BB 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic

Exam-1 material

Fermentations

Exam-2 material

Pyruvate
- pyruvate dehydrogenase (ox-decarbox; S-ester)

Krebs' Cycle
- How did he figure it out?
- Overview
- 8 Steps
  - Citrate Synthase (C-C)
  - Aconitase (=, -OH)
  - Isocitrate dehydrogenase (ox-decarbox; =O)
  - Ketoglutarate dehydrogenase (ox-decarbox; S-ester)
  - Succinyl-CoA synthetase (sub-level phos)
  - Succinate dehydrogenase (=)
  - Fumarase (-OH)
  - Malate dehydrogenase (=O)
- Energetics
- Regulation
- Summary

Oxidative Phosphorylation
- Energetics (−0.16 V needed for making ATP)
- Mitochondria
- Transport (2.4 kcal/mol needed to transport H^+ out)
- Electron transport
  - Discovery
  - Four Complexes
    - Complex I: NADH → $CoQH_2$
    - Complex II: Succinate → $CoQH_2$
    - Complex III: $CoQH_2$ → Cytochrome C (Fe^{2+})
    - Complex IV: Cytochrome C (Fe^{2+}) → H_2O
- Chemiosmotic theory: Phosphorylation
  - ATPase
  - Mitchell Hypothesis
  - Binding-Change Model
  - Connection to the proton motive force
  - Net ATP production
  - Regulation

Exam-2 material

Exam-3 material

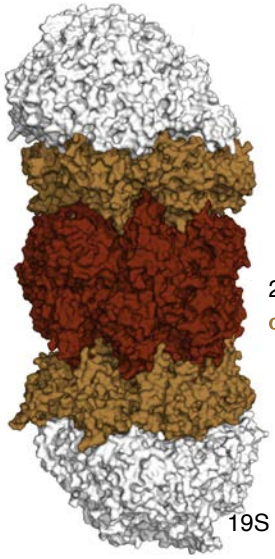
Catabolism: Lipid Degradation
- Digestion and storage
- FOUR stages lipid catabolism
  - Mobilization from adipose tissues
  - Activation of fatty acids
  - Transport into mitochondria
  - Oxidation
    - Saturated
    - Unsaturated
    - Odd-chain
- Ketone Bodies
- Oxidation in other organelles

Catabolism: Nitrogenous
- Digestion
- Inside of cells
  - Protein turnover
    - Ubiquitin
    - Proteosome
- Urea Cycle
- Amino-acid Degradation
- Nucleotide Degradation

Protein Catabolism

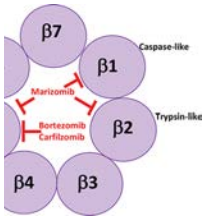
Protein Turnover (within cell): THE 26S PROTEOSOME

19S = $\alpha_6\beta_3\gamma_{10}$



20S = $\alpha_7\beta_{14}\alpha_7$

19S = $\alpha_6\beta_3\gamma_{10}$

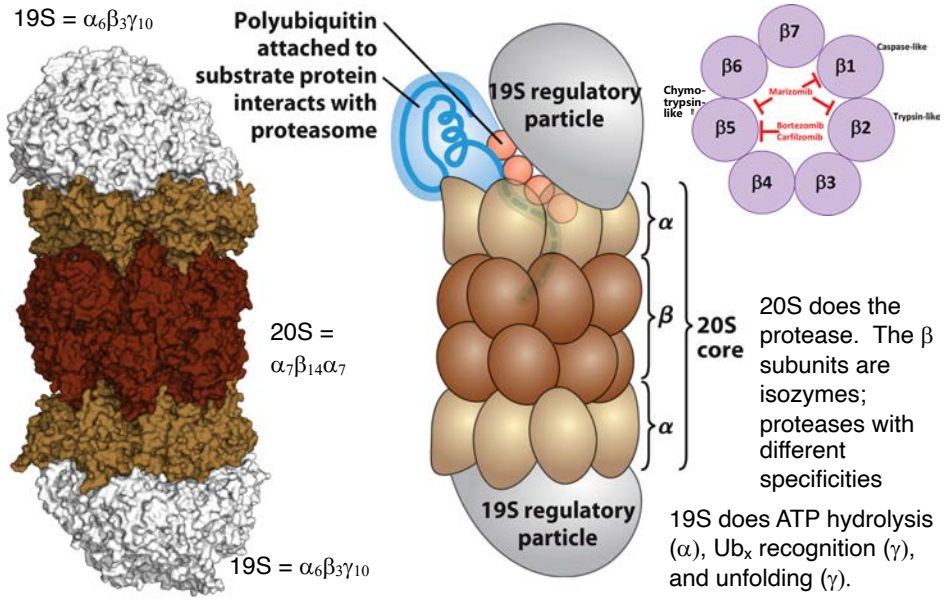


20S does the protease. The β subunits are isozymes; proteases with different specificities

es ATP hydrolysis
x recognition (γ),
folding (γ).

Protein Catabolism

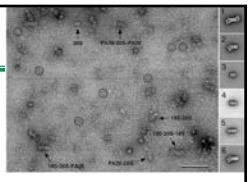
Protein Turnover (within cell): THE 26S PROTEOSOME

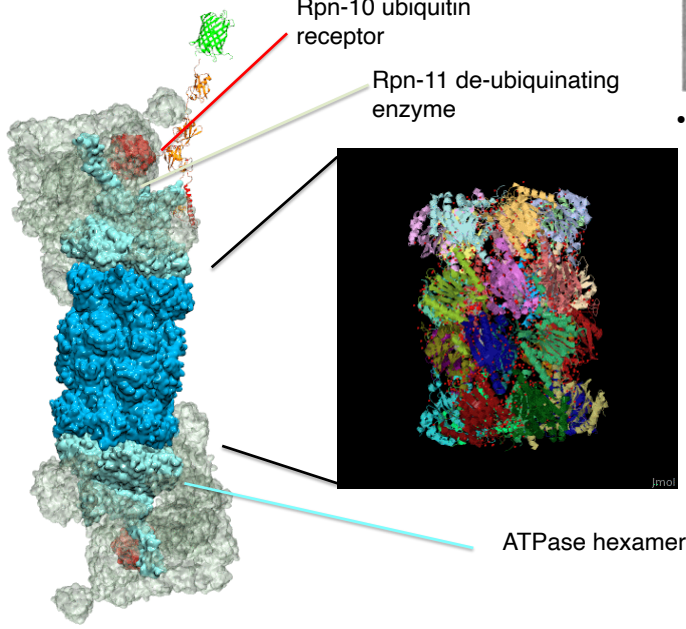


20S does the protease. The β subunits are isozymes; proteases with different specificities

19S does ATP hydrolysis (α), Ub_x recognition (γ), and unfolding (γ).

Protein Catabolism





- Cryo-EM was used to attain these structures within the last 5 years.

Protein Catabolism

Fate of Amino Acids:

Catabolism
Anabolism

Protein Biosynthesis

Oxidation

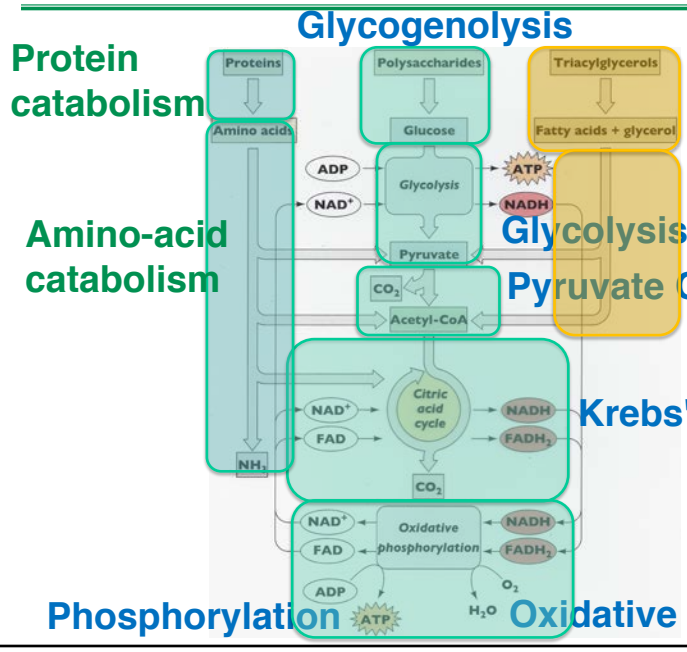
Once broken down to amino acid, all types of protein are treated the same way depending on the organism's energy needs:

1. Recycled into new proteins
2. Oxidized for energy
   - There are two separate aspects:
     - removal of amino group (urea cycle)
     - entry into central metabolism (glycolysis, citric acid cycle)

We'll call this "Amino Acid Degradation or Catabolism"

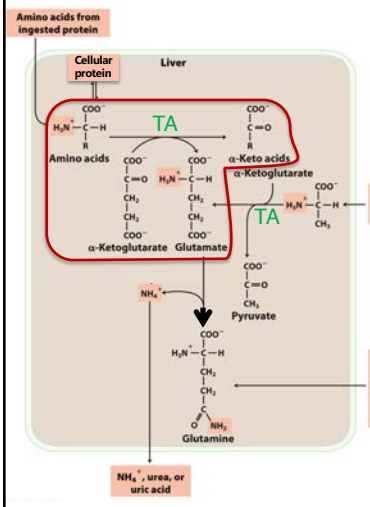
Amino Acid Catabolism

Nitrogen Catabolism



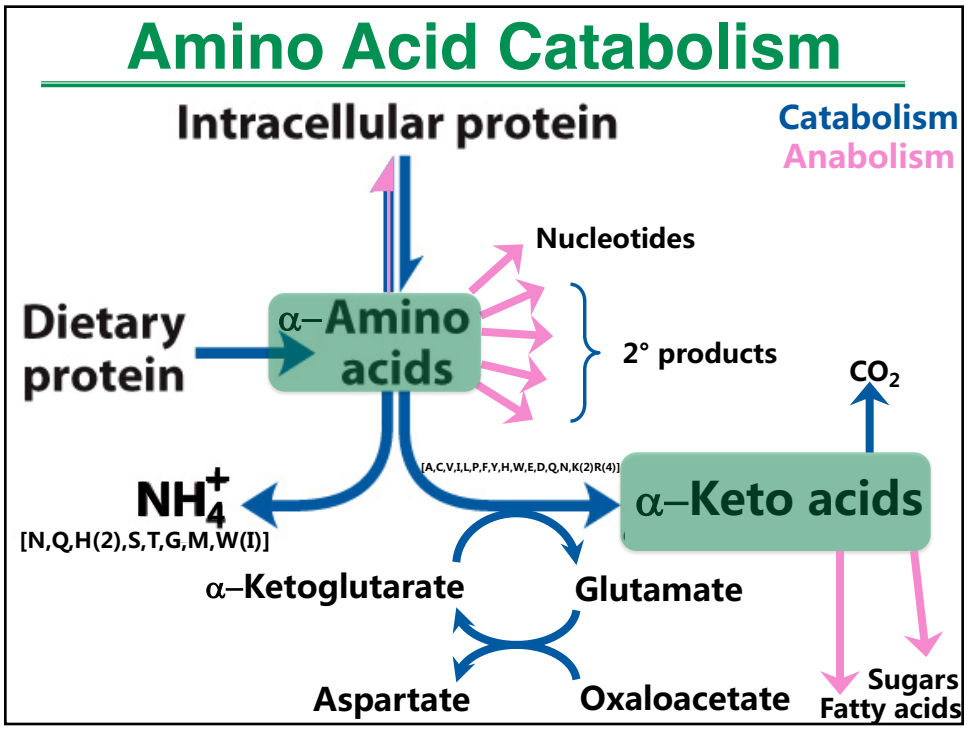
Amino Acid Catabolism

Step 1: Removal of the Amino Group



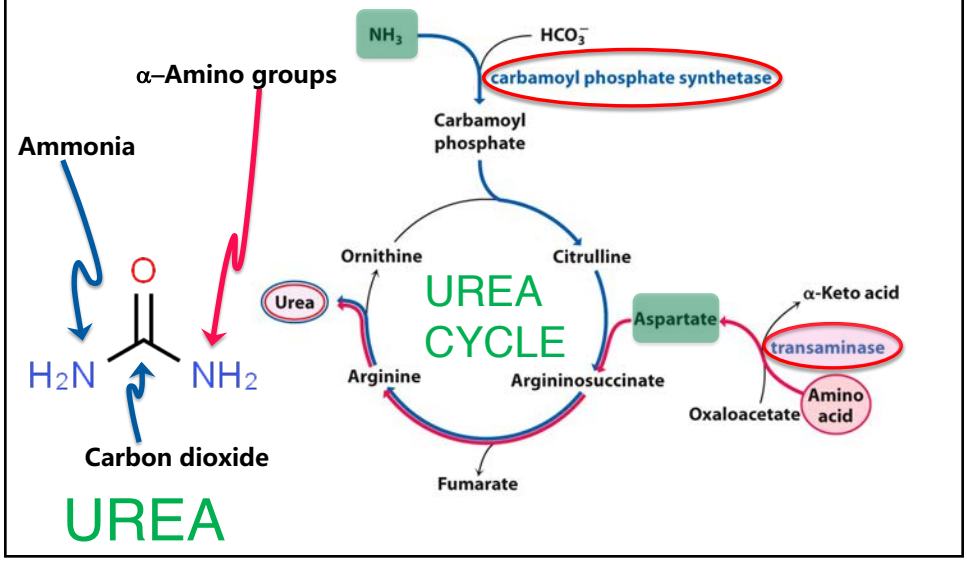
- Release of free ammonia, which is toxic.
- Instead of Ammonia, nitrogen is captured by a series of trans-aminations (TA).
- Transaminations allow transfer of an amine to a common metabolite (e.g., α-ketoglutarate) and generate a traffickable amino acid (e.g., glutamate).
  - Catalysis by aminotransferases (TA):
  - Uses the pyridoxal phosphate (PLP) cofactor
  - Typically, α-ketoglutarate accepts amino groups.
    - Transfer of one amine to α-ketoglutarate results in synthesis of glutamate (e.g., transamination).
    - Transfer of a second amine results in synthesis of glutamine (e.g., glutamine synthetase).
  - L-Glutamine acts as a temporary storage of nitrogen and a way of de-toxifying the ammonia
  - L-Glutamine can donate the amino group when needed for amino acid biosynthesis.

Amino Acid Catabolism



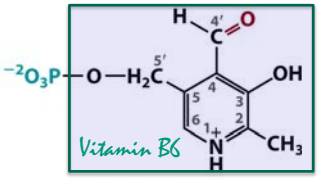
Amino Acid Catabolism

What is the fate of the free ammonia and Asp?

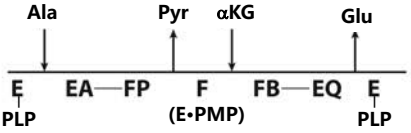


Amino Acid Catabolism: Transamination*

- Catalyzed by aminotransferases*
- Uses the pyridoxal phosphate cofactor
- Typically, either oxaloacetate or α-ketoglutarate accept amino groups.
  - Transfer of the α-amino group to α-ketoglutarate results in synthesis of glutamate (e.g., transamination).

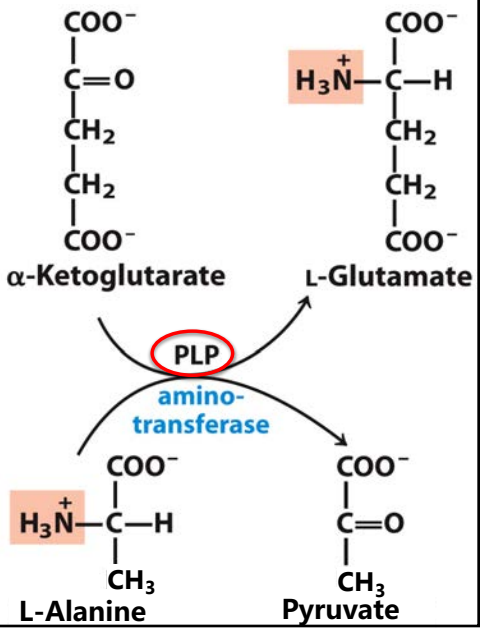


Pyridoxal-5′-phosphate (PLP)

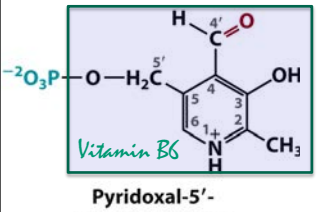


*same thing

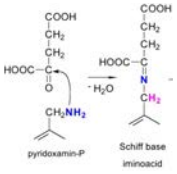
Ping-pong bi bi

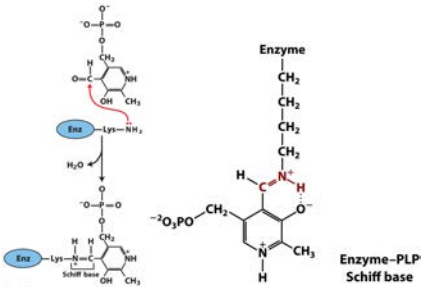


Amino Acid Catabolism: Transamination

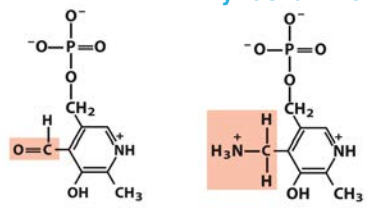


Pyridoxal-5′-phosphate (PLP)





Structure of Pyridoxal Phosphate and Pyridoxamine Phosphate



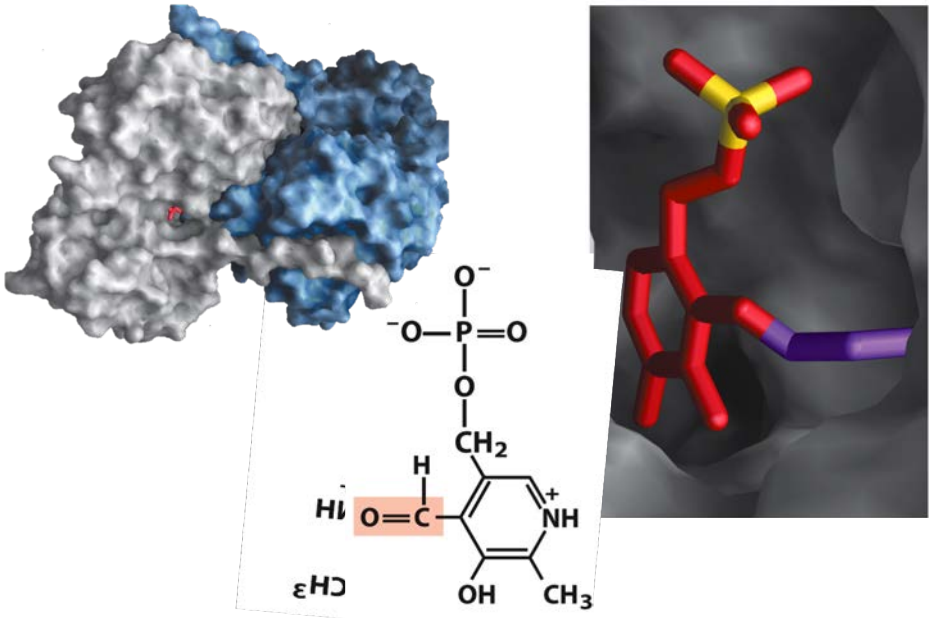
Pyridoxal phosphate (PLP)

Pyridoxamine phosphate

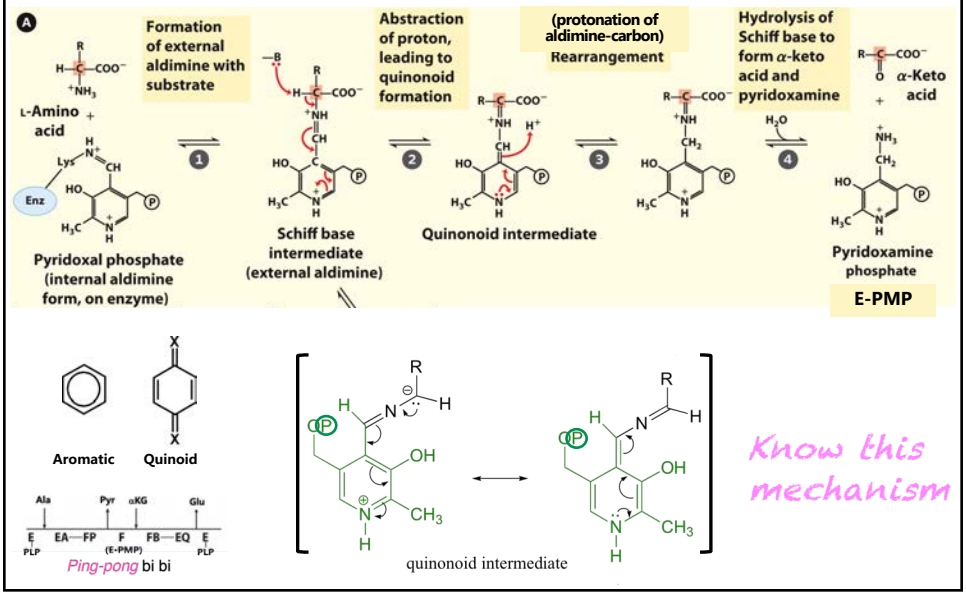
- Intermediate, enzyme-bound carrier of amino groups
- The linkage is made via a nucleophilic attack of the amino group of an active-site lysine.
- Making an internal aldimine

- Aminated form can react reversibly with carbonyl groups to make a Schiff base.
- Aldehyde form can react reversibly with amino groups to make a Schiff base…… which is how its attached to the enzyme as a prosthetic group.

Amino Acid Catabolism: Transamination

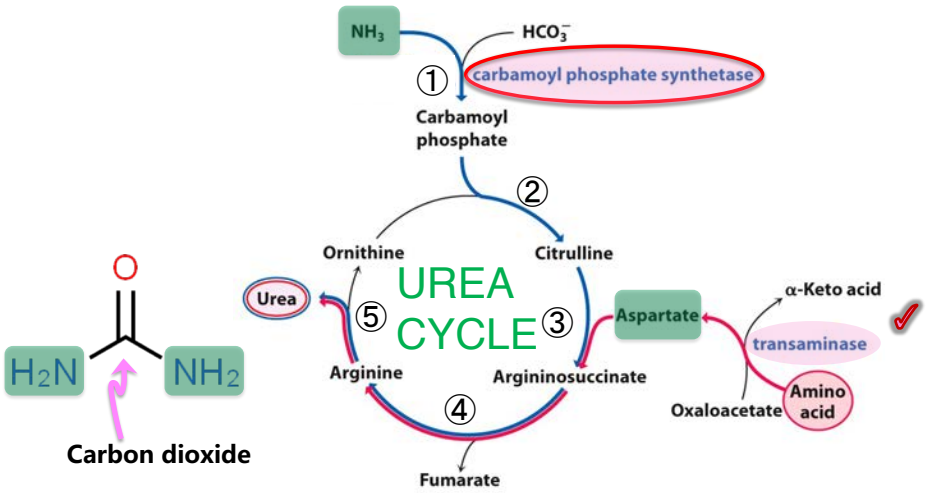


Amino Acid Catabolism: Transamination



Amino Acid Catabolism: Urea Cycle

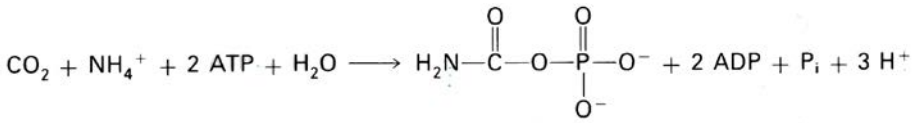
Synthesis of Carbamoyl Phosphate



Amino Acid Catabolism: Urea Cycle

① Synthesis of Carbamoyl Phosphate

- The first nitrogen-acquiring reaction of the urea cycle



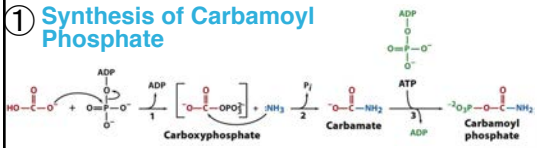
Carbamoyl phosphate

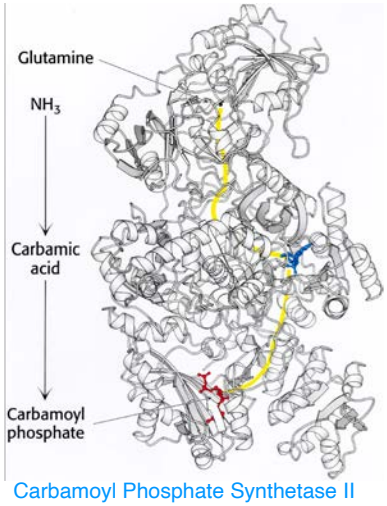
Carbamoyl Phosphate Synthetase I

- Excess CO_2, ATP, and ammonia is present in liver mitochondria. This is where the activation of both waste products occur (the majority of the other urea-cycle reactions occur within the cytosol, mostly in the liver).

Amino Acid Catabolism: Urea Cycle

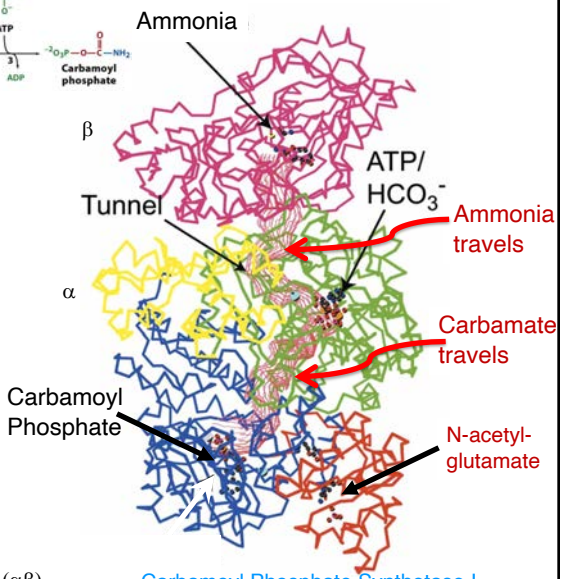
① Synthesis of Carbamoyl Phosphate





Carbamoyl Phosphate Synthetase II

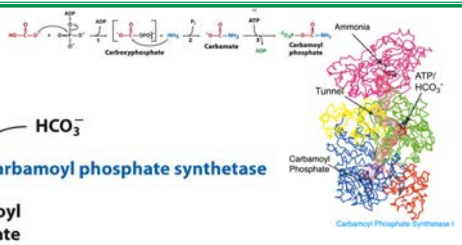
$(\alpha\beta)_3$



Carbamoyl Phosphate Synthetase I

Amino Acid Catabolism: Urea Cycle

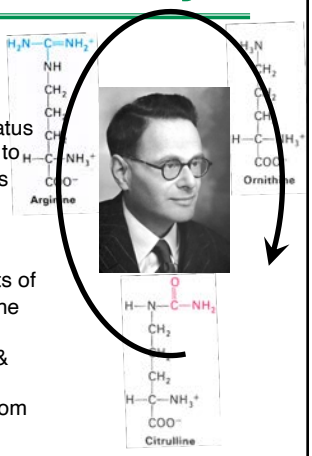
The Urea Cycle

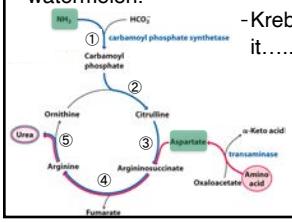


Amino Acid Catabolism: Urea Cycle

The Urea Cycle: Evidence for a cycle

- Already known that Arg gives rise to Urea and Orn ⑤
- How do CO_2 and NH_3 get into Arg?
- Enter H. Krebs, who studies metabolism using the Warburg apparatus
- Urease (recently purified by Sumner in 1925) can hydrolyzed urea to CO_2 + 2 NH_3; Could measure CO_2 from urea by Warburg apparatus
- Using liver slices, which amino acids gave rise to urea?
- ….. Most gave some
- Then as a control, he tried Orn
- Added Orn to prep: extraordinary occurrence of "catalytic" amounts of urea!! Orn, which is the product, will give rise to more urea than the Orn added, and at a 7-30x increased rate: "catalytic"
- In the library, Krebs looked for intermediates that might have CO_2 & NH_3 stuck to Orn: citrulline
- Dr. Wada in Japan had just published the purification of citrulline from watermelon.





- Krebs attained 10 mg citrulline and added it……same catalytic phenomenon!!

First cyclic process!
……lead to lots of notariety

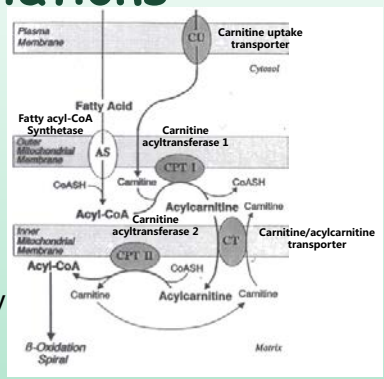
Clinical Correlations

Carnitine Deficiencies

- Underlying these disorders is the fact that the kidney does not reabsorb carnitine
- Two classes of carnitine deficiency:

1) Primary – low levels in muscle, kidney, heart, and plasma

2) Secondary – accumulation of acyl-carnitine, which are excreted by the kidney



- Primary Carnitine Deficiency caused by reduced activity of the Carnitine uptake transporter on the plasma membrane.
- The most common form is a secondary deficiency caused by defects in CPT-II. These mutations are mild and lead only to a partial loss of activity. Similar symptoms can arise for mild defects in other enzymes in β-oxidation*.
- The less common forms arise from more severe mutations in CPT-II or the liver form of CPT-I. Death by age 3 is not uncommon. Mutations in CT or muscle CPT-1 are presumably embryonically lethal.

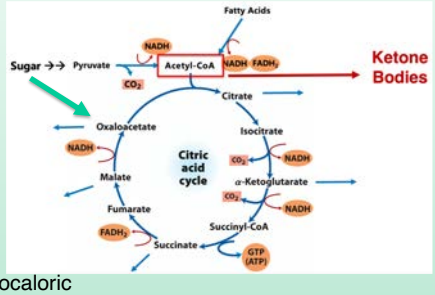
- Symptoms range widely.; from muscle cramps & weakness to severe weakness & death
- Treatment with dietary carnitine often helps, but also minimize long-chain fatty acids and minimize fasting (conditions that require FA oxidation)

*among these are the fairly common and more well known deficiencies in acyl-CoA dehydrogenases; VLCAD, LCAC, MCAD, and SCAD

Clinical Correlations

The Atkins Diet

- A controversial diet of high protein & fat, but low carbohydrate (<20 g/d) was developed by Dr. Robert Atkins.
- The claim was that this diet is better for weight loss than a low-fat diet
- Many clinical trails have born this out:



For example 322 individuals with >31 BMI on two isocaloric diets. Those on Atkins lost >10.4 lbs. compared to a group on low-fat/high carbohydrate diet who lost 6.4 lbs

- It has been concluded to be safe and effective.

- Why it works is still being debated:

- Ketone body levels can change 100-200 fold, sometimes leading to hyperketonemia of diabetics. Brain uses ketone bodies and glucose, which is made from protein

1) Works by mobilization of FA from adipose, and due to the lack of sugar, ketone bodies are produced. Urine levels rise, and there is acetone in the breath. This loss of carbon in this way is thought to be the reason for the weight loss.
2) Others has noted that it might be from the reduction of appetite from the high fat.