SHEET NO. 21

METABOLISM

DOCTOR 2019 | MEDICINE | JU

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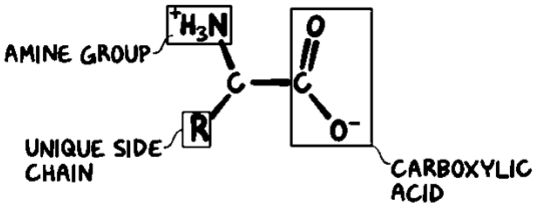
♥ Hello everyone! This sheet is for Dr. Nafeths' 2nd lecture. It was written based on Wednesday's lecture. The first 20 minutes have been discussed in the previous sheet.

In the previous lecture, we had an overview about amino acids, their Sources (diet, remodeling, or synthesized de novo), how proteins are broken down into amino acids (Lysosome or Ub-proteasome system), how are amino acids absorbed into cells. Make sure you understand all of them :)
NOW, we will talk about amino acids inside the cells…

Amino Acid Metabolism

Amino acids are the building blocks of proteins, each amino acid has a nitrogen-containing amino group, carboxylic acid, and unique side chain.



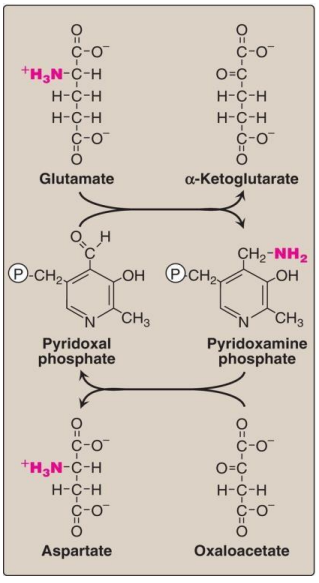
Whenever we talk about an amino acid, we look at it as two parts: nitrogen and the rest of the chain, due to the high importance of nitrogen in our bodies.
➔ Amino acids metabolism occurs in two-phases: (overview)
First: Getting rid of the nitrogen-containing amino group, and freeing up ammonia which is toxic to the cell, thus the peripheral tissues transfer it to the liver where it will be converted to form urea then excreted in the urine (urea cycle). To do that, cells introduce amino acids in **transamination reactions** (transferring ammonia) with keto acids such as; α-ketoglutarate, this results in generating intermediates like pyruvate and glutamate. Glutamate can then be **oxidatively deaminated**.

Second: Conversion of the carbon skeletons to common intermediates of energy-producing metabolic pathways

Now, let's dig deeper into the first phase of amino acid metabolism, NITROGEN REMOVAL FROM AMINO ACIDS, 4 steps: Transamination, Oxidative Deamination, Ammonia Transport to the Liver AND Urea Cycle.

1.Transamination

Transferring the amino group from an amino acid to a keto acid to produce another amino acid and another keto acid, catalyzed by **Aminotransferases (transaminases)** enzyme.
Transaminase enzyme needs the co-enzyme **pyridoxal phosphate (vitamin B6).**
We have a huge number of transaminases enzymes in the cytosol and mitochondria; to help in metabolism of specific amino acids like:
(1) non-essential amino acids which we can synthesis in our bodies.
(2) non-coding amino acids that don't participate in the forming of protein.



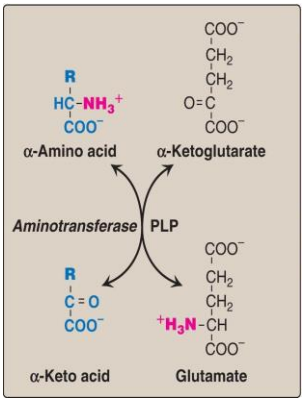
❖ Mechanism of the action:

Aminotransferases act by transferring the **amino group** of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino acid. In other words, removing the amino group from the amino acid and putting it into a keto acid with the help of pyridoxine to produce pyridoxamine phosphate co-enzyme, resulting in converting the amino acid to its corresponding keto acid which means generating an amino acid from another one. Given careful look at this figure

- Transamination reaction is reversible and under equilibrium

❖ The specificity of Aminotransferases:

The **specificity** of individual enzymes determines the specific amino acid that serves as the amino group donor.



ALMOST ALWAYS... In the active site of aminotransferases, transferring amino group from an amino acid to **α-ketoglutarate** (keto acid with 5 carbons) produces:

1) corresponding **keto acid** for the amino acid (**its structure and name depend on the original amino acid**)
2) corresponding **amino acid** for the keto acid (which is **Glutamate**).

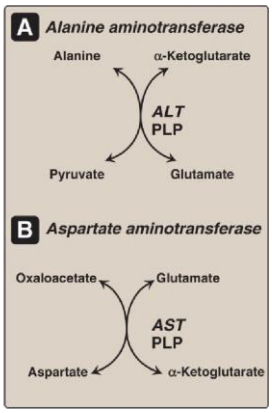
The idea here that all keto acids are converted to one type of amino acid which is glutamate**.** So that, our body deals with only one type of amino acid (glutamate) in the remaining steps of metabolism rather than dealing with several amino acids.

N.B. We said that **α-ketoglutarate** is mostly the accepter of the amino group but there is an exception, **oxaloacetate** can act as an acceptor of the amino group, being converted to aspartate.
Oxaloacetate→ Aspartate rather than α-ketoglutarate→ Glutamate

Important amino acids with their corresponding keto acids

	Amino acid	Corresponding Keto acid	# carbons
1.	Alanine	pyruvate	3C
2.	Aspartate	Oxaloacetate	4C
3.	Glutamate	α-ketoglutarate	5C

❖ In this figure:



A. Alanine aminotransferase (ALT) → transfer amino group from Alanine to α-ketoglutarate.

B. Aspartate aminotransferase (AST) → transfer amino group from Aspartate to α-ketoglutarate.

These two enzymes are important liver enzymes, through them, the liver activity can be checked. (We will discuss this soon)

Question you may ask...
Can all amino acids get transaminated? Of course, not
Explanation: We know that we have:
- Essential amino acids → we can't synthesize them in our body; thus, we get them from the diet
- Non-essential amino acids → can be synthesized in our bodies.

So, if it's possible (while it is not possible) that all amino acids undergo transamination then we can synthesize all amino acids in our bodies either essential or non- essential.

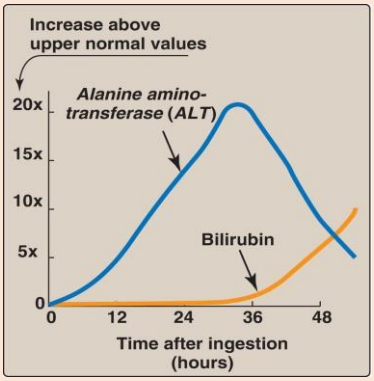
e.g., **Lysine** can't undergo transamination reaction.

Helpful video (overview)
https://drive.google.com/file/d/1RBhbhZjirhlvKQHttRhBDVIDP-bqvfwS/view?usp=sharing

Clinical Hint: Hepatic Disease

ALT & AST are liver enzymes concentrated intracellularly; they are used as indicators for liver disease.
Normally, there is a small concentration of ALT & AST in the **BLOOD**, that's because of the normal hepatic cell turnover → release their content to the blood, their conc. In healthy individual termed as normal concentration.
BUT, in the case of unusual break down of hepatic cells due to any hepatic abnormality (viral hepatitis B or C, Fibrosis, Cirrhosis, Fatty liver disease, Alcoholic liver disease or any Drug that intoxicate the liver), if this abnormality led to extensive cell necrosis, then leakage of hepatic cells contents to the blood will occur → ALT & AST concentration increases in the blood → abnormal concentration.
BY taking a **blood sample** you can know whether ALT & AST conc. Is normal or not, and detect if the patient is ill or well.

N.B.
We use these enzymes for early detection of liver disease; their elevation (by 20 folds) in blood can be detected before the appearance of other signs such as bilirubin.
As you can see in the figure, bilirubin will start to increase dramatically after 36 hours but, much earlier of that the concentration of ALT will get increased and can be examined to detect liver abnormalities.



What is the difference between ALT & AST?
ALT is more specific but AST is more sensitive, what does this mean?

- **ALT** exists in all body's tissues but it is much more **concentrated in the liver** compared to other tissues.
  → its elevation in the blood indicates hepatic disease so it's **specific to the liver**.
  ALT is the most specific enzyme to detect liver abnormalities.

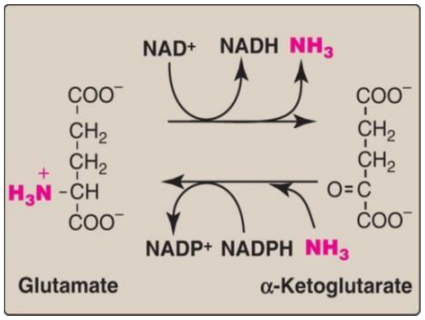
- **AST** exists in the liver, heart and other organs (present in higher conc. than ALT in the liver)
  → its elevation in the blood indicates either hepatic problem or heart problem such as heart attack so it's not specific to the liver but its conc. is higher in the liver, meaning that it is **more sensitive**. i.e., When your patient is determined to have a high level of AST, you cannot assert that he has a heart or kidney disorder, rather you have to diagnose your patient through the clinical signs.

To sum up..
Both ALT & AST are indicators for liver disorders, AST is detected first but it is not specific to the liver, ALT is detected later but is specific to the liver.

ALT & AST→ Definitive indication of hepatic abnormalities.
ALT → Definitive indication of hepatic abnormalities.
AST→ It is may indicate hepatic abnormalities or other abnormalities.

2.Oxidative Deamination

This is the second step in the nitrogen removal process from amino acids. First, we converted all amino acids to glutamate.
Now, glutamate will undergo an oxidative deamination reaction to free up the amino group into free ammonia in the solution and producing a keto acid.



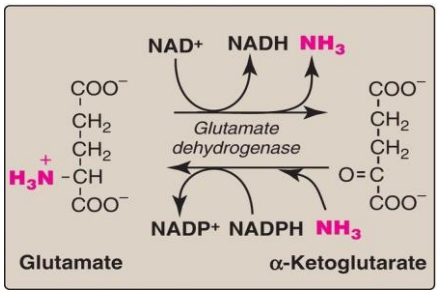
It's so smart that our body converts all amino acids to glutamate, it just needs one dehydrogenase enzyme which is **glutamate dehydrogenase** to do this oxidative reaction rather than having many dehydrogenase enzymes for every single amino acid.

❖ Mechanism of the action:

This step is reversible and under equilibrium, thus, the direction of the reaction depends on the concentration of:

- Glutamate /α-ketoglutarate ratio
- NADP+ / NADPH ratio
- NAD+ / NADH ratio
- Free ammonia

e.g., when we have high concentration of Glutamate→reaction goes into the forward direction (Oxidation) and when we have a high concentration of α-ketoglutarate→reaction goes into the backward direction (Reduction).

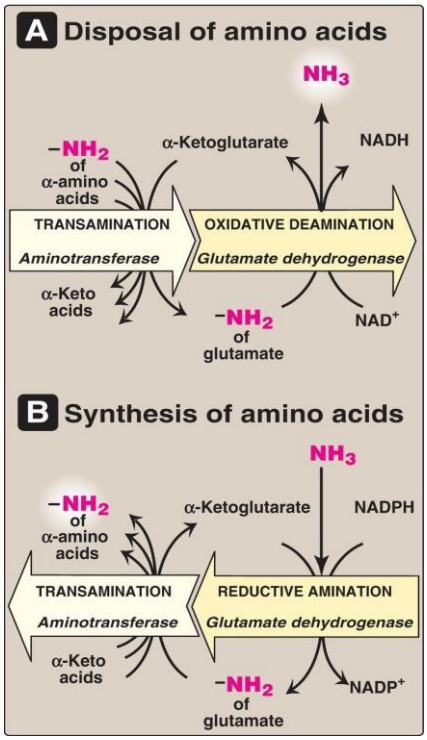


A. Oxidative deamination (forward direction) GLU → α-KG

When glutamates concentration is high it binds to the active site of glutamate dehydrogenase enzyme, it needs NAD+ as a co-enzyme to produce α-ketoglutarate, and energy in the form of NADH. It is an exogenous reaction. The amino group is freed up to the solution either in the form of ammonia (NH3) or ammonium ion (NH4+). NH3 can pass the cell membrane, NH4+ can't, because of the membrane's hydrophobicity.

B. Reductive amination (backward direction) α-KG → GLU

When α-ketoglutarate concentration is high it binds to the active site of glutamate dehydrogenase enzyme, it needs NADPH as an energy source and amino group from the solution to produce Glutamate and NADP+. It is an endogenous reaction.



Question you may ask ..
Why is **NADH** produced in the Oxidation reaction, while in the Reduction reaction **NADPH** is consumed?

- In Oxidation, glutamate (which has an amino group) will bind to the active site, and this binding takes more space in the active site, so, unphosphorylated, NAD+ will bind with it as a co-enzyme to produce NADH.
- In Reduction, α-ketoglutarate (which is slightly smaller since it doesn't have an amino group) will bind to the active site, and this binding takes less space, so, phosphorylated, NADPH is more suitable for providing energy.

❖ Glutamate dehydrogenase (GDH)

The enzyme that catalyzes both directions of the reaction; forward and backward. It takes its name from the forward reaction **(GLU → α-KG).**

Glutamate dehydrogenase allosteric regulators:

- **GTP** is an indication of high energy, so it inhibits Oxidative Deamination ↑ GTP>>↑ energy>>↓OD
- **ADP** indicates low energy so it activates Oxidative Deamination↑ ADP>>↓ energy>>↑OD

N.B. **NOT** glutamate is the only amino acid that can undergo this reaction, other amino acids can do too **BUT** glutamate is the most active one.

Notes you probably know:
ATP→ Energy production
GTP→ Protein synthesis
CTP→ Lipid biosynthesis
UTP→ carbohydrate metabolism.

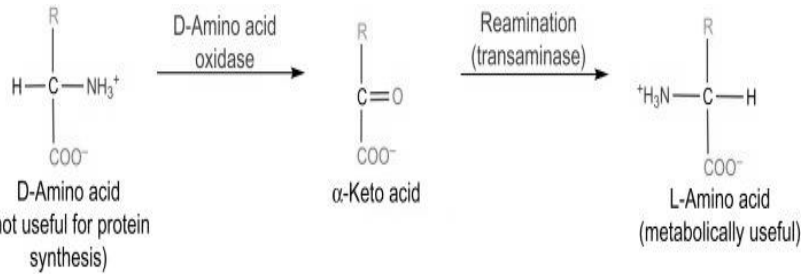
❖ D-Amino acid & L-Amino acid

Although the majority of amino acids that are in our body and we can synthesize proteins ONLY from them are L-Amino acids, **BUT** we have D-Amino acids in a **very low concentration** from the diet.

- L-Amino acids are oxidatively deaminated by **Glutamate dehydrogenase,** but D-Amino acids are not. So, what will happen to D-amino acids when we eat? They'll be converted to L-amino acids, How?

- **D-Amino acid oxidase (DAO):**
  - ✓ **An** enzyme found in the intestines.
  - ✓ catalyzes **Oxidative Deamination reaction** of **D-Amino acids**, which occurs in the peroxisomes of the liver and kidney cells.
  - ✓ FAD dependent, considered as an energy producing reaction (in the form of FADH2).
  - ✓ converts D-amino acids to α-keto acids, producing NH_3, and hydrogen peroxide.
  - ✓ The α-keto acids can go through the general pathways of amino acid metabolism and be reanimated to L-isomers or catabolized for energy.
  - ✓ DAO also converts glycine to glyoxylate.
  - ✓ DAO degrades D-serine (the isomeric form of serine) that modulates N-methyl-D-aspartate (NMDA)-type glutamate receptors.
  - ✓ Increased DAO activity has been linked to increased susceptibility to **schizophrenia** (a serious mental disorder in which people interpret reality abnormally).

- **L-Amino acid oxidase (LAO):**
  - ✓ **an** enzyme found in snake venom.
  - ✓ catalyzes **Oxidative Deamination reaction** to **L-Amino acid.**
  - ✓ converts L-amino acid to α-keto acids producing NH3, and hydrogen peroxide.
  - ✓ This reaction is very toxic, due to the high conc. of L-Amino acids in our body, which leads to high production of NH3 and hydrogen peroxide. That's why Snakebite is mostly fatal.



3. Ammonia Transport to the Liver

In the second step, we freed up the amino group in the form of free ammonia this step occurs in all peripheral tissues but primarily in the liver and kidney *check the note*. Ammonia is very toxic especially to the CNS, it might lead to death, so its concentration in the blood **must** remain very low.
how can we get rid of ammonia in the tissues?
Ammonia must be transferred to the liver to get converted into a less toxic molecule called **urea** then excreting it in urine. But it can't be transferred as free ammonia, thus, there are two processes in which ammonia is transported to the liver: glutamate-glutamine cycle and Glucose-alanine cycle.
Check the figure below

N.B.
-**All** peripheral tissues metabolize amino acids and produce ammonia (primarily the liver); because any cell can receive an amino acid and degrade it and produce ammonia (which will then be transferred to the liver forming urea) to synthesize proteins. Thus, all cells undergo transamination and oxidative deamination.
-Liver cannot metabolize Branched-chain amino acids,

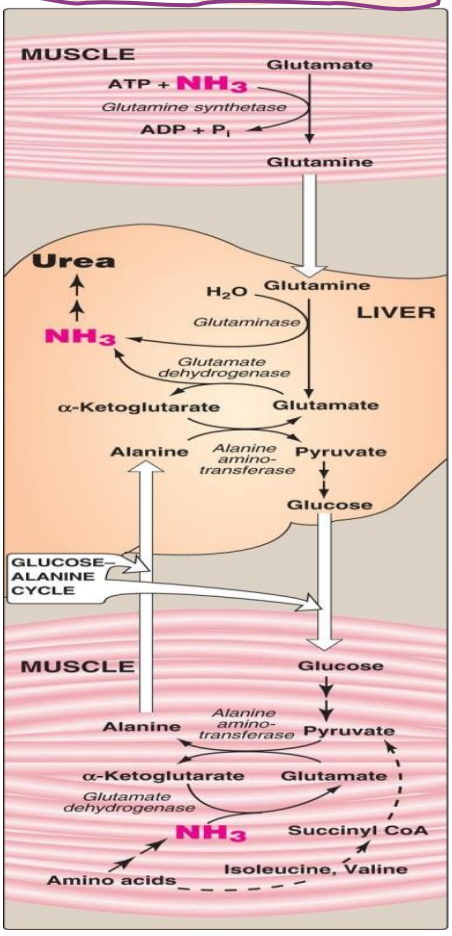
✓ Glutamate-Glutamine cycle

1. **In the tissues** (e.g., muscles) → Converting glutamate into glutamine by adding free ammonia to glutamate (Amidation reaction), this reaction is catalyzed by Glutamine synthetase and it needs ATP.
   Glutamine synthetase act like **Glutamate dehydrogenase**, they both fix free ammonia on a chemical structure. These are two of three special enzymes in our body that can fix ammonia.
2. **In the liver** → Converting glutamine into glutamate by removing the amino group, this reaction is catalyzed by glutaminase.
   Now, other than free ammonia in the liver, we have glutamate, it either undergoes Transamination or Oxidative Deamination.

✓ Glucose- alanine cycle

1. **In the tissues** (e.g., muscles) → two reactions separately occur
   (1) glucose is converted to pyruvate through glycolysis,
   (2) α-KG undergoes reductive amination and by fixing NH3 on it, glutamate is produced. Glutamate then participates in the transamination reaction to produce Alanine from pyruvate. Alanine can drive Safely in the circulation until it reaches the liver.
2. **In the liver**→ alanine undergoes transamination being converted to pyruvate, and producing glutamate. Glutamate then is oxidatively deaminated producing free ammonia.

In the liver now, we have a pool of free ammonia from both cycles. Ammonia must be converted into urea through the urea cycle.

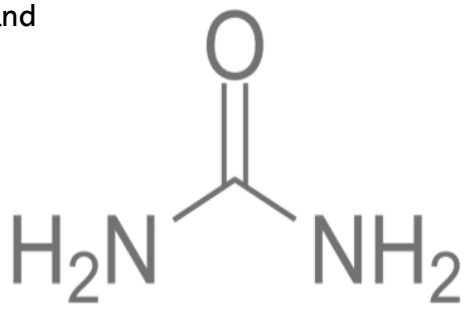


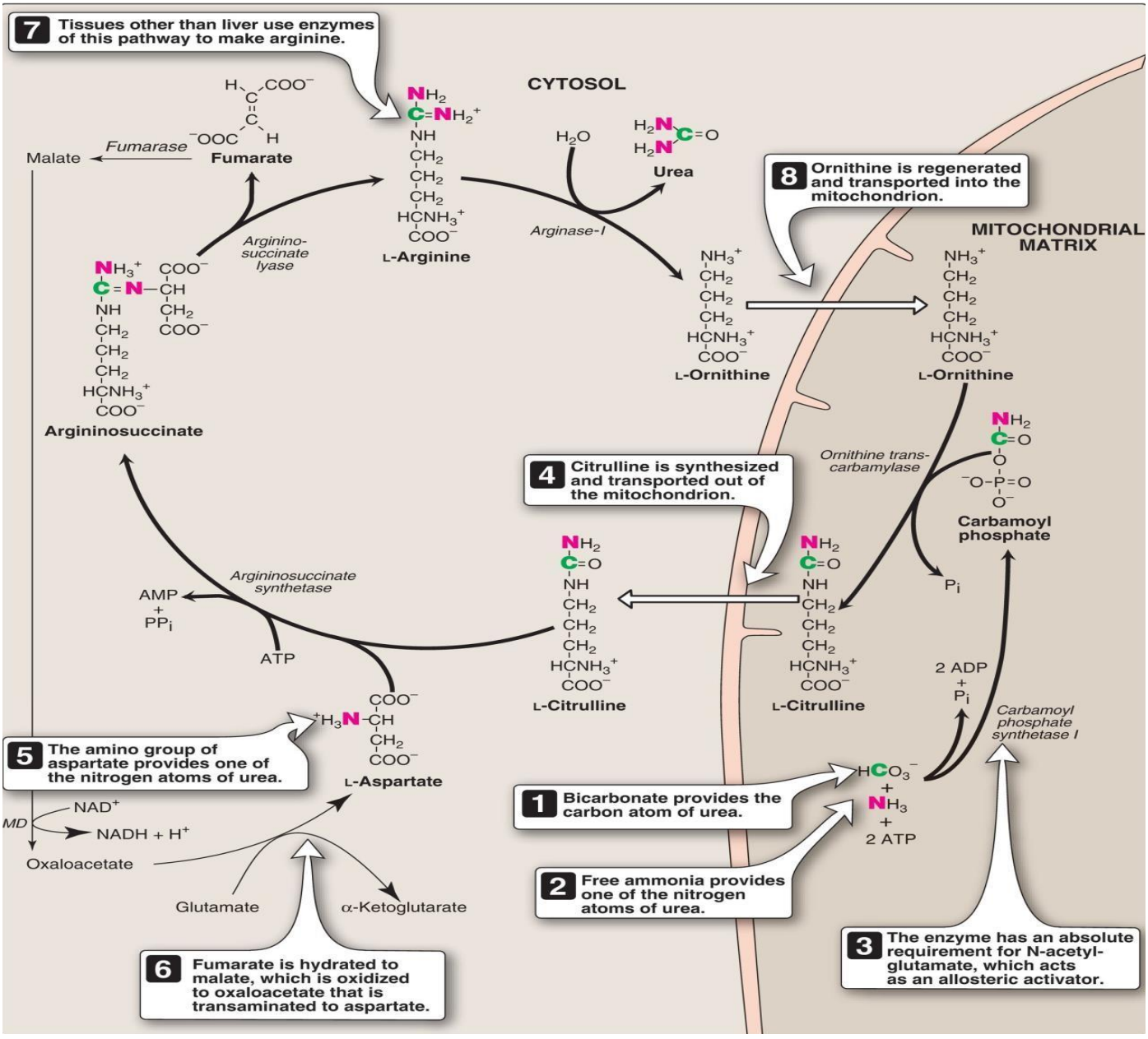
The whole story until now:
absorption of huge number of amino acids that reached the cells → transamination and funneling them into glutamate → glutamate is oxidatively deaminated to produce free ammonia → loading this free ammonia either on pyruvate or on glutamate producing alanine or glutamine, respectively → transferring alanine and glutamine into the circulation reaching the liver, once they are in the liver the process is reversed through GDH producing free ammonia again.

Enzymes which can fix free ammonia into a chemical structure: (3) Rare enzymes in our bodies
- Glutamine synthetase
- Glutamate dehydrogenase
- Carbamoyl phosphate synthetase I (in urea cycle)

4.UREA CYCLE

- It's a series of enzymatic reactions that convert ammonia to urea.
- In the third step, ammonia reached the liver from all body tissues through two cycles. Now ammonia will go through the urea cycle to be converted into urea, which is a simple molecule composed of a carbonyl group and two amino groups; i.e., two nitrogen atoms; since urea is responsible of nitrogen excretion outside the body. So The **Structure Fits Function.**
- The first nitrogen in the structure of urea comes from glutamate and the second one comes from Aspartate.
- Carbon and oxygen from bicarbonate which is soluble CO2.
- The source of CO2 is the Krebs cycle (in mitochondria).
- **The first two reactions occur in the mitochondria, the last three in the cytosol.**



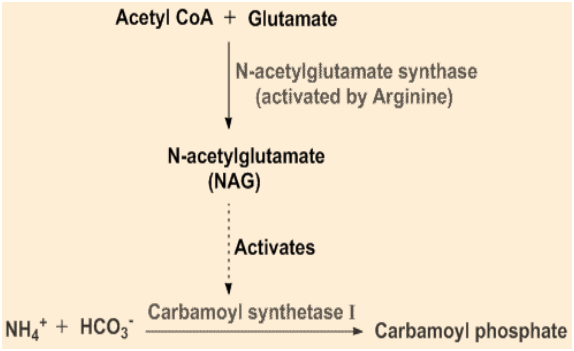


Helpful video (urea cycle)

https://drive.google.com/file/d/1MxMKZzoMXWh59J0xblkRL_zSI_51Fluv/view?usp=sharing

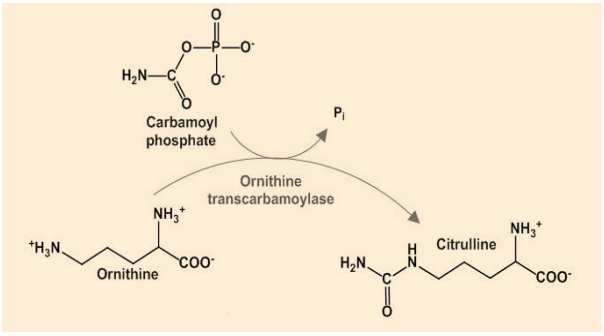
❖ Mechanism of the action:

1. Carbamoyl phosphate formation



- Free ammonia from the oxidative deamination fuse with bicarbonate (containing CO2) from the Krebs cycle and with a phosphate group, producing a simple structure called **carbamoyl phosphate**.
- It's a very energy-requiring reaction, needs 2ATP molecules.
- Rate-limiting step (NAG).
- **Carbamoyl phosphate synthetase I (CPS I) is the** enzyme that catalyzes this step, it is the third enzyme which can fix free ammonia into a chemical structure. ^^
- carbamoyl phosphate consists of CO2, NH3, and phosphate group from ATP.
- CPS I requires N-acetyl glutamate (NAG) as a positive allosteric activator.
- NAG is synthesized from glutamate and acetyl CoA group, bound to the nitrogen, by the enzyme N-acetyl glutamate synthase (NAGS) (which gets activated by arginine).
- Regarding NAGS, the substrate is an amino acid (glutamate) and the activator is also an amino acid (arginine) so, this enzyme gets activated after a protein-rich meal.

2. Citrulline formation

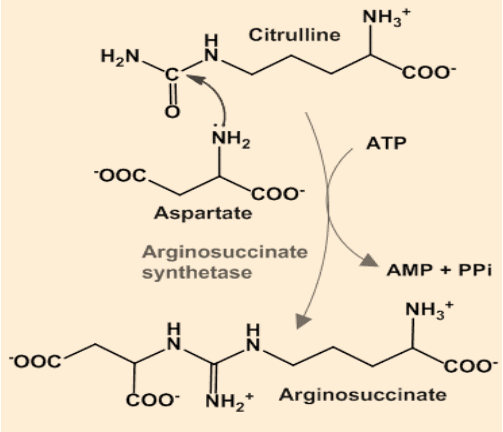


- In this step, **carbamoyl phosphate** (CO2, NH3, Pi) will bind with **Ornithine** to give **Citrulline** and release the phosphate group (which provides energy for the reaction to occur) through hydrolytic reaction.
- **Ornithine** is a dibasic amino acid; i.e., has a carboxyl group and an amino group & non-translated amino acid.
- **Citrulline** is also dibasic amino acid.
- The enzyme **Ornithine transcarbamoylase (OTC)** catalyzes this reaction.
- **Citrulline** consists of CO2, NH3, and Ornithine.

Remember! the previous two reactions occur in mitochondria, now Citrulline should get out to the cytosol; this happens through what is called an: **Antiporter for Ornithine and Citrulline** in the mitochondrial membrane. For every Citrulline that leaving the mitochondria, an Ornithine enters in exchange.

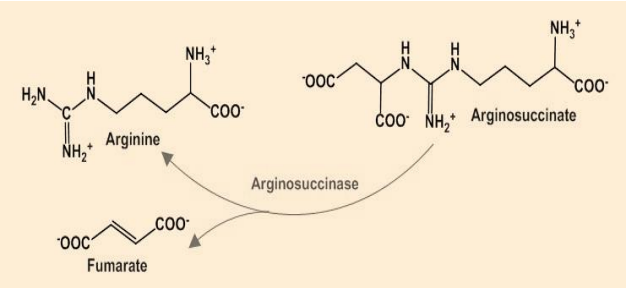
Before we move on, make sure that you understand the concept of the cycle; the beginning molecule should be regenerated again, the beginning molecule here is **Ornithine** chick the whole figure above so, Ornithine is continuously regenerated outside the mitochondria (at the end of the cycle) and consumed inside it (at the beginning of the cycle, when binding to carbamoyl phosphate).

3. Argininosuccinate formation and cleavage



- Free **Citrulline** in the cytosol will bind with **Aspartate** to produce **Arginosuccinate**.
- **Arginosuccinate Synthetase** enzyme catalyzes this step.
- Aspartates amino group will attach to the carbonyl group on Citrulline.
- A huge amount of energy is needed in the form of ATP → it will be converted into AMP and pyrophosphate; which will get hydrolyzed.
- Aspartate is 4 carbon units, which will be oxidized.

- Arginosuccinate (which is formed from binding Citrulline and Aspartate) will be broken again into **Arginine** and **Fumarate**.
- Fumarate is simply Aspartate that lost its amino group.
- **Arginosuccinase enzyme** catalyzes this step.

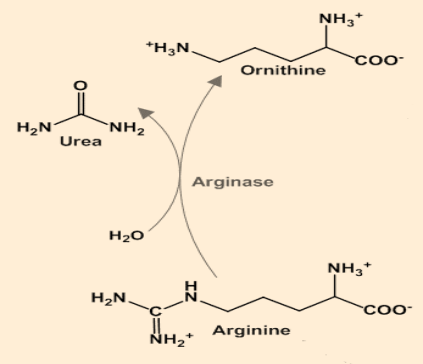


Fumarate is converted into malate (in the cytosol) so, it can enter the mitochondria through (malate-aspartate shuttle) where it acts as an intermediate of energy-producing metabolic pathways, producing energy in the form of NADH, thus, compensating some of the lost energy in the urea cycle.

Glutamate is the indirect source of nitrogen in urea molecules:
1st nitrogen from free ammonia excreted from amino acids, **indirectly from glutamate**.
2nd nitrogen from Aspartate, which was formed through transamination between OAA and glutamate which provides the amino group **also indirectly from glutamate**.

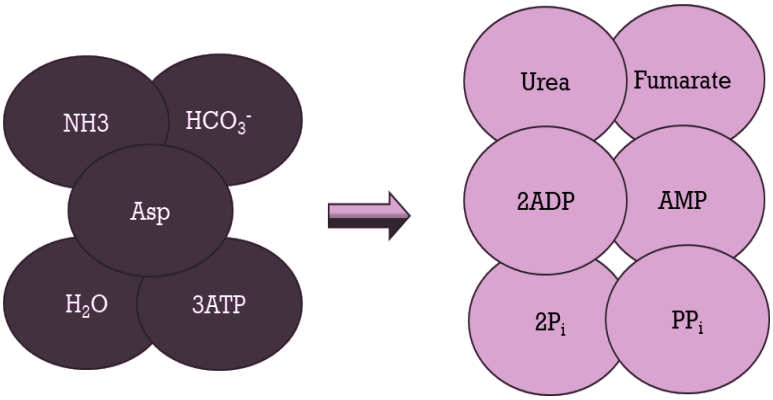
4. Arginine cleavage and Fate of urea

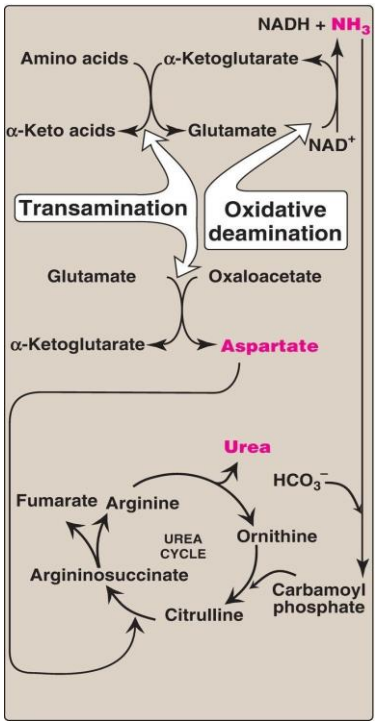
- In the last step, we will break Arginine into **Urea** and **Ornithine** by Arginase enzyme.
- Arginase enzyme has many isomers present in all body tissues in small amount for their own function, but **Arginase I** which catalyzes this reaction is Liver-specific; that's why urea cycle occurs only in liver cells.



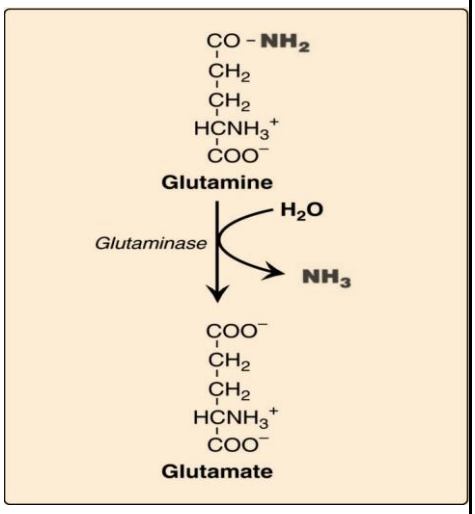
❖ Stoichiometry of Urea cycle

- Synthesis of urea is irreversible
- Free ammonia and aspartate
- Glutamate is the immediate precursor of both ammonia in urea.





❖ AMMONIA METABOLISM



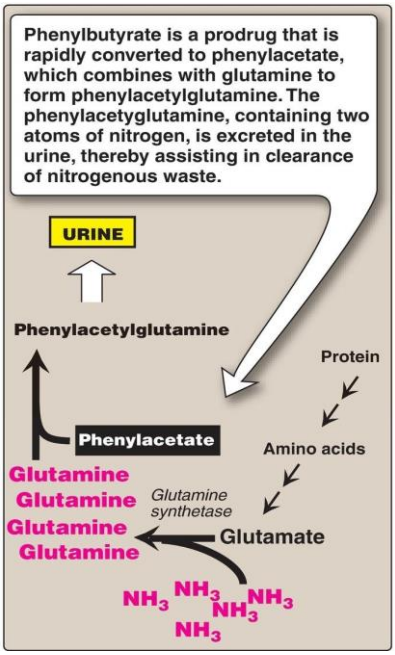
- Ammonia is produced by all tissues, either from branched-chain amino acids metabolism in the muscles or by amino acids metabolism in the liver.
- Disposed of, primarily by: the formation of urea.
- The level of ammonia in the blood must be kept very low; it is very toxic (hyperammonemia causes CNS diseases)
- Sources of ammonia:
  - AAs (liver, SCAA)
  - Glutamine (BCAA); Acid-base balance (kidneys); urea (liver)
  - intestinal glutaminase; An enzyme present in the intestine that converts glutamine into glutamate, and releases amino groups in the form of ammonia. (gluconeogenesis)
  - Intestinal bacteria: bacterial urease; this enzyme breaks down urea back into free ammonia It is found in bacteria, not in humans.
  - Amines: diet and hormones or (NTs); MAO (monoamine oxidase)
  - Purines and pyrimidines

❖ Hyperammonemia

- A metabolic disturbance characterized by an excess of ammonia in the blood.
- Normal Levels of ammonia in the blood is low than (5–35µmol/l)
- Can be >1,000 µmol/l; it is a medical emergency (tremors, slurring of speech, somnolence (drowsiness), vomiting, cerebral edema, and blurring of vision) that may lead to coma and death
- Problems with the urea cycle can be either Acquired, ex: liver disease, or Congenital, ex: Deficiency in one of the enzymes.
- Congenital Overall incidence of ~1:25,000 live births e.g.

- **OTC deficiency (the most common)**
  OTC enzyme catalyze the 2nd step in the urea cycle (carbamoyl phosphate+ Ornithine→Citrulline), it's X-linked; affects males more than females. All of the other urea cycle disorders follow an autosomal-recessive inheritance pattern

- **Arginase deficiency (less severe)**
  Arginase I enzyme catalyzes the last step in the urea cycle (breaking Arginine into Urea and Ornithine). Arginase deficiency is an inherited disorder that causes **Argininemia**, which is less severe than other disorders; why? Because Arginine (just like urea) contains 2 nitrogen atoms and could be excreted in the urine and Reduces excess nitrogen in the body.



❖ Treatment

To treat these deficiencies, we can give the patient **Phenylbutyrate** which turns into **Phenylacetate** in the body, **Phenylacetate** bind to **glutamine** forming **Phenylacetylglutamine** which contains 2 nitrogen atoms and can be excreted in the urine.

Phenylbutyrate → Phenylacetate
Phenylacetate + glutamine → Phenylacetylglutamine (excreted in the urine)

Whenever you feel sad just remember that there are billions of cells in your body and all they care about you :)

HAVE FUN ♥