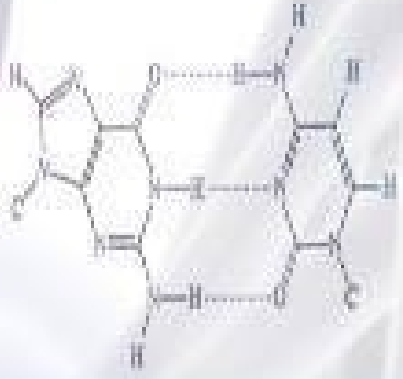
Medical Committee
The University of Jordan

SLIDE SHEET

SLIDE : 28

DR.NAME: Dr. Nafeth

Biochemistry



Majida Al-Foqaraa'

Metabolism
Protein & Amino Acid

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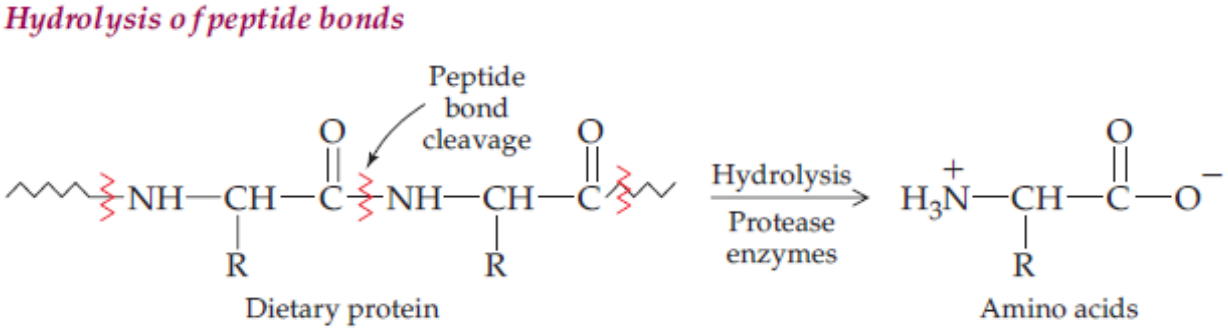
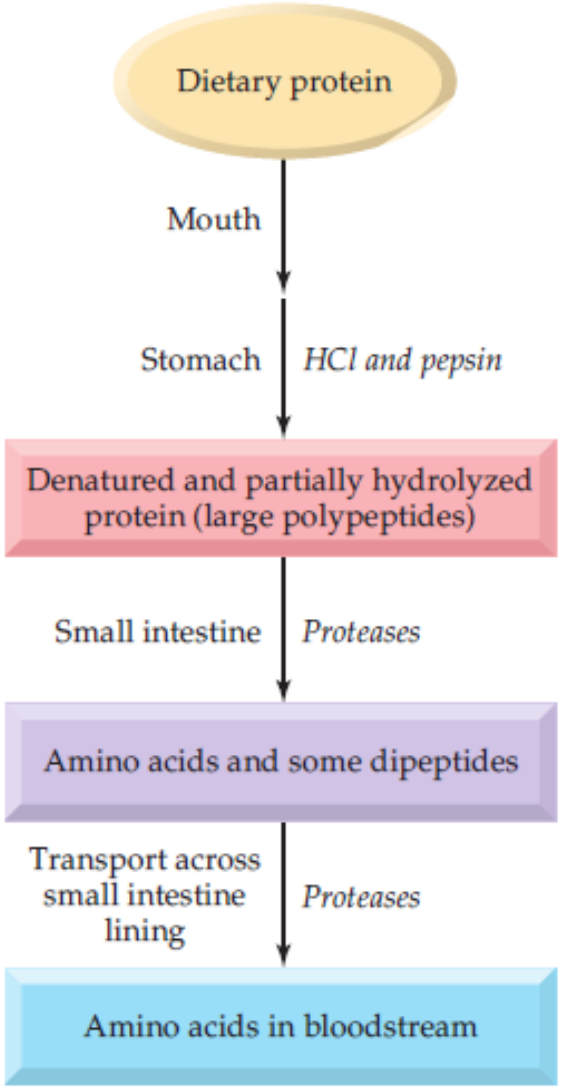
Digestion of Proteins

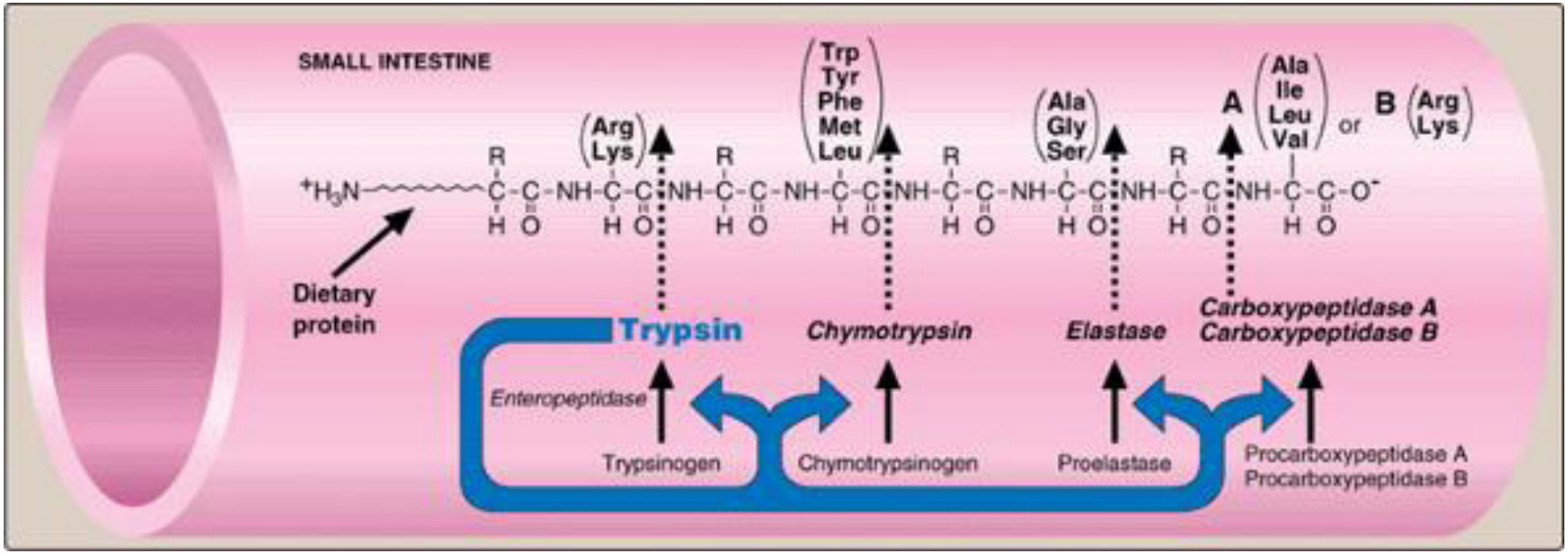
- What is protein digestion?
- Where digestion begins?
- Stomach; HCl (2-3) & pepsinogen (polypeptides)
  - Acid and autocatalytic activation





Digestion of Protein

- Pancreatic (specific). Secretion mediated by cholecystokinin & secretin
- Enteropeptidase (mucosal) activates trypsinogen (the common activator of all the pancreatic zymogens). Result in amino acids and small peptides
- Intestinal lining enzymes, aminopeptidases



Absorption & transport of amino acids & dipeptides

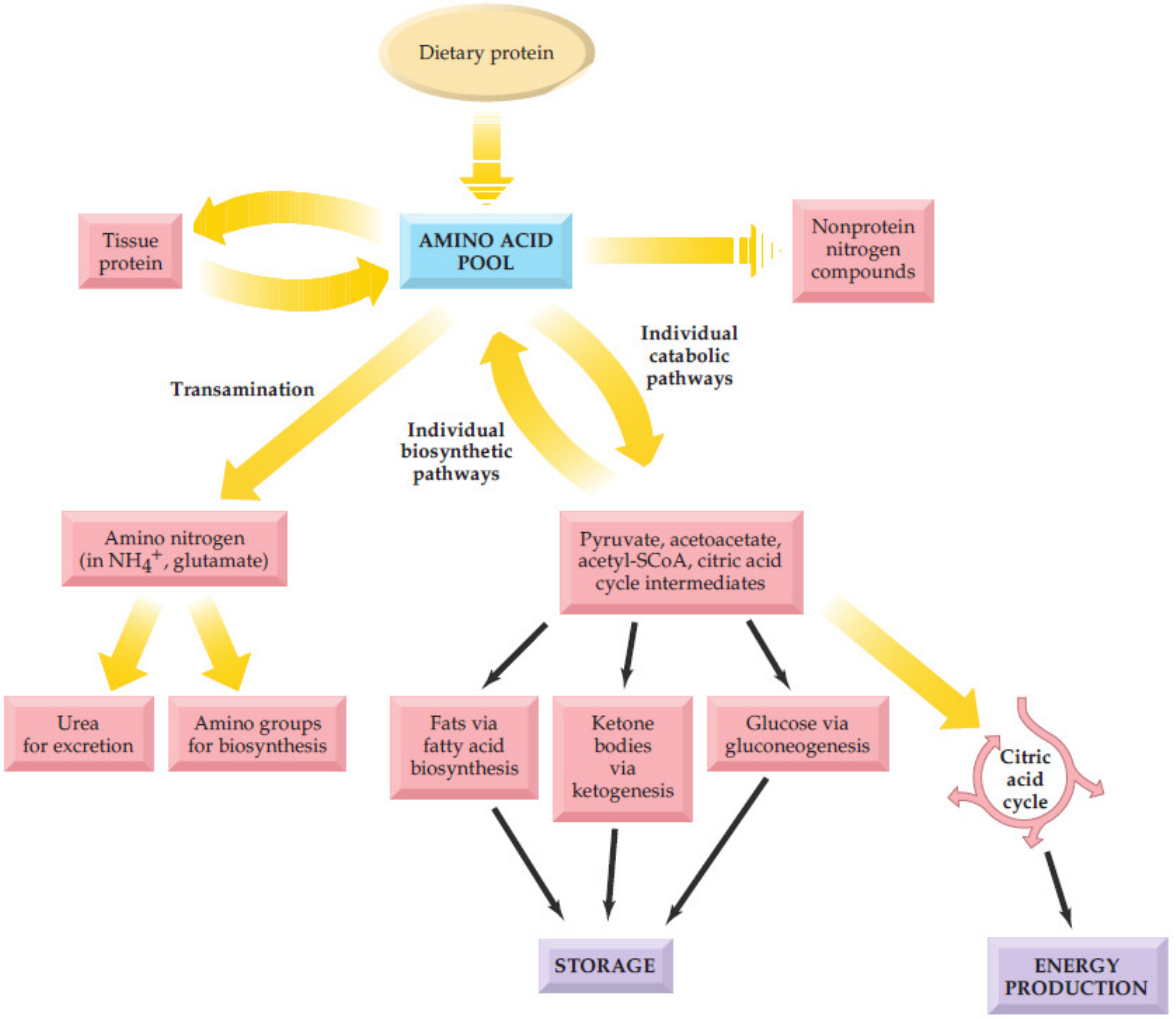
- Free amino acids (Na^+-cotransport)
- Di- & tripeptides (H^+-cotransport) followed by hydrolysis
- **Only free amino acids are found in the portal vein**
- From cells to the bloodstream through active transport
- Active transport is grouped (at least 7 transport systems); excess in one can cause deficiency in another!
- The small intestine & the proximal tubule of the kidney have common transport systems for amino acid uptake

Amino Acid Metabolism - overview

- Amino acids are unlike fats and carbohydrates!
- Three sources; diet, synthesis, degradation
- Three destinations; synthesis, precursors, conversion
- Catabolism is of two phases; the amino group & the carbon skeleton
- Each amino acid is degraded via its own unique pathway but the general scheme is similar
- The metabolism involves two important concepts: amino acid pool and protein turn-over

Amino Acid Pool

- Where is it? How much is it? (≈100g)
- Supply: degradation, diet, synthesis
- Demand: synthesis, precursors, conversion
- In healthy, well-fed individuals, steady state, nitrogen balance

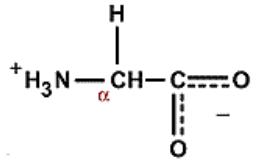


Protein Turn-over

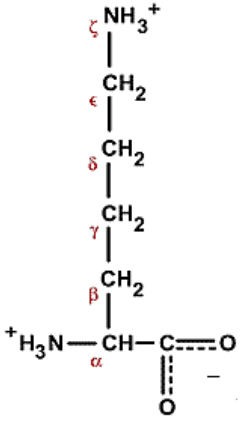
- Most proteins in the body are in a constant remodeling.
- What regulates protein concentration in cells? synthesis or degradation?
- Turn over is about 300-400 g of protein per day, varies widely for individual proteins (minutes, hours, days, weeks, years)
- Two protein degradation pathways; ATP-dependent vs. independent, proteasomes vs. lysosomes, intracellular vs. extracellular

ATP dependent

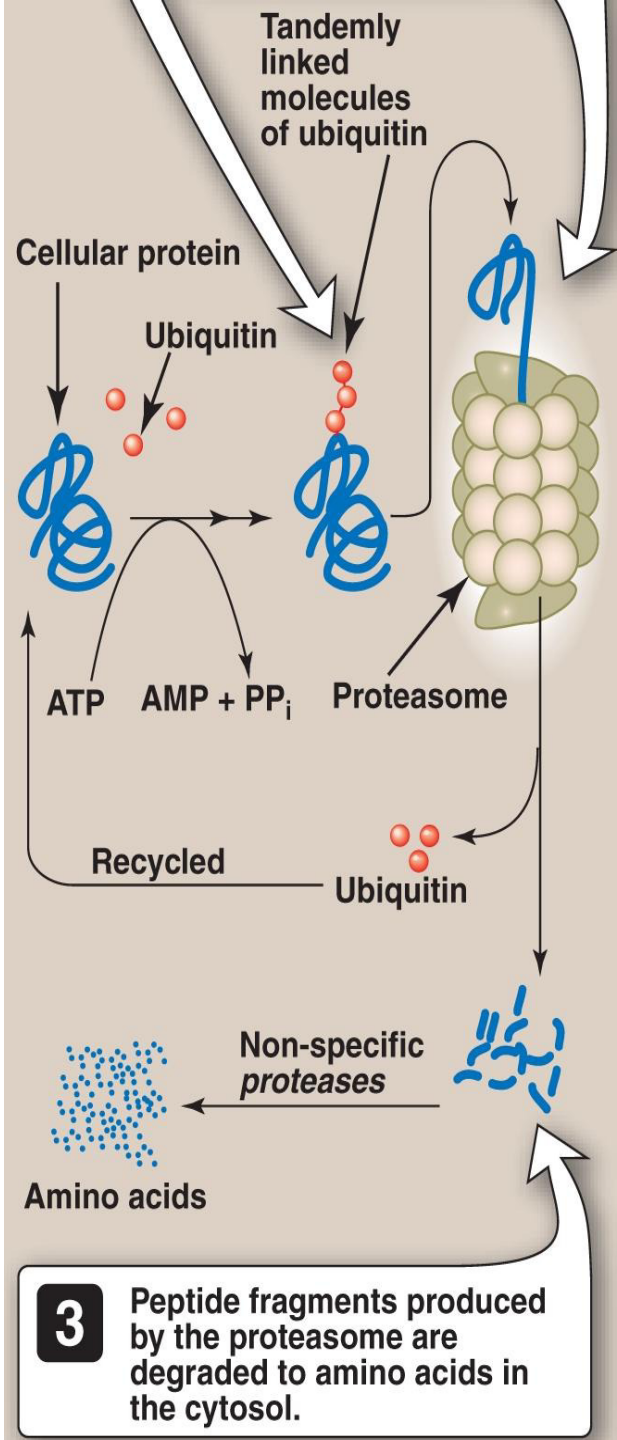
- Ubiquitin-proteasome proteolytic pathway
- Ubiquitin is a small globular protein, non-enzymic



- Covalent attachment, polyubiquitination, proteasome complex, ubiquitins are recycled
- Is protein degradation random?



- Signals can be: oxidation of aa., ubiquitination, N-terminal residue; Ser (20 hrs) vs. Asp (3 min), PEST sequences are short lived

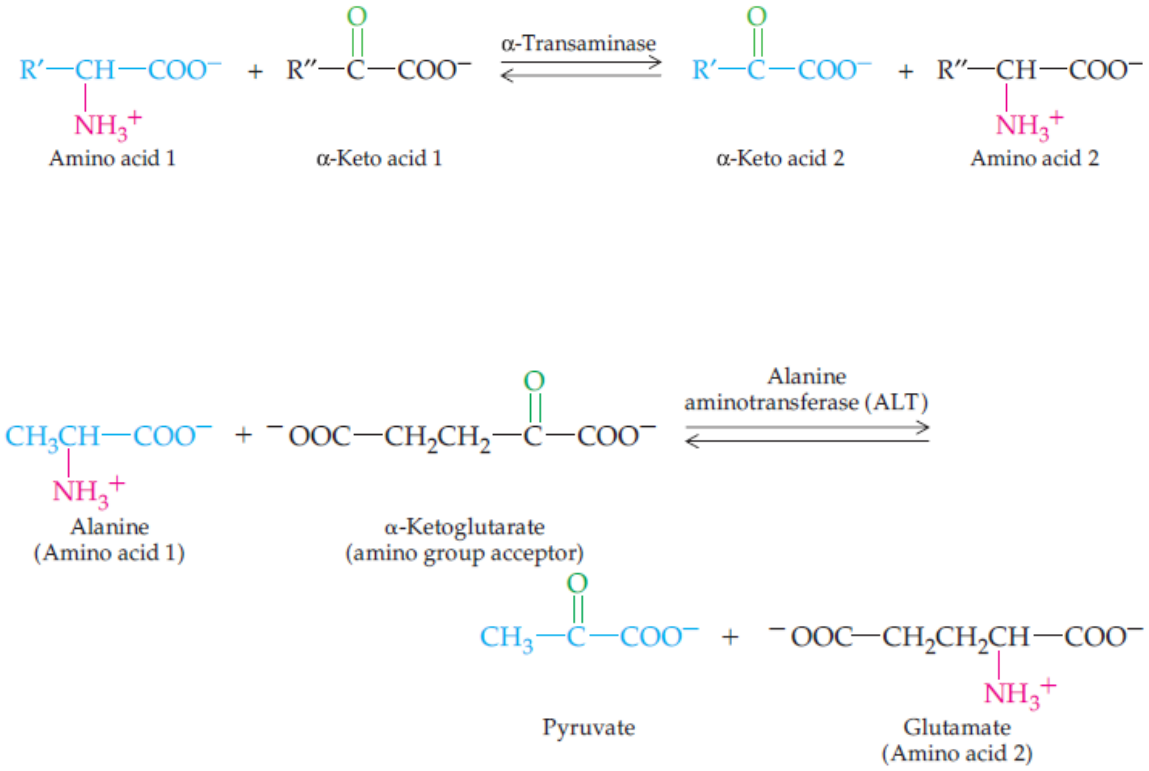


General scheme for amino acid catabolism

- Removal of amino group → Use of nitrogen (synthesis of N-compounds → Passage of nitrogen (urea cycle) → carbon atoms convert to compounds (citric acid cycle or glucose formation)
- No storage of nitrogen-containing compounds & ammonia is toxic to cells
- N-containing compounds (NO, hormones, neurotransmitters, NAD^+, heme, purine & pyrimidine bases)
- Carbon portion that converted to TCA cycle compounds is available for several pathways; energy (10-20%), TAGs (lipogenesis), glycogen (gluconeogenesis & glycogen synthesis), or ketone bodies

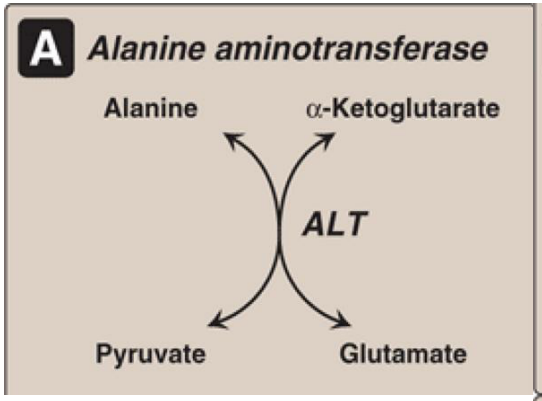
Catabolism of The Amino Group

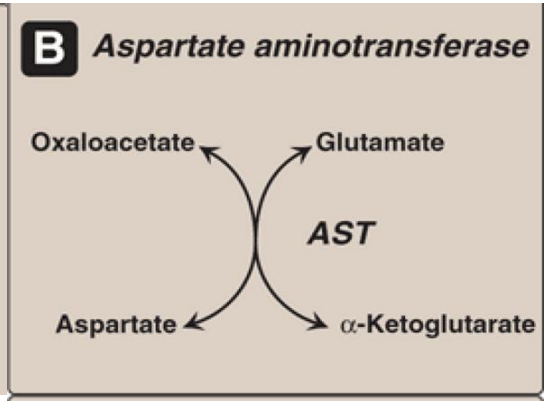
- Removal of amino group
- Transamination
- Several aminotransferases (transaminases)
- Most are specific for α-ketogluterate, easily reversible depending on concentrations (regulation)



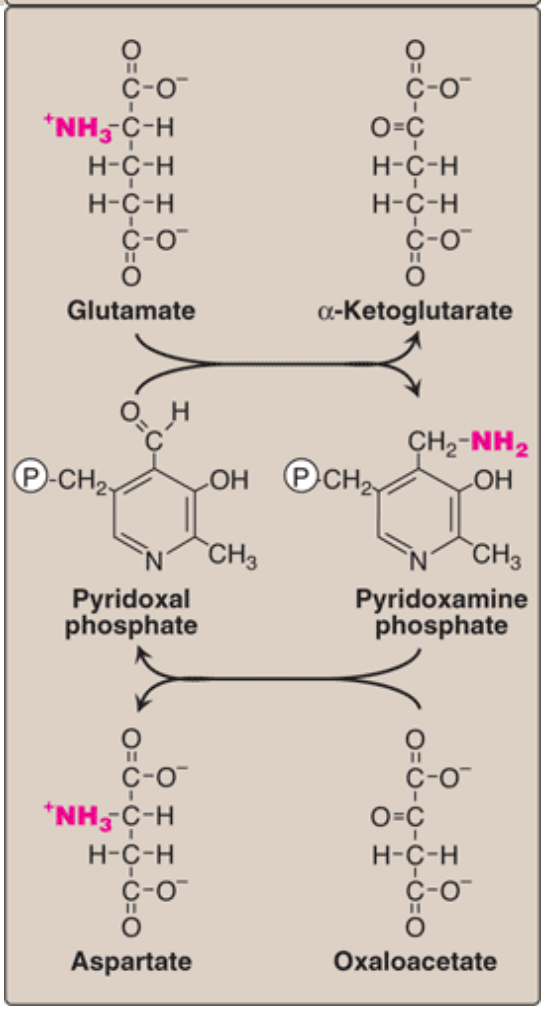
- The products are an α-keto acid (derived from the original amino acid) & glutamate

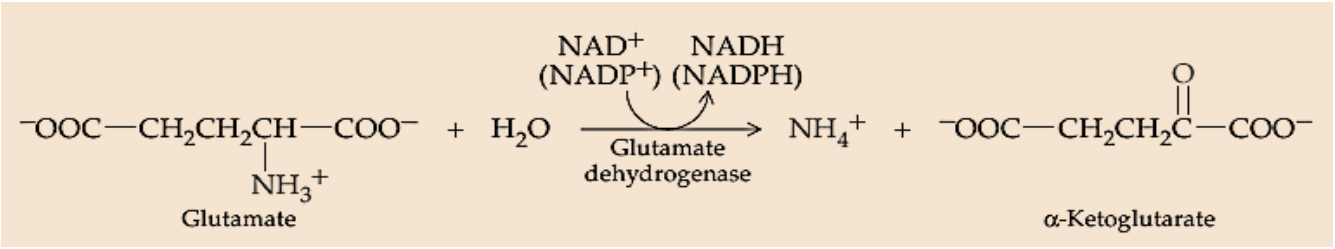
Catabolism of The Amino Group





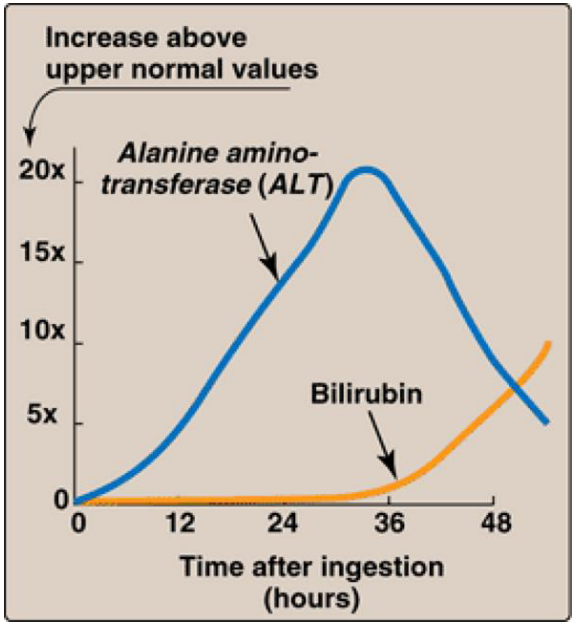
- Glutamate deamination
  - Oxidative deamination (α-ketogluterate)
  - Transamination (nonessential amino acids)
- Aminotransferases are specific (1 or few)
- The two most important are; ALT & AST
- All require PLP coenzyme (B_6)
- Aminotransferases reactions are in a state of equilibrium, aid in catabolism & anabolism

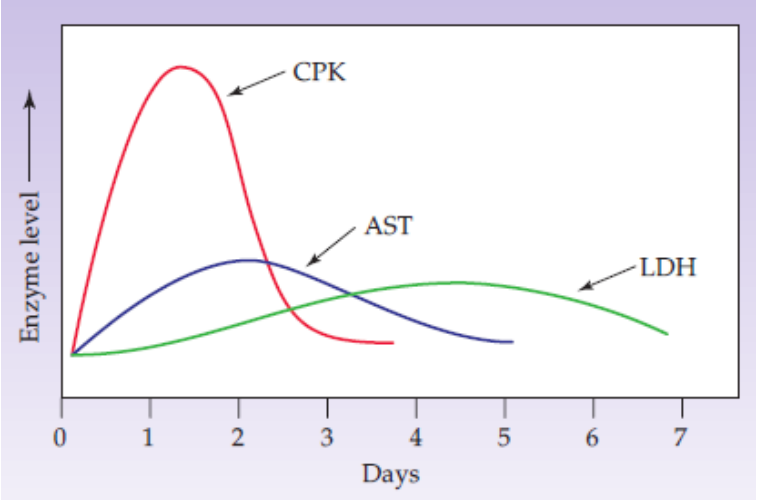




Diagnostic value of plasma aminotransferases

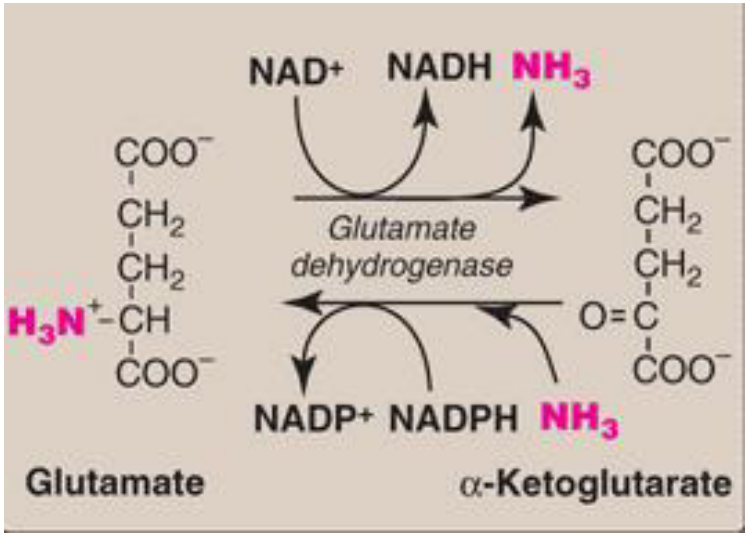
- AST & ALT
  - Conditions that cause extensive cell necrosis
    - Viral hepatitis, toxic injury, & prolonged circulatory collapse
  - ALT is more specific for liver disease
  - AST is more sensitive for liver disease
  - Elevated serum bilirubin
  - Nonhepatic disease: Aminotransferases may be elevated but they are clinically different





Oxidative deamination of amino acids: Glutamate DH

- The result is free amonia
- Glutamate is the only amino acid that undergoes rapid oxidative deamination
  - Amino groups of most aa. can be released as ammonia
- Occurs primarily in liver & kidney
- Provides α-ketogluterate (energy) & ammonia (urea)
- Use either NAD^+ or $NADP^+$ as a coenzyme

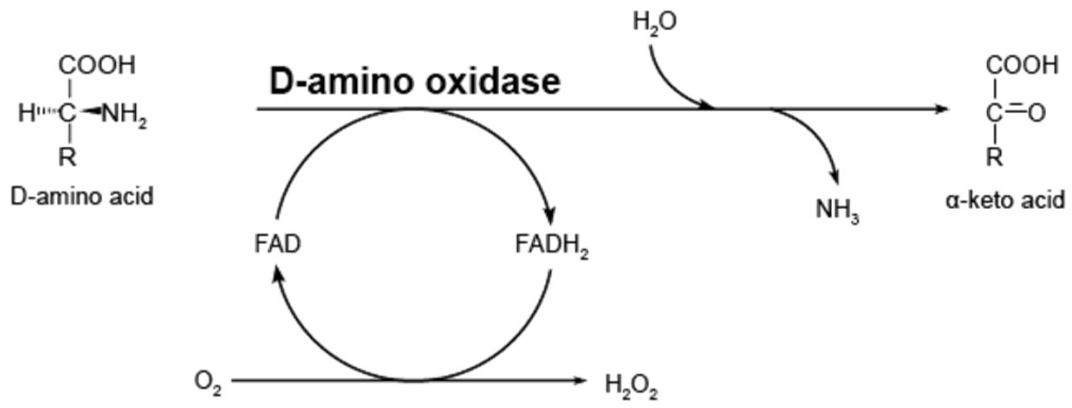


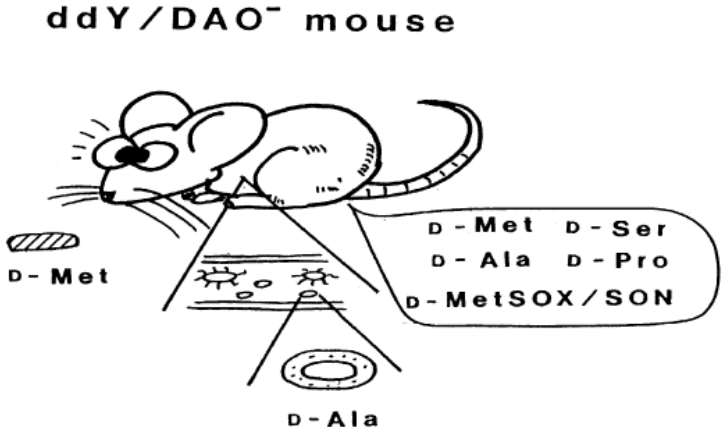
- ADP is an allosteric activator

- The direction depends on: conc. of glutamate, α-ketoglutarate, & ammonia, and the ratio of oxidized to reduced coenzymes

Oxidative deamination of amino acids: D-amino acid Oxidase

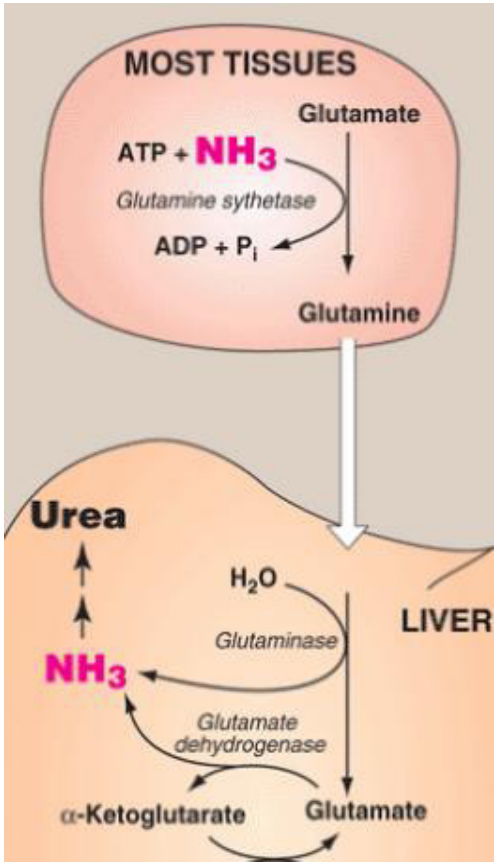
- Plants & microorganisms
- FAD-dependent peroxisomal enzyme
- Catalyzes oxidative deamination
- Results in α-keto acids that can either be reaminated to L-isomers, or catabolized for energy

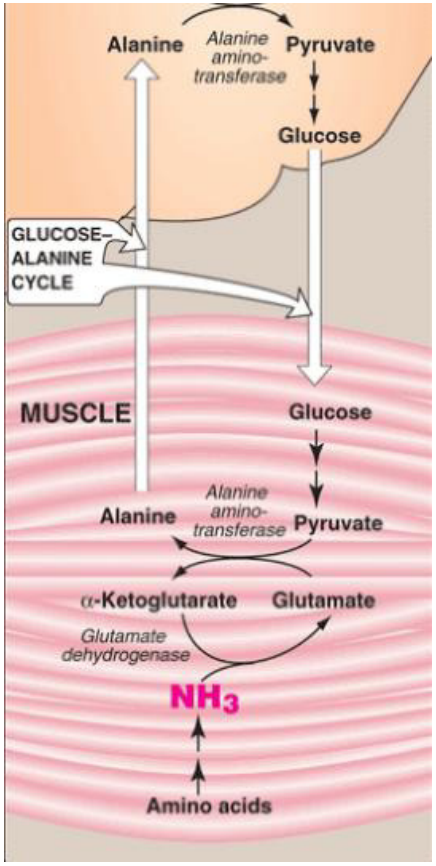




Transport of ammonia to the liver

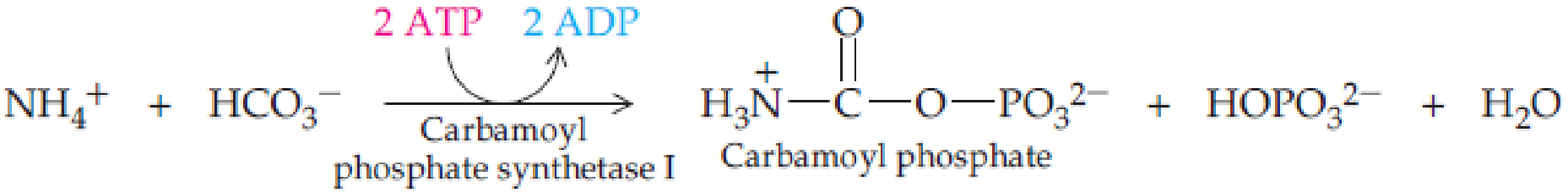
- Two mechanisms;
- 1. Glutamine synthetase
  - ✓In most tissues → blood → liver (glutaminase)
- 2. Glucose-alanine cycle,
  - ✓Primarily by muscle
  - ✓Pyruvate transamination (alanine) → blood → liver →
    - ○Transamination (puruvate)
    - ○Gluconeogenesis (glucose), back to muscle





The Urea Cycle

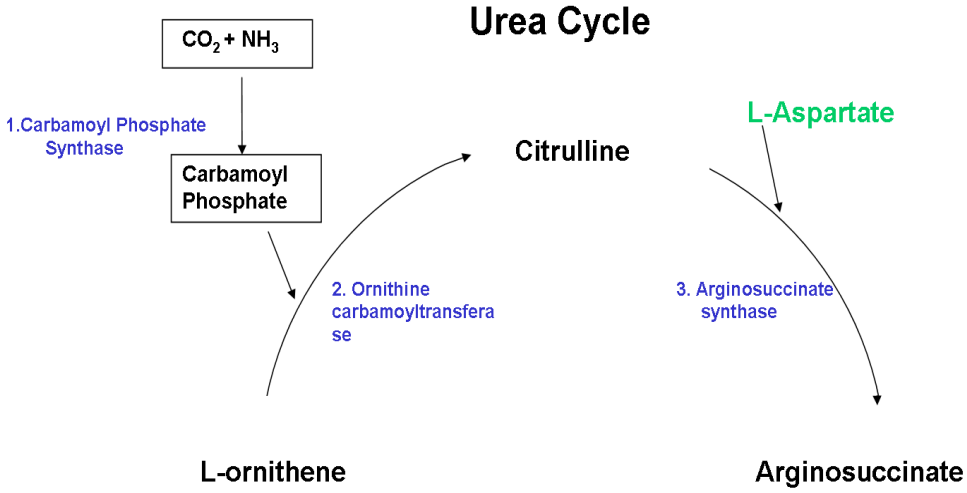
- What is it? Why do we need it? What fish do? Is it applicable in mammals? What mammals do?
- Where does it occur? Where does it go? Is energy needed?
- Accounts for 90% of the nitrogen-containing components of urine



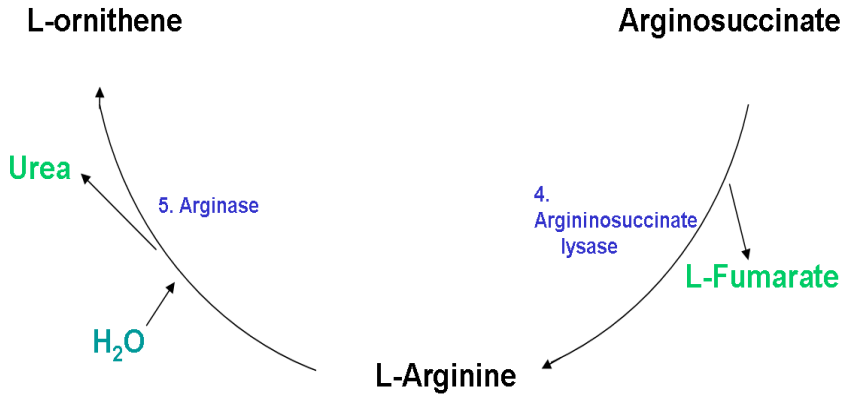
UREA CYCLE

- STEPS 2 AND 3: Building Up a Reactive Intermediate

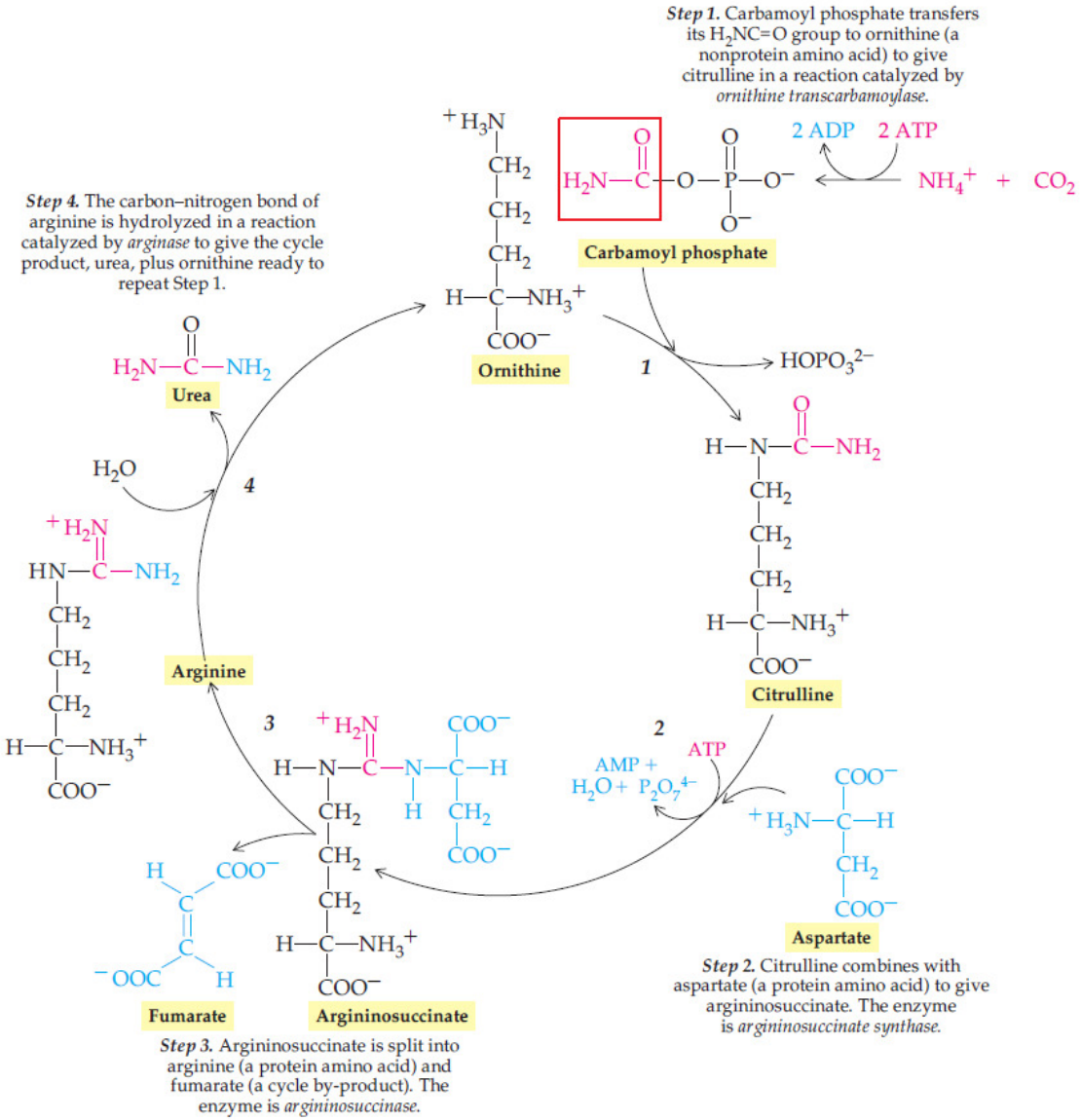
- C & O of urea are CO_2 derived
- Nitrogens is provided primarily by glutamate
- 1+2 mitochondrial
- 3-5 cytosolic
- N-acetylglutamate (positive allosteric activator)



- STEPS 4 AND 5: Cleavage and Hydrolysis



Occurs almost exclusively in the liver



Fumarate is hydrated to malate (TCA, or oxidized to oxaloacetate in cytosol) (aspartate, glucose)

A third ATP is consumed

Ornithine & citrulline are basic amino acids

The release of the high-energy P drives the reaction

Amino group of aspartate provides the second N

Net Result of the Urea Cycle

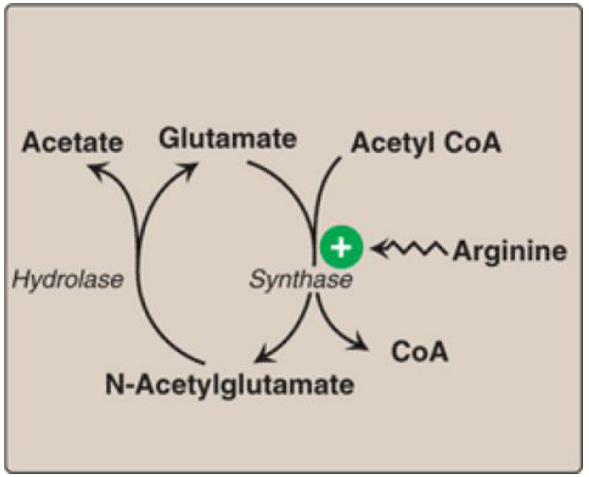
$Asp + NH_3 + CO_2 + 3ATP \rightarrow Urea + Fumarate + 2ADP + AMP + 3H_2O$

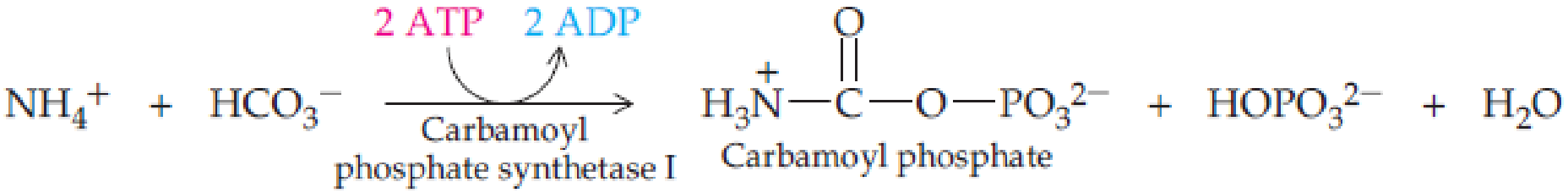
- Breaking of four high-energy phosphate bonds (large –ΔG, irreversible)
- Production of fumarate
- Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) & aspartate nitrogen (through transamination of oxaloacetate by AST)
- Formation of urea from the C of CO_2, NH_4^+, and aspartate, followed by biological elimination through urine
- Small portion to the intestine, cleaved to CO_2 and NH_3 by **bacterial urease**, feces and blood, kidney failure, hyperammonemia, neomycin

Regulation of the Urea Cycle

Asp + NH_3 + CO_2 + 3ATP → Urea + Fumarate + 2ADP + AMP + $3H_2O$

- Regulation occurs at the level of the rate-limiting step (CPSI)
- N-Acetylglutamate is synthesized by N-acetylglutamate synthase, arginine is an activator (fed state)





Metabolism of Ammonia

- Ammonia is produced by all tissues, slightly elevated concentrations (hyperammonemia) are toxic to CNS

- Sources of ammonia: amino acids (food and transamination with oxidative deamination), kidneys (renal glutaminase and glutamate dehydrogenase), Intestinal glutaminase, bacterial urease, catecholamines, nitrogenous bases

- Transport of ammonia: very low levels in the blood, rapid action of liver, alanine or glutamine (primarily in muscle, liver and brain, glutamine synthetase), deaminated by glutaminase

- Disposal of ammonia: urea in liver, to kidneys, urine

Diseases

- Hyperammonemia:
  - Normal serum ammonia (5–50 µmol/L)
  - Liver compromised (genetic or acquired): can reach 1000 µmol/L)!
  - Medical emergency (CNS toxicity)
- Acquired: either acute (viral hepatitis, ischemia, or hepatotoxins), cirrhosis (alcoholism), or chronic hepatitis
- Hereditary: five enzymes, (1:30,000 live births), autosomal recessive. Ornithine transcarbamoylase deficiency (X-linked, most common, males)
- Immediate treatment (hemodialysis) vs. long-term treatment (low-protein diet and frequent small meals)