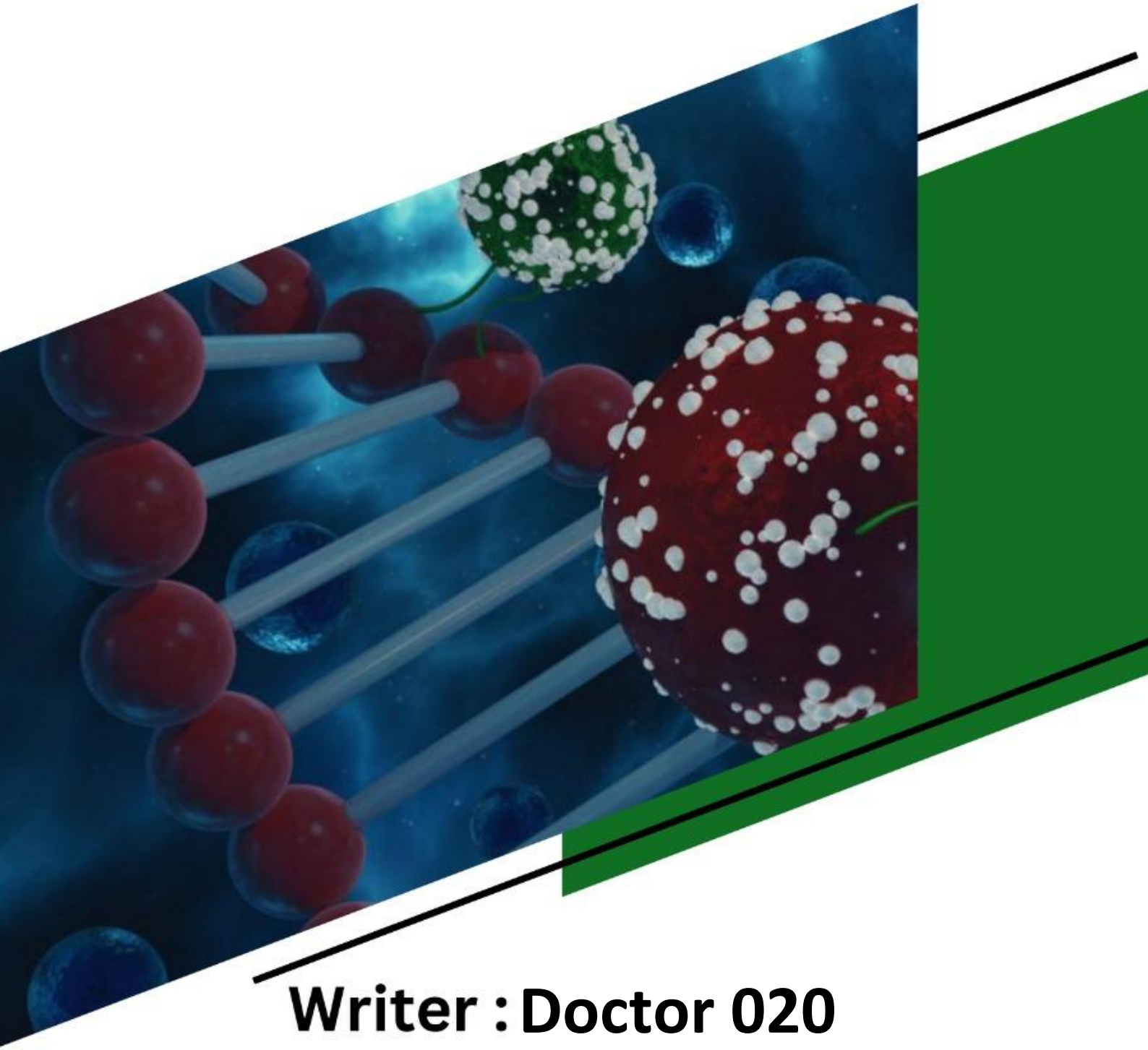
Doctor 021

METABOLISM

Sheet no. 28



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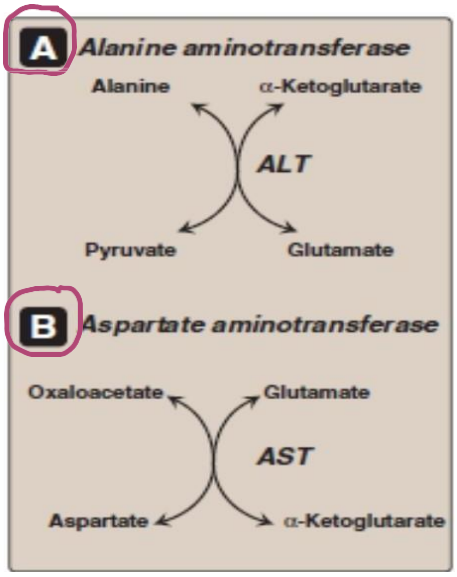
Last lecture we discussed the structure of Amino Acids which has the **alpha carbon** attached to **4 groups:** Amino group (NH3 +), Carboxyl group (COO-), Hydrogen and R group. Today we will talk about new section of Amino Acid that deals with common pathways and common structures between these A.A and how they get metabolized. Our main concern is the **Nitrogen** of the(backbone) Amino group -not the nitrogen in the side chain- because it is a source of Nitrogen, and this nitrogen is going to be released as Ammonia (NH_3) which is a very toxic compound that target CNS, so we must be careful when we are dealing with these compounds. Also we have to maintain this nitrogen in balance (no increasing ordecreasing) because we need it in the synthesis of many other compounds not only A.A, when we have A.A we have to degrade it or to use it in gluconeogenesis or we use it in in protein synthesis, synthesis of nitrogen containing compound like: catecholamine, serotonin, melatonin, keratin, histamine. Because AA can't be stored .

Transamination:

The first step in metabolism and degradation of A.A

Transamination Substrate specificity of aminotransferases: Each aminotransferase (AT) is specific for one or a few amino group donors. The most important ATs are: Alanine Aminotransferase (ALT) Aspartate Aminotransferase (AST) The equilibrium constant of transamination reactions is near one. Keq=1 means the reaction functions in both amino acid degradation and biosynthesis according to the cellular needs.



Generally the transamination process is about transferring (not removing) an amino group (NH_3+) from a nitrogen containing compound to a certain recipient by a certain Aminotransferase (AT). In the case of transamination of AA, we transfer amino group from the AA to **α-ketoglutarate (recipient).**

*When an AA loses its amino group, it still have its carboxyl group, hydrogen and R group which is called it **α-keto acid**.

Simply:

Amino Acid – Amino group = α-keto acid

*So (α-keto acid) is a product from thetransamination reaction.

^ Each AA has its own α-keto acid due to the differences in the R groups.

^accordingly, there are different α-keto acids derived from different AA.

So, each aminotransferase (AT) is specific for one or few amino group donors.

α-ketoglutarate is a TCA's cycle intermediate.

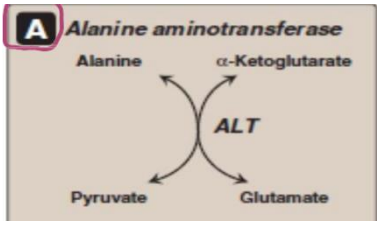
*The common characteristic between the transamination of all A.A is that they have the same recipient which is **α-ketoglutarate.** Recall that the structure of α-ketoglutarate (5 carbons, 1 carbonyl group, 2 carboxylic groups) adding amino group to it transforming it into GLUTAMATE (AA)

What is the first step? Transfer the amino group from any amino acid to the α-ketoglutarate whichbecomes Glutamate.

What will happen to the amino acid that lost its amino group? It will become α-keto acid.

Alanine aminotransferase (ALT)

ALT is present in many tissues. The enzyme catalyzes the transfer of the amino group of alanine to α-ketoglutarate Reaction products: pyruvate and glutamate.The reaction is reversible. During amino acid catabolism, ALT functions in the direction of glutamate synthesis. Glu acts as a "collector" of nitrogen from Ala.

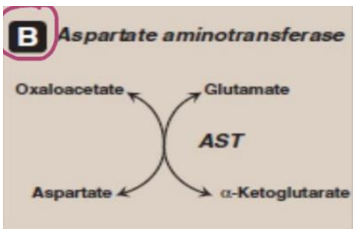


Alanine Amino Transferase (ALT) another name is Alanine Transaminase.

ALT transfers the amino group from Alanine to α-ketoglutarate which become glutamate and the leftover of Alanine is called pyruvate (α-keto acid of alanine is pyruvate). Pyruvate can be used as a gluconeogenesis intermediate.

Aspartate aminotransferase (AST)

AST does not funnel amino groups to form Glu, During amino acid catabolism, AST transfers amino groups from glutamate to oxaloacetate, forming aspartate. Aspartate is used as a source of nitrogen in the urea cycle The AST reaction is reversible



Aspartate Amino transferase (AST) transfers the amino group of Aspartate to

α-ketoglutarate which becomes Glutamate, and the leftover of Aspartate is called

Oxaloacetate (oxaloacetate is the α-keto acid of aspartate). Oxaloacetate can be used as a gluconeogenesis intermediate.

There are other enzymes for other amino acids that have the same mechanism, but we will focus on thesetwo enzymes (ALT & AST), because they have medical significance.

The reaction of ALT goes in both directions (degradation pathway of alanine or synthetic pathway for alanine), forward and backward (reversible), but **it favors the forward direction** which is the synthesis of glutamate and conversion of Alanineto pyruvate, so the amino group of the Alanine funnels (تتجمع وتتمركز) **into** glutamate(As a collector of nitrogen from alanine)

Actually, we are converting all AA into glutamate!

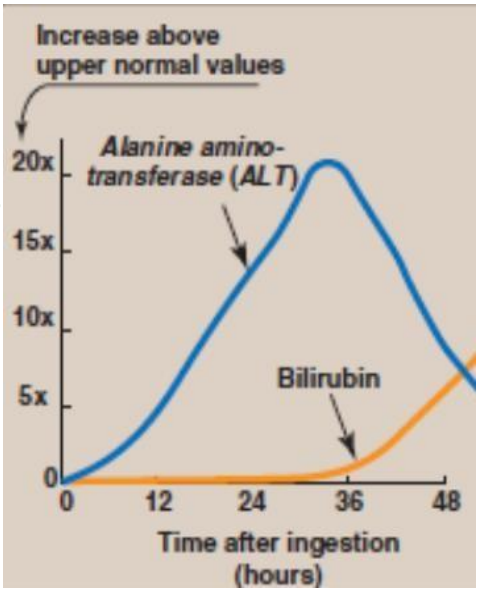
In this situation we are producing pyruvate by degrading amino acids, and we are degrading them because we're in the **starvation situation** to produce energy and provide gluconeogenesis with its own intermediates (e.g., oxaloacetate and pyruvate).

The reaction of AST goes in both directions (reversible), but **generally favors the backward direction**, so during AA catabolism AST transfers amino group from glutamate to oxaloacetate forming aspartate.

In this situation we didn't degrade the Aspartate, we produced it, **why is this happening?**

Because this Aspartate will be used in another pathway related to amino acids metabolism which is the Urea Cycle! So the amino group of Aspartate doesn't funnel into glutamate.

Clinical hint: Diagnostic value of plasma aminotransferases



ATs are normally intracellular enzymes but low levels in the plasma represent the release of cellular contents during normal cell turnover. AST and ALT have a diagnostic value when found in the plasma.

a. Liver disease: Plasma AST and ALT are elevated Examples: severe viral hepatitis, toxic injury, and prolonged circulatory collapse. ALT is more specific than AST for liver disease AST is more sensitive because the liver contains larger amounts of AST.

b. No hepatic disease: MI and muscle disorders.

ALT is specific to **L**iver which means it is mostly expressed in hepatocytes.

AST can be expressed by different cell types BUT it is sensitive indicator in every tissue that it is expressed in. for example, if a patient has a problem in the liver (hepatitis, hepatic cancer... etc.) there will be destruction in the cells so they go

Underapoptosis or necrosis and releases their contents, these contents are taken by the blood stream. So, taking a blood sample from the patient and doing a**diagnostic lab test called "Liver Function Test (LFT)"** which has serve different parameters and variables measured through this test. (ALT&AST) are two of these parameters. If ALT or AST has highlevels in the blood sample that indicates a big destruction in the liver because AST and ALT normally found in the blood in low levels.

*High levels of ALT indicate that destruction is happening in liver, so ALT is a **specific indicator.**

*High levels of AST indicate that destruction is happening in many tissues not only the liver (e.g., muscle disorders, myocardialinfraction), so AST is a **sensitive indicator**.

→ Specificity VS sensitivity:

Specificity Deals with the location of disruption, while sensitivity deals with the amount, so small destruction in the organ can reflect a high amount of this enzyme.

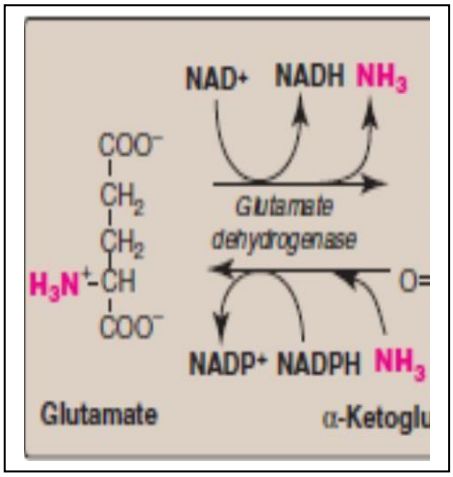
→Relation between specificity & sensitivity:

Sensitivity and specificity are inversely proportional, once specificity is increased, thesensitivity is decreased etc.

After the transamination of all AA to **GLUTAMATE**, the next step is removal of this amino group in the form of ammonia (NH_3) in a step called: OXIDATIVE 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.



Reaction products:

1. α-keto acids that can enter the central pathway of energy metabolism

2. Ammonia, the source of nitrogen in urea synthesis.

Allosteric regulators of glutamate dehydrogenase: GTP is an allosteric inhibitor ADP is an activator. 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.

All amino acids are converted into glutamate because Glutamate is the only AA that undergoes rapid oxidative deamination.

Oxidative = loss of hydrogen.

deamination=removal of amino

*Oxidative deamination by glutamate dehydrogenase result in the liberation of amino group as a free ammonia. So, the products of this reaction can either enter the central pathway of energy metabolism (**α- ketoglutarate**) or enter the urea cycle as a source of Nitrogen(**ammonia**).

*Reactions occur primarily in the liver and thekidney.

→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 asa free ammonia (no recipient).

→Glutamate Dehydrogenase:

*Catalyzes the forward and backward reactions but with different co-factors(observe the picture above):

→**In the forward reaction**: (converting Glutamate to α-ketoglutarate) using **NAD+** as a coenzyme which is reduced to **NADH,** and glutamate is oxidized by losing H.

←**In the backward reaction**: (converting α-ketoglutarate to Glutamate) using **NADPH** as a coenzyme which is oxidized to **NADP+,** and α-ketoglutarate is reducedby gaining H.

→Allosteric regulators:

GTP is an inhibitor for glutamate dehydrogenase because GTP is high energyindicator, so there is no need to degrade AA.

ADP is an activator for glutamate dehydrogenase because ADP is low energyindicator, so there is a need to degrade AA.

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 pathways that discussed before is applied for **L- amino acids** which normally present in our bodies.

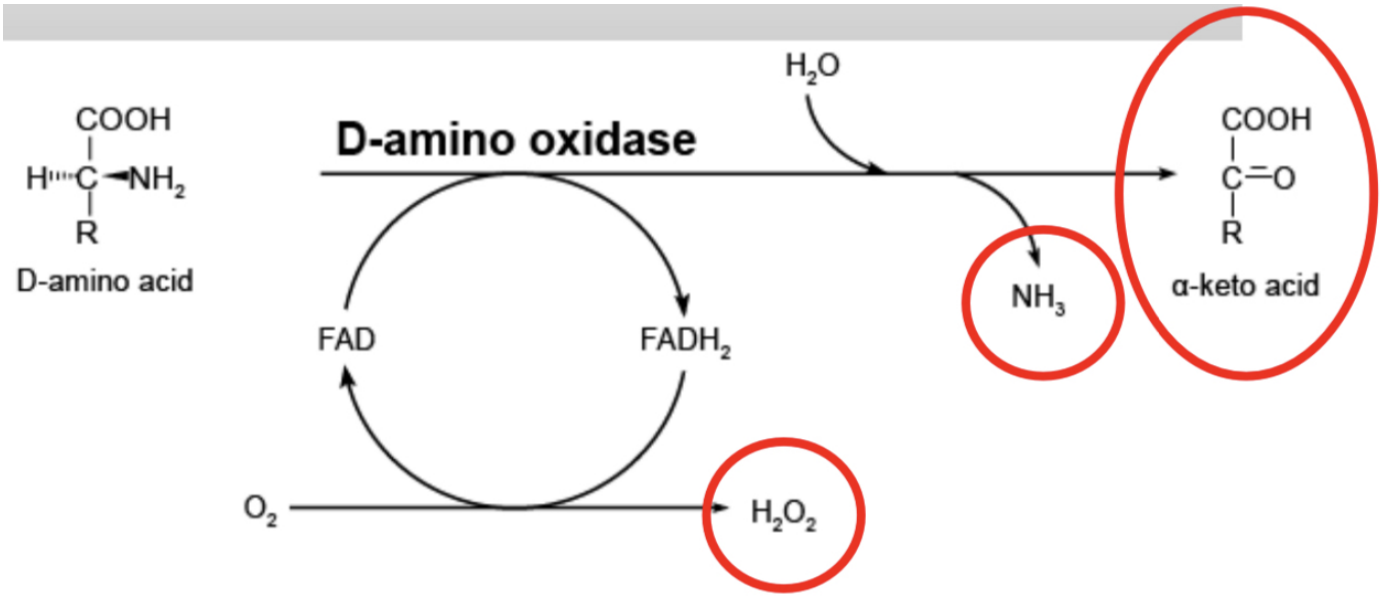
D-amino acids can get to our bodies from different sources, and they can be metabolized by the kidney and the liver.

D-amino oxidase (DAO) is a FAD-dependent peroxisomal enzyme that catalyzes the oxidative deamination of D-amino acid. Same mechanism explained above with different co-factor which is **FAD** reduced to **$FADH_2$** and producing H_2O_2 (ROS) from O_2.

Increased DAO activity has been linked to increased susceptibility to schizophrenia (مرض الفصام) But the mechanism is unknown.

There is L-amino oxidase which is a component of **snake venoms** which will affect the deamination of AA.

Now let's move on and see what will happen to ammonia that was released inoxidation deamination.



Metabolism of ammonia

Sources of ammonia

1. **From glutamine: Most of this ammonia is excreted into the urine as NH4 + (acid –base balance)**

Most of the ammonia is excreted into urine as NH4+ (acid-base balance).

Why glutamine not glutamate? because this is the format in which ammonia that was released through oxidative deamination is going to be transported through blood stream by adding it on glutamate to be glutamine by glutamine synthase.

2. **From bacterial action in the intestine: Ammonia is formed from 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.**

Ammonia is formed from urea by bacterial urease(degradation of urea to ammonia through intestine to blood stream) in the intestinal lumen. This ammonia can be absorbed from the intestine by the portal vein and is converted again to urea by the liver.

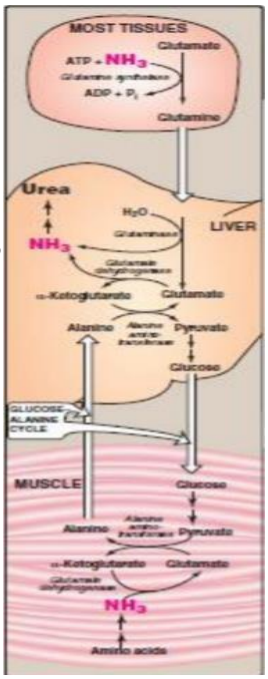
3. **From amines: Amines in the diet, and monoamines that act as hormones or neurotransmitters, give rise to NH3 by amine oxidase**

Amines in diet, and monoamines that acts as hormones and neurotransmitters give rise to ammonia 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 Intestine, kidney

In the catabolism of purines and pyrimidines, amino group attached to the rings are released as NH_3.

Transport of ammonia to the liver NH3 is transported from peripheral tissues to liver for conversion to urea.



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 easilybut there is a problem in that.

The problem is that Ammonia is a very toxic molecule, 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.

Two mechanisms for ammonia transport:

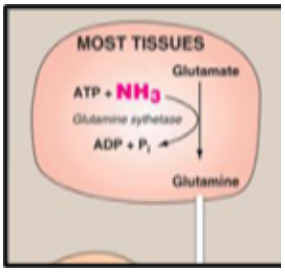
1. By glutamine synthetase that combines NH3 with Glu to form Gln

- Found in most tissues

- Requires energy

- A nontoxic transport form of ammonia

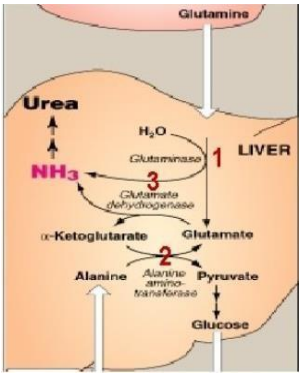
- The resulting glutamine is transported in the blood to the liver to be cleaved by glutaminase to produce glutamate and free ammonia



Glutamine synthetase combines ammonia to glutamate to form glutamine. This reaction is ATP-dependent→ using ATP to avoid toxicity.

$NH_3 + \text{Glutamate} = \text{Glutamine}$

After we have hidden the ammonia in glutamine. Glutamineis transported from the peripheral tissues by blood and reaches to the liver. Once glutamine reaches the liver, we remove **ammonia,** so it's converted again to glutamate.



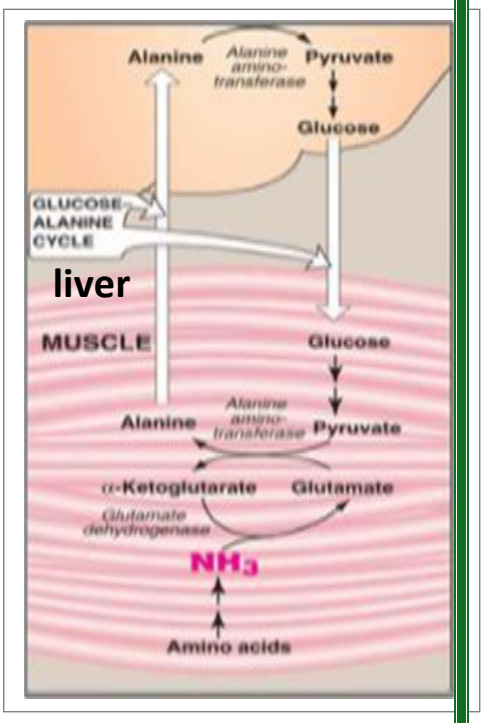
Then we remove another amino group from glutamate by oxidative deamination which gives **α-ketoglutarate** and **ammonia**, this ammonia can then enter to urea cycle. (See the pic) Note: 1 glutamine gives 2 ammonia.

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)

Ammonia is transported in the blood from **muscles** to **hepatocytes** in a form of **Alanine.**

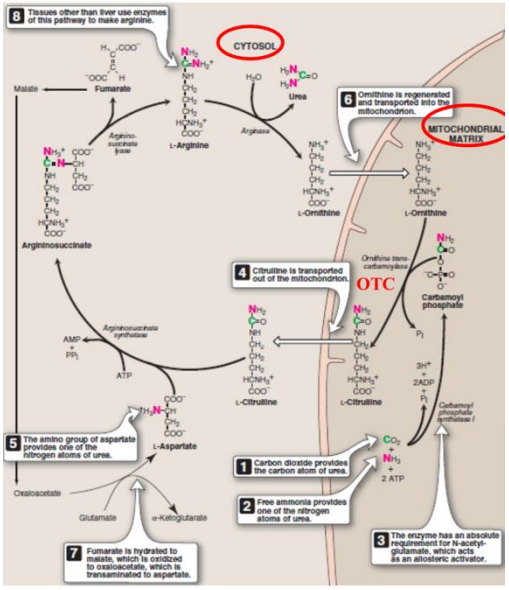
In muscles, amination reaction occur to pyruvate converting it to alanine. Alanine gets out of the muscles to the blood stream then to hepatocytes. Once alanine reaches the liver, ALT enzyme works onAlanine removing (ammonia → urea cycle) and the leftover is pyruvate, this pyruvate can be used in gluconeogenesis producing glucose in the hepatocytes. Then glucose leaves the liver and go back to muscles. The whole process is called "**glucose-alanine cycle".** (See the picture)→

Note: 1 Alanine gives 1 ammonia.



UREA CYCLE

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 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 CO2. Urea is produced by the liver Urea is transported in the blood to the kidneys for excretion in the urine.

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.

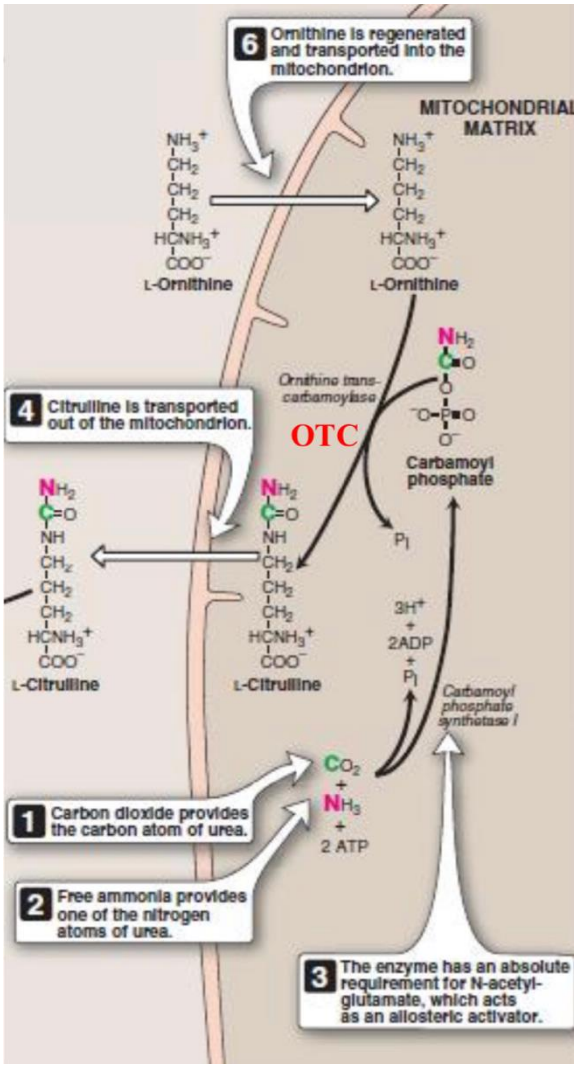
Reactions of urea cycle occur in two phases:

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 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.



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.

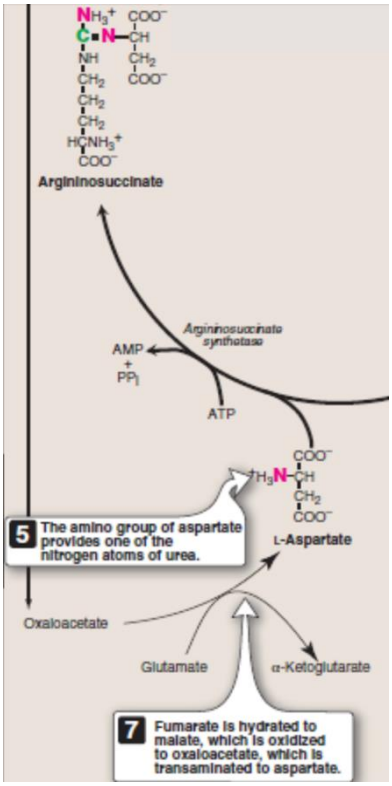
L-citrulline leaves mitochondria to cytosol.

Cytosol:

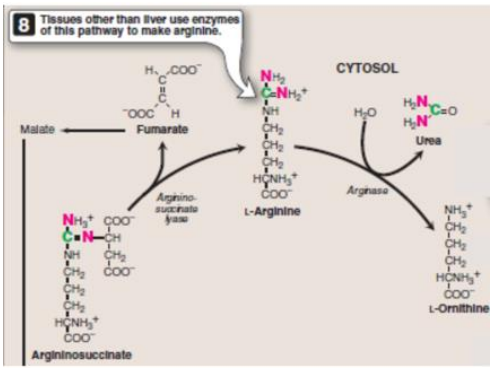
Building phase continues →L-citrulline interacts with aspartate producing Argininosuccinate the largest intermediate in urea cycle. This step is catalyzed by arginiosuccinate synthetase (ATP-dependent step) through transferring ATP to AMP and pyrophosphate. Recall that Aspartate aminotransferase (AST) favors the backward reaction Æ 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 Notice that: carbamoyl phosphate consists of carbonyl, phosphate, and amine group. Source of CO2 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. OCT enzyme is the most common urea cycle enzyme effected by genetic mutations. L-ornithine is non-coding AA.

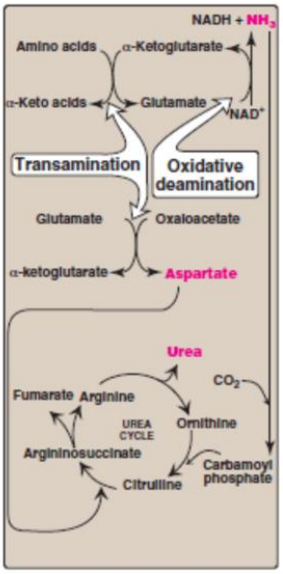
Transfer amino group from glutamate to oxaloacetate forming aspartate that supplies the previous step. 2) L-Arginine (AA with a fork like structure consist of 2 amine groups). *Urea simply consisted of carbonyl attached to 2 amino groups. *Notice that the green carbon attached to 2 amine groups in arginine 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:

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 the urea cycle

N-Acetylglutamate 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.

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.

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 of Hyperammonemia:

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

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.