Biochemistry Team 435

جامعة الملك سعود
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UREA CYCLE

* Please check out this link to know if there are any changes or additions.

Revised by

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Color index: Important | Doctors notes | Further explanation.

OBJECTIVES:

-Understand the reactions for removal of α-amino group of amino acids and formation of ammonia .
-Identify the importance of blood transport of ammonia to the liver in the form of glutamine/alanine.
-Understand the importance of conversion of ammonia into urea by the liver through urea cycle.
-Identify urea as the major form for the disposal of amino groups derived from amino acids.
-Identify the causes (hereditary & acquired), clinical manifestations and management of hyperammonemia.

BACKGROUND

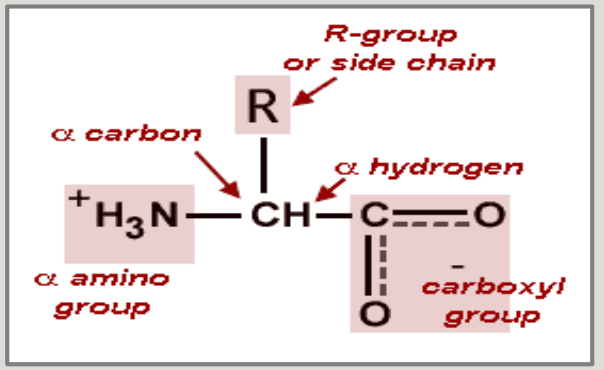
- Unlike glucose (stored as Glycogen) and fatty acids (stored as Triacylglycerol), amino acids are **NOT** stored by the body.
- Amino acids in excess of biosynthetic needs are degraded.

Degradation of amino acids involves:

- **Removal of α-amino group** → **Ammonia (NH_3)**
- **Remaining carbon skeleton** → **Energy metabolism**

Amino acids are very stable and they're protected from degradation by the presence of alpha amino group. Once alpha amino group is removed it becomes unstable & very active, so its converted to energy.

- As we know Amino acids composed of: Carbon skeleton and **α-amino group.**
- The presence of the **α-amino group** protect the amino acid from Oxidative breakdown.
- Removing the **α-amino group** is essential for producing energy from any amino acid.
- Removal of this **α-amino group** will convert the amino acid (Nitrogen) into Ammonia which is toxic and carbon skeleton which will be used in energy metabolism.



This picture is Extra

REMOVAL OF ALPHA-AMINO GROUP, FORMATION OF AMMONIA AND ITS TRANSPORT TO LIVER

A: Removal of α-amino group of amino acids and formation of ammonia:

- 1-Transamination to **glutamate**
- 2-Oxidative deamination of glutamate

- All the amino group from all amino acids are transferred to ONE single molecule which is **glutamate**.
- Once all amino groups are in glutamate, they're removed as ammonia by oxidative Deamination .

B: Blood transport of ammonia <u>into liver:</u>

- 1-in the form of **glutamine** (**most tissue**)
- 2-in the form of **alanine** (**muscle**)

- Ammonia produced in tissues is very toxic. It's like a criminal that can't be transported freely!
- It needs a police car (glutamine & alanine) to deliver it to the police station(the LIVER) to be disposed.
- **Why the liver?** Because it's the only place which have the enzymes needed for turning ammonia into urea (non-toxic)

A- REMOVAL OF ALPHA-AMINO GROUP & FORMATION OF AMMONIA

Amino groups of amino acids are **funneled** to **glutamate** by **transamination** reactions with α-ketoglutarate

Glutamate is unique. It is the **ONLY** amino acid that undergoes rapid oxidative deamination.

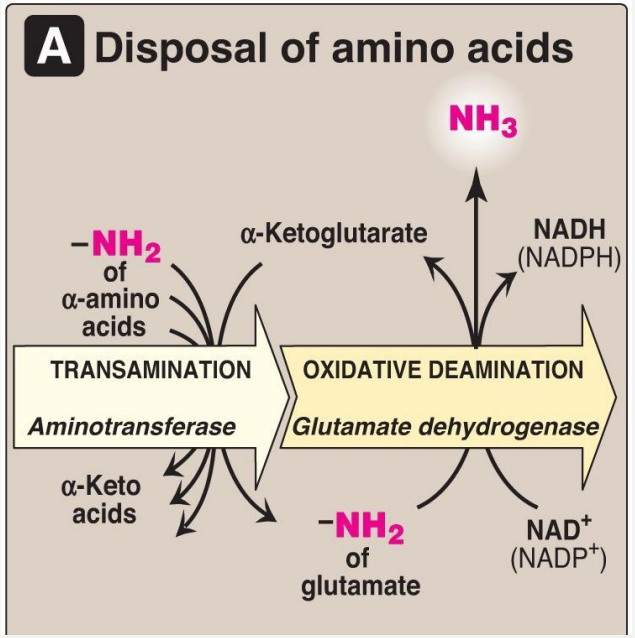
Oxidative deamination of glutamate will: release NH_3 and re-generate α-ketoglutarate.

1- Transamination:

- The first step in the catabolism of the amino acid is the transfer of their **α-amino group** to **α-Ketoglutarate (the acceptor),** producing an **α-Keto acid** and **Glutamate.** This transfer of amino groups catalyzed by **Aminotransferase. (or Transaminase).**
- The Glutamate can be Oxidatively deaminated (Rapidly).

2-Oxidative deamination:

- Result in the release of **the amino group** as **free ammonia**, provide **α-Keto acid** that can enter the central pathways of energy metabolism and ammonia, which is a source of nitrogen in hepatic urea synthesis.
- Didn't understand? Read the next slides :)



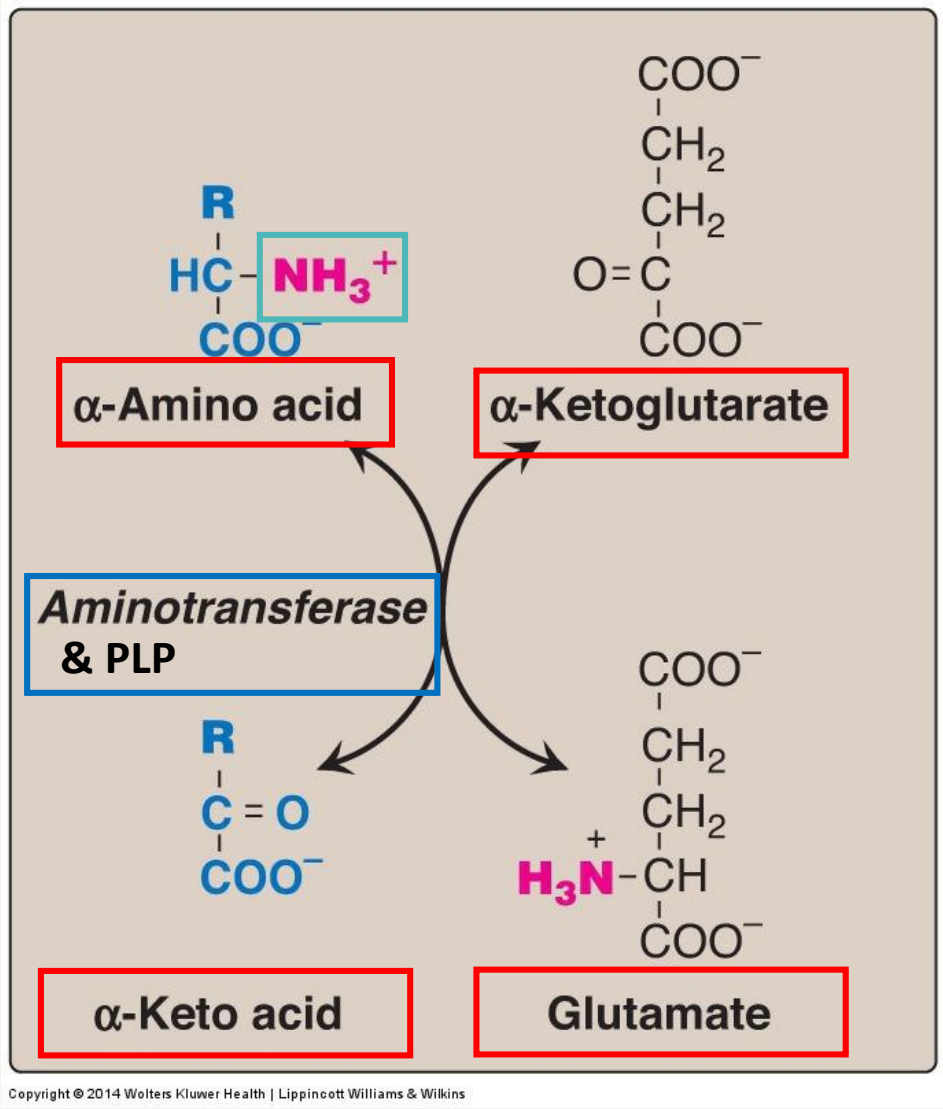
1-TRANSAMINATION

-This is a reaction between **α-amino acid** and **α-Ketoglutarate** to form **α-keto acid** and **Glutamate,** by the enzyme: **Aminotransferase and** the co-enzyme: **PLP.**

- α-amino group (NH3) transported from the amino acid to the **α-ketoglutarate .**

- Amino acid give → α-keto acid
- α-Ketoglutarate give → Glutamate

➢ **PLP: Pyridoxal phosphate, a co-enzyme that is derived from vitamin B6 (the active form of vit B6).**



TRANSAMINATION BY ALT & AST

- Same as last slide but here we'll take Alanine and Oxaloacetate as examples of amino acids:

A. Alanine Aminotransferase (ALT): (Reversible reaction)

1- α-amino group transported by ALT and PLP from **Alanine** (donor) to **α-Ketoglutarate** (acceptor).

2- Forming **Pyruvate** (carbon skeleton) (from alanine), **and Glutamate** (from α-Ketoglutarate).

B. Aspartate Aminotransferase: (Reversible reaction)

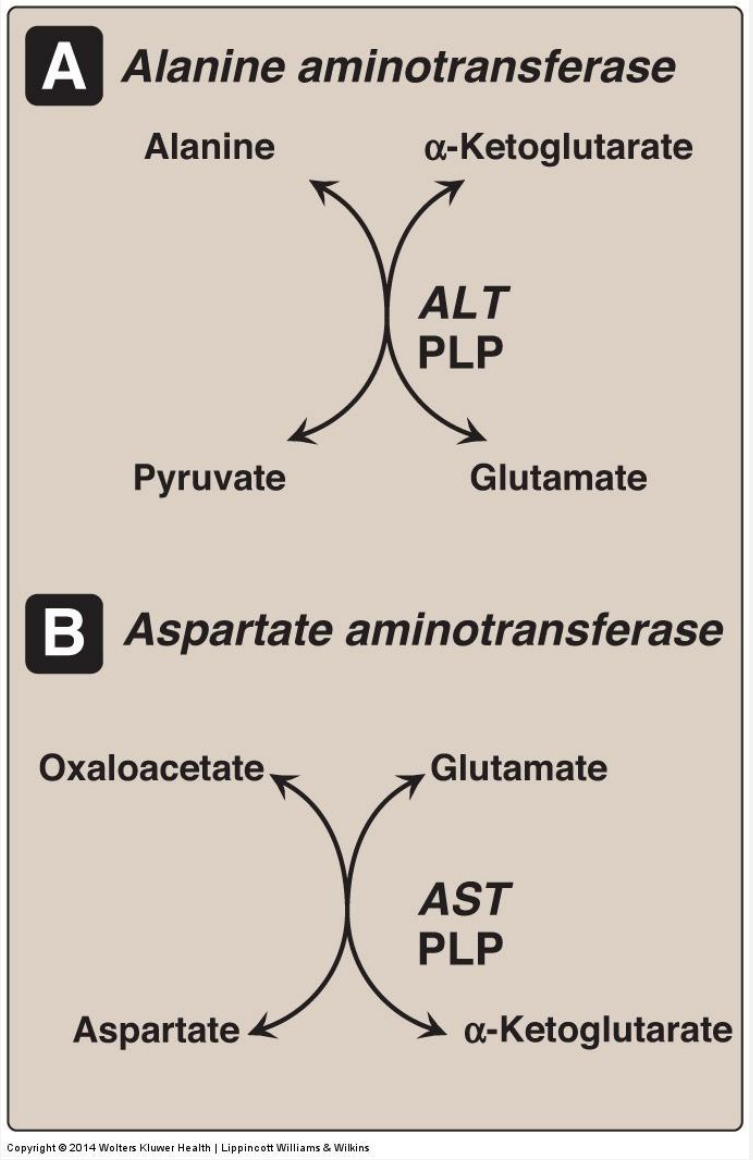
-This reaction is an exception to the rule that aminotransferases funnel amino groups to form glutamate.

1-During amino acid catabolism, AST transfers amino groups from **Glutamate** to **Oxaloacetate.**

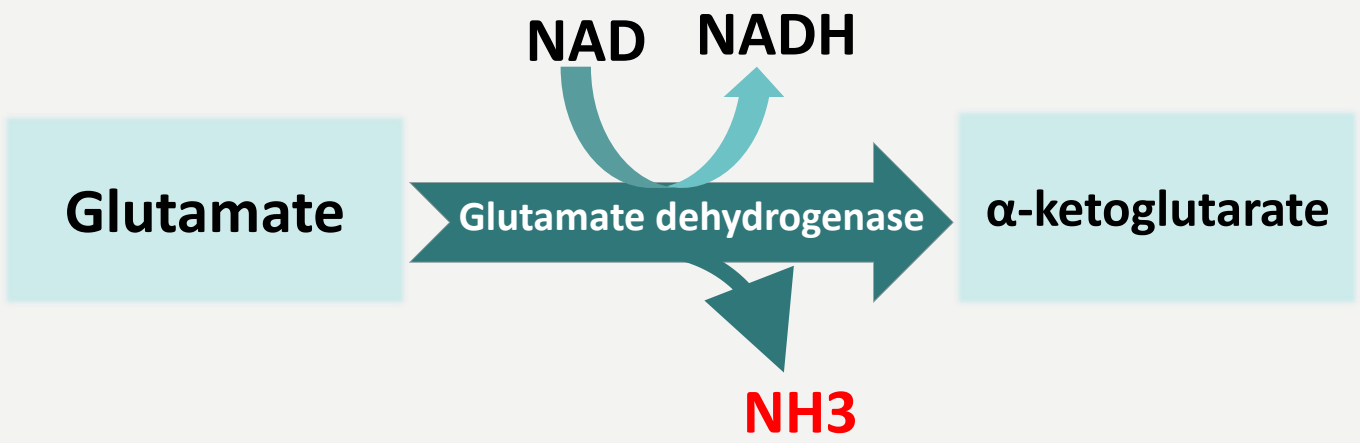
2-Forming **Aspartate** and **α-Ketoglutarate**.

يعني -عكس الباقين- يأخذ القروب من الجلوتاميت بدال ما يعطيها إياه.

Normally, aspartate is the donor. It donates its amino group to alpha-kitoglutarate to form glutamate. BUT in urea cycle it works the other way, producing ASPARTATE because we need it more than glutamate in urea cycle. urea has 2 nitrogen (one from ammonia the other is from **aspartate**) **.**

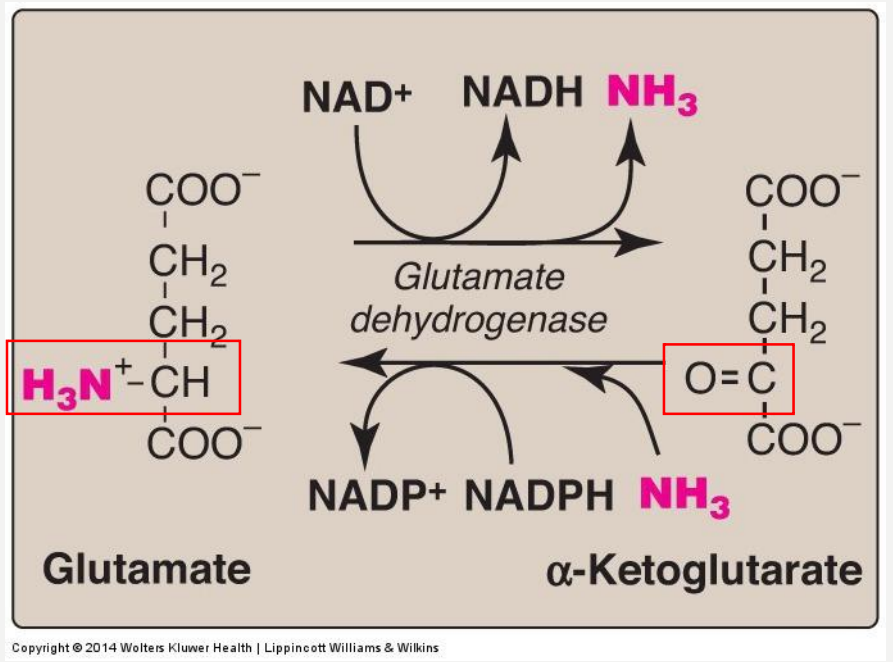


OXIDATIVE DEAMINATION



- This reaction occurs primarily in kidneys and liver.
- Glutamate is unique, because it is the only amino acid that undergoes rapid oxidative deamination.
- The Sequential action of **transamination** (resulting in the transfer of amino groups from most amino acids to α-ketoglutarate to produce glutamate) and **Oxidative deamination of Glutamate (regenerating α-ketoglutarate)** provide a pathway whereby the amino groups can be released as **ammonia**.

This picture is Extra



*لاحظوا المربعات الحمراء في الصورة ، هي الي تمثل الفرق بين الجلوتاميت والألفا كيتوجلوتاريت.
خلال هذا التفاعل ، الأمونيا راح تنفصل عن الجلوتاميت وتشيل معها ذرة هيدروجين وتعطيها "ناد" كشكر له على تحريرها.

Notice that α-Amino group are charged, Ammonia are not.
Charged molecules are difficult to diffuse through cell membrane. As for Ammonia, it has no charge! Therefore it can easily diffuse to tissues (very toxic). it can lead to mental retardation if it gets into the CNS. That's why it's not transported freely to the liver!

B- TRANSPORT OF NH_3 FROM PERIPHERAL TISSUES INTO THE LIVER

- Ammonia is produced by all tissues, and **the main disposal way is via formation of urea in liver**.
- Blood level of NH_3 must be kept very low, otherwise, hyperammonemia and CNS toxicity will occur **(NH_3 is toxic to CNS)**
- To solve this problem, NH_3 is transported from peripheral tissues to the liver **via formation of:**

-Glutamine (most tissues)

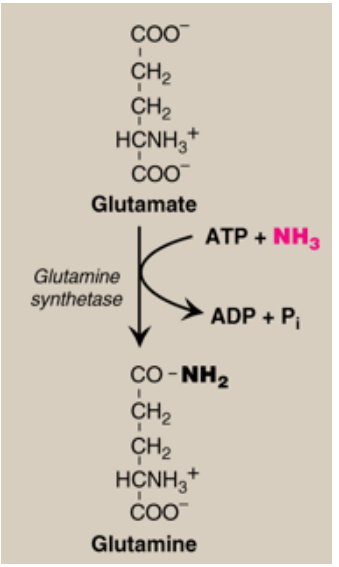
NOT glutamate!!

From most peripheral tissues:

NH_3 is transported Into the liver through forming glutamine by **glutamine synthetase**.

❖ This reaction uses glutamine Synthase to combine ammonia with Glutamate to form Glutamine (nontoxic transport of ammonia), then this glutamine is transported in the blood to the liver where it is cleaved by Glutaminase to produce Glutamate nd free ammonia → Ammonia converted to Urea (nontoxic).

لاحظوا أننا هنا أضفنا الأمينو قروب إلى الجلوتاميت فتكون عندنا الجلوتامين، يعني : ألفا كيتوجلوتاريت +أمينو قروب ← جلوتاميت.
جلوتاميت + أمينو قروب ← جلوتامين.



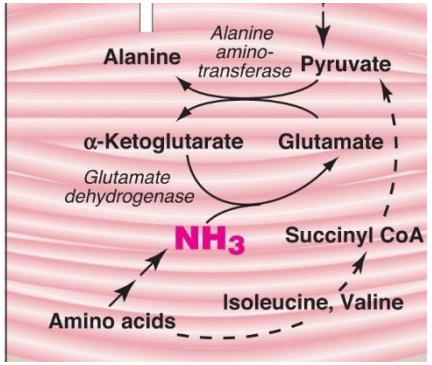
Alanine (muscle)

From the muscle:

First, NH_3 will be transferred into α-ketoglutarate to form glutamate

Then, glutamate will give its amino group to pyruvate to form alanine by ALT (alanine aminotransferase)

Therefore, NH_3 is **transported from muscle into the liver through forming alanine**



☺: بيجي بالإختبار
يتم نقل الأمونيا من العضلات للكبد عن طريق؟

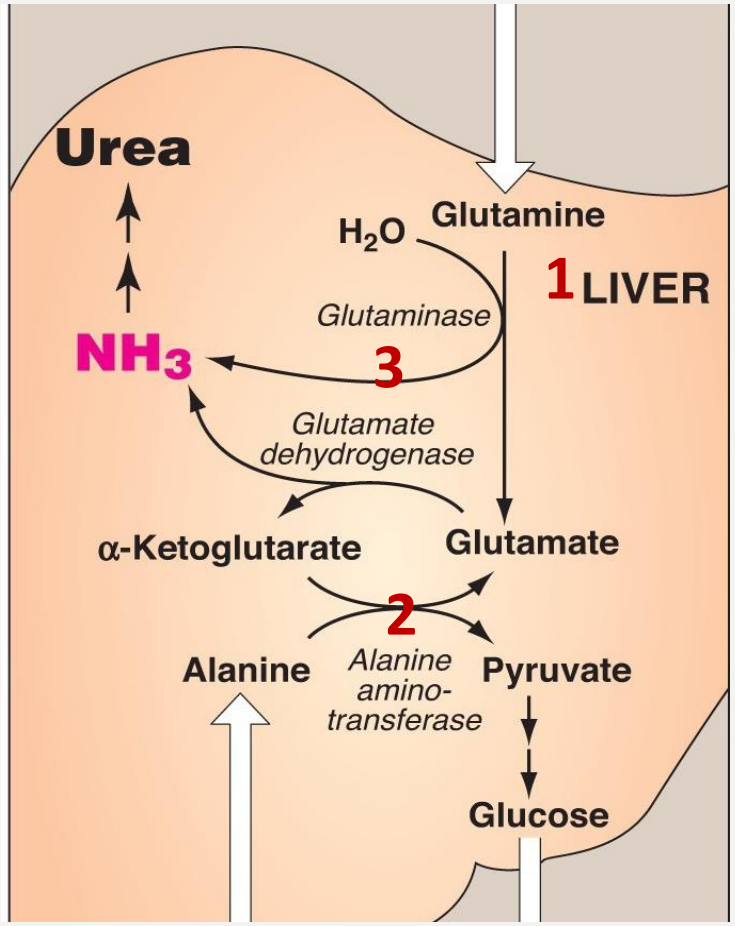
RELEASE OF AMMONIA IN THE LIVER

In the liver:

1. **Glutamine** is converted back into **glutamate** by **glutaminase**.

2. **Alanine** will give its amino group to **α-ketoglutarate** to form **glutamate** by **ALT.**

3. **Glutamate** is converted into **α-ketoglutarate** and releasing NH_3 by **glutamate dehydrogenase**.



في الليفر، الألانين والجلوتامين بيرجعون مرة ثانية إلى جلوتاميت:
- الجلوتامين راح يتحول لجلوتاميت ويطلع أمونيا.
- الألفا كيتوجلوتاريت يأخذ أمينو قروب من الألانين ويتحول لجلوتاميت، والألانين راح يتحول بدوره إلى بايروفيت (ثم إلى جلوكوز ، ثم يرجع للعضلات عشان يعطيها طاقة وهكذا).
- في النهاية ، كل الجلوتاميت المتكون راح يتحول مرة أخرى إلى ألفا كيتوجلوتاريت وينتج لنا الأمونيا الي تدخل في اليوريا سايكل.

The enzymes here are very important. Memorize them.
Question can be: How can we get ammonia in hepatocyte?

•Urea is the major form for disposal of amino groups derived from amino acids.

1.What is "Urea"?

Urea cycle

Site

•**Urea cycle occurs in the liver.**

Formation

•One nitrogen of urea is from NH_3 and the other nitrogen from aspartate.

يعني كل ذرتي النيتروجين كان مصدرها الأول هو الجلوتاميت. ليه؟ لأن الاسبرتيت متكون من الجلوتاميت والامونيا جابها الجلوتاميت.
مافهمتوا؟ راجعوا الترانزامينشين :)

Fate of urea

To the kidneys

Most of urea will take this route and get excreted in the **urine**

Urea is mainly excreted by kidneys.

A small part goes to the intestine, The bacteria in intestine has urease activity. It will break urea into ammonia and Co2. this ammonia is either lost in feces or reabsorbed into blood and transported by glutamine to the liver to synthesis urea again.

To the intestine

- A small portion will go to the **intestine** and get catalyzed by the enzyme urease to release : **NH_3 + CO_2**.
- The **NH_3** then will be lost in the feces or will be reabsorbed into the blood and go to the liver again and so on.

❖ **The action of intestinal urease to form NH_3 is clinically significant in renal failure:**

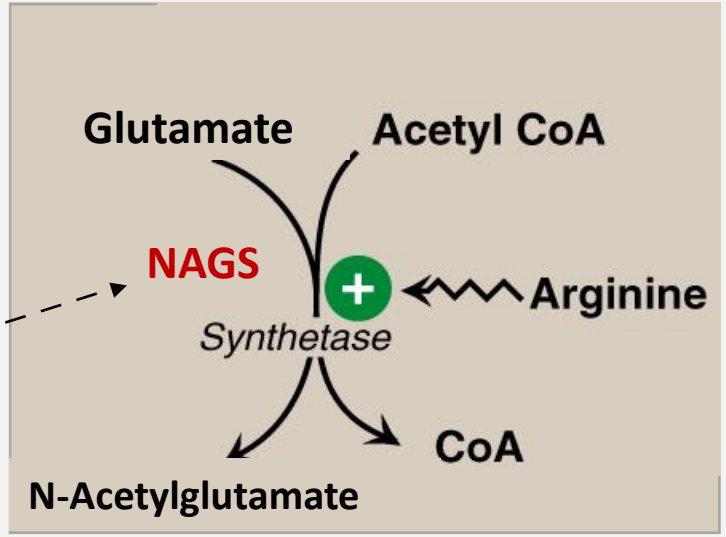
Renal failure (thus no excretion of urea) → increased Blood urea → increased urea to intestine (so urease will act on it) → increased NH3 blood level → **Acquired hyperammonemia.**

UREA CYCLE (IMPORTANT SLIDE)

The five enzymes of urea cycle

- **Carbamoyl phosphate synthetase I (CPSI)**
- **Argininosuccinate lyase (ASL)**
- **Argininosuccinate synthase (ASS)**
- **Ornithine transcarbamoylase (OCT)**
- **Arginase**

- CPSI is the **RATE-LIMITING ENZYME** of urea cycle (so its presence is necessary for the cycle to begin).
  - ❖ **Allosteric activator of CPSI**: N-Acetylglutamate.



- ❖ **N-Acetylglutamate is synthesized by:**
  N-Acetylglutamate synthetase (NAGS) in presence of **arginine.**
- ❖ **Treatment of NAGS deficiency: Carbaglue**, a CPS1 activator. which is a drug that mimics the action of NAGS.

The most important enzyme in urea cycle is CPS1. it's the rate limiting enzyme. Either it accelerate the synthesis of urea or slows it down. **How ?**
CPS1 will be activated by:
N-acetylglutamate: It's a product that increases when there's a lot of arginine by diet. That means when we eat arginine it will activate the synthesis of N-acetylglutamate by NAGS enzyme. Thus, activating CPS1 to speed up the synthesis of urea to get rid of ammonia.

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UREA CYCLE

- Urea cycle happens **in the liver.** Part of it takes place in mitochondria and the rest in cytosol.
- As mentioned, The urea's structure contains two nitrogen(one from ammonia and the other from aspartate).
- **The cycle starts with:**
  - **1**- The first nitrogen from ammonia combines with carbon dioxide to form carbamoyl phosphate by the enzyme CPS1.
  - **2**- Carbamoyl phosphate will condense with L-ornithine to form L-citrulline by OCT enzyme.
  - **3**- L-citrulline will diffuse from mitochondria to cytosol to act with L-aspartate (which is the second nitrogen forming urea) by ASS enzyme to give us Argininosuccinate
  - **4**- By another enzyme called ASL we'll have L-arginine & fumarate.
  - **5-** the unique enzyme of the liver ARGINASE will finally form urea! and regenerate ornithine.

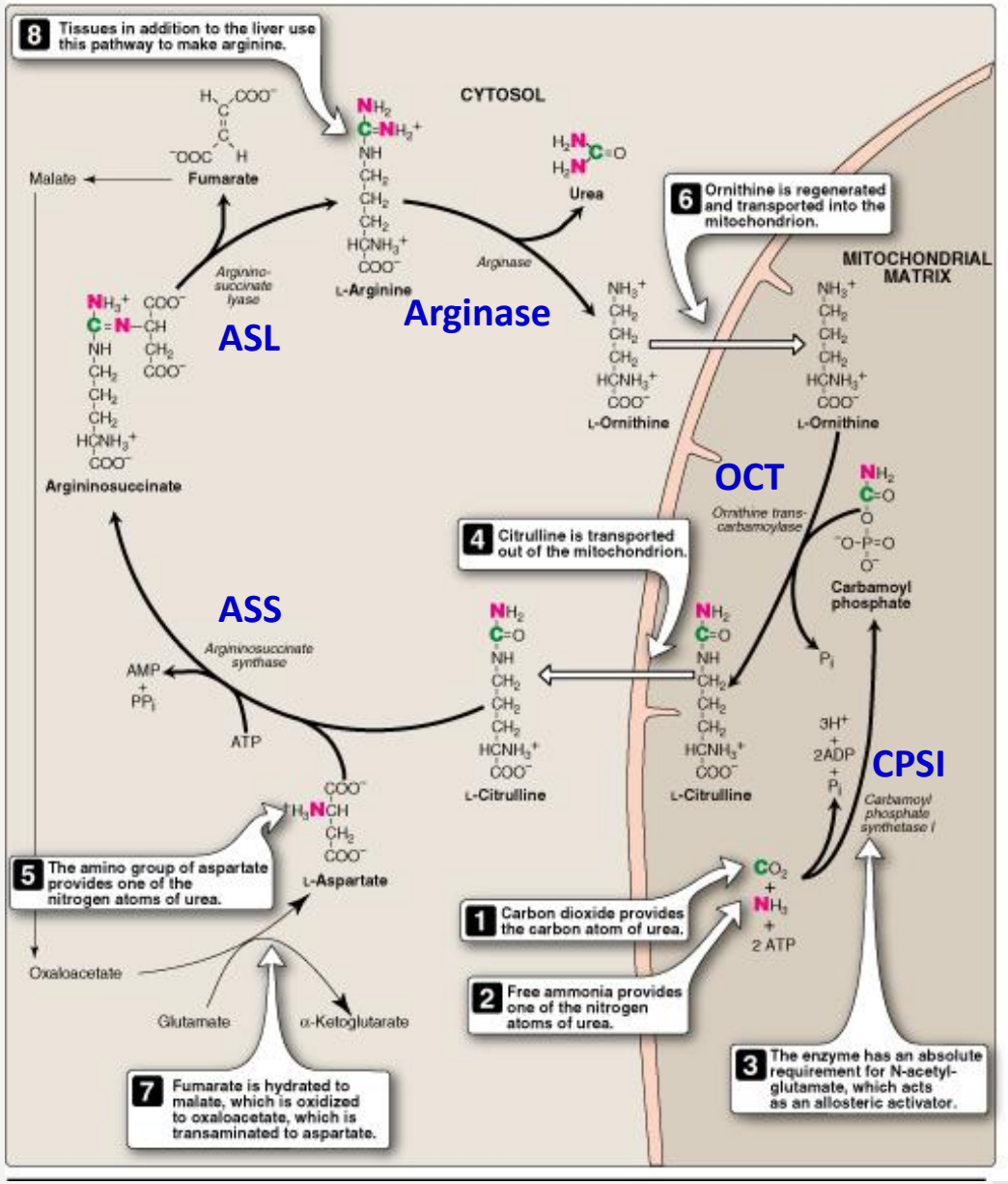
اهم شيء تعرفونه عن urea cycle:

1-urea cycle has 5 enzymes.

2- CPS1 is the rate limiting enzyme.

3- The most common enzyme to be deficient is OCT.

4-the activator of urea cycle is N-acetylglutamate.

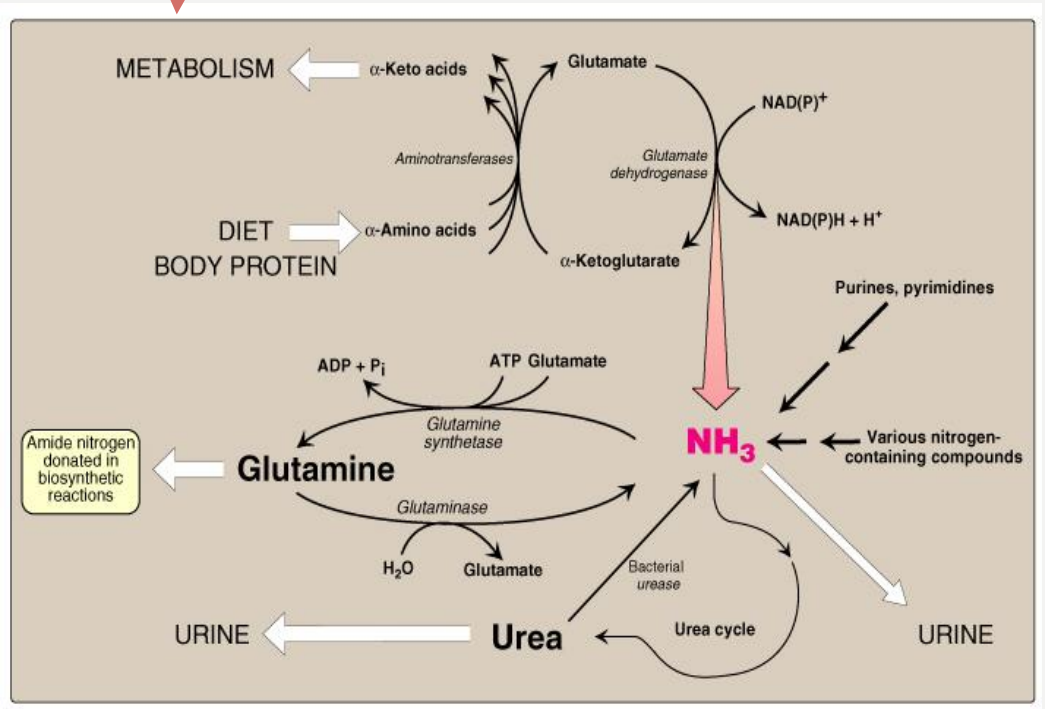


Urea cycle

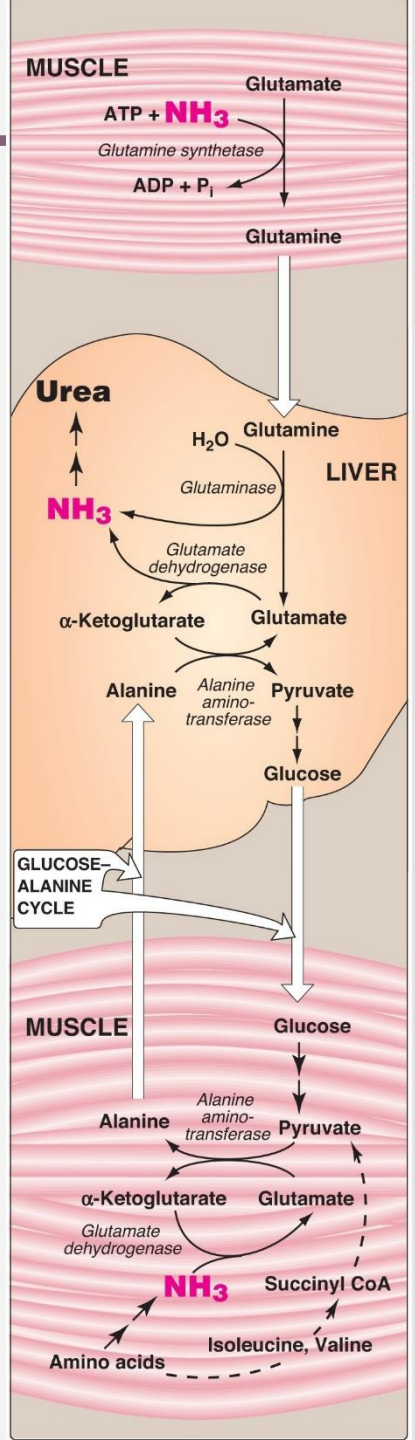
SOURCES AND FATE OF AMMONIA

- These pics summarize the "journey" of the ammonia and urea:

Blood transport of NH3 from peripheral tissues (in the form of glutamine and alanine) into the liver and the release of NH3 back in the liver to start the urea cycle.



Normal blood level of ammonia: 5 – 50 µmol/L



Hyperammonemia

Aquired hyperammonemia

Due to :

1- Liver diseases

- **Acute:** e.g. Viral hepatitis or hepatotoxic.
- **Chronic:** Cirrhosis by hepatitis or alcoholism.

2- Renal Failure

Inherited hyperammonemia

Genetic deficiencies of any of **the 5 enzymes** of urea cycle or the activator enzyme for CPSI.

Ornithine transcarbamoylase (OCT)

- **X-linked recessive.**
- **Most common of congenital hyperammonemia .**
- **Marked decrease of citrulline and arginine.**

CPSI, ASS, ASL, arginase or NAGS.

Deficiencies of any of these enzymes **autosomal recessive.**

Cirrhosis may result in formation of collateral circulation around the liver → portal blood is shunted directly into the systemic circulation and does not have access to the liver → the conversion of ammonia to urea is severely impaired →elevated levels of ammonia.

❖ Clinical Presentation of Hyperammonemia:

- Lethargy and somnolence (drowsiness)
- Tremors
- Vomiting and cerebral edema
- Convulsions
- Coma and death

MANAGEMENT OF HYPERAMMONEMIA

Management of Hyperammonemia

1-Protein restriction

2-Volume repletion to maintain renal function
Use 10% dextrose in water but **limit the use of normal saline.**

3-Ammonia removal by **hemodialysis** and/or **drugs**.

4-Avoid drugs that **increase protein catabolism** (eg, glucocorticoids) or **inhibit urea synthesis** (eg, valproic acid), or **have direct hepatotoxicity.**

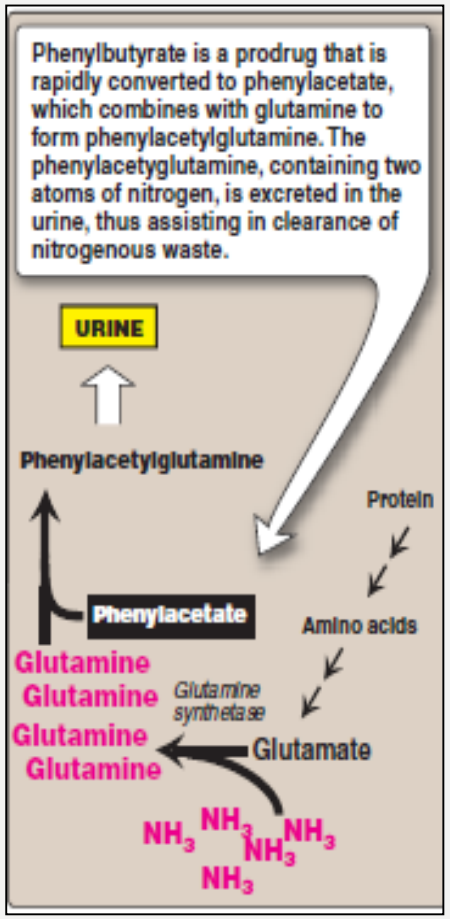
When a patient presents with hyperammoniemia and they checked the 5 enzymes of urea cycle and they were all normal, they'll have to check the NAGS enzyme! Because when there's no N-acetylglutamate, the urea cycle will be very slow leading to accumulation of ammonia

When a baby comes with hyperammoniemia, the first enzyme they check is OCT before anything else.

Treatment of hyperammonemia

Activators to CPSI (**Carglumic acid "Carbaglu"**)

Drugs that scavenge ammonia by creating an alternate pathway to excrete N2- precursors:



Oral sodium phenyl butyrate (Buphenyl)

- Buphenyl is a Prodrug that is converted to phenylacetate.
- Phenylacetate condenses with glutamine forming phenylacetylglutamine that is excreted in urine.

البوفينل بيتحول إلى فينايل اسيتيت ثم راح يرتبط مع الجلوتامين ويكون فينايل اسيتايل جلوتامين (يحتوي على ذرتين نيتروجين) ويخرج مع البول ، بمعنى أن البوفينل يوفر طريقة أخرى للتخلص من النيتروجين بدون تكوين الامونيا

I.V. Sodium phenylacetate & sodium benzoate (**Ammonul**)

I.V. Arginine

for all UCDs (urea cycle enzyme deficiency) except UCD due to arginase deficiency which is the enzyme that act on arginine, so deficieny in this enzyme will lead to accumulatation of arginine in blood (argininemia).

Summary (IMORTANT Notes) – الملخص الشامل موجود بالداونلود سنتر

How do we get rid of amino acids?

A-Transmination:	B-Oxidative deamination:
By: ALT & AST. Amino groups of amino acids are **funneled to glutamate** by transamination reactions with α-ketoglutarate	**By: Glutamate dehydrogenase.** - **NH3** will be produced. - Regeneration of α-ketoglutarate.

Transport of NH_3 from peripheral tissues into the liver

Glutamine (from most tissues → liver)	Alanine (from muscles → liver)

In the liver (one of the questions: how do we get ammonia in hepatocytes?):

Glutamine is converted back into **glutamate** by **glutaminase**	Alanine will give its amino group to **α-ketoglutarate** to form **glutamate** by **ALT.**	Glutamate is converted into **α-ketoglutarate** and releasing NH_3 by **glutamate dehydrogenase**

Urea Cycle enzymes:

Carbamoyl phosphate synthetase I (CPSI)	Argininosuccinate lyase (ASL)	Argininosuccinate synthase (ASS)	Ornithine transcarbamoylase (OCT)	Arginase
RATE-LIMITING ENZYME **Allosteric activator:** N-Acetylglutamate. **This activator is synthesized by:N-Acetylglutamate synthetase (NAGS)** in presence of **arginine.** **NAGs defiency is treated by: Carbaglue.**			**The most common enzyme to be deficient .**	

Fate of urea: **A- To the kidneys (Mostly).** B- To the intestine.

Hyper-ammonemia	
	A- Aquired (liver diseases – Renal failure) . **B- Inherited** (all 5 enzymes are autosomal recessive **except OCT which is X-linked recessive!!!!)**
	Treatement: **Oral sodium phenyl butyrate (Buphenyl)** - a Prodrug that is converted to phenylacetate.

Check your understanding!

Q1: In order to remove amino group as ammonia by oxidative deamination it should transferred to:

A. Alanine.
B. Glutamate.
C. Glycogen.
D. None of the above.

Q2: Ammonia is transported from tissue to the liver by:

A. Glutamine.
B. Aspartate.
C. Alanine.
D. Glutamate.

Q3: Transamination is a reaction between α-amino acid and α-ketoglutarate to form:

A. Glutamate only.
B. PLP.
C. α-Keto acid + glutamate.
D. α-Keto acid only.

Q4: Where does the urea cycle occur:

A. Liver.
B. Kidney.
C. Blood.
D. Tissues.

Q5: During amino acid catabolism which one of the following transfers amino groups from glutamate to oxaloacetate:

A. PLP.
B. ALT.
C. PMP.
D. AST.

Q6: Which one of the liver enzymes will finally form urea and regenerate ornithine:

A. ALT.
B. Alkaline phosphate.
C. Arginase.
D. AST.

Q7: A baby come with hyperammoniemia which enzyme we need to check first:

A. CPS1.
B. OCT.
C. ALT.
D. ASL.

1.B 2.A 3.C 4.A 5.D 6.C 7.B

Check your understanding!

Q8: Which one the following is the rate limiting enzyme of urea cycle:

A. CPS1.
B. OCT.
C. Arginase.
D. ASL.

Q9: The action of intestinal urease to form NH3 is clinically significant in:

A. Liver failure.
B. Hepatic toxicity.
C. Renal injury.
D. Renal faliure.

Q10: The most common enzyme deficient and cause congenital hyperammoniemia is:

A. Ornithine transcarbamoylase.
B. Argininosuccinate lyase.
C. Argininosuccinate synthase.
D. Carbamoyl phosphate synthetase I.

Q11: In the management of hyperammoniemia the patient should avoid which one the following drugs:

A. Glucocorticoids.
B. Valproic acid.
C. A&B.
D. Buphenyl.

Q12: Carbaglu is given to treat:

A. Hyperammoniemia secondary to renal faliure.
B. Hyperammoniemia secondary to NAGS.
C. Hyperammoniemia secondary to CPS1 deficiency.
D. Hyperammoniemia secondary to.

8.A 9.D 10.A 11.C 12.B

Done by:

- شهد العنزي.
- عبدالله الغزي.
- دانيا الهنداوي.
- منيرة الحسيني.
- مروج الحربي.
- عبدالله الشنيفي.
- أحمد الرويلي.

Resources:

- 435's slides and 434's notes.
- Lippincott's illustrated reviews: Biochemistry – sixth edition.

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