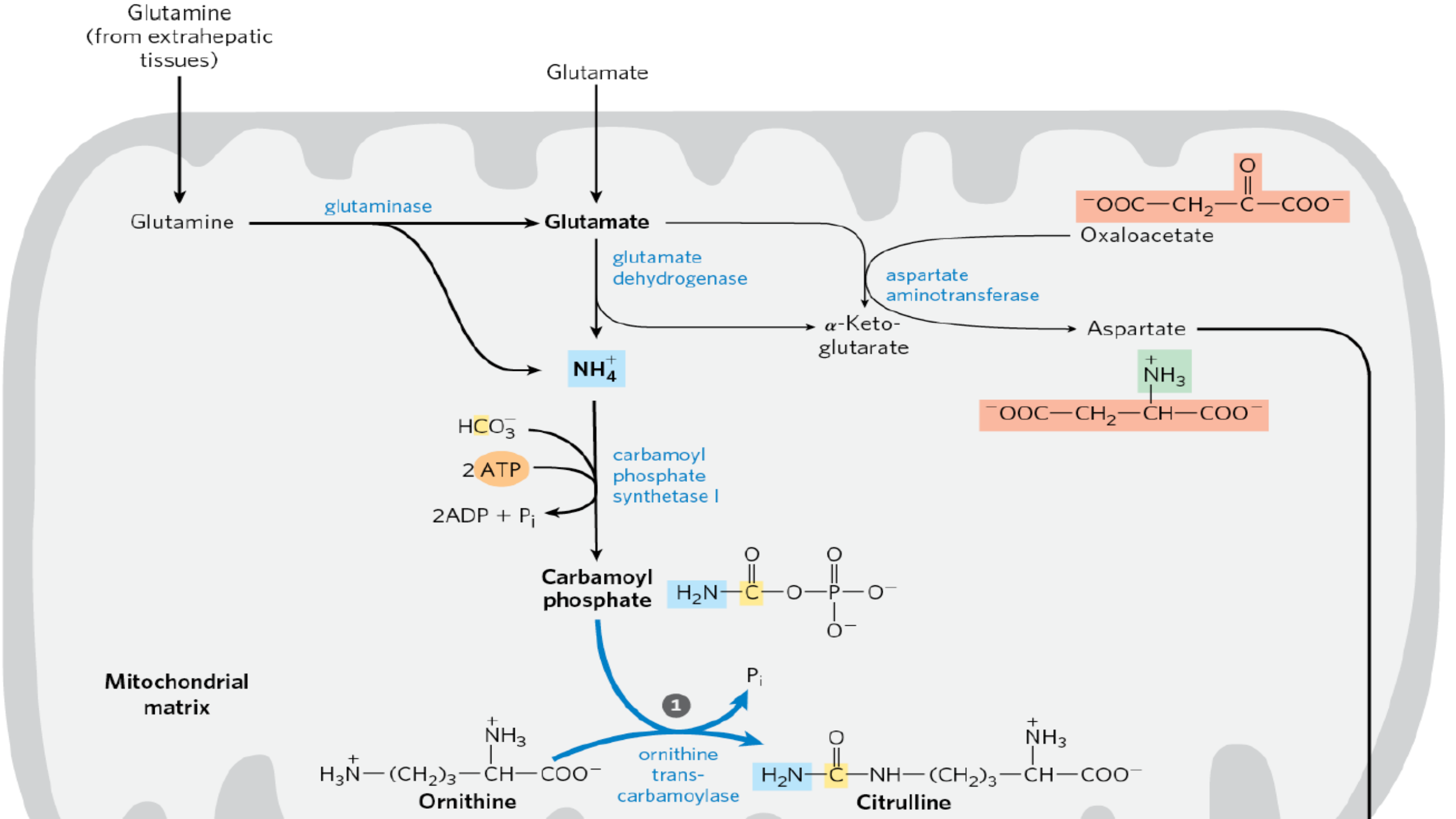
- Ammonia is highly toxic to the CNS; it is converted to non-toxic urea in the **liver only.**
  - Toxicity of ammonia may be explained by withdrawal of α-ketoglutarate from citric acid cycle to form glutamate and glutamine. In addition, it may alter the neurotransmitters in the CNS.

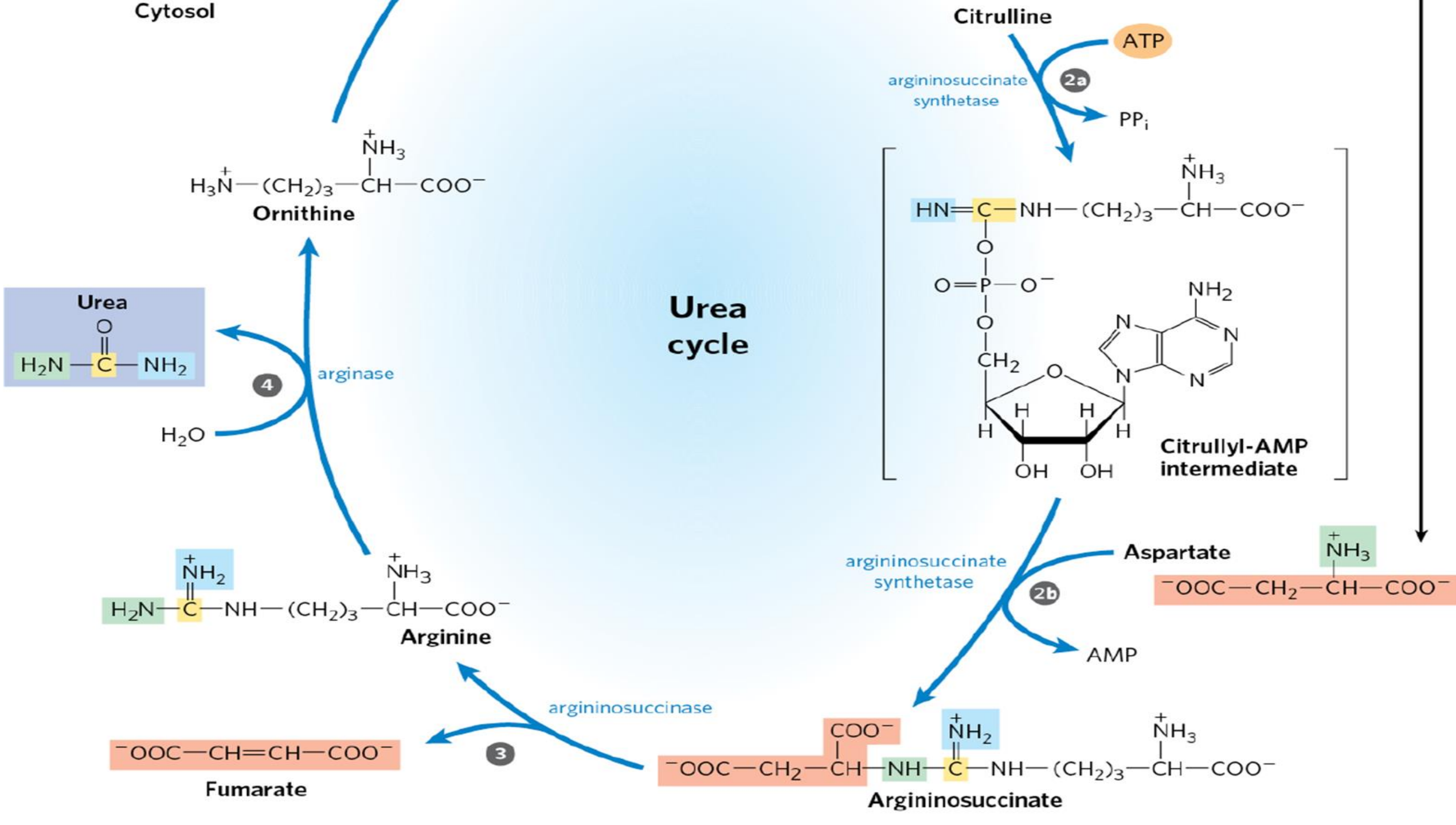
Urea Cycle

- The urea cycle begins inside **liver mitochondria**, but three of the subsequent steps take place in the cytosol.

- The **first amino group** to enter the urea cycle is derived from **ammonia** in the mitochondrial matrix.

- The liver also receives some ammonia via the portal vein from the intestine, from the bacterial oxidation of amino acids.


Glutamine
(from extrahepatic
tissues)
Glutamate
Glutamine
glutaminase
Glutamate
glutamate
dehydrogenase
Oxaloacetate
aspartate
aminotransferase
α-Keto-
glutarate
Aspartate
NH4+
HCO3−
2 ATP
2ADP + Pi
carbamoyl
phosphate
synthetase I
Carbamoyl
phosphate
Mitochondrial
matrix
Pi
1
ornithine
trans-
carbamoylase
Ornithine
Citrulline

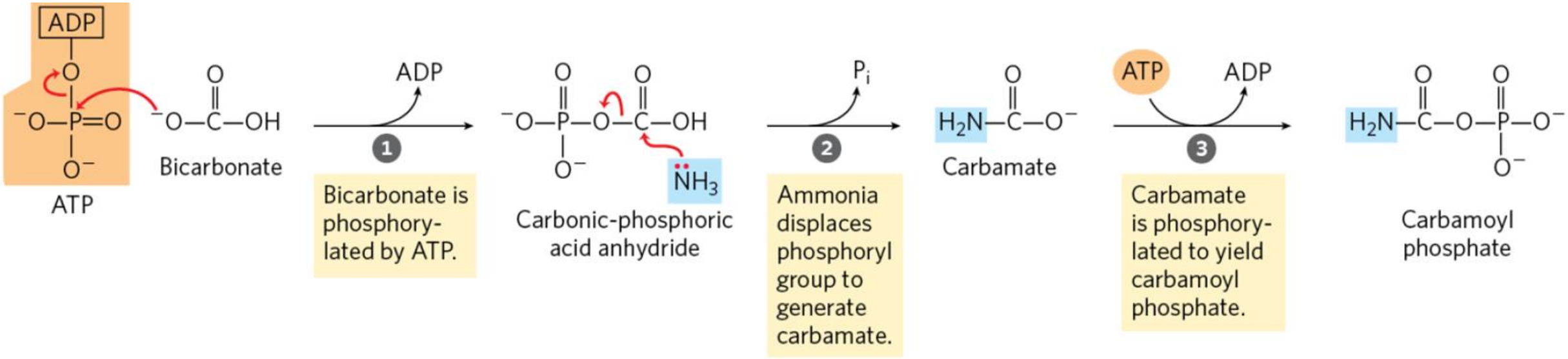

Cytosol
Ornithine
Urea
arginase
4
H_2O
Arginine
argininosuccinase
3
Fumarate
Urea cycle
Citrulline
ATP
argininosuccinate synthetase
2a
PP_i
Citrullyl-AMP intermediate
Aspartate
argininosuccinate synthetase
2b
AMP
Argininosuccinate

Carbamoyl Phosphate

- Whatever its source, the NH^{+}_{4} generated in liver mitochondria is immediately used, together with CO_2 (as HCO^{-}_{3}) to form **carbamoyl phosphate** in the matrix.

- This ATP-dependent reaction is catalyzed by **carbamoyl phosphate synthetase I**, a regulatory enzyme.
  - ❑The mitochondrial form of the enzyme is distinct from the cytosolic (II) form, which has a separate function in pyrimidine biosynthesis.

Carbamoyl Phosphate

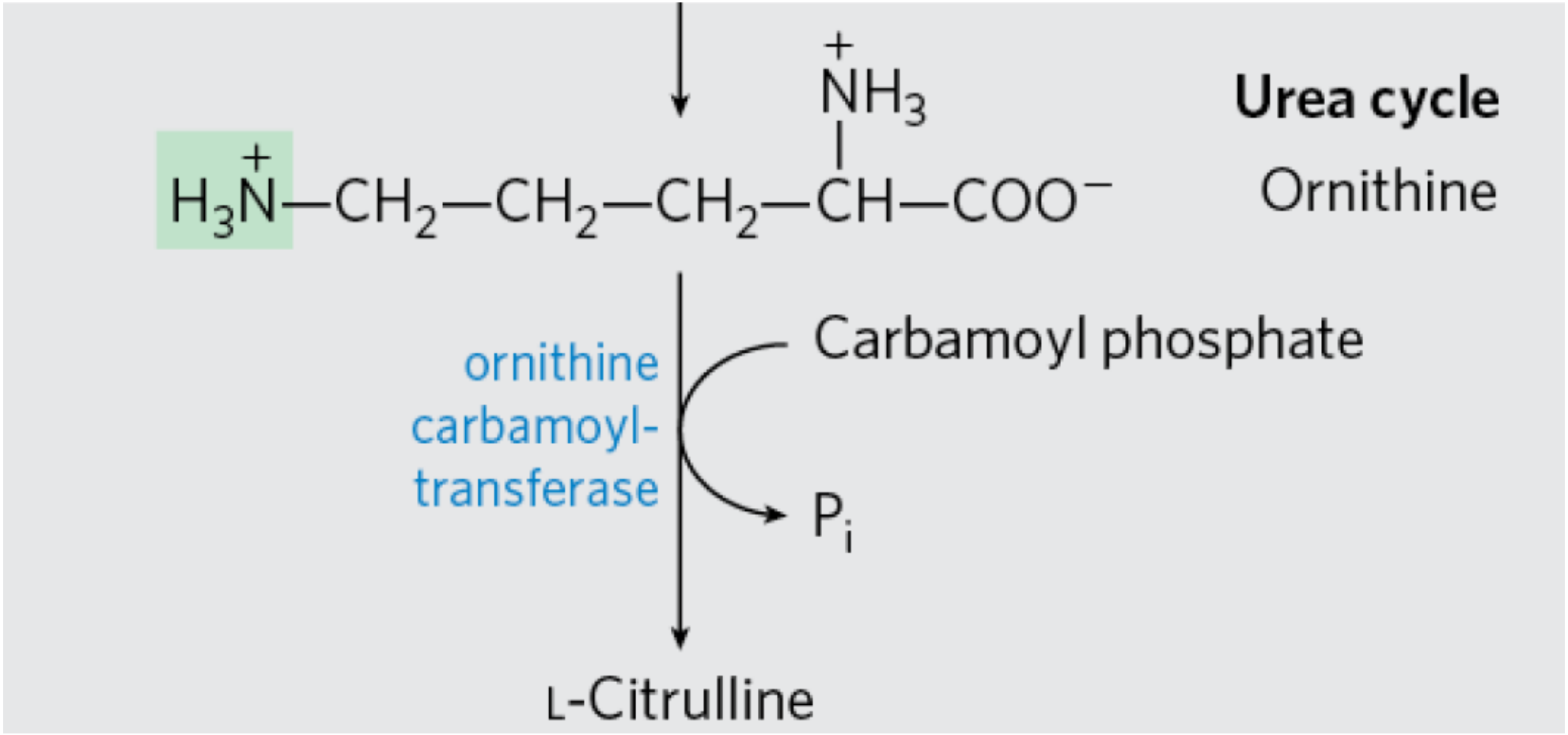


Citrulline

- Carbamoyl phosphate donates its carbamoyl group to ornithine to form citrulline, with the release of Pi.
- The reaction is catalyzed by **ornithine transcarbamoylase**.
- The citrulline produced in the urea cycle **passes** from the **mitochondrion** to the **cytosol**.
  - The citrulline transported out of the mitochondrion is not diluted into the general pool of metabolites in the cytosol but is passed directly to the active site of argininosuccinate synthetase.

- Ornithine is not one of the 20 common amino acids found in proteins, but it is a key intermediate in arginine biosynthesis and nitrogen metabolism in general.

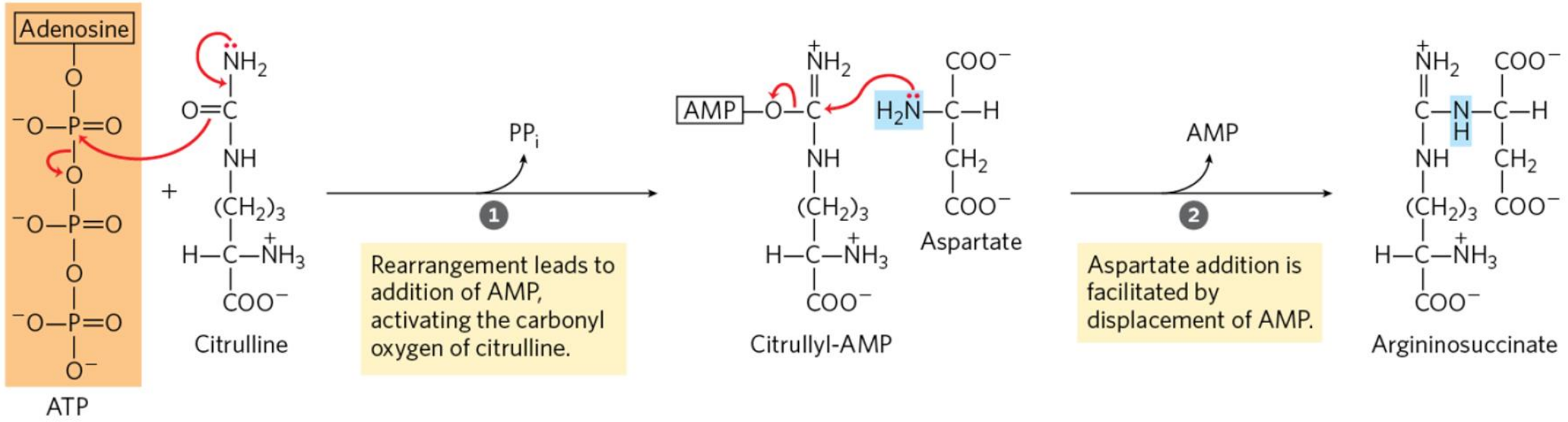
Citrulline


Urea cycle
Ornithine
$H_3\overset{+}{N}-CH_2-CH_2-CH_2-\underset{\overset{+}{N}H_3}{CH}-COO^-$
ornithine carbamoyl-transferase
Carbamoyl phosphate
P_i
L-Citrulline

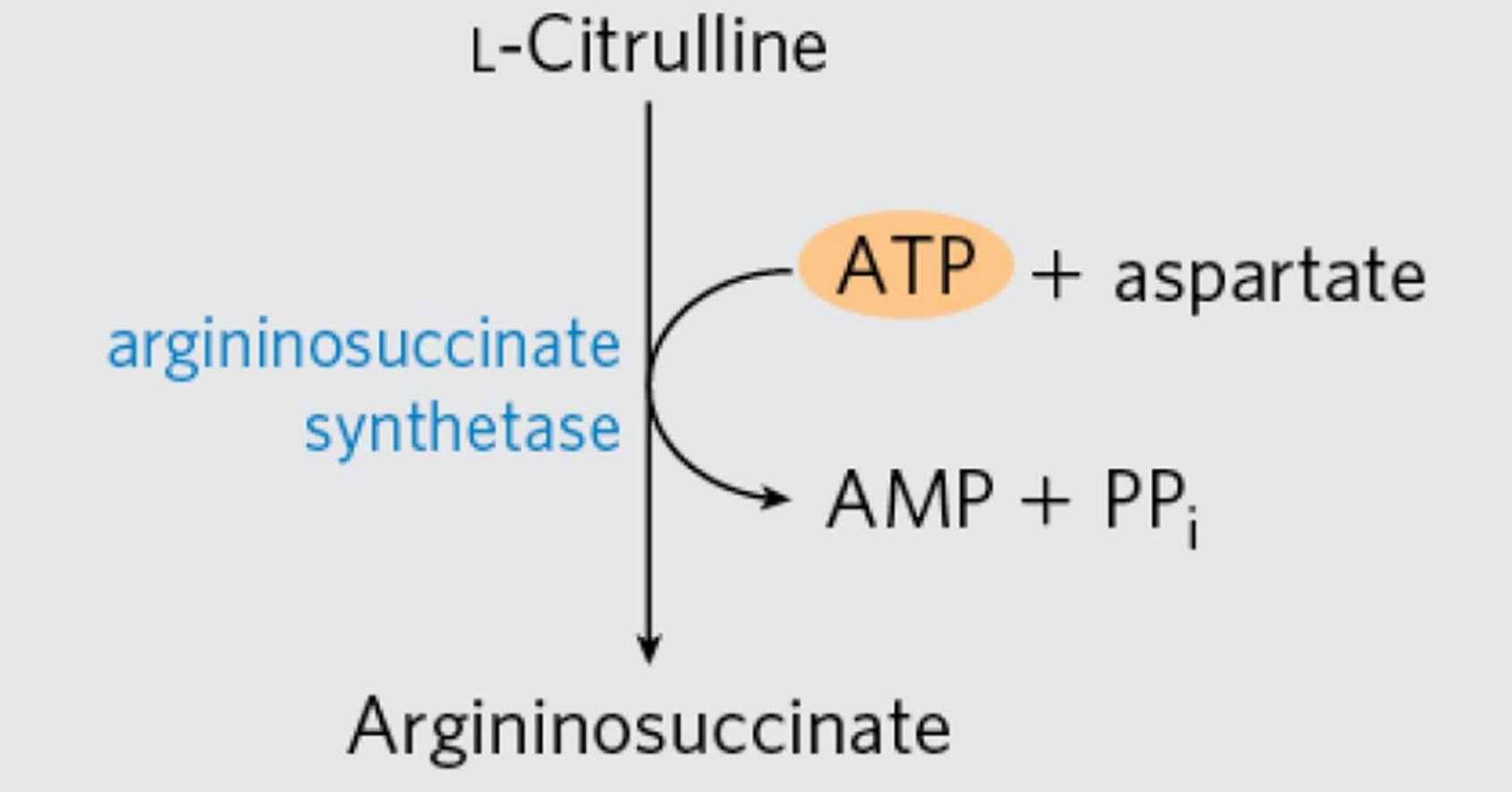
Argininosuccinate

- The next two steps bring in the **second** amino group, featuring **aspartate as the amino group donor.**
- The aspartate is generated in the mitochondria by transamination between glutamate and oxaloacetate, and then transported into the cytosol.
- A condensation reaction between the amino group of aspartate and the ureido (carbonyl) group of citrulline forms argininosuccinate.
- This cytosolic reaction, catalyzed by **argininosuccinate synthetase**, requires ATP and proceeds through a citrullyl-AMP intermediate.
  - This is the third and final molecule of ATP consumed in the formation of urea.

Argininosuccinate



Argininosuccinate

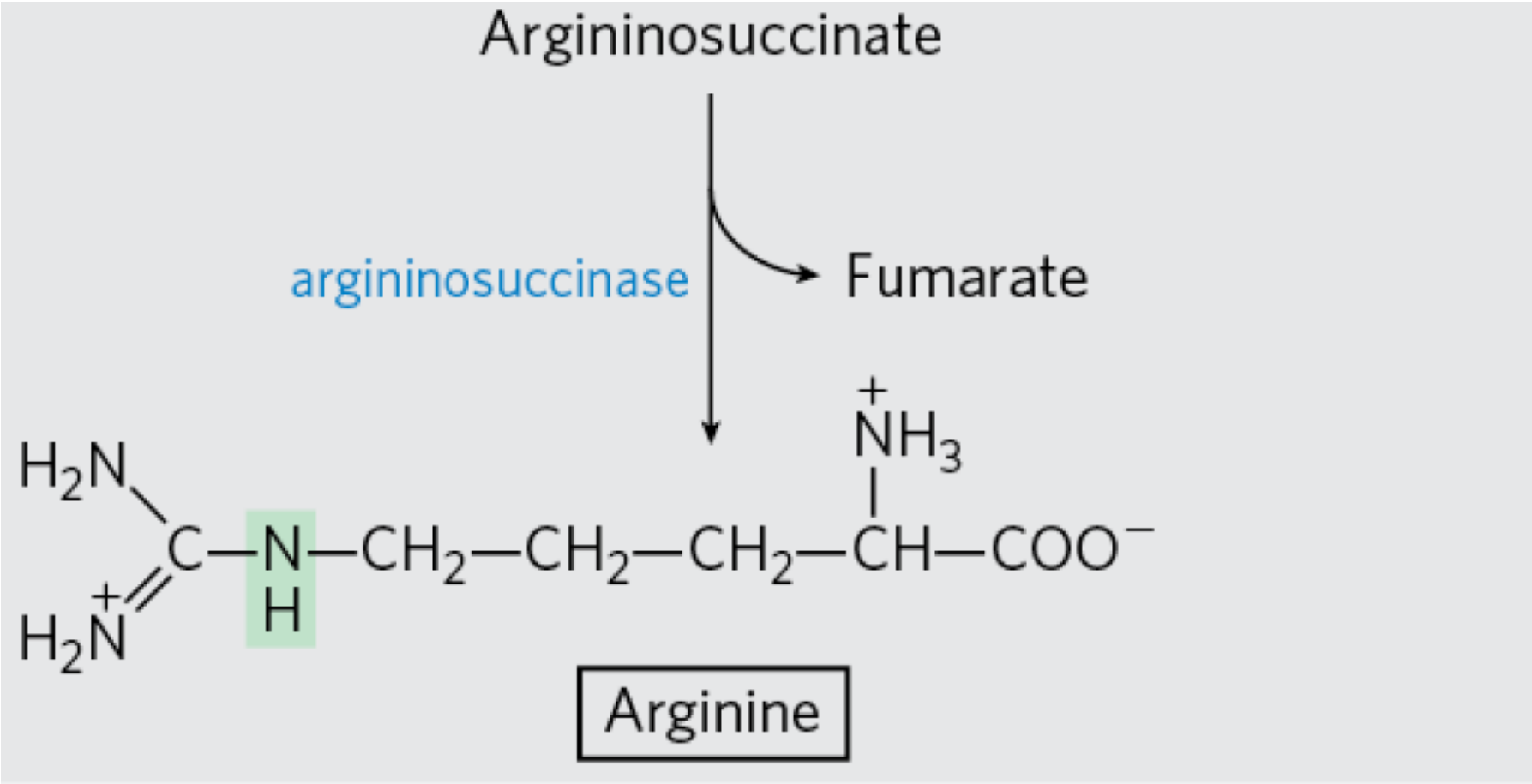


Arginine

- The **argininosuccinate** is then cleaved by **argininosuccinase** to form free **arginine** and **fumarate**.

- Fumarate is then converted to malate before entering mitochondria to join the pool of citric acid cycle intermediates.
  - This is the only reversible step in the urea cycle.

Arginine


Argininosuccinate
argininosuccinase
Fumarate
H_2N
C—N—CH_2—CH_2—CH_2—CH—COO^-
H
H_2N^+
NH_3^+
Arginine

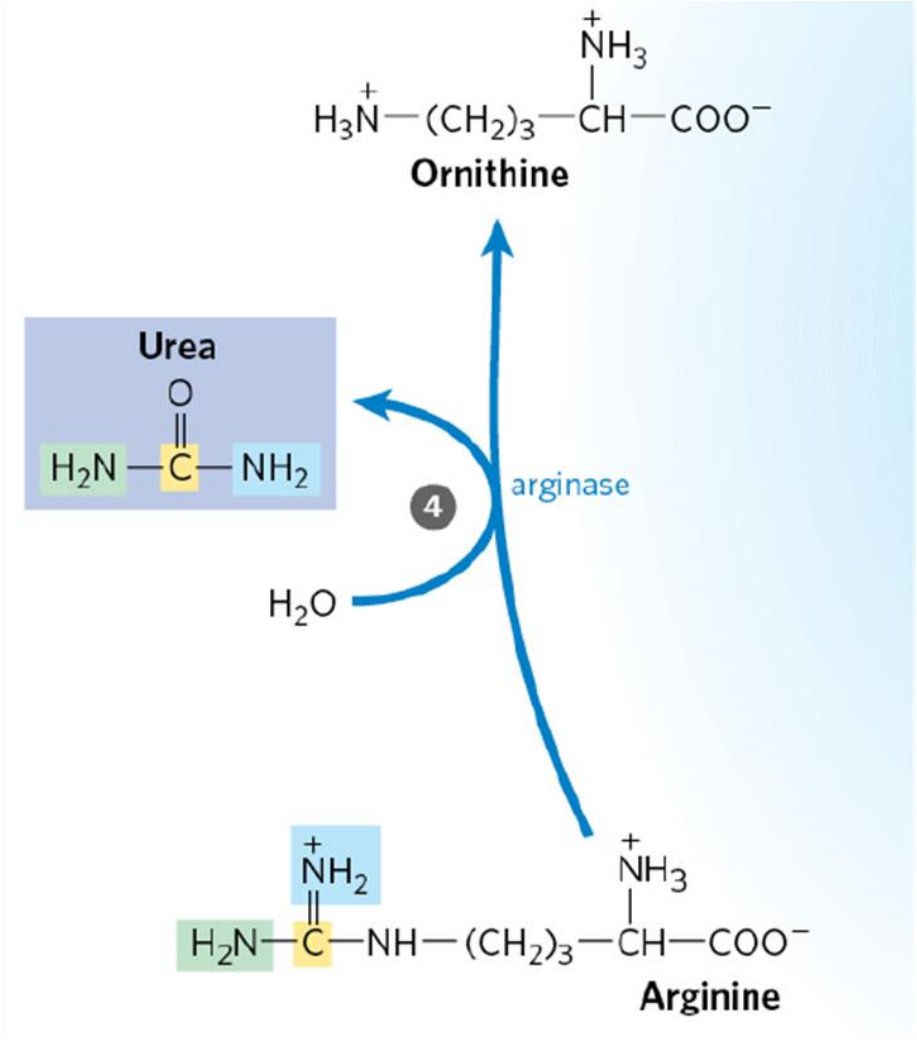
Urea

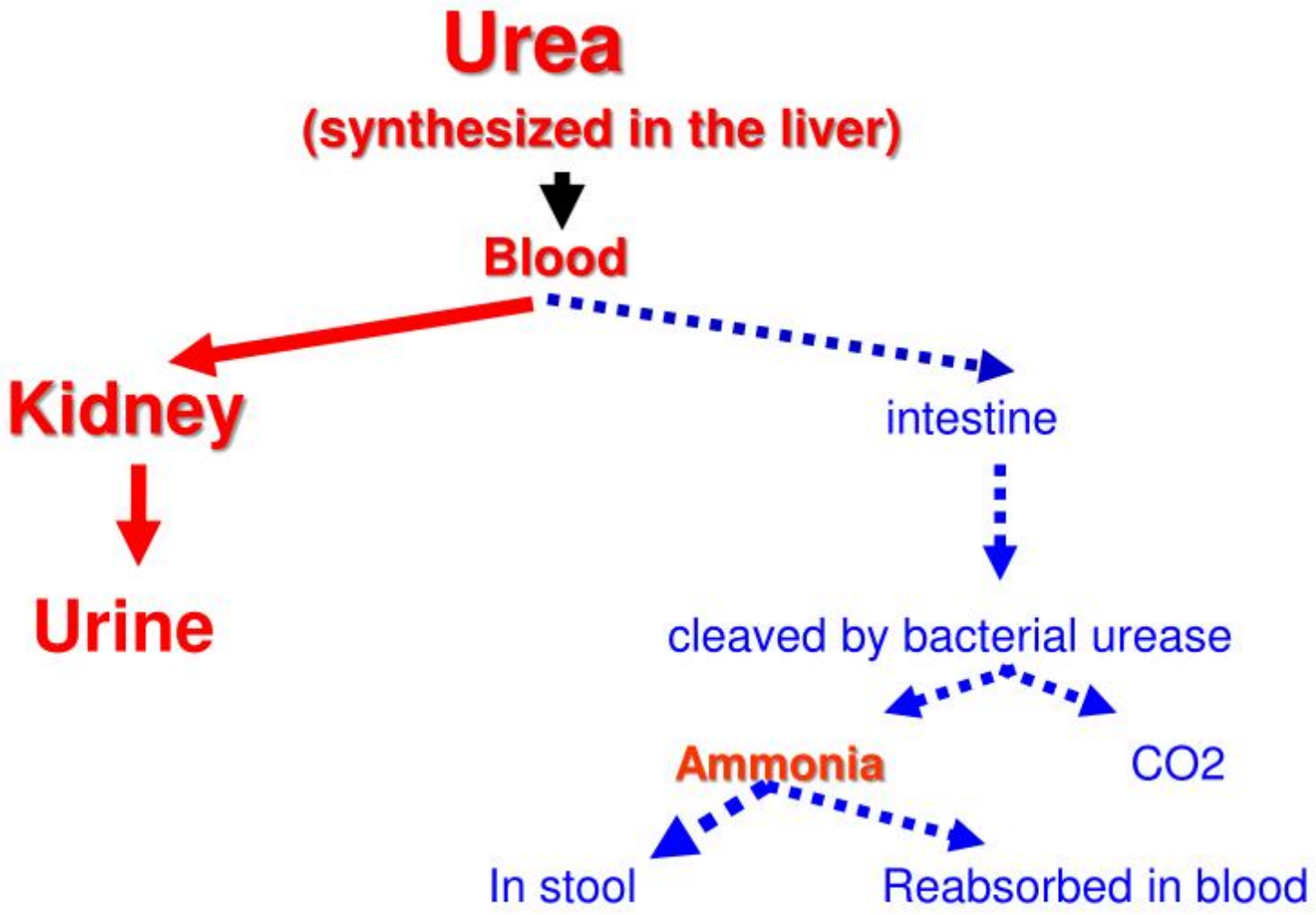
- In the last reaction of the urea cycle, the cytosolic enzyme **arginase** cleaves **arginine** to yield **urea** and **ornithine**.

- Only **urea** is released into the general cytosolic pool of metabolites.

- **Ornithine** is transported into the **mitochondrion** to initiate another round of the urea cycle.

Urea




Urea
(synthesized in the liver)
Blood
Kidney
Urine
intestine
cleaved by bacterial urease
Ammonia
CO2
In stool
Reabsorbed in blood

Fate of Urea

- A portion of the urea diffuses from the blood into the intestine and is cleaved to CO_2 and ammonia by bacterial **urease**.
- The ammonia is partly lost in the feces and is partly reabsorbed into the blood.
- After ammonia is absorbed, it goes back to the liver where it re forms urea.

Fate of Urea

- However, in **hepatic failure** ammonia remains in the blood, leading to hyperammonemia and ammonia intoxication.

- A portion of the urea goes, via the blood to the kidneys to be excreted in the urine.

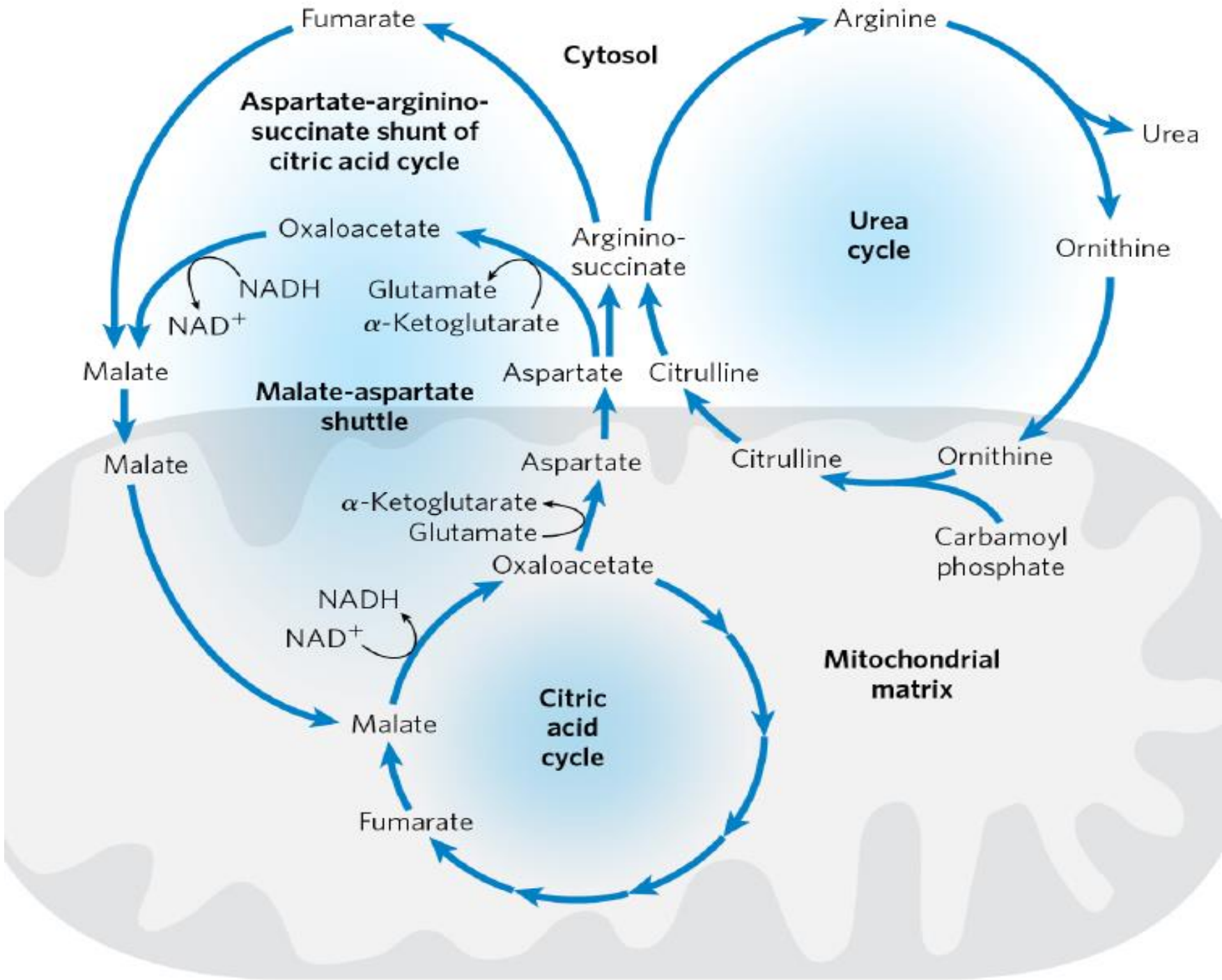
- Little amount of urea is excreted in sweat.

Link between Urea Cycle & Krebs Cycle

- The **fumarate** produced in the argininosuccinase reaction is also an intermediate of the citric acid cycle.

- **Aspartate** formed in mitochondria by transamination between oxaloacetate and glutamate can be transported to the cytosol, where it serves as nitrogen donor in the urea cycle reaction catalyzed by **argininosuccinate synthetase**.

- However, each cycle can operate independently, and communication between them depends on the transport of key intermediates between the mitochondrion and cytosol.

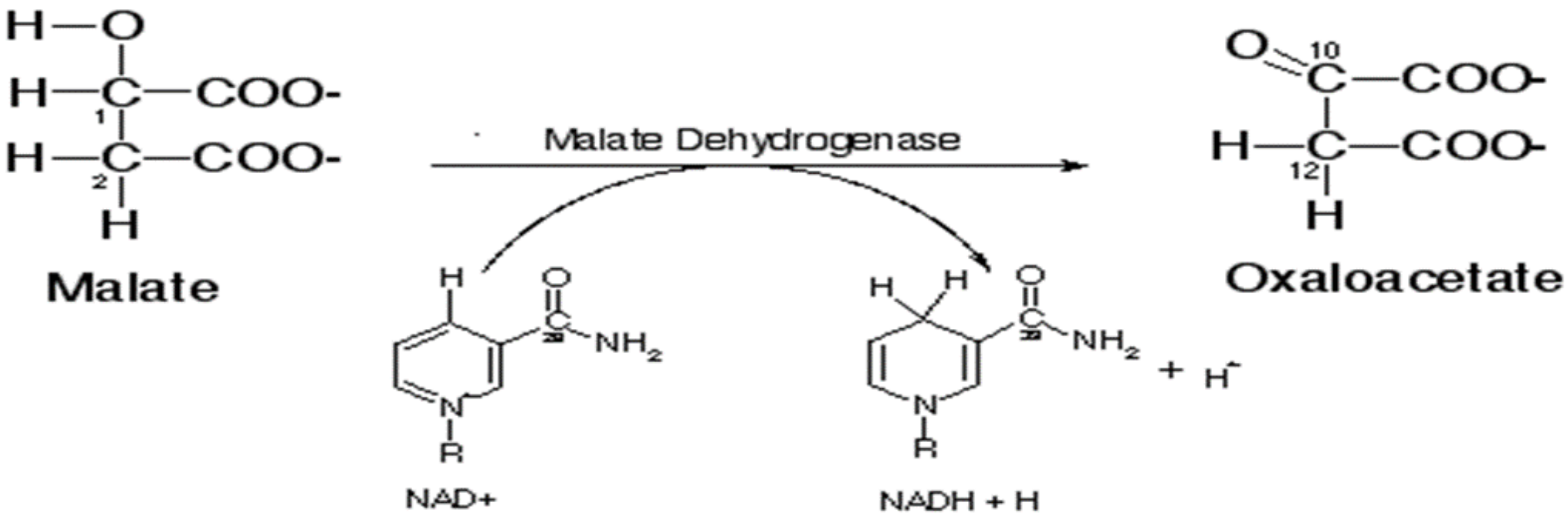

Fumarate
Cytosol
Arginine
Aspartate-arginino-succinate shunt of citric acid cycle
Urea
Urea cycle
Oxaloacetate
Arginino-succinate
Ornithine
NADH
NAD+
Glutamate
α-Ketoglutarate
Malate
Aspartate
Citrulline
Malate-aspartate shuttle
Malate
Aspartate
Citrulline
Ornithine
α-Ketoglutarate
Glutamate
Carbamoyl phosphate
Oxaloacetate
NADH
NAD+
Mitochondrial matrix
Citric acid cycle
Malate
Fumarate

Energetic Cost of Urea Synthesis

- Synthesis of one molecule of urea requires four high-energy phosphate groups.
- Two ATP molecules are required to make carbamoyl phosphate
- One ATP to make argininosuccinate — ATP undergoing a pyrophosphate cleavage to AMP and PPi , which is hydrolyzed to two Pi.

Energetic Cost of Urea Synthesis

- However! The fumarate generated by the urea cycle is converted to malate, and the malate is transported into the mitochondrion.
- Inside the mitochondrial matrix, NADH is generated in the **malate dehydrogenase** reaction.
- Each NADH molecule can generate up to 2.5 ATP during mitochondrial respiration, greatly reducing the overall energetic cost of urea synthesis.


Malate
Malate Dehydrogenase
Oxaloacetate
NAD+
NADH + H
+ H+

The Activity of the Urea Cycle Is Regulated at Two Levels

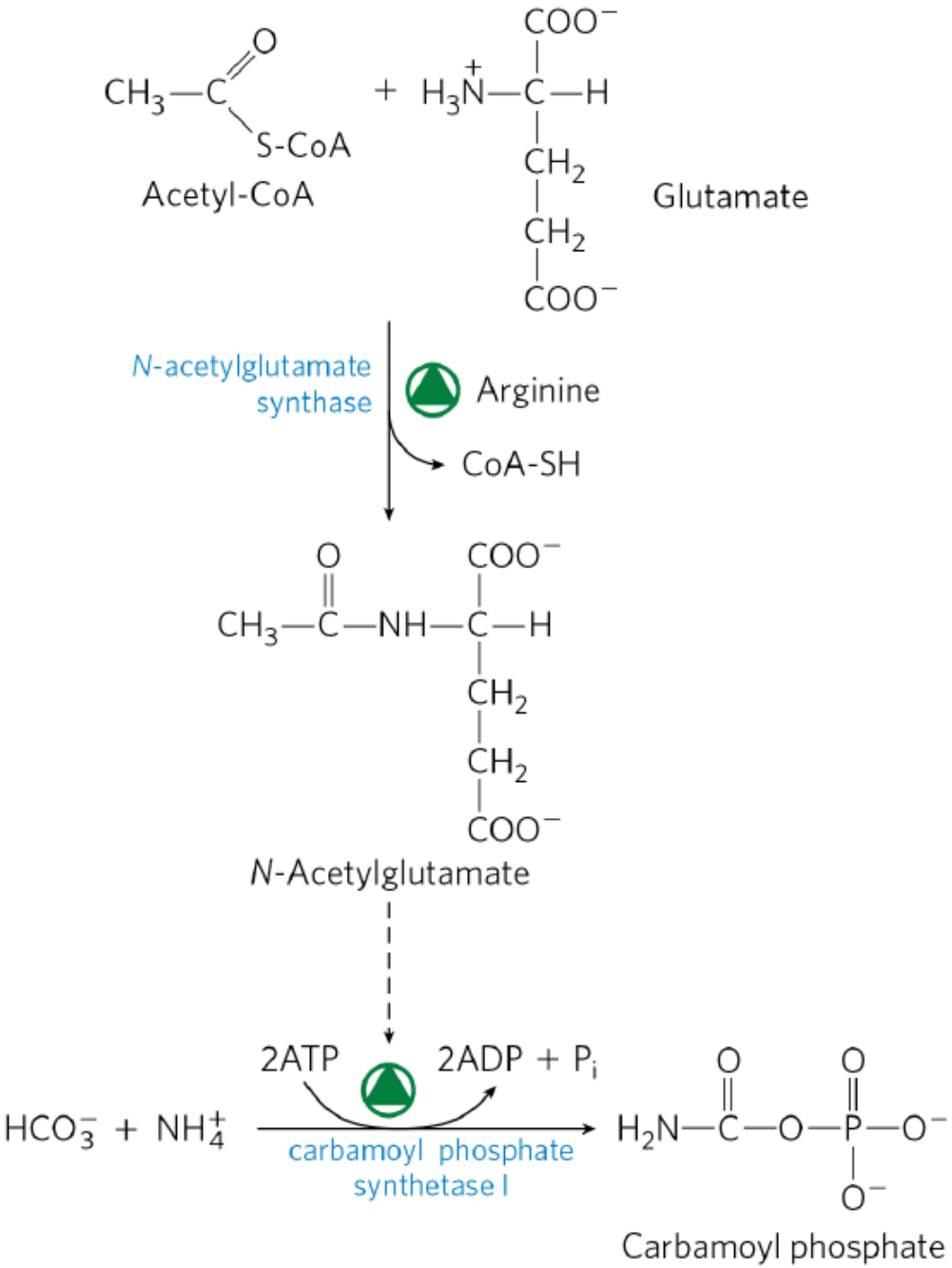
- When the **dietary intake** is primarily protein, the carbon skeletons of amino acids are used for fuel, producing much urea from the excess amino groups.

- During prolonged starvation, when breakdown of muscle protein begins to supply much of the organism's metabolic energy, urea production also increases substantially.

- Protein-free diets produce lower levels of urea cycle enzymes.

The Activity of the Urea Cycle Is Regulated at Two Levels

- **Carbamoyl phosphate synthetase I**, is allosterically activated by **N-acetylglutamate.**
- **N-acetylglutamate** is synthesized from **acetyl-CoA** and glutamate by **N-acetylglutamate synthase**.
- It increases the affinity of CPS I for ATP.
- **Arginine** an activator of **N-acetylglutamate synthase**, and thus an **activator of the urea cycle**.



Hyperammonemia

- The capacity of the hepatic urea cycle exceeds the normal rates of ammonia generation, and the levels of blood ammonia are normally low (5–35 μmol/l).

- When liver function is compromised, due either to genetic defects of the urea cycle or liver disease, blood levels can be >1,000 μmol/l.
  - Such hyperammonemia is a medical emergency, because ammonia has a direct neurotoxic effect on the CNS.

- Elevated concentrations of ammonia in the blood cause the symptoms of ammonia intoxication, which include tremors, slurring of speech, somnolence (drowsiness), vomiting, cerebral edema, and blurring of vision.

Hyperammonaemia

- In patients with **kidney failure**, plasma **urea** levels are **elevated**, promoting a greater transfer of urea from blood into the gut.

- The intestinal action of **urease** on this urea becomes a clinically important source of ammonia, contributing to the hyperammonemia often seen in these patients.

- Oral administration of antibiotics reduces the number of intestinal bacteria responsible for this ammonia production.

Acquired Hyperammonaemia

- Liver disease is a common cause of acquired hyperammonemia in adults and may be due, for example, to **viral hepatitis** or to **hepatotoxins** such as **alcohol**.

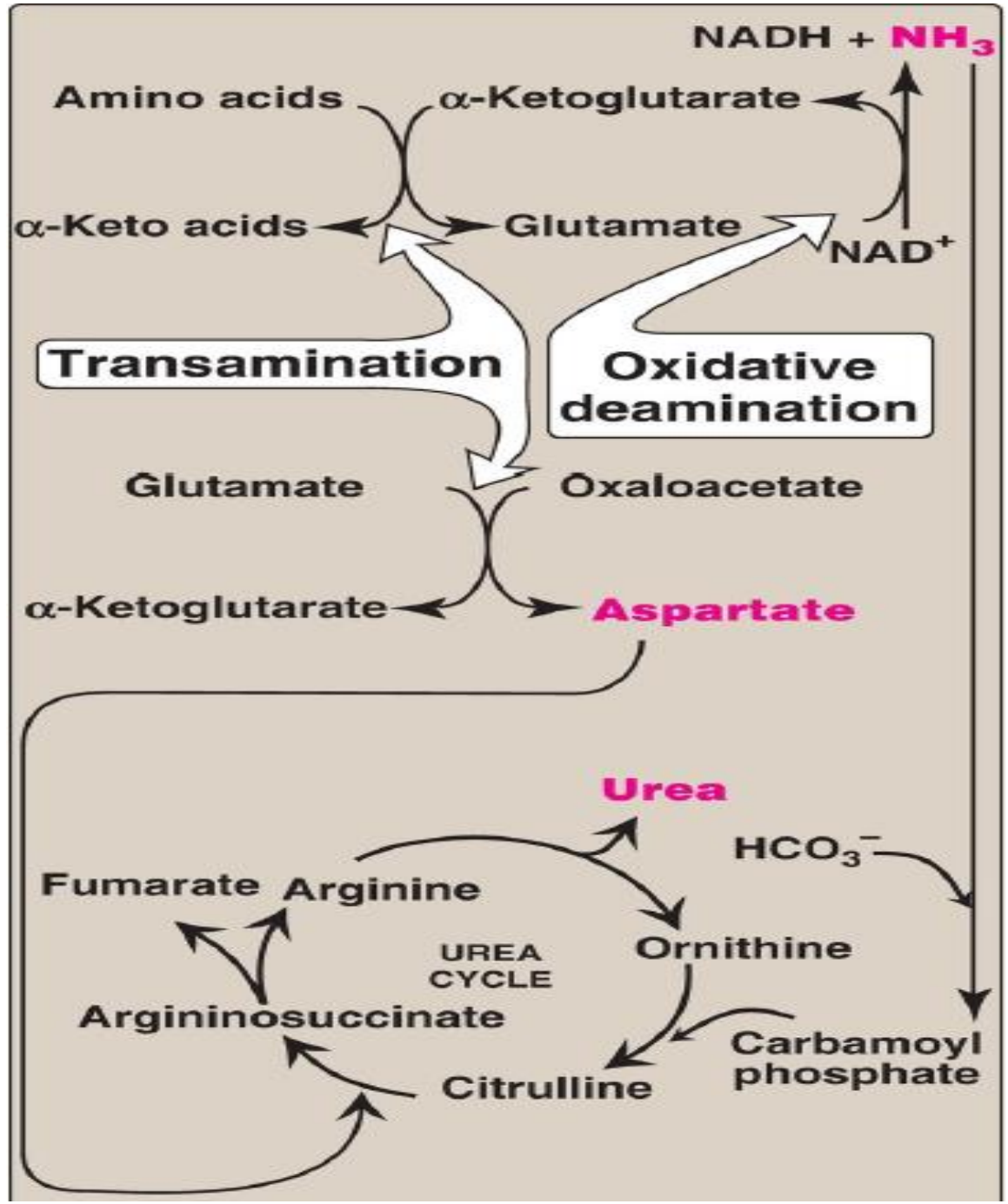
- Cirrhosis of the liver may result in formation of collateral circulation around the liver.
- As a result, portal blood is shunted directly into the systemic circulation and does not have access to the liver.
- Therefore, the conversion of ammonia to urea is severely impaired, leading to elevated levels of ammonia.

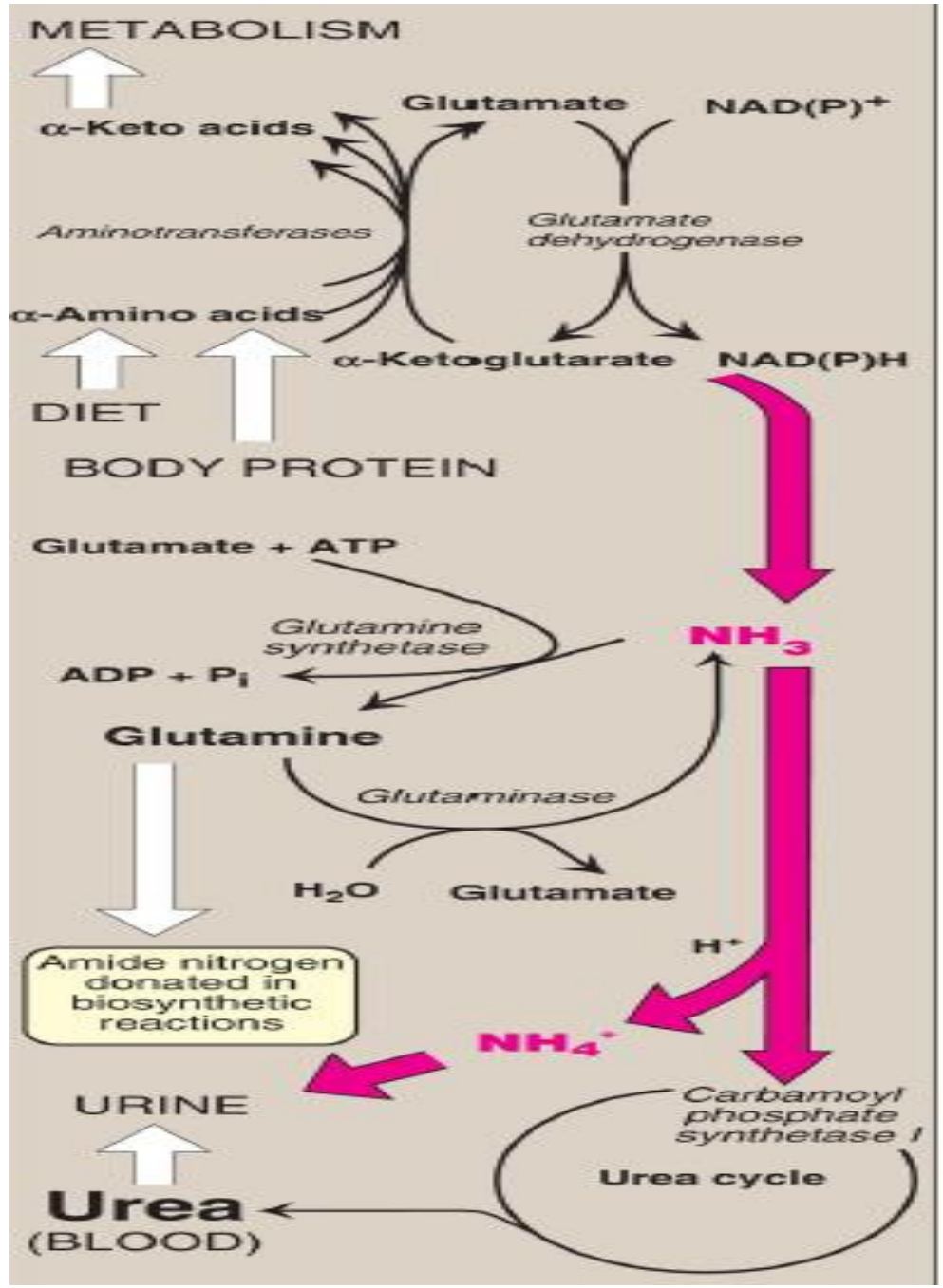
Congenital Hyperammonemia

- Genetic deficiencies of each of the five enzymes of the urea cycle (and of NAGS) have been described, with an overall incidence of ~1:25,000 live births.
- X-linked **OTC deficiency** is the most common of these disorders, predominantly affecting males, although female carriers may become symptomatic.
- The failure to synthesize urea leads to hyperammonemia during the first weeks following birth.
- Low protein diet with sufficient arginine and energy by frequent feeding can minimize brain damage since ammonia levels do not increase very high.

Disorders of Urea Cycle

Diseases	Enzyme deficit	Features
Hyperammonemia type I	CPS-I	Very high NH_3 levels in blood. Autosomal recessive. Mental retardation. Incidence is 1 in 100,000.
Hyperammonemia type II	(OTC) Ornithine transcarbamoylase	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 transporter 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.
Citrullinemia	Argininosuccinate synthetase	Autosomal recessive inheritance. High blood levels of ammonia and citrulline. Citrullinuria (1-2 g/day).
Argininosuccinic aciduria	Argininosuccinate lyase	Argininosuccinate in blood and urine. Friable brittle tufted hair (Trichorrhexis nodosa). Incidence 3/200,000
Hyperargininemia	Arginase	Arginine increased in blood and CSF. Instead of arginine, cysteine and lysine are lost in urine. Incidence 1 in 100,000


NADH + NH3
Amino acids
α-Ketoglutarate
α-Keto acids
Glutamate
NAD+
Transamination
Oxidative deamination
Glutamate
Oxaloacetate
α-Ketoglutarate
Aspartate
Urea
HCO3−
Fumarate
Arginine
Ornithine
UREA CYCLE
Argininosuccinate
Carbamoyl phosphate
Citrulline


METABOLISM
α-Keto acids
Glutamate
NAD(P)+
Aminotransferases
Glutamate dehydrogenase
α-Amino acids
α-Ketoglutarate
NAD(P)H
DIET
BODY PROTEIN
Glutamate + ATP
Glutamine synthetase
ADP + Pi
NH3
Glutamine
Glutaminase
H2O
Glutamate
H+
Amide nitrogen donated in biosynthetic reactions
NH4+
URINE
Carbamoyl phosphate synthetase I
Urea cycle
Urea
(BLOOD)