Urea and creatinine metabolism

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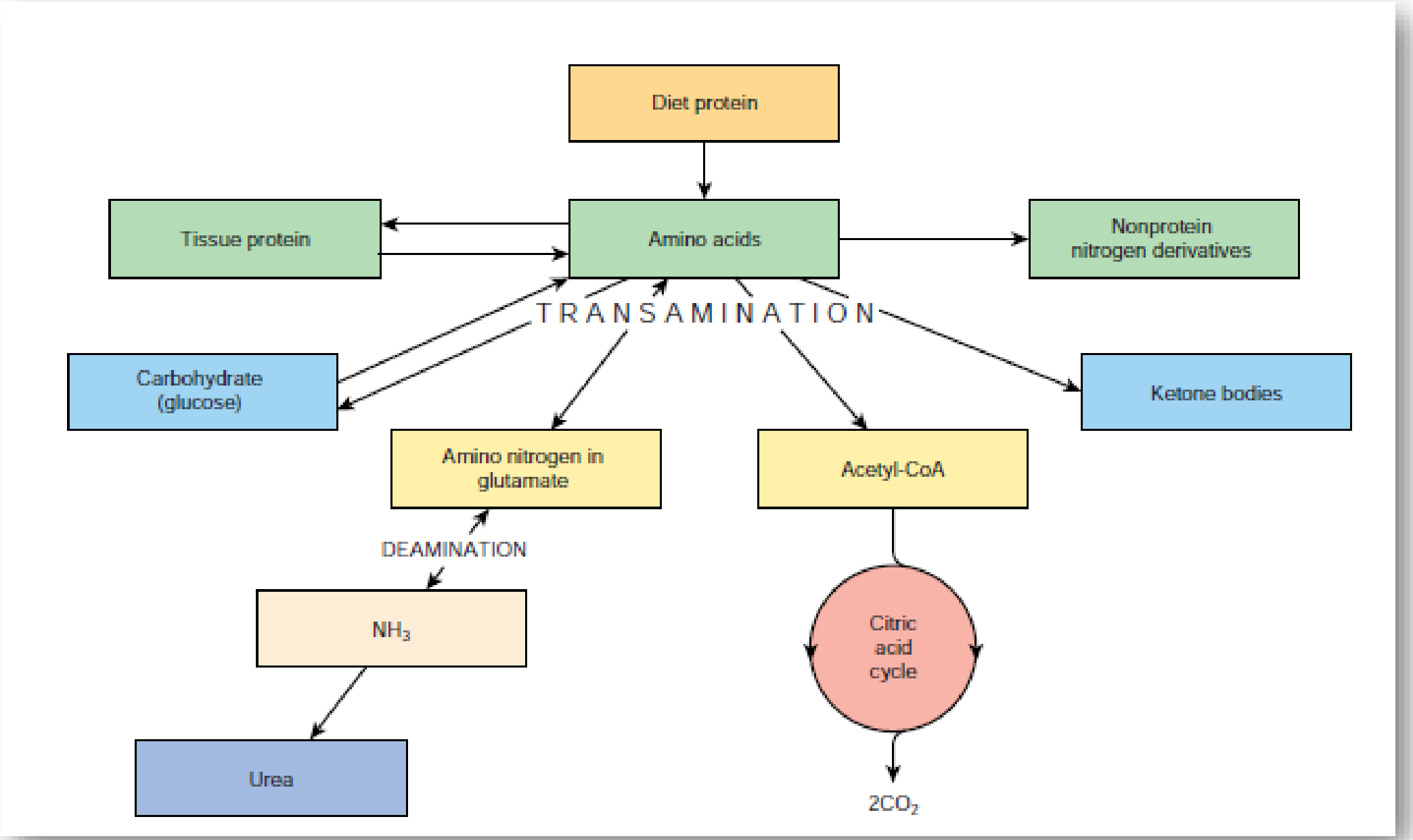
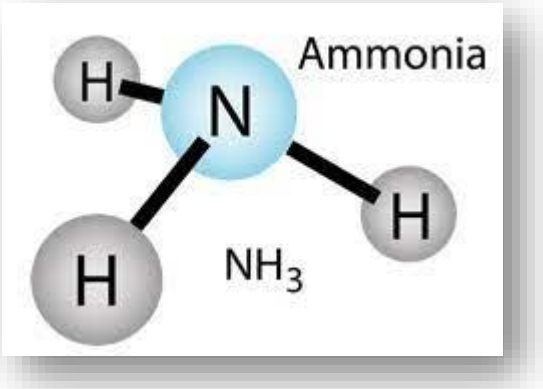
40	**Special aspects of renal metabolism. Role of kidney in acid base balance. (Biochemistry 1+2)**	1. Discuss urea and creatinine metabolism/ cycle. 2. Understand the basic principles on the role of kidney in the regulation of hydrogen ions and bicarbonate buffer system to understand abnormalities in urine composition. 3. Discuss amino acids absorption by the kidney and their disorders 4. Discuss normal and abnormal composition of urine 5. Interpret the results of routine urine analysis


Diet protein
Tissue protein
Amino acids
Nonprotein nitrogen derivatives
TRANSAMINATION
Carbohydrate (glucose)
Ketone bodies
Amino nitrogen in glutamate
Acetyl-CoA
DEAMINATION
NH_3
Citric acid cycle
Urea
$2CO_2$

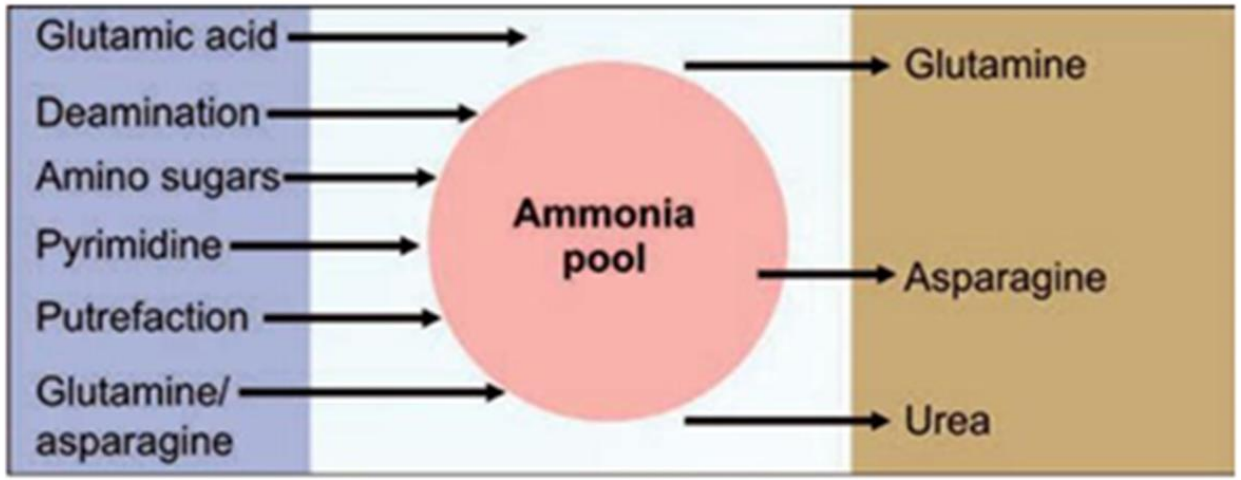
Nitrogen metabolism

- An adult consuming 100g of protein/d excretes 16.5g nitrogen/d; 95% in urine and 5% in faeces (v small amounts in sweat & other routes)
- **Nitrogen balance:** quantitative difference btwn nitrogen intake & output
- **Positive nitrogen balance:** intake > output
  - Growth, muscular training, pregnancy, recovery from –ve nitrogen balance
- **Negative nitrogen balance:** output > intake
  - Inadequate protein diet, loss of protein, increased protein catabolism
- **Nitrogen equilibrium** → output = intake
  - Normal healthy adult on an adequate diet

Ammonia

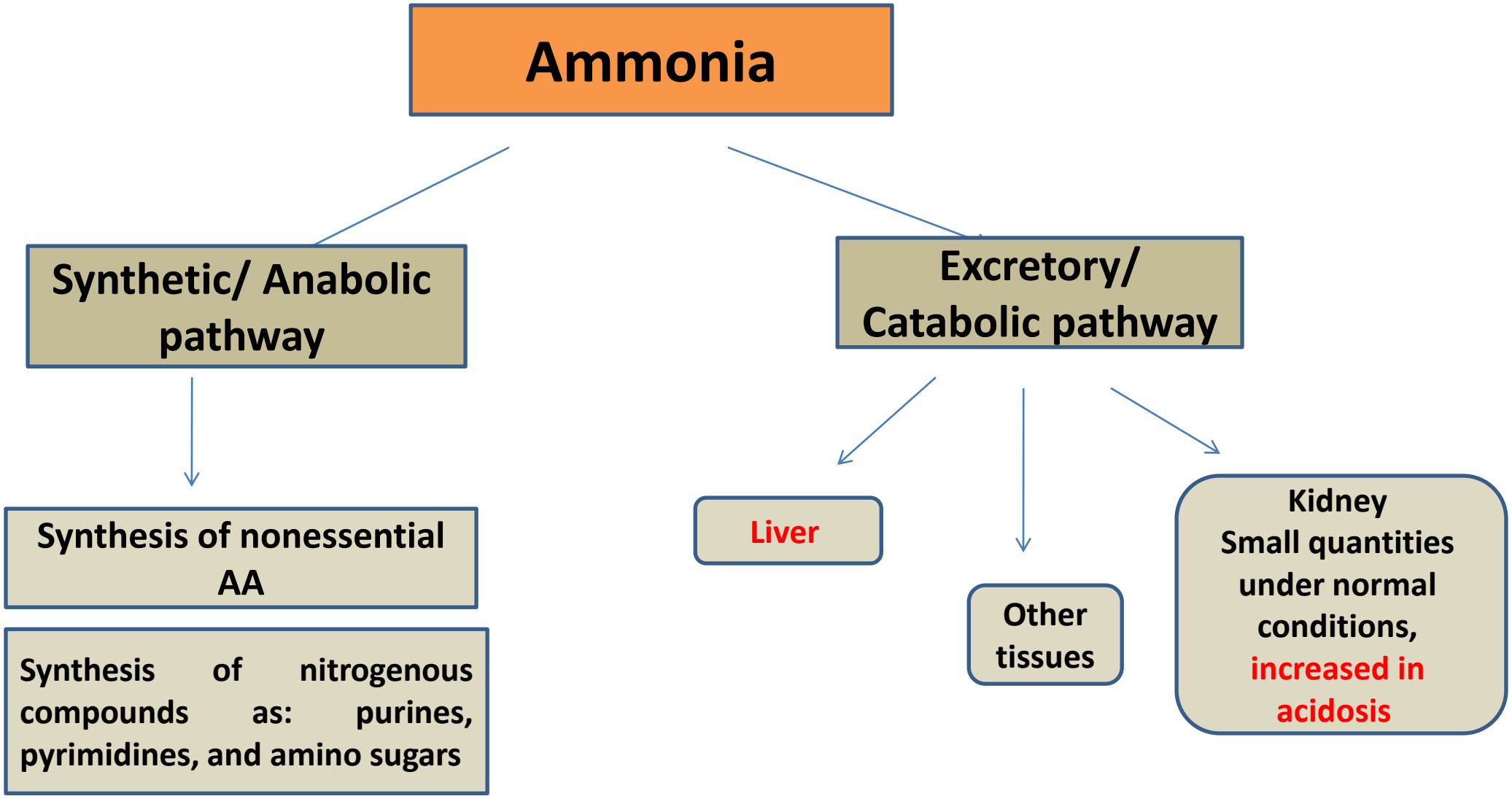


- Universal participant in amino acid synthesis and catabolism (deamination)
- Accumulation in abnormal concentrations → **toxic effects**
- Ammonia must be eliminated as soon as it is formed



Sources and fate of ammonia

Fate of the ammonia removed



- **Fate of products of deamination:**

(A) Fate of the ammonia removed

(A) Fate of the carbon skeleton

Ammonia transport from tissues to liver/ kidney

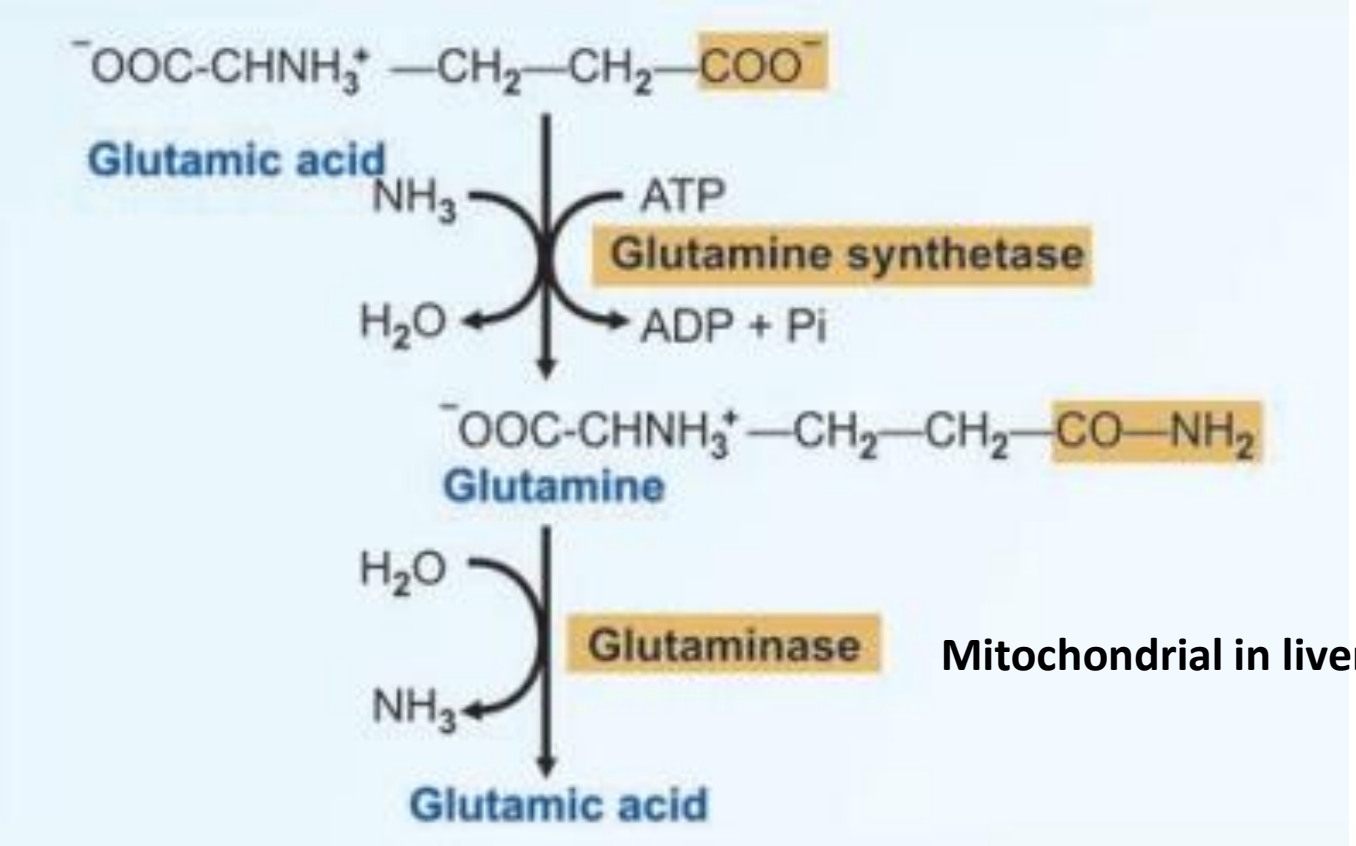
- Inside the cells of almost all tissues, the transamination of amino acids → glutamic acid

- **First line of Defense (Trapping of ammonia):** Being highly toxic, ammonia should be eliminated or detoxified, as and when it is formed
    - Even very minute quantity of ammonia may produce toxicity in central nervous system

- Intracellular ammonia is immediately trapped by glutamic acid to form **glutamine**, especially in brain cells
    - The glutamine is then transported to liver, where the reaction is reversed by the enzyme **glutaminase**

- Aspartic acid may also undergo similar reaction to form **asparagine**

Ammonia trapping as glutamine



Glutamate is critical to intracellular AA metabolism

Glutamate synthetase: mitochondrial enzyme, high concentration in brain, liver & kidney

Mitochondrial in liver, kidney

Catabolic and excretory pathways:

- Being highly toxic to tissues, the ammonia produced **in excess of the requirements for anabolic purposes** is rapidly disposed of

- The method of disposal depends upon the tissue in which deamination occurs

A- In the liver:

The liver is the main site of deamination of amino acids

- Most of the ammonia released (via glutaminase) is converted to urea
- The urea formed goes via the blood to the kidneys to be excreted in urine

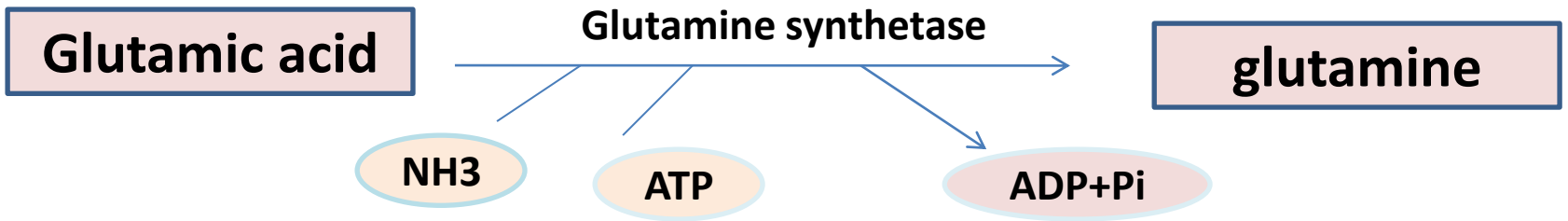
B- In the kidneys:

The ammonia resulting from the deamination of AA in the kidneys is directly excreted in urine

This accounts for about 40% of the urinary ammonia

C- In extrarenal tissues:

The ammonia resulting from the deamination of AA in extrarenal tissues, particularly the brain, is converted to glutamine



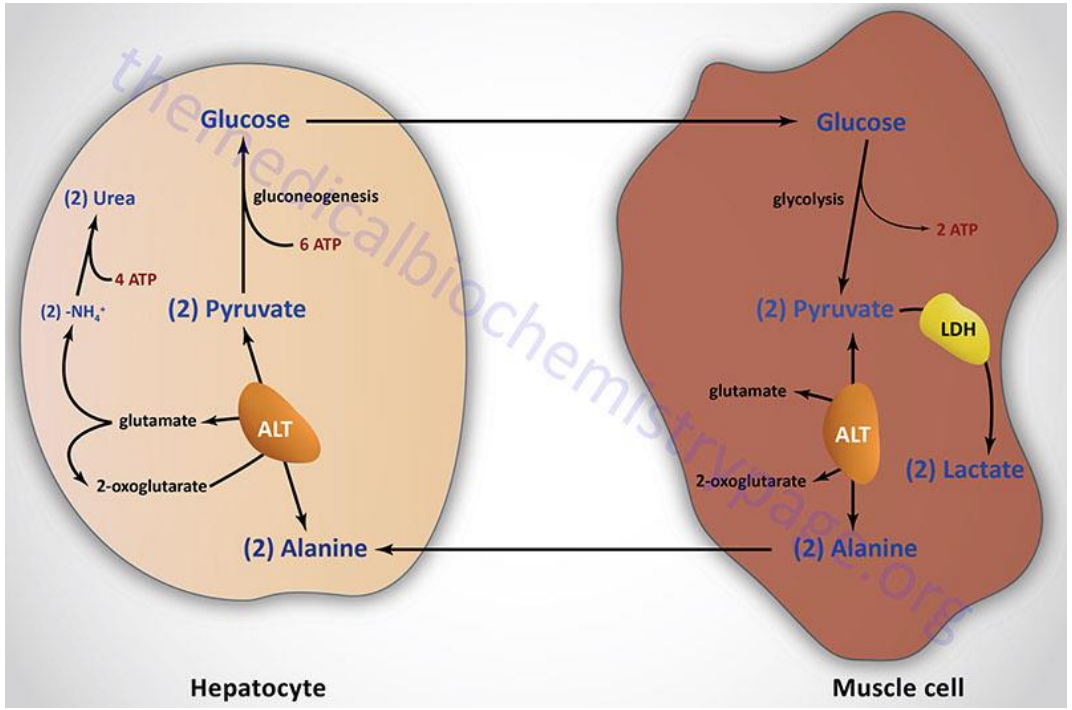
- Glutamine goes, via the blood, to the kidneys where it becomes hydrolyzed by **glutaminase** into **glutamic acid** and **ammonia**
  - The ammonia is excreted in urine, accounting for about 60% of urinary ammonia
  - This amount increases in acidosis (forms salts with metabolic acids) → counteracting acidosis

- Glutamic acid acts as the link between amino groups of amino acids and ammonia
- The concentration of glutamic acid in blood is 10 times more than other amino acids

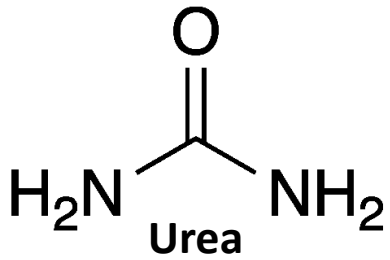
Glutamine is the transport forms of ammonia from brain and intestine to liver; while alanine is the transport form from muscle

Glucose-Alanine cycle

- Alanine is transported from muscle to liver, transaminated → pyruvate → glucose
- Glucose can enter glycolytic pathway to form pyruvate which is transaminated → alanine
- Glucose-alanine cycle is of primary importance in conditions of starvation
- **Importance**
  - Transfer if 3C of pyruvate to the liver to give glucose
  - Transfer of NH3 in non-toxic form from muscle to liver to be converted to urea

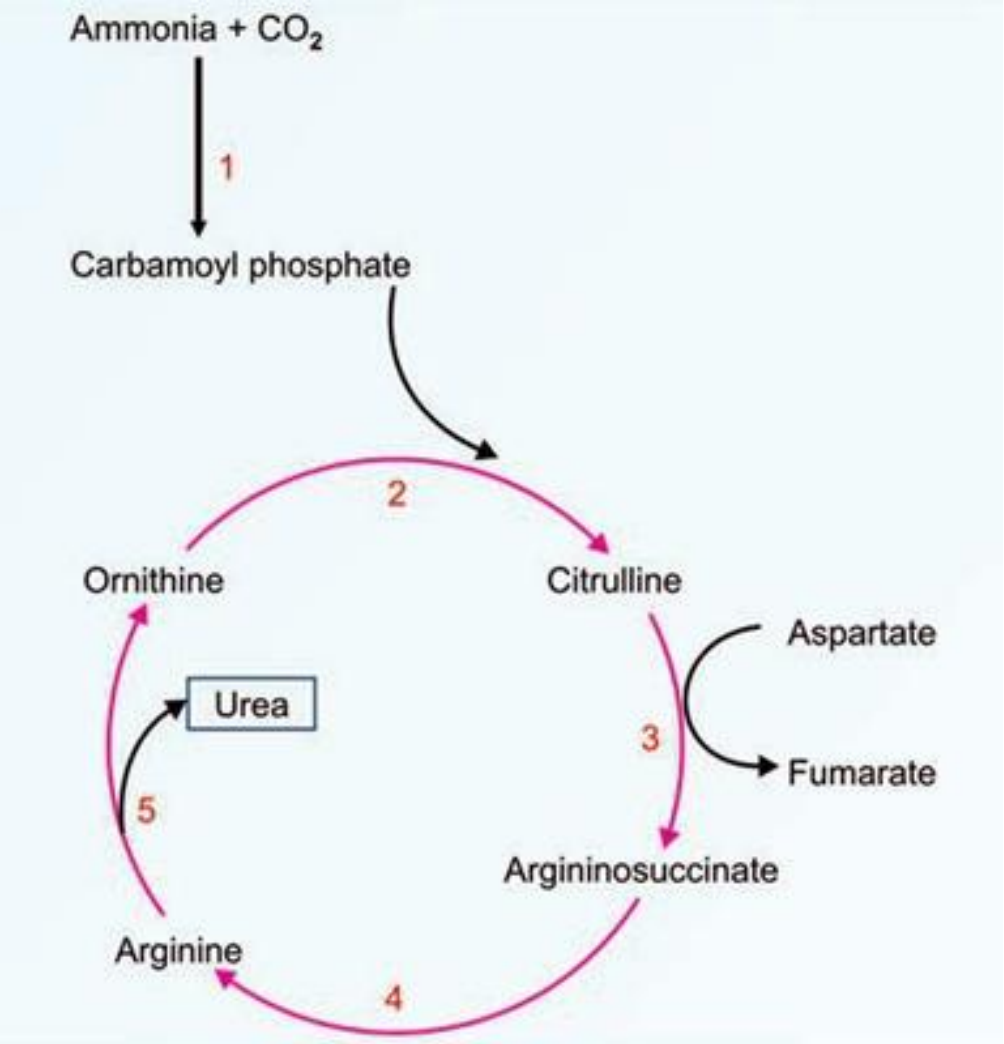


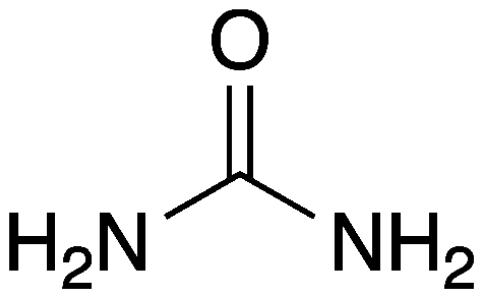
Urea cycle



- Urea is the main way of excretion of ammonia resulting from the deamination of AA
- Ammonia is highly toxic to the CNS; it is converted to non toxic urea in the **liver only**
  - Urea is water soluble easily excreted by the kidneys in urine. **Urea is the main end product of protein (amino acids) metabolism**
- Plasma urea is 15-45 (20-40) mg/dl, it is formed in the liver and transported in blood to the kidney to be excreted in urine (urinary urea is 15-45 (20-40) g/day)
- Urea **cycle** is known as **Krebs–Henseleit** cycle (5 reactions, 1-2 in mitochondria; 3-5 in cytosol)
- As ornithine is the first member of the reaction, it is also called as **Ornithine cycle**
- The two nitrogen atoms of urea are derived from two different sources:
  - one from ammonia; and
  - the other directly from the alpha amino group of aspartic acid

Urea cycle



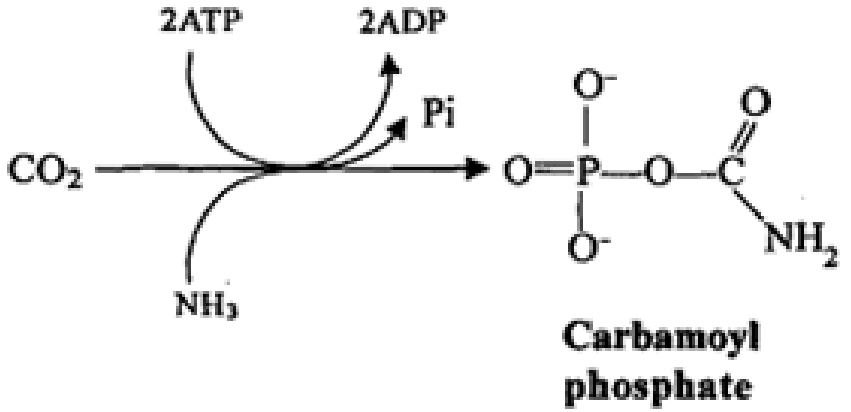


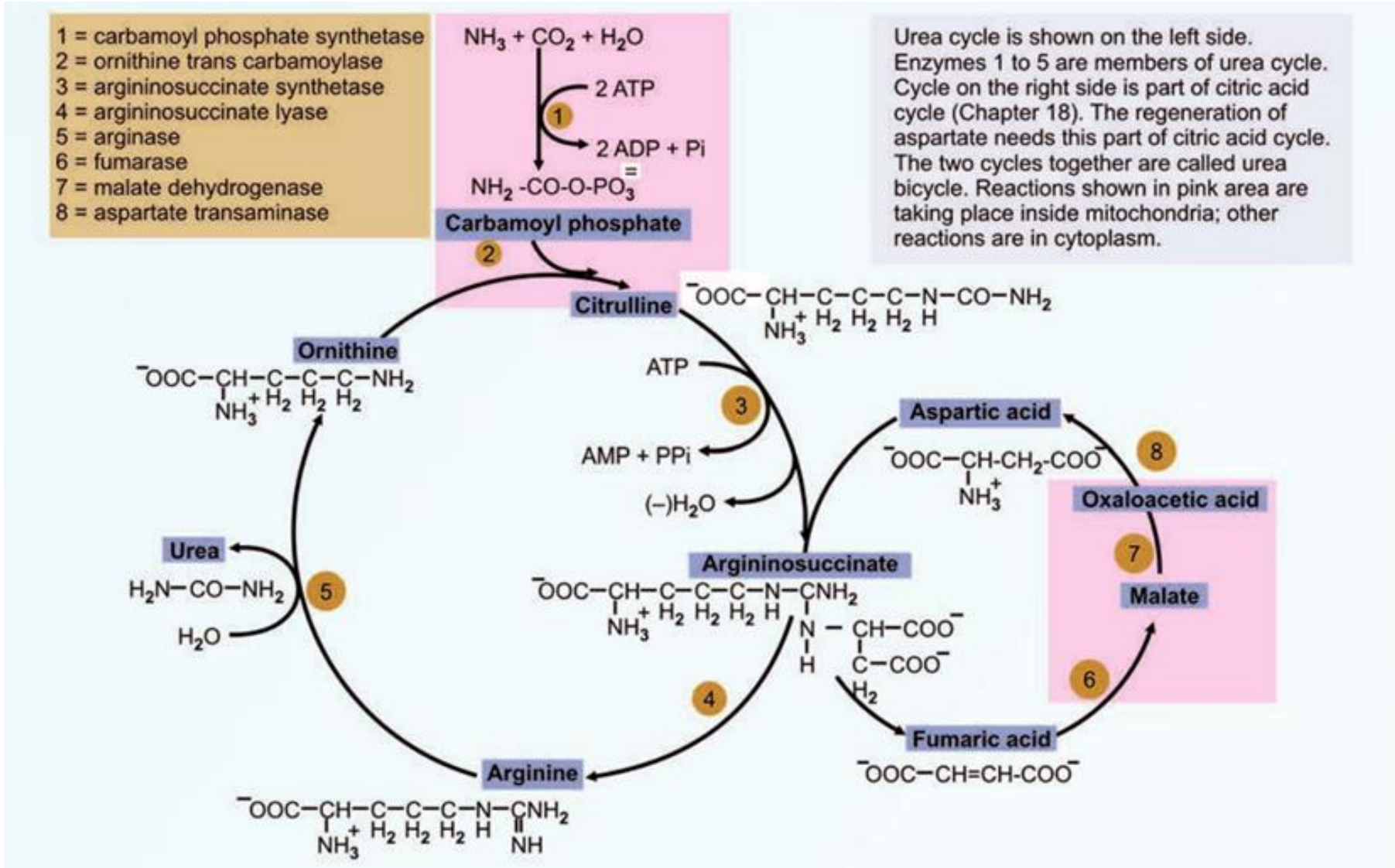
Urea

Urea cycle, summary. Note that aspartate enters and fumarate leaves at different steps

Step 1. Formation of Carbamoyl Phosphate

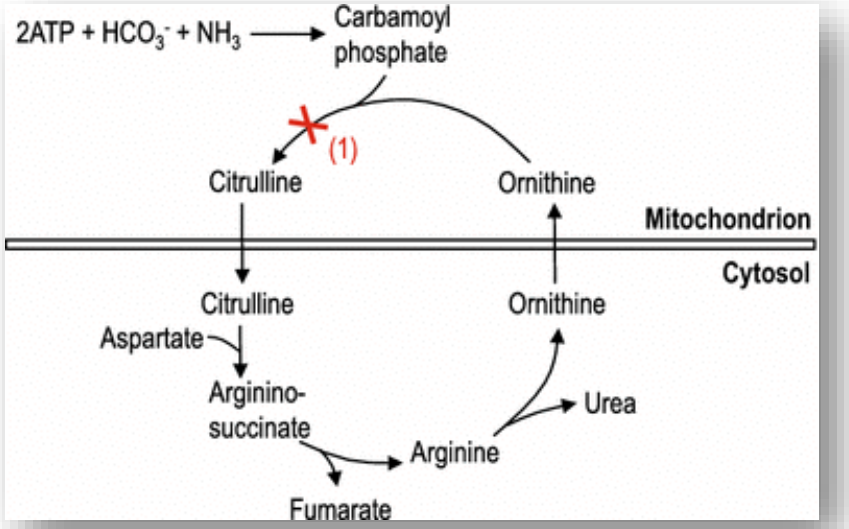
- One molecule of ammonia condenses with CO2 in the presence of **two molecules of ATP** → form carbamoyl phosphate
- The reaction is catalysed by the mitochondrial enzyme **carbamoyl phosphate synthetase-I (CPS-I)**
- An entirely different cytoplasmic enzyme, carbamoyl phosphate synthetase-II, (CPS-II) is involved in pyrimidine nucleotide synthesis
- **CPS-I reaction is the rate-limiting step in urea formation (**It is irreversible and allosterically regulated)



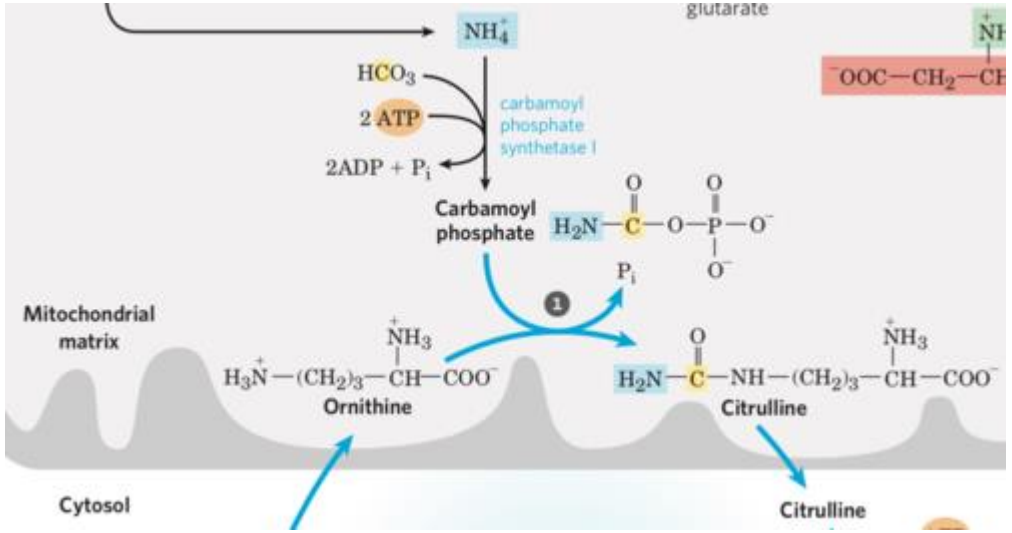


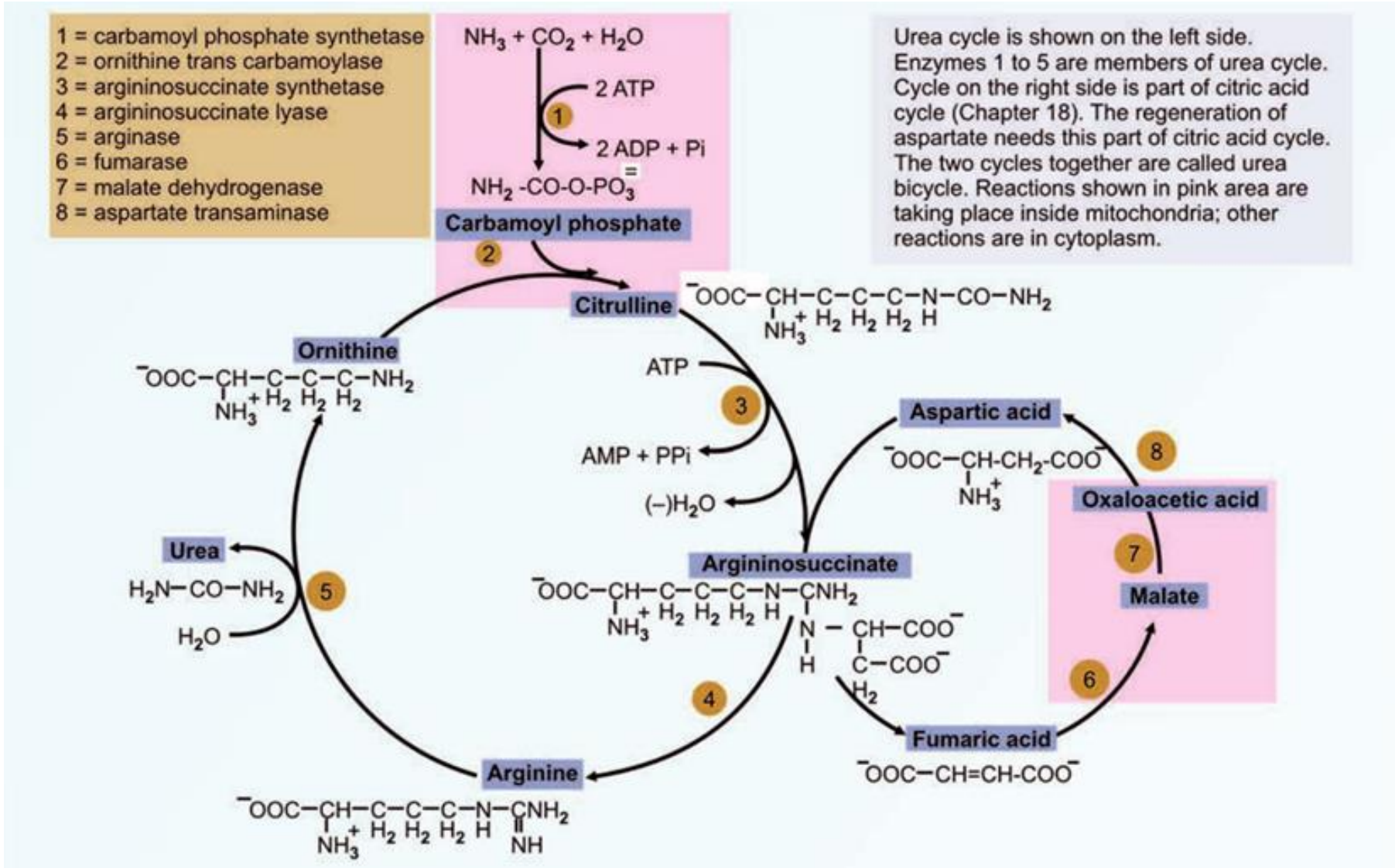
Urea cycle and its relation with citric acid cycle

Step 2. Formation of Citrulline



- The second reaction is also **mitochondrial**
- The carbamoyl group is transferred to the NH_2 group of ornithine by **ornithine transcarbamoylase** (OTC)
- Citrulline leaves the mitochondria and further reactions are taking place in cytoplasm

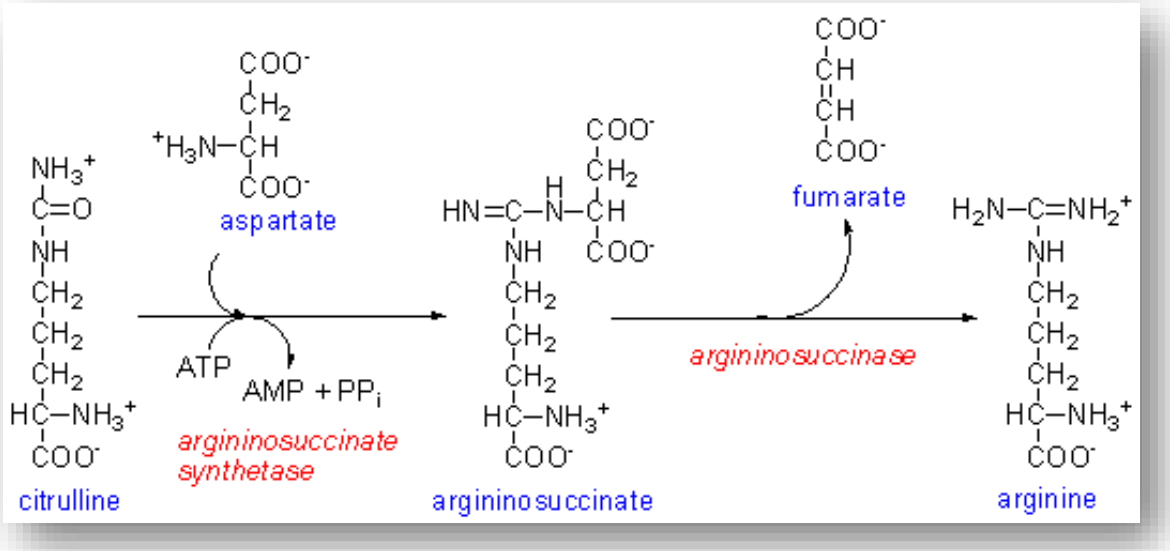


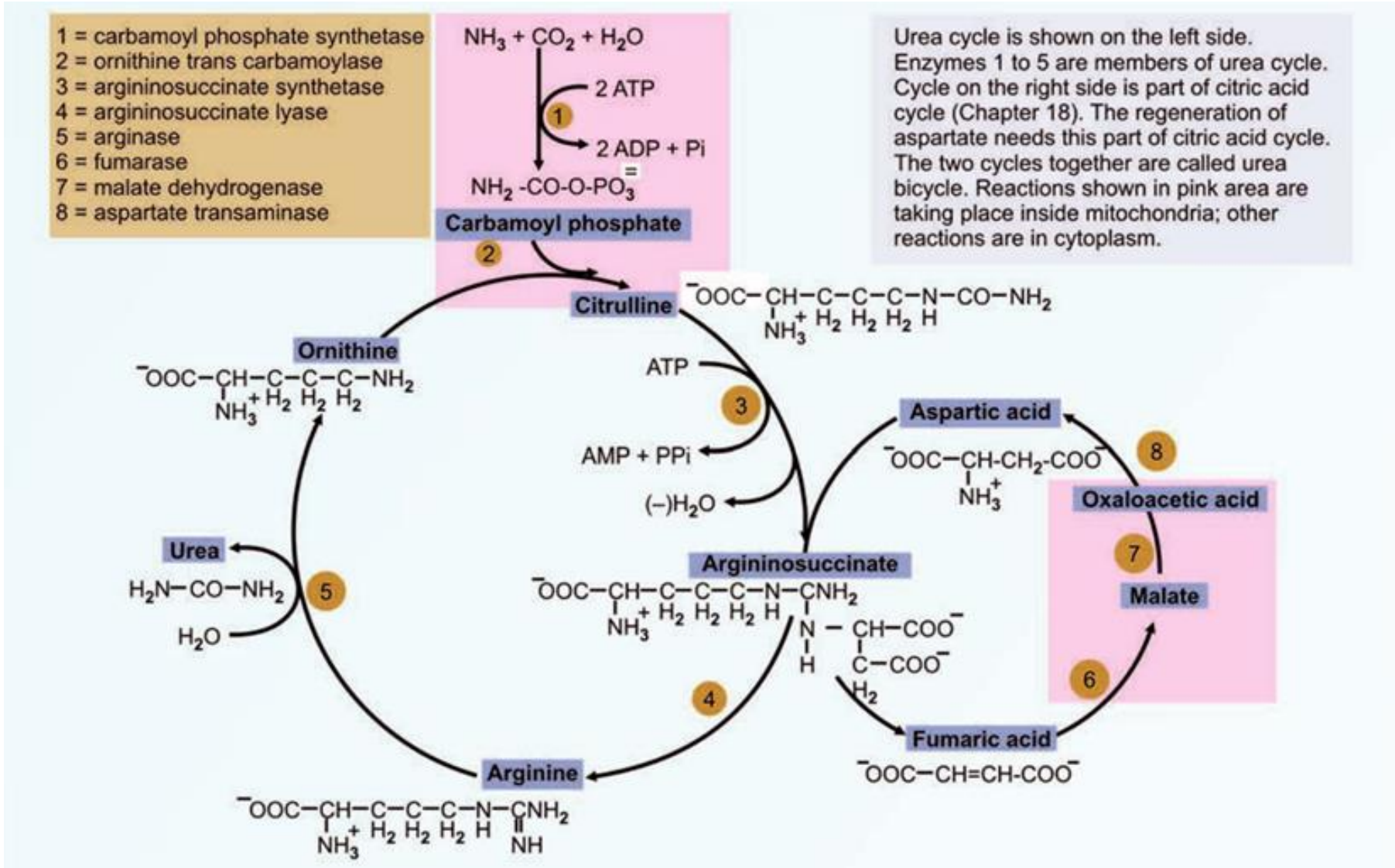


Urea cycle and its relation with citric acid cycle

Step 3. Formation of Argininosuccinate

- One molecule of aspartic acid adds to citrulline forming a carbon to nitrogen bond which provides the 2nd nitrogen atom of urea
- **Argininosuccinate synthetase** catalyzes the reaction
- This needs hydrolysis of ATP to AMP level, so "**two relatively high energy phosphate bonds"** are utilized
- The PPi is an inhibitor of this step

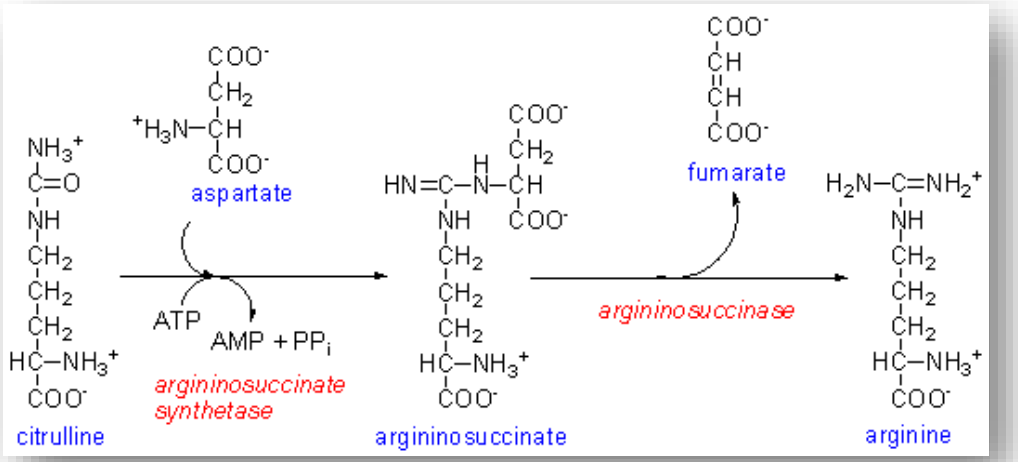


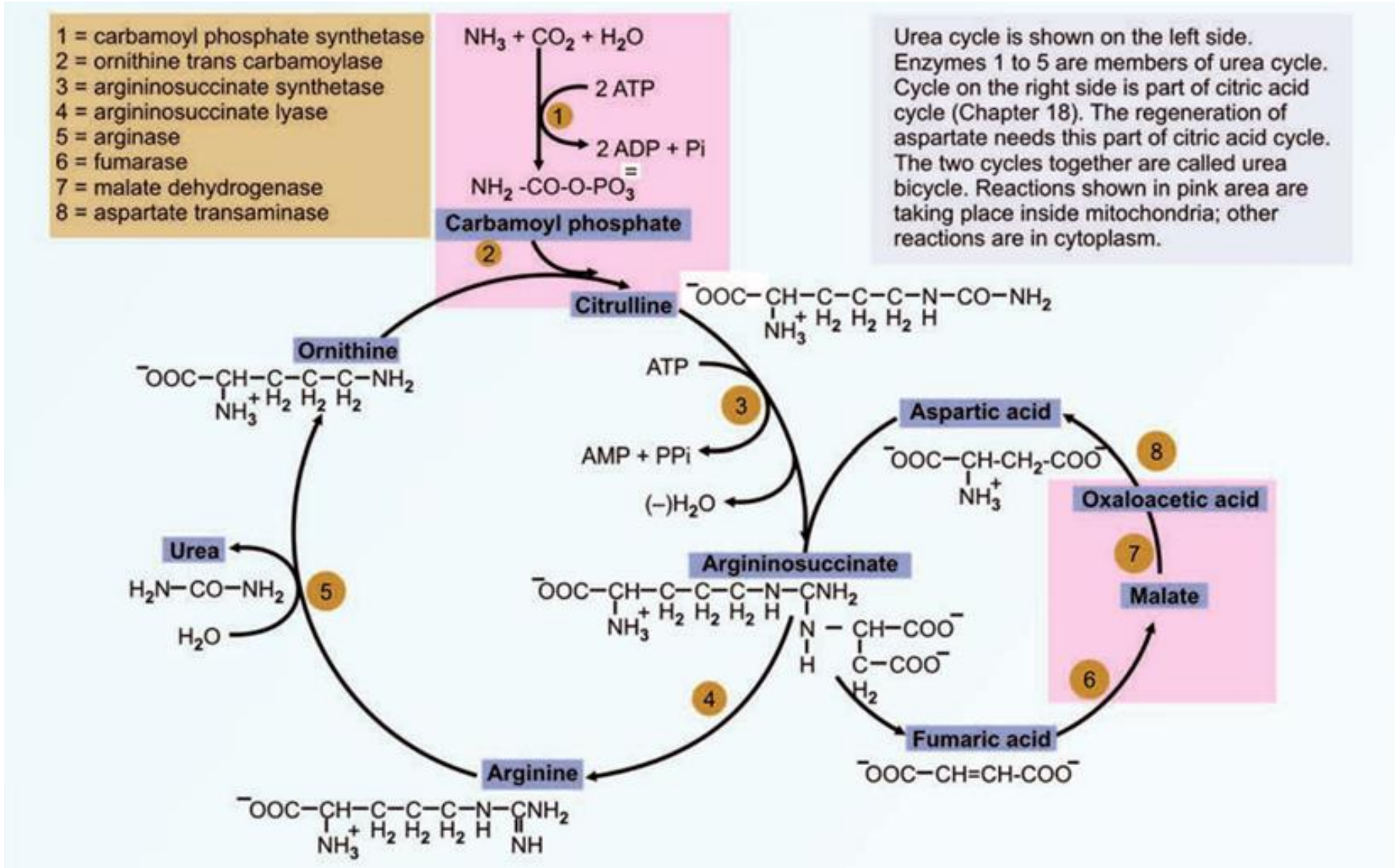


Urea cycle and its relation with citric acid cycle

Step 4. Formation of Arginine

- Argininosuccinate is cleaved by **argininosuccinate lyase** (argininosuccinase) to arginine and fumarate
- The enzyme is inhibited by fumarate
  - But this is avoided by the cytoplasmic localization of the enzyme
- The fumarate formed may be funneled into TCA cycle to be converted to malate and then to oxaloacetate to be transaminated to aspartate
- Thus the urea cycle is linked to TCA cycle through fumarate
- The 3rd and 4th steps taken together may be summarized as:
  - Citrulline + aspartate → Arginine + fumarate

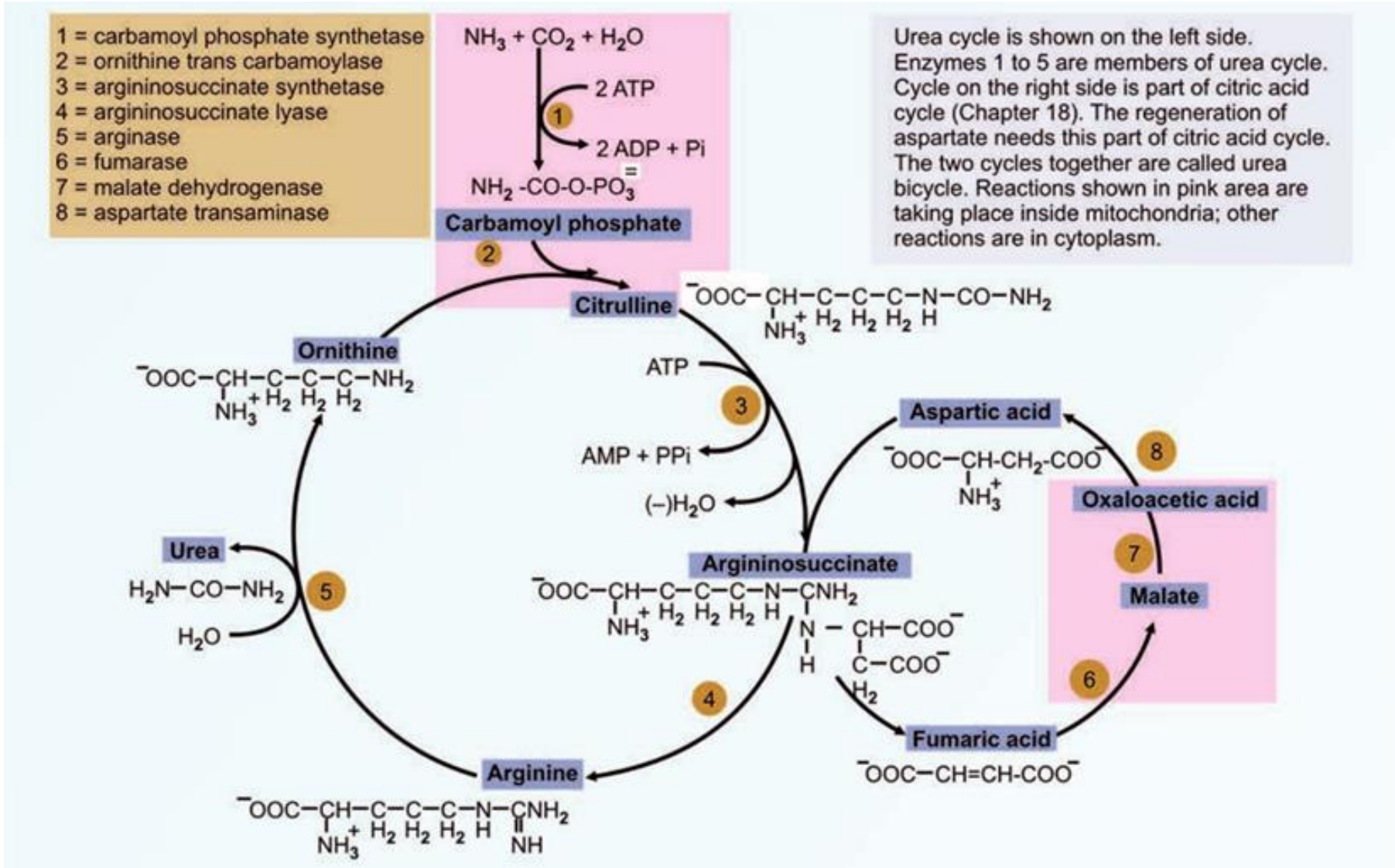




Urea cycle and its relation with citric acid cycle

Step 5. Formation of Urea

- The final reaction of the cycle is the hydrolysis of arginine to urea + ornithine by **arginase**
    - The ornithine returns to the mitochondria to react with another molecule of carbamoyl phosphate so that the cycle will proceed
    - Thus, ornithine may be considered as a catalyst which enters the reaction and is regenerated



Urea cycle and its relation with citric acid cycle

Energetics of Urea Cycle

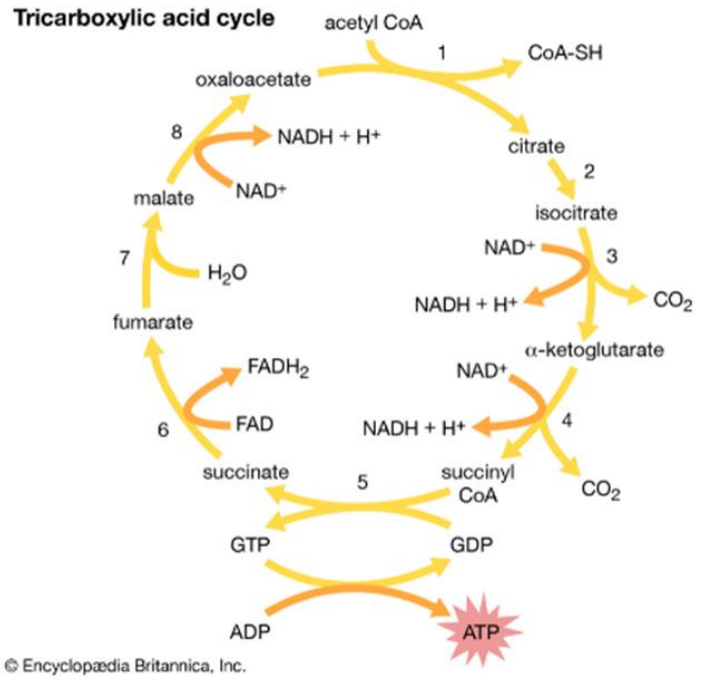
- The overall reaction may be summarized as:

  NH_3 + CO_2 + Aspartate → Urea + fumarate

- During these reactions, 2 ATPs 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**
- However, fumarate formed in the 4th step may be converted to malate
  - Malate when oxidised to oxaloacetate produces 1 NADH equivalent to 2.5 ATP (new system)
- 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**"

Relationship between urea cycle and tricarboxylic acid cycle (Kerbs cycle):

- Fumarate produced in urea cycle can be oxidized in Kerbs cycle to oxaloacetate which by transamination give aspartate needed for urea synthesis
- Co2 needed in urea formation is derived mainly from TCA cycle
- ATP needed in urea formation is derived from TCA cycle



Regulation of urea cycle:

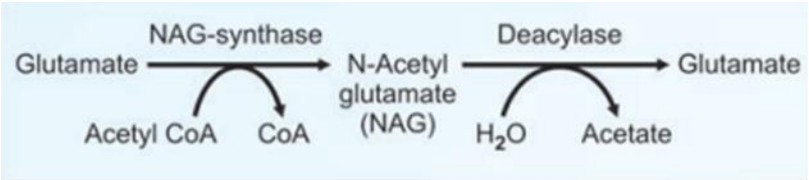
- **Corse control → Effect of feeding and fasting:** the enzymes of urea cycle are:
  - increased by high protein diet
  - decreased by low protein diet

- **Fine control** → N-acetylglutamate acts as activator for carbamoyl phosphate synthetase I (CPS I) which is inactive in its absence

Compartmentalization

- The urea cycle enzymes are located in such a way that the 1st two enzymes are in the mitochondrial matrix
  - The inhibitory effect of fumarate on its own formation is minimized because argininosuccinate lyase is in the cytoplasm, while fumarase is in mitochondria

↑↑ AA → ↑↑transdeamination → ↑↑ glutamate which combines with acetyl coA forming N-acety glutamate



NAG synthesis and breakdown

Diagnostic importance of plasma urea determination:

- Plasma urea is one of the kidney function tests

- Plasma urea is increased in kidney diseases like renal failure (uremia)

- In liver failure: liver cells cannot convert ammonia to urea so there will be:
  - **hyperammonemia** (ammonia intoxication); and
  - **urea is decreased**

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
- As a general description, disorders of urea cycle are characterized by **hyperammonemia, encephalopathy**
- Clinical symptoms include vomiting, irritability, lethargy and severe mental retardation (if untreated)
- Infants appear normal at birth, but within days progressive lethargy sets in
- Treatment is more or less similar in the different types of disorders
  - Low protein diet by frequent feeding can minimize brain damage since ammonia levels do not increase very high

Disorders of Urea Cycle

- **Brain is very sensitive to ammonia, ↑ ammonia leads to:**
  - Ammonia will combine with α−ketoglutaric acid forming glutamate and glutamine →
    - ↓ energy production by Krebs cycle in brain leading to brain damage
  - ↑ levels of glutamine → ↑ osmotic pressure in the astrocytes → which become swollen
  - **+ other mechanisms**

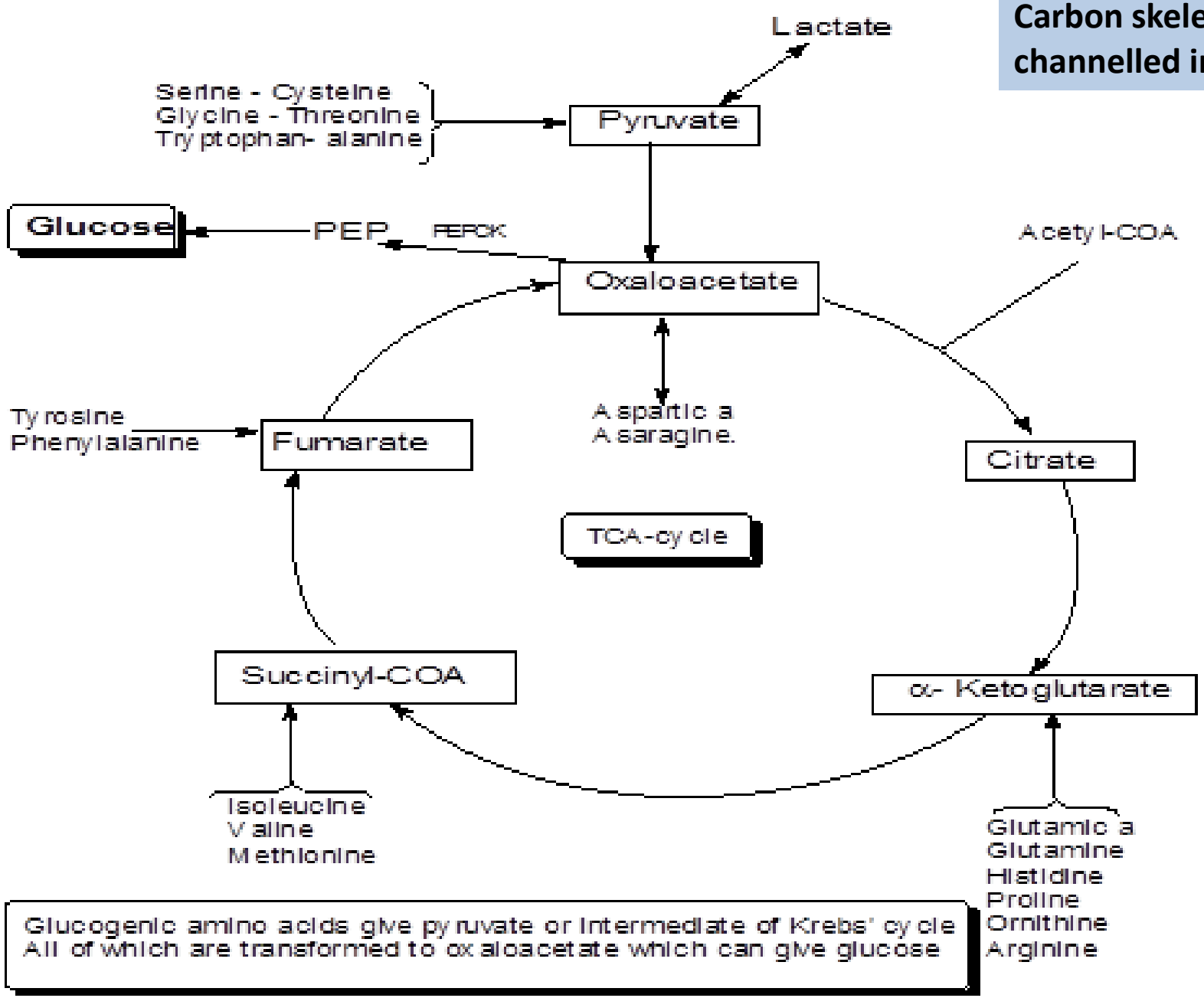
Fate of carbon skeleton of amino acids

- **Ketogenic AA:** produce acetyl coA or aceto-acetyl coA used in ketogenesis
    - Leucine
    - Lysine

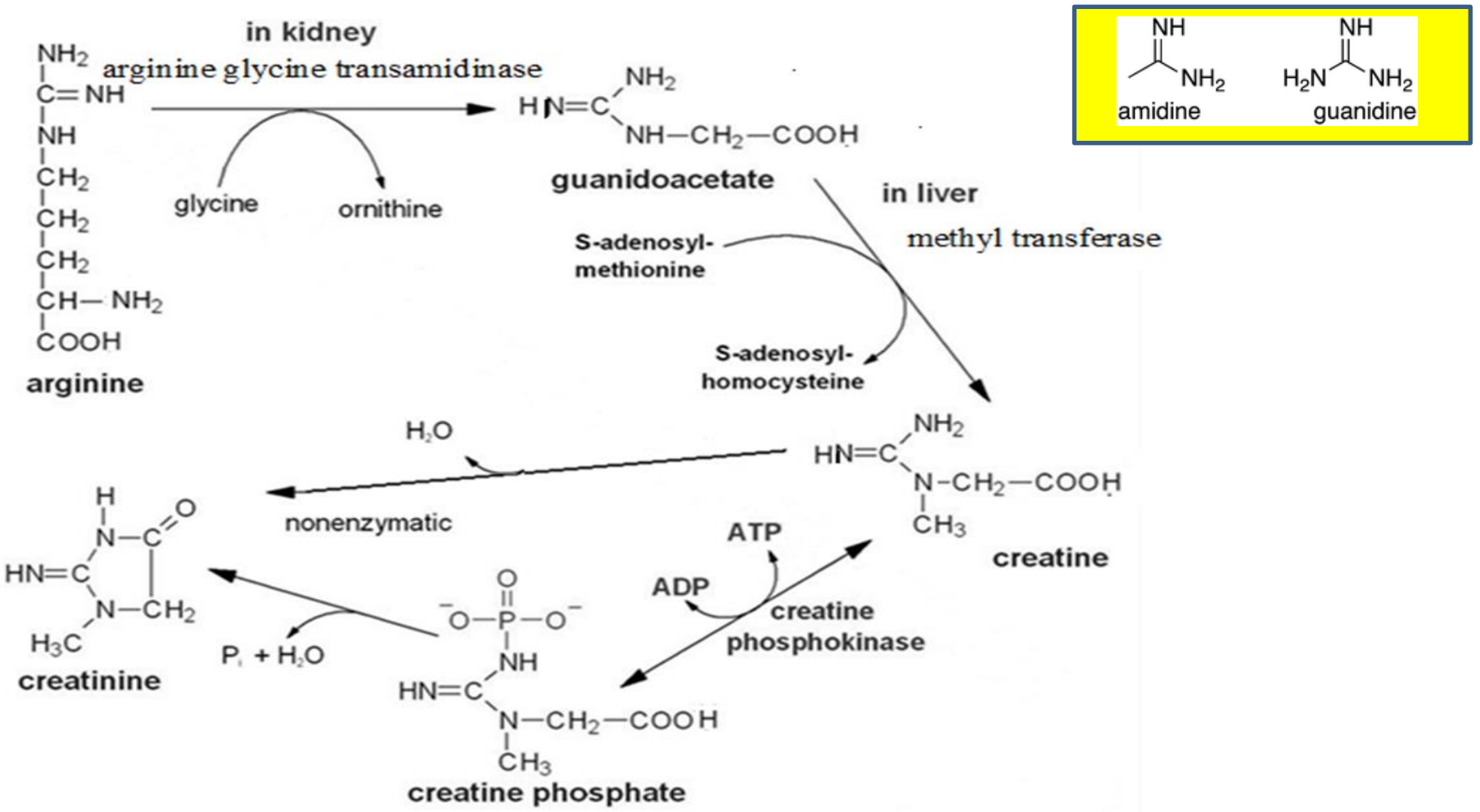
- **Glucogenic and ketogenic AA:** can give both glucose & ketone bodies
    - Tyrosine
    - Phenylalanine
    - Tryptophan
    - Isoleucine

- **Glucogenic AA:** rest of AA (14)

Carbon skeletons of the 20 AA are channelled into 7 molecules only



Creatine and Creatinine Metabolism



- **Creatine** is methyl guanido acetic acid. It is a NPN compound.
- It is widely distributed in our tissues: mainly (98%) in muscles as phosphocreatine (= phosphagen).
- **Creatinine** is creatine anhydride, it is the excretory product of creatine.
- The transamidinase reaction occurs in the kidney.
- The methyl transferase reaction occurs in the liver.

- The creatine goes via blood to different tissues mainly to the muscles (98% of the body creatine).
- Androgen (male sex hormones e.g. testosterone) increase the uptake and retention of creatine by muscles.

Function :

❖ Creatine forms creatine phosphate (phosphagen) which is the main storage form of energy in the cells .

❖ During muscular exercise, ATP is consumed rapidly to ADP. ATP is formed quickly at the expense of creatine phosphate by reversal of the CPK reaction. This occurs before glycoysis starts to produce ATP. i.e. maintain ATP during 1st few minutes of muscle contraction.

- Creatinine (the creatine anhydride) is formed from creatine or creatine phosphate.
- About 2% of body creatine is converted to creatinine . The amount of creatinine excreted for each individual is nearly constant (1-2 g/ day) and it is related to muscle mass.
- **The normal serum creatinine level 0.6- 1.2 mg/dl.**
- Plasma creatinine level increases in cases of kidney diseases and it is a good index for renal functions as its level is not affected by diet.