Revised & Approved

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BIOCHEMISTRY TEAM
439

MED439
KING SAUD UNIVERSITY

Urea cycle

Editing File

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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 and fatty acids, amino acids are not stored by the body.

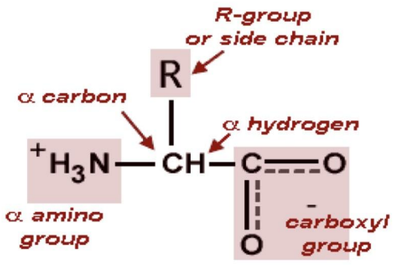
Amino acids in excess of biosynthetic needs are degraded.

Degradation of amino acids involves:

- **Removal of α-amino group** → **Ammonia (NH_3)** That's what we will talk about it in this lecture
- **Remaining carbon skeleton** → **Energy metabolism** As alpha keto glutarate

#435

- 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.



Removal of α-amino group, formation of ammonia and its transport to liver

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

1. Transamination to glutamate.
2. Oxidative deamination of glutamate

Oxidative deamination of glutamate is done to release the ammonia in the liver

Blood transport of ammonia into liver:

1. In the form of glutamine (most tissue). Glutamine = glutamate + nitrogen
2. In the form of alanine (muscle).

The presence of the α-amino group keeps amino acids safely locked away from oxidative breakdown. Removing the α-amino group is essential for producing energy from any amino acid and is an obligatory step in the catabolism of all amino acids. Once removed, this nitrogen can be incorporated into other compounds or excreted as urea, with the carbon skeletons being metabolized. This section describes transamination and oxidative deamination, reactions that ultimately provide ammonia and aspartate, the two sources of urea nitrogen

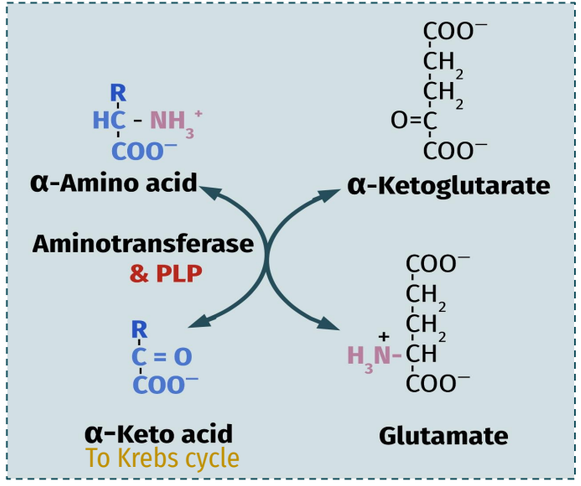
A: Removal of α-amino group & formation of ammonia

1 Transamination to glutamate

- **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.**
  - Glutamate is the only amino acid that is able to be deaminated rapidly, other amino acids can undergo deamination but they'll take more time.
  - Ammonia is a toxic waste and we're trying to get rid of it as fast as possible, that's why glutamate is unique.

Click here for original picture

Important



- PLP: Pyridoxal phosphate , a co-enzyme that is derived from vitamin B6.
- Aminotransferases: ALT & AST.

Examples

Transamination by ALT

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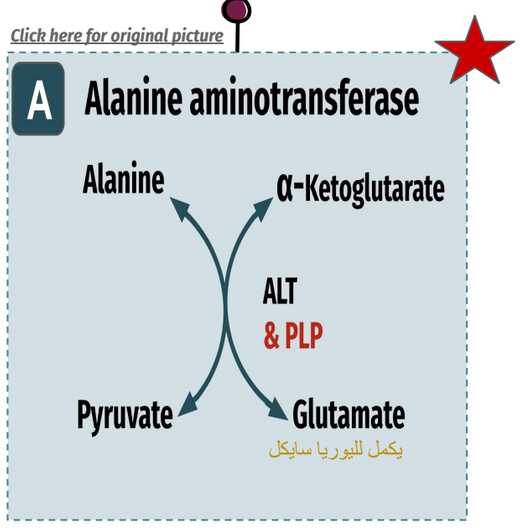

Alanine + a-ketoglutarate —(ALT & PLP)→ **Glutamate + Pyruvate**

- In the muscles.
- There's no AST in the muscles

Transamination by AST

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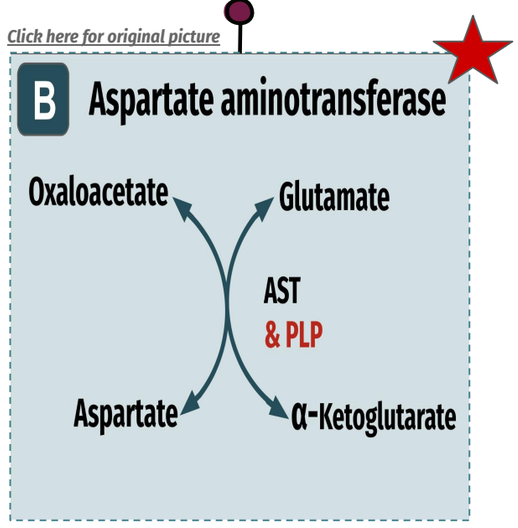

Aspartate + a-ketoglutarate —(AST + PLP)→ **Glutamate + oxaloacetate**

2 Oxidative deamination of glutamate

- **Oxidative deamination of glutamate will release NH_3 and re-generate α-ketoglutarate.**
  - To remove the amino group from glutamate, it undergoes deamination. Deamination involves reducing NAD to NADH (gains H) and oxidising glutamate to α-Ketoglutarate by the Oxidative enzyme glutamate dehydrogenase. The reaction is called oxidation- reduction reaction. This result in the removal of ammonia, and the regeneration of α Ketoglutarate. #437

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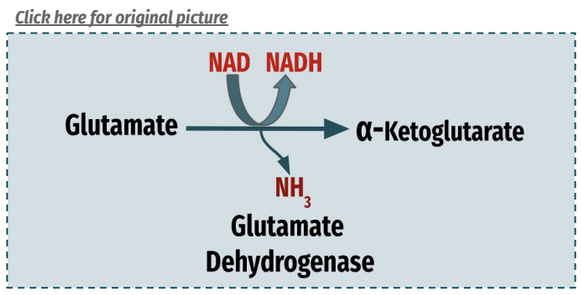

Summary:

Removal of α-amino group of amino acid & formation of ammonia.

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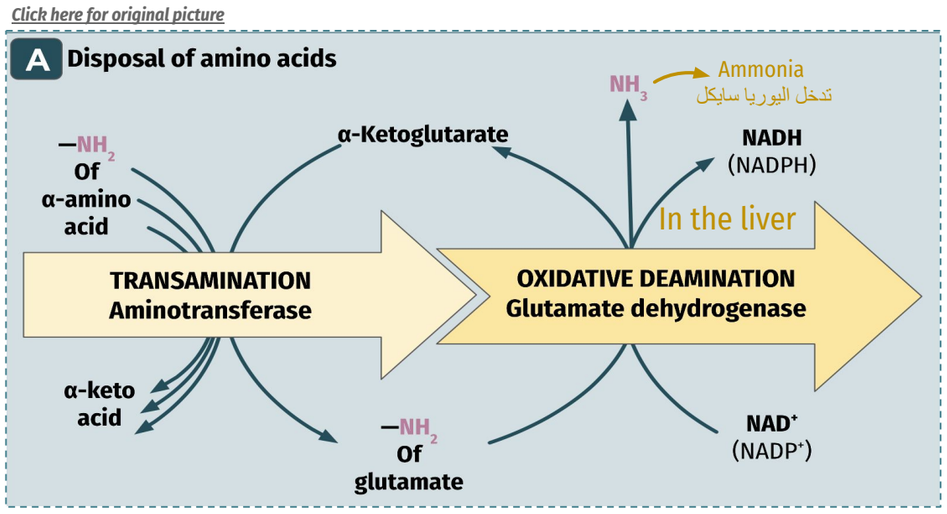


This process occurs in 2 steps:
1- amino acids donate amino group to a- ketoglutarate to form glutamate(**Transamination**)

2- Glutamate by the action of Glutamate dehydrogenase will release Ammonia (NH3) and becomes a-ketoglutarate (**Oxidative deamination**)

B: Transport of NH_3 from peripheral tissues into the liver

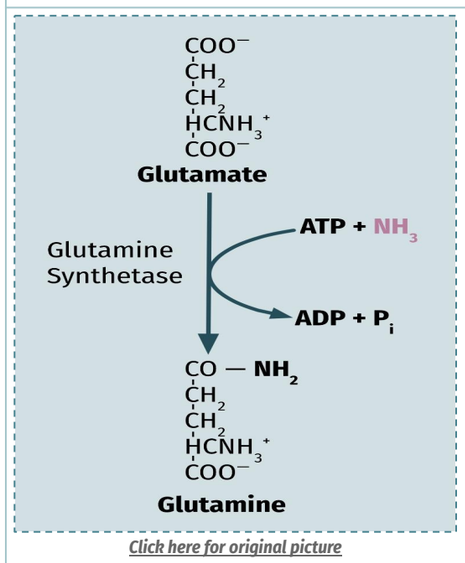
1. Ammonia is produced by all tissues and the main disposal is via formation of urea in liver.
2. Blood level of NH_3 must be kept very low, otherwise, hyperammonemia and CNS toxic it will occur ($NH_3$is toxic to CNS).
3. To solve this problem, $NH_3$is transported from peripheral tissues to the liver via formation of: **Glutamine (most tissues) & Alanine (muscle)**

نعامل الـNH_3 وكأنه مشاغب مانقدر نخليه يروح لحاله لازم معه احد يوصله للمكان المناسب:)

Transport of NH_3 from peripheral tissues into the liver

From most peripheral tissues

- NH_3 is transported Into the liver through forming **glutamine** by **glutamine synthetase** Needs ATP



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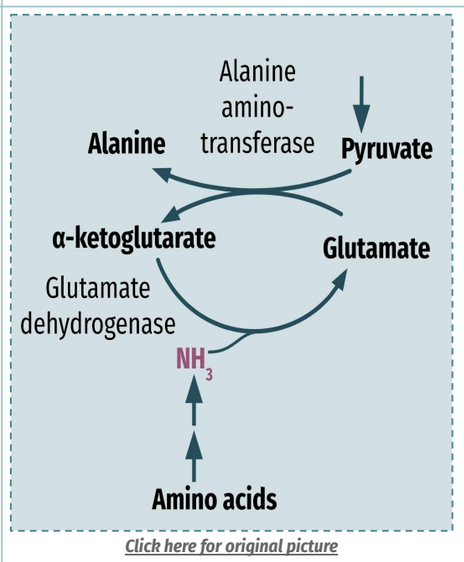
Glutamate can not leave the tissue to the blood, it has to be converted to glutamine, by adding ammonia to the glutamate by the help of glutamine synthetase enzyme.
Glutamine = glutamate + NH3

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



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From all body tissues except the muscles ammonia is transported as glutamine

Ammonia is transported from the muscles as Alanine due to the lack of AST

متوقع اننا نشوف الامونيا في الدم على هيئة
glutamine or Alanine
فقط وما نشوفها بأي صيغة اخرى
Alanine from the muscles
Glutamine from other body tissues

Release of ammonia from glutamine and alanine in the liver

In the Liver:

In the liver the alanine and the glutamine are all going to be converted into glutamate

1- Glutamine → is converted 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.**

Summary:
Blood transport of NH_3 from peripheral tissues (in the form of glutamine and alanine) into the liver and the release of NH_3 back in the liver to start the urea cycle.

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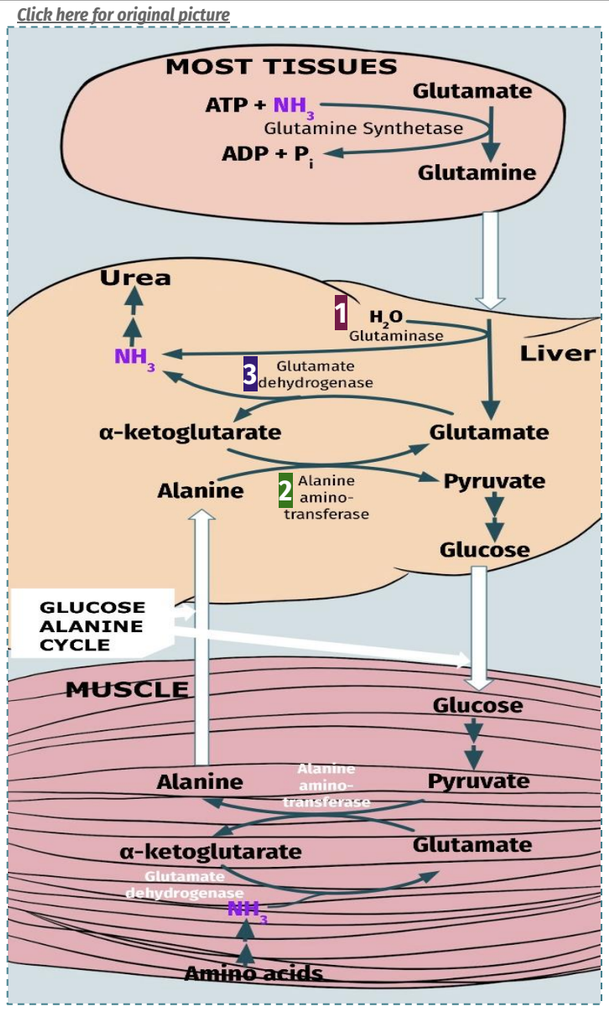


Urea Cycle

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

Urea cycle occurs in the liver.

One nitrogen of urea is from NH3 and the other nitrogen from aspartate.

Urea is transported in the blood to the kidneys for excretion in urine.
الـUrea هي Untoxic فعادي
تروح للـ Blood

Urea is composed of 2 nitrogens and 1 carbon
The carbon source is CO_2, the first N is from the ammonia, the second N is from aspartate

The five enzymes of urea cycle: Important

Enzyme	Location	Note
1- Carbamoyl phosphate synthetase I	Mitochondria	CO_2 (النواة لتكوين اليوريا) + NH3 (in the liver) = carbamoyl phosphate by carbamoyl phosphate synthetase I enzyme (CPSI) **CO_2 + NH3** —CPSI→ **Carbamoyl phosphate**
2- Ornithine transcarbamoylase (OCT) Can be abbreviated to OTC, both are correct		The carbamoyl phosphate من التفاعل اللي فوق reacts with ornithine by the OCT enzyme and form citrulline (citrulline is a non proteinogenic amino acid) **Carbamoyl phosphate + ornithine** —OCT→ **Citrulline**
3- Argininosuccinate synthase	Cytosol	Citrulline from the previous reaction has no function in the mitochondria, لازم يروح السايتوسول Citrulline reacts with the aspartate (aspartate provides the second nitrogen atom to the urea) in the cytosol and form argininosuccinate **Citrulline + aspartate** —Argininosuccinate synthetase→ **Argininosuccinate**
4- Argininosuccinate lyase		Argininosuccinate will be lysed to fumarate and arginine *arginine is a non essential amino acid* Fumarate will be used in the Krebs cycle **Argininosuccinate** —Argininosuccinate lyase→ **Fumarate + Arginine**
5- Arginase		Arginine (مربط الفرس) in the presence of arginase enzyme will be converted into ornithine + urea (as a byproduct), ومن هنا تروح اليوريا للدم وبعدين للكلى **Arginine** —Arginase→ **Ornithine + urea** Ornithine يدخل يوريا سايكل ثانية

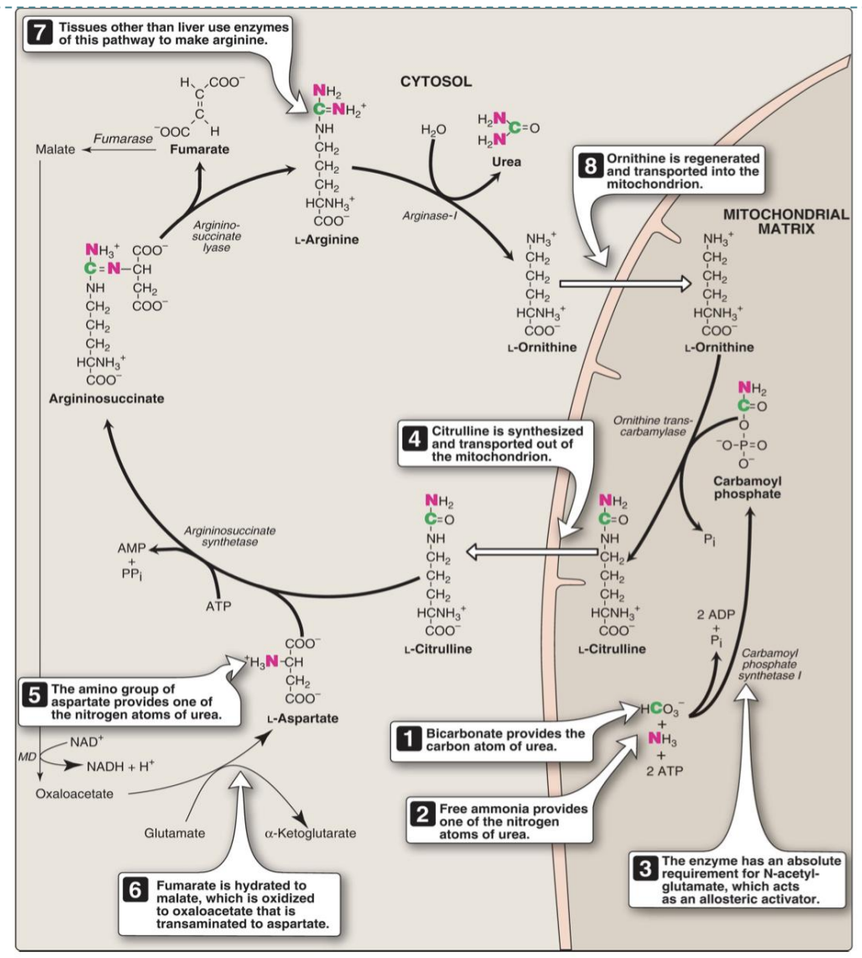


Figure 19.14 Reactions of the urea cycle. [Note: An antiporter transports citrulline and ornithine across the inner mitochondrial membrane.] ADP = adenosine diphosphate; AMP = adenosine monophosphate; PP_i = pyrophosphate; P_i = inorganic phosphate; NAD(H) = nicotinamide adenine dinucleotide; ***MD*** = ***malate dehydrogenase***.

Urea Cycle: regulation

Rate-limiting enzyme of urea cycle:

Carbamoyl phosphate synthetase I (CPSI)

CPSI depends on the food, if we eat too much protein this enzyme will be very active

Allosteric activator of CPSI:

N-Acetylglutamate

N-Acetylglutamate is synthesized by:

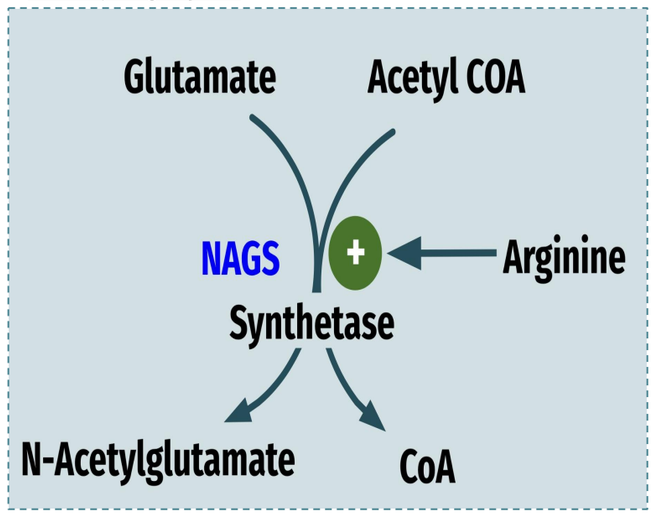
N-Acetylglutamate synthetase (NAGS) in presence of arginine

بدون هذا الانزيم **NAGS** الـ **N-Acetylglutamate** ماراح يشتغل وتبعًا له **CPSI**
ماراح يشتغل ايضًا فبالتالي الـ **Urec Cycle** ماراح تبدأ وعشان نعالج هذي المشكله
يعطى الشخص بديل اللي هو **Carbaglu**
الـ**NAGS** مهم وهو يعتبر الانزيم الـ**6** اضافه للانزيمات الـ**5** اللي فوق

NAGS deficiency is efficiently treated with:

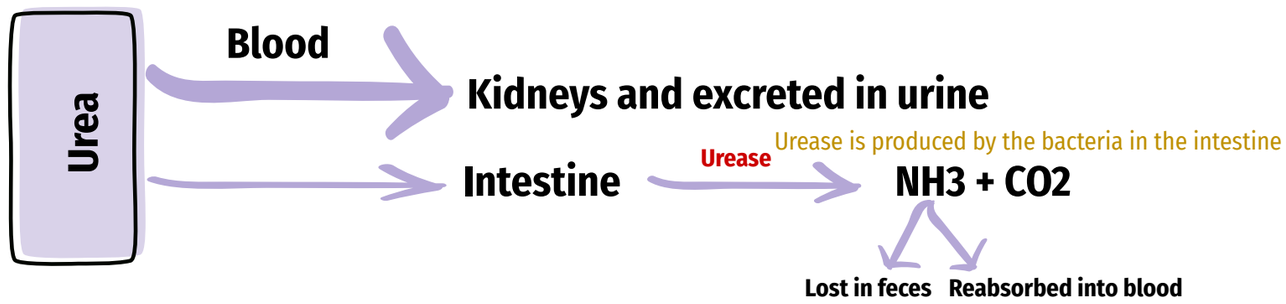
Carbaglu, a CPS1 activator Carbaglu mimics NAGS (analog)

Click here for original picture



Glutamate + acetyl CoA —NAGS + arginine→ N-acetylglutamate + CoA

Fate of Urea

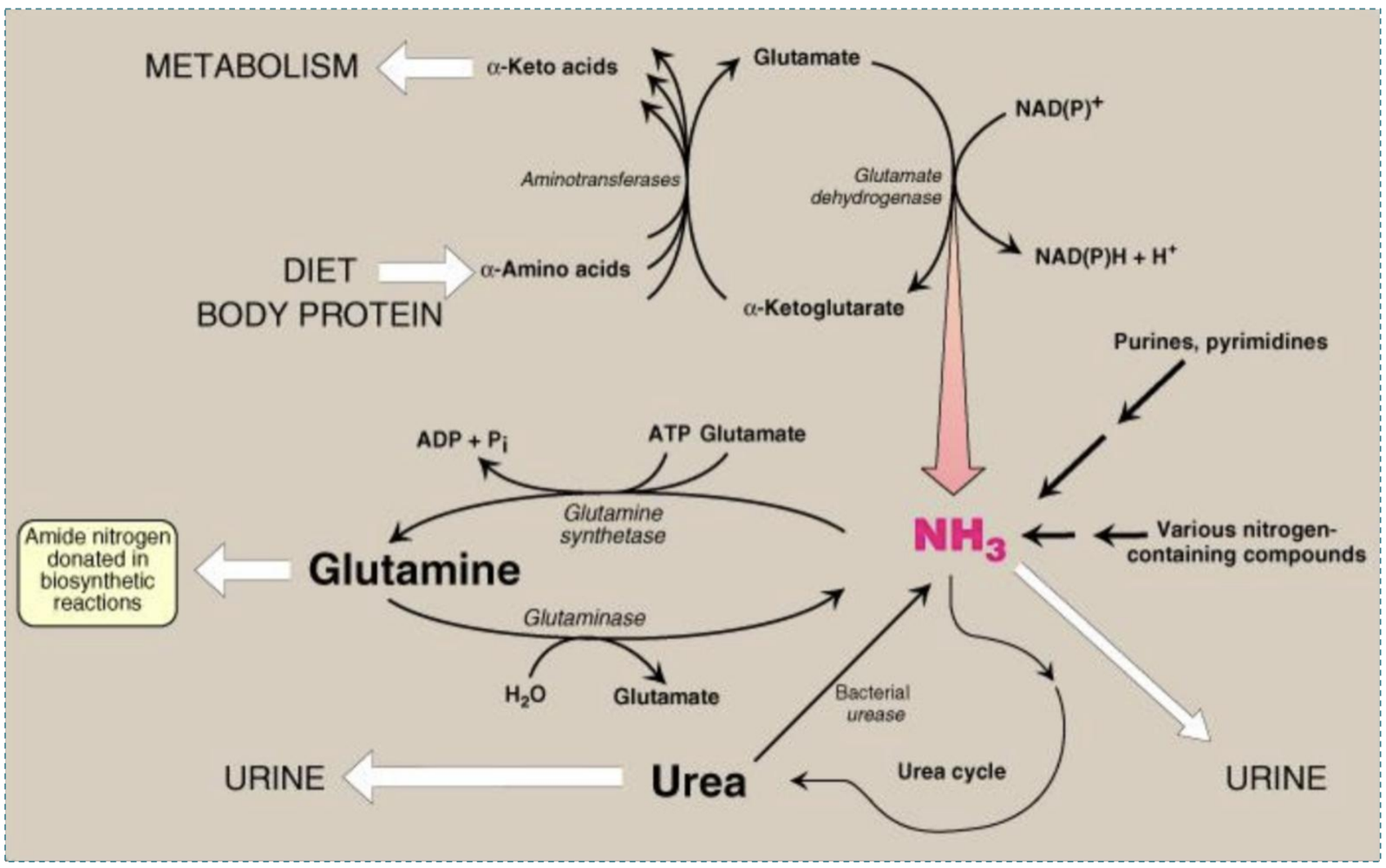


في الحالات الطبيعية الطريق الرئيسي لليوريا هو الكلية لذلك السهم أكبر وكمية
بسيطة فقط تروح عن طريق الامعاء
لكن في حال صار فيه فشل كلوي يصير الطريق الرئيسي لليوريا هو الامعاء

The action of intestinal urease to form NH3 is clinically significant in renal failure: (important)

Renal failure	→	Increase Blood urea	→	Increase Urea to intestine	→	**Urease enzyme**	→	increase NH3 blood level	→	**Acquired (hyperammonemia)**

Sources and Fates of Ammonia



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

Hyperammonemia

Cusses		
Acquired hyperammonemia	**1. Liver diseases:** Urea cycle happens in the liver so if we have liver dysfunction for any reason ,we won't be able to get rid of ammonia	- Acute: Viral hepatitis or hepatotoxic. - Chronic: Cirrhosis by hepatitis or alcoholism.
	2. Renal failure	Urea is mainly transported to the kidneys, and a **portion** of the urea is transported to the intestine and in the intestine it's cleaved by urease into NH3 and CO2. The **NH3** is partly lost in the feces and **partly reabsorbed** into the blood. In patients with kidney failure the **portion** of the urea that is transported to intestine will increase leading to an increase in the **reabsorbed** amount of **NH3** which will lead to hyperammonemia
Inherited hyperammonemia	Genetic deficiencies of any of the 5 enzymes of urea cycle or the activator enzyme for CPSI: **CPSI, OTC, ASS, ASL, arginase or NAGS**	Ornithine transcarbamoylase deficency: ● X-linked recessive. ★ **Most common of congenital hyperammonemia Marked decrease of citrulline and arginine.**
		Others: Autosomal recessive

Clinical Presentation of Hyperammonemia

- Lethargy and somnolence.
- Tremors.
- Vomiting and cerebral edema.
- Convulsions.
- Coma and death.

Management of Hyperammonemia

1. Protein restriction. الاساس في تكوين الامونيا هو البروتين
2. Volume repletion to maintain renal function Use 10% dextrose in water but limit the use of normal saline.
3. Ammonia removal by hemodialysis &/or drugs.
4. Avoid drugs that increase protein catabolism (eg, glucocorticoids) or inhibit urea synthesis (eg, valproic acid), or have direct hepatotoxicity.

Drug Treatment of Hyperammonemia Important ★

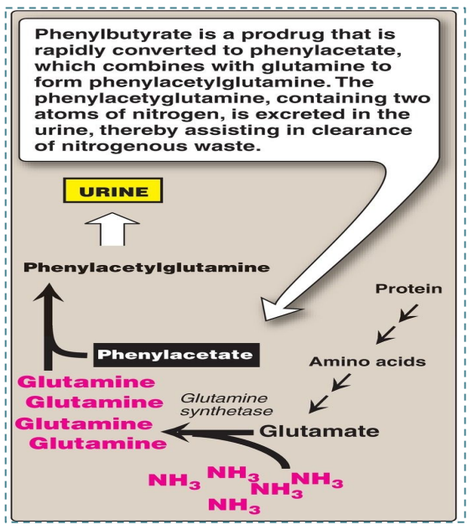
A) Drugs that scavenge ammonia by creating an alternate pathway to excrete N2- precursors:	1. I.V. Sodium phenylacetate & sodium benzoate (**Ammonul**). Ammonia scavenger 2. **Oral sodium phenylbutyrate (Buphenyl).** 3. I.V. Arginine: for all UCDs (urea cycle enzyme deficiency) except UCD due to arginase deficiency which is the enzyme that act on arginine, so deficieny in this enzyme will lead to accumulatation of arginine in blood(**argininemia**).
B) Activators to CPSI (Carglumic acid "Carbaglu"): For hyperammonemia due to NAGS deficiency	

Sodium phenyl butyrate (Buphenyl) Important

Sodium phenyl butyrate (Buphenyl):

- Prodrug that is converted to phenylacetate
- Phenylacetate condenses with glutamine (Not glutamate) forming phenylacetylglutamine that is excreted in urine

الـ Buphenyl بيتحول الى Phenylacetae ثم راح يرتبط مع glutamine و يكوّن Plenylacetylglutamine (يحتوي على ذرتين من النيتروجين)
بمعنى أن phenylacetylglutamine يعمل نفس ال urea زي ال product حقها ويصير له excretion في الـ Urin



Summary

<table>
<tr><th colspan="3">Urea Cycle</th></tr>
<tr><td>The five enzymes of urea cycle:</td><td colspan="2">1. Carbamoyl Phosphate synthetase (CPSI).
2. Ornithine Transcarbamylase (OTC).
3. Argininosuccinate Synthase (ASS).
4. Argininosuccinate Lyase (ASL).
5. Arginase.</td></tr>
<tr><td>Regulation of urea cycle</td><td colspan="2">• Rate-limiting enzyme of urea cycle: Carbamoyl phosphate synthetase I (CPSI).
• Allosteric activator of CPSI: N-Acetylglutamate.
• N-Acetylglutamate is synthesized by: N-Acetylglutamate synthetase (NAGS) in presence of arginine.
• NAGS deficiency is efficiently treated with Carbaglu, a CPS1 activator.</td></tr>
<tr><td>Fate of urea</td><td colspan="2">1. Kidneys = excreted in urine.
2. Intestine = CO_2 + NH_3 (lost in feces or reabsorbed into blood).</td></tr>
<tr><td rowspan="2">Hyperammonemia</td><td>Causes:</td><td>1. Acquired (liver diseases – Renal failure).
2. Inherited (all the enzymes are autosomal
recessive except OTC which is X-linked
recessive).</td></tr>
<tr><td>Clinical Presentation of Hyperammonemia:</td><td>• Lethargy and somnolence.
• Tremors.
• Vomiting and cerebral edema.
• Convulsions.
• Coma and death.</td></tr>
<tr><td></td><td>Drug Treatment of Hyperammonemia:</td><td>A) Drugs that scavenge ammonia:
- I.V. Sodium phenylacetate & sodium benzoate (Ammonul).
- Oral sodium phenylbutyrate (Buphenyl).
- I.V. Arginine.

B) Activators to CPSI.</td></tr>
</table>

MCQs

1- Glutamine is converted into glutamate by :			
A- Glutaminase	B- Glutamate dehydrogenase	C- ALT	D- Glutamine synthetase
2- Blood transport of NH_3 from peripheral tissues into the liver in the form of :			
A- glutamine	B- pyruvate	C- Glutamate	D- A&C
3- NH_3 is transported Into the liver through forming glutamine by :			
A- Glutaminase	B- Glutamate dehydrogenase	C- ALT	D- Glutamine synthetase
4-Carbaglue is a treatment of which of the following?			
A-Hyperammonemia secondaryto renal failure	B- Hyperammonemia secondary to CPS1 deficiency	C-Hyperammonemia secondaryto OCT deficiency	D-Hyperammonemia secondary to NAGS deficiency.
5- The most common enzyme deficient and cause congenital hyperammonemia is?			
A- Argininosuccinate lyase	B-Carbamoyl phosphate synthetase I	C-Ornithine transcarbamoylase	D-Glutaminase
6-Which one of the following is the rate limiting enzyme of urea cycle?			
A-Arginase	B- CPSI	C-Argininosuccinate Lyase	D-OCT

Answers key

1- A 2- A 3- D 4- D 5- C 6- B

SAQs

1- Explain the steps of Releasing of ammonia from glutamine and alanine in the liver.

1. Glutamine is converted 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.

2- 35 y.o patient was diagnosed with hyperammonemia, which drugs he must avoid it?

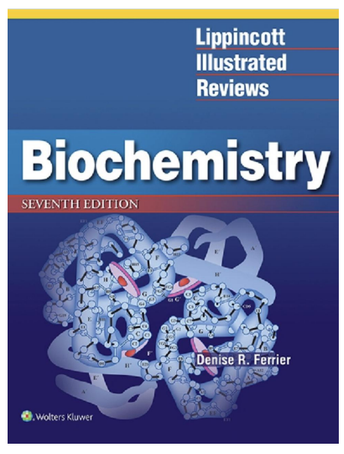
Valproic acid and glucocorticoids.

3- Mention the conditions which can cause Hyperammonemia ?

Slide 9

Resources

Click on the book to download the resource



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Special thanks to Fahad AlAjmi for designing our team's logo.