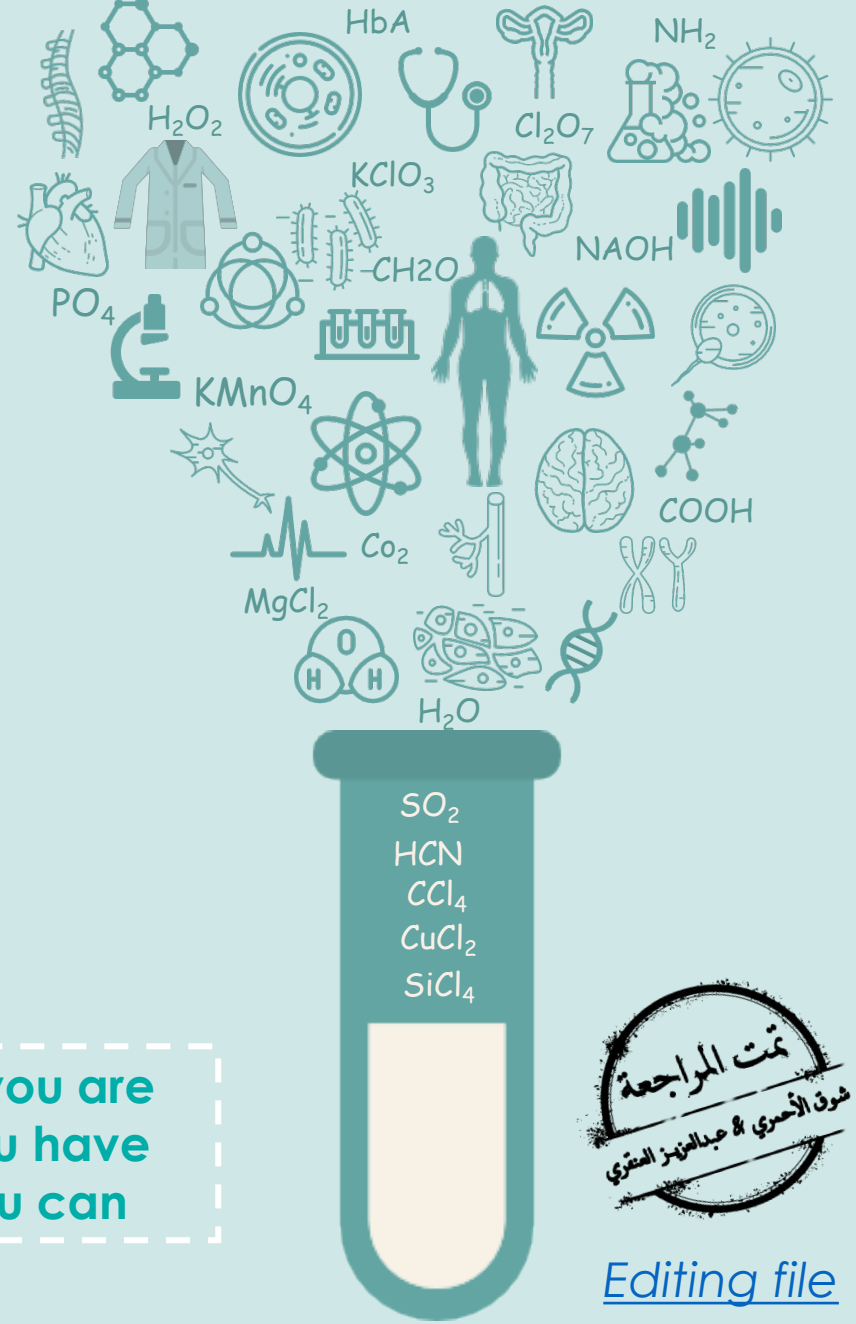


Important Doctors slides

Extra Information **Doctors notes**

Biochemistry

Urea Cycle

Start where you are
Use what you have
Do what you can

تمت المراجعة
شوق الأحمري & عبدالعزيز العتري

Editing file

OBJECTIVES

Upon completion of this lecture, the students should be able to :

Understand the reactions for removal of α-amino group of amino acids and formation of ammonia

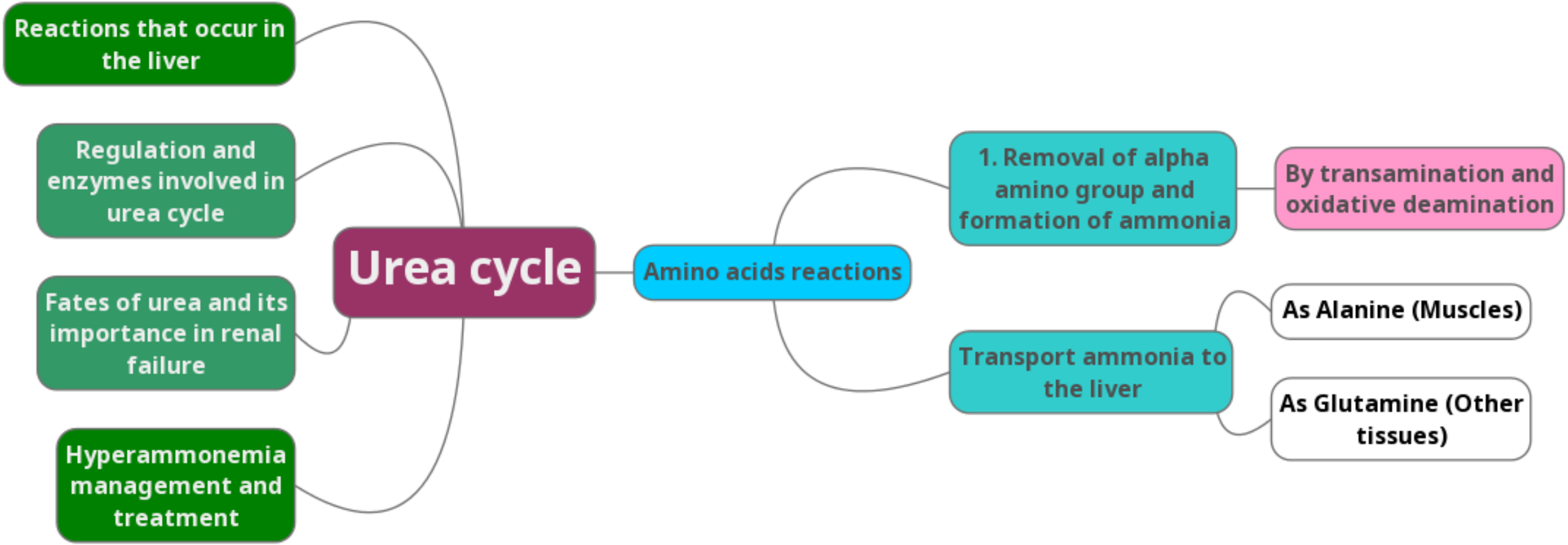
Identify the importance of blood transport of ammonia to the liver in the form of glutamine/alanine

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

Overview


Urea cycle
Reactions that occur in the liver
Regulation and enzymes involved in urea cycle
Fates of urea and its importance in renal failure
Hyperammonemia management and treatment
Amino acids reactions
1. Removal of alpha amino group and formation of ammonia
By transamination and oxidative deamination
Transport ammonia to the liver
As Alanine (Muscles)
As Glutamine (Other tissues)

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)
- Remaining carbon skeleton → Energy metabolism

Urea cycle happens only in the hepatocytes because we have unique enzyme such as Arginase that is present only in liver, that's why pts with liver cirrhosis have high levels of ammonia

Urea cycle is basically the formation of urea from the Ammonia of the Amino acids (occurs in the liver)

So our main goal is to get the Ammonia (which is toxic) from amino acids of different tissues to reach the liver where it will be converted to urea (which is not toxic)

First the amino acid undergoes transamination reaction and oxidative deamination to form this ammonia
Second, this toxic ammonia is transported to the liver in the form of glutamine and Alanine
Third, upon reaching the liver ,urea cycle can begin and we can convert ammonia to urea .

Removal of α-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

B : Blood transport of ammonia into liver

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

Removal of amino group make it active because its presence on the carbon skeleton (within the amino acid) stabilize it and make it not active.

A: α-amino group removal & ammonia formation

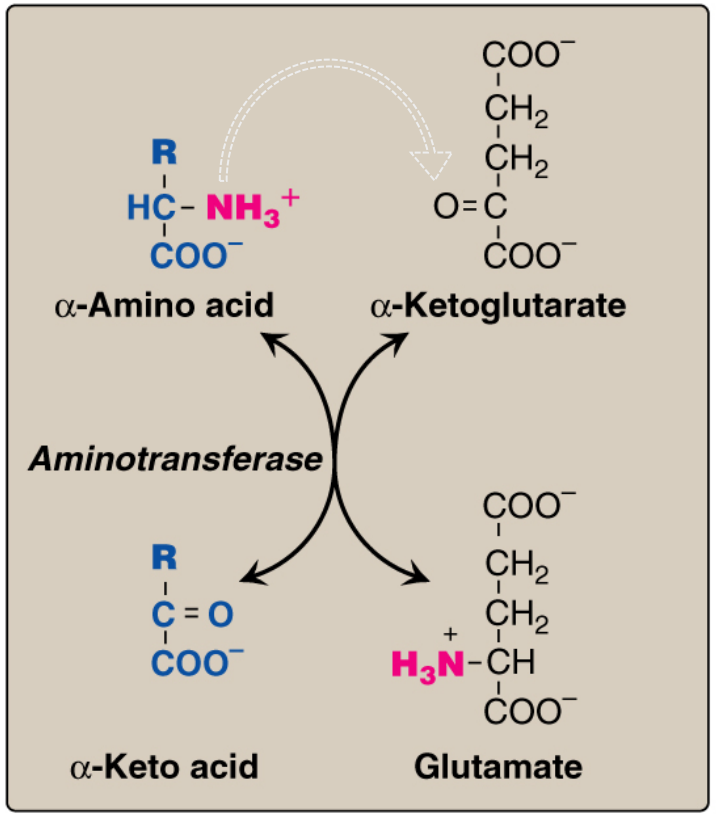
- Amino groups of amino acids are funneled to glutamate (Why?) by transamination reactions with α-ketoglutarate
- Because Glutamate is unique. It is the only amino acid that undergoes rapid oxidative deamination
- Oxidative deamination of glutamate will release NH3 and re-generate α-ketoglutarate

Transamination in this picture where amino acids donate their amino group to alpha-ketoglutarate to become glutamate and therefore the amino acid become alpha keto acid (keto acid is a general term) each amino acid has its own keto acid for example alanine's keto acid is pyruvate

The reason why Amino groups of amino acids are funneled to glutamate is the unique feature that it has which is the rapid oxidative deamination

Important

Transamination



PLP: Pyridoxal phosphate, a co-enzyme that is derived from vitamin B6

These reactions are bidirectional

Transamination

Here is an example to clarify

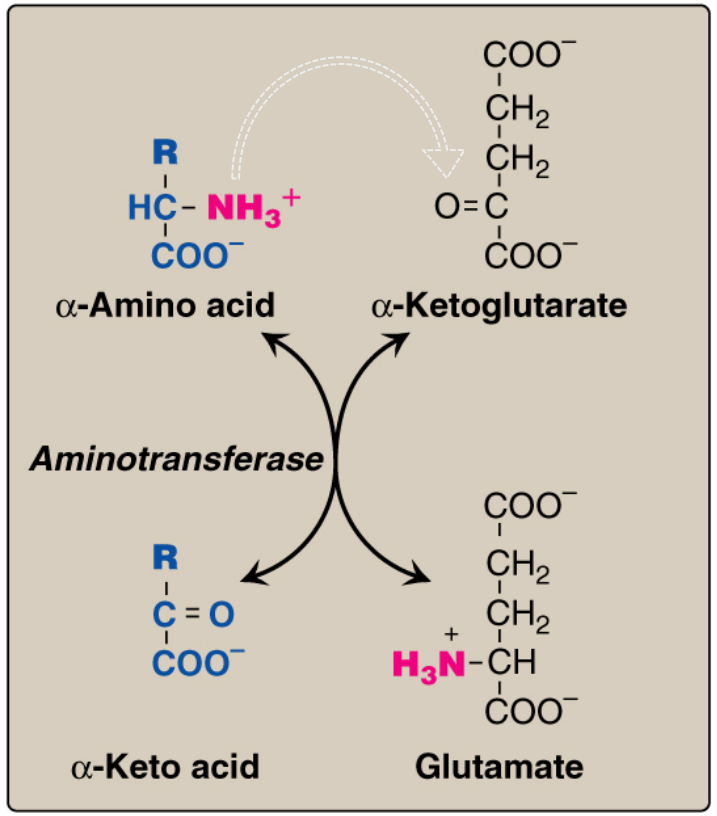
Glycine (amino acid) donates its amino group (NH3+) to alpha-ketoglutarate

Gives

Alpha-Keto acid

Glutamate

After the loss of the amino group the amino acids becomes an alpha - keto acid (carbon skeleton)

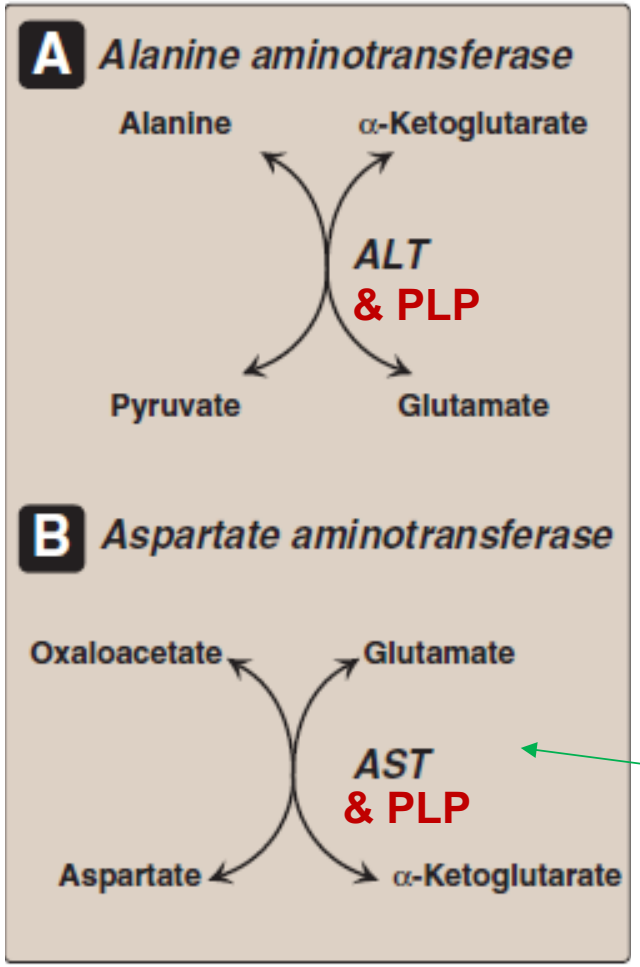


Mainly:
The ketoglutarate here is the the receiver, it receives the (NH3) from amino acids by an enzyme called aminotransferase. The alpha ketoglutarate with the amino group is called (Glutamate).
The amino acid that lost it's amino group is generally called (alpha keto acid)

A: α-amino group removal & ammonia formation

Important

Transamination by ALT & AST



Pyruvate is the keto acid of Alanine.
Oxaloacetate is the keto acid of aspartate

Alanine is converted into pyruvate after the removal of an amino group by alanine aminotransferase enzyme

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The enzyme name consist of the name of the substrate that donate alanine and "aminotransferase" which is the function of the enzyme

---

If we removed amino group :

1. Alanine becomes Pyruvate
2. Aspartate becomes Oxaloacetate

Glutamate here can donate amino group to Oxaloacetate to give Aspartate

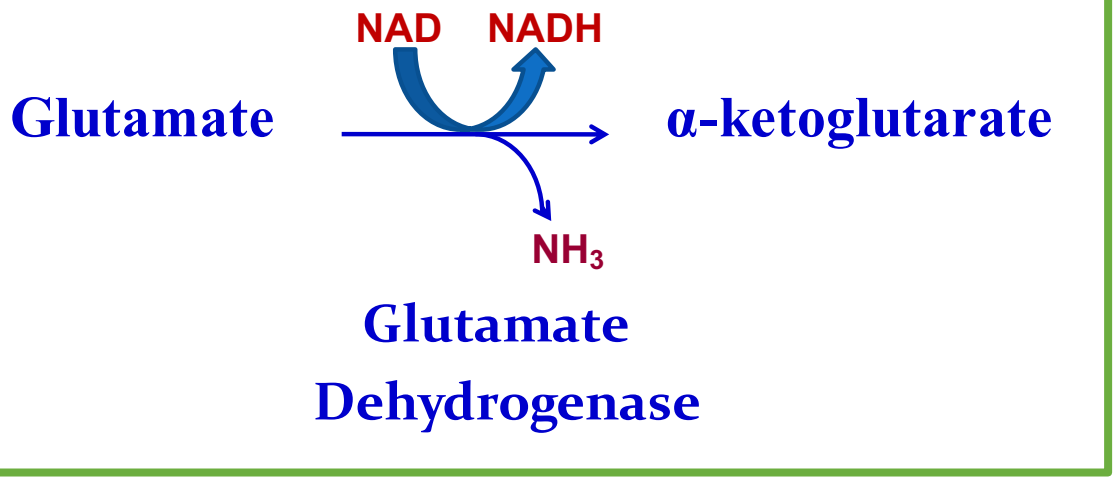
Oxaloacetate → Becomes Aspartate

Glutamate → Becomes alpha-ketoglutarate

Normally all amino acids go through the formation of glutamate direction, except in this reaction the glutamate is the donator.

A: α-amino group removal & ammonia formation

Oxidative Deamination



By the action of glutamate dehydrogenase the glutamate gets oxidized into Alpha keto glutarate and NAD gets reduced to NADH

The purpose of this reaction is to produce free ammonia (NH3) and its really important to know the enzyme that mediate this reaction (Glutamate dehydrogenase)

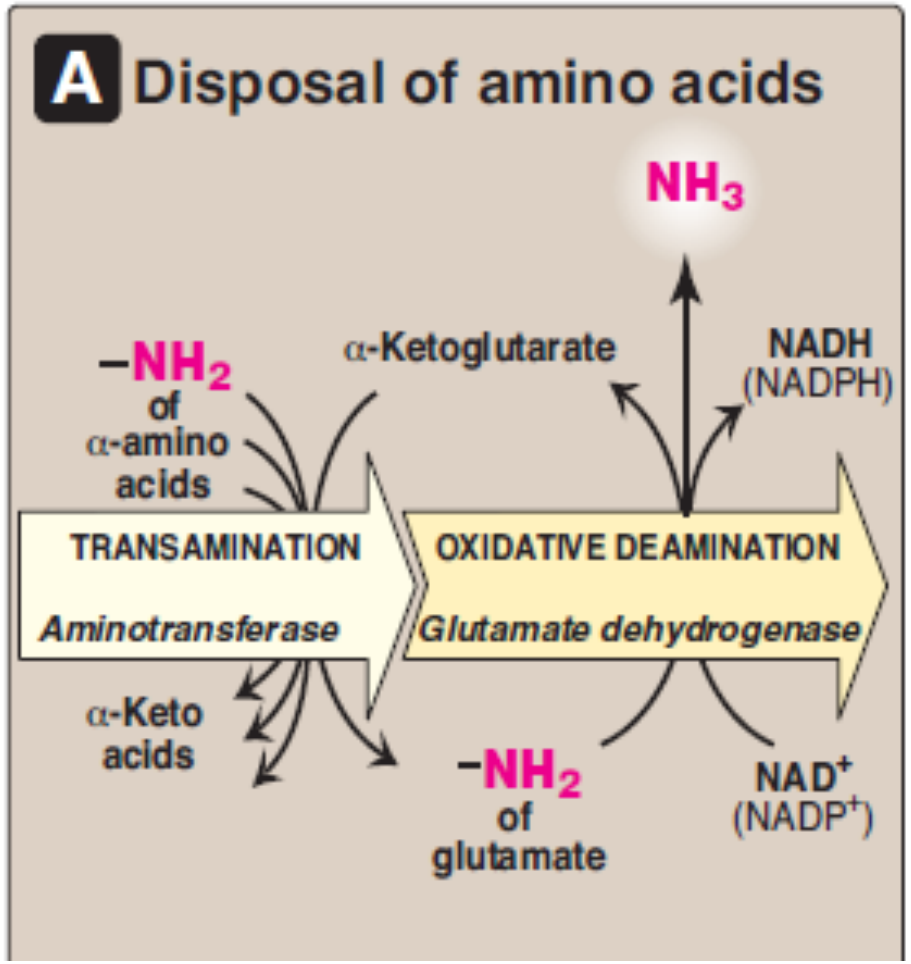
The amino groups(NH3+) from amino acids are funneled into glutamate and as we mentioned it is unique because it can be rapidly oxidative deaminated

The regeneration of alpha-ketoglutarate in this reaction because we need it as an recipient of amino group in the previous transamination reactions .

NH3 group that results is toxic because it is not positively nor negatively charged which means it's free and can diffuse freely in the blood and can cross the blood brain barrier which leads to CNS toxicity

A: α-amino group removal & ammonia formation

Summary



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)

Question: How can we remove the amino group from amino acid and form Ammonia ?
Answer : By Transamination and oxidative deamination

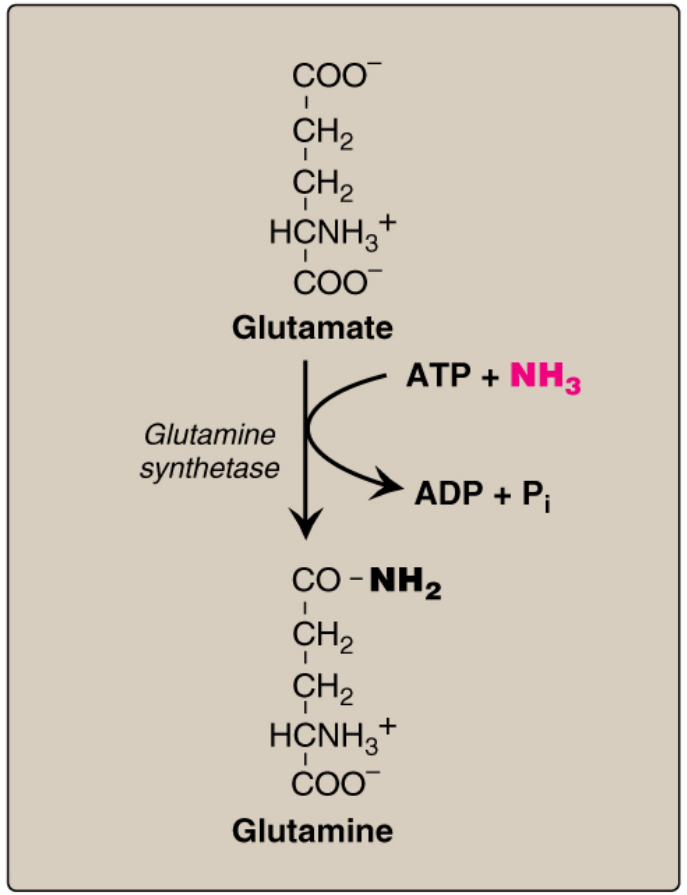
B: Transport of NH3 from peripheral tissues into the liver

- NH3 needs to reach the liver because the liver contains the enzyme required to convert ammonia to urea
- 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)

Ammonia should not be left alone in the blood because it can cause CNS toxicity Therefore it is transported to the liver in the form of Glutamine and alanine

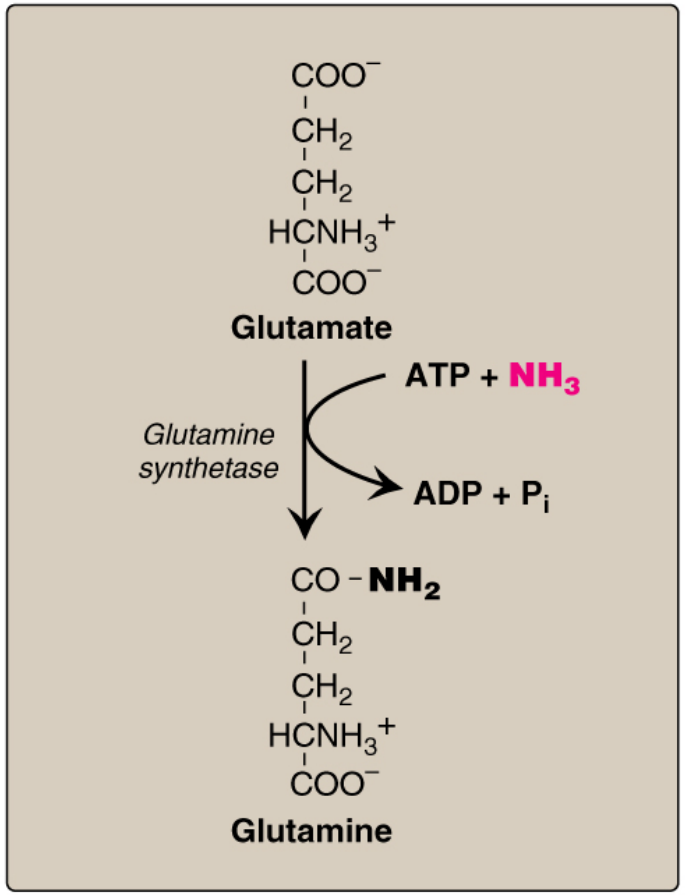
Transport of NH3 from peripheral tissues into the liver



From most peripheral tissues NH3 is transported Into the liver through forming glutamine by glutamine synthetase

Explanation

Transport of NH3 from peripheral tissues into the liver



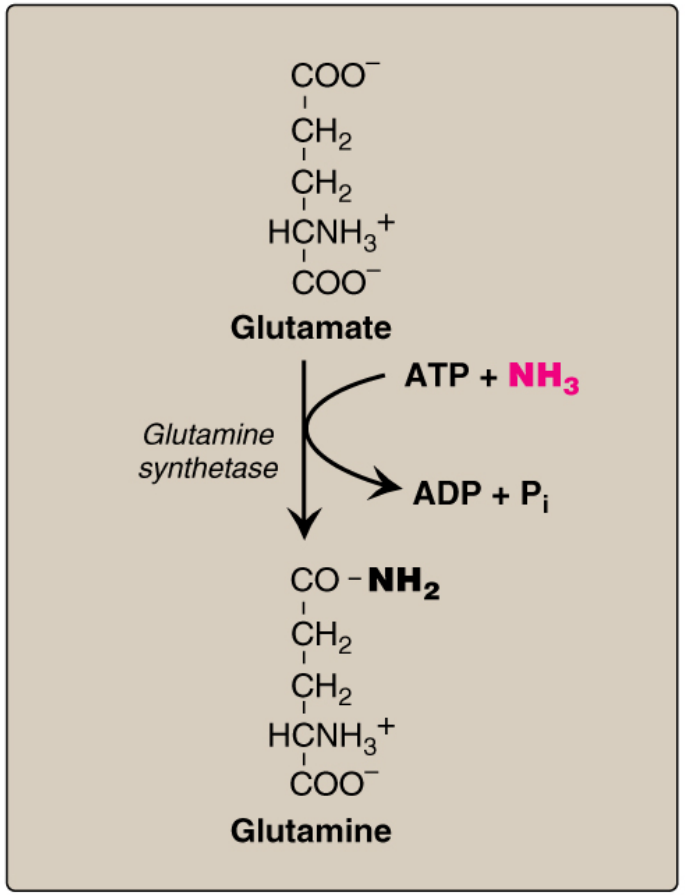
So now the ammonia is added to glutamate with the action of glutamine synthetase will form glutamine

Glutamate + NH3 (Glutamine synthetase) = Glutamine

Now it is safe to transport the ammonia to the liver
(this happens in most peripheral tissues)
هذه العملية تصير عشان نخلي نقل الامونيا للكبد يتم بصورة آمنة

B: Transport of NH3 from peripheral tissues into the liver

From muscles to the liver



From just the muscles First, NH3 will be transferred into α-ketoglutarate to form glutamate
Then, glutamate will give its amino group to pyruvate to form alanine by ALT
Therefore, NH3 is transported from muscle into the liver through forming alanine

Here we will package the ammonia into alanine in order for it to be safely transported from the muscles to the liver, but this requires 2 steps
1- this ammonia is combined to a-ketoglutarate to form glutamate
2- glutamate gives of its amino group to pyruvate and forms Alanine

B: Transport of NH3 from peripheral tissues into the liver

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.

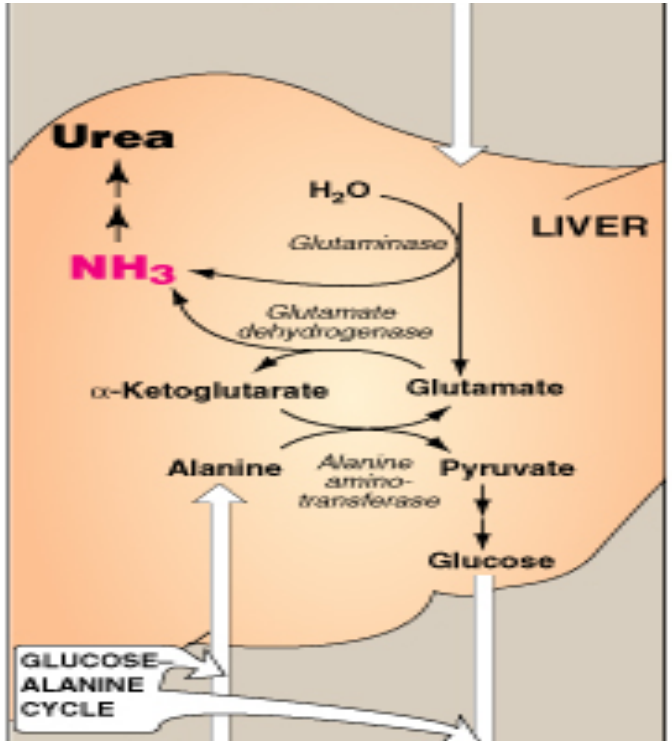
3. Glutamate is converted into α-ketoglutarate and releasing NH3 by glutamate dehydrogenase.

Summary
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

The liver is just interested in ammonia So now the Glutamine and Alanine will be broken down to release ammonia .

The action of glutaminase releases ammonia also .

In the third step ammonia is released from glutamate by oxidative deamination ..
And now after ammonia reached the liver urea cycle begins



Urea Cycle

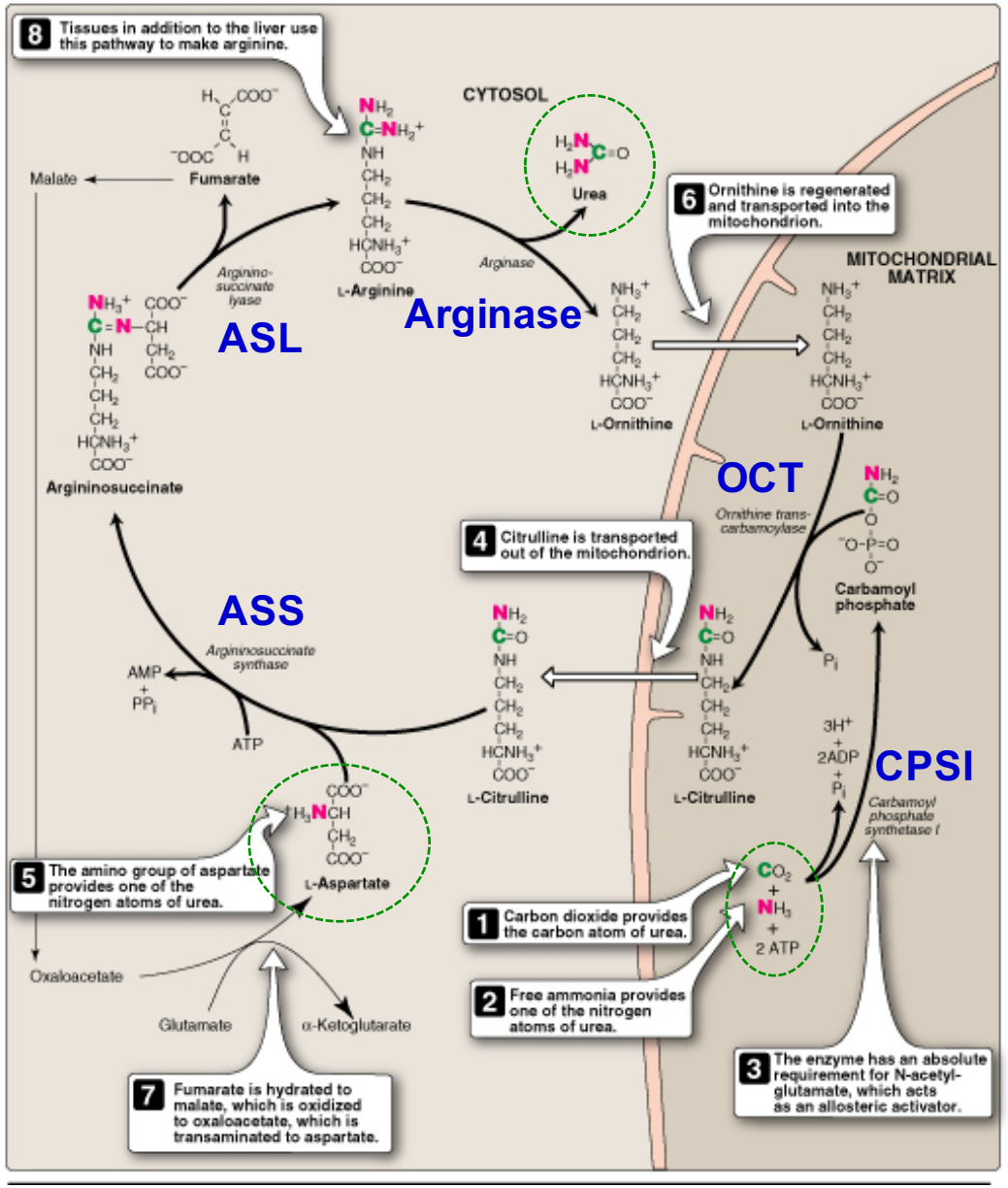
Urea has two amino groups, one from the aspartate and the other from the ammonia

- 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

The five enzymes involved in urea cycle are :

1. Carbamoyl phosphate synthetase I
2. Ornithine transcarbamoylase (OCT)
3. Argininosuccinate synthase
4. Argininosuccinate lyase
5. Arginase (Unique)

Enzyme 1 & 2 are present in the mitochondria while the rest in the cytosol



Urea Cycle : important explanation

It has several steps starting from ammonia presence in the liver

1-$NH_3 + CO_2 + 2\ ATP \xrightarrow{CPSI}$ Carbamoyl phosphate

2-Carbamoyl phosphate + L-Ornithine $\xrightarrow{OCT}$ L-Citrulline

3-L-Citrulline diffuses through mitochondrial wall to the cytosol

4-L-Citrulline combines with Aspartate to form Argininosuccinate by Argininosuccinate synthetase

5-Argininosuccinate by Argininosuccinate lyase gives L-Arginine

6-L-Arginine by Arginase gives urea

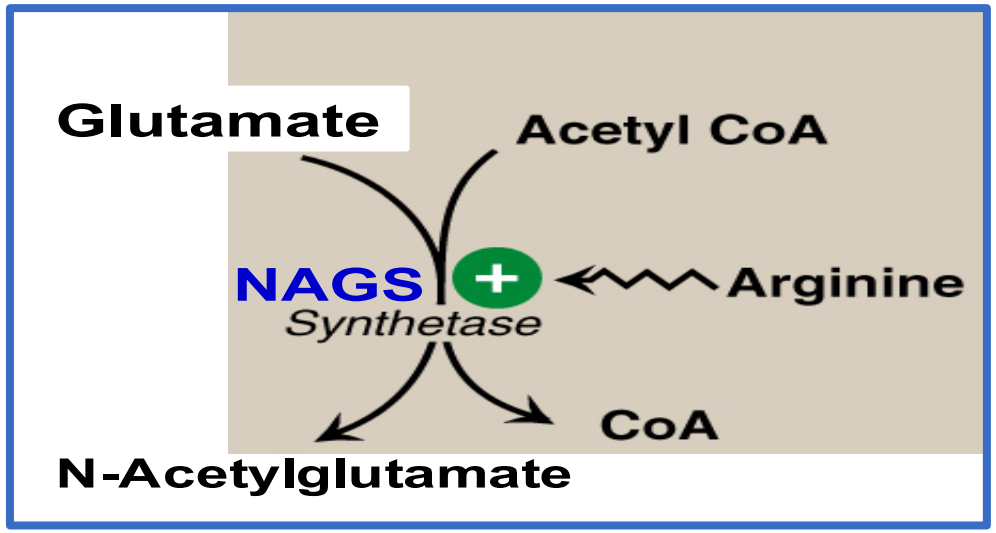
Urea Cycle

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

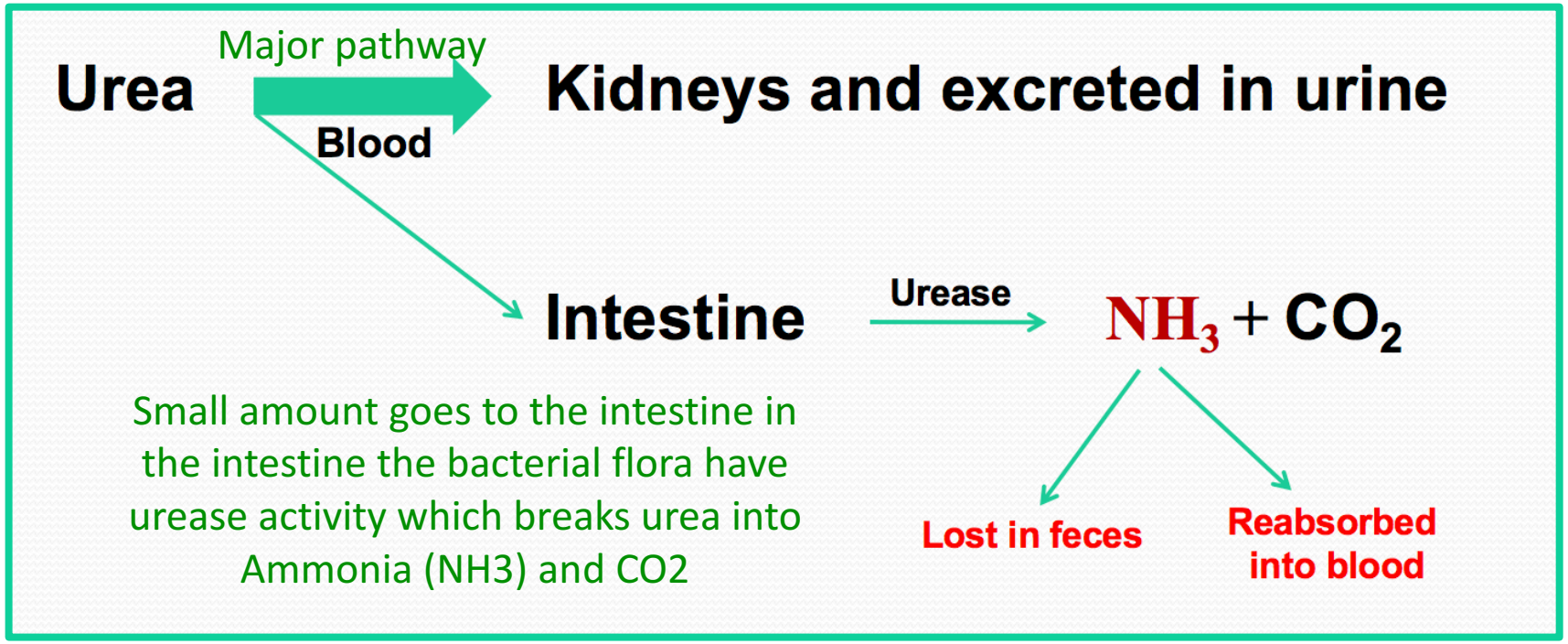
NAGS deficiency is efficiently treated with **Carbaglue**, a CPS1 activator



Here it shows you how the N-Acetyl glutamate is formed which is an allosteric activator for CPSI

Glutamate + Acetyl CoA in the presence of Arginine forms N-acetyl glutamate by the action of N-acetyl glutamate synthetase (NAGS)

Fate of urea

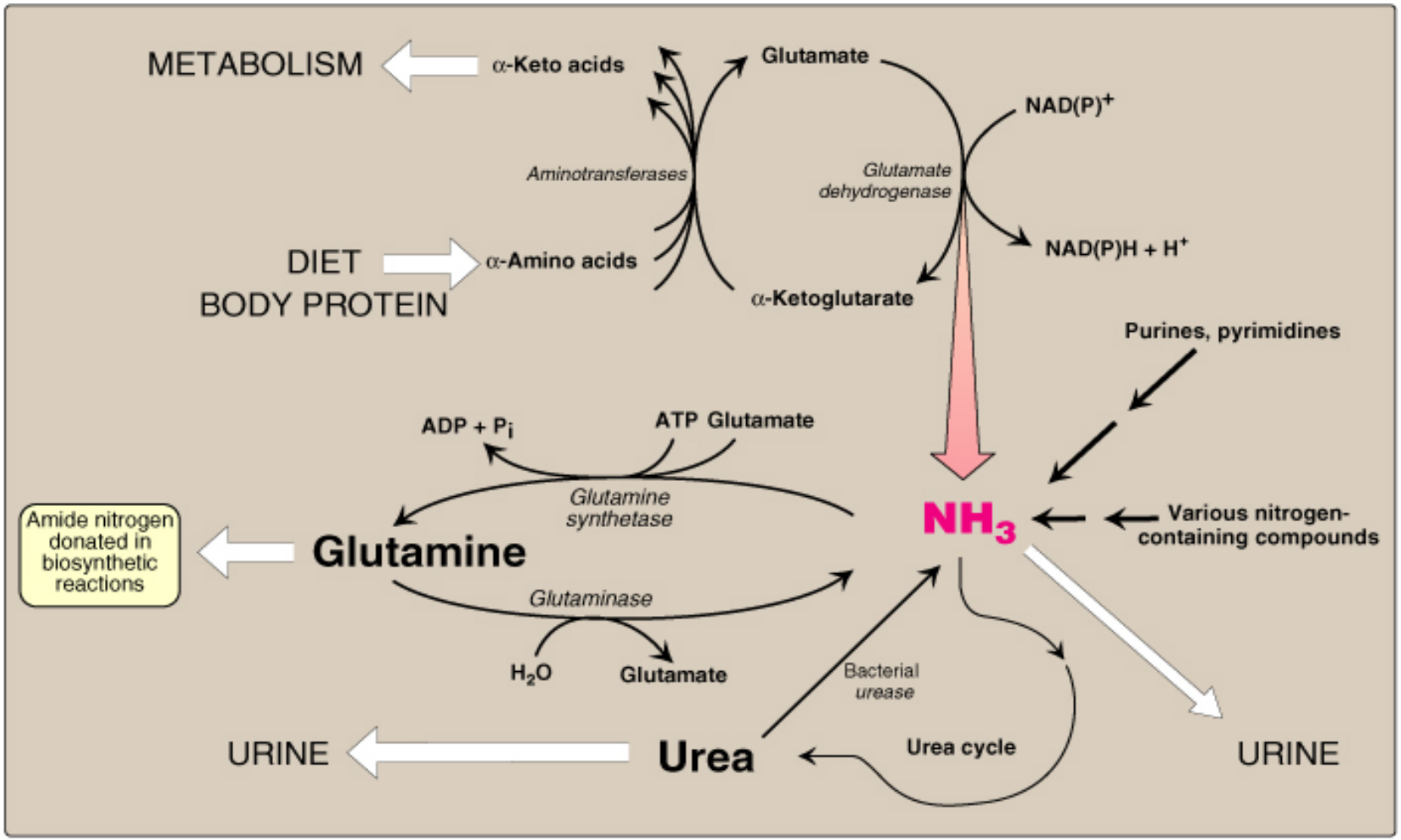


In normal health conditions, the NH3 results which is in small amounts won't affect us

In renal failure , the major pathway to the kidneys will be inhibited and more urea will go to the intestine , this leads to large amounts of ammonia that are reabsorbed into the blood(acquired hyperammonemia). This NH3 can diffuse into BBB and cause CNS toxicity

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

Sources and Fates of Ammonia



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

Hyperammonemia

❖ Acquired hyperammonemia:

1. Liver diseases:
Acute: Viral hepatitis or hepatotoxic
Chronic: Cirrhosis by hepatitis or alcoholism
2. Renal failure

❖ Inherited hyperammonemia:

A. Genetic deficiencies of any of the 5 enzymes of urea cycle or the activator enzyme for CPSI :
CPSI, OTC, ASS, ASL, arginase or NAGS

B. Ornithine transcarbamoylase deficiency:
X-linked recessive
Most common of congenital hyperammonemia
Marked decrease of citrulline and arginine.

C. Others : Autosomal recessive

Clinical presentation of hyperammonemia

CNS problems mainly

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

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

Management & treatment of hyperammonemia

Management

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

If N-acetylglutamate synthetase (NAGS) is deficient , we can't form N- acetylglutamate (NAG)which is an allosteric activator of CPSI. We can give a medication called Carglumic acid(carbaglu) that can directly activate CPSI to enhance the urea cycle

Treatment

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

1. I.V. Sodium phenylacetate & sodium benzoate (Ammonul)
2. Oral sodium phenyl butyrate (Buphenyl)
3. I.V. Arginine: for all UCDs except UCD due to arginase deficiency (argininemia)

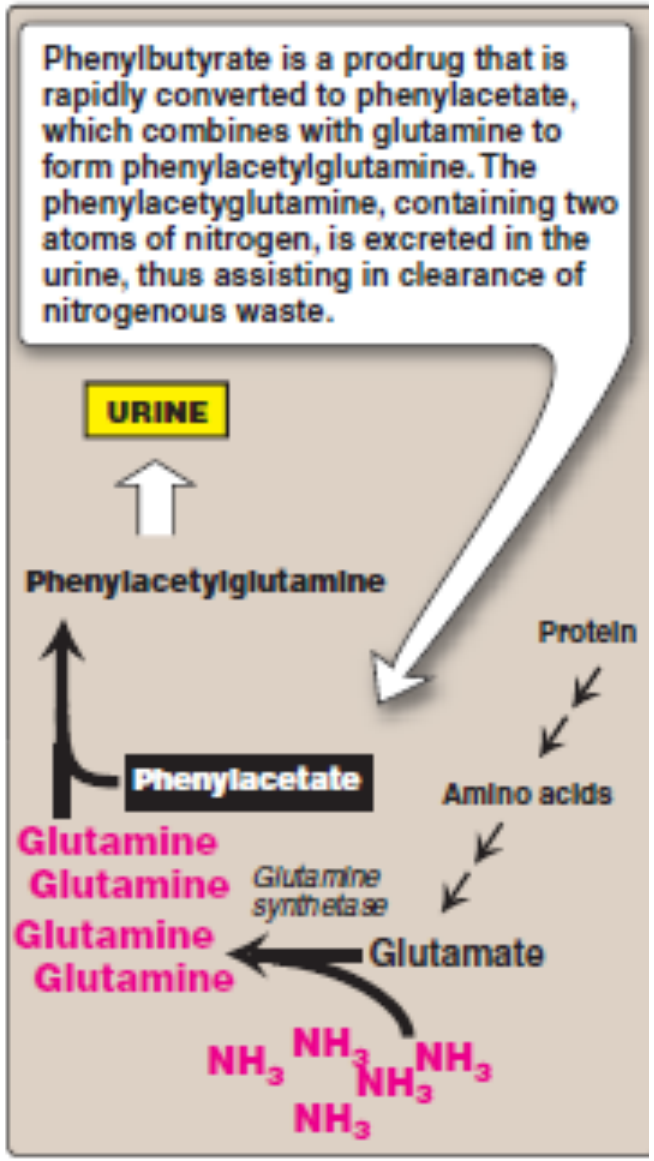
B. Activators to CPSI (Carglumic acid “Carbaglu”): For hyperammoniemia due to NAGS deficiency

Sodium phenyl butyrate (Buphenyl)

Sodium phenyl butyrate (Buphenyl):
Pro-drug that is converted to phenylacetate.

Phenylacetate condenses with glutamine forming phenylacetylglutamine that is excreted in urine

No urea formation here, there is direct excretion of ammonia into urine in the form of phenyl-acetyl-glutamine



Summary

summary	
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	B-Oxidative deamination: in liver **By: Glutamate dehydrogenase. IMP** - The glutamate will release **NH3** - Regenerate α-ketoglutarate
Transport of NH_3 from peripheral tissues into the liver	
Glutamine (from most tissues → liver) Glutamine formed by glutamine synthetase	**Alanine** (from muscles → 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 **NH_3** by **glutamate dehydrogenase**	

Summary

Enzyme of urea :	**1. Carbamoyl phosphate synthetase I (CPSI)**	**2. Ornithine transcarbamoylase (OCT)**	
	3. Argininosuccinate synthase (ASS)	**4. Argininosuccinate lyase (ASL)**	**5. Arginase**
Regulation of urea cycle	- RATE-LIMITING ENZYMEof 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: A- To the kidneys (Mostly) excreted in urine . B- To the intestine by urease gives NH_3(lost in feces or reabsorbed into blood + CO_2			
Hyperammonemia	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		

QUIZ

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 secondary to renal failure.
B. Hyperammonemia secondary to CPS1 deficiency.
C. Hyperammonemia secondary to OCT deficiency
D. Hyperammonemia secondary to NAGS deficiency.

Q6 : Amino Group of Amino Acids are funneled to Glutamine by ?

A. Transamination
B. Oxidative Deamination
C. Decarboxylation
D. Hydrolysis

QUIZ

Q7 : Mention the conditions which can cause Hyperammonemia ?

Inherited: Ornithine transcarbamoylase deficiency and others Autosomal dominant Acquired: Liver disease like Viral Hepatitis, Hepatotoxic ex Alcohol, Cirrhosis Renal Failure

Q8 : What is the normal blood level of Ammonia?

5 – 50 µmol/L

Q9 : Interpret signs and symptoms of hyperammonemia ?

Ammonia is a toxic product and it can cross the Blood brain barrier, causing:

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

Q10 : What is the enzyme required for the oxidative deamination of glutamate ?

Glutamate dehydrogenase

Suggestions and recommendations

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

Biochemistry Team 436

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THANK YOU

FOR CHECKING OUR WORK

PLEASE CONTACT US IF YOU HAVE ANY ISSUE

- Lippincott's Illusrated Reviews Biochemistry 6th E

Review the notes

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