DOCTOR 2020 | JU

METABOLISM

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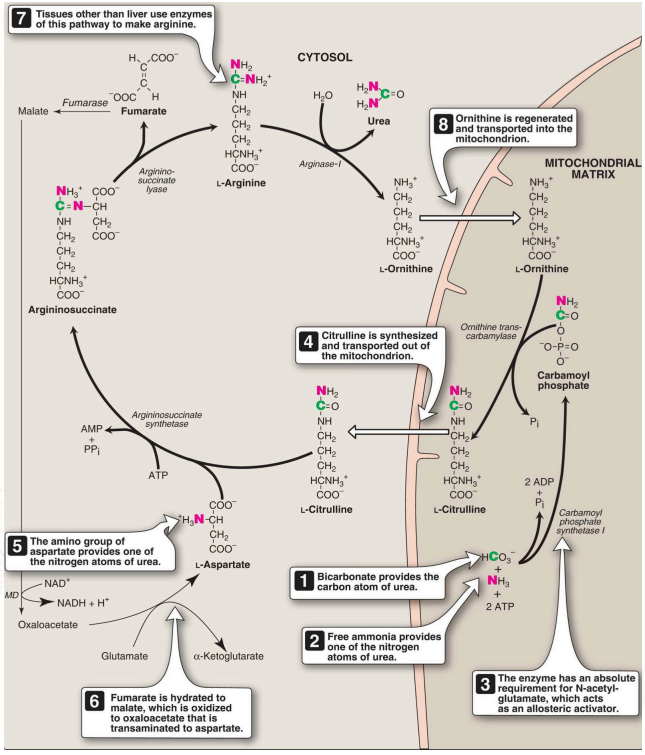
The toxic free ammonia molecule released from **oxidative deamination reaction** by **glutamate dehydrogenase** enzyme is transported from peripheral tissues through blood to hepatocytes by 2 mechanisms explained in the previous lecture. Now ammonia in hepatocytes and ready to be converted into urea by **UREA CYCLE** the main topic to be discussed in this lecture.

UREA CYCLE:



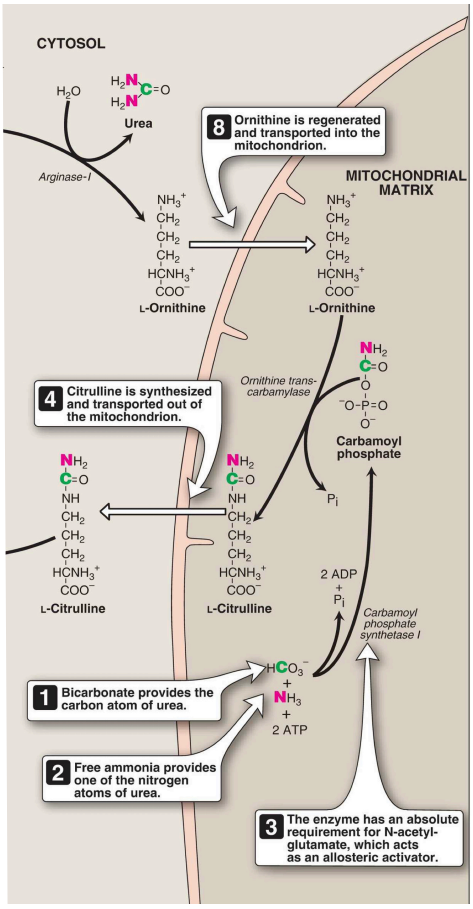
- As explained before the free ammonia produced from oxidative deamination of Glutamate which is converted into urea- less toxic molecule- in liver **(hepatocytes)**. Urea can be safely transported through blood to kidneys to be excreted in urine. Accordingly, urea is the major disposal form of amino groups derived from AAs (urea accounts for about **90%** of the nitrogen containing compounds of urine).

- Urea cycle and TCA cycle are similar in principle, both need a starting material →starting material interacts with the last intermediate in the cycle to initiate series of reactions. Also, none of the intermediates involved are produced or consumed unless these intermediates are used in other biochemical pathways. Urea cycle is different from TCA cycle that urea cycle consumes energy in the form of ATP to get rid of the toxic ammonia, occur **only** in **hepatocytes** and all steps of this cycle are irreversible**.** However, TCA cycle is efficient in producing energy, occur in all tissues and few steps of TCA are irreversible.

- Reactions of urea cycle occur in two places in hepatocytes: 1) cytosol. 2) mitochondria.

- Reactions of urea cycle occur in two phases:
  The first phase: building up intermediate from smaller ones.
  The second phase: breaking down intermediates into smaller ones.

Mitochondria:



- Ammonia doesn't enter the cycle in the form of NH_3+ so there is a preoperative step for ammonia in mitochondria→Ammonia interacts with CO_2 and 2 ATP producing **carbamoyl phosphate** (the starting material of urea cycle). this preoperative step is catalyzed by **carbamoyl phosphate synthetase I**.
  - Notice that: carbamoyl phosphate consists of carbonyl, phosphate, and amine group.

    Source of CO_2 is decarboxylation reactions of cellular respiration.

    2 ATP are used for 2 purposes: 1) as source of phosphate 2) to provide the reaction with energy.

    synthetase needs energy in the form of ATP.
- The first step of urea cycle and building phase start now. **L-ornithine** (last intermediate in the cycle) interacts with **carbamoyl phosphate** producing **L-citrulline**. This step is catalyzed by **ornithine trans-carbamoylase (OCT)** enzyme, releasing an inorganic phosphate Pi.
  - OCT enzyme is the most common urea cycle enzyme effected by genetic mutations.
    L-ornithine is non-coding AA.
- **L-citrulline** leaves mitochondria to cytosol.

Cytosol: observe picture below

- Building phase continues →**L-citrulline** interacts with **aspartate** producing **Argininosuccinate** the largest intermediate in urea cycle. This step is catalyzed by **arginiosuccinate synthetase** (ATP-dependent step) through transferring ATP to AMP and pyrophosphate.
  - Recall that Aspartate aminotransferase (AST) favors the backward reaction → synthesizing of aspartate rather than degrading it by transferring ammonia from glutamate to oxaloacetate forming aspartate.
- Second phase starts and breaking down is occur to **Argininosuccinate** by **Arginiosuccinate lyse** forming 2 molecules 1) fumarate which is a TCA cycle intermediate→ complete the reaction to malate then oxaloacetate after that AST

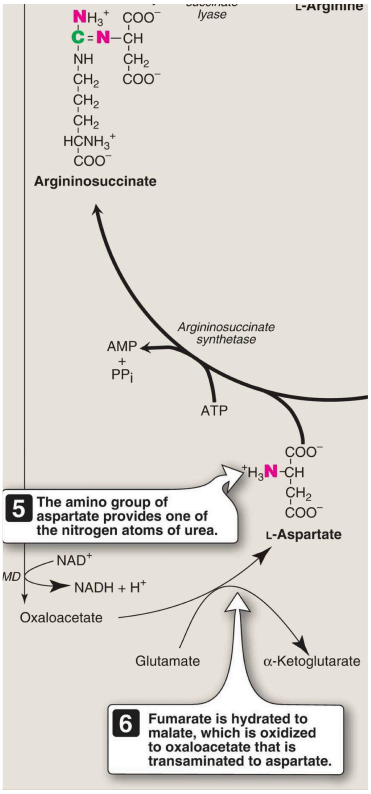
transfer ammino group from glutamate to oxaloacetate forming aspartate that supplies the previous step. 2) **L-Arginine** (AA with a fork like structure consist of 2 amine groups).

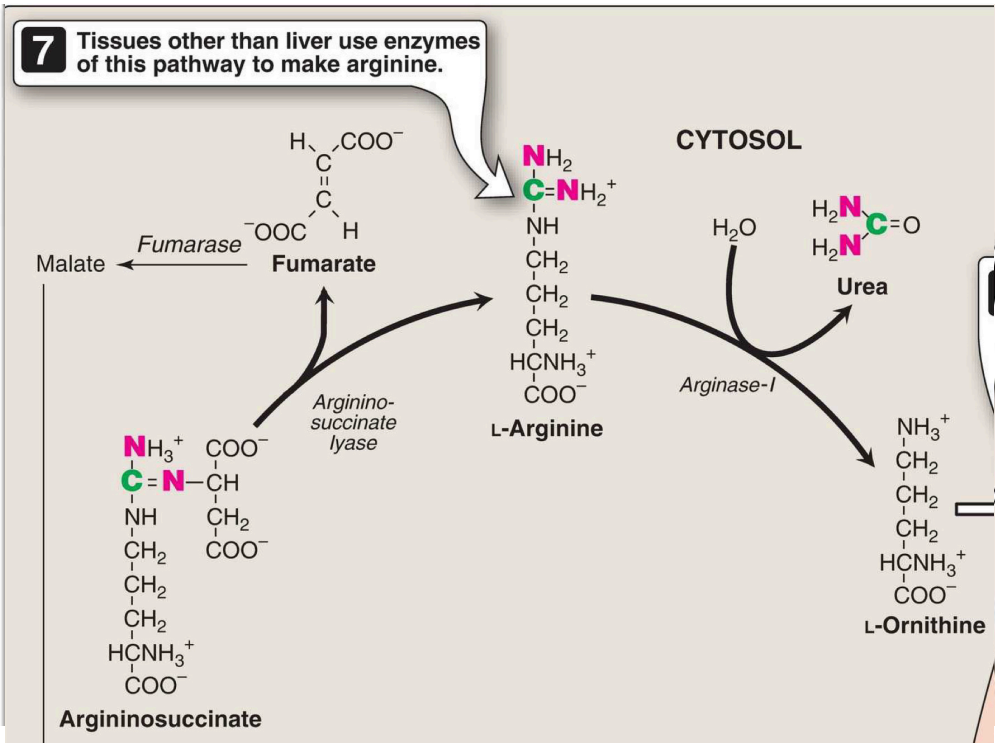
*Urea simply consisted of carbonyl attached to 2 ammino groups.

*Notice that the green carbon attached to 2 amine groups in arginine structure is going to be converted into urea, but urea has a carbonyl group.

- Breaking down phase ends and **L-Arginine** is degraded into **L-ornithine** and **Urea** as a side product. This step is catalyzed by **arginase** → adding water molecule forming the carbonyl group of urea.

free ammonia released from Oxidative deamination of glutamate and Aspartate produced from transamination of oxaloacetate by AST are the sources of nitrogen in UREA





Overall stoichiometry of the urea cycle:

The synthesis of urea is irreversible, with a large, negative ΔG for each urea molecule:

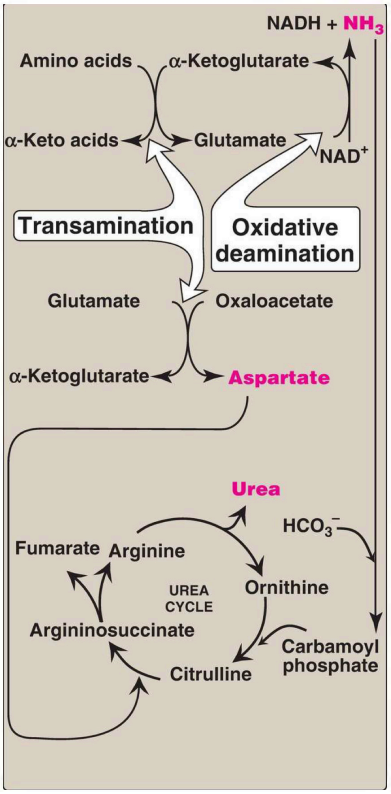
$$\text{Aspartate} + NH_3 + CO_2 + 3\,ATP + H_2O \longrightarrow$$
$$\text{urea} + \text{fumarate} + 2\,ADP + AMP + 2\,P_i + PP_i$$

1. Four high-energy P-bonds

2. One nitrogen of the urea molecule is supplied by free NH3
3. The other nitrogen is supplied by aspartate.

4. Glutamate is the precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (through transamination of oxaloacetate by AST).

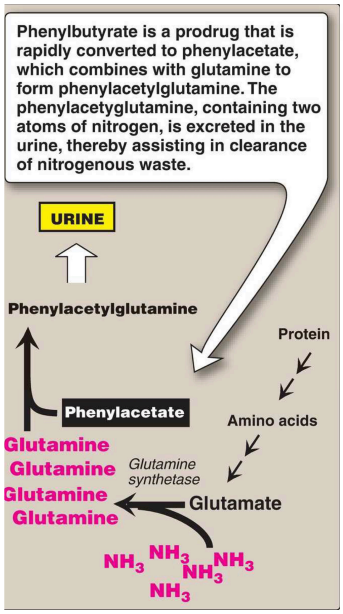
Regulation of urea cycle:



Urea cycle doesn't work permanently but it is stimulated and activated by several conditions.

- <u>Protein rich diet activates urea cycle</u>: more proteins mean more AAs, and since AAs can't be stored in large amounts, our bodies begin to use these AAs in different pathways. In well fed state our bodies use AAs in synthesizing proteins and other molecules. In starvation state our bodies start to degrade amino acid, even if our bodies in well fed state and get AAs beyond our needs the body is going to degrade them → more degradation means more AAs and more activation for urea cycle to get rid of nitrogen.
- <u>N-Acetylglutamate (modified glutamate) is an essential activator for carbamoyl phosphate synthetase I (The rate-limiting step in the urea cycle)</u>. The intrahepatic concentration of N-acetylglutamate increases after a protein-rich meal (more glutamate and arginine are provided leading to more oxidative deamination to glutamate and more ammonia molecules are released accordingly more activation for urea cycle).
- <u>Arginine is an activator for N-Acetylglutamate synthesis.</u>

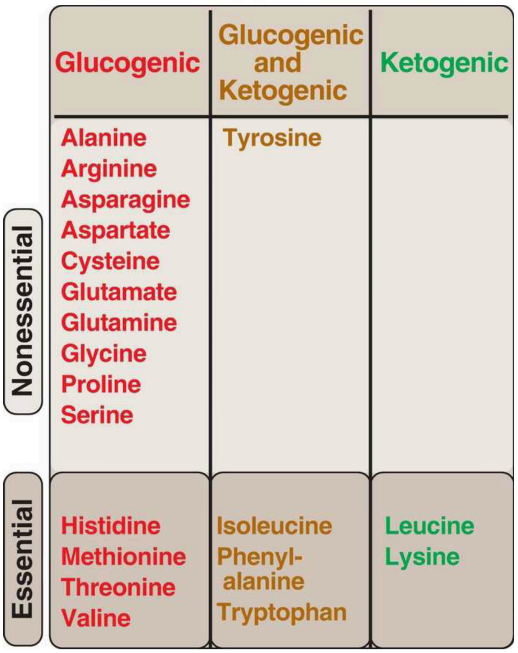
Clinical hint: Hyperammonemia.



- NH3 has a neurotoxic effect on the CNS (tremors, slurring of speech, somnolence, vomiting, cerebral edema, and blurring of vision). At high concentrations, ammonia can cause coma and death.
- Hyperammonemia means accumulation of ammonia due to disruptions in urea cycle.
- Types of hyperammonemia:
  Acquired hyperammonemia: Liver disease due to viral hepatitis, or to hepatotoxins such as alcohol→ malfunction in hepatocytes leads to accumulation of ammonia due to disruption in urea cycle.
  Congenital hyperammonemia: Genetic deficiencies of any of the five enzymes of the urea cycle leads to failure to synthesize urea.
- Treatment: restriction of dietary protein, as well as administration of compounds that bind covalently to AAs, producing nitrogen-containing molecules that are excreted in the urine.

هون موضوع جديد الدكتورة شرحت مقدمه عنه و حتكمله في المجاضرة القادمة

GLUCOGENIC AND KETOGENIC AMINO ACIDS:



- All previous pathways in AAs metabolism concentrate on the ammonia and how to get rid of it safely. The other parts of AAs like the carboxyl group and R chain are considered as a part of carbon skeleton, so this carbon skeleton is going to be degraded after the removal of ammonia.
- The diversity in the carbon skeleton is huge due to the diversity in the R chain. R chains are different from each other in functional groups and reactivity.
- To facilitate carbon skeleton degradation→ AAs are classified according to the Seven intermediates are produced during AA catabolism **(oxaloacetate, pyruvate, α-ketoglutarate, fumarate, succinyl coenzyme A (CoA), acetyl CoA, and acetoacetate).**

	Glucogenic	Ketogenic	Glucogenic & ketogenic
Intermediates Produced during AAs catabolism	catabolism yields pyruvate or one of the TCA cycle intermediates that can be used as substrates for gluconeogenesis in the liver and kidney.	catabolism yields either acetoacetate (a type of ketone bodies) or one of its precursors (acetyl CoA or acetoacetyl CoA).	Catabolism yields to either pyruvate/one of the TCA cycle intermediates or acetoacetate and its precursors (acetyl CoA or acetoacetyl CoA).
Essential AAs	هيهم بالصورة فوق 😉 الدكتورة ما قرأتهم-	Leucine and lysine	Isoleucine, phenylalanine, tryptophan.
Non-essential AAs	Same above	----------	Tyrosine.
Notes	Most AAs are glucogenic.	عشان تربطوهم التنين عندهم حرف L .	Phenylalanine tryptophan and tyrosine share in the benzene ring.

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