# Protein and Amino Acid Metabolism

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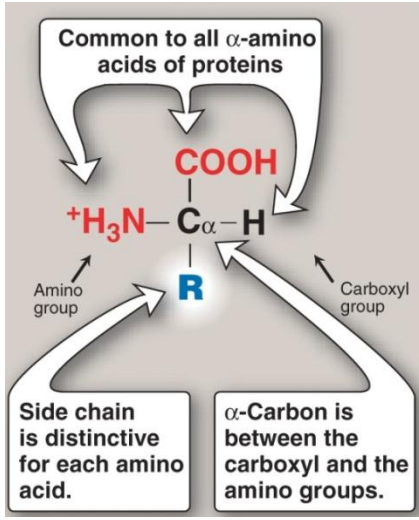
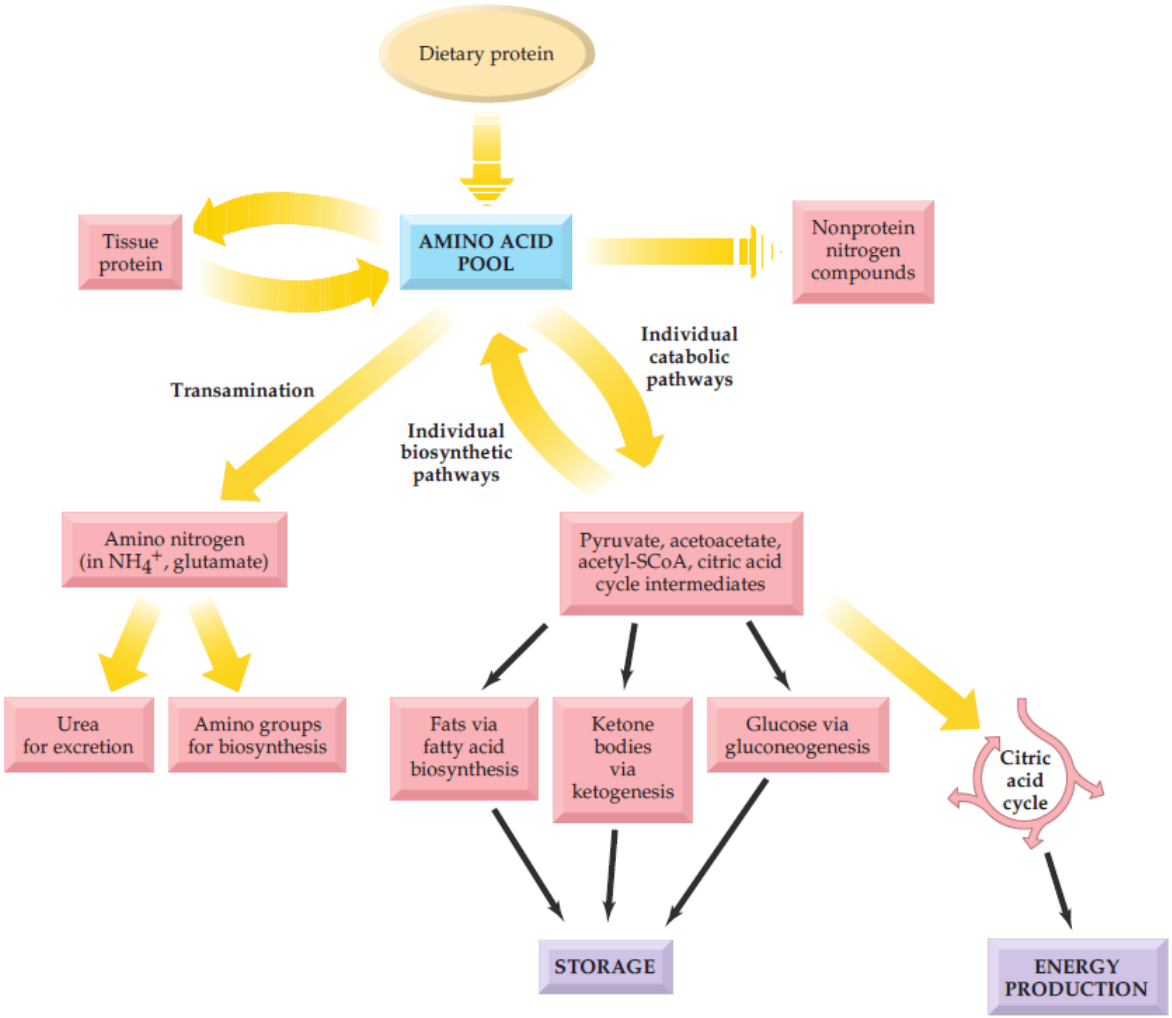
# Amino Acid Metabolism - overview

- 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 and 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 turnover

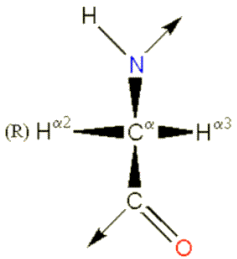
- Most proteins in the body are in a constant remodeling
- What regulates protein concentration in cells?

- 1. Rate of turnover: In healthy adults, constant total protein (300–400 g / day), varies widely for individual proteins (minutes, hours, days, weeks, years)

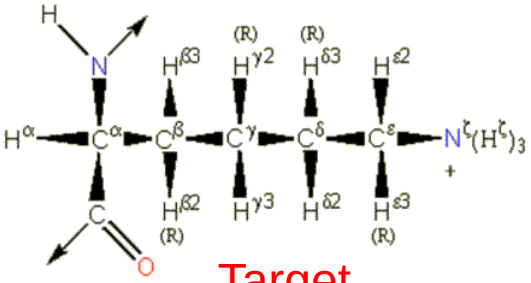
- 2. Protein degradation:
  - ATP-dependent vs. ATP-independent
  - Proteasomes vs. lysosomes (acid hydrolases)
  - Intracellular vs. extracellular

# ATP dependent

- Ubiquitin-proteasome proteolytic pathway: ubiquitin (small protein, globular, non-enzymic)
- What is Ubiquitination? polyubiquitin chain? Proteasome?

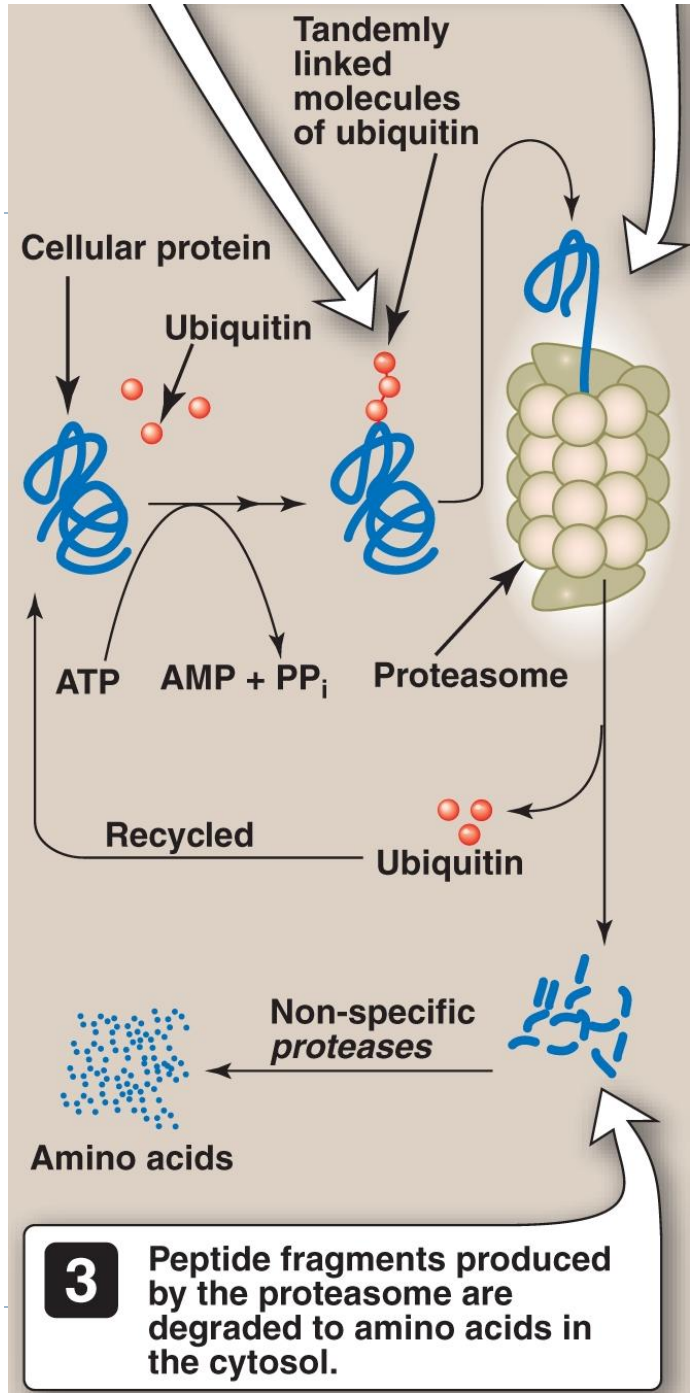


Ubiquitin



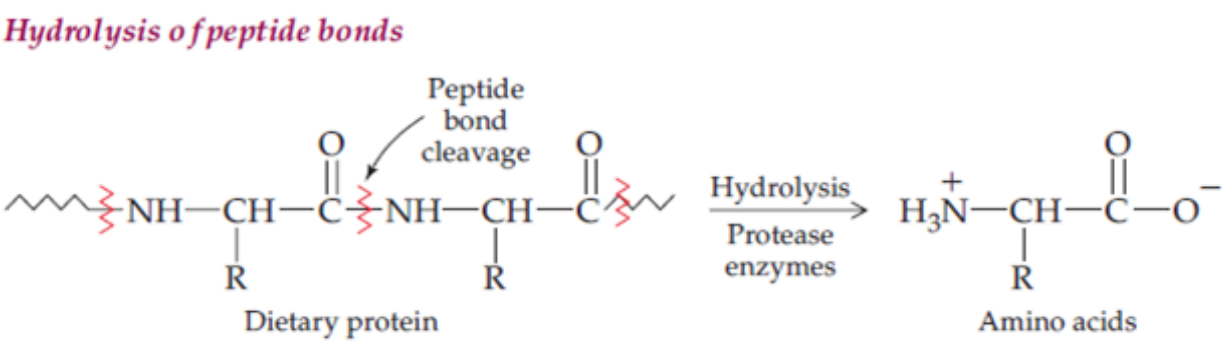
Target

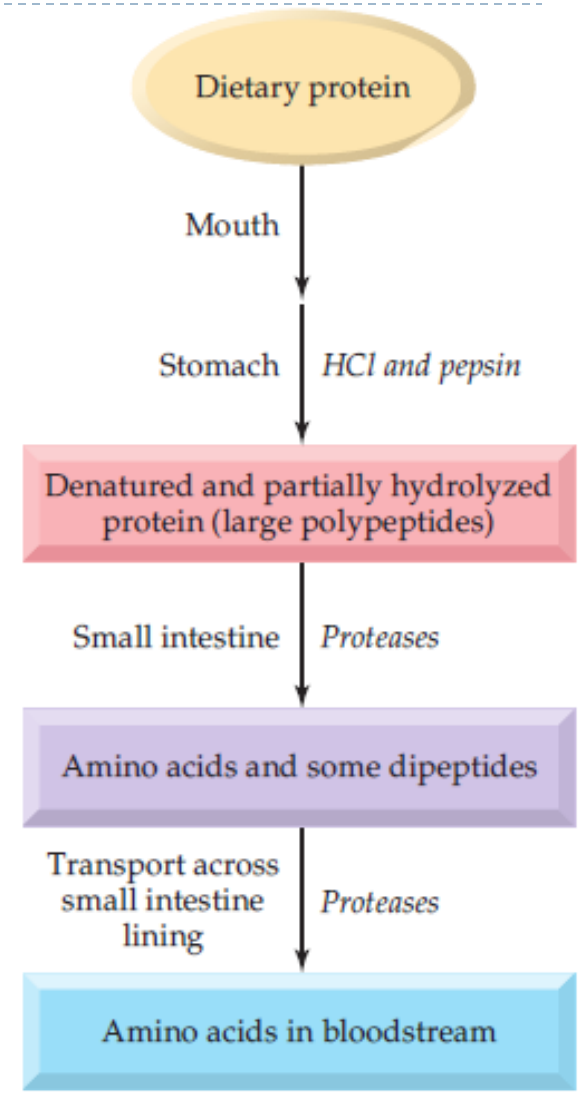
- Chemical signals for protein degradation
  - Is it random?
  - Oxidation of amino acids, ubiquitination, N-terminal residue (Ser >20 hrs vs. Asp ≈ 3min), PEST sequences are short lived



# Digestion of Protein

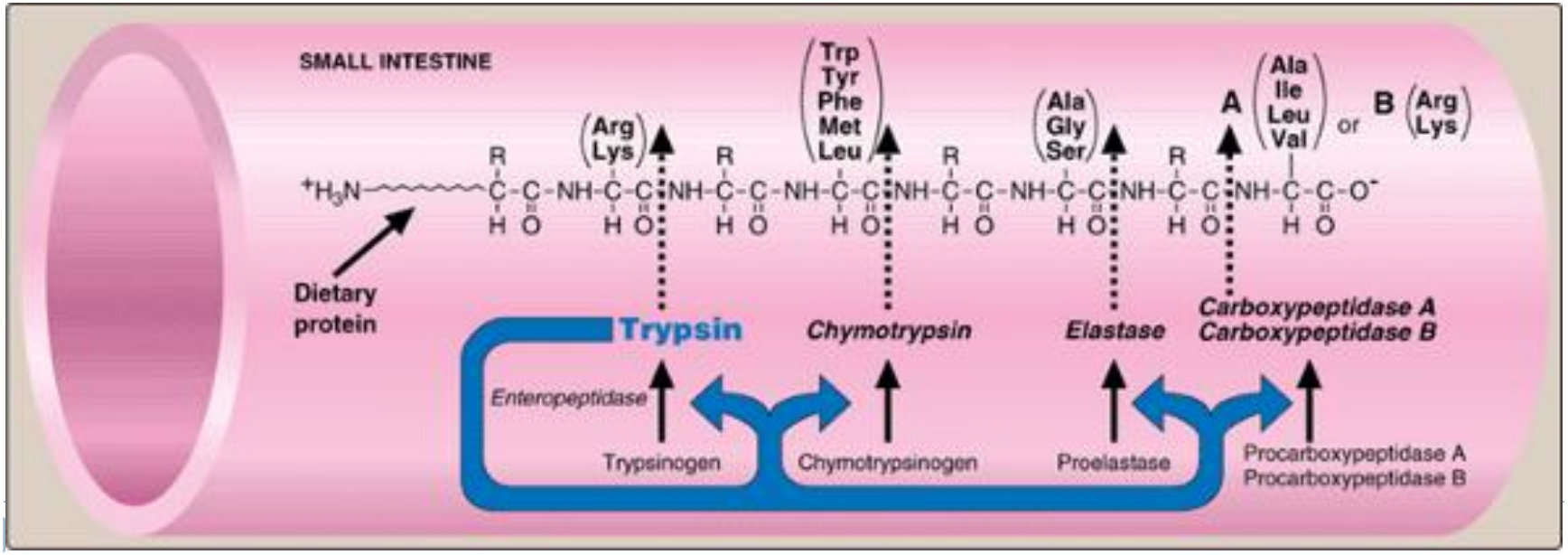
- What is protein digestion? (70–100) g/day
- Where digestion begins?
- Stomach; HCl (2-3) and pepsinogen (polypeptides). Acid and autocatalytic activation.



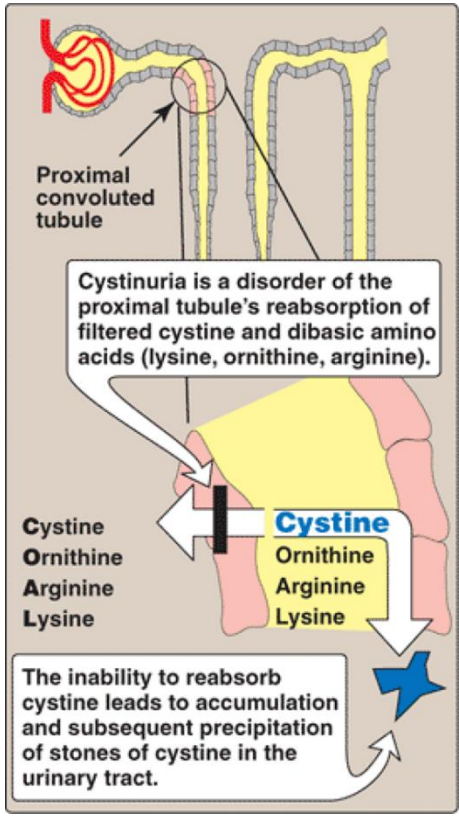
# Digestion of Protein

- Pancreatic (specificity). Secretion mediated by cholecystokinin and 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 and transport of amino acids and dipeptides

- Free amino acids ($Na^+$-cotransport) vs. di- and tripeptides ($H^+$-cotransport) followed by hydrolysis.
- Only free amino acids are found in the portal vein.
- From cells to the bloodstream
- Free amino acids extracellular is significantly lower than that within the cells of the body (ATP)
- Active transport is grouped (at least 7 transport systems); excess in one can cause deficiency in another!
- The small intestine and the proximal tubule of the kidney have common transport systems for amino acid reuptake
- COAL system is deficient in cystinurea, 1/7000, the most common inherited genetic diseases of amino acids, cystine kidney stones

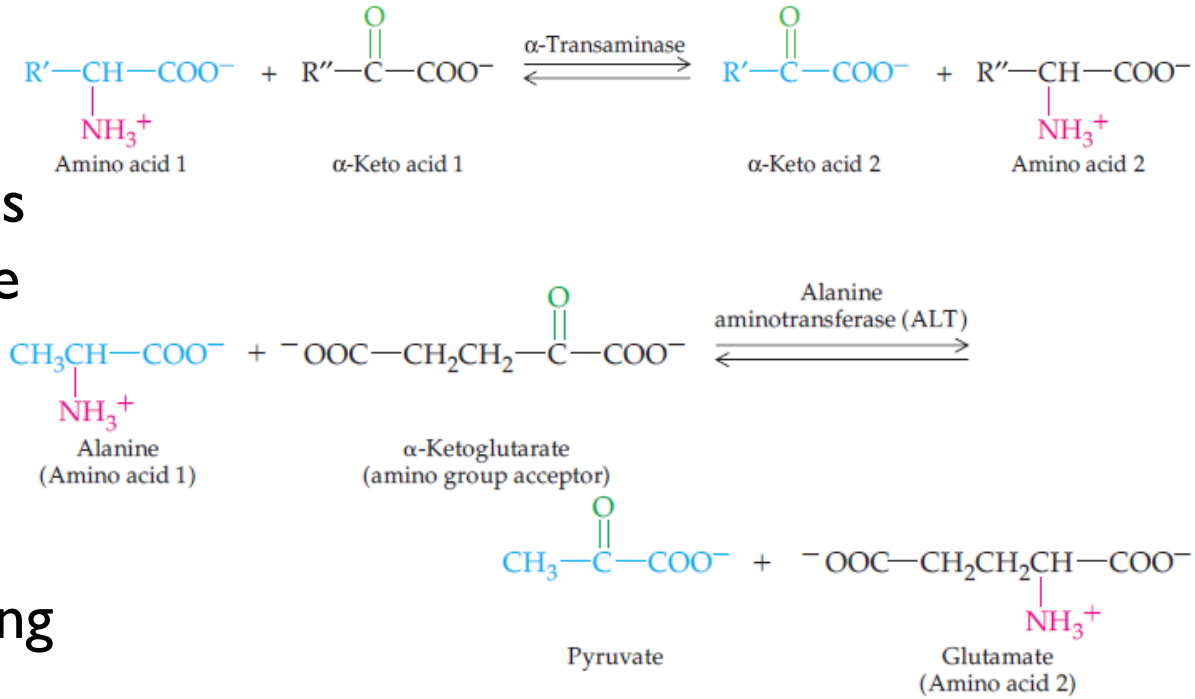


# General scheme for amino acid catabolism

- Removal of the amino group → Use of nitrogen in synthesis of new nitrogen compounds → Passage of nitrogen into the urea cycle → Incorporation of the carbon atoms into compounds that can enter the citric acid cycle or glucose formation
- No storage of nitrogen-containing compounds & ammonia is toxic to cells. So, amino nitrogen has only two fates: incorporation into urea, or used in synthesis of compounds (NO, hormones, neurotransmitters, $NAD^+$, heme, purine & pyrimidine bases)
- The carbon portion of the amino acid is converted to TCA cycle compounds; from there they are available for several alternative pathways; energy (10-20%), triacylglycerols (lipogenesis), glycogen (gluconeogenesis and glycogen synthesis), or ketone bodies

# Catabolism of The Amino Group

- Removal of the amino group; transamination, several aminotransferases (transaminases), most are specific for α-ketogluterate

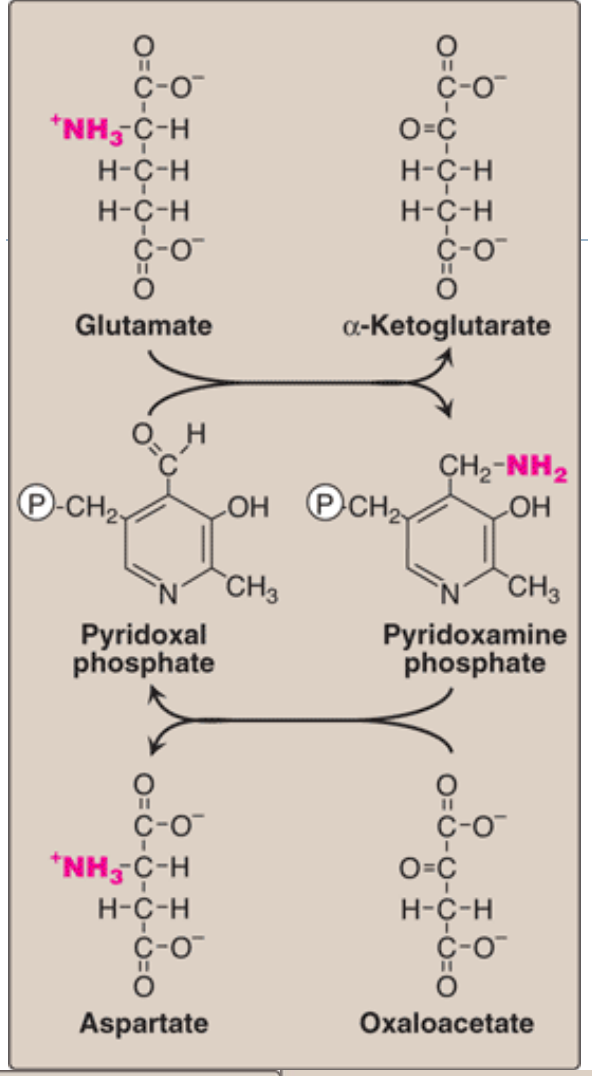


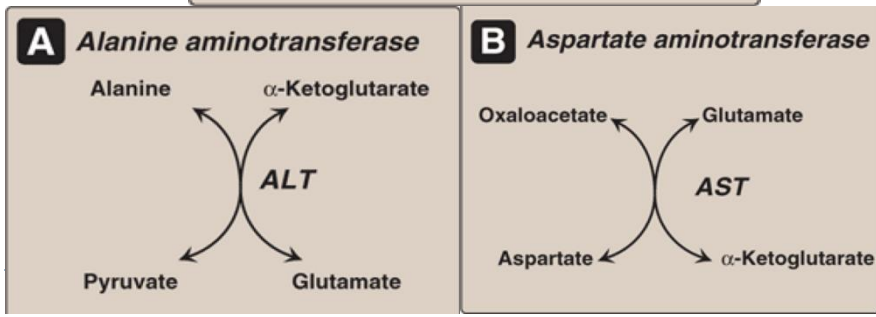

- Easily reversible depending on concentrations (regulation)

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

# Catabolism of The Amino Group

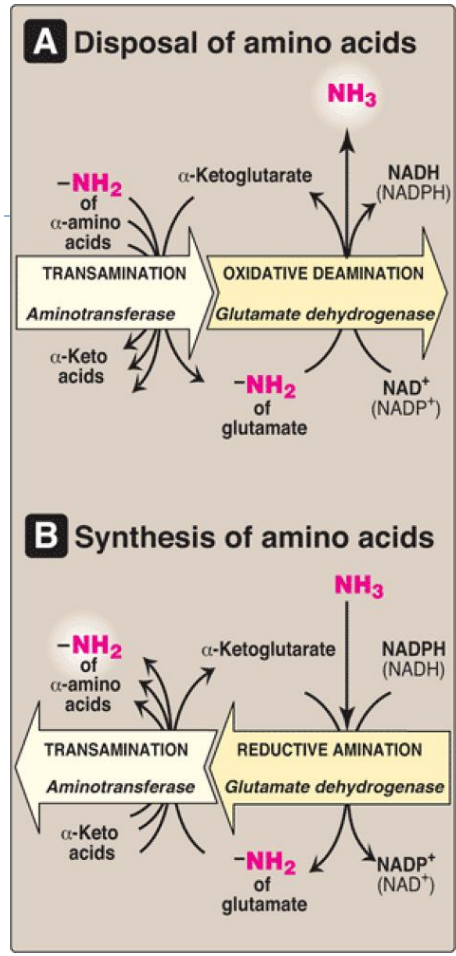
- All aminotransferases require the coenzyme pyridoxal phosphate coenzyme (a derivative of vitamin $B_6$)
- For most transamination reactions, the equilibrium constant is near one, allowing the reaction to function in both amino acid degradation and biosynthesis
- Aminotransferases are specific for one or, at most, a few amino group donors
- The two most important are; ALT and AST

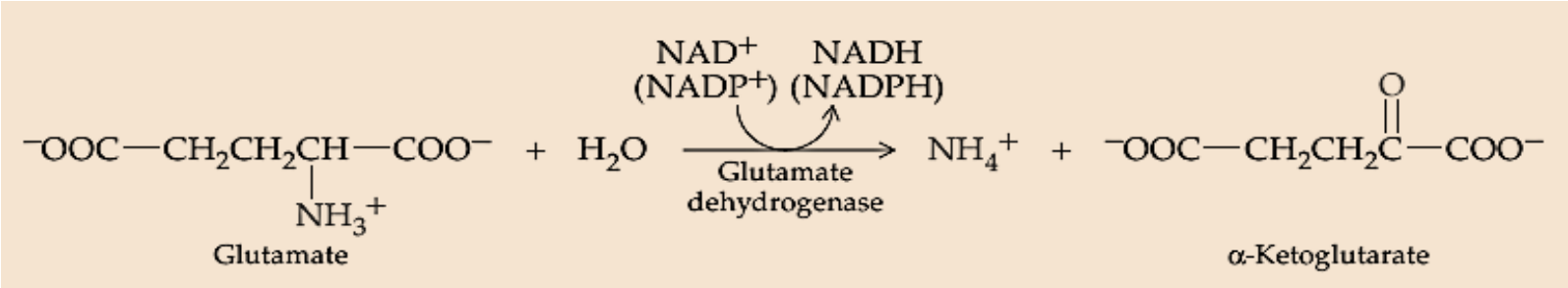


# Oxidative deamination of amino acids: Glutamate DH

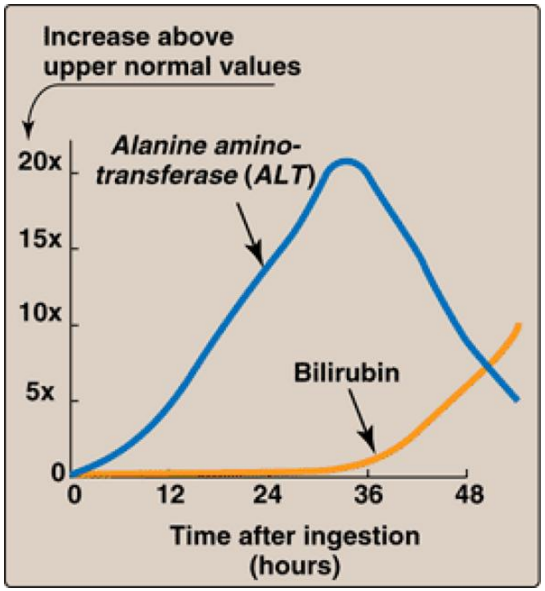
- Glutamate is the only amino acid that undergoes rapid oxidative deamination
- Glutamate either oxidatively deaminated (α-ketogluterate) or transaminated (nonessential amino acids)
- It can use either $NAD^+$ or $NADP^+$ ($NAD^+$ in oxidative deamination and NADPH in reductive amination)
- The direction of the reaction depends on the relative concentrations of glutamate, α-ketoglutarate, & ammonia, and the ratio of oxidized to reduced coenzymes
- Allosterically regulated: GTP inhibitor, ADP activator



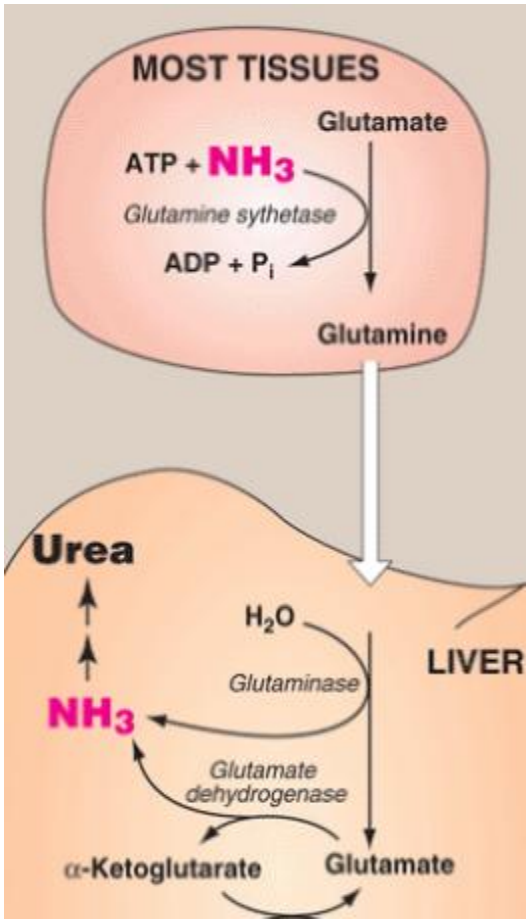
# Diagnostic value of plasma aminotransferases

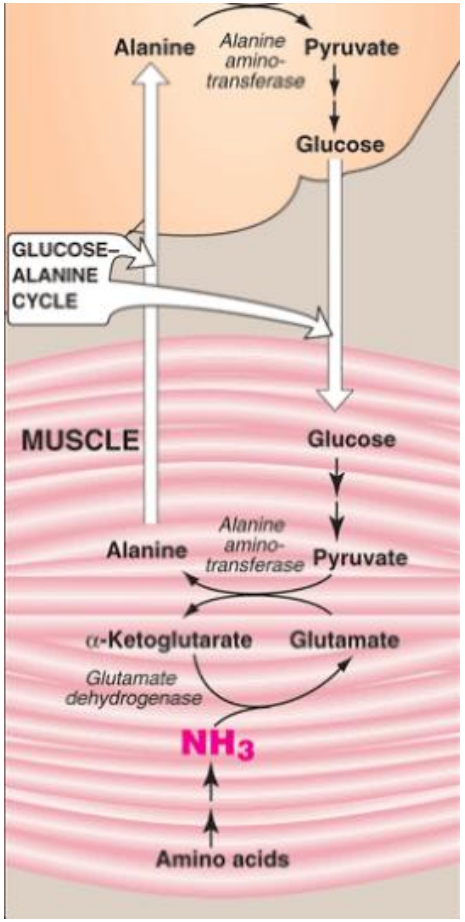
- AST and ALT
  - Particularly in conditions that cause extensive cell necrosis, such as severe viral hepatitis, toxic injury, and prolonged circulatory collapse
  - ALT is more specific for liver disease
  - AST is more sensitive for liver disease
  - Elevated serum bilirubin results from hepatocellular damage that decreases the hepatic conjugation and excretion of bilirubin
  - Nonhepatic disease: Aminotransferases may be elevated but they are clinically different



# Transport of ammonia to the liver

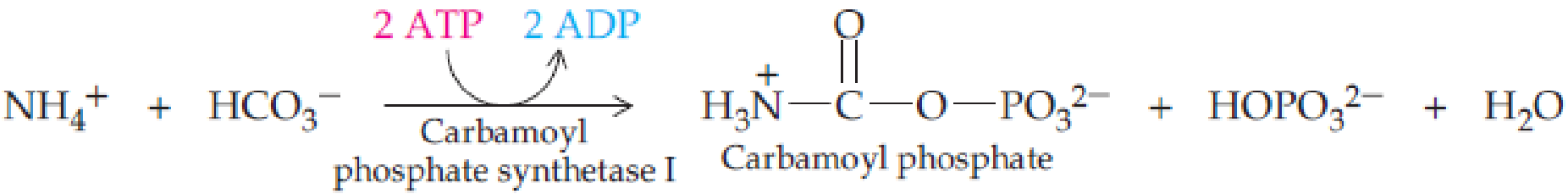
- Two mechanisms;
- 1. Glutamine synthetase, in most tissues, in blood to liver, glutaminase
- 2. Glucose-alanine cycle, primarily by muscle, pyruvate transamination (alanine), in blood to liver, another transamination (puruvate), gluconeogenesis (glucose), back to muscle



# The Urea Cycle

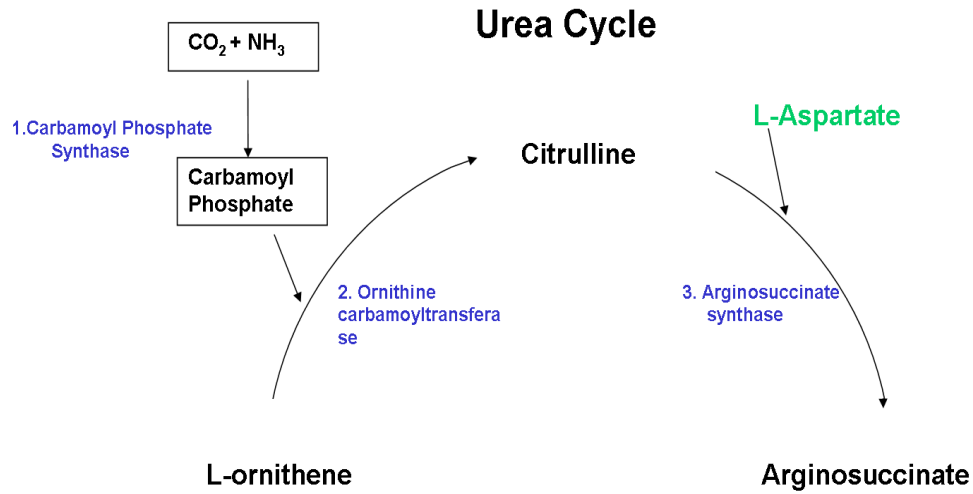
- What is it? Why do we need it? What fish do? Is it applicable in mammals? What mammals do?
- Where does it occur? Where does it go? Is energy needed?
- Accounts for 90% of the nitrogen-containing components of urine



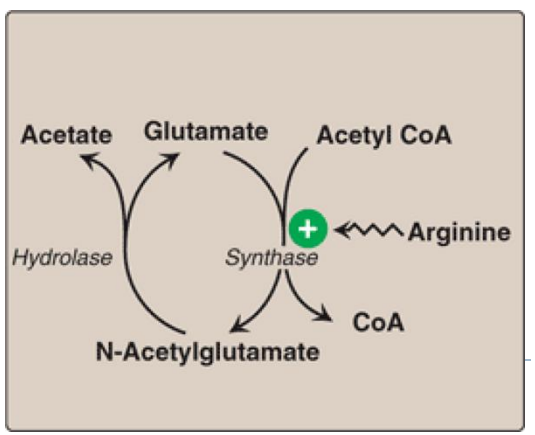
# UREA CYCLE

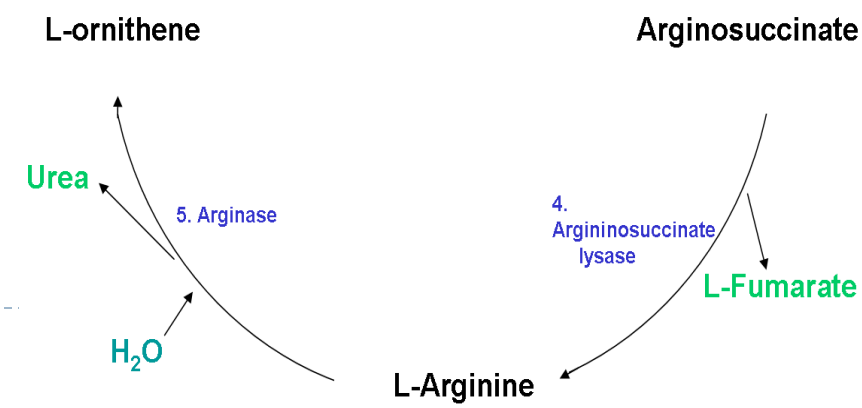
- STEPS 2 AND 3: Building Up a Reactive Intermediate

- The carbon and oxygen of urea are derived from $CO_2$.
- Ammonia in carbamoyl phosphate is provided primarily by glutamate
- 1+2 mitochondrial, 3-5 cytosolic
- N-acetylglutamate (positive allosteric activator).

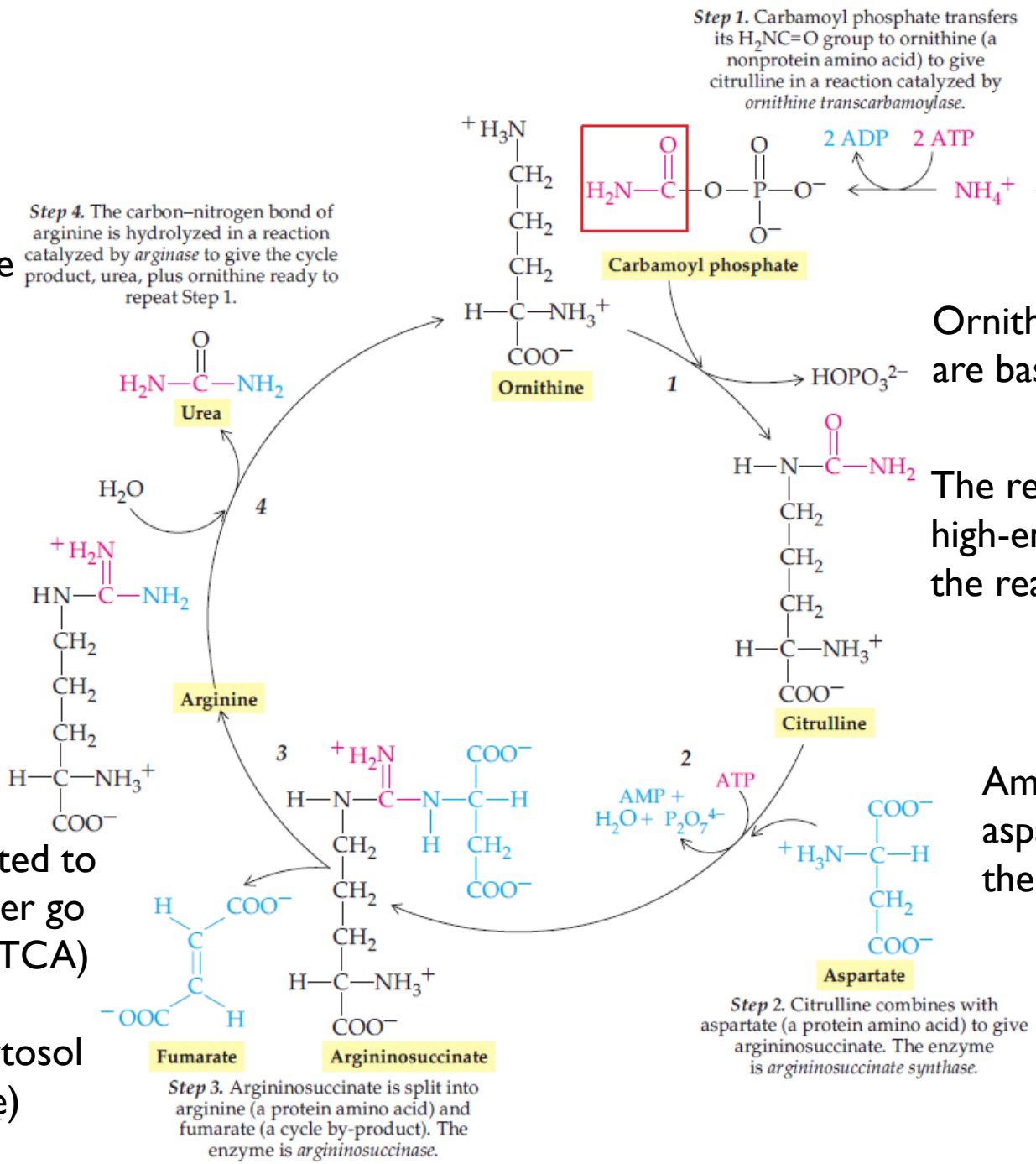



- STEPS 4 AND 5: Cleavage and Hydrolysis



occurs almost exclusively in the liver




Ornithine & citrulline are basic amino acids

The release of the high-energy P drives the reaction

Amino group of aspartate provides the second N

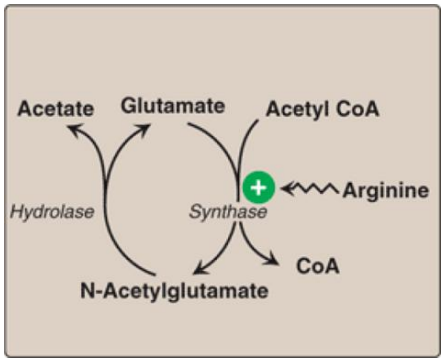
A third ATP is consumed

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

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

# *Net Result and regulation of the Urea Cycle*

- Breaking of four high-energy phosphate bonds (large $-\Delta G$, irreversible)
- Production of fumarate
- Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) and 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 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 & 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), when liver compromised (genetic or acquired), ammonia (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)