BCH 447

Estimation Of Arginase Activity In Liver Extract

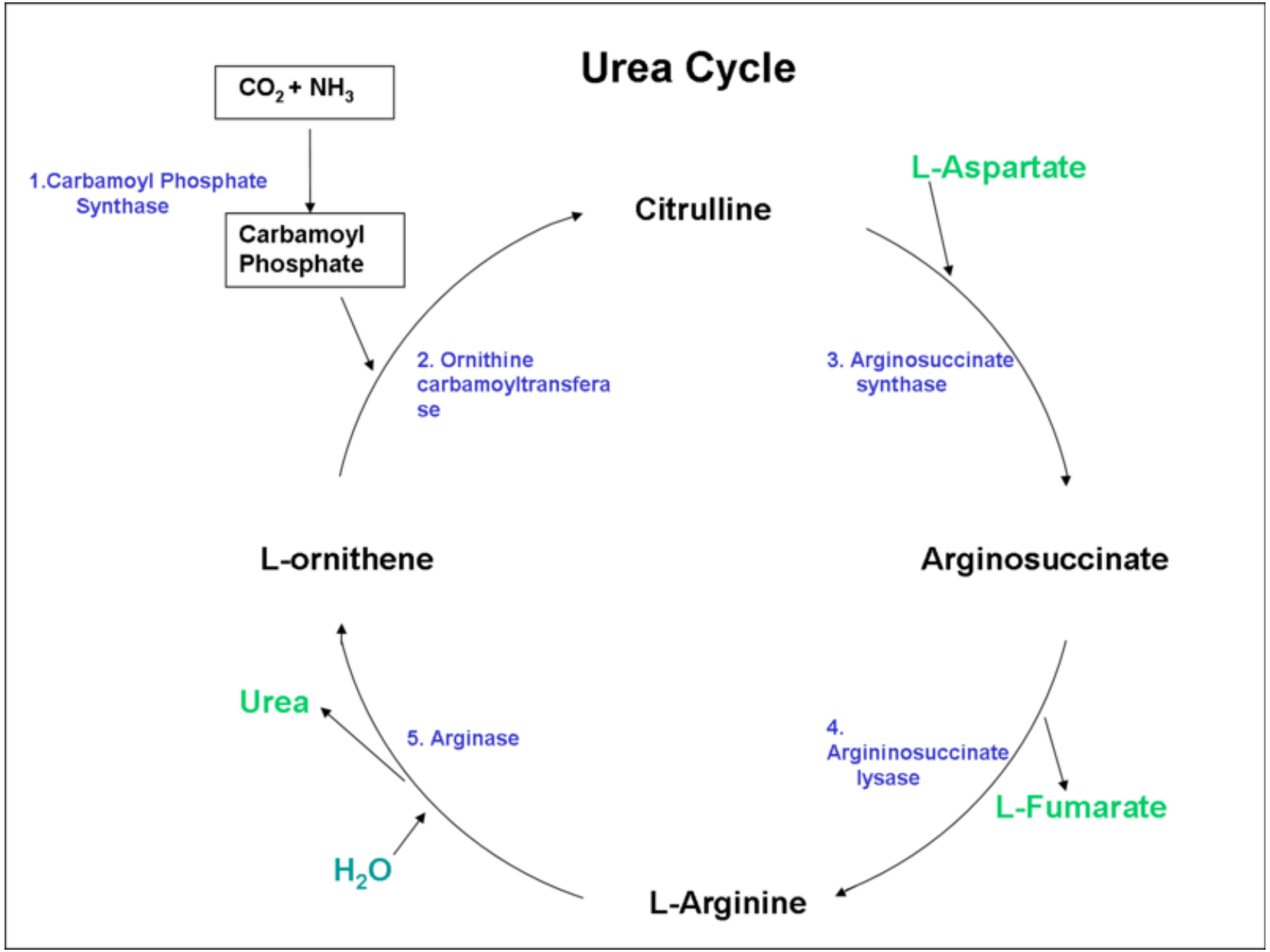
- Introduction:

- Ammonia is a product of oxidative deamination of amino acids.

- It is toxic in even small amount and it must be removed from the body.

- Arginase is one of the important enzymes in urea cycle which is the major disposal form of amino groups derived from amino acids.

- Urea cycle catalyzed by a set of enzymes (Five enzymes) present in the liver ,and then is transported in the blood to the kidneys for excretion.


Urea Cycle
$CO_2 + NH_3$
1.Carbamoyl Phosphate Synthase
Carbamoyl Phosphate
2. Ornithine carbamoyltransfera se
Citrulline
L-Aspartate
3. Arginosuccinate synthase
Arginosuccinate
4. Argininosuccinate lysase
L-Fumarate
L-Arginine
5. Arginase
Urea
H_2O
L-ornithene

- Principle:

-The arginase enzyme catalyzes **the fifth reaction** in the urea cycle, the enzyme is present **exclusively in the liver .**

-Arginase catalyzes the hydrolytic cleavage of the guanidine group of Arginine to regenerate ornithine and urea.

Arginine ↔ Urea + Ornithine

-Two isozymes of this Enzyme exist ,

-First ; Arginase I **(In cytoplasm)** for functions of urea cycle,

- Second; Arginase II to regulate the arginine/ornithine concentration in the cell **(In mitochondria).**

- Arginase requires a two-molecules metal of **Co^{2+} and Mn^{2+}** for it's activation while **ornithine and lysine** are potent inhibitors.

-The activity of the enzyme is determined by **measuring the amount of urea produced**, urea is reacted with the reagent iso-nitrosopropiophenone and heated in boiling water, leading to the production of a red color compound which is measured by spectrophotometry at 520nm.

Urea + iso-nitrosopropiophenone $\xrightarrow{\text{boiling water bath}}$ **red color compound**

- Question:

- What are the causes of high blood ammonia level?