VNIVERSITAT DE VALÈNCIA

LESSON 29. METABOLISM OF AMINO ACIDS (III): CATABOLISM OF AMINO ACIDS. TRANSPORT OF AMMONIA AND THE UREA CYCLE.

Amparo Galán Albiñana

Biochemistry and Molecular Biology

Degree in Medicine

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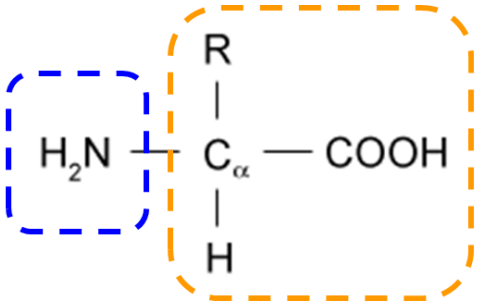
amparo.galan@uv.es

Department of
Biochemistry and Molecular Biology



AMINO ACIDS

PROTEINS

Structural blocks (amino acids)
Plastic or structural function

Protein turnover

Lesson 27

BIOMOLECULES

Prosthetic groups

Neurotransmitters

Biogenic amines

Purines and pirimidine bases

Lesson 30

DEGRADATION

Amino group loss

NH_3 + Carbon chain

Urea cycle

Lesson 29

Glucose
CO_2
Acetil-CoA
Ketone bodies

Lesson 28

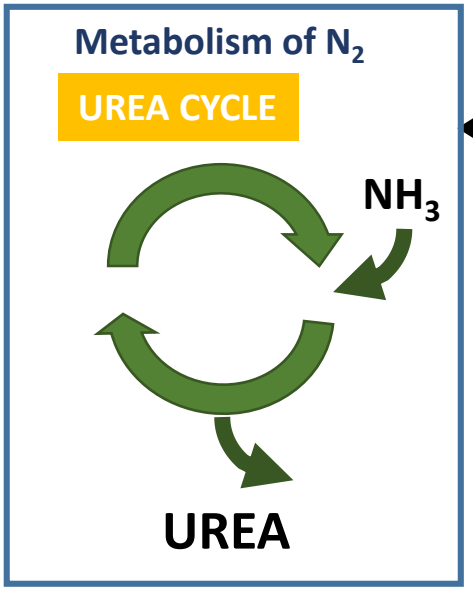
Amino acids cannot be stored or secreted.
Site of tissue degradation: liver and, occasionally, skeletal muscle.

INDEX

1. ORIGIN OF AMMONIA AND TRANSPORT TO THE LIVER

CATABOLISM OF AMINO ACIDS

TRANSAMINATION
OXIDATIVE DEAMINATION



CARBON CHAIN

2) Metabolism of the carbon chain that is recycled

1) The elimination and metabolism of N from amino acids that is ultimately excreted in urine and faeces

1. AMINO GROUP LOSS. ORIGIN OF AMMONIA AND TRANSPORT TO THE LIVER

NEED AND BIOCHEMICAL MECHANISMS FOR NH_3/NH_4^+ ELIMINATION

Ammonia is toxic for the organism

Part of the NH_3 generated is reincorporated into the biosynthesis of other compounds. However, the excess is toxic and must be eliminated. The catabolic production of NH_4^+ involves a biochemical problem because ammonium is highly toxic. The brain is particularly sensitive, which causes cognitive impairment, ataxia and epileptic episodes. In extreme cases, there is cerebral oedema and death. As roughly 98% of N is in its protonated (+) form (NH_4^+) in the blood, it cannot cross the plasma membrane. However, the small amount of NH_3 (2%) present can cross all membranes, including the blood-brain barrier. This allows it to enter cells, where much of it is converted into its protonated form (NH_4^+) and may accumulate.

Ammonia excretion occurs differently in different organisms. In humans it is mostly excreted through the urea cycle.

The pathway by which NH_4^+ is converted into urea was the first metabolic cycle to be described. It was discovered by Sir Hans Krebs (1932).

Urea is the main way in which amino groups are eliminated from amino acids.

1. AMINO GROUP LOSS. ORIGIN OF AMMONIA AND TRANSPORT TO THE LIVER

1. Excess NH_3/NH_4^+ is toxic and must be eliminated from the organism through the formation of urea (non-toxic) in the liver that is later excreted in the kidneys.

2. NH_3/NH_4^+ cannot be delivered freely in the bloodstream from the peripheral tissues to the liver. It requires specific transport mechanisms in the bloodstream:

- Glutamine (highly concentrated amino acid in plasma)
- Glucose-alanine cycle muscle specific transport (high content in proteins)

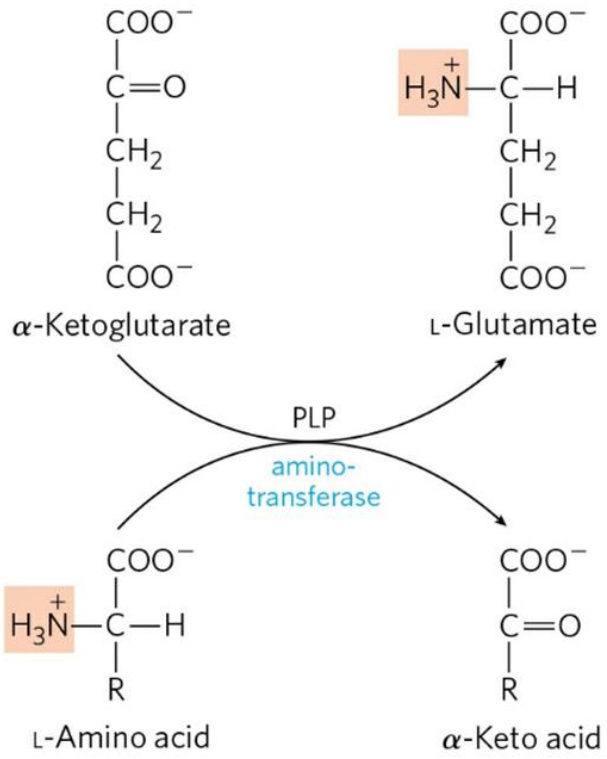
3. In the liver, NH_3 is extracted from glutamine and glucose-alanine cycle to produce urea.

Other minority sources that produce NH_3:

1. Kidneys generate NH_3 from renal glutamine through the action of glutaminase and glutamine dehydrogenase (GDH). Most from this ammonium is directly excreted through urine as free NH_4^+
2. Intestinal glutaminase generates NH_3, which circulates to the liver.
3. Intestinal bacteria have bacterial urease, which produces NH_3, which is delivered to the liver through portal transport.
4. Amines from the diet or from hormones or neurotransmitters of other origin (biogenic amines) give rise to ammonia after monoaminoxidase catabolism.
5. Purine and pyrimidine catabolism.

1.1 TRANSAMINATION REACTIONS

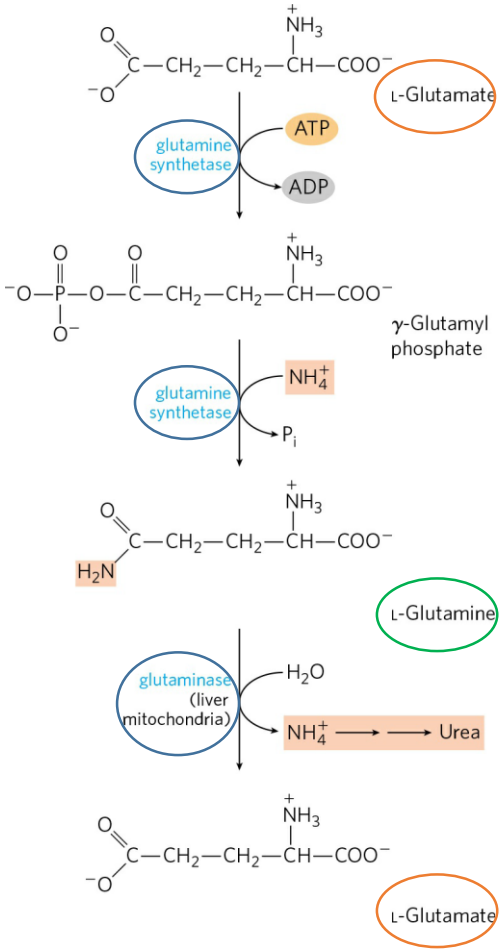
1. TRANSAMINATION
2. OXIDATIVE DEAMINATION



1. Transamination reaction:
Glutamate formation from α-ketoglutarate

- **All tissues (liver and other tissues)**
- **Aminotransferases (transaminases):**
  specific aminotransferase for each amino acid
- **Prosthetic group: pyridoxal phosphate (PLP) (derived from vitamin B6)**
- **Reversible reactions (according to requirements)**

AMMONIUM TRANSPORT IN THE BLOOD TO THE LIVER



TISSUES*

* All tissues except muscle

Specific amino transferases

R-CH-COO$^-$ + α-KG ⟹ α-AA + **Glu**

Ammonium is transported in the form of Glutamine.
Excess ammonium in the tissues is added to Glutamate to form Glutamine, a process catalyzed by the enzyme glutamine synthetase.

glutamine synthetase

Glu + $NH4^+$ ⟹ **Gln**

After transport through bloodstream, Glutamine enters the liver and is released into the mitochondria by the enzyme glutaminase to again form Glutamate.

LIVER (mitochondria)
glutaminase

Gln ⟹ **Glu** + $NH4^+$ ⟹ **Urea cycle**

1.2 TRANSPORT TO THE LIVER

Glutamine transports ammonia in the blood from all tissues except the muscle.

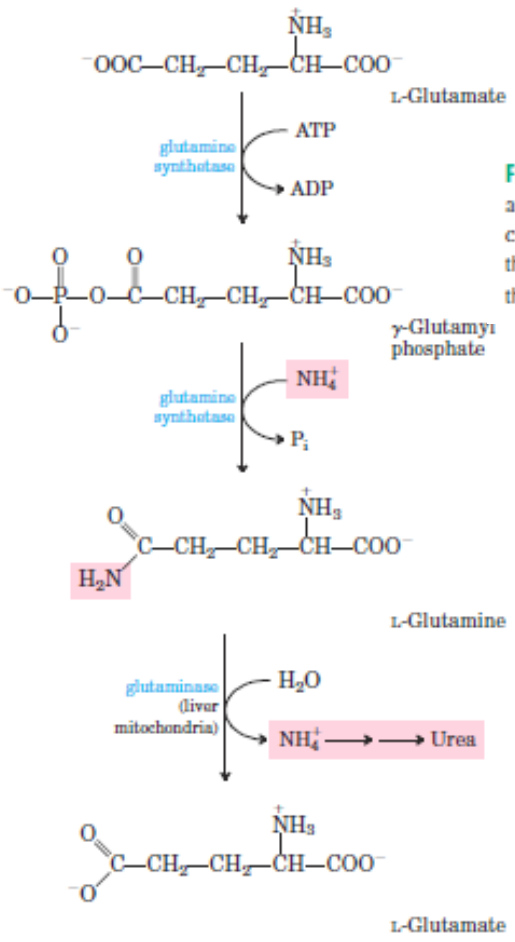


FIGURE 18–8 Ammonia transport in the form of glutamine. Excess ammonia in tissues is added to glutamate to form glutamine, a process catalyzed by glutamine synthetase. After transport in the bloodstream, the glutamine enters the liver and NH_4^+ is liberated in mitochondria by the enzyme glutaminase.

Alanine transports ammonia from the muscle to the liver.

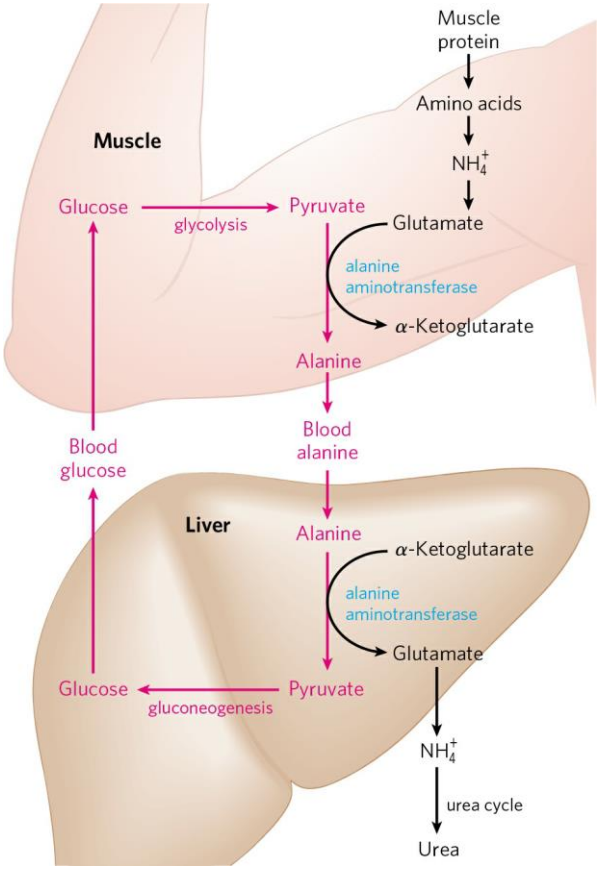
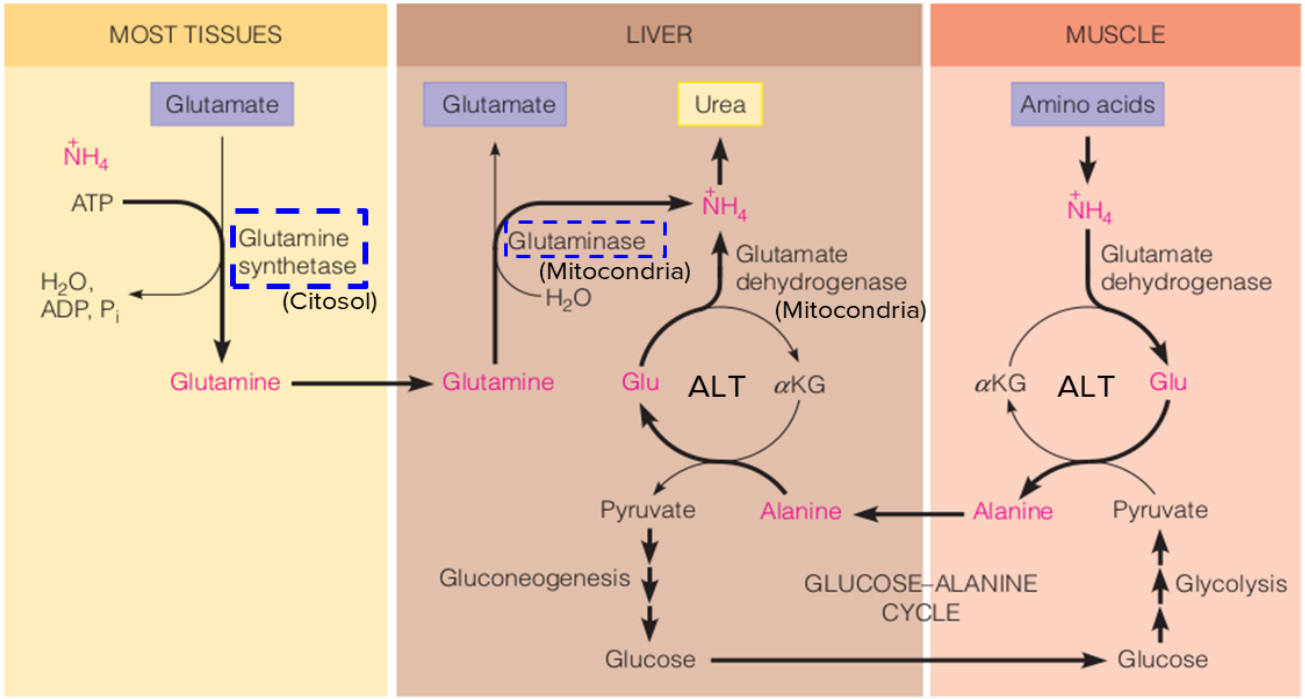


FIGURE 18–9 Glucose-alanine cycle. Alanine serves as a carrier of ammonia and of the carbon skeleton of pyruvate from skeletal muscle to liver. The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.

Lehninger

1.2 TRANSPORT TO THE LIVER

Ammonia produced in extra-hepatic tissues is transported to the liver in the form of glutamine and alanine



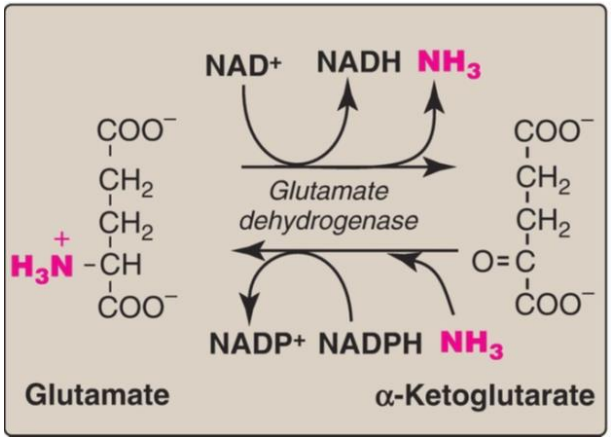
Ammonia transport to the liver (liver mitochondria)

Glutaminase → **Glutamate**

Alanine amino transferase (ALT) → **Glutamate**

1.3 LOSS OF AMINO GROUP

1. TRANSAMINATION
2. OXIDATIVE DEAMINATION

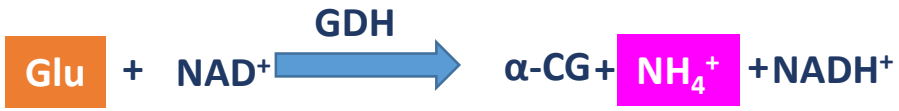


Once in the mitochondrial liver, glutamate suffers ammonia group loss by oxidative deamination

2. Oxidative deamination

Glutamate releases its amino group into the liver (mitochondria)

2. Oxidative deamination of the glutamate (release of ammonium ion ($NH4^+$))



Enzyme: Glutamate dehydrogenase (GDH)

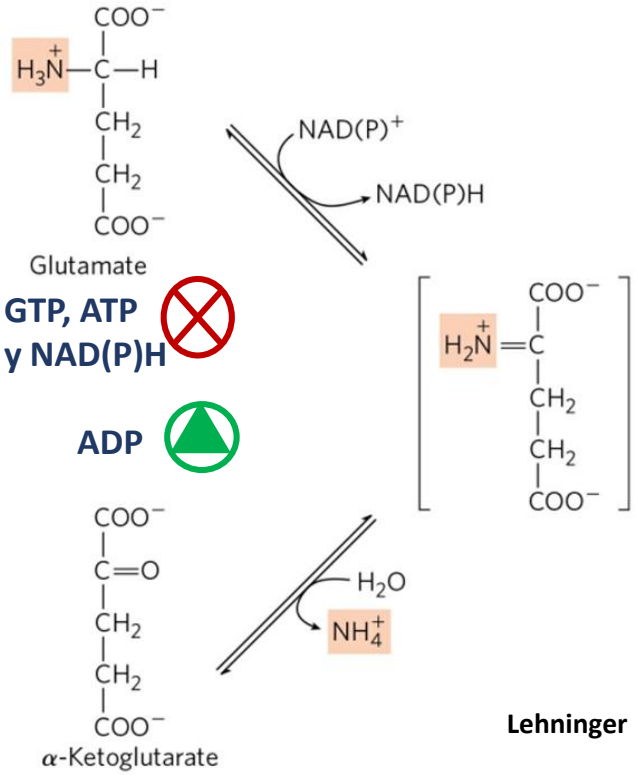
Reversible Reaction

Mitochondria in the liver

Cofactor: NAD^+ or $NADP^+$

Release of $NH4^+$ → **Toxic**

Urea cycle



Lehninger

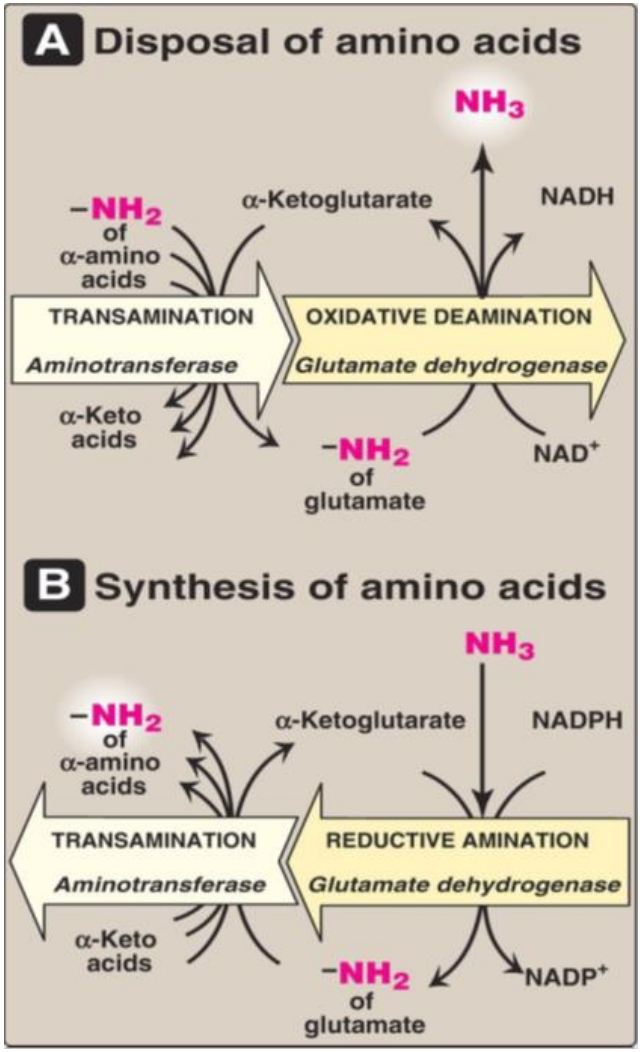
1.3 LOSS OF AMINO GROUP

1. TRANSAMINATION
2. OXIDATIVE DEAMINATION

2. Oxidative deamination

Glutamate is incorporated into **non-essential amino acid** synthesis as a donor of amino groups that fuse with α-ketoacids or incorporates NH_3 in the **urea cycle**.

Amino acid catabolism



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The direction of the reaction depends on the relative concentrations of **glutamate**, **α-ketoglutarate** and **ammonia** and the ratio between oxidized coenzymes and reduced coenzymes.

Amino acid synthesis

1.3 LOSS OF AMINO GROUP

Minoritarian amino group (NH_3) loss mechanisms

1. OXIDATIVE DEAMINATION OF D-amino acids

D-amino acid oxidase:

-oxidises D-amino acids from the diet
-produces α-ketoacids, ammonia (NH_4^+) and hydrogen peroxide (H_2O_2)
The reaction is catalysed catalysed in liver and kidney peroxisomes and uses as a cofactor flavin adenine dinucleotide (FAD)

2. DEHYDRATION

Serine dehydratase

Serine $\xrightarrow{PLP}$ Pyruvate+ NH_4^++ H_2O

Threonine dehydratase

Threonine $\xrightarrow{PLP}$ α-Ketobutyrate+ NH_4^++ H_2O

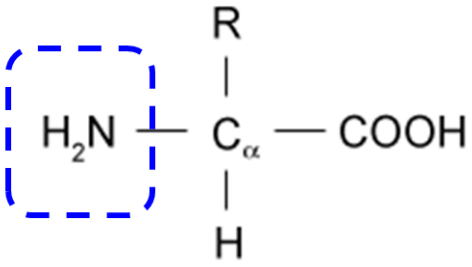
3. DESULFURIZATION

Cysteine $\longrightarrow$ Pyruvate+ NH_4^++ SH_2

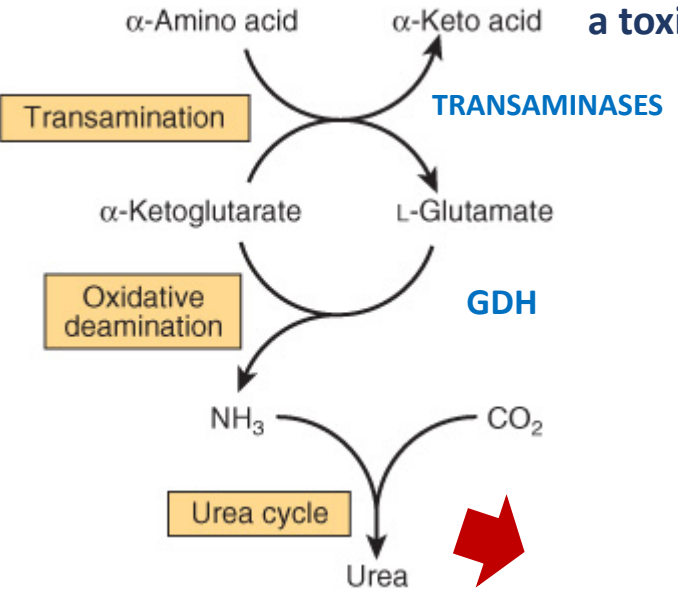
1.3 LOSS OF AMINO GROUP

TRANSAMINATION + OXIDATIVE DEAMINATION

TRANSDEAMINATION



Most NH_3 is managed by transamination reactions that take place in the cytoplasm of all the cells in the body followed by transport to the liver in the form of Glutamine and Alanine and later oxidative deamination in the mitochondria of hepatocytes. The two components of the reaction are physically far away but physiologically coupled in a reaction known as transdeamination. Finally, the amino groups enter the urea cycle to convert a toxic molecule, NH_3, into a non-toxic disposable molecule: urea.



AMINO TRANSFERASES

1. R-CH-COO + α-CG → α-AA + Glu

GDH

2. Glu + NAD^+ → α-CG + NH_4^+ + $NADH^+$

OXIDATIVE DEAMINATION

UREA

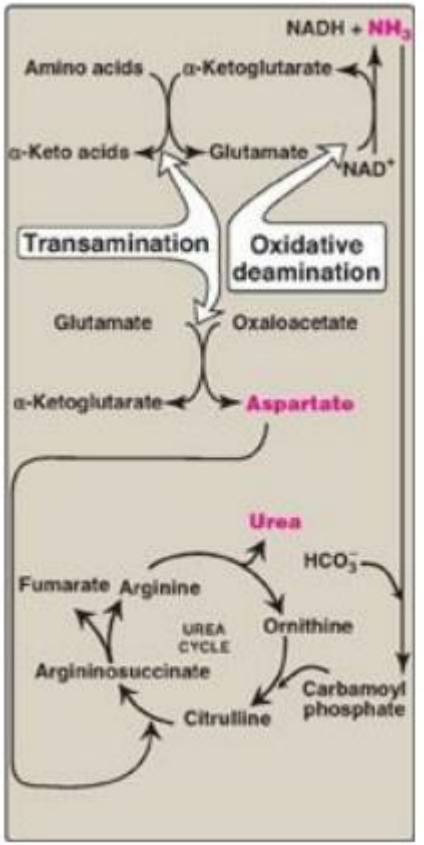
$H_2N\text{-}C(=O)\text{-}NH_2$

Liver mitochondria

Urea cycle: Biosynthesis of urea from ammonia (NH_3)

General stoichiometry

$NH_3 + HCO_3^- + \text{aspartate} + 3\ ATP + H_2O \rightarrow$
$\text{urea} + \text{fumarate} + 2\ ADP + 2\ Pi + AMP + PPi$



1. Main pathway to eliminate NH_3. 2 N per UREA* molecule
2. Urea is synthesized in the liver
3. Different reactions in different cell compartments:
   Two in the mitochondria and the other three in the cytosol.
4. High energy expense: four phosphate bonds (urea synthesis is irreversible).

3 ATP ⟶ **AMP+PPi+2ADP+2Pi**

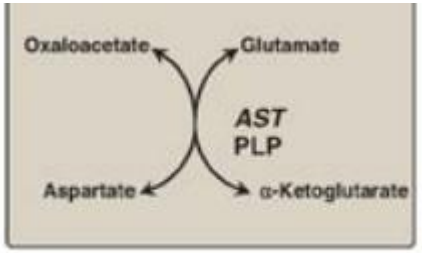
UREA

$H_2N\text{-}C\text{-}NH_2$ (O)

*2N: come from Aspartate and NH_3

Glutamate can be a precursor of either:

1. ammonia (oxidative deamination by GDH), or
2. nitrogen from aspartate (through transamination of oxaloacetate from AST).

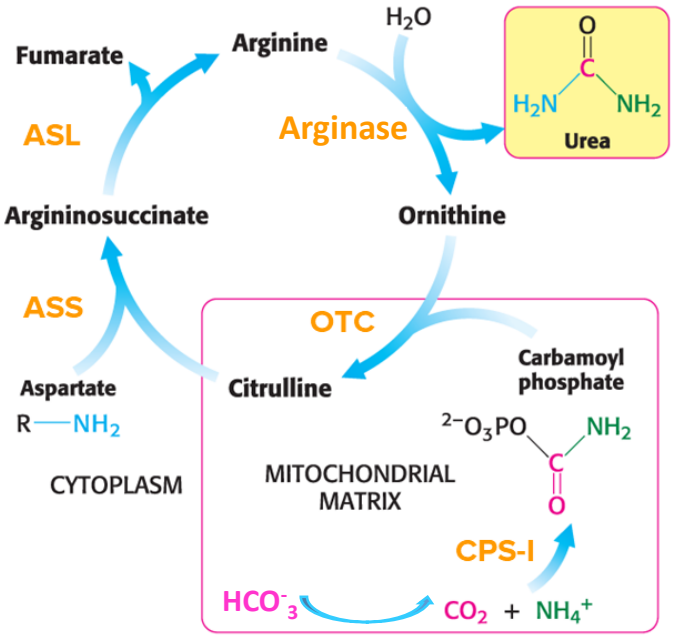


Aspartate: carbon chain for fumarate

HCO_3^-: C for urea

2. BIOSYNTHESIS OF UREA: CHEMICAL REACTIONS AND REGULATION

- Urea cycle (non-toxic): cyclic route → Condensation reactions of molecules to gather, in one molecule, two ammonia (from Glu and Asp) and one carboxyl: Urea, small molecule, non-toxic and easily discarded in the urine.
- Liver: mitochondria and cytosol
- Five enzymes:
  - Carbamoyl-phosphate synthetase I (CPS-I)
  - Ornithine transcarbamylase (OTC)
  - Argininosuccinate synthase (ASS)
  - Argininosuccinate lyase (ASL)
  - Arginase
- One amino group comes from NH_4^+ and the other comes from Aspartate
- Functions:
  - to prevent the accumulation of toxic nitrogenous compounds.
  - to participate in the *de novo* synthesis of Arginine.



2. BIOSYNTHESIS OF UREA: CHEMICAL REACTIONS AND REGULATION

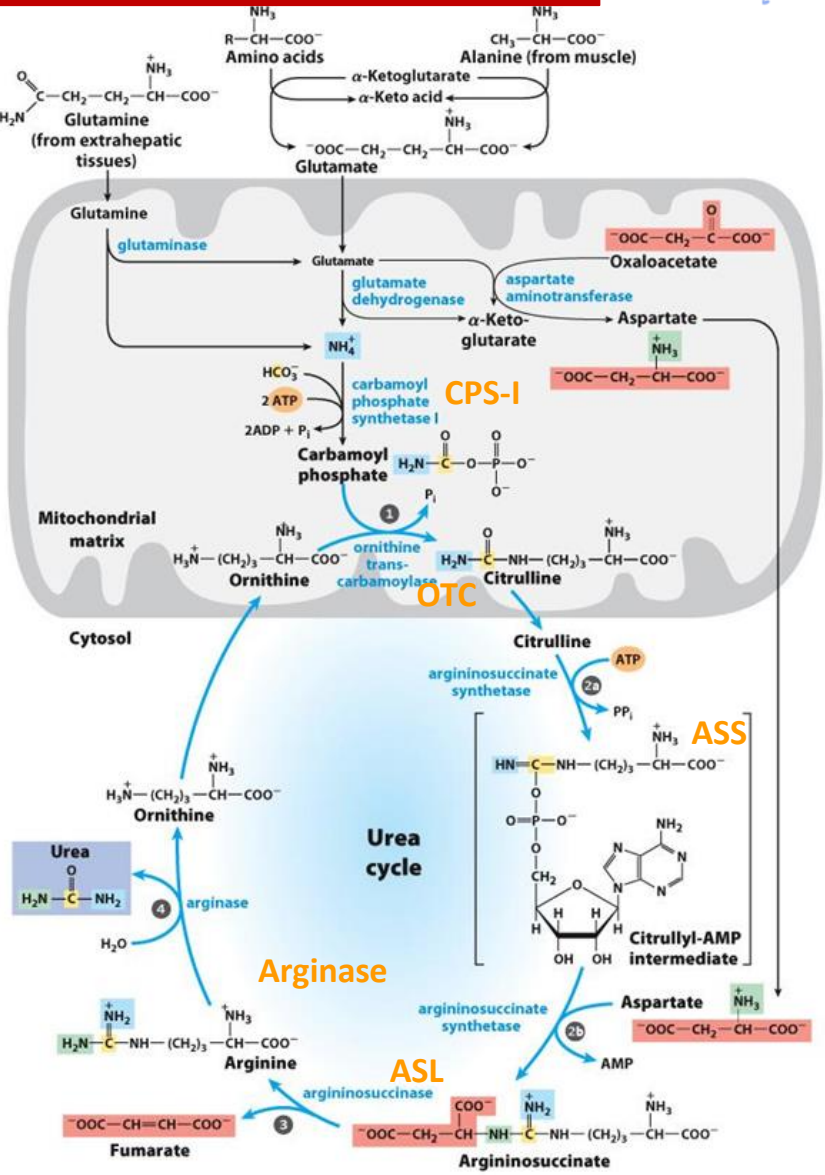
Global reaction

$HCO_3^- + NH4^+ + 3ATP + Aspartate + H_2O$

$\rightarrow$ Urea + 2ADP + 4Pi + AMP + Fumarate

Mitochondria

Cytosol



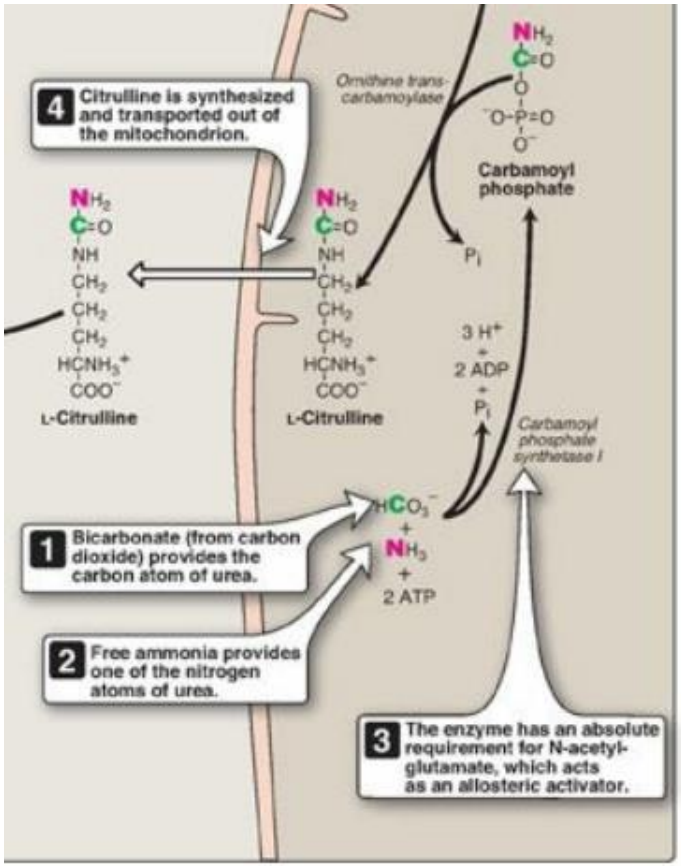
Urea biosynthesis takes place in five chemical reactions in two cellular compartments

MITOCHONDRIAL MATRIX:

1. **CARBAMOYL PHOSPHATE SYNTHASE I (CPS-I)** Synthesis of **Carbamoyl phosphate, CPS-I** needs **NAG (N-acetyl-glutamate)** to be allosterically activated.

2 ATP hydrolysis needed

HCO_3^- + NH_4^+ —(2 ATP → 2ADP+Pi)→ $H_2N\text{-}C\text{-}Pi$ (O)



2. **ORNITHINE TRANSCARBAMYLASE (OTC):** Synthesis of **L-Citrulline** through carbamoyl transference to ornithine and Pi is released. **Citrulline** is transported to cytosol.

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CYTOSOL:

3. ARGININOSUCCINATE SYNTHASE (ASS):

Argininosuccinate synthesis combining Citrulline and Aspartate.

L-Citrulline + Aspartate —(ATP → AMP+PPi)→ Argininosuccinate

4. ARGININOSUCCINATE LYASE (ASL):

Dissociation of Argininosuccinate to Arginine and Fumarate

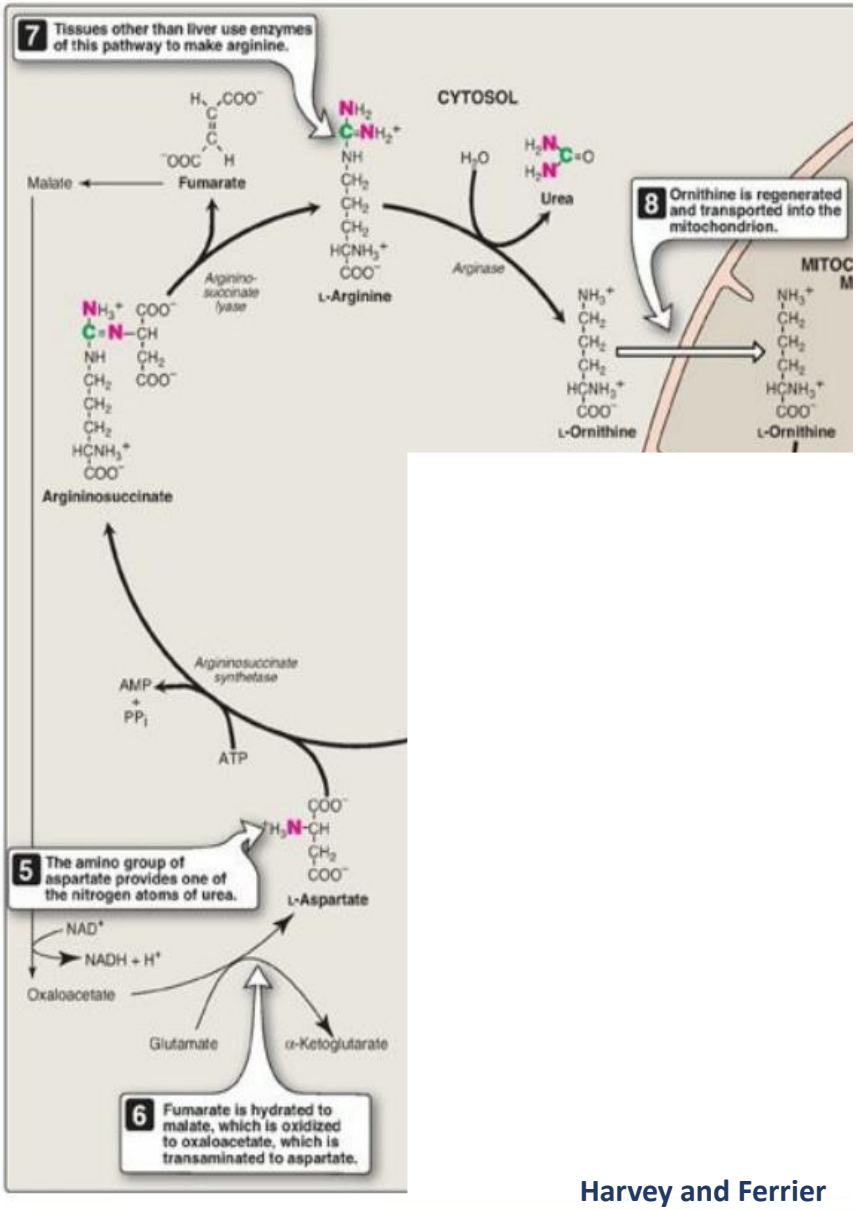
5. ARGINASE-I (ARGI):

Dissociation of Arginine to produce Ornithine and urea

Only the liver can dissociate Arginine and produce urea

Ornithine is transported to mitochondria

Ornithine and Citrulline can move through the internal mitochondrial membrane by means of an anti-carrier.



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REGULATORY MECHANISMS OF THE UREA CYCLE:

Short-term regulatory mechanisms:

CARBAMOYL PHOSPHATE SYNTEHASE (CPSI)

Allosteric regulation:

-Allosteric enzyme limiting step in the urea cycle

-Positive regulator: N-Acetylglutamate (NAG)

-Depends on substrate availability: NH_4^+

Long-term regulatory mechanisms:

High-protein content diets, diabetes

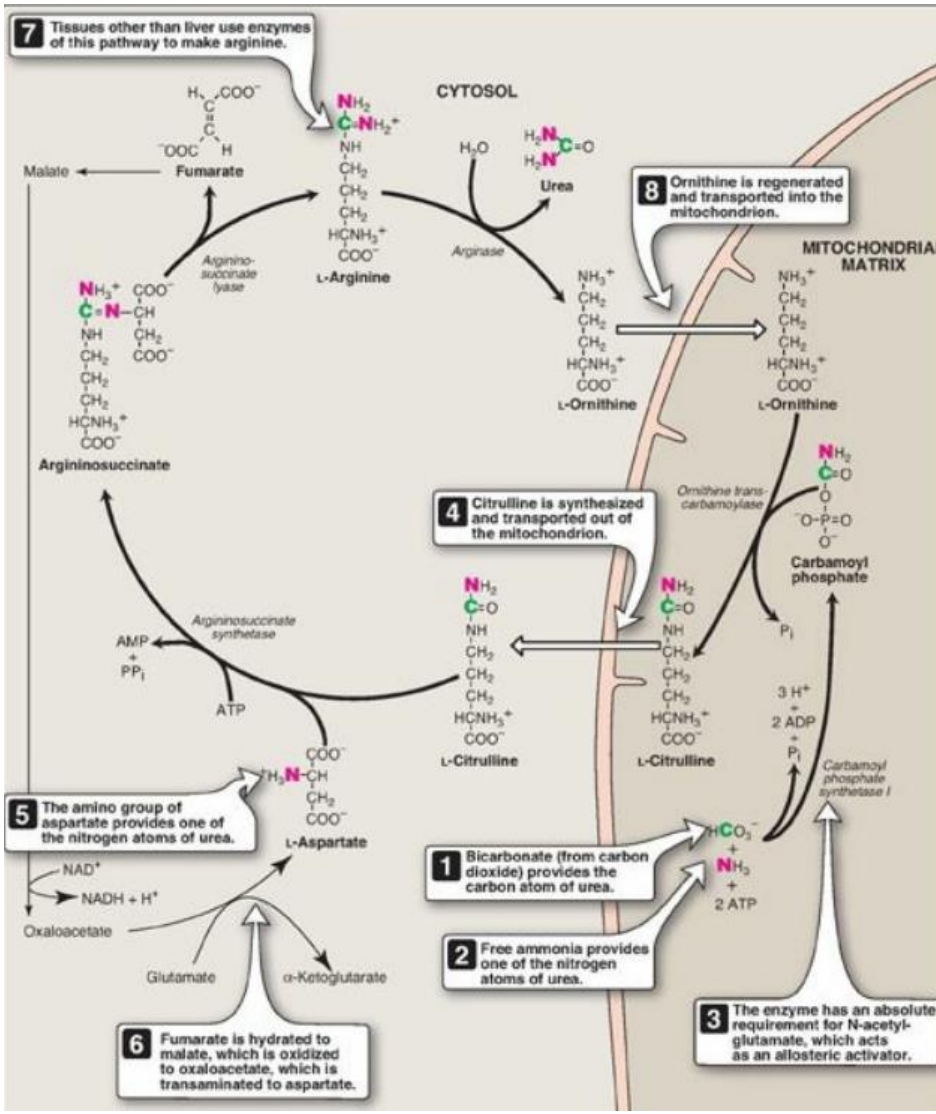
(use of proteins as the source of energy) or

severe fasting (destruction of body proteins):

Increase the activity in the cycle:

Complete increase in all 5 Enzymes

SYNTHESIS OF THE 5 ENZYMES



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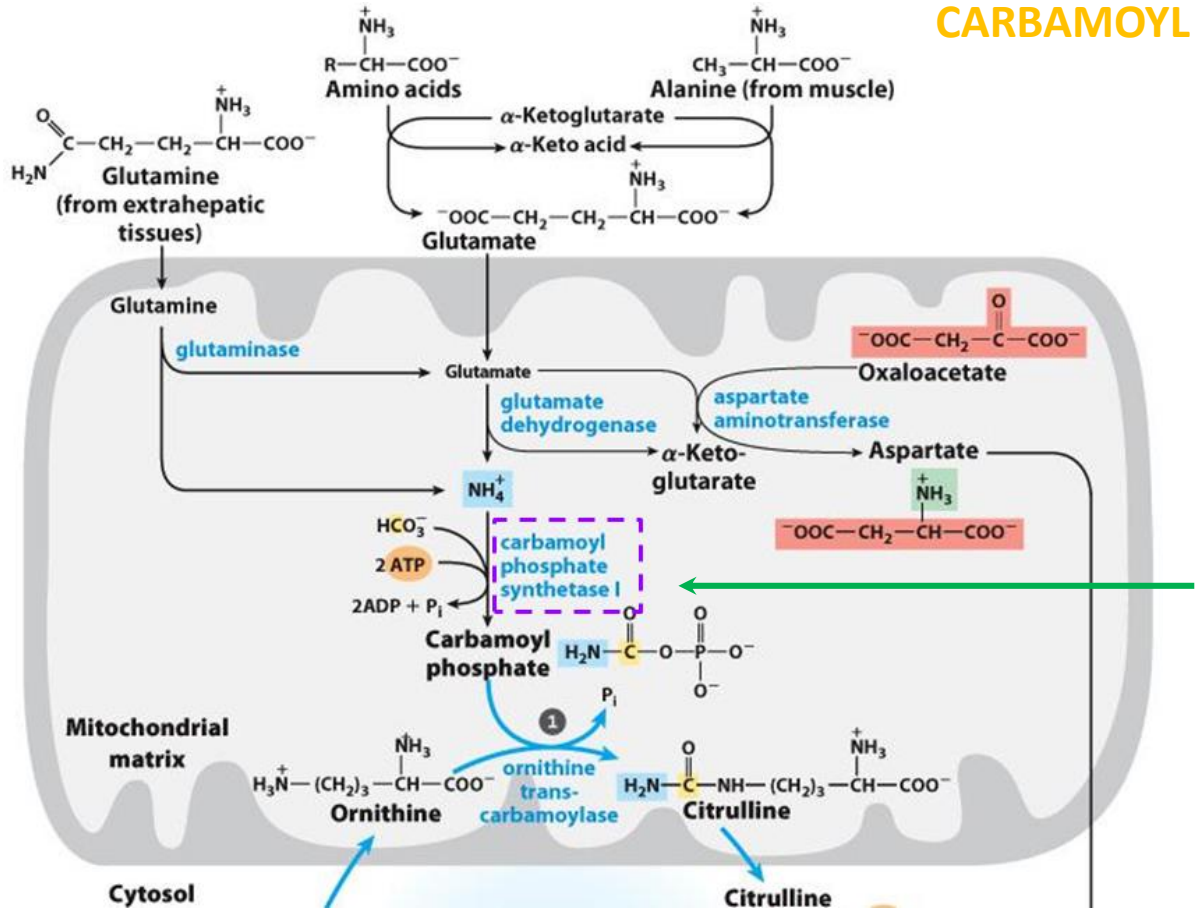
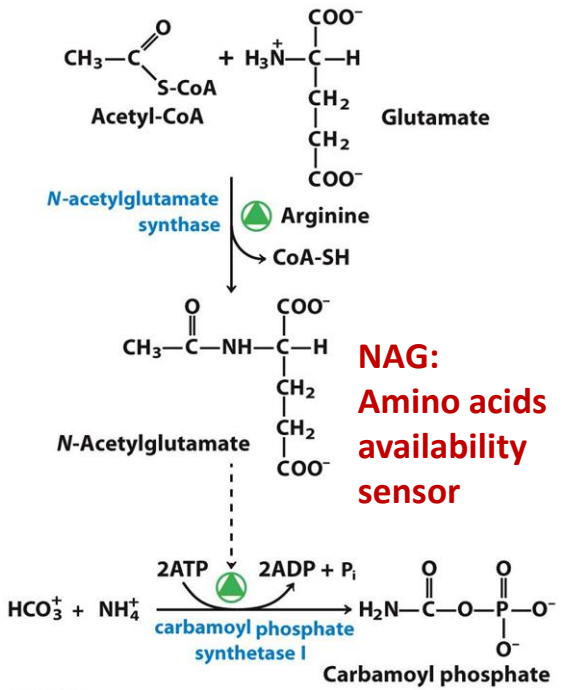
REGULATORY MECHANISMS OF THE UREA CYCLE:

Short-term regulation



CARBAMOYL PHOSPHATE SYNTETHASE (CPSI)

Short-term regulation



NAG: Amino acids availability sensor

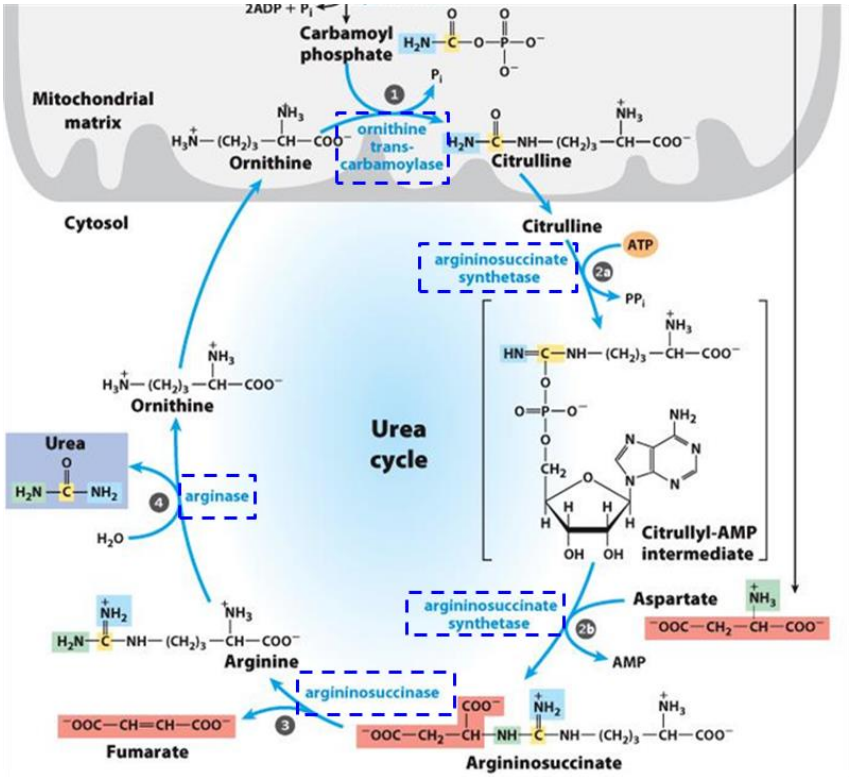
Essentially irreversible. CPSI is allosterically activated by N-Acetylglutamate (NAG) and is synthesized from glutamate and acetyl-CoA.

REGULATORY MECHANISMS OF THE UREA CYCLE:

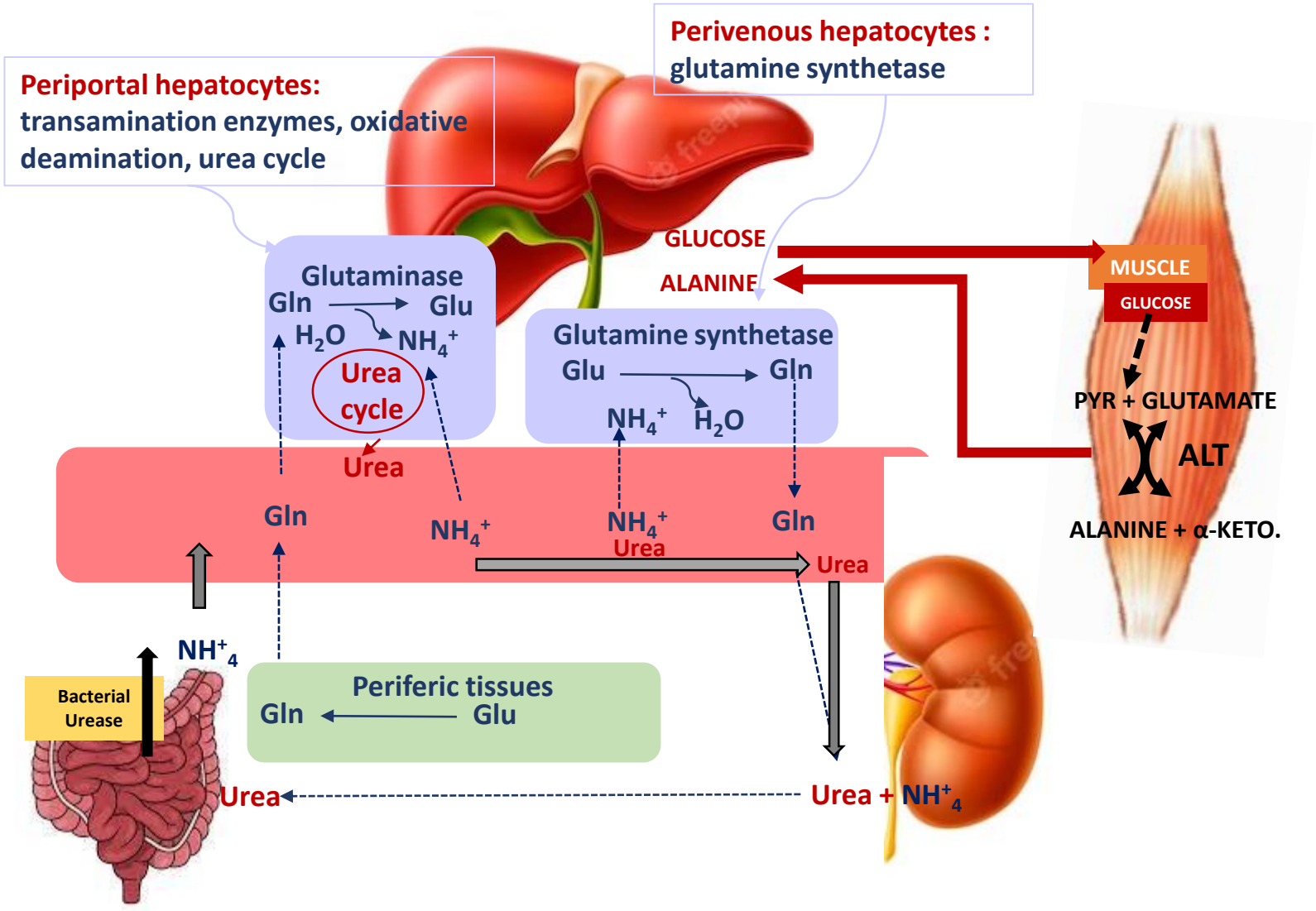
Long-term regulation

- The regulation is transcriptional.
- Protein-rich diet or starvation increases all five enzyme levels.

High-protein diets, diabetes
(use of proteins as a source of energy) or severe fasting (destruction of body proteins) increase the activity in the cycle:
Complete increase in the 5 ENZYME LEVELS



Inter-tissue ammonia transport



Urine is made up as follows: 2-3% N is free NH_4^+, 85% N is urea, and all the rest is creatinine, creatine and uric acid.

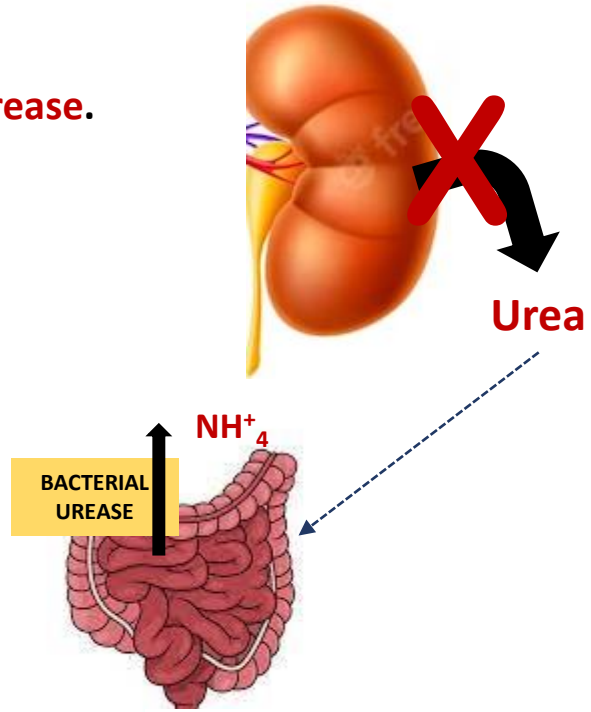
Inter-tissue ammonia transport

Urea destination:

-Kidney: Urea transport to the kidney to be excreted.
-Intestine: Urea is degraded to CO_2 and NH_3 by bacterial urease.

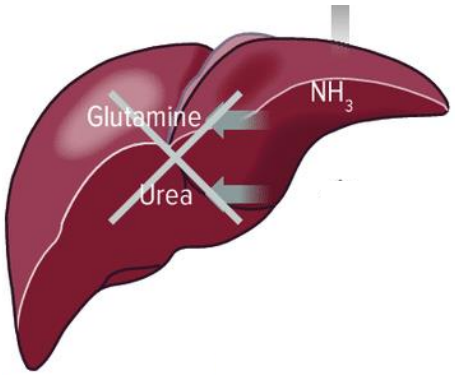
Renal insufficiency: high blood urea levels that pass into faeces.

A large amount of bacterial NH_3 is generated that can pass into the blood and induce hyperammonaemia.



The ammonia concentration in blood is 30-60 µM.
Urine:

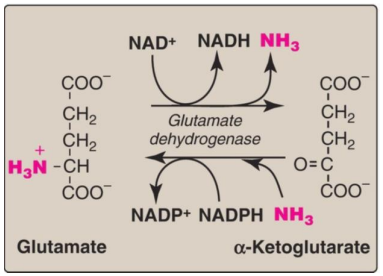
2-3% N is free NH_4^+,
85% N is urea, and
the rest is creatinine, creatine and uric acid.



Malfunction of urea cycle due to deficiency in any of its enzymes or to liver pathology (defective urea cycle, alcoholic cirrhosis) produces hyperammonaemia.

Inter-tissue ammonia transport

Hyperammonaemia: blurred vision, loss of consciousness, lethargy, brain injuries, coma, cerebral oedema, increased blood pressure.



Potential causes that produce these symptoms:

α-ketoglutarate levels decrease (↓) by pushing GDH reaction to the synthesis of Glutamate ↑ and Glutamine synthetase to the synthesis of Glutamine ↑ (both enzymes are abundant in nervous tissue).

glutamine synthetase

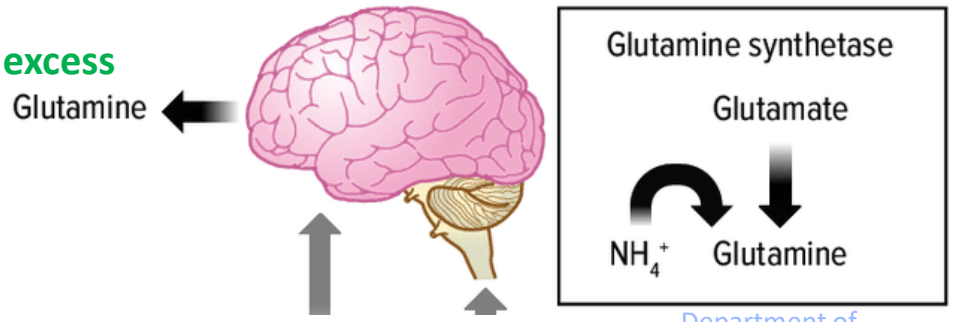
Glu + $NH4^+$ → Gln

A large concentration of NH_4^+ sequesters α-ketoglutarate in the form of Glu and depletes the Krebs cycle. ATP production decreases.

A large concentration of NH_4^+ decreases the NADH/NAD^+ ratio, thus decreasing ATP production.

→ Brain (nervous system): loss of consciousness, lethargy, coma

Glutamate excess provokes glutamine excess in astrocytes and is toxic.



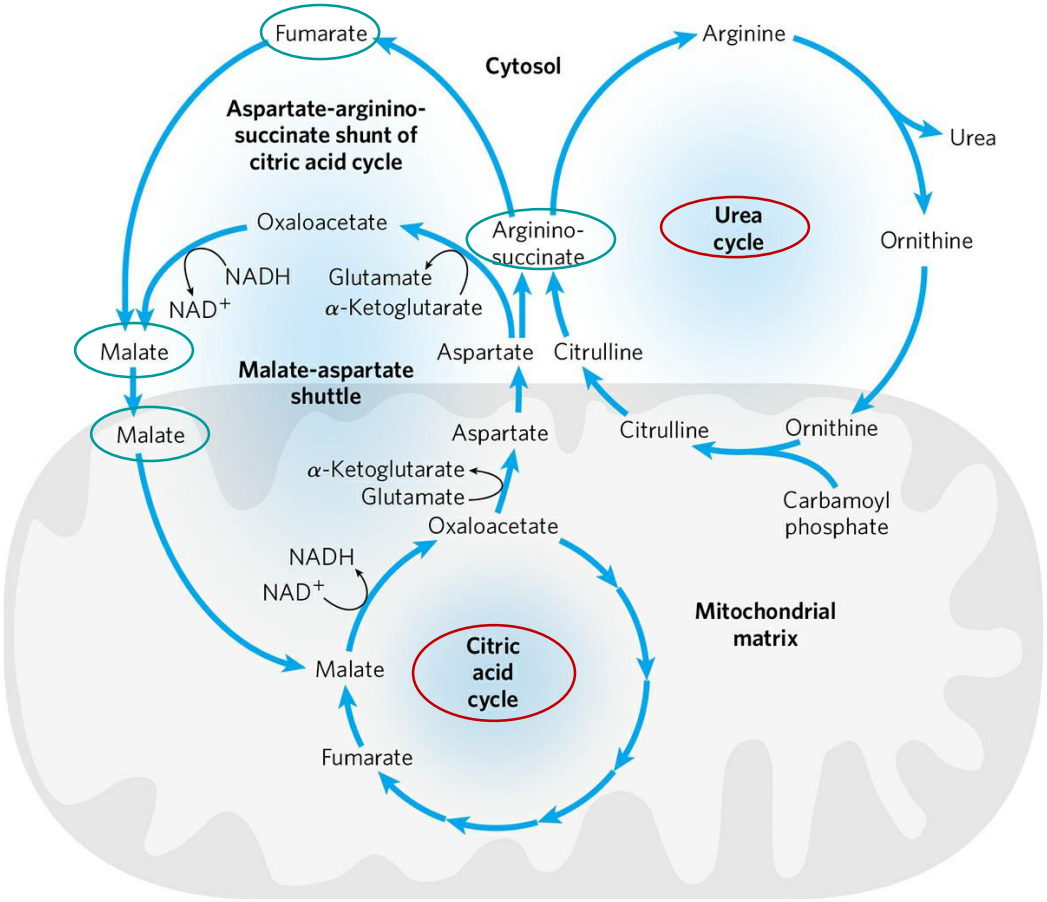
The urea cycle and the citric acid cycle (the Krebs cycle) can be connected. Destination of Fumarate

The cycles are interconnected and have been named the "Krebs bicycle". Pathways connecting these two cycles are known as the aspartate-argininosuccinate shunt of the citric acid cycle. These effectively connect the amino and carbon chains of the amino acids.

Some enzymes from the citric acid cycle, such as fumarase and malate dehydrogenase, have cytosolic and mitochondrial isoenzymes.

Fumarate produced in the cytosol, from the urea cycle, purine biosynthesis or other processes, can be converted into cytosolic malate, which can be used in the cytosol or be transported to mitochondria to enter the citric acid cycle.

These processes are further intertwined with the malate-aspartate shuttle, a set of reactions that brings reducing equivalents into the mitochondria.



3. INTERRELATIONSHIP BETWEEN UREA SYNTHESIS AND CITRIC ACID CYCLE

Destination of Fumarate

Fumarate hydrates to Malate and provides a link to different metabolic pathways.

CYTOSOL

Fumarate → (Fumarase) → Malate → (MD (Cytosolic)) → Oxaloacetate → (AST) → Aspartate → Urea cycle

Malate-Aspartate shuttle

MITOCHONDRIA

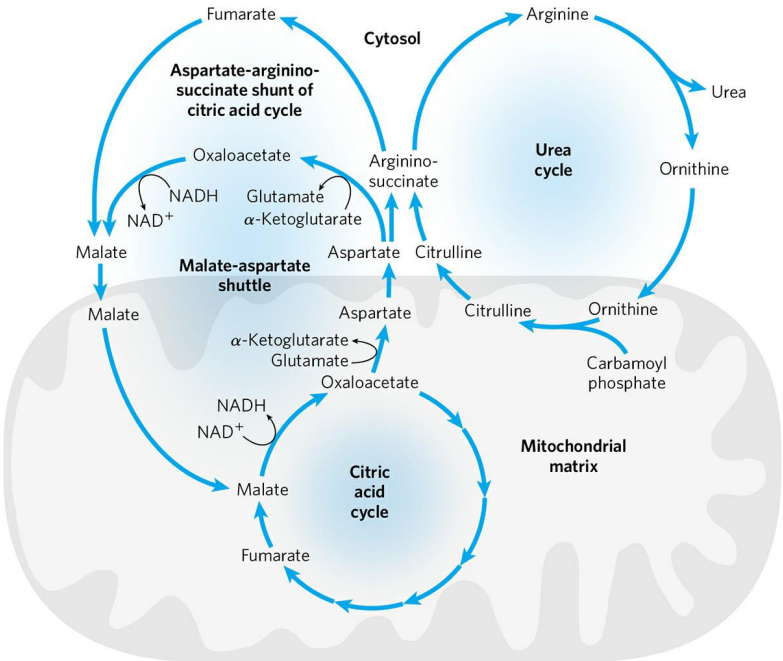
Malate → CITRIC ACID CYCLE → Oxaloacetate (NADH)

Oxaloacetate → (AST) → Aspartate

ATP

Gluconeogenesis

Cyclic



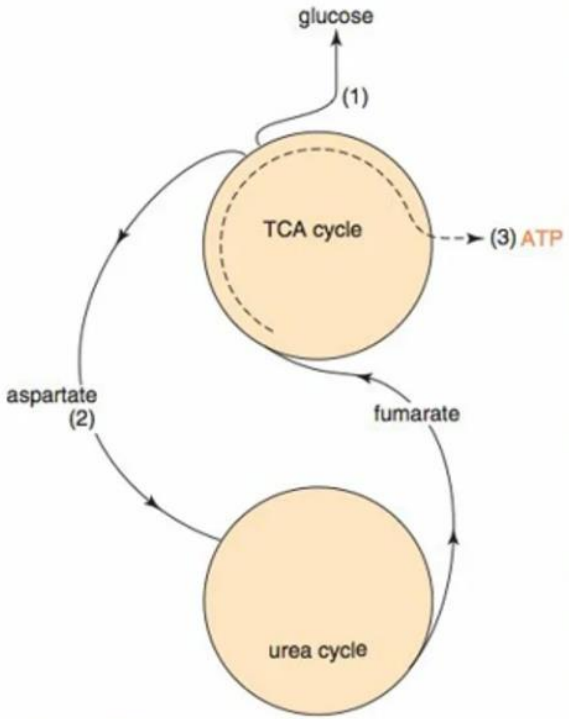
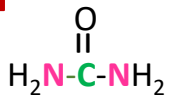


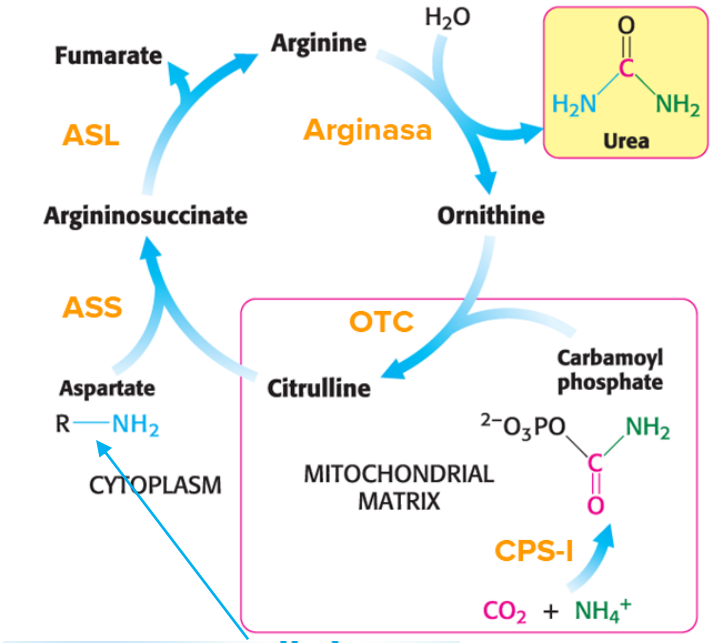
Figure 19.24 **Fumarate from the urea cycle is a source of glucose (1), aspartate (2), or energy (3).**

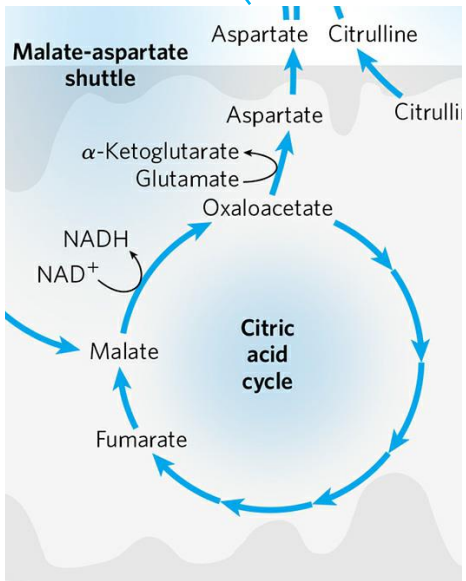
Devlin

BIOSYNTHESIS OF UREA: SUMMARY

UREA







MITOCHONDRIAL MATRIX

1. **Urea** synthesis takes place in five enzymatic reactions
   - in mitochondria (CPS-I, OTC)
   - in cytosol (ASS, ASL, Arginase)

2. **Two molecules** of **N** are discarded, four **phosphate** bonds **(3 ATP)** are required.

3. **Fumarate** synthesis and **Aspartate** and **CO_2** are used.

4. **CPSI** is allosterically controlled by N-acetyl glutamate **(NAG).**

5. Interconnection by the **Krebs cycle** :
   Fumarate→Malate→ **Oxaloacetate: Urea cycle**
   Fumarate→Malate→ **Mitochondrial shuttle**
   Malate-Aspartate→ **Oxaloacetate: Gluconeogenesis and energy**

6. **Urea** diffuses to the kidney and is excreted. However, in **renal failure** it increases in plasma and drifts to the intestine to produce **hyperammonemia** due to **bacterial urease**.

Department of
Biochemistry and Molecular Biology