Metabolism

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NOTE:

- After long fasted state or starvation , the body starts synthesizing glucose by Gluconeogenesis using these amino acids . These amino acids could be excess free amino acids(not stored) or from the degradation of proteins which is obvious people who are in hunger strike where their muscle mass decreases dramatically.
- The degradation of amino acids produces ammonia which is a toxic compound, which is the focus of amino acids degradation.

NOTE:

- Firstly , ammonia is transferred From any amino acids to common recipient which is alpha ketoglutarate (Krebs cycle intermediate) Which then becomes glutamate
  - alpha ketoglutarate is the alpha ketoacid of glutamate

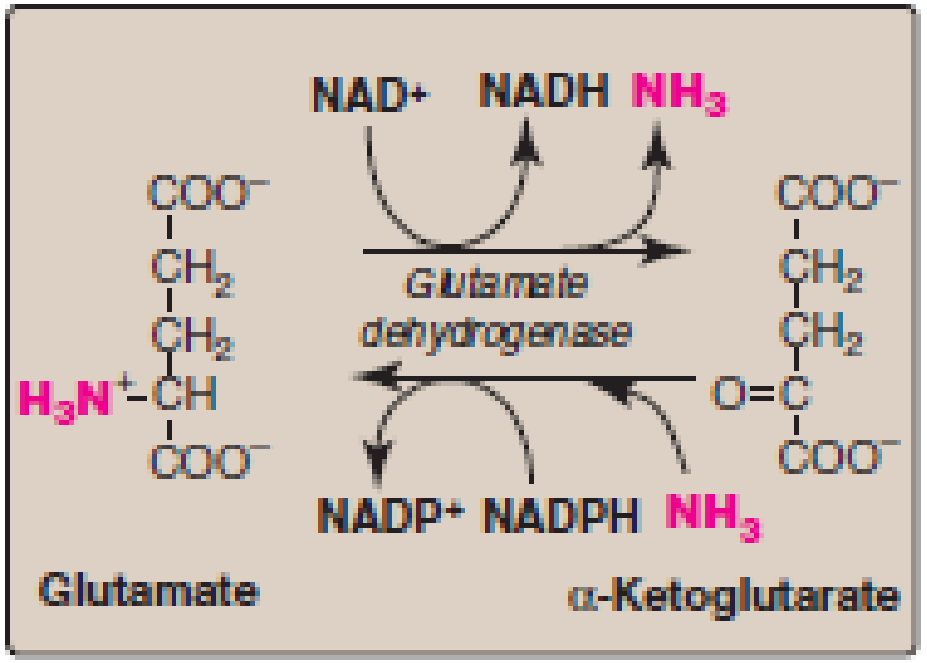
- The second step is oxidative deamination.
  - All amino acids are converted into glutamate because Glutamate is the only AA that undergoes rapid oxidative deamination (oxidation+deamination).

Oxidative deamination of amino acids

Oxidative deamination by glutamate dehydrogenase results in the liberation of the amino group as **free ammonia (NH3)**

Glutamate is the only amino acid that undergoes rapid oxidative deamination

Reactions occur primarily in the liver and kidney.



Allosteric regulators of glutamate dehydrogenase:

GTP is an allosteric inhibitor
ADP is an activator.

Reaction products:

1. α-keto acids that can enter the central pathway of energy metabolism
2. ammonia, the source of nitrogen in urea synthesis.

The complement in this slide:

- transamination VS oxidative deamination:
  Transamination: transferring amino group from AA to a recipient (α-ketoglutarate).
  Oxidative deamination: removing amino group from glutamate and releasing it as a free ammonia (no recipient).

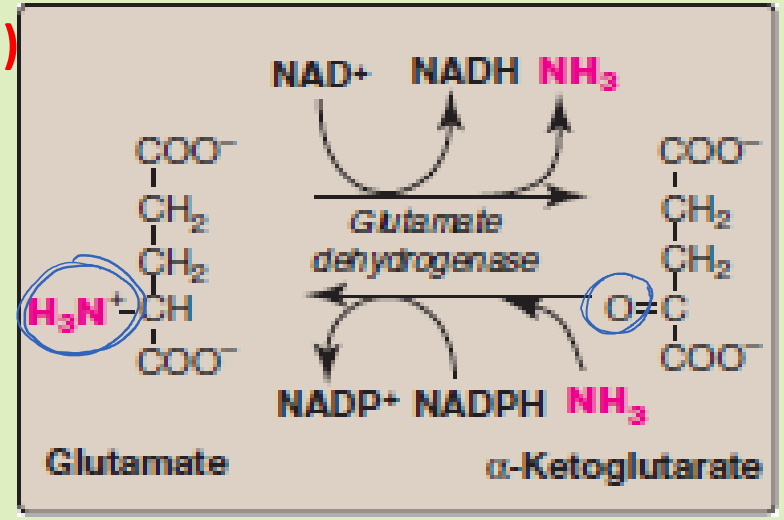
The complement in this slide:

Glutamate Dehydrogenase:

*Catalyzes the forward and backward reactions but with different co-factors:

▪forward Oxidative deamination reaction : (Glutamate → α-ketoglutarate)
Using NAD+ as a coenzyme which is reduced to NADH,
and glutamate is oxidized by losing and NH3 is removed.

▪backward reductive amination reaction : (α-ketoglutarate→Glutamate)
Using NADPH as a coenzyme which is oxidized to NADP+,
And α-ketoglutarate is reduced by gaining H and NH3 is added.



*Allosteric regulators of glutamate dehydrogenase :
Inhibitor : GTP indicates high energy state, so there is no need to degrade AA.
Activator : ADP indicates low energy state, so there is a need to degrade AA.
_AA is not the first source of energy

The pathways that discussed before is applied for L- amino acids which normally presented in our bodies

D-Amino acid oxidase

D-Amino acids are found in plants and in the cell walls of microorganisms

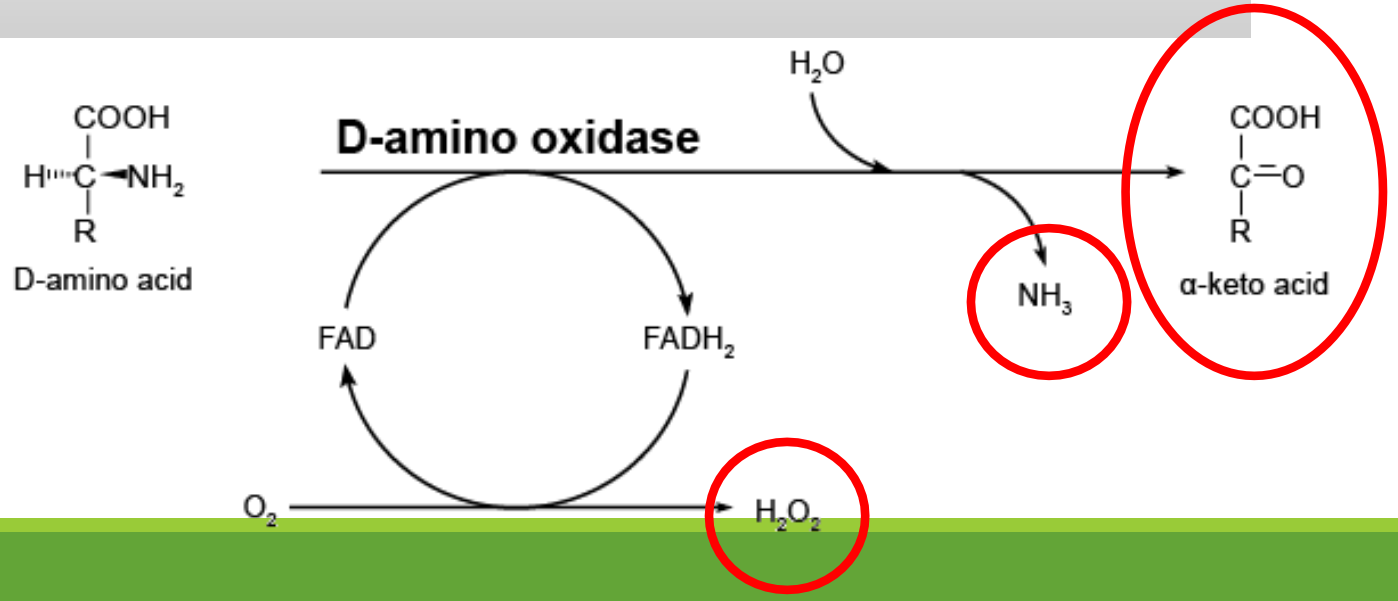
No D-amino acids in mammalian proteins

D-Amino acid metabolism by the kidney and liver.

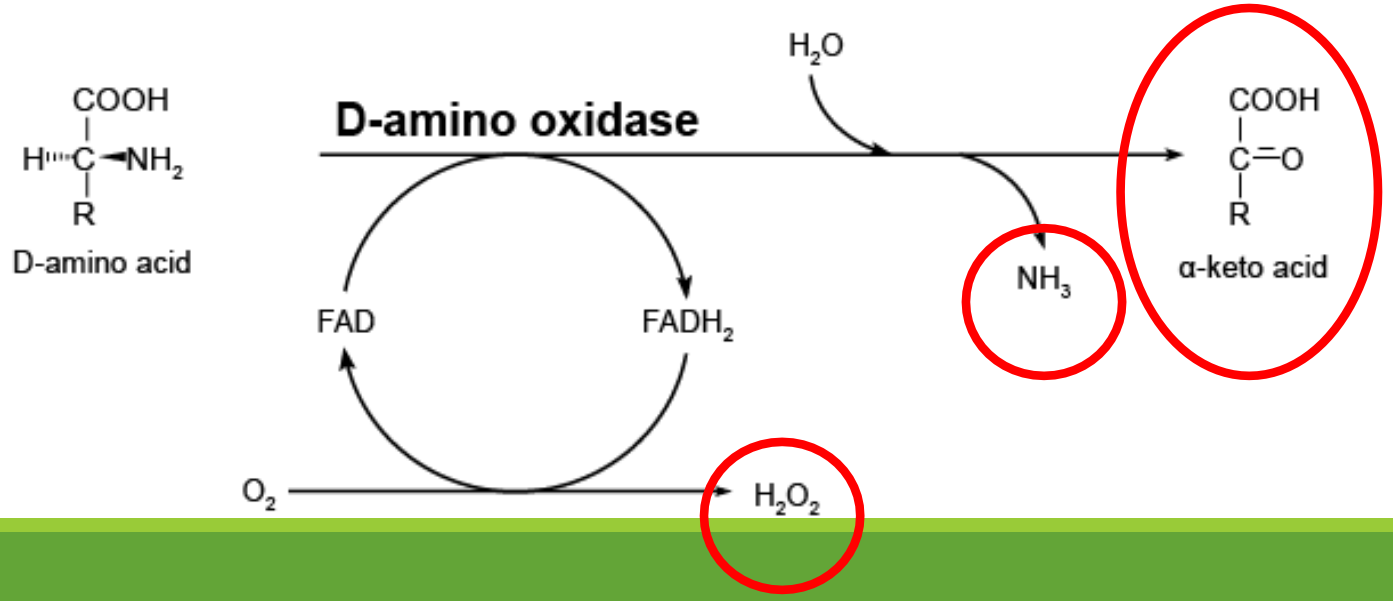
D-Amino acid oxidase (DAO) is an FAD-dependent peroxisomal enzyme that catalyzes the oxidative deamination of D-amino acids

Increased **DAO** activity has been linked to increased susceptibility to **schizophrenia**.

L-amino acid oxidases are components of several **snake venoms**.



- **The complement in this slide:**
- **D-amino oxidase (DAO) catalyzes the oxidative deamination of D-amino acids without involving a transamination step. The enzyme uses FAD as a cofactor, which is reduced to FADH2 during the reaction. This process generates hydrogen peroxide (H2O2), which is a reactive oxygen species (ROS), from molecular oxygen (O2).**



Metabolism of ammonia

- **NOTE:** Now let's move on and see what will happen to ammonia that was released in oxidative deamination .

Sources of ammonia

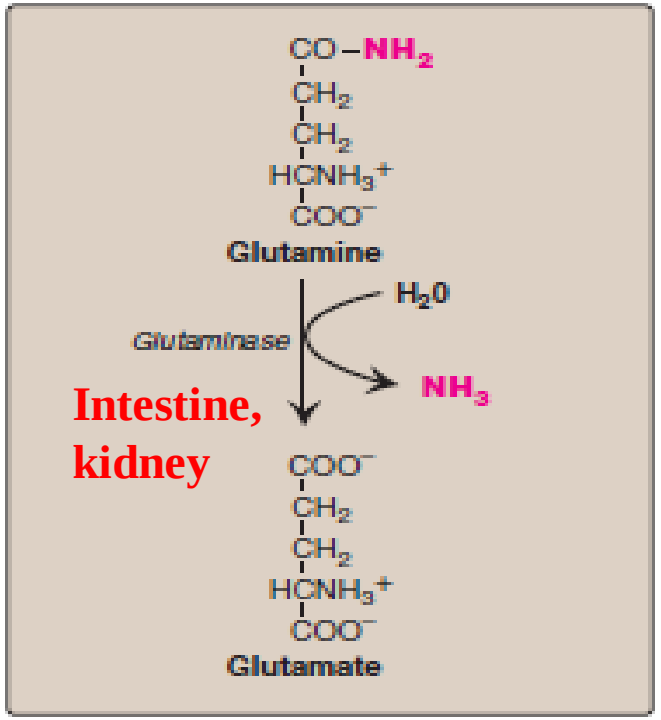
1. **From glutamine:** Most of this ammonia is excreted into the urine as NH_4^+ (acid –base balance

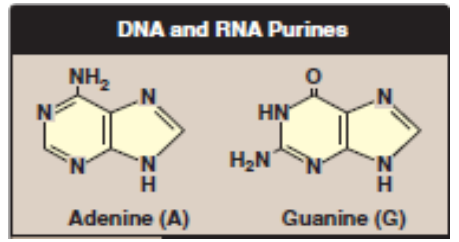
2. **From bacterial action in the intestine:** Ammonia is formedFrom urea in the intestinal lumen by the bacterial urease.

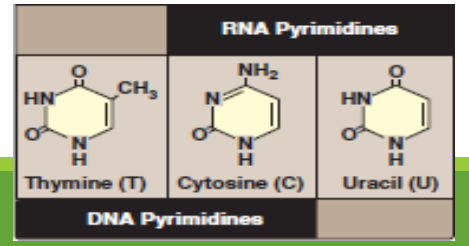
This NH3 is absorbed from the intestine by the portal vein and is converted to urea by the liver.

3.From amines: Amines in the diet, and monoamines that act as hormones or neurotransmitters, give rise to NH3 by **amine oxidase**

4.From purines and pyrimidines: In the catabolism of purines and pyrimidines, amino groups attached to the rings are released as NH3

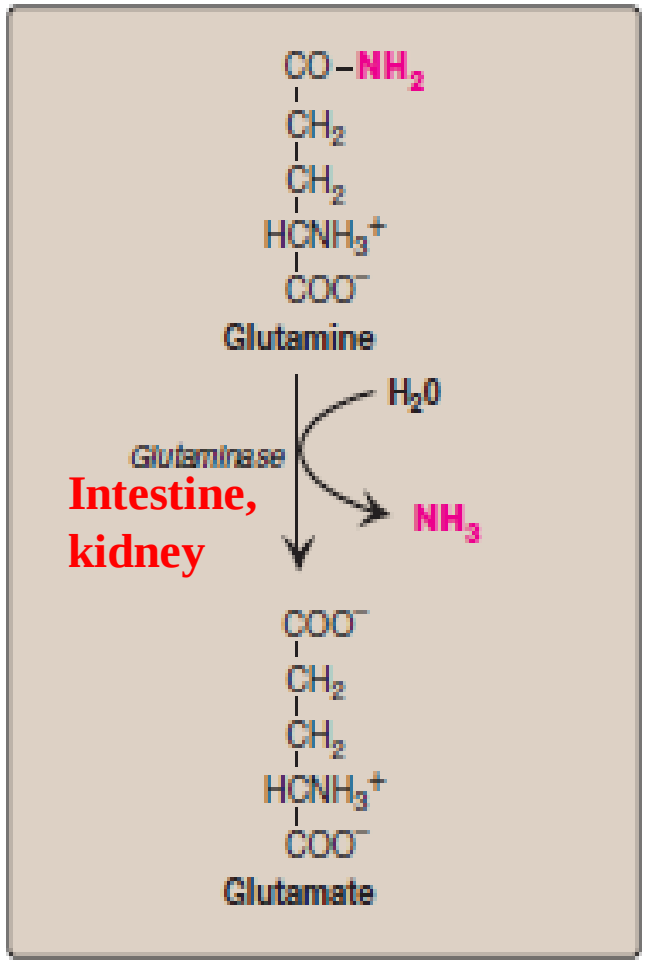






Sources of ammonia

1. **From glutamine (amino acid):** Most of this ammonia is excreted into the urine as NH_4^+ (acid –base balance)

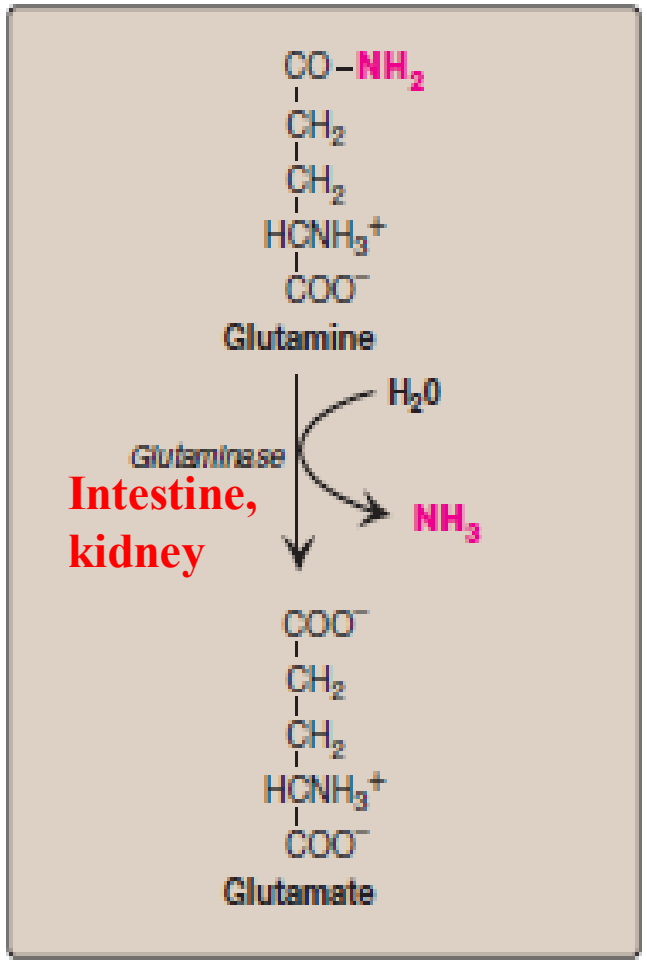


- **NOTE:** why glutamine not glutamate??Glutamine is the preferred transport form in the bloodstream of ammonia because it is less toxic. Ammonia is first produced through oxidative deamination in different cell types. Subsequently, this ammonia is combined with glutamate to form glutamine by glutamine synthase . Glutamine serves as a safe carrier for transporting ammonia to the kidneys, where it can be readily excreted in urine as NH4+ to help maintain acid-base balance. And it could go to hepatocytes where ammonia could be released again and converted to less toxic compound urea.

Sources of ammonia

2. From bacterial action in the intestine(normal flora): Ammonia is formedFrom urea in the intestinal lumen by the bacterial urease. This NH3 is absorbed from the intestine by the portal vein and is converted to urea by the liver.

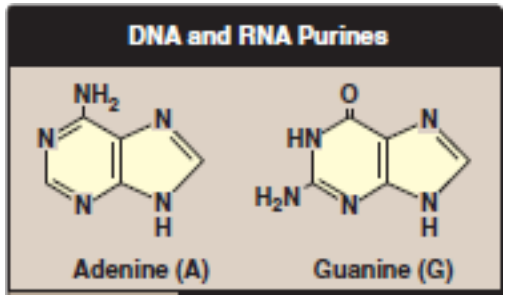
- **NOTE:** Ammonia can be formed from urea through the action of bacterial urease in the intestinal lumen. This process involves the degradation of urea to ammonia, which can then be absorbed into the bloodstream from the intestines.



Sources of ammonia

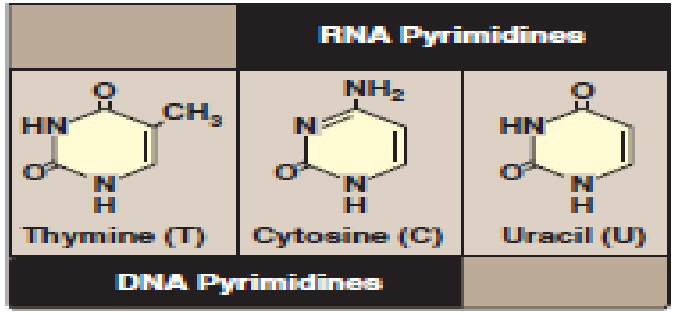
3.From amines: Amines in the diet, and monoamines that act as hormones or neurotransmitters, give rise to NH3 by **amine oxidase**

- **NOTE:**. Different compounds in our bodies (hormones or neurotransmitters) when they are metabolised, they Release NH3 By amino oxidase



4.From purines and pyrimidines: In the catabolism of purines and pyrimidines, amino groups attached to the rings are released as NH3

- **NOTE: We will discuss later on the metabolism of nitrogenous bases where** NH3 is released .



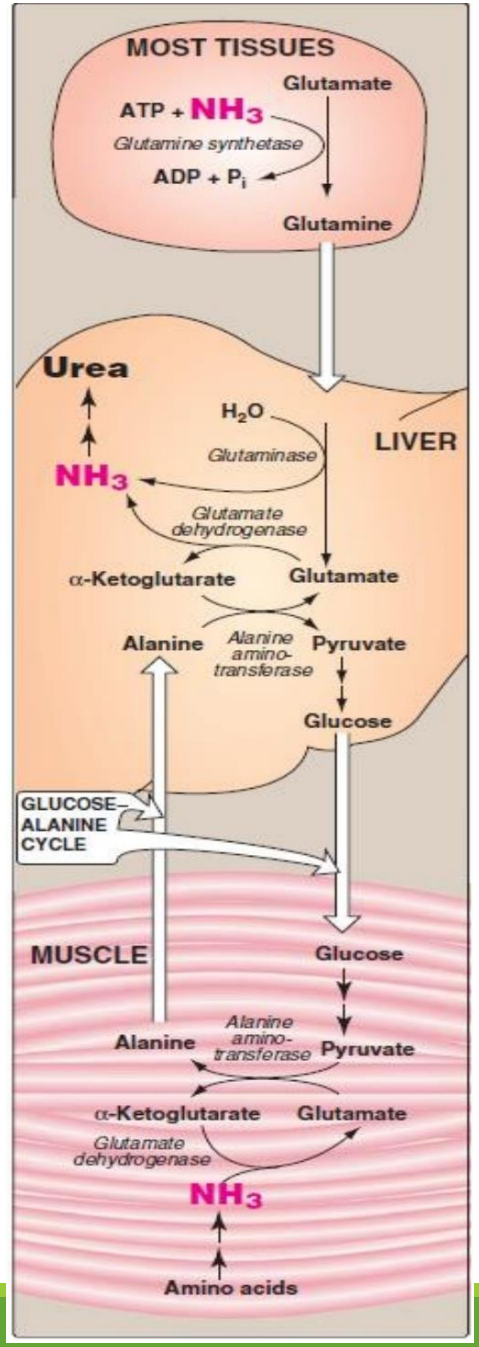
Transport of ammonia to the liver

NH_3 is transported from peripheral tissues to liver for conversion to urea.

Two mechanisms for ammonia transport:

1. By glutamine synthetase that combines NH_3 with Glu to form Gln
2. By transamination of pyruvate to form alanine

- **Note:**The ammonia that was formed from oxidation deamination in different tissues, is going to be converted to urea to become less toxic. This takes place only in hepatocytes, so ammonia **must be transported from peripheral tissues to the hepatocytes (liver)**.
- .Ammonia is a hydrophilic molecule (polar), so it can move easily but there is a problem in that. The problem is that Ammonia is a very toxic molecule, The whole water in our bodies is not enough to dilute ammonia to not toxic concentration. so while it is moving in the blood to reach the liver, it causes toxicity to the blood. So, what is the solution for such situation?
- -Transferring ammonia in a hidden form (AA) so it won't cause toxicity to the blood .

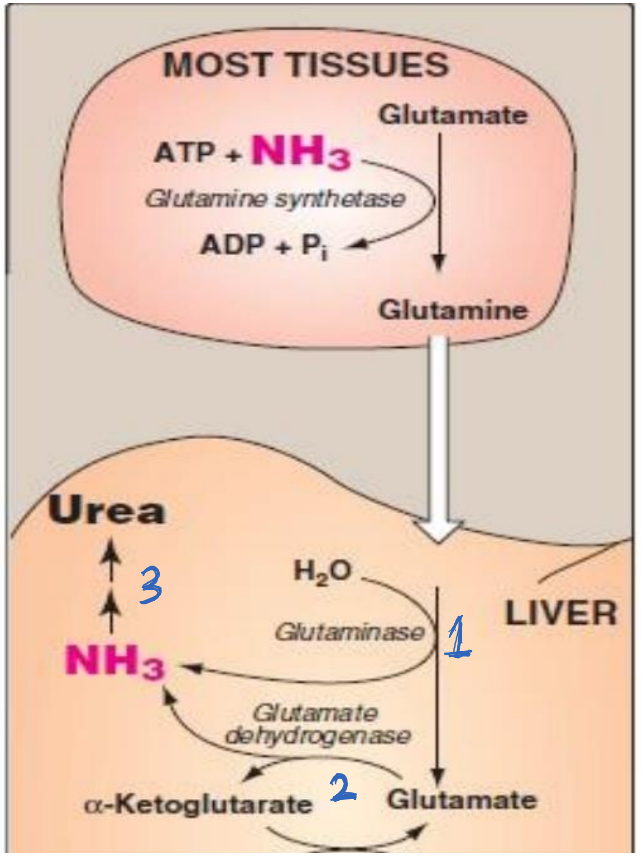


Transport of ammonia to the liver

Two mechanisms for ammonia transport:

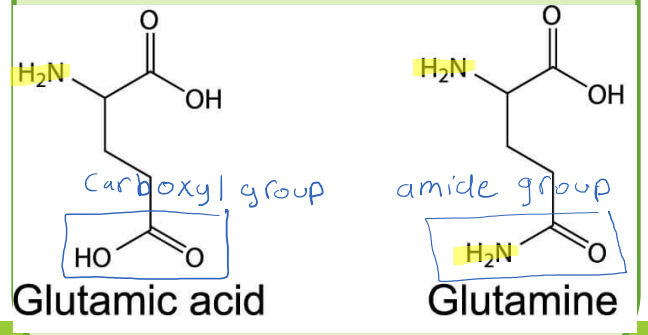
1. By **glutamine synthetase** that combines NH_3 with Glu to form Gln

- Found in most tissues(**the major transport mechanism**)
- Requires energy (**ATP dependent**)
- A nontoxic transport form of ammonia
- The resulting glutamine is transported in the blood to the liver to be cleaved by **glutaminase** to produce glutamate and free ammonia



- **Note: Glutamine synthetase combines ammonia to glutamate to form glutamine. NH_3+Glutamate + ATP →Glutamine**
- **Glutamine is transported from the peripheral tissues by blood and reaches to the liver. Once glutamine reaches the liver, [1]glutaminase removes ammonia converts glutamine again to glutamate.**
  [2]Then another amino group is removed from glutamate by oxidative deamination which gives α-ketoglutarate and ammonia, this [3]ammonia can then enter to urea cycle..
- **Glutamate has two carboxyl groups while glutamine has two NH3 groups,** Note: 1 glutamine gives 2 ammonia.

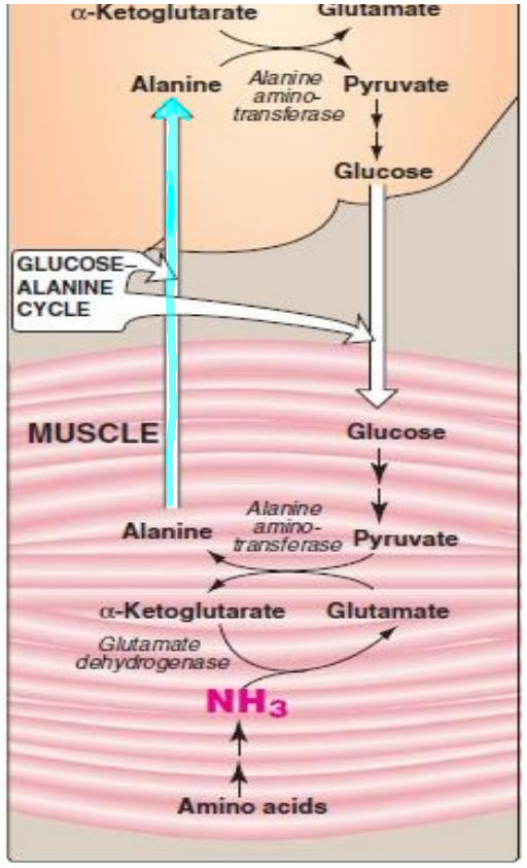
- **Attachment:**



Transport of ammonia to the liver

2. By transamination of pyruvate to form **alanine**

- **Primarily in muscles**
- Alanine is transported by the blood to the liver to be converted to pyruvate by transamination.
- Pyruvate can be used in gluconeogenesis (glucose-alanine cycle)



Note:

Ammonia is transported in the blood from muscles to hepatocytes in a form of Alanine. In muscles, amination reaction occur to pyruvate converting it to alanine.

Alanine gets out of the muscles to the blood stream then to hepatocytes. Once alanine reaches the liver, ALT enzyme works on Alanine removing (ammonia → urea cycle) and the leftover is pyruvate, this pyruvate can be used in gluconeogenesis producing glucose in the hepatocytes (so take 2 advantages of alanine by transporting ammonia and pyruvate in the same time to hepatocytes) Then glucose leaves the liver and go back to muscles. The whole process is called "glucose-alanine cycle".

Note: 1 Alanine gives 1 ammonia.

UREA CYCLE

Urea is a major disposal form of amino groups derived from AAs.

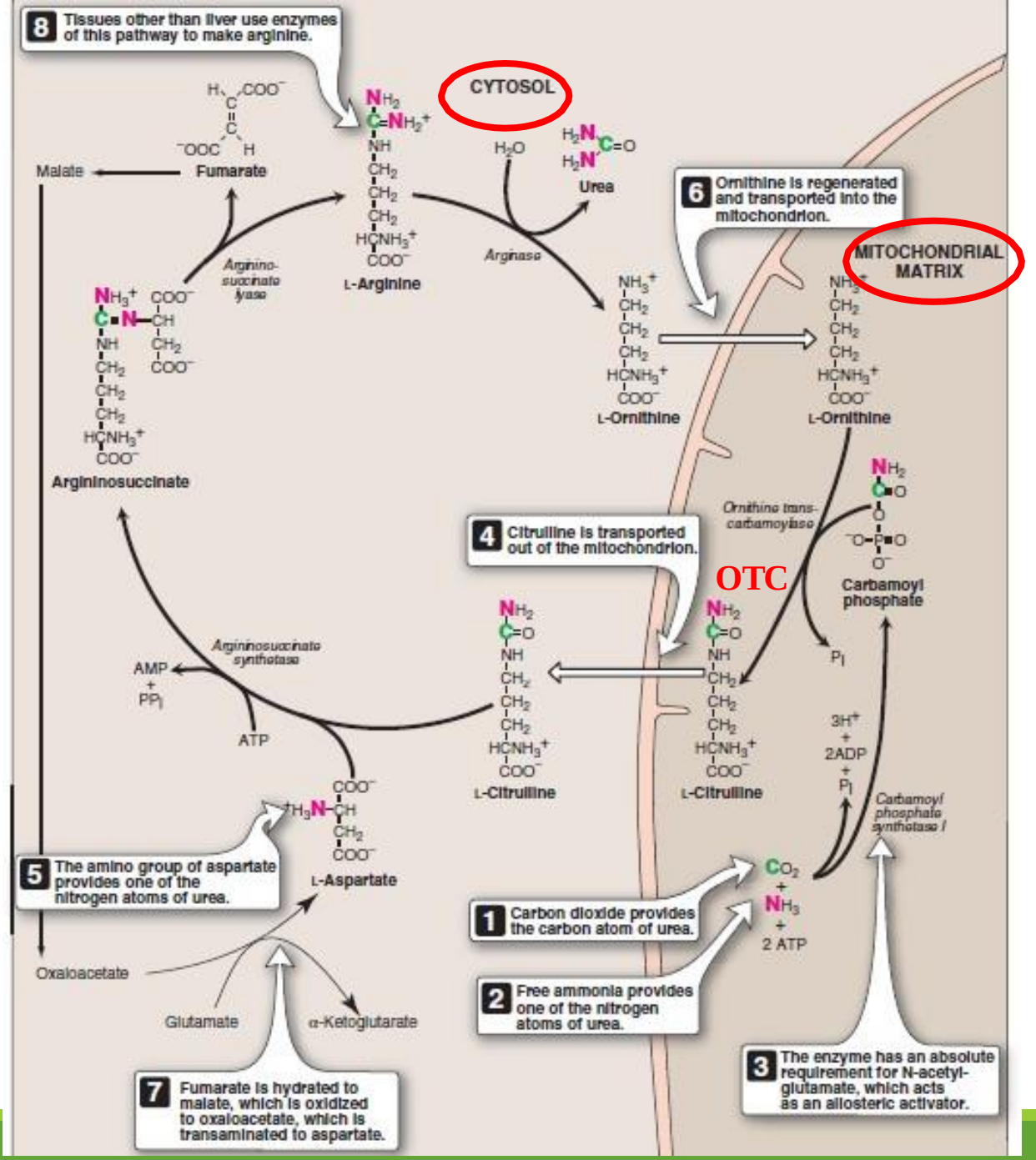
Urea accounts for about 90% of the N-containing components of urine.

One N of urea molecule is supplied by free ammonia (from oxidative deamination of Glu), and the other N by Asp.

The C and O of urea are derived from CO_2.

Urea is produced by the liver

Urea is transported in the blood to the kidneys for excretion in the urine.

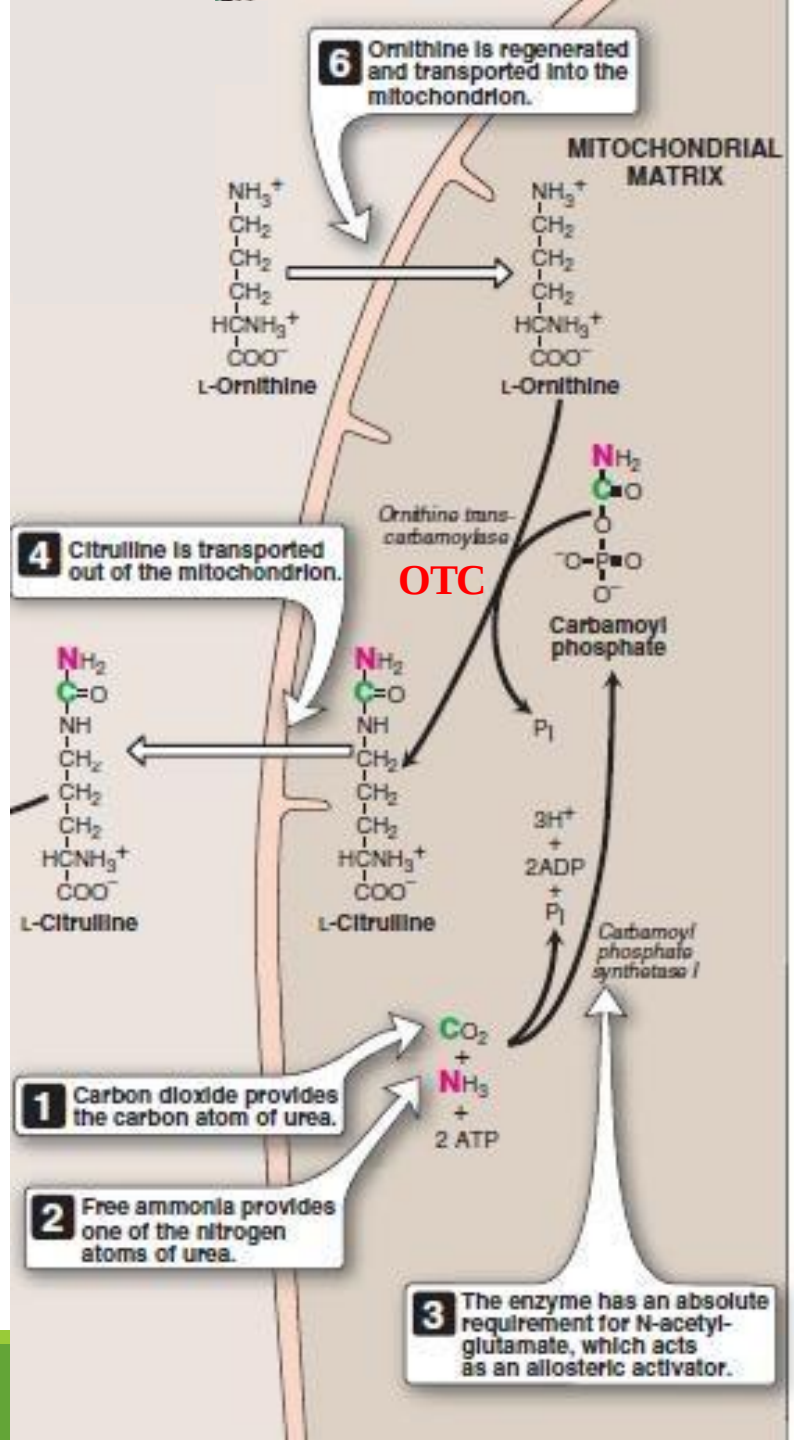


The complement in this slide:

- Urea cycle and TCA cycle are similar in principle, both need a 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 intermediates from smaller ones to form large molecules.
  The second phase: breaking down intermediates into smaller ones.

In the mitochondria

- **NOTE:** Ammonia doesn't enter the cycle in the form of NH3+ so there is a preoperative step for ammonia in mitochondria---> Ammonia interacts with CO2 and 2 ATP producing **carbamoyl phosphate (the starting material of urea cycle)**. This preoperative step is catalyzed by **carbamoyl phosphate synthetase I**. *synthetase indicates that there is a need for energy.
- 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 (OTC) enzyme**, releasing an inorganic phosphate Pi.
- ornithine trans carbamoylase(OTC) enzyme= transfer carbamoyl + "lase" removing phosphate
- **L-citrulline leaves mitochondria to cytosol.**
- NOTE: Ornithine and citruline are amino acids.

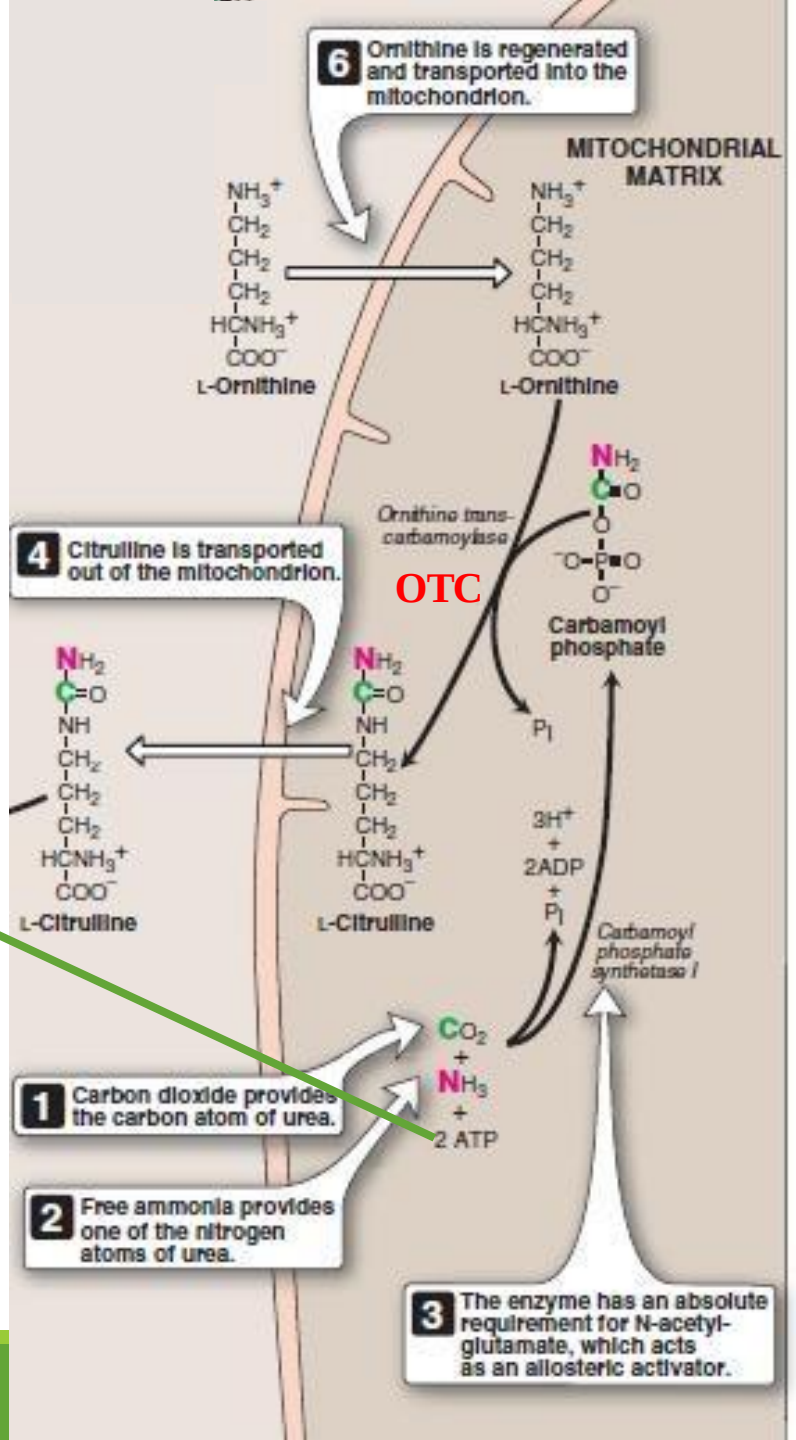


In the mitochondria

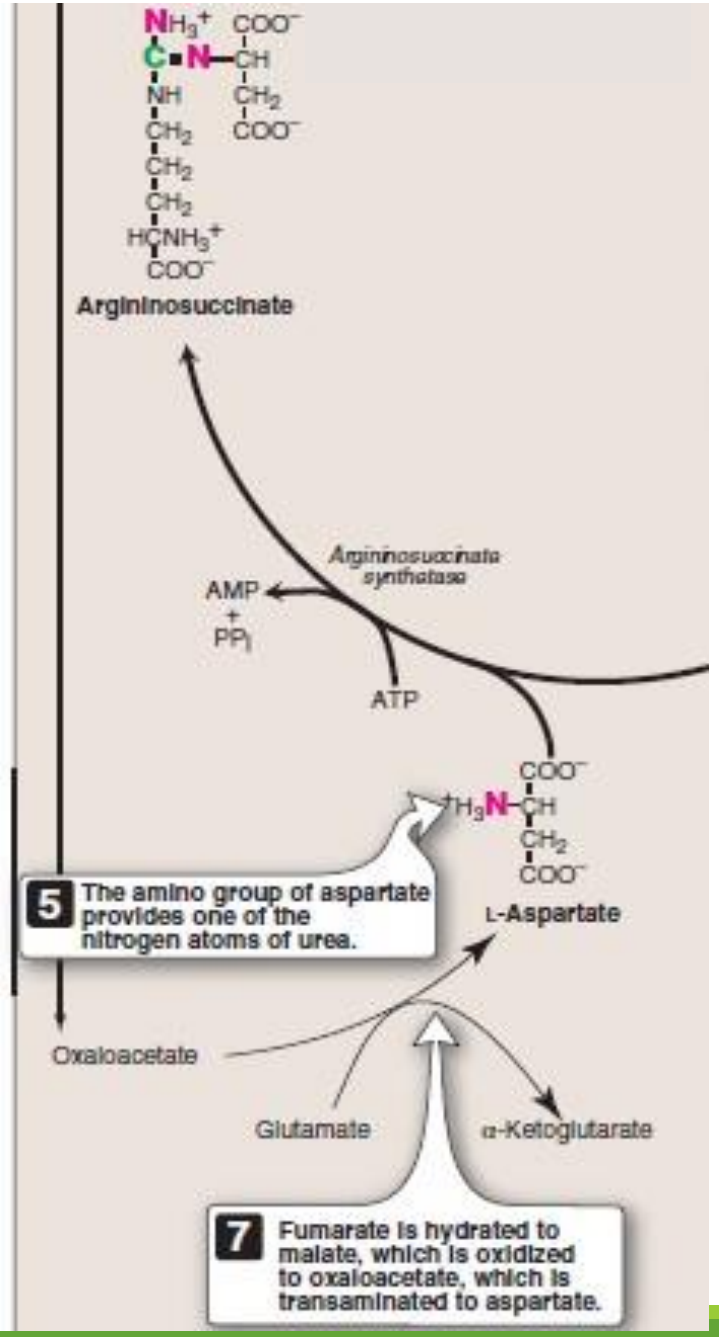
NOTE:

but there are 2 ATP how is that?

Hold on a second, the first ATP have two functions: 1) enter the structure of the product. 2) give energy to the reaction. But this energy doesn't enough to make the reaction happen and that's why we add another ATP to get the enough energy to start the reaction.



In the cytosol



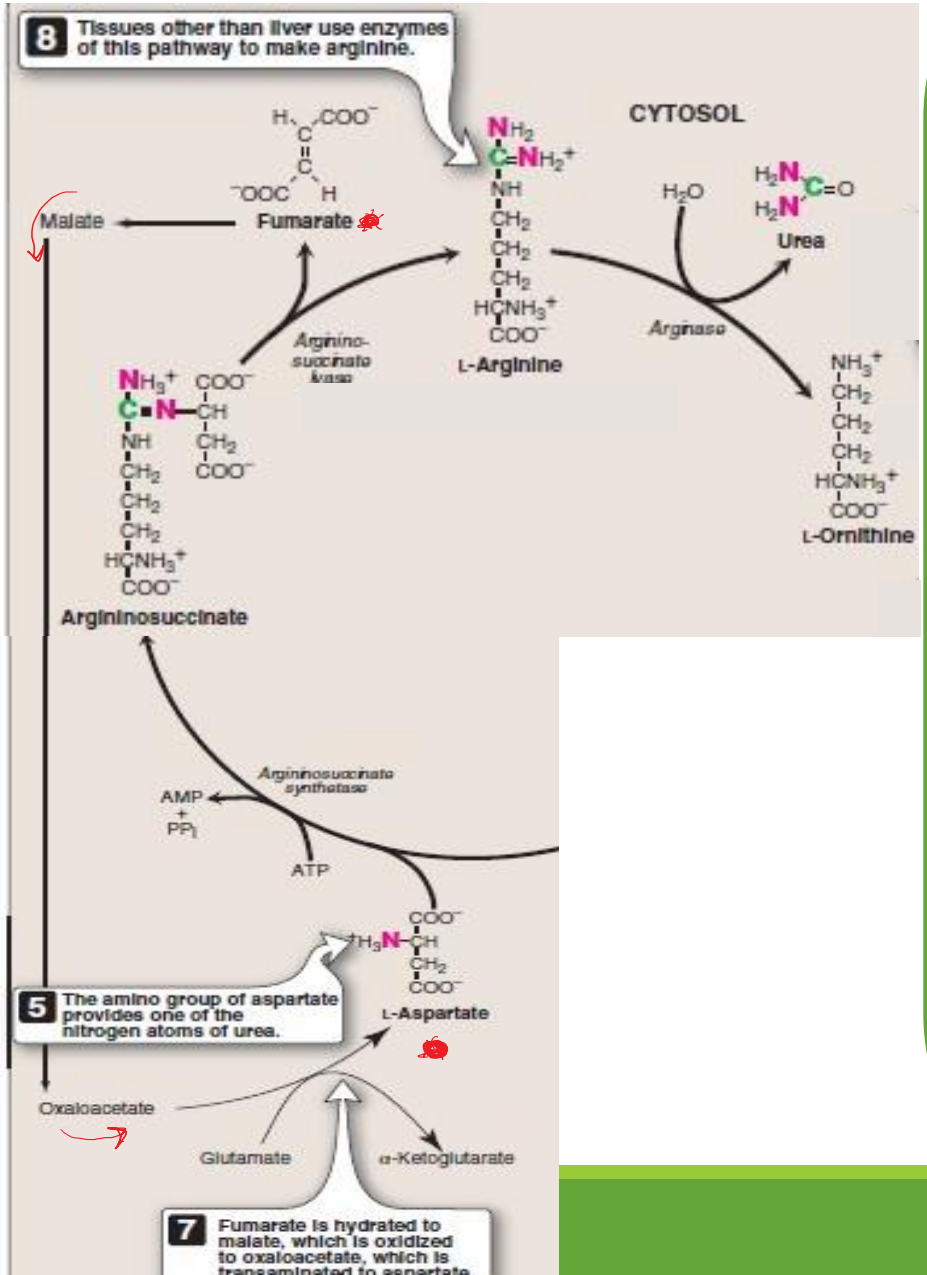
- **The complementing in this slide:**

Building phase continues…

L-citrulline interacts with aspartate producing Argininosuccinate the largest intermediate in urea cycle. This step is catalyzed by **Argininosuccinate synthetase** (ATP-dependent step) through transferring ATP to AMP and pyrophosphate PPi

*Recall: so the aspartate is consumed in this step which explains why Aspartate aminotransferase (AST) Favors the backward reaction synthesizing of aspartate rather than degrading it

In the cytosol



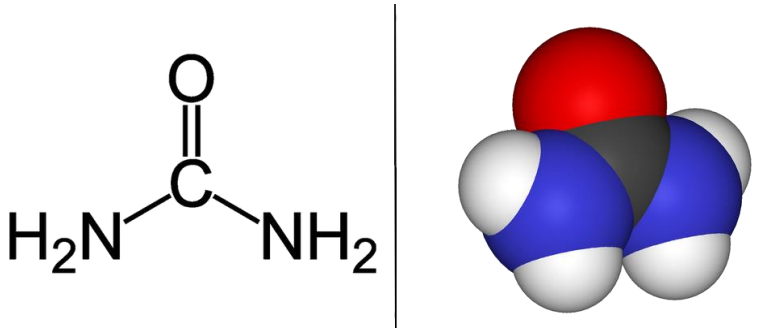
NOTE:

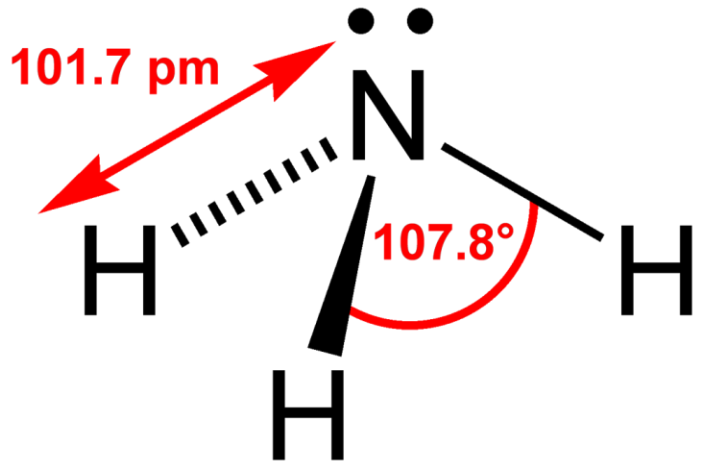
Second phase starts

- breaking down Argininosuccinate by Argininosuccinatelyse forming 2 molecules : [1] fumarate which is a TCA cycle intermediate(this a connection point between TCA and urea cycle) complete the reaction to malate then oxaloacetate that can be used to make aspartate and enters the urea cycle again. Transfer amino 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) . this part (NH2 --C= NH2+)is going to be used to form urea

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

- 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.
- The end





NOTE:

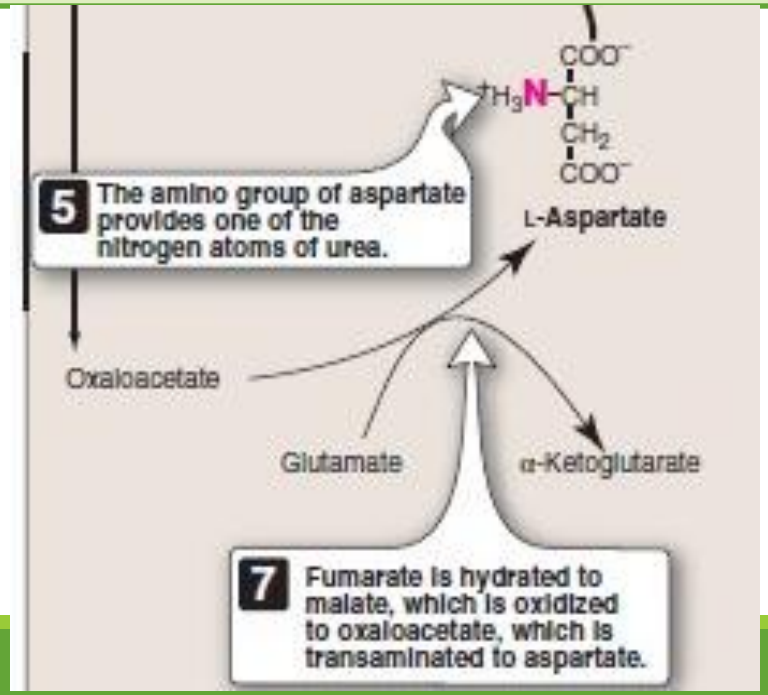
- Urea molecule has 2 amino groups while ammonia has only one.

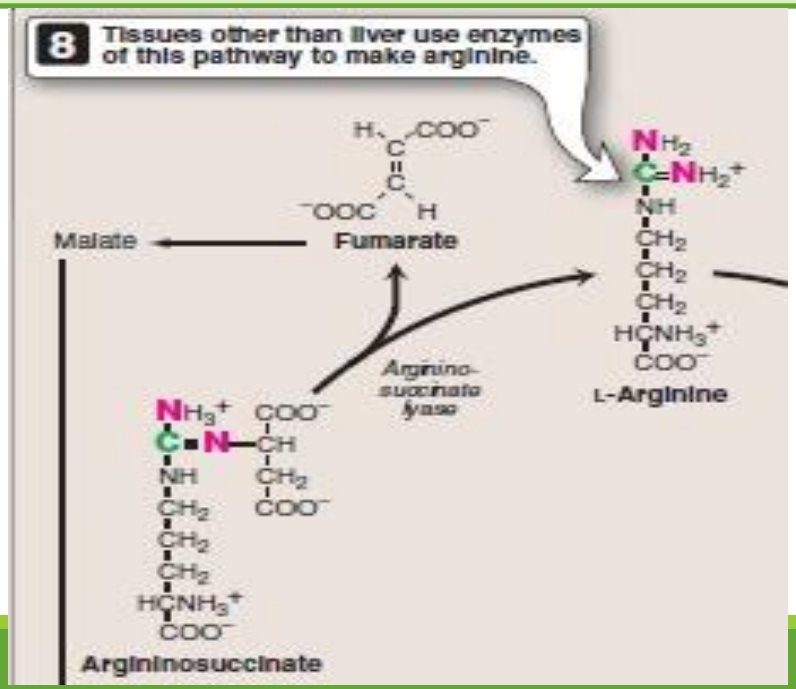
What are the sources of amino groups in Urea

1. Free ammonia released from Oxidative deamination of glutamate
2. Aspartate produced from transamination of oxaloacetate by AST

This pathway is converting not degrading ammonia (small) to UREA (large) , it also consumes energy rather than producing.

- **NOTE:** the aspartate is not like the feedback activation but it we can use to repeat the cycle but we can get it from everywhere so at the end this process will repeat and consume amino group by the convert the oxaloacetate to aspartate, and in the production process I am transferring and amine group but at this time I take it from the amine group not the opposite so that will give an abundant source to use the aspartate in my cycle. So keep in your mind that the fumarate that produce from the urea cycle is not make enough to use it as aspartate because I need amino group from the glutamate and that by the AST reaction but the reaction on the opposite direction to make me more aspartate





Overall stoichiometry of the urea cycle

$$\text{Aspartate} + NH_3 + CO_2 + 3\,ATP + H_2O \longrightarrow$$

$$\text{urea} + \text{fumarate} + 2\,ADP + AMP + 2\,P_i + PP_i$$

The synthesis of urea is irreversible, with a large, negative ΔG

For each urea molecule:

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).

The complement in this slide:

- if I sum up the reaction to get the overall the stichometry of the urea cycle, the intermediates once it is products and once it is reactant and all the things that enter or exit and if it not an intermediate it will appear in the final equation
- There is also the CO2, NH3 and the 2 ATP to be in the synthesis, also there is the aspartate that get in the synthesis of the arginosuccinate that produce by arginosuccinate lyase –that will form the fumarate as a byproduct then malate – an arginine and after that we add the water in the arginine to produce the urea and Ornithine.
- The formation of the urea is the last step in the cycle by arginase enzyme

So what happened in the ATP ???

- The 2 ATP goes in the mitochondria to the carbamoyl phosphate synthetase 1 to produce the 2ADP
- the third ATP consumed when we added the aspartate to get in the cycle but it produce AMP and Ppi ((that then it will cleaved and hydrolyzed to use the energy from it))
- So after all of that we can confirm that this cycle consume the energy of four phosphate groups ((2 Pi + Ppi))

Regulation of the urea cycle

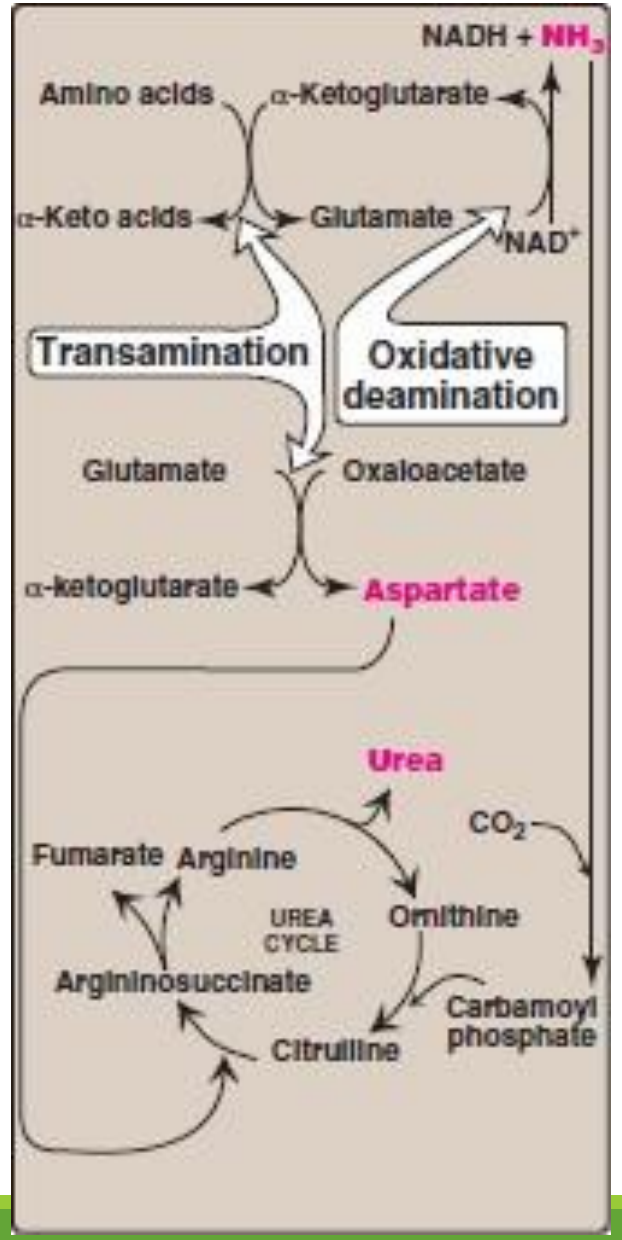
N-Acetyl glutamate is an essential **activator** for carbamoyl phosphate synthetase I—the rate-limiting step in the urea cycle

Arginine is an **activator** for N-Acetylglutamate synthesis

The intrahepatic concentration of N-Acetylglutamate increases after a protein-rich meal (more glutamate and arginine are provided)

More protein in diet leads to increased urea synthesis rate

And that will lead to exhausted your liver



The complement in this slide:

- Urea cycle doesn't work permanently but it is stimulated and activated by several conditions.
- Protein rich diet activates urea cycle: 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.
- the urea cycle and then if we go down of levels of molecules, they activators are the N-Acetylglutamate, if we really have excess molecule of glutamate then we will degrade it
- The one who activate the N-Acetylglutamate is the arginine amino acid, so we called it the activator of the activator that activate the urea cycle

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.

Types:

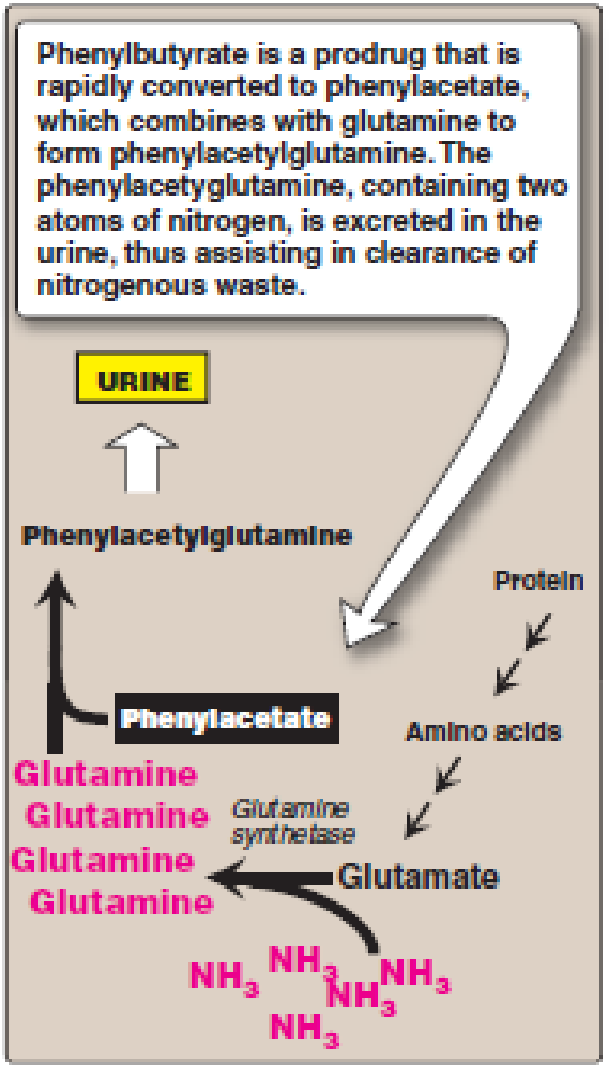
Acquired hyperammonemia: Liver disease due to viral hepatitis, or to hepatotoxins such as alcohol.

Congenital hyperammonemia: Genetic deficiencies of any of the five enzymes of the urea cycle leads to failure to synthesize urea

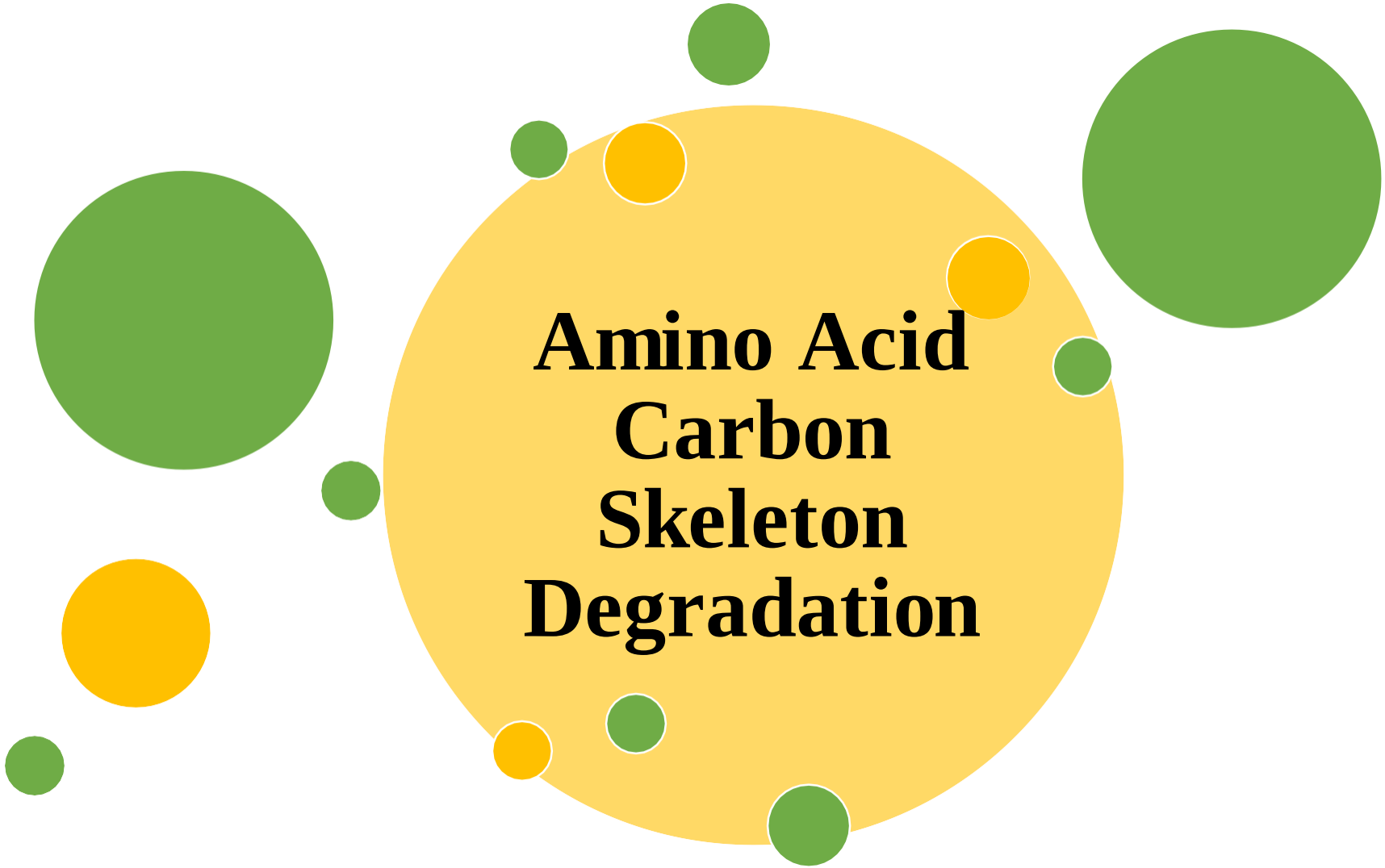
The overall prevalence estimated to be 1:25,000 live births.

Ornithine transcarbamoylase deficiency is the most common

Treatment: restriction of dietary protein, administration of compounds that bind covalently to AAs, producing nitrogen-containing molecules that are excreted in the urine



- **The compliment of this slide:**
  - **The ammonia is a very toxic molecule, even when we use one of the process that get red of the toxicity that called the dilution o the molecule the toxicity of the ammonia still there.**
  - **So, I must get red of it, but if I not, then these toxic will lead to affect on the CNS and make symptoms like (the chills, vomiting and other toxic effect on it)**


Amino Acid
Carbon
Skeleton
Degradation

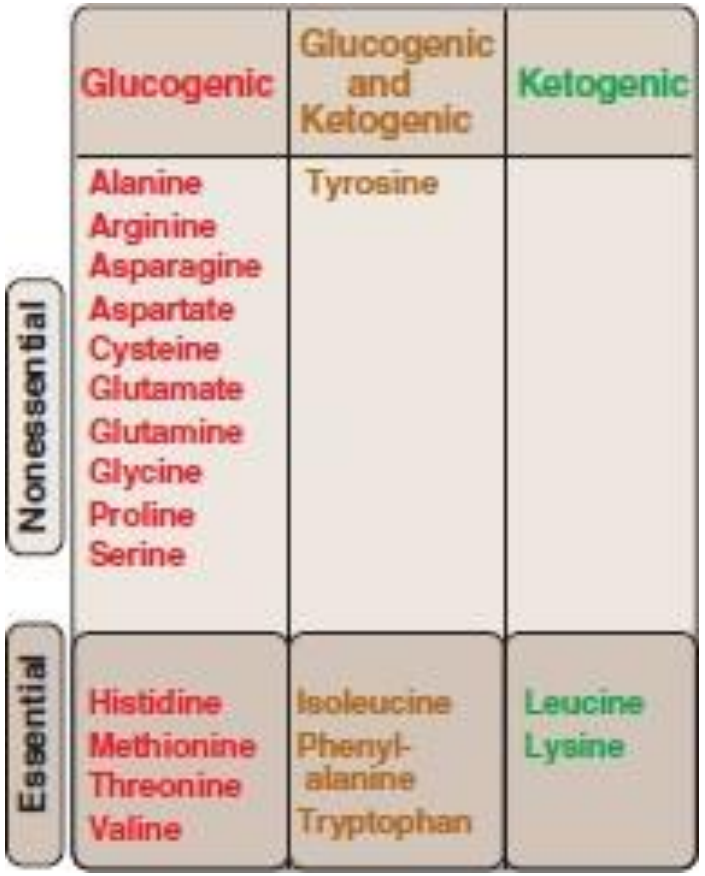
GLUCOGENIC AND KETOGENIC AMINO ACIDS

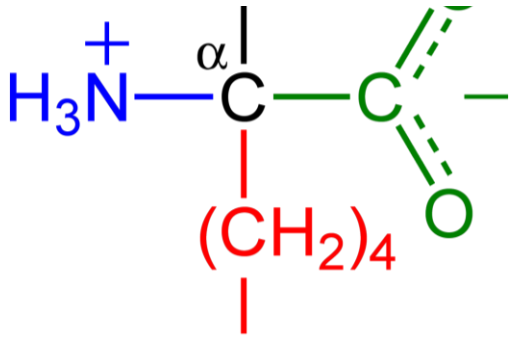
Seven intermediates are produced during AA catabolism (**oxaloacetate, pyruvate, *α*-ketoglutarate, fumarate, succinyl coenzyme A (CoA), acetyl CoA, and acetoacetate**).

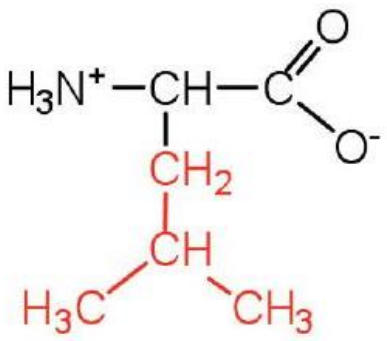
Glucogenic amino acids catabolism yields pyruvate or one of the TCA cycle intermediates that can be used as substrates for gluconeogenesis in the liver and kidney.

Ketogenic amino acids catabolism yields either acetoacetate (a type of ketone bodies) or one of its precursors (acetyl CoA or acetoacetyl CoA).

Other ketone bodies are 3-hydroxybutyrate and acetone





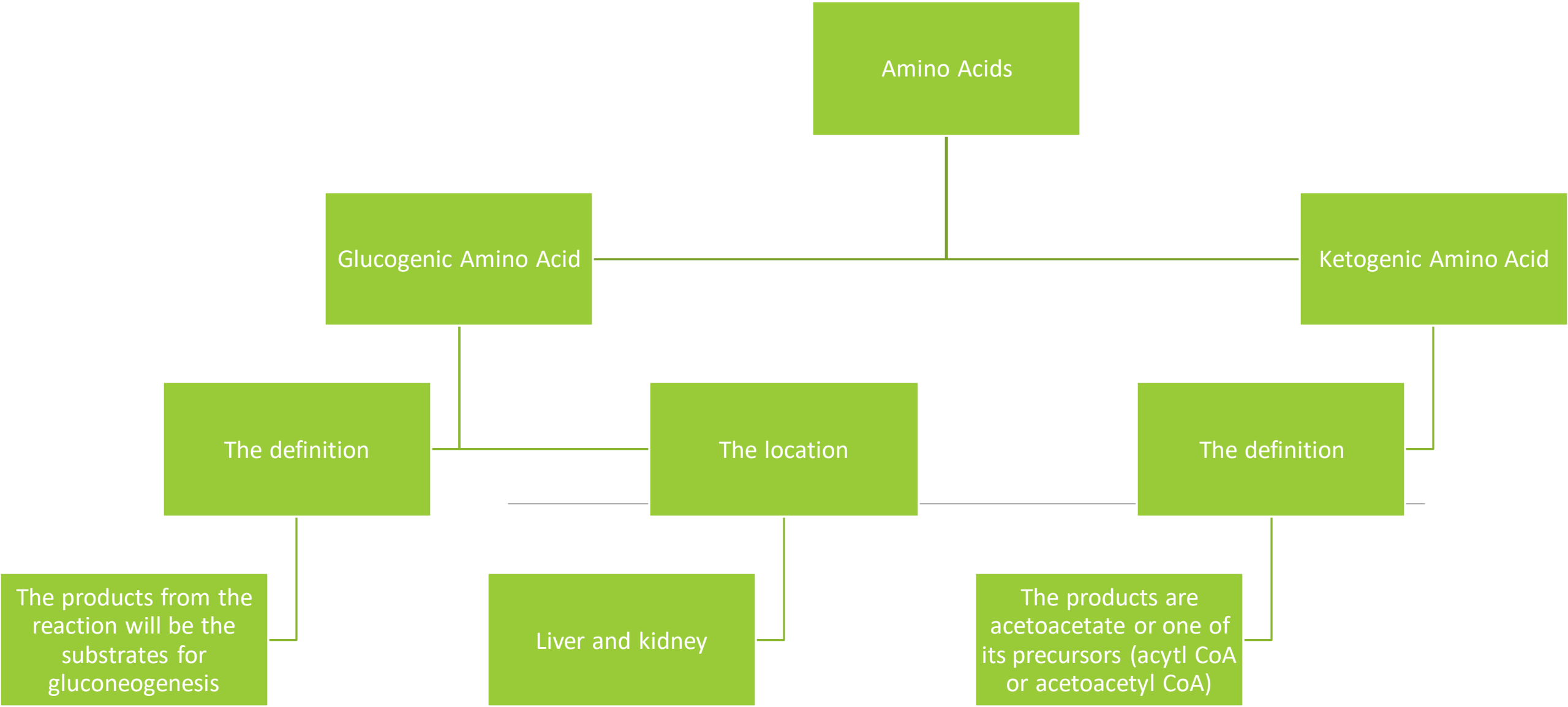


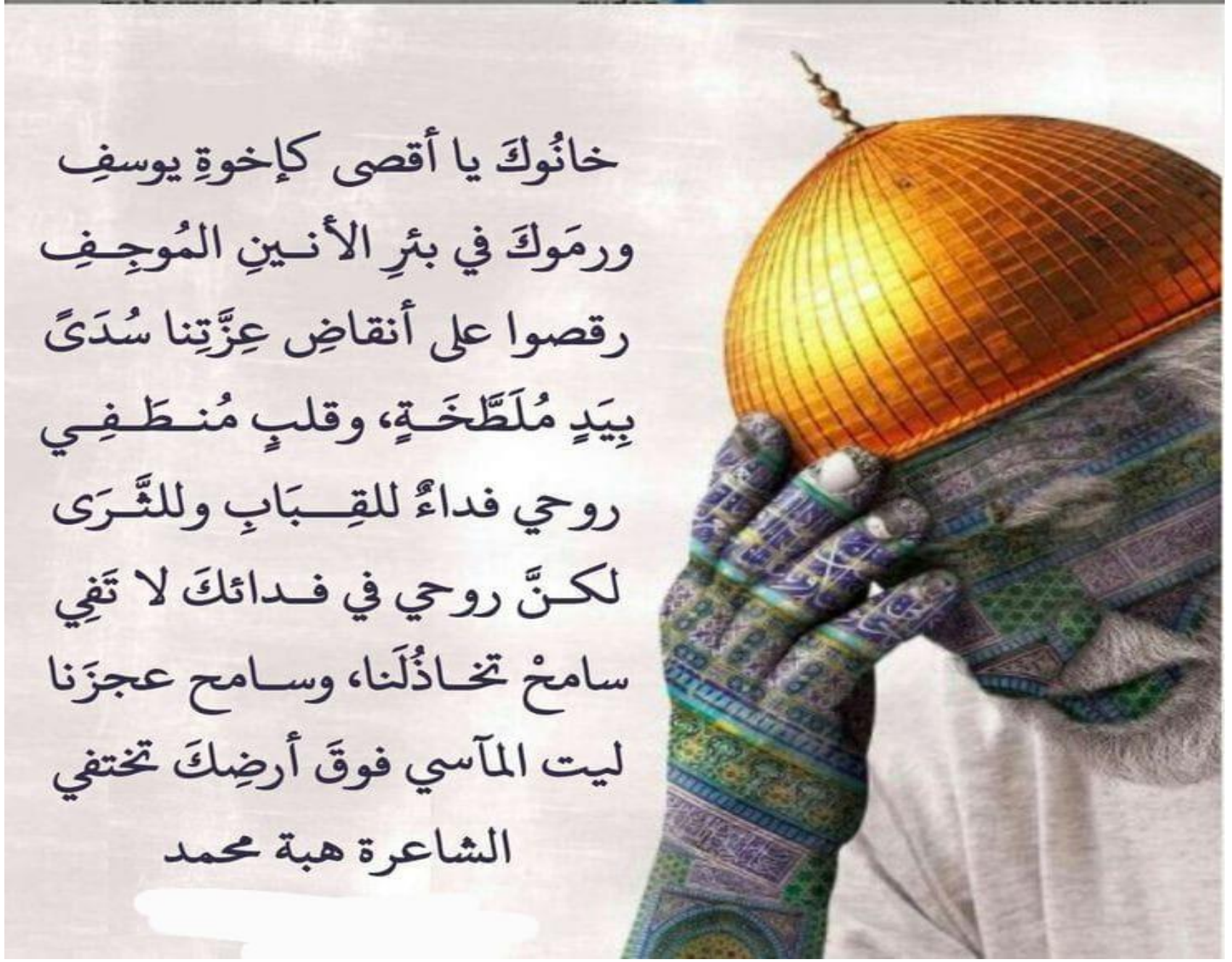
The compliment of this slide:

- the introduction about the processes that have the degradation of the carbon skeleton and the R chain and expect multiple pathway to degrade them in different chains and may produce pyruvate or Krebs cycle in different intermediate to make the glycolysis or the gluconeogenesis depends the body needs so that's why the amino acid are glucogenic
- If the amino acid is degraded to acytl co A or acetoacetyl co A and this used synthesized to make the ketone bodies and then I considered the amino acid is ketogenic.

But you ask your self is there any amino acid that considered the amino acids that make different final products?

- Yes!! , it may be the final products they belong the same group and a few examples of amino acids that can make both final products the glucogenic and ketogenic.
- The exclusively 2 ketogenic amino acids are the Lucine and Lysine ((remember they are both have the letter L))
- however, the amino acids that maybe glucogenic or ketogenic are all the aromatic ((phenylalanine, tyrosine, tryptophan and the isoleucine))
- Otherwise, the most amino acids are glucogenic amino acids


Amino Acids
Glucogenic Amino Acid
Ketogenic Amino Acid
The definition
The location
The definition
The products from the reaction will be the substrates for gluconeogenesis
Liver and kidney
The products are acetoacetate or one of its precursors (acytl CoA or acetoacetyl CoA)


خانُوكَ يا أقصى كإخوةِ يوسفِ
ورمَوكَ في بئرِ الأنـينِ المُوجِـفِ
رقصوا على أنقاضِ عِزَّتِنا سُدَىً
بِيَدٍ مُلَطَّخَـةٍ، وقلبٍ مُنـطَـفِـي
روحي فداءٌ للقِـــبَابِ وللثَّـرَى
لكـنَّ روحي في فـدائكَ لا تَفِي
سامحْ تخـاذُلَنا، وسـامح عجزَنا
ليت المآسي فوقَ أرضِكَ تختفي
الشاعرة هبة محمد