Metabolism of ammonia

Question: What are the sources of ammonia in our body?

Ammonia is produced in the human body through a variety of processes, including:

- ***Amino acid metabolism:*** During protein breakdown, amino acids produce ammonia.
- ***Intestinal bacteria:*** Healthy bacteria in the intestines produce ammonia when digesting protein.
- ***Renal ammoniagenesis:*** Ammonia is produced in the kidneys through the metabolism of glutamine.
- ***Purine and pyrimidine metabolism:*** Ammonia is produced from the amino groups of nitrogenous bases like purine and pyrimidine.

Ammonia is a normal waste product in the body, but it must be removed because high levels can be toxic to the central nervous system. The liver converts ammonia into urea, another waste product, which is then removed from the body.

Metabolism of ammonia

Question: Why body converts ammonia to urea?

The body converts ammonia to urea to remove toxic waste from the body and make it less harmful to excrete:

Ammonia is toxic:

- Ammonia is a waste product created when the body breaks down amino acids and other nitrogenous compounds. If left in the body, ammonia can become toxic to the brain.

Urea is less toxic:

- Urea is a nitrogenous waste that's less toxic than ammonia and requires less water to remove from the body.

The urea cycle

- *The liver produces enzymes that convert ammonia into urea through a process called the urea cycle. The urea is then excreted in urine.*

Metabolism of ammonia

Question: Why is ammonia toxic to brain?

- Overpower the brain's defenses

  Ammonia can trigger a molecular chain reaction that short-circuits the transport of potassium into the brain's glial cells. This causes potassium to accumulate around nerve cells, which can lead to cell death.

- Change the polarity of cell membranes

  Ammonia can change the polarity of brain cell membranes, which affects the transport of substances across the membrane

Production, transport, storage and excretion of ammonia

In the human body, **ammonia is transported and stored in the form of glutamine, a non-toxic amino acid**:

- Production: Ammonia is produced in the gut and kidneys.
- Transport: Amino acids carry ammonia to the liver in the form of amino groups.
- Storage: In the liver, ammonia is converted to glutamine, which is then transported by the blood to other tissues.
- Unloading: In other tissues, glutaminase breaks down glutamine to release ammonia.
- Disposal: The liver treats ammonia as a waste product and disposes of it in a few ways:
    - Urea: Ammonia is packaged as urea in the liver and transported to the kidneys via the bloodstream. The kidneys eliminate urea in urine.
    - Excretion: Ammonia produced in the kidneys is excreted in urine as ammonium ions.

Metabolism of ammonia

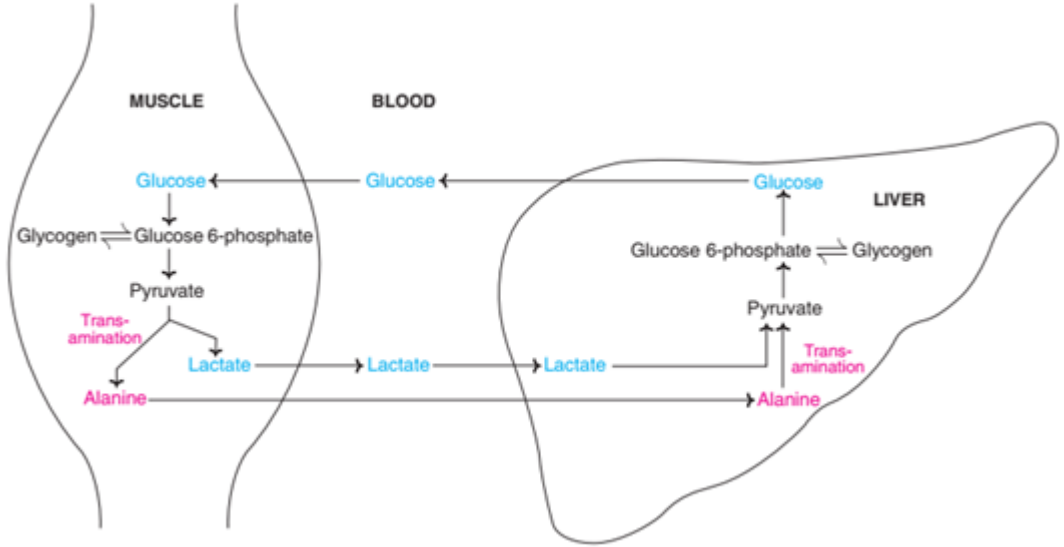
- Ammonia is constantly being liberated in the metabolism of amino acids (mostly) and other nitrogenous compounds.
- At the physiological pH, **ammonia exists as ammonium (NH^{+4}) ion.**

I. Formation of ammonia:

- The production of NH_3 occurs from
  - ✓ the amino acids (transamination and deamination),
  - ✓ biogenic amines,
  - ✓ amino group of purines and pyrimidines and
  - ✓ by the action of intestinal bacteria (urease) on urea.

II. Transport and storage of NH_3

- Despite a regular and constant production of NH_3 from various tissues, its concentration in the circulation is surprisingly low (normal plasma 10-20 mg/dl).
- This is mostly because the body has an efficient mechanism for NH_3 transport and its immediate utilization for urea synthesis.
- The transport of ammonia between various tissues and the liver mostly occurs in the form of **glutamine** or **alanine** and not as free ammonia. Alanine is important for NH_3 transport from muscle to liver by glucose-alanine cycle



Role of glutamine

- Glutamine is a **storehouse of NH_3.**
- It is present at the highest concentration **(8 mg/dl in adults)** in blood among the amino acids.
- Glutamine serves as a **storage** and **transport** form of NH_3.
- Its synthesis mostly occurs in liver, brain and muscle.
- Ammonia is removed from the brain predominantly as glutamine.
- Glutamine is **freely diffusible** in tissues, hence easily transported.
- Glutamine synthetase (a mitochondrial enzyme) is responsible for the synthesis of glutamine from glutamate and ammonia.
- This reaction is unidirectional and requires ATP and Mg^{2+} ions.
- Glutamine can be deaminated by hydrolysis to release ammonia by glutaminase an enzyme mostly found in kidney and intestinal cells.

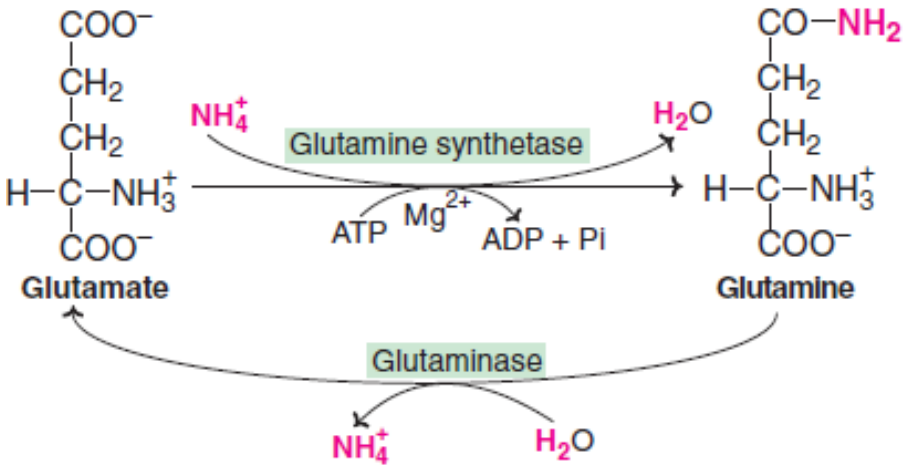


Fig. 15.8 : *Synthesis of glutamine and its conversion to glutamate. (**Note :** The reactions are independent and irreversible).*

III. Functions of ammonia

- Ammonia is not just a waste product of nitrogen metabolism.
- It is involved (directly or via glutamine) for the **synthesis** of many compounds in the body.
- These include
  - **Nonessential amino acids,**
  - **purines,**
  - **pyrimidines,**
  - amino sugars,
  - Ammonium ions (NH_4^+) are very important to maintain **acid-base balance** of the body.

IV. Disposal of ammonia

- The organisms, during the course of evolution, have developed different mechanisms for the disposal of ammonia from the body.
- The animals in this regard are of three different types:
  - **Ammoniotelic :** The aquatic animals dispose off NH_3 into the surrounding water.
  - **Uricotelic :** Ammonia is converted mostly to uric acid e.g. reptiles and birds.
  - **Ureotelic :** The mammals including man convert NH_3 to urea. Urea is a non-toxic and soluble compound, hence easily excreted.

V. Toxicity of ammonia

- Even a marginal elevation in the blood ammonia concentration is **harmful to the brain.**
- Ammonia, when it accumulates in the body, results in **slurring of speech and blurring of the vision** and causes tremors.
- It may lead to coma and, finally, death, if not corrected.

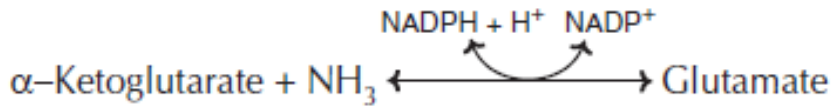
✓ **Hyperammonemia :**

- Elevation in blood NH_3 level may be genetic or acquired.
- Impairment in urea synthesis due to a defect in any one of the five enzymes is described in urea synthesis.
- All these disorders lead to hyperammonemia and cause **hepatic coma** and **mental retardation**.
- The acquired hyperammonemia may be due to hepatitis, alcoholism etc. where the urea synthesis becomes defective, hence NH_3 accumulates.

Ammonia toxicity occurs when the body's ability to eliminate ammonia is overwhelmed by the amount of ammonia in the blood. This can happen when the body produces too much ammonia or when it can't eliminate enough.

	Ammonia toxicity
Symptoms	Irritability, lack of energy, vomiting, headache, loss of muscle coordination, confusion, mood swings, seizures
Causes	Overproduction of ammonia, such as in congenital hyperammonemia, or under-elimination, such as in liver cirrhosis
Blood ammonia levels	Usually less than 50 micromoles per liter (micromol/L) in healthy adults
Toxicity levels	100 micromol/L can lead to changes in consciousness, 200 micromol/L is associated with coma and convulsions
Exposure	Inhaling anhydrous ammonia gas or vapors, ingesting ammonia-containing liquids, or direct contact with anhydrous ammonia gas
Other effects	Skeletal muscle metabolic derangements, impaired protein synthesis, increased autophagy, protein nitration

Exposure to high concentrations of ammonia in the air can also cause immediate burning of the eyes, nose, throat, and respiratory tract. This can lead to lung damage, respiratory distress or failure, and even death.



Explanation for NH_3 toxicity

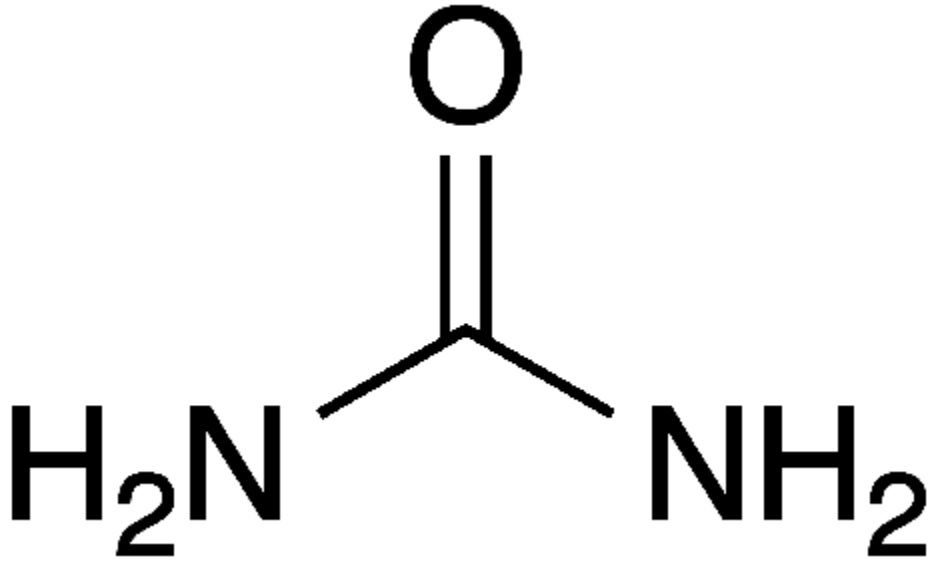
- The reaction catalyzed by glutamate dehydrogenase probably explains the toxic affects of NH_3 in brain.
- Accumulation of NH_3 shifts the equilibrium to the right with more glutamate formation, hence more utilization of alpha-ketoglutarate.
- Alpha- Ketoglutarate is a key intermediate in TCA cycle and its depleted levels impair the TCA cycle.
- The net result is that production of energy (ATP) by the brain is reduced. The toxic effects of NH_3 on brain are, therefore, due to impairment in ATP formation".

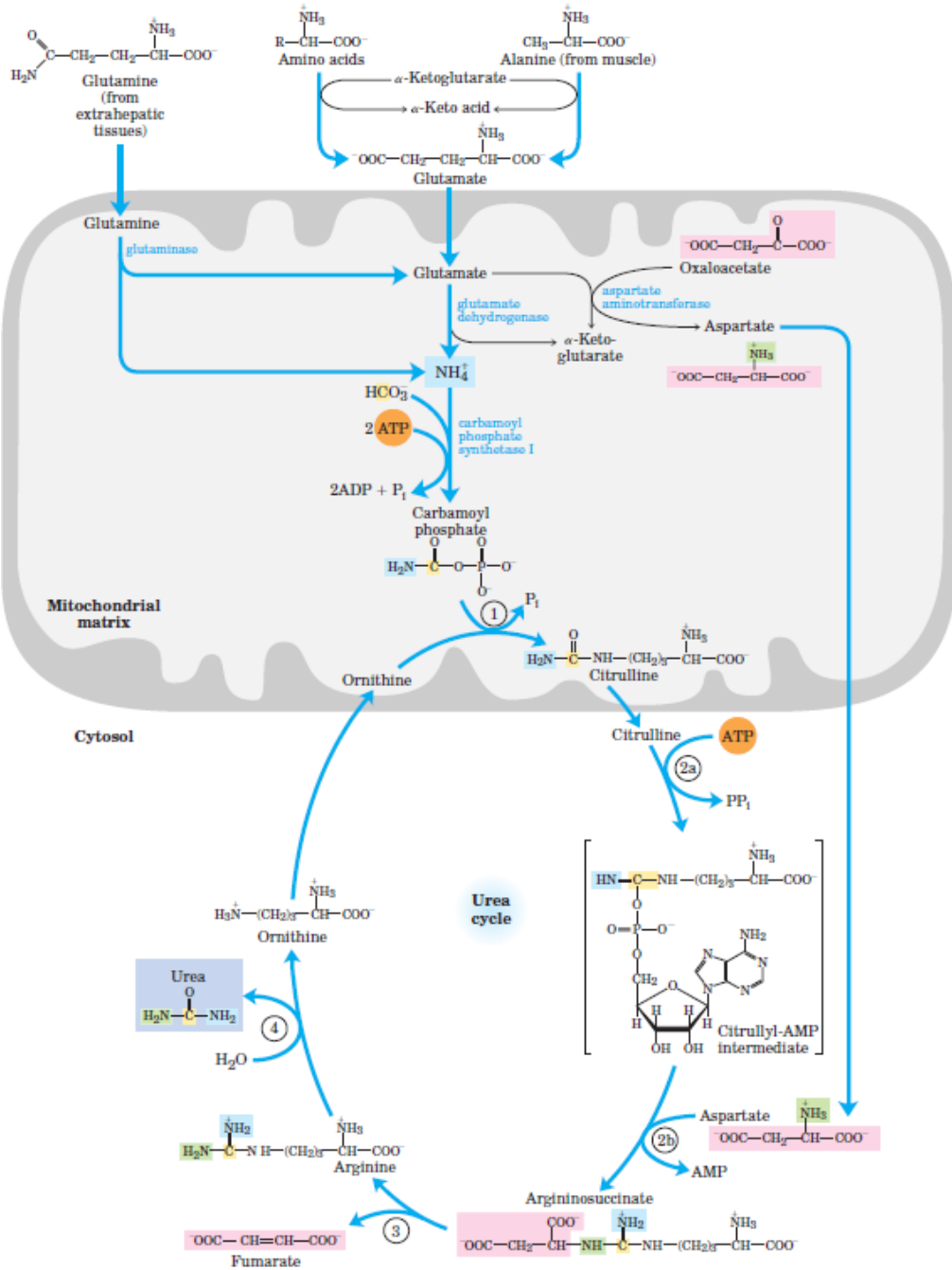
Trapping and elimination of ammonia

When the plasma level of ammonia is highly elevated, intravenous administration of sodium benzoate and phenyl lactate is done. These compounds can respectively condense with glycine and glutamate to form water soluble products that can be easily excreted. By this way, ammonia can be trapped and removed from the body. In some instances of toxic hyperammonemia, haemodialysis may become necessary.

Urea Cycle

- **Urea** is the **end product of protein metabolism** (amino acid metabolism). The
- nitrogen of amino acids, converted to ammonia (as described above), is toxic to the body. It is
- converted to urea and detoxified. As such, urea accounts for 80-90% of the nitrogen containing substances excreted in urine.
- 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**.
- The individual reactions, however, were described in more detail later on by **Ratner and Cohen.**
- Urea has **two amino (NH_2) groups**, one derived **from NH_3** and the other **from aspartate.**
- Carbon atom is supplied by CO2. 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**.




Glutamine (from extrahepatic tissues)
Amino acids
α-Ketoglutarate
α-Keto acid
Alanine (from muscle)
Glutamate
Glutamine
glutaminase
Glutamate
Oxaloacetate
aspartate aminotransferase
Aspartate
glutamate dehydrogenase
α-Keto-glutarate
NH4+
HCO3
2 ATP
carbamoyl phosphate synthetase I
2ADP + Pi
Carbamoyl phosphate
Mitochondrial matrix
Pi
1
Ornithine
Citrulline
Cytosol
Citrulline
ATP
2a
PPi
Citrullyl-AMP intermediate
Urea cycle
Ornithine
Urea
4
H2O
Aspartate
2b
AMP
Argininosuccinate
Arginine
3
Fumarate

Synthesis of carbamoyl phosphate

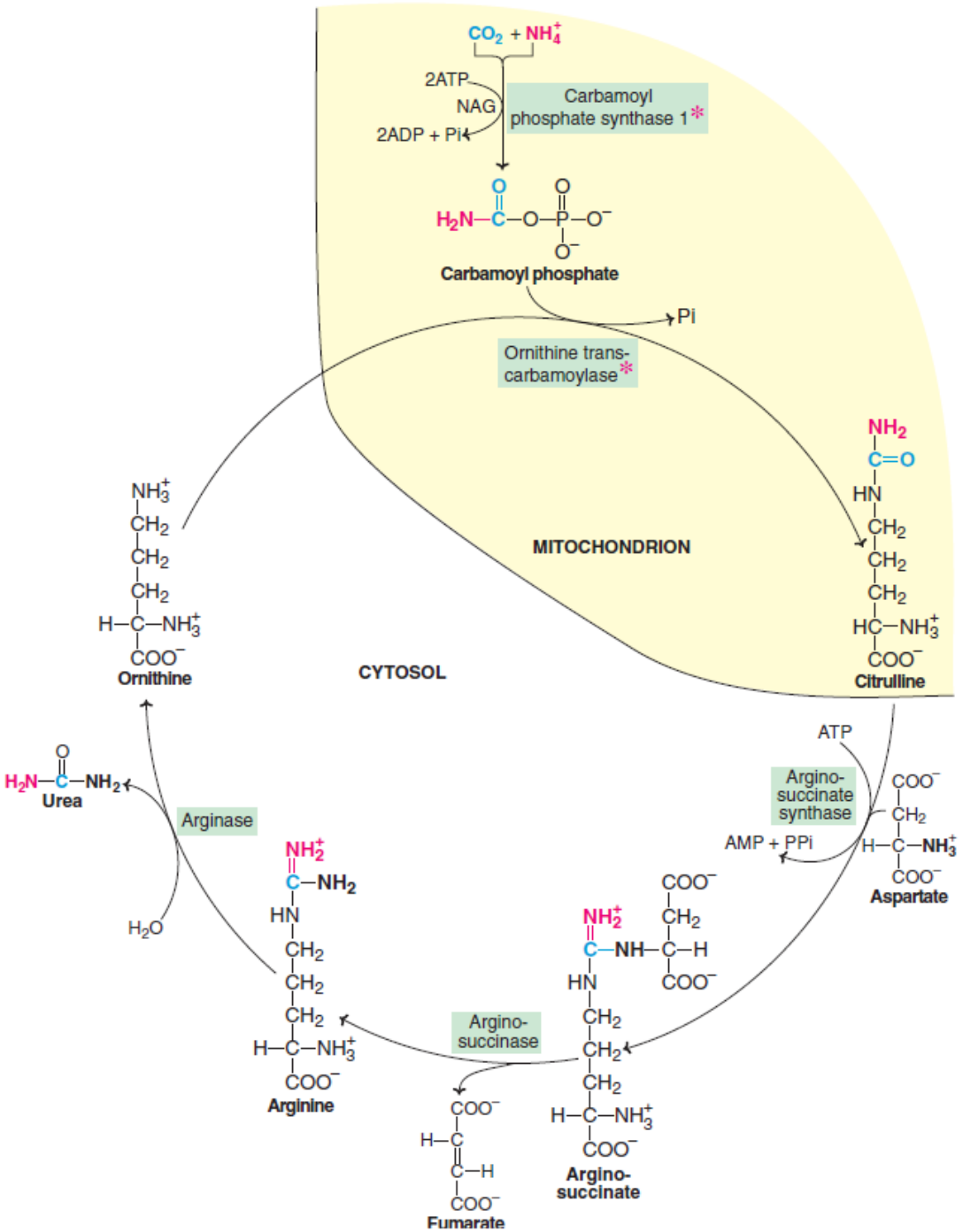
- ✓ Carbamoyl phosphate synthase I (CPS I) of mitochondria catalyzes the condensation of NH^{4+} ions with CO_2 to form carbamoyl phosphate.
- ✓ This step consumes two ATP and is **irreversible**, and **rate-limiting**.
- ✓ CPS I requires **N-acetyl glutamate** for its activity.

Formation of citrulline

- ✓ Citrulline is synthesized from carbamoyl phosphate and ornithine by ornithine transcarbamoylase.
- ✓ Ornithine is regenerated and used in urea cycle.
- ✓ Therefore, its role is comparable to that of oxaloacetate in citric acid cycle.

Synthesis of arginosuccinate

- ✓ Arginosuccinate synthase condenses citrulline with aspartate to produce arginosuccinate.
- ✓ The second amino group of urea is incorporated in this reaction.
- ✓ This step requires ATP which is cleaved to AMP and pyrophosphate (PPi).
- ✓ The latter is immediately broken down to inorganic phosphate (Pi).



Cleavage of arginosuccinate :

- ✓ Arginosuccinase cleaves arginosuccinate to give arginine and fumarate.
- ✓ Arginine is the immediate precursor for urea.
- ✓ Fumarate liberated here provides a connecting link with TCA cycle, gluconeogenesis etc.

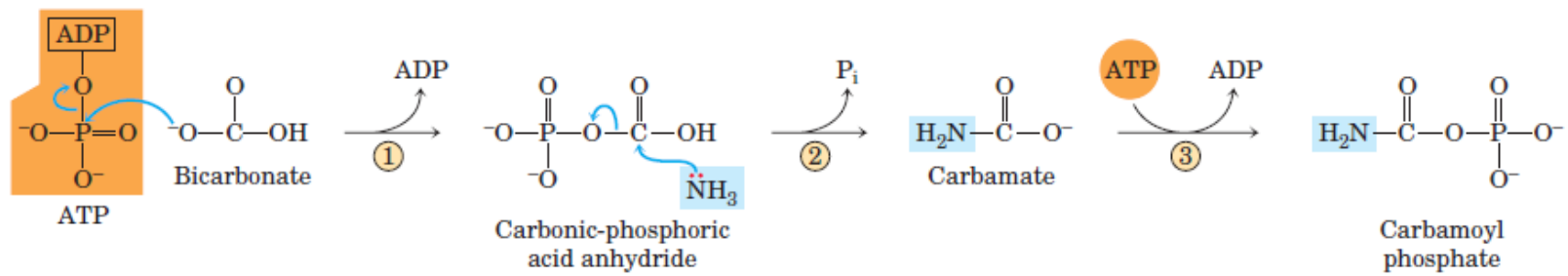
Formation of urea :

- ✓ Arginase is the fifth and final enzyme that cleaves arginine to yield urea and ornithine.
- ✓ Ornithine, so regenerated, enters mitochondria for its reuse in the urea cycle.
- ✓ Arginase is activated by Co^{2+} and Mn^{2+}.
- ✓ Ornithine and 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.
- ✓ For this reason, arginine synthesis may occur to varying degrees in many tissues. But only the liver can ultimately produce urea.

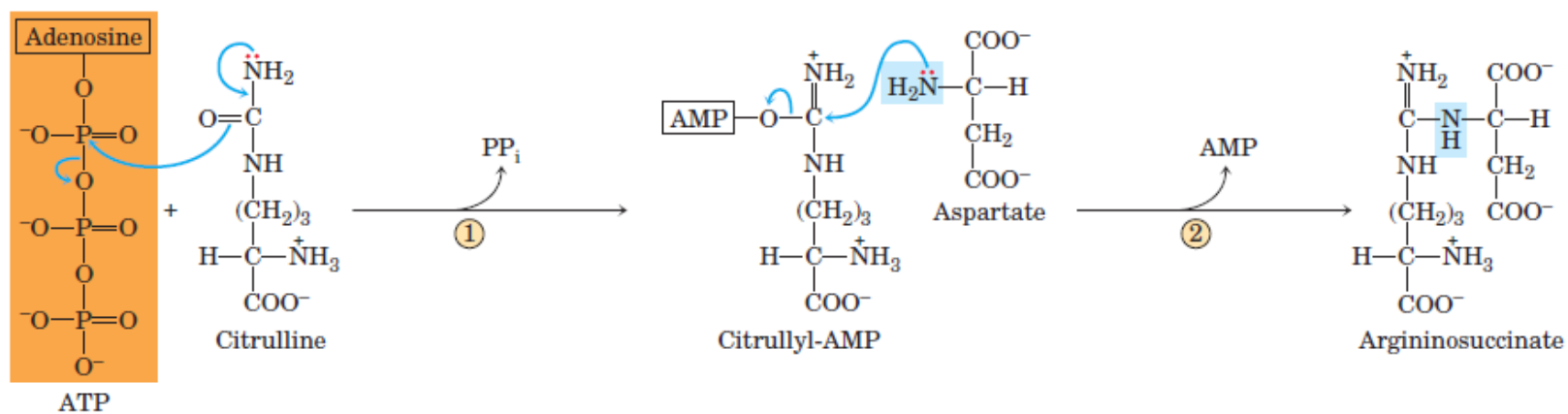
Overall reaction and energetics

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***.

$$NH_4^+ + CO_2 + \text{Aspartate} + 3ATP \longrightarrow \text{Urea} + \text{Fumarate} + 2\ ADP + 2\ Pi + AMP + PPi$$

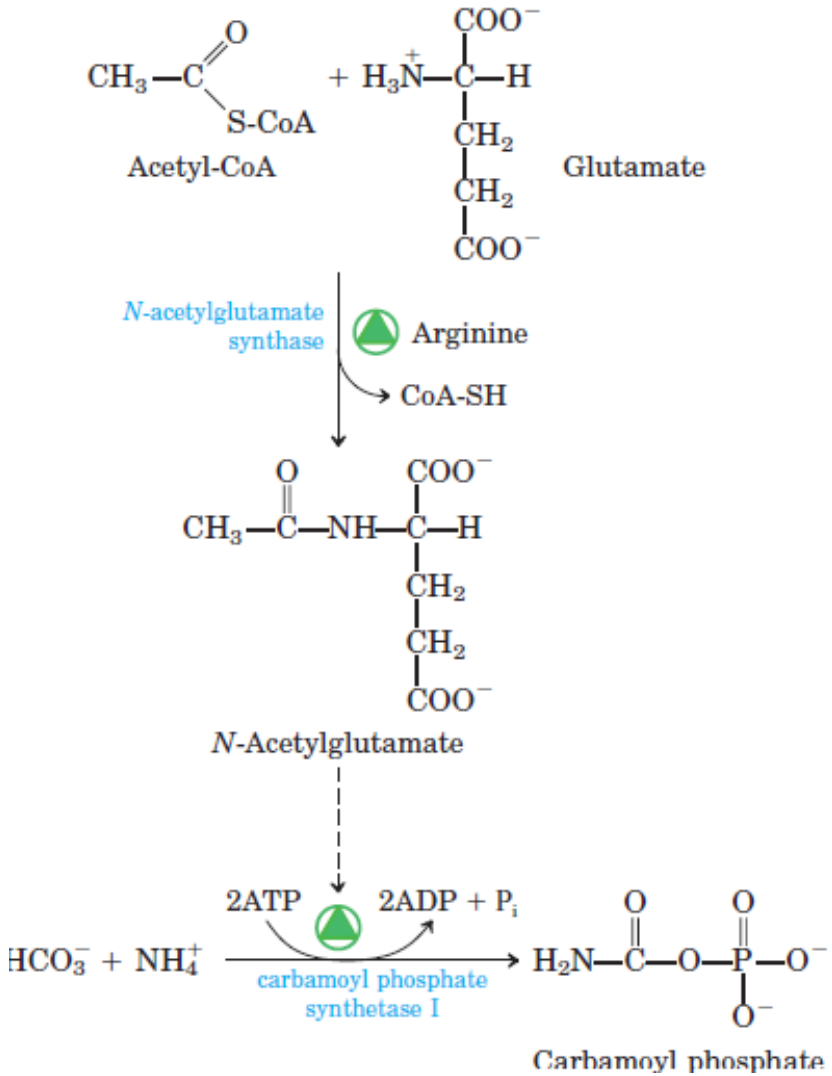


(a)



Regulation of urea cycle

- The first reaction catalysed by **carbamoyl phosphate synthase I** (CPS I) is **rate-limiting** 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.***
- The rate of urea synthesis in liver is correlated with the concentration of N-acetylglutamate.
- The consumption of a protein-rich meal increases the level of NAG in liver, leading to enhanced urea synthesis.
- The remaining four enzymes of urea cycle are mostly controlled by the concentration of their respective substrates.



Disposal of urea

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

Integration between urea cycle and TCA cycle

Urea cycle is linked with TCA cycle in three different ways. This is regarded as **bicyclic integration** between the two cycles.

- The production of **fumarate** in urea cycle is the most important integrating point with TCA cycle. Fumarate is converted to malate and then to oxaloacetate in TCA cycle. Oxaloacetate undergoes transamination to produce aspartate which enters urea cycle. Here, it combines with citrulline to produce arginosuccinate. Oxaloacetate is an important metabolite which can combine with acetyl CoA to form citrate and get finally oxidized. Oxaloacetate can also serve as a precursor for the synthesis of glucose (gluconeogenesis).
- ATP (12) are generated in the TCA cycle while **ATP** (4) are utilized for urea synthesis.
- Citric acid cycle is an important metabolic pathway for the complete oxidation of various metabolites to CO_2 and H_2O. The **CO_2** liberated in TCA cycle (in the mitochondria) can be utilized in urea cycle.

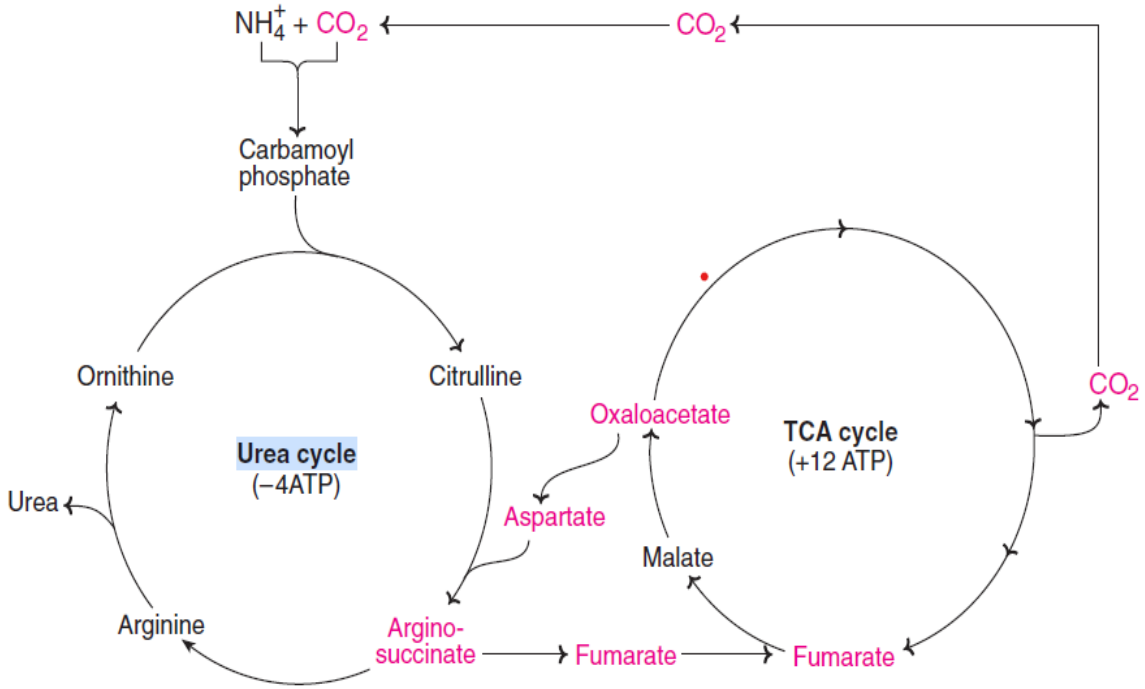


Fig. 15.12 : Interrelation between urea and tricarboxylic acid (TCA) cycle (Depicted in blue colour).