"اللَّهُمَّ لا سَهْلَ إلاَّ ما جَعَلْتَهُ سَهْلاً، وأنْتَ تَجْعَلُ الحَزْنَ إذَا شِئْتَ سَهْلاً "

Urea Cycle

Biochemistry Team 437

Color index:
Doctors slides
Doctor's notes
Extra information
Highlights

GNT block

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

We advise you to study liver function test lecture before this lecture!

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[1] → Ammonia (NH_3)
  - Remaining carbon skeleton → Energy metabolism

Sources of amino acid in the body:

1) Diet
2) Protein turnover
3) Biosynthesis such as nonessential amino acid

[1]It involves 2 reactions:
1-Transamination
2-Deamination

Introduction:

Proteins are made of two main parts:

- Carbon skeleton, which gives us the energy.
- Amino group, which protects the amino acid from being metabolized.

If i want to degrade the amino acid, i have to remove the amino group in the form of ammonia, but because the ammonia is toxic we also have to get rid of it.

↦ To get rid of it, I must first transport it to the liver, then in the liver the ammonia will be converted to urea and then excreted.

In this lecture, we will discuss:

1) Removal of amino group from the amino acid
2) Transport of ammonia through the blood to the liver
3) Conversion of ammonia to urea by the urea cycle

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

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

1. Transamination to glutamate (Except in the Muscles → to Alanine)
2. Oxidative deamination of glutamate

- Amino groups of amino acids are funneled to glutamate (Why?) by transamination reactions with α-ketoglutarate
- Because Glutamate is unique. It is the only amino acid that undergoes rapid oxidative deamination
- Oxidative deamination of glutamate will release NH3 and re-generate α-ketoglutarate

B) Blood transport of ammonia into liver[2]:

1. In the form of glutamine[1] (most tissue)
2. In the form of alanine (muscle[3])

- Ammonia is produced by all tissues and the main disposal is via formation of urea in liver
- Blood level of NH3 must be kept very low, otherwise, hyperammonemia and CNS toxicity will occur (NH3 is toxic to CNS)
- To solve this problem, NH3 is transported from peripheral tissues to the liver via formation of:
  - Glutamine (most tissues)
  - Alanine (muscle)

[1] Dibasic amino acid, the only transporter of glutamate molecules from other tissues to liver.

[2] Ammonia is very toxic so we cant let it circulate freely in the blood, we have to carry it to the liver in another form.

[3] Because the final product of anaerobic metabolism is pyruvate which is a main component of alanine.

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

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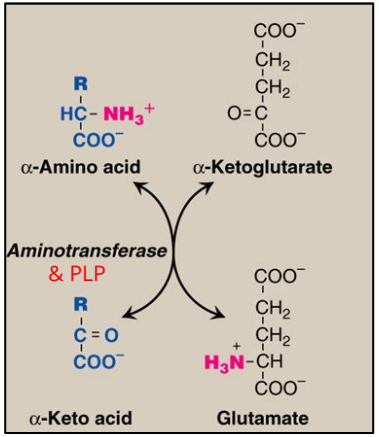
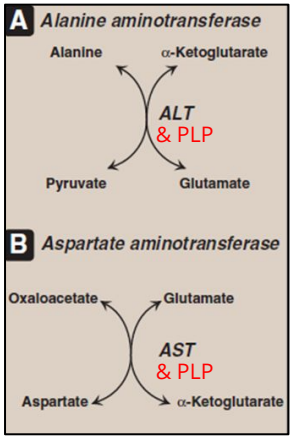
Transamination[1]

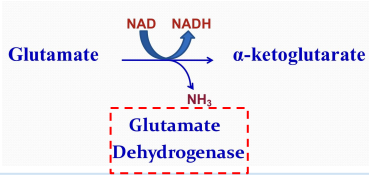


PLP: Pyridoxal phosphate, a coenzyme that is derived from vitamin B6

Transamination by ALT[2] & AST[3]



Oxidative Deamination[4]

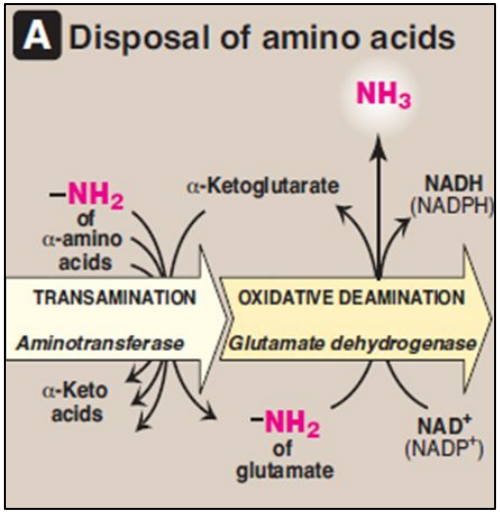


Pathways are important.

[1]The amino group is transferred from the α-amino acid to **α-Ketoglutarate (acceptor of amino groups)** by **Aminotransferase with the help of PLP**, forming **glutamate** and α-keto acid. Note that the reaction is bidirectional.
Two common examples for this reaction is transamination of alanine and aspartate:
[2]The amino group is transferred from the alanine to the α-Ketoglutarate (acceptor of amino groups) by ALT with the help of PLP, forming glutamate and pyruvate
[3]The amino group is transferred from the aspartate to α-Ketoglutarate (acceptor of amino groups) by AST with the help of PLP, forming glutamate and oxaloacetate. Note that the reaction can go in the other direction where the amino group is transferred from glutamate to oxaloacetate forming aspartate which is needed for urea cycle.
[4] 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 enzyme **glutamate dehydrogenase.** The reaction is called **oxidation- reduction reaction.** This result in the **removal of ammonia, and the regeneration of α-Ketoglutarate.**

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

Summary[1]



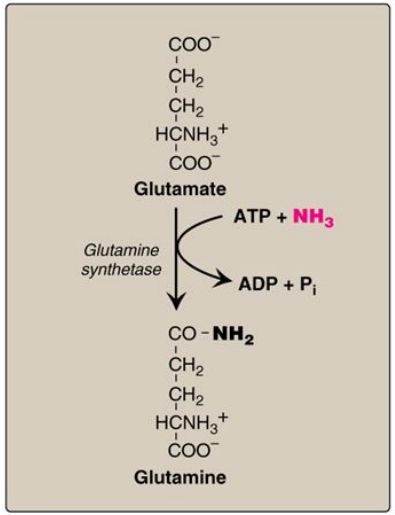
[1] Transamination is done in tissues, deamination is done in liver.

B) Blood transport of ammonia into liver:

Ammonia is very toxic so we cant let it circulate freely in the blood, we have to carry it to the liver in another form

From most peripheral tissues:

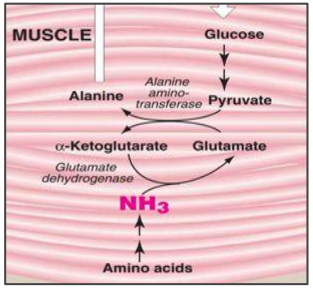
NH_3 is transported Into the liver through forming glutamine by glutamine synthetase[1]



[1]Turn glutamate into glutamine "glutamic acid" by adding ammonia
Requires ATP "any synthetase requires ATP"

From the muscle:

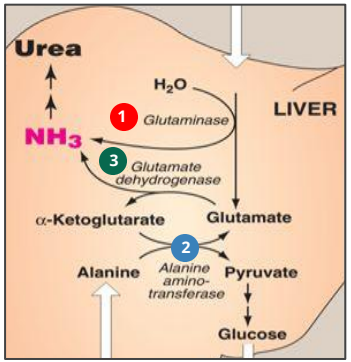
- First, NH_3 will be transferred into α-ketoglutarate to form glutamate
- Then, glutamate will give its amino group to pyruvate to form alanine by ALT
- Therefore, NH_3 is transported from muscle into the liver through forming alanine



Release of ammonia from glutamine and alanine in the liver

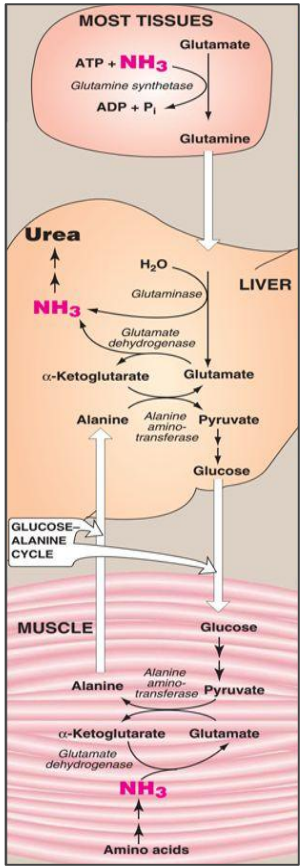
1. *Glutamine* is converted into glutamate by glutaminase.
2. *Alanine* will give its amino group to α-ketoglutarate to form glutamate by ALT.
3. *Glutamate* is converted into α-ketoglutarate and releasing NH_3[1] by glutamate dehydrogenase.

[1] we call this process deamination.



Summary

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



Urea cycle

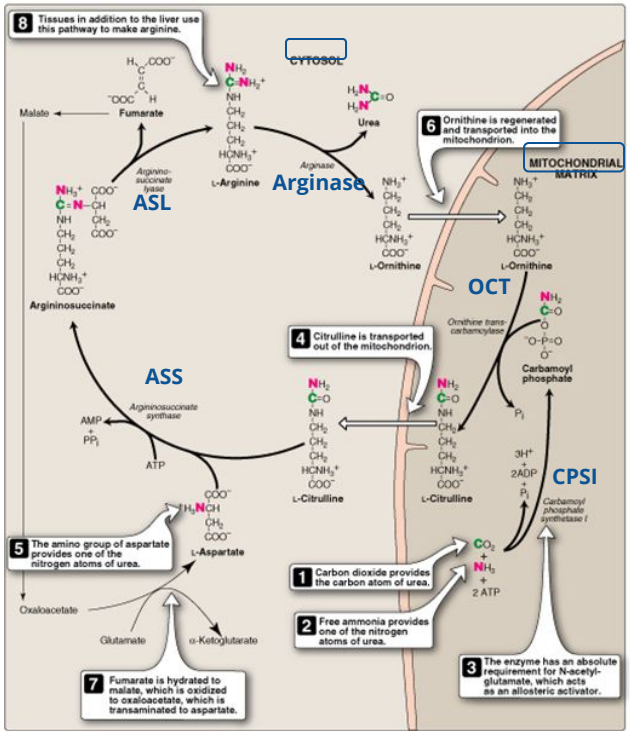
- Urea is the major form for disposal of amino groups derived from amino acids
- Urea cycle occurs in the liver
- **One nitrogen of Urea is from NH3 (Ammonia) and the other nitrogen is from aspartate.**
- Urea is transported in the blood to the kidney for excretion in urine.

The five enzymes of urea cycle:

- Carbamoyl phosphate synthetase I[1] } Mitochondria
- Ornithine transcarbamylase (OCT)[2] } Mitochondria
- Argininosuccinate synthase } Cytoplasm
- Argininosuccinate lyase } Cytoplasm
- Arginase[3] } Cytoplasm

tip

It is important to memorize the 5 main enzymes and the one activator!



1- Rate limiting enzyme, needs N-acetyl glutamate to be activated. “activator”
When i eat more protein, I will have More arginine and more glutamate, which will increases n acetyl glutamate, which in turn increases the production of urea
2- Alternative name :Ornithine carbamoyltransferase that's why we call it “OCT”
Could also be called (OTC)
3- Present only in the liver, this is why the urea cycle happens only in the liver

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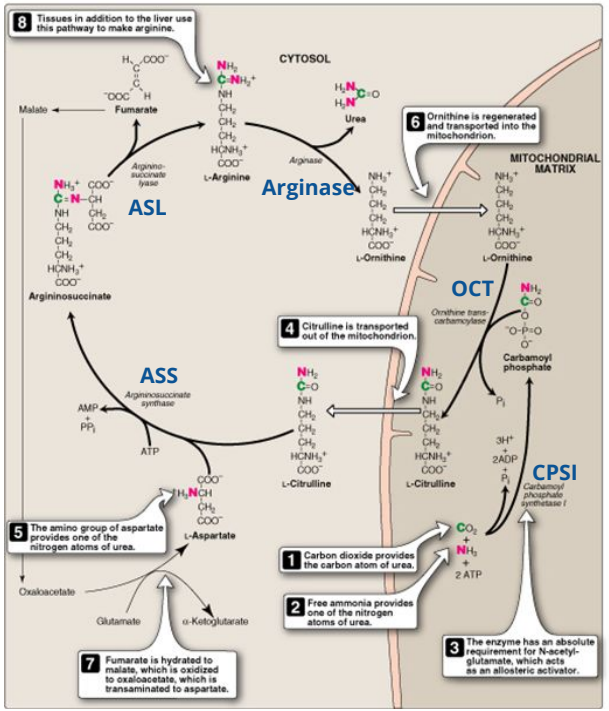
Important to understand & memorize

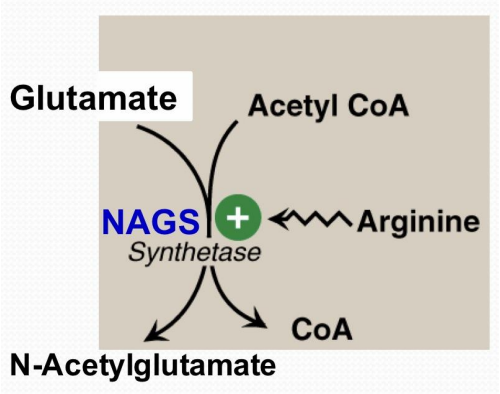
Urea cycle :-

1. The ammonia in the presence of ATP & CO2 will create the Urea backbone with the use of Carbamoyl phosphate synthetase I Enzyme in the presence of N-Acetylglutamate as an activator will convert it to Carbamoyl Phosphate
2. Carbamoyl Phosphate in the presence of Ornithine and OTC Enzyme (Ornithine transcarbamylase) will convert it to Citrulline
3. Citrulline will leave the mitochondria to go to the cytosol where a nitrogen group will be added to it from aspartate by Argininosuccinate synthase which will convert it to Argininosuccinate
4. Argininosuccinate will be converted to arginine by Argininosuccinate lyase
5. Arginine will be converted to Ornithine by Arginase. This is the MOST IMPORTANT step as it will lead to the release of urea & The cycle precursor (The Ornithine)
6. Ornithine will leave the cytosol to go the mitochondria to start another Cycle



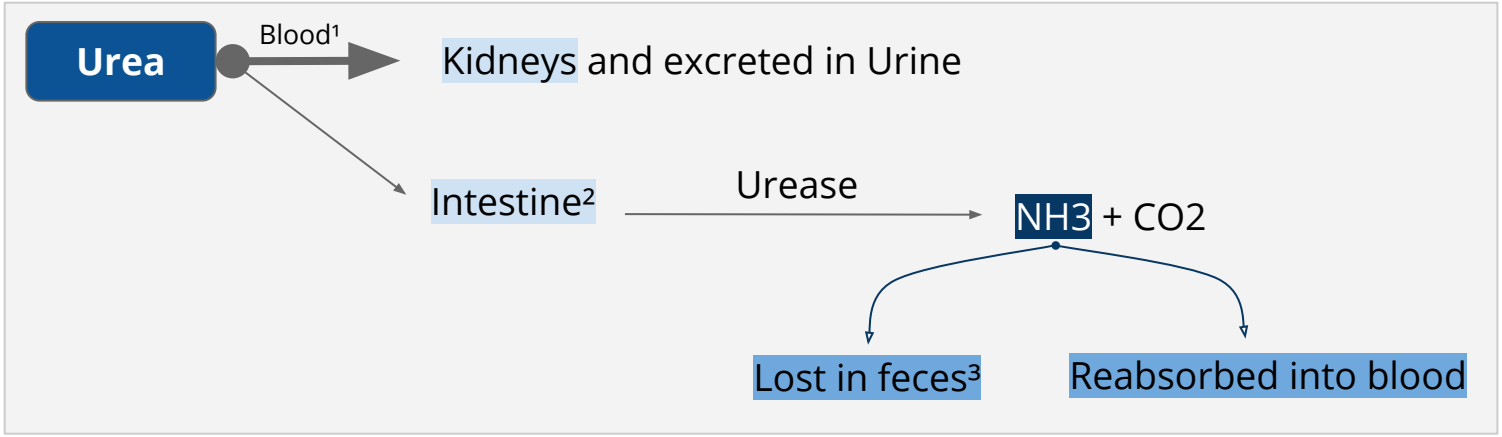
Urea Cycle: Regulation

Rate-limiting[1] 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:
Carbaglue, a CPS1 activator



[1] هي إنزيمات تحدد مسار التفاعل، و بدونها ما راح تمشي السايكل
وجود Acetyl CoA و Glutamate و بوجود كمية كافية من ال Arginine كـ Activator للتفاعل و وجود إنزيم N-Acetylglutamate synthetase (الإنزيم السادس و الأخير في اليوريا سايكل) يتم إنتاج الـ N-Acetylglutamate (الناتج وهو أهم شيء) والي بدوره يحفز (CPSI)

Fate of Urea



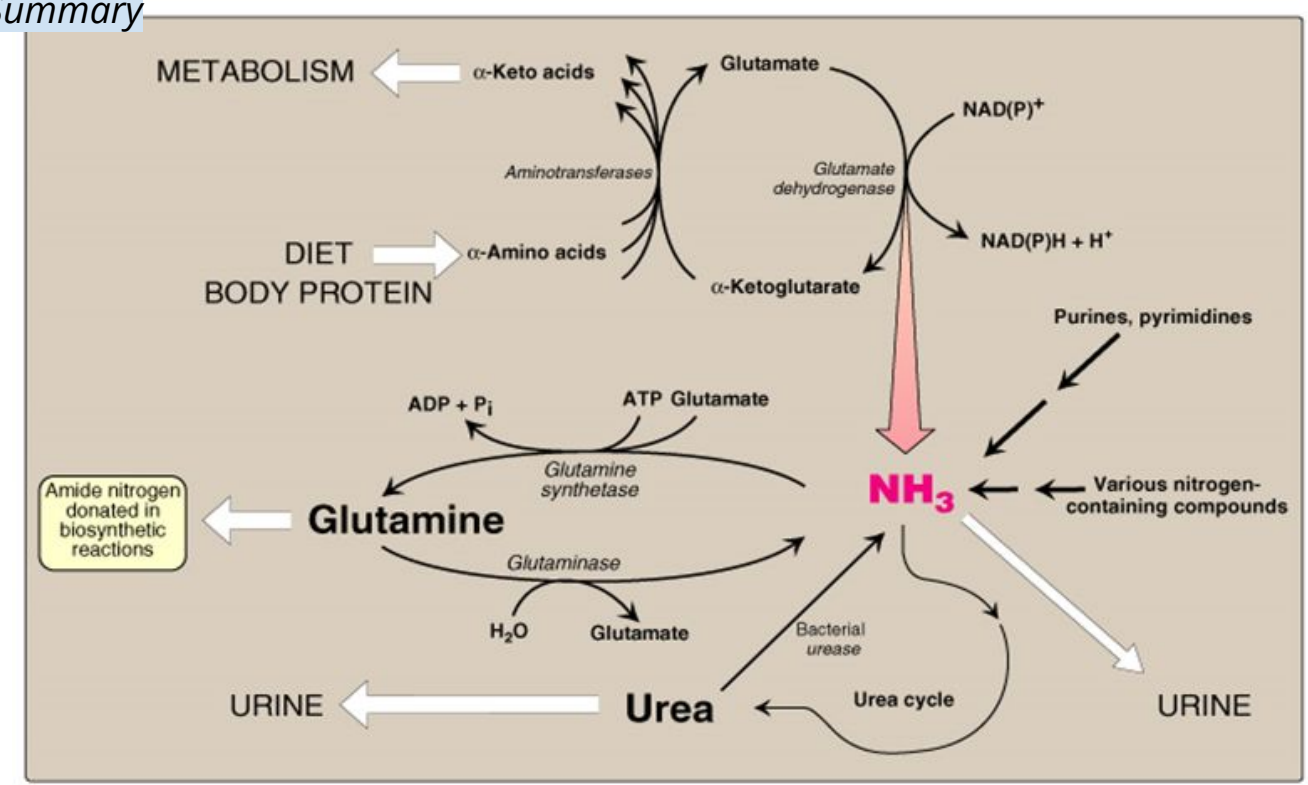
The action of intestinal Urease to form NH3 is clinically significant in renal failure:

Renal Failure → ↑ Blood Urea → ↑Urea to intestine —Urease→ ↑ NH3 blood level (acquired hyperammonemia)[4]

[1] Majority
[2] Minority
[3] Mostly
[4] Secondary to Kidney Failure

Sources and fates of Ammonia

Summary



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

Hyperammonemia

Acquired Hyperammonemia:

- Liver diseases:
  - Acute: viral hepatitis or hepatotoxic
  - Chronic: cirrhosis by hepatitis or alcoholism
- Renal failure: high urease activity, high urea in blood

Inherited Hyperammonemia:

Genetic deficiencies of any of the 5 enzymes of urea cycle or the activator enzyme for CPS I:
CPSI, OTC, ASS, ASL, Arginase, NAGS

Ornithine transcarbamylase deficiency

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

Other: autosomal recessive

Clinical presentation of Hyperammonemia:

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

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

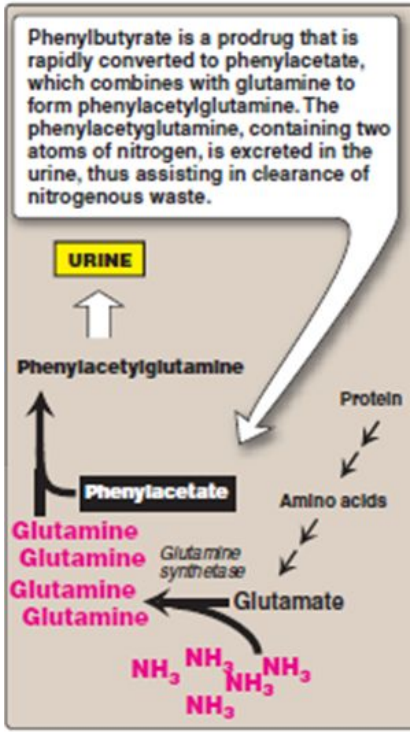
Drug treatment of hyperammonemia

- Drugs that scavenge ammonia by creating an alternate pathway to excrete N_2- precursors:
  1. I.V. Sodium phenylacetate & sodium benzoate (Ammonul)
  2. Oral sodium phenylbutyrate (Buphenyl)
  3. I.V. Arginine: for all UCDs except UCD due to arginase deficiency (argininemia)
- Activators to CPSI (Carglumic acid “Carbaglu”):
  - For hyperammonemia due to NAGS deficiency

Sodium phenylbutyrate (Buphenyl)

Sodium phenylbutyrate (Buphenyl):

- Prodrug that is converted to phenylacetate.
- Phenylacetate condenses with glutamine (in blood) forming phenylacetylglutamine that is excreted in urine.



Summary

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Removal of α-amino group, formation of ammonia	
Transamination: By: **ALT & AST** Amino groups of amino acids are funneled to **glutamate** by transamination reactions with **α-ketoglutarate**.	Oxidative deamination: in **liver** **By: Glutamate dehydrogenase.** • The glutamate will release NH. • Regenerate α-ketoglutarate.
Transport of NH3 from peripheral tissues into the liver	
Glutamine (from most tissues to liver). Glutamine formed by **glutamine synthetase**	**Alanine** (from muscles to liver).

Release of ammonia from glutamine and alanine in the liver		
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 NH3 by **glutamate dehydrogenase**

Summary

Enzyme of urea	1.Carbamoyl phosphate synthetase (CPSI)		2. Ornithine transcarbamoylase (OCT)
	3. Argininosuccinate synthase (ASS)	4. Arginase	5. Argininosuccinatelyase (ASL)
Regulation of urea cycle	RATE-LIMITING ENZYME of urea cycle		**CPSI**
	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
Fate of urea	To the kidneys (Mostly) excreted in urine		To the intestine by urease gives NH_3 (lost in feces or reabsorbed into blood) + CO_2
Hyperammonemia	A- Acquired (liver diseases – Renal failure)		
	B- Inherited (all 5 enzymes are autosomal recessive except **OCT which is X-linked recessive**		
	Treatment: Oral sodium phenyl butyrate (Buphenyl): a Prodrug that is converted to phenylacetate		

MCQs:

Q1: NH3 is transported from muscles to liver by?
A. Glutamate B. Glutamine
C. Alanine D. B&C

Q2: Glutamine is converted into glutamate by?
A. Alanine aminotransferase(ALT)
B.Glutamate dehydrogenase
C. Arginase
D. Glutaminase

Q3: One of this enzymes are not included in urea cycle?
A. Ornithine transcarbamoylase(OCT)
B. Glutaminase
C. Argininosuccinate synthase
D. Carbamoyl phosphate synthetase I

Q4: The most common enzyme deficient and cause congenital hyperammonemia is?
A. Argininosuccinate lyase
B. Carbamoyl phosphate synthetase I
C. Ornithine transcarbamoylase
D. Glutaminase

Q5: 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- D
4- C
3- B
2- D
1- C

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- معن شكر
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- عبدالرحمن التركي
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