بسم الله الرحمٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | Final 16

Urea Cycle

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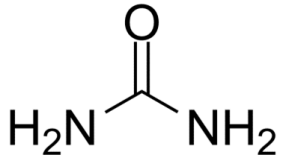
Art of the liver

UREA CYCLE

- We have reproduced the ammonia inside the liver also we agreed upon having a lot of CO2 inside the liver represented by the high metabolic rate (citric acid cycles) producing CO2 in excess amount.

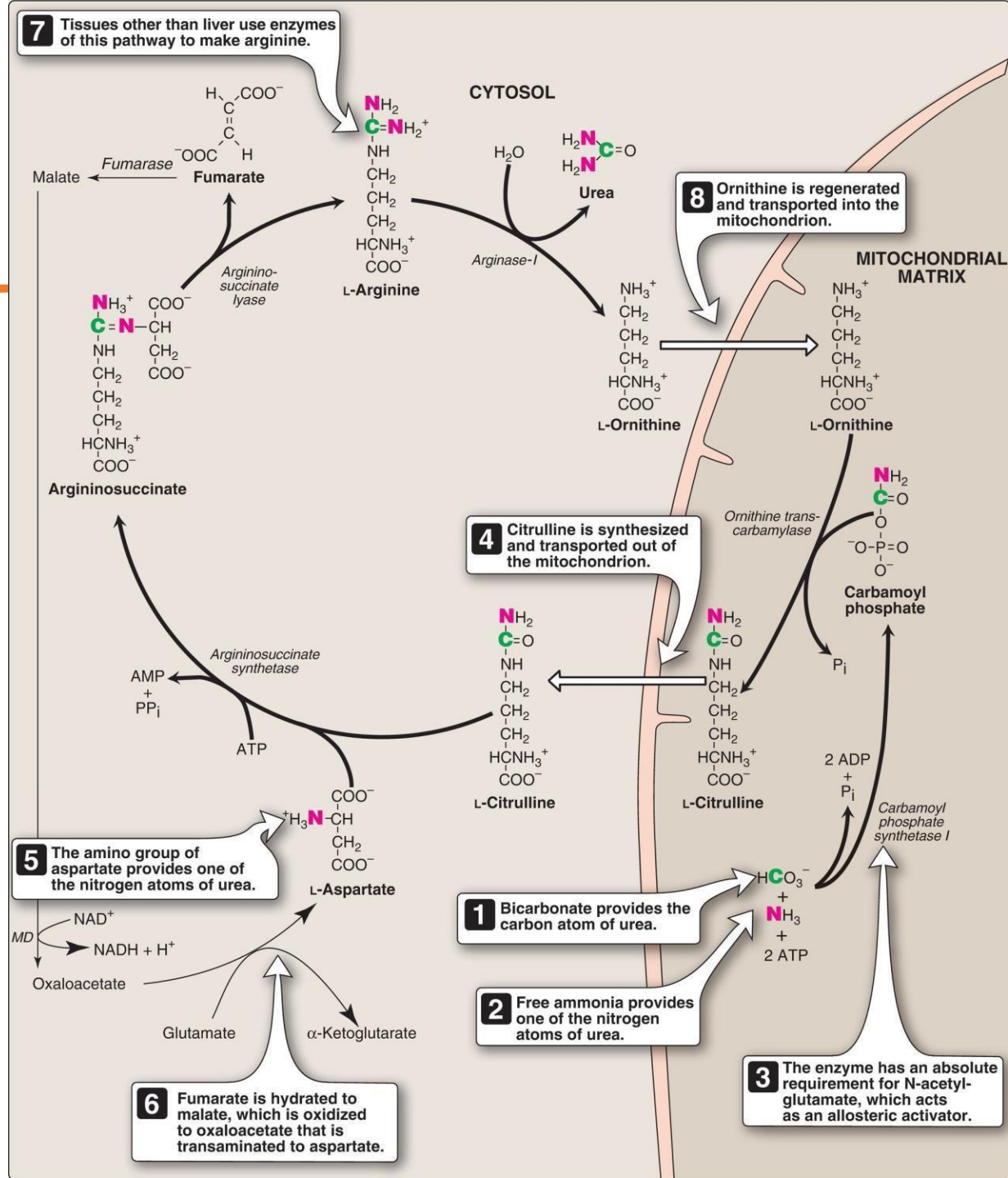
What happens inside the mitochondria of the liver cell ?

UREA CYCLE



- ~90% of the nitrogen- containing components of urine
- Sources of urea atoms
- Glutamate is the immediate precursor of both ammonia
- Mitochondria or cytosol?
- Reactions!

ammonia and CO2 are coupled together (energy requiring reactions) it requires 2 ATP to produce carbamoyl phosphate

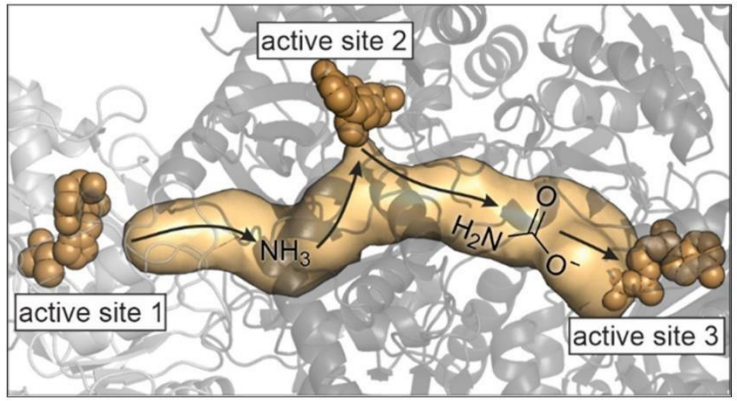


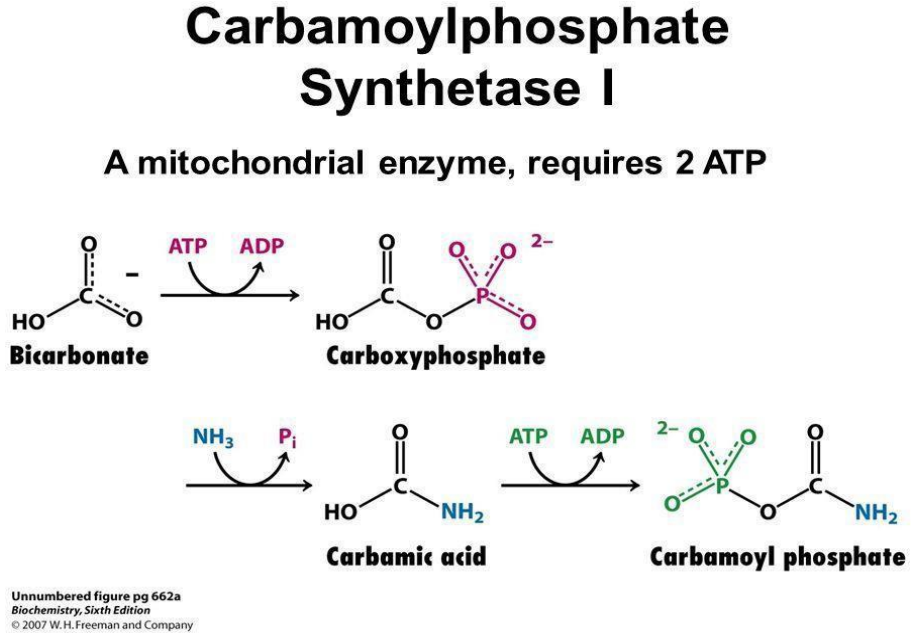
Urea cycle

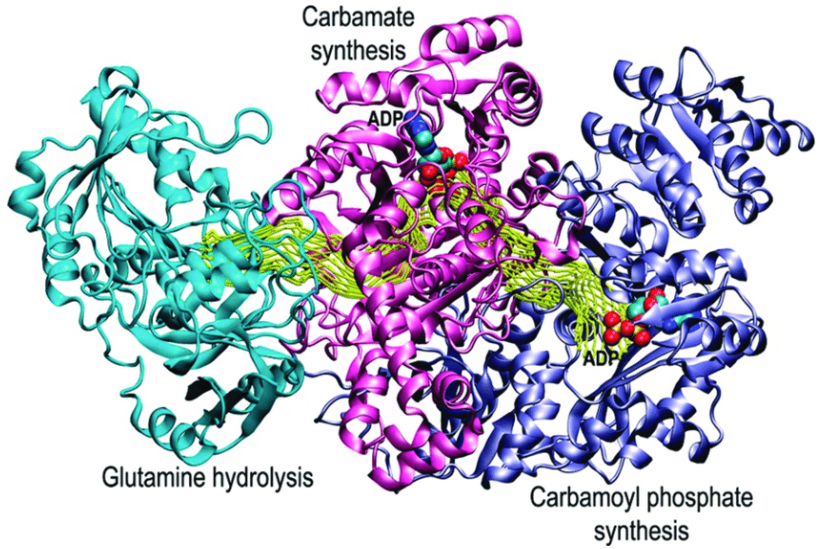
- Carbamate is the combination of free amino group and CO2 if its connected with phosphates it produces carbamoyl phosphate
- The production a urea in the liver is something smart!
- Urea structure is great because it has 2 Nitrogen atoms and one carbon atom, purpose of urea is to excrete nitrogen and minimize the loss of carbon
- It occurs in liver and in little amount in the kidney

Carbamoyl Phosphate Synthetase I: The Gateway Enzyme

- **Structure:** 150 kDa, homodimer
- **Domains:** NH_3 binding, ATP binding, allosteric
- **Reaction Mechanism:**
  - ATP + HCO_3^- → carboxyphosphate
  - Carboxyphosphate + NH_3 → carbamate
  - Carbamate + ATP → carbamoyl phosphate







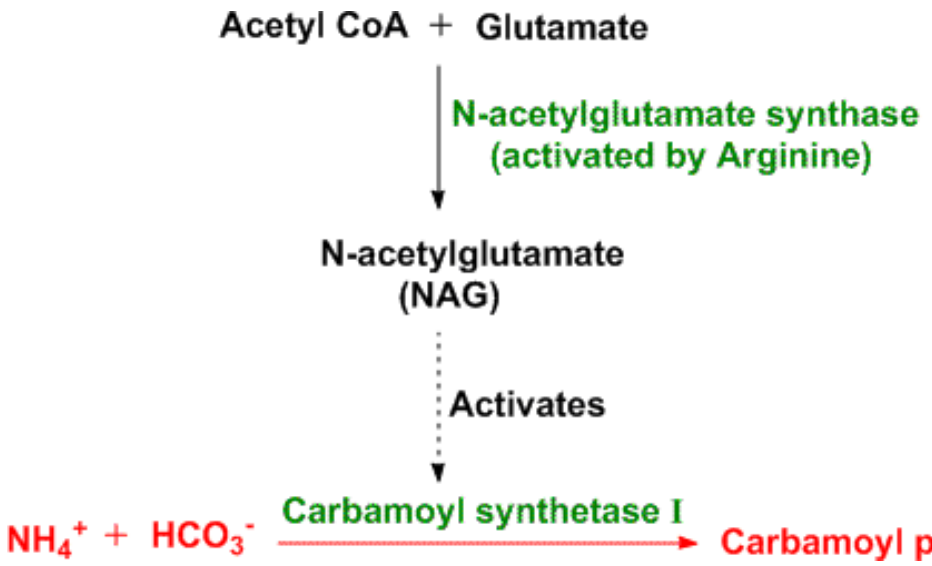
Production of carbamoyl phosphate

- As mentioned before it's an energy requiring reaction it requires 2 ATP molecules products are 2 ADP and 2 inorganic phosphates
- Carbamoyl phosphate synthetase I catalysis this step, a mitochondrial enzyme its activated by the allosteric activator is N-acetylglutamate it catalysis the rate limiting step in urea cycle

Carbamoyl Phosphate Synthetase I: The Gateway Enzyme

- **N-Acetylglutamate (NAG):**
  - Synthesis: Acetyl-CoA + glutamate
  - Regulation: Arginine activates NAG synthase
  - Clinical: NAG synthase deficiency mimics CPS I deficiency

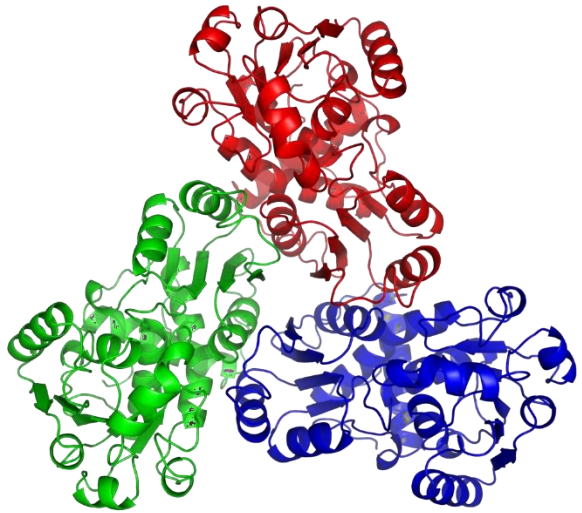
Glutamate is in excess during protein input ,acetyl group attaches to the nitrogen of the glutamate catalyzed by NAG synthase



Arginine is present in high concentration in protein diet

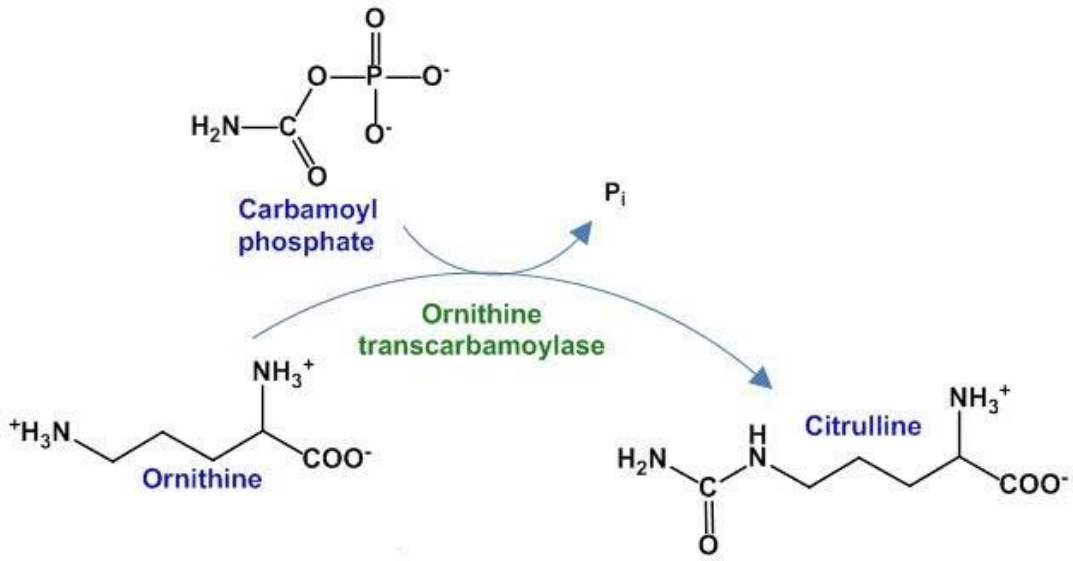
Ornithine Transcarbamoylase: Mitochondrial Commitment

Structure: Trimer, each subunit 36 kDa



Deficiency (OTC):

- Incidence: 1:14,000 live births
- Genetics: X-linked dominant (male lethal in utero without treatment)
- Presentation: Male neonates: crisis at 24-72h; Females: variable
- Diagnosis: NH_3↑, Gln↑, Cit↓ in urine



Glutamine transport nitrogen from peripheral tissue to the liver.

- Carbamoyl phosphate (mitochondrial) binds to ornithine (basic amino acid) (not involved in proteins structures) citrulline is formed, inorganic phosphate is releases this reaction is catalyzed by ornithine transcarbamoylase which transfer the carbamoyl group to ornithine (OTC) it might be genetically effected
- Inborn errors of amino acid metabolism is mostly rare (x-linked)

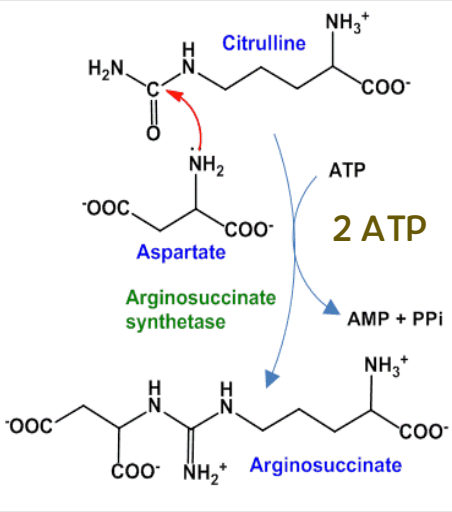
Aspartate becomes acidic on both sides

Cytosolic Enzymes: Linking to TCA Cycle

Argininosuccinate Synthetase:

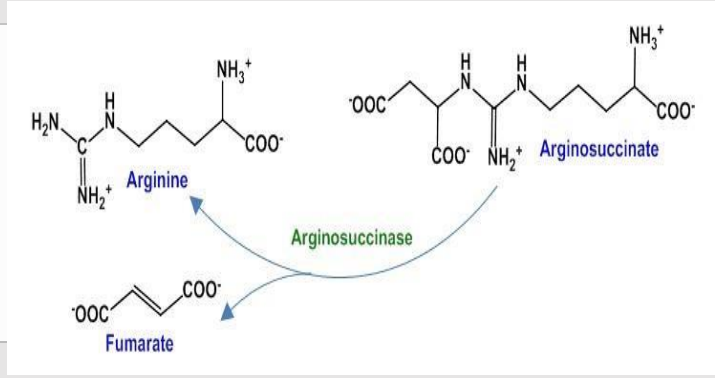
Energy requiring reaction

- Reaction: Citrulline + aspartate (4C (oxaloacetate)) + ATP → argininosuccinate + AMP + PPi
- Citrullinemia Type I: Cit↑↑ (>1000 µM), NH_3↑



Argininosuccinate Lyase:

- Reaction: Argininosuccinate → arginine + fumarate (4C)
- Argininosuccinic aciduria: ↑ASA in blood/urine



Aspartate enters the cycle to be released as fumarate(to provide an amino group (arginine))

How many ATP molecules in urea cycle do we use ?

3

How many ATP equivalents?

4

CPS I uses 2ATP, Argininosuccinate synthetase uses 1 ATP

ATP equivalence in the number of high energy phosphate bonds consumed

CPS I =2 ATP equivalents

Argininosuccinate synthetase= 2ATP equivalents

PPi is immediately broken into 2 Pi

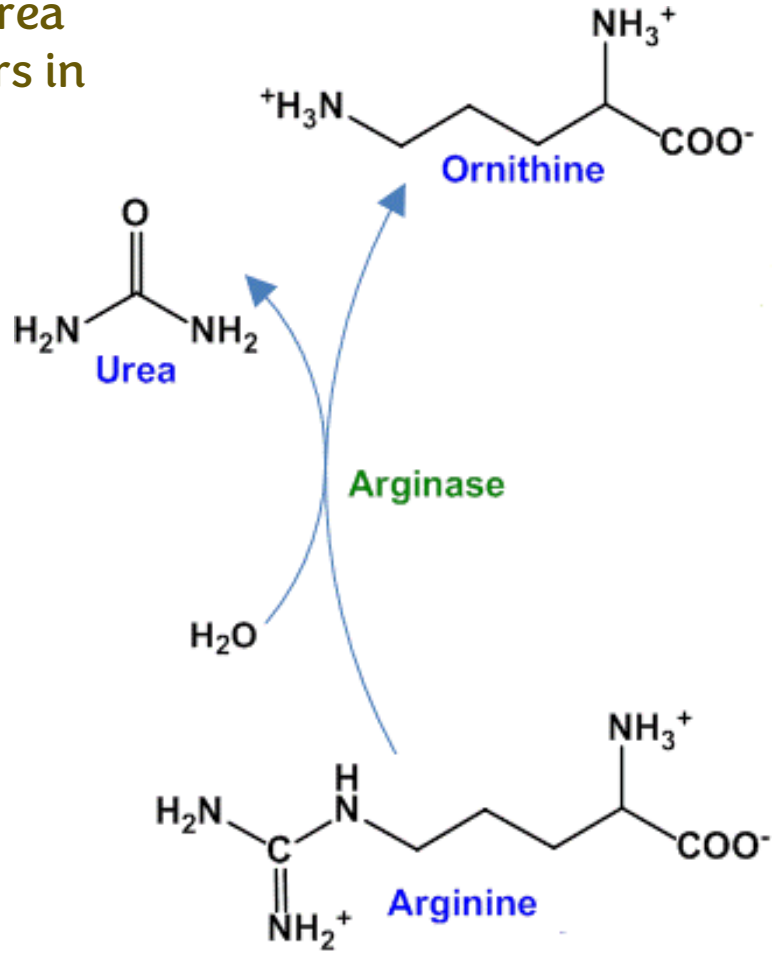
Ornithine is taken by an antiporter to the mitochondria and exits as citrulline

Arginine cleavage and Fate of urea

isoforms

- Arginase-I: virtually exclusive to the liver
- Reaction: Arginine → ornithine + urea
- Other tissues, such as the kidney
- Blood to urine; small intestine (urease); kidney failure (hyperammonemia and antibiotics)

This why urea cycle occurs in the liver



Arginase-2 is found in the kidney(not that effective as arginase-1) as a backup.

Production of argininosuccinate, fumarate with arginine, breaking down of arginine they occur in the cytosol. First 2 steps occurs in the mitochondria.

Irreversible like krebs (large negative delta G)

Urea Cycle Energetics: The Metabolic Cost

- **Direct ATP consumption:**
  - CPS I: 2 ATP → 2 ADP + Pi (equivalent to 2 ATP)
  - ASS: ATP → AMP + PPi (PPi → 2Pi, equivalent to 2 ATP)
  - Total: 4 ATP equivalents per urea
- **Indirect costs:**
  - Transport (ornithine/citrulline antiporter)
  - Aspartate regeneration (malate dehydrogenase) oxaloacetate
- **Percentage of hepatic ATP:** ~15% in fed state, increases with protein load

Urea can be broken down to ammonia if its concentration is high and enzyme (urease) is available. But we don't have this enzyme, urease is found in bacteria(intestines)

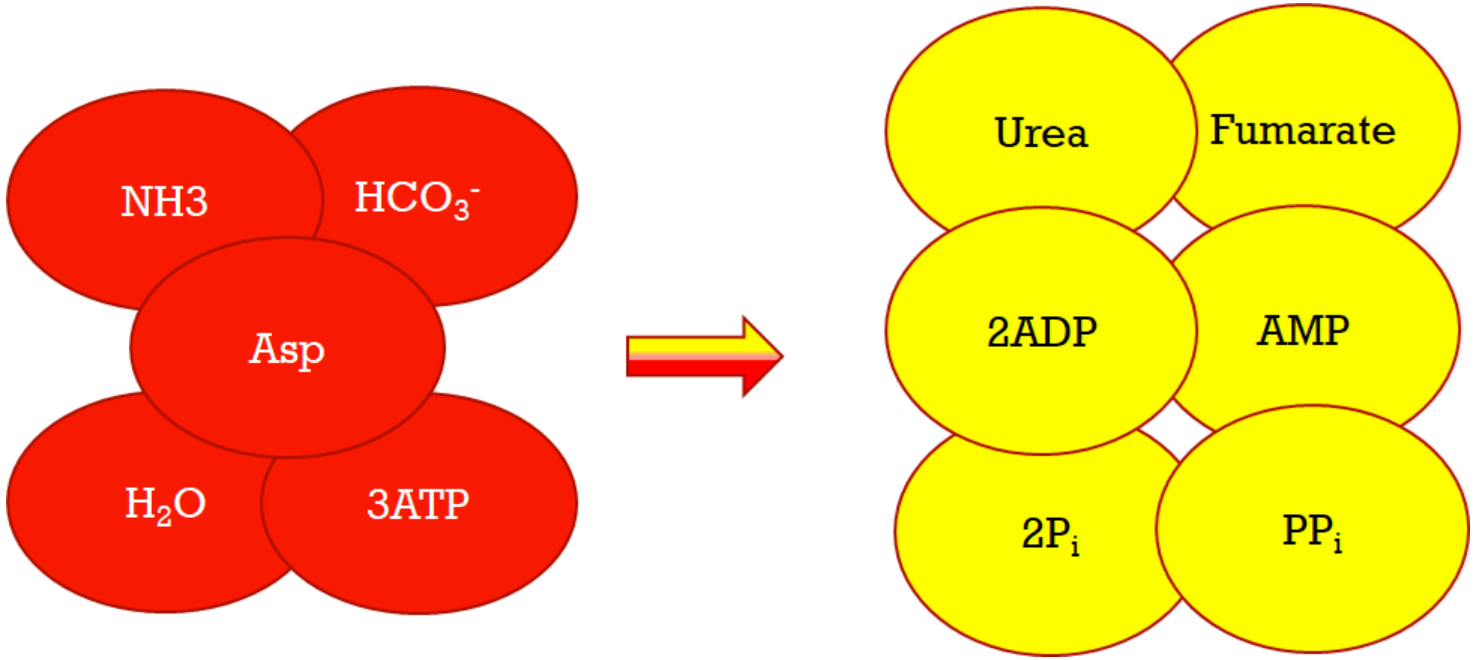
If urea exceeds normal limits accordingly ammonia will exceeds normal limits (toxic)

Kidney failure patients have higher amounts of urea (urea removed by kidneys) they are given antibiotics to kill some of the bacteria

Deficiency in arginase is the least toxic urea cycle defect (arginase 2) in kidneys

Overall stoichiometry

- Synthesis of urea is irreversible, why?
- Free ammonia and aspartate
- Glutamate is the immediate precursor of both ammonia

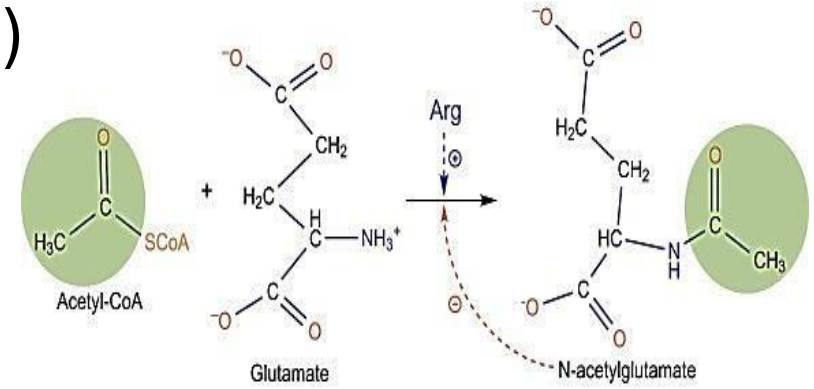


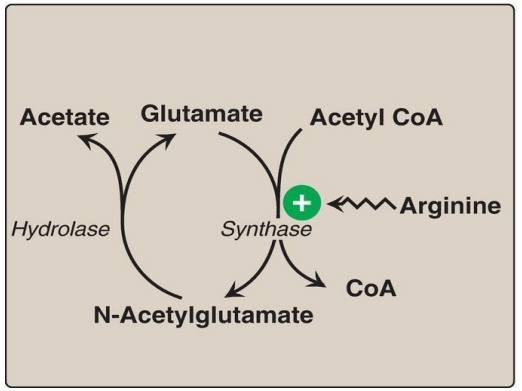
Regulation: Short, Intermediate, and Long-term

- Short-term (seconds-minutes)

Important, specific

  - NAG activation of CPS I
  - Substrate availability





- Intermediate-term (hours):
  - Covalent modification (phosphorylation/dephosphorylation)
  - Allosteric regulation by amino acids
- Long-term (days):
  - Enzyme induction by protein intake (2-3-fold increase)
  - Transcriptional regulation via hormone response elements

Causes hyperammonemia

- Levels are normally low (5–35 µmol/l)
- Can be >1,000 µmol/l; medical emergency (tremors, speech slurring, drowsiness, vomiting, cerebral edema, blurring of vision, coma and death)
- Acquired: liver disease
- Congenital:
  - Overall incidence of ~1:25,000 live births
  - X-linked OTC deficiency is the most common (M vs. F)
  - All urea cycle disorders are of autosomal-recessive inheritance except OTC deficiency
  - Arginase deficiency is less severe!

Hyperammonemia: Pathophysiology cascade

1. NH_3 crosses blood-brain barrier
2. Astrocyte glutamine synthesis: Glu + NH_3 → Gln (glutamine synthetase)
3. Osmotic effects: Gln accumulation → astrocyte swelling Because its soluble(swollen cells)
4. Energy depletion: α-KG depletion → TCA cycle impairment As Glu is converted to Gln
5. Neurotransmitter imbalance: Glu↑, GABA↓
6. Oxidative stress: Mitochondrial dysfunction
7. Cerebral edema: Intracranial pressure ↑

Diagnostic Approach to Hyperammonemia

- Confirm hyperammonemia (NH_3 > 50 µM in neonate, > 35 µM in child/adult) Blood test
- Link to acid-base status (Respiratory alkalosis or Metabolic acidosis)
- Plasma amino acids:
  - Glutamine > 1000 µM suggests UCD
  - Citrulline: Absent (OTC, CPS), very high (citrullinemia), moderate (ASA)
- Urine organic acids
- Enzyme assays/genetic testing

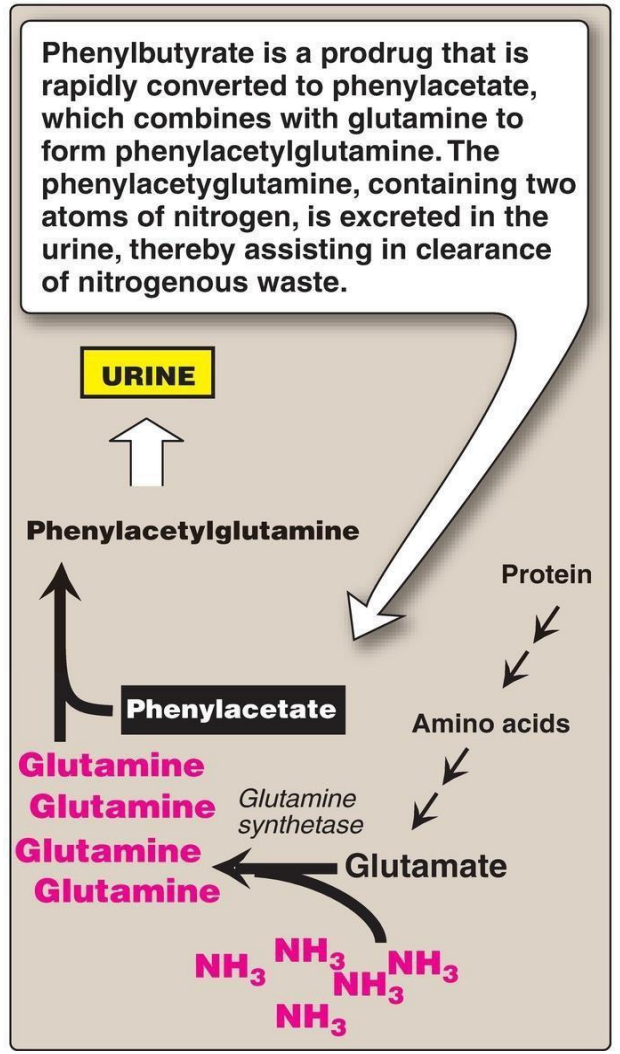
Medical emergency (fatal) above 500 can reach up to a thousand causes neurological problems (coma, death).
Causes can be genetic or aquired anything that effect the liver(hepatitis,alcohol)

Management

You have to decrease the amount of ammonia in the blood smartest way was by producing urea

- Acute Management Protocol
  - acute chronic
- Stop all/decrease protein intake
- IV dextrose (suppress catabolism)
- IV lipids (provide calories)
- Specific therapy:
  - Sodium phenylacetate
  - Arginine (except arginase deficiency)
  - Hemodialysis for NH_3 > 500 µM or clinical deterioration

Take supplements if urea cycle is effected such as phenylbutyrate converts to phenylacetate
Coupled inside the blood (glutamine)



رسالة من الفريق العلمي:

﴿وَلَا تَخَافِي وَلَا تَحْزَنِي﴾

من الدعاء لا تنسونا (:

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide **#10**	Citrulline transcarbamoylase	Ornithine transcarbamoylase
V1 → V2			