31

Biochemistry 2

Doctor 2018 | Medicine | JU

Sheet ○ Slides

Done By

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Contributed in the Scientific Correction

Obada froukh

Contributed in the Grammatical Correction

Obada froukh

Doctor

Diala

إلى كل عمو وخالتو بدرسوا الشيت....تحية طيبة وبعد،،

نظراً للظروف الراهنة أحيّكِ لوصولك الشيت 31 ،وطبعاً الشيت سهلة بس يا عمو وخالتو جهزوا كاسة نسكافيه أو قهوة أو أي شيء من هذا القبيل أو كاسة شاي أو عصير بارد أو كاسة مي أو كاسة فاضية واربطوا الأحزمة ولا حول ولا قوة إلا بالله.

زائد بنحبكم كثير دفعة القطاعات ولا تنسوا بتقدروا تسألونا بأي وقت، والحق ع المدرب. ☺

Quick recap:

*Amino acid has an alpha carbon which is connected to amino group, carboxyl group, hydrogen atom and R group which differs from an amino acid to another.

Sources of AAs:

*Diet (exogenous proteins).

*Degradation of proteins (endogenous proteins).

*De novo synthesis (synthesis of nonessential AAs from metabolic intermediates).

Depletion Routes:

*synthesis of another proteins.

*Conversion of AAs to other compounds such as (Glucose, fatty acids…. etc.)

* AAs consumed as precursors of nitrogen containing small molecules.

(input of proteins-whatever the source) = (output of proteins-whatever the route of depletion) = constant amount.

*Some amino acids can share the same metabolic pathway and other amino acids can have specific pathways that are related to their R groups.

*What is the first step of Amino Acids degradation??

سؤال صعب، سؤال يراوني
☺
الجواب تحت

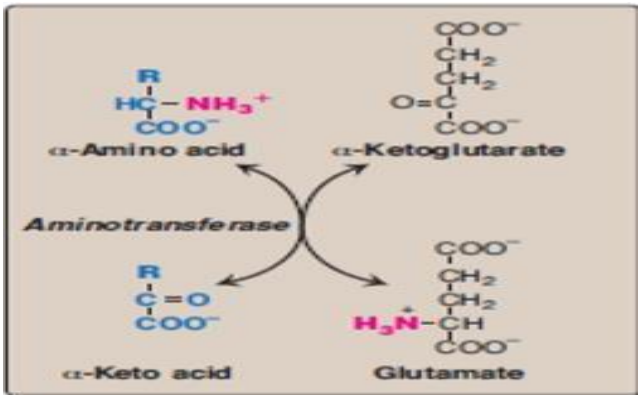
Removal Of Nitrogen From Amino Acids

Amino group is a source of nitrogen,which leads to form ammonia-highly toxic compound- so I have to deal with nitrogen and MAINTAINING THE BALANCE OF IT inside the body.The ammonia is converted to less toxic substance"UREA" prior to excretion in urine by the kidneys (I need to remove the amino group to avoid converting it to ammonia and the excess of ammonia in the blood will cause hyperammonemia).

The first step in the removal of amino group is:

TRANSAMINATION: the funneling of amino groups to glutamate.

*Any AA in the catabolism pathway will donate its Amino group to α-Keto acid specefically α-Ketoglutarate and once α-ketoglutarate acceptes the amino group, it becomes glutamate and the leftover of AA is α-Keto acid (depending on what type of AA we started with),so the products of transamination are GLUTAMATE&α-KETO ACID.



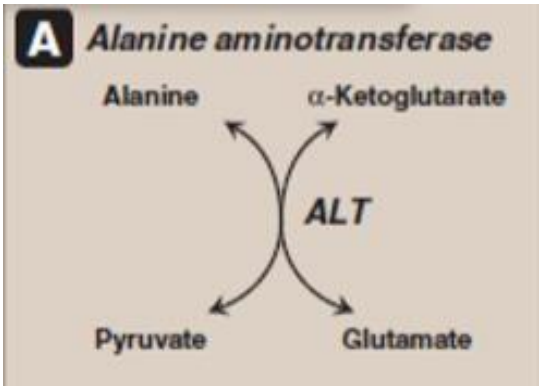
Glutamate produced by transamination can be oxidatively deaminated or used as an amino group donor in the synthesis of nonessential AAs

The equilibrium constant of transamination reactions is near one which means the reaction functions in both amino acid degradation and biosynthesis according to the cellular needs.

Substrate specificity of aminotransferase: each aminotransferase (AT) is specific for one or a few amino group donor.

The most 2 important ATs are:

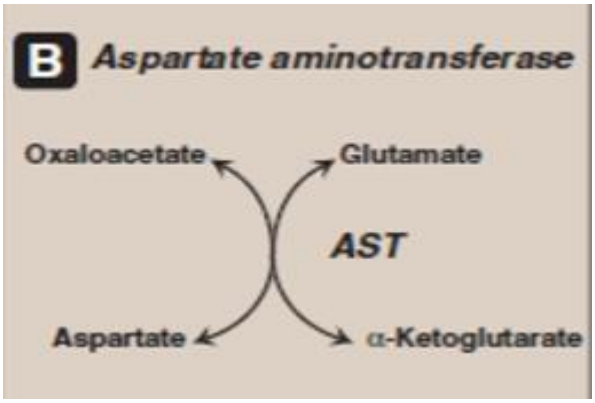
1- Alanine Aminotransferase (ALT):



is a transaminase enzyme catalyzes the transfer of amino group of alanine to α-ketoglutarate resulting in the formation of pyruvate and glutamate, it is found in many tissues, acts reversibly -ALT catalyzes the rxn in both direction depending on the need wheather I need to degrade it or I need it to synthesize protein but the preferable direction in amino acids catabolism toward pyruvate. Glutamate,in effect, acts as a collector of nitrogen from alanine.

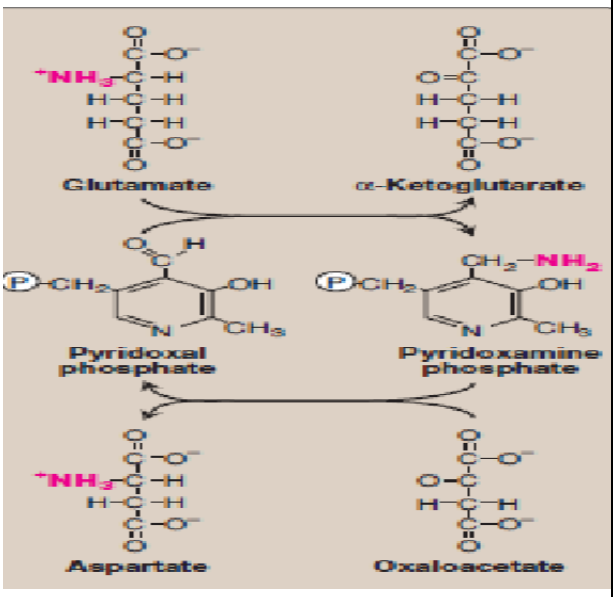
2- Aspartate Aminotransferase (AST):

"it's an exception for ATs work" transfers amino group from glutamate to oxaloacetate (prefer to consume oxaloacetate) resulting in the formation of aspartate, which is used as a source of nitrogen in the urea cycle, but it is not an intermediate of the cycle.



Mechanism of action of ATs:

All ATs require pyridoxal phosphate (PP) as a coenzyme (vitamin B6 derivative), AT transfers the amino group from amino acid to the pyridoxal part of the coenzyme, which is covalently linked to the amino group of a specific lysine residue at the active site of the enzyme to generate pyridoxamine phosphate, the pyridoxamine phosphate then reacts with an α-keto acid to form an amino acid and regenerate the original coenzyme.



Clinical hint: Diagnostic value of plasma aminotransferases:

AST and ALT have a diagnostic value when found in the plasma so when they are elevated it may indicate problems, such as: severe viral hepatitis, toxic injury, and prolonged circulatory collapse.

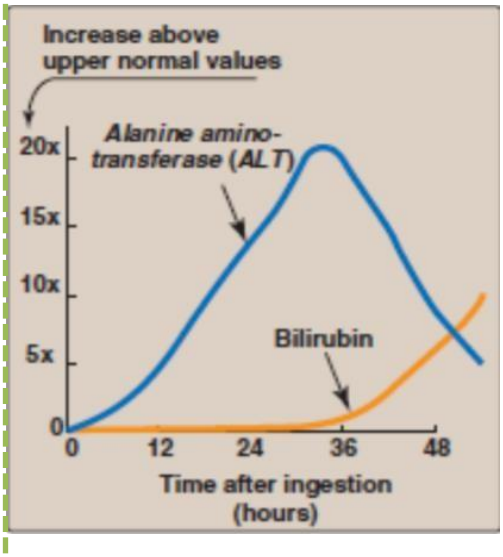
Increasing of ALT in plasma mainly related for Liver damage or Liver disease.

Why???!!! B/c it's specific for Liver

Increasing of AST it isn't necessary to have a problem in the liver it may MI or muscle disorder (Nonhepatic diseases).

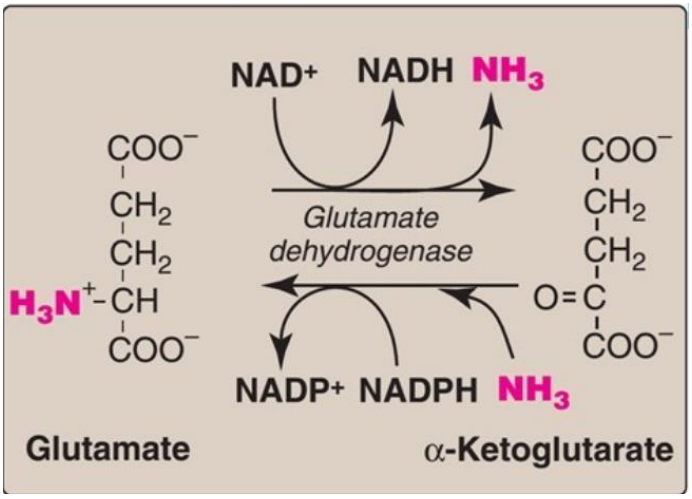
AST is more sensitive marker than ALT to liver function.

To test the liver function there is series of tests it isn't only one test.



The next step of removal of N from amino acid is →

Oxidative Deamination (complete removal of N):



Oxidative deamination by glutamate dehydrogenase results in the liberation of the amino group as ammonia (NH3), the ammonia generated in this process can then be neutralized into urea via the urea cycle, these rxns happen mainly in liver and kidneys.

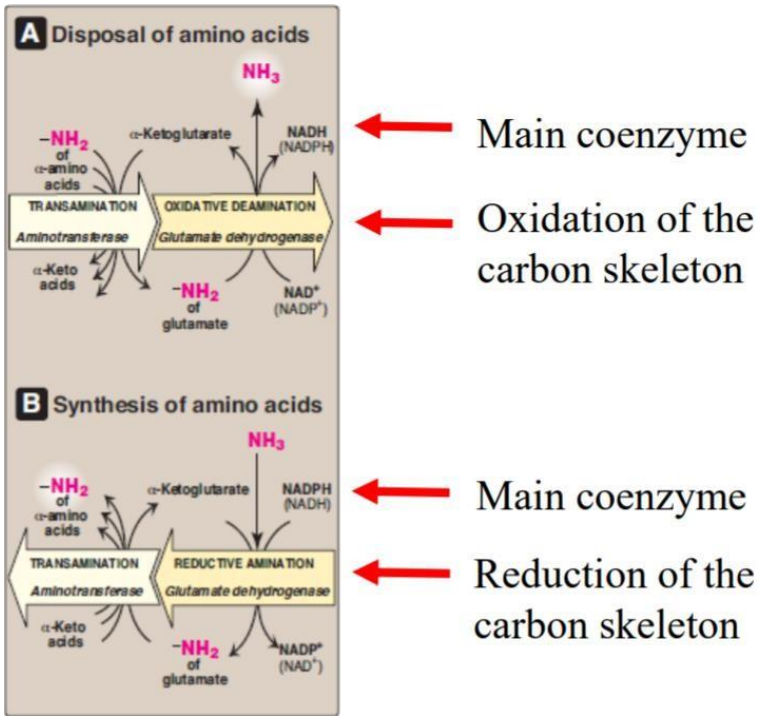
*They provide α-keto acids that can enter the central pathway of energy metabolism, and ammonia, which is a source of nitrogen in urea synthesis.

*GDH acts on both directions and the difference between forward and backward direction is the conzyme (NAD+ or NADPH).

***NAD+** is used mainly in oxidative deamination ,lossing of Ammonia coupled with the oxidation of the carbon skeleton.

***NADPH** is used mainly in reductive amination, gaining of ammonia coupled with the reduction of the carbon skeleton.

*Direction of rxns depends on the relative concentrations of glutamate, α-ketoglutarate, and ammonia, and the ratio of oxidized to reduced co-enzymes so after ingesting a meal that contains protein, glutamate levels in the liver will increase, thus, more AA degradation and NH3 formation.



Allosteric Regulator of GDH:

GTP: is an allosteric inhibitor because when it's present in high concentrations, it indicates high energy state in the cell. (so we don't need to degrade AAs to get energy because it is already high).

ADP: is an a activator, when ADP levels are high that indicates low level of energy in the cell, amino acid degradation will increase, facilitating energy production from the carbon skeletons derived from amino acids.

D-Amino Acids:

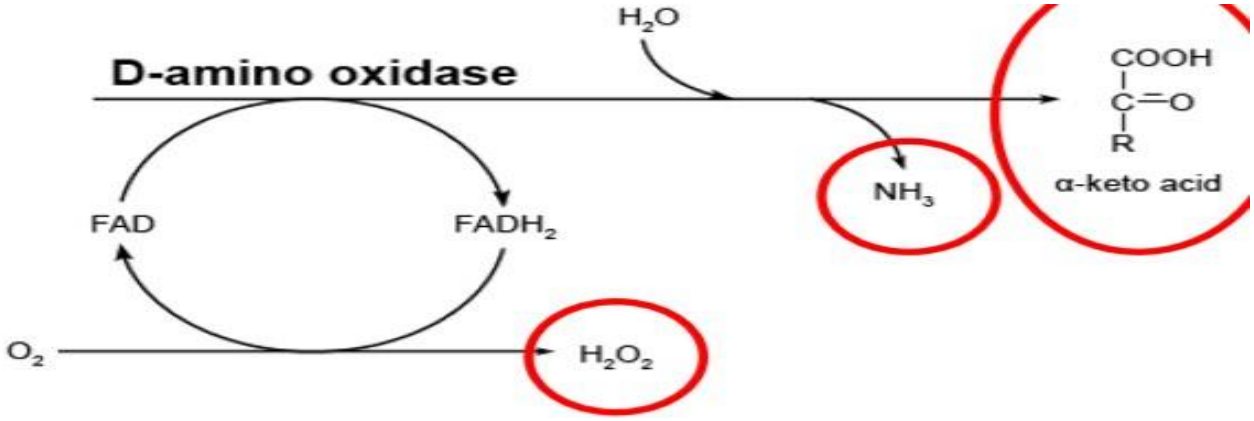
All amino acids that we deal with ,previously, are in L-conformation,what about D-Amino Acids??

*D-Amino acids are found in plants and in the cell walls of microorganisms, but they aren't used in the synthesis of mammalian proteins, anyway the D AAs are found in diet and they metabolised 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 producing α-keto acid and Ammonia and associated with the release H2O2.

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

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



Metabolism of ammonia:

*Ammonia is produced by all tissues during the metabolism of different compounds, NH3 is disposed of primarily by formation of urea in the liver.

The level of ammonia in the blood must be kept very low, (hyperammonemia is toxic to the CNS).*

*A portion of the free ammonia is excreted in the urine, but most is used in the synthesis of urea which is quantitatively the most important route for disposing of nitrogen from the body.

Sources of ammonia

The amino acids are not the only source of ammonia, there are other sources that can produce ammonia, which are:

1- **purines and pyrimidines:** In the catabolism of purines and pyrimidines, amino groups attached to the rings are released as NH3.
2- **Amines** (compounds contain amine group): Amines in the diet, and monoamines that act as hormones or neurotransmitters, give rise to NH3 by amine oxidase.
3- **intestinal bacteria:** 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.
4- **Glutamine:** Most of this ammonia is excreted into the urine as NH4 + (acid –base balance).

You may wonder! Why glutamine not glutamate?

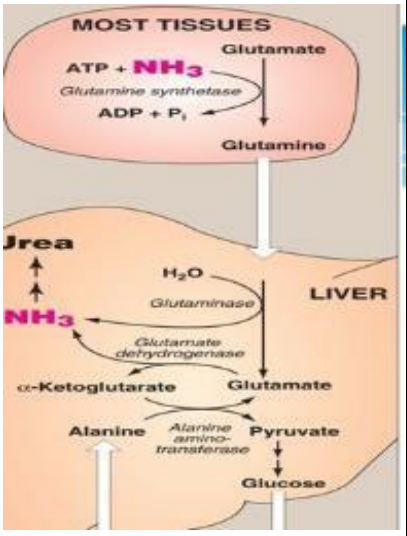
We previously convert all types of A.As into glutamate, so why do we have to convert glutamate into glutamine?

Because Gln works as a transporter for ammonia!

The previous reactions (transamination, deamination) happens in many places other than hepatocytes; because the aminotransferases (ALT, AST) are presented and expressed in many tissues, so the ammonia is produced in peripheral tissues, and it needs to be transported through the blood to hepatocytes for the reactions of urea cycle to be happened (because urea cycle only occurs in the liver).

So we have two mechanisms for ammonia transport:

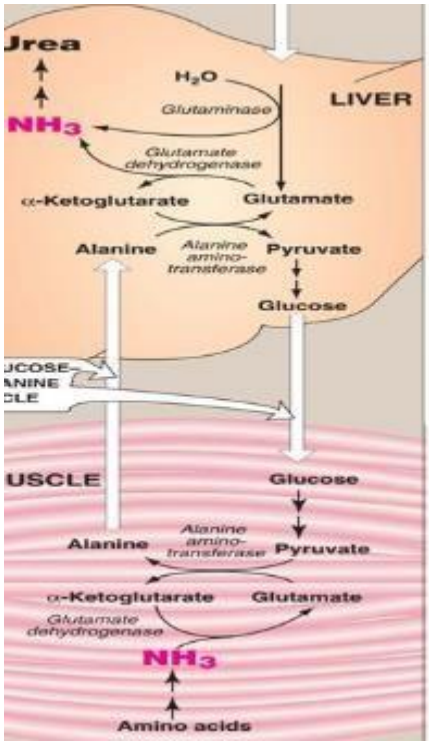
1. <u>The transport that happens from **most tissues**:</u>



Because the ammonia is highly toxic to the blood, it combines with **glutamate** to form **glutamine** (temporarily) by **glutamine synthetase** (so it requires energy).

The resulting glutamine is transported in the blood to the liver to be cleaved by **glutaminase** to produce glutamate and free ammonia, and glutamate is converted into **α-ketoglutarate** and free ammonia by oxidative deamination (That will produce two ammonia molecules).

2. The transport that happens from **muscles**:



Pyruvate undergoes transamination to form **alanine**, then the alanine is transported by the blood to the liver where it converted back to pyruvate by transamination.

- Pyruvate can be used in gluconeogenesis to produce glucose, then the glucose is transported by the blood to provide energy for muscles. This cycle is called **(glucose-alanine cycle)** ➔ It is one of the methods that our body uses to compensate for glucose deficiency (amino acid degradation).

NOW; the ammonia is in the liver and it's ready to be converted to urea by:

UREA CYCLE

The *urea cycle* begins in the *mitochondria* of hepatocytes and ends in the *cytoplasm*.

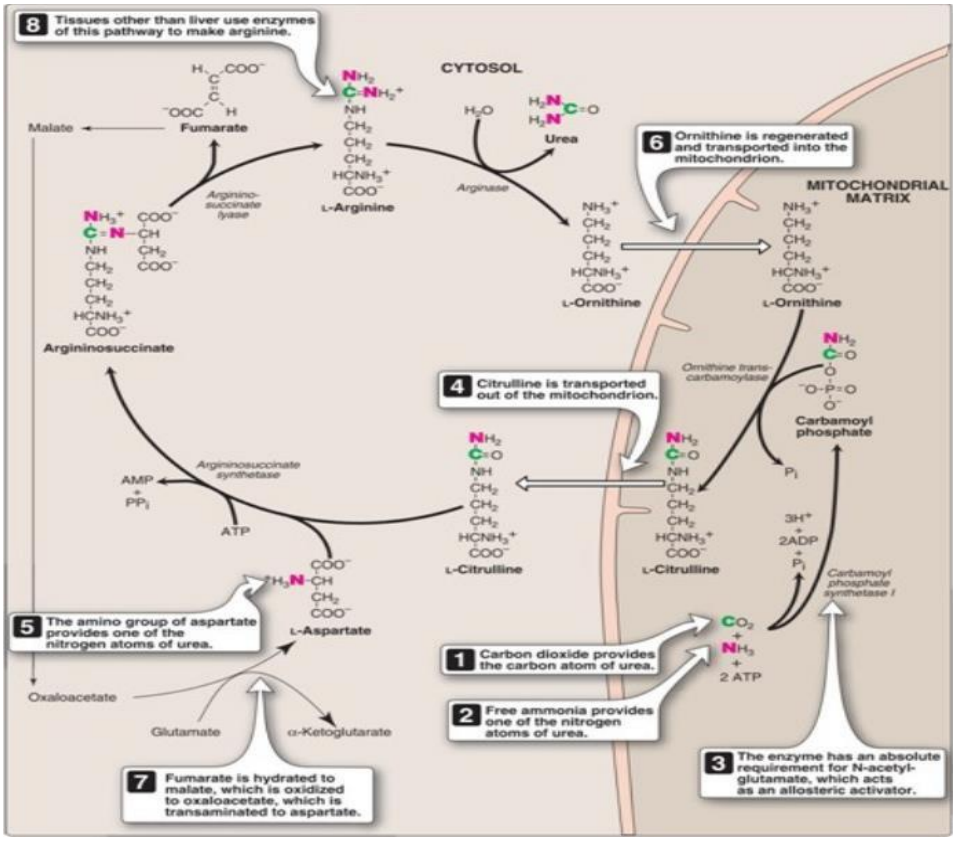
Urea is a major disposal form of amino groups derived from AAs.

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 of urea is derived from CO2, but the oxygen atom is derived from H2O.

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



PHASE I **Building Up a Reactive Intermediate**	**reactants**	**Type of the reaction**	**products**	**Enzyme that catalyze the reaction**	**comments**
	Ammonia (NH3) + HCO3- + 2ATP	***Combination reaction***	**carbamoyl phosphate** +2ADP +P_i	**carbamoyl phosphate synthetase I**	The process of creating the starting molecule **(carbamoyl phosphate)**
1	**carbamoyl phosphate (**the starting molecule) interact with **ornithine** (the last molecule)	***Combination reaction***	**Citrulline** Accompanied by the release of inorganic phosphate → P_i	**ornithine transcarbamylase** **OTC**	** ornithine is an amino acid, which is not found in protein structure.
2	**Citrulline is** released from mitochondria to cytosol and reacts with **Aspartate** that comes from outside the cycle.	***combination reaction***	**Argininosuccinate** The largest intermediate in urea cycle	**Argininosuccinate synthetase** Which needs energy by the consumption of ATP. ATP → AMP + PP_i	That's why the reaction of AST favors the production rather than the consumption of the Asp.
PHASE II **Cleavage and Hydrolysis** **3**	Degradation of **Argininosuccinate**	***Cleavage***	**Fumarate & arginine**	**argininosuccinate lyase**	Fumarate is Krebs cycle intermediate. It will enter the Krebs cycle and hydrated to malate that oxidized to oxaloacetate which Is transaminated to Asp (supply for urea cycle).
4	Hydrolysis of **Arg** by H_2O. **Arg** is a basic amino acid Which is characterized by the presence of two nitrogen molecules branched at the end.	***hydrolysis***	H_2O works as a source of oxygen And **Arg** as source of 2 nitrogen and carbon molecules **In UREA**	**Arginase -1**	***The remainder of the arginine is the ornithine (the last molecule) that will interact with carbamoyl phosphate (the starting molecule) And the cycle*** .

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 (use energy rather than produce), with a large negative ΔG

For each urea molecule:

1. Four high-energy P-bonds →from 3ATP molecules 2 from producing carbamoyl phosphate and the other one from producing Argininosuccinate.

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.

N-Acetylglutamate is synthesized from acetyl coenzyme A and glutamate by N-acetylglutamate synthase

Arginine is an activator for N-Acetylglutamate synthesis

Arginine activates → **N-Acetylglutamat**e synthesis that will activates → **carbamoyl phosphate** synthesis → then activates the **urea cycle**.

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.

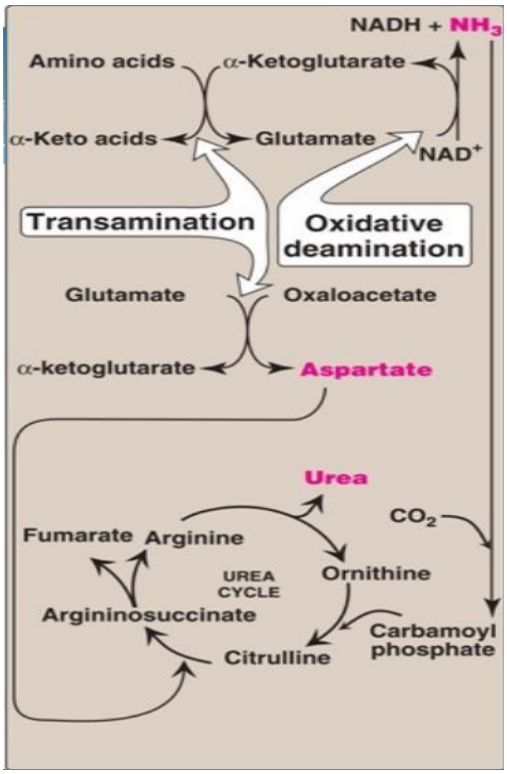
Clinical hint: Hyperammonemia

If liver function is compromised (genetic defects of the urea cycle or liver disease), NH3 blood levels can rise above 1,000 µmol/L (medical emergency)

** The levels of serum ammonia are normally low (5–35 µmol/L).

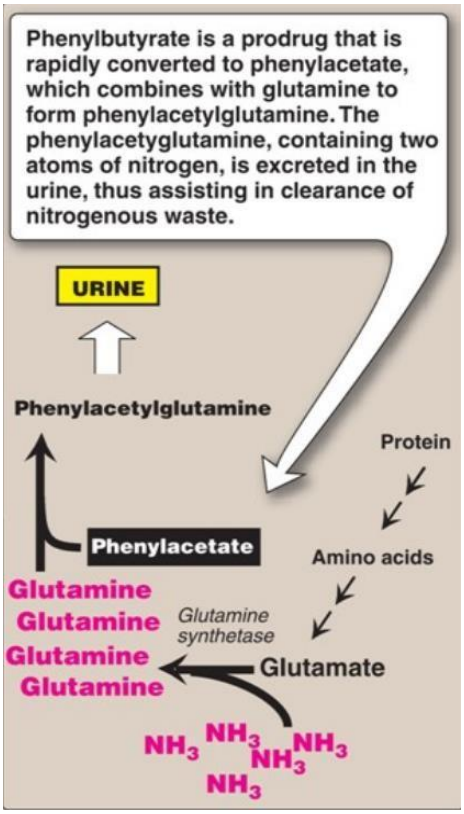
So the ammonia molecules that reach the liver are not efficiently converted to urea, so they will accumulate in high concentrations and reach the toxic level.

- 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.

The most common Genetic deficiencies of urea cycle enzymes is the Ornithine transcarbamylase OTC deficiency, which is X-linked (males).

All of the other urea cycle disorders follow an AR inheritance pattern.

Treatment:

- ✓ Restriction of dietary protein.
- ✓ Administration of compounds that bind covalently to AAs, producing nitrogen-containing molecule that are excreted in the urine.

Don't listen to other stories, make your own one