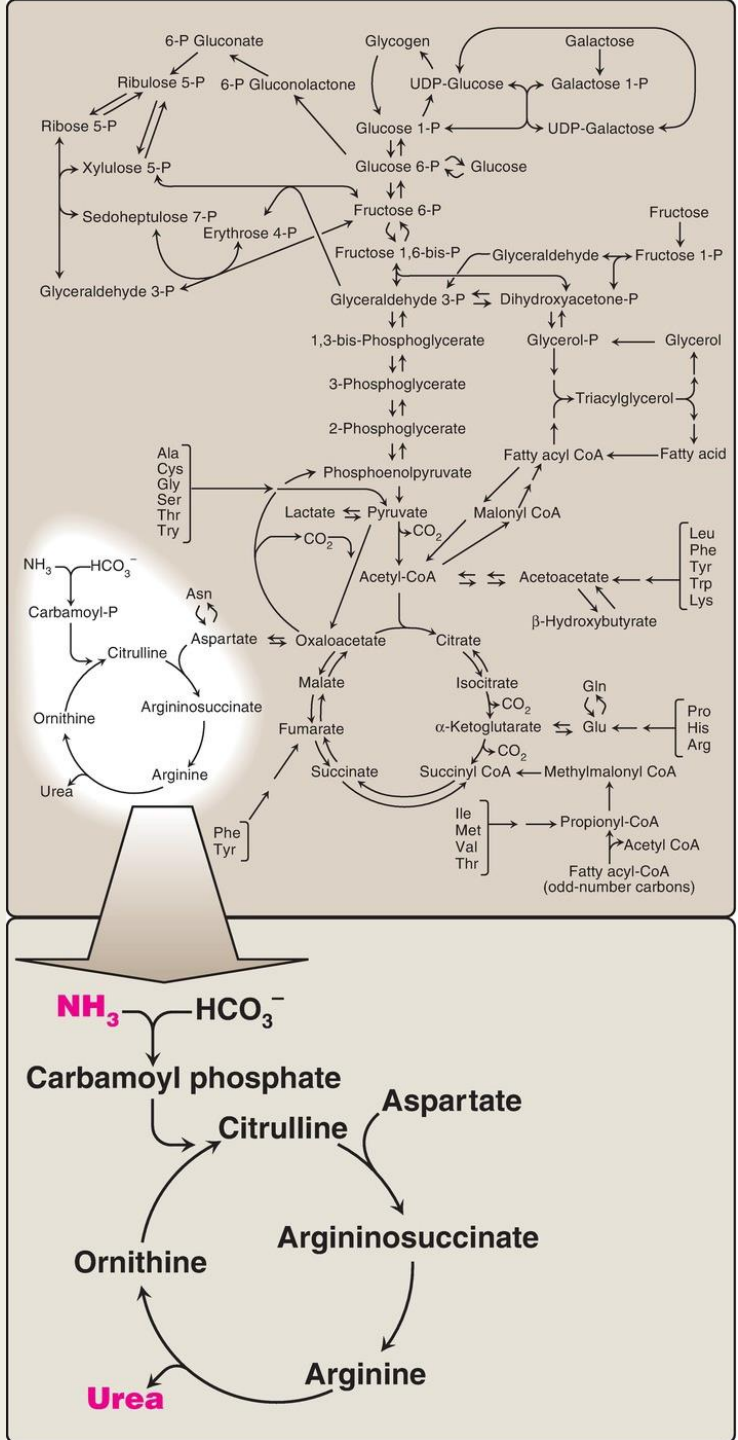
AMINO ACIDS

Nitrogen Disposal

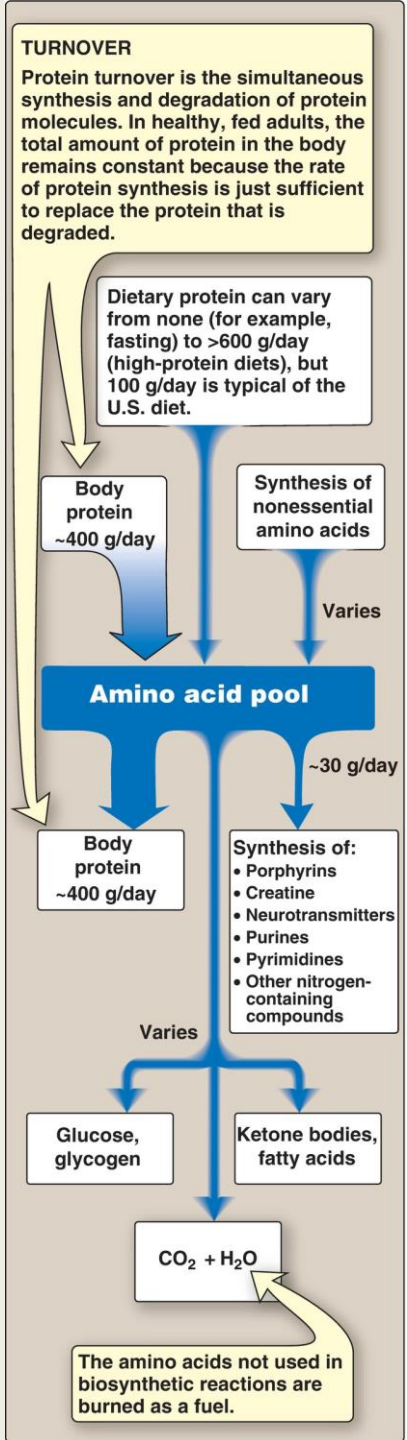
OVERVIEW

- Unlike fats and carbohydrates
- The first phase
  - *A portion excreted in the urine, but most as urea*
- The second phase
  - *Metabolized to carbon dioxide (CO_2) and water (H_2O), glucose, fatty acids, or ketone bodies by the central pathways of metabolism*



II. OVERALL NITROGEN METABOLISM
A. Amino acid pool

- What is it?
- Sources? 3
- Depletion? 3
- ~90–100 g (~12 kg in a 70-kg man)
- In healthy, well-fed individuals (steady state), and the individual is said to be in nitrogen balance



II. OVERALL NITROGEN METABOLISM
B. Protein turnover

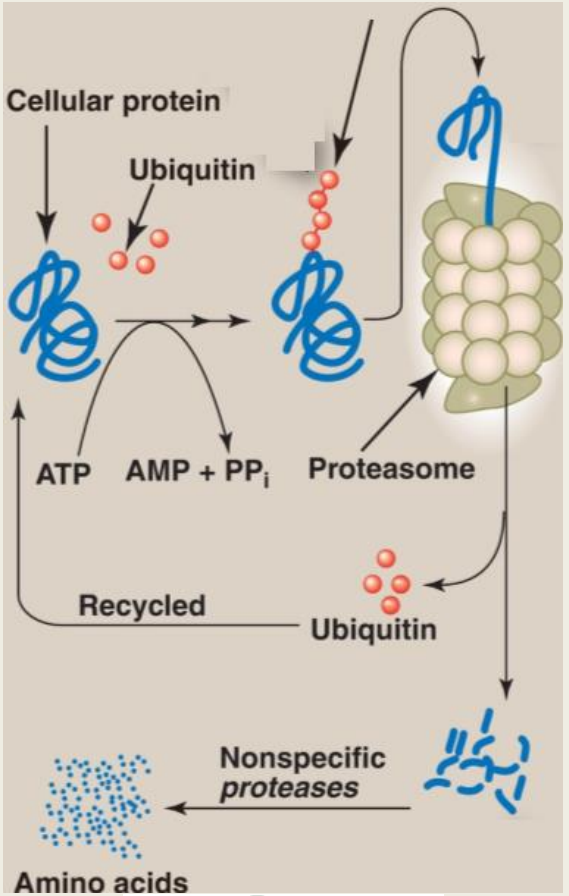
- Vary from a protein to another
- 1. Rate: the total amount of protein in the body remains constant (hydrolysis and resynthesis of 300–400 g of body protein daily)
- The rate of protein turnover varies:
  - *Shortlived proteins (regulatory proteins and misfolded proteins, minutes or hours)*
  - *Long-lived proteins (days to weeks, majority of proteins in the cell)*
  - *Structural proteins (months or years)*

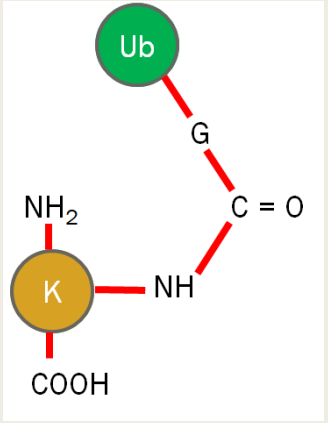
II. OVERALL NITROGEN METABOLISM
B. Protein turnover

- 2. Protein degradation:
  - *ATP-dependent ubiquitin (Ub)–proteasome system of the cytosol (selective, damaged or short-lived proteins)*
  - *ATP-independent degradative enzyme system of the lysosomes (acid hydrolases, nonselective, autophagy and heterophagy)*

Ubiquitin–proteasome system

- A small, globular, nonenzymic, highly conserved
- Covalent attachment, ATP (hydrolytic enzymes), enzyme catalyzed (E1 activates, E2 conjugates, E3 ligates)
- There are many more *E3* proteins (*E1* or *E2)*
- A polyubiquitin chain (4 or more) is recognized
- Proteasome: large, macromolecular, barrel-shaped, proteolytic complex (unfolds, deubiquitinates, and cuts)
- Cytosolic proteases (amino acid pool)
- Ub is recycled



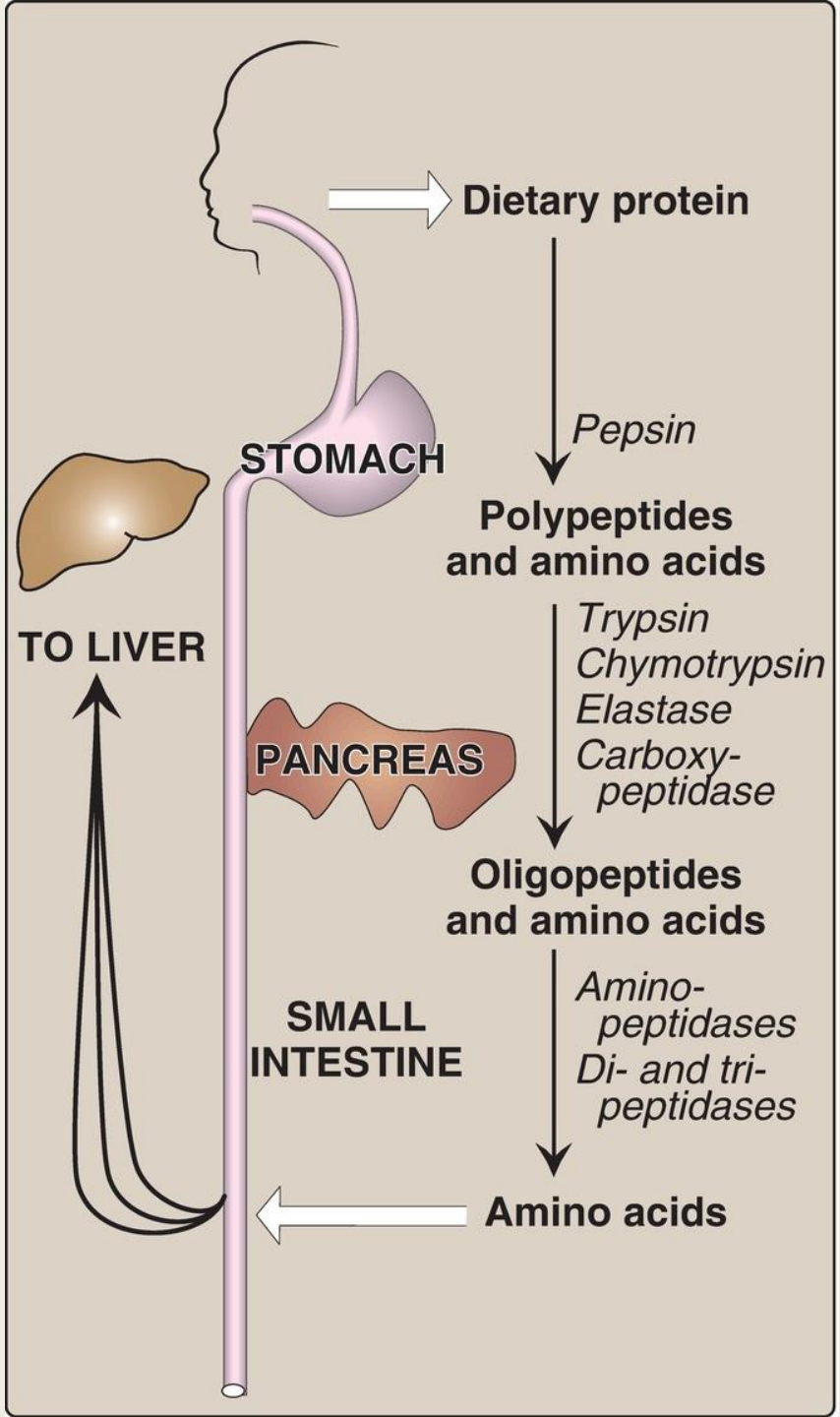


Degradation signals

- Is protein degradation a random process? Why? How?
- Recognized and bound by an *E3*
- N-end rule, and ranges from minutes to hours.
- Destabilizing (Arg, acetylated alanine), Stabilizing (Ser)
- Proteins rich PEST sequences are rapidly ubiquitinated and degraded

III. DIETARY PROTEIN DIGESTION

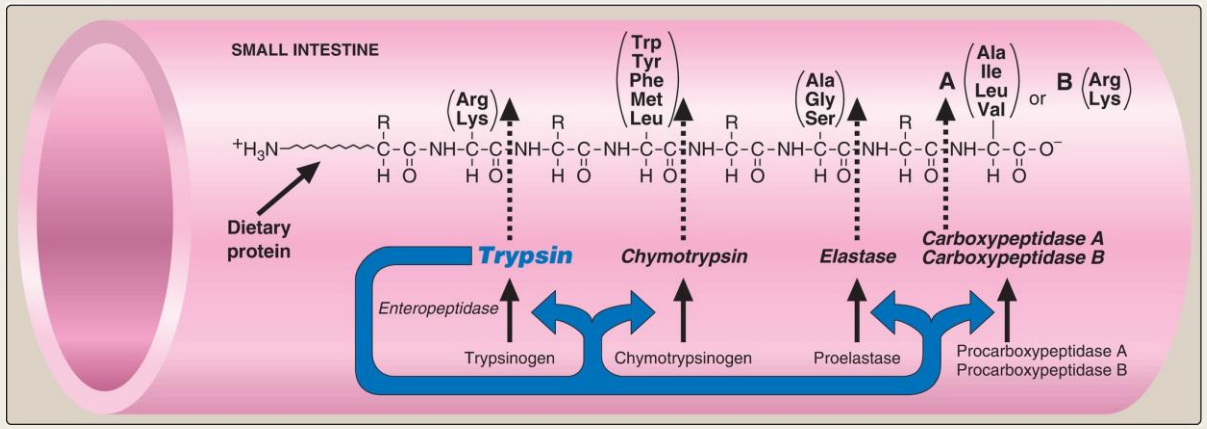
- 70–100 g/day (USA)
- Proteins' hydrolyses (stomach, pancreas, small intestines)
- Stomach: HCl function? Pepsinogen to pepsin!
- Pancreas: endopeptidases and exopeptidases
- How pH changes? secretin → Bicarbonate

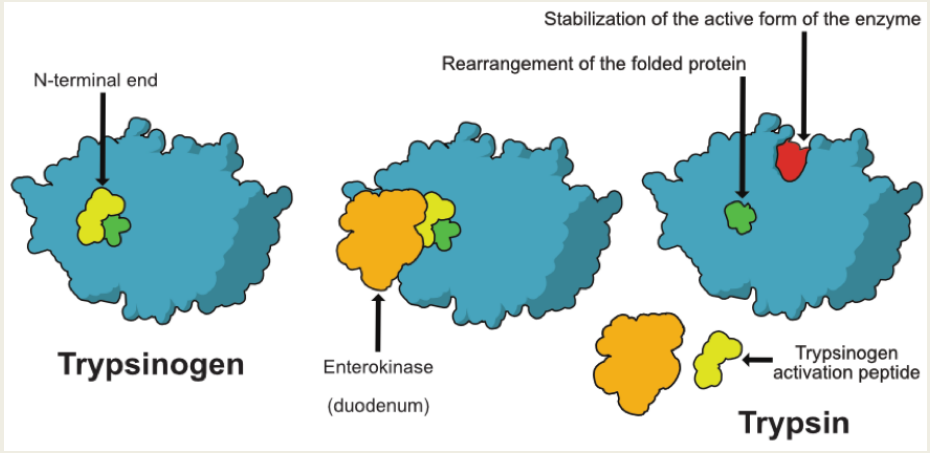


Digestion by pancreatic enzymes

Enzyme	Specificity
Trypsin	C-terminal to R, K, but not if next to P
Chymotrypsin	C-terminal to F, Y, W but not if next to P
Elastase	C-terminal to A, G, S, V, but not if next to P
Pepsin	N-terminal to L, F, W, Y, but not when next to P

- Specificity
- Zymogen release: cholecystokinin
- Zymogen activation: Enteropeptidase (enterokinase), hexapeptide from the N-terminus of trypsinogen
- Trypsin takes over



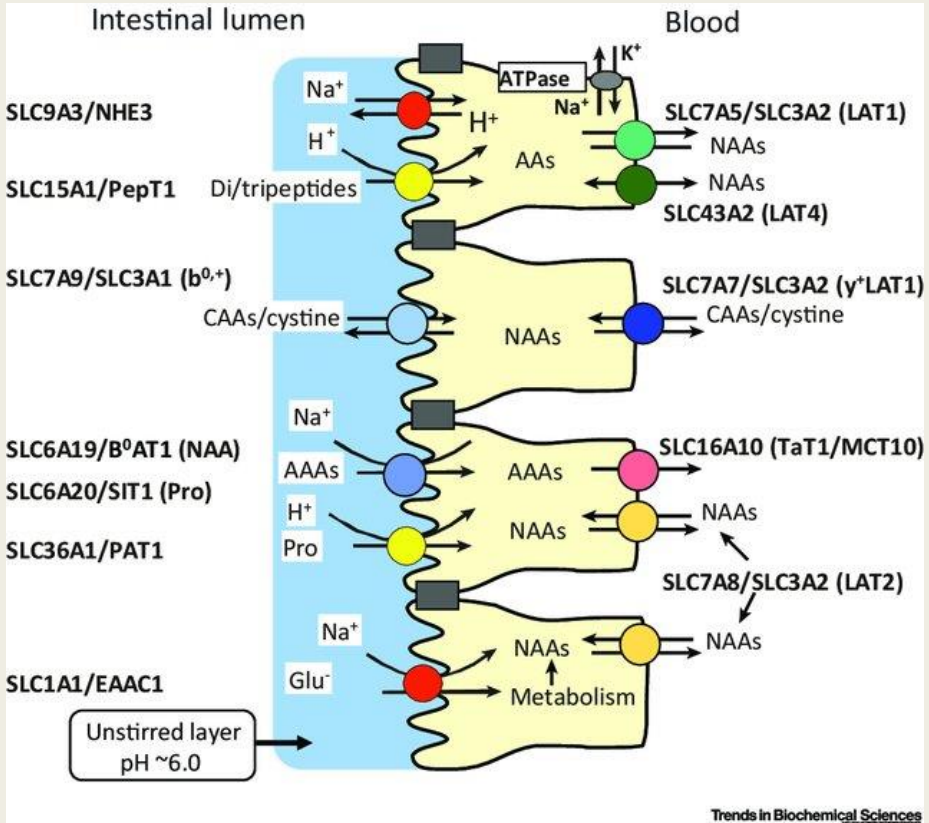


Digestion abnormalities

- Causes of deficiency? chronic pancreatitis, cystic fibrosis, or surgical removal of the pancreas
- Steatorrhea, undigested protein
- Celiac disease: immune-mediated damage to the small intestine in response to ingestion of gluten (wheat, rye)

Digestion of oligopeptides by small intestine enzymes

- *Aminopeptidase (exopeptidase)*

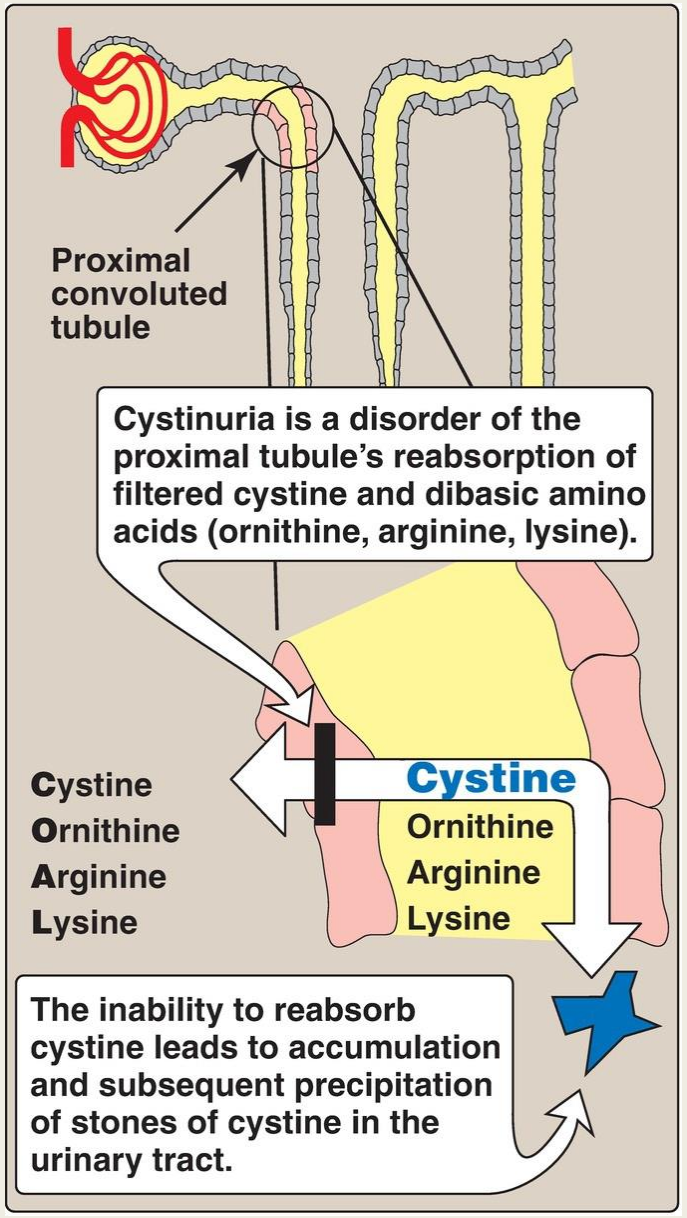


Amino acid and small peptide intestinal absorption

- Most free amino acids: Na^+- dependent secondary active transport
- At least seven different transport systems; overlapping amino acid specificities
- Di- and tripeptides: a proton-linked peptide transporter (PepT1)
- Free amino acids are released into the portal system: Na^+-independent transporters
- These amino acids are either metabolized by the liver (BCAA) or released into the general circulation

Absorption abnormalities

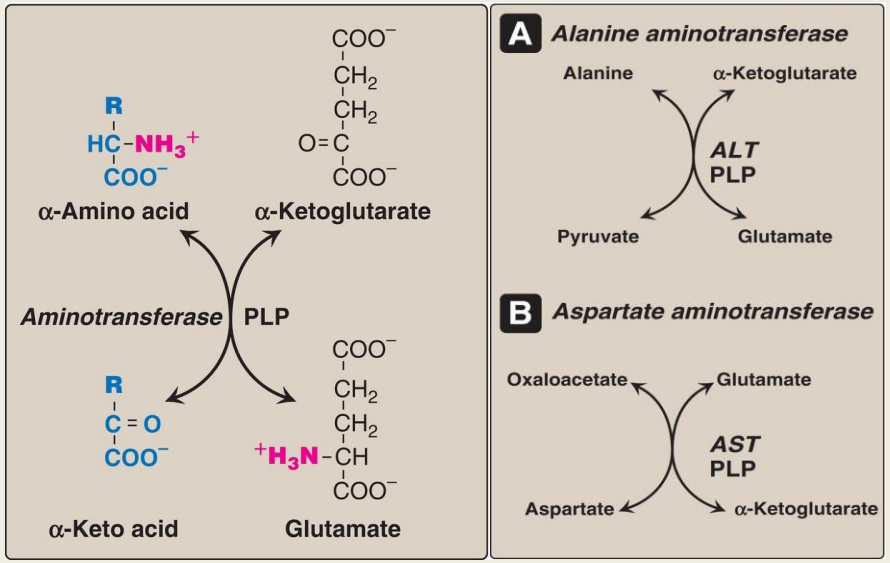
- Small intestines and kidney proximal tubules
- Defect consequences?
- Cystinuria: inherited; defective COAL; 1 in 7,000; the most common genetic error of amino acid transport
- Clinically: kidney stones (calculi), hydration,
- Hartnup disorder: defective neutral amino acid transporter (NAAT, tryptophan)

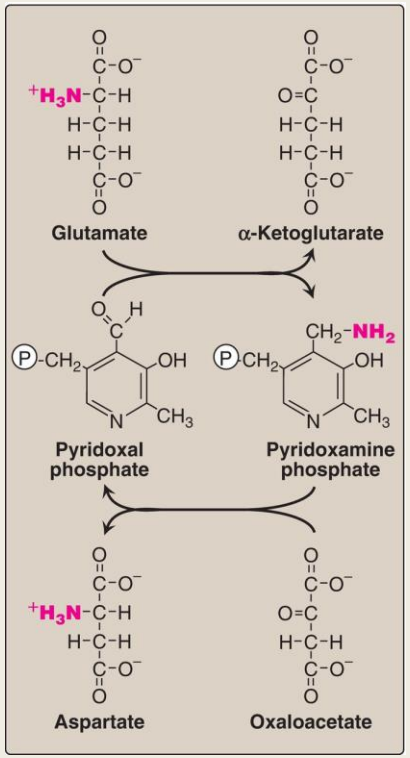


IV. NITROGEN REMOVAL FROM AMINO ACIDS

This section describes transamination and oxidative deamination, reactions that ultimately provide ammonia and aspartate, the two sources of urea nitrogen

Transamination: Funneling amino groups to glutamate

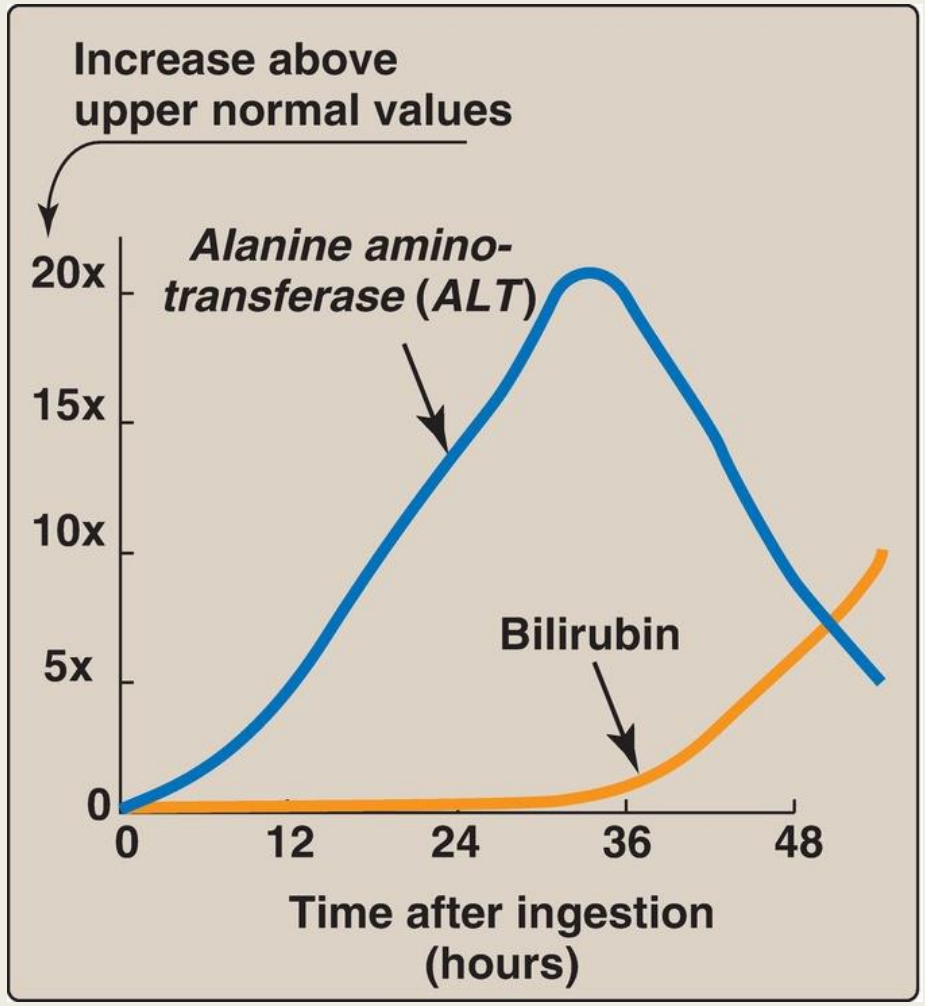




- Glutamate: oxidative deamination or amino group donor
- *Aminotransferases* (*transaminases*): cytosol and mitochondria; lysine and threonine (deamination)
- Substrate specificity (one or few); naming; reversibility, Keq ≈ 1,
- ALT
- AST: exception (aspartate); urea cycle
- Mechanism: PLP

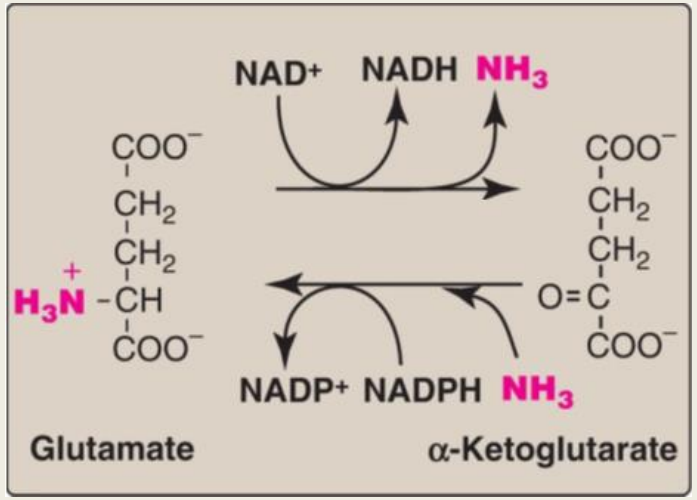
Hepatic disease

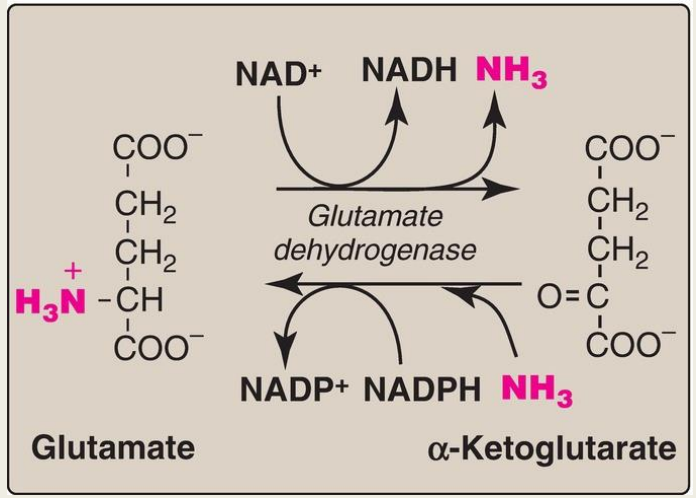
- Nearly all hepatic diseases esp. extensive cell necrosis, (viral hepatitis, toxic injury, and prolonged circulatory collapse)
- *ALT* is more specific than *AST* for liver disease but the latter is more sensitive
- Liver toxin
- Nonhepatic disease



Oxidative deamination: Amino group removal

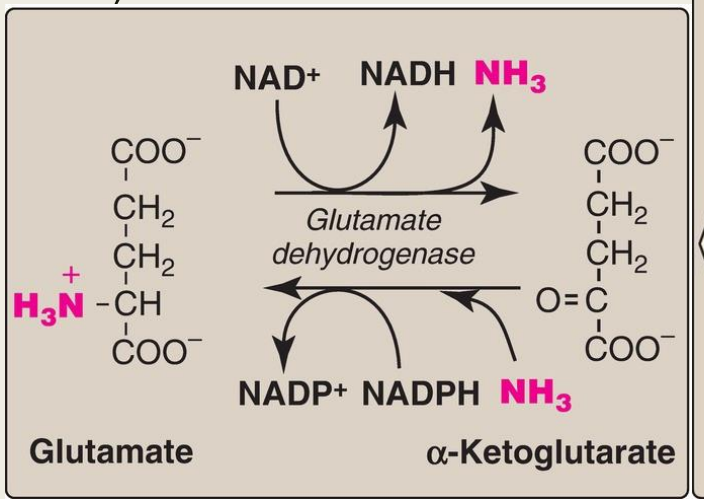
- Free ammonia
- Primarily in the liver and kidney
- They provide α-keto acids
- NH4+ or NH3 that crosses membranes

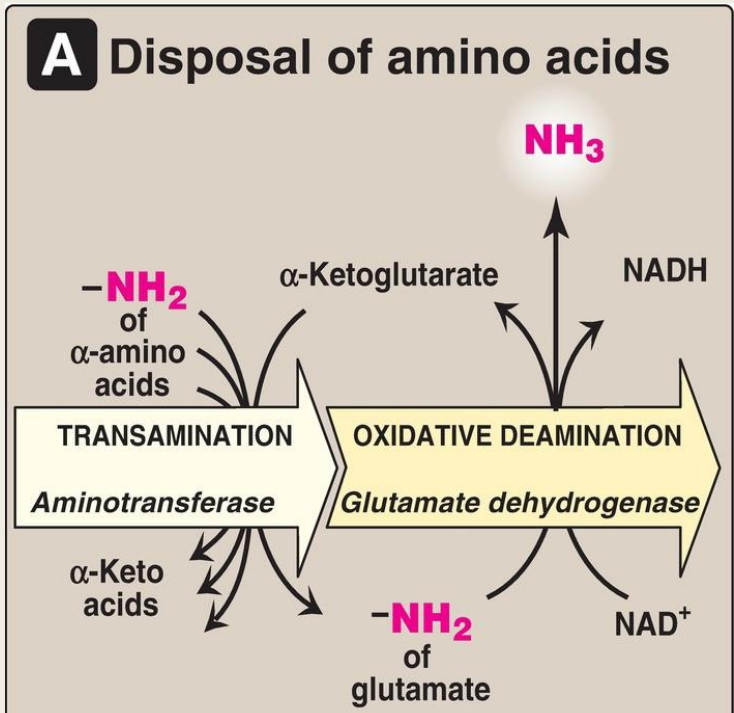


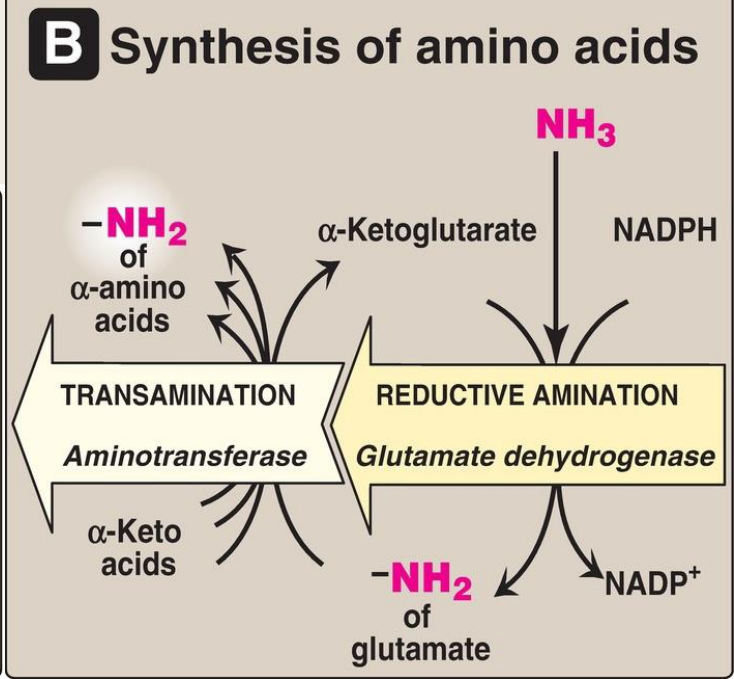


Glutamate dehydrogenase

- Rapid oxidative deamination
- Regenerating α-ketoglutarate
- NAD+ or NADPH (Oxidative deamination or reductive amination)
- Reaction direction
- Allosteric regulators: GTP?; ADP?;





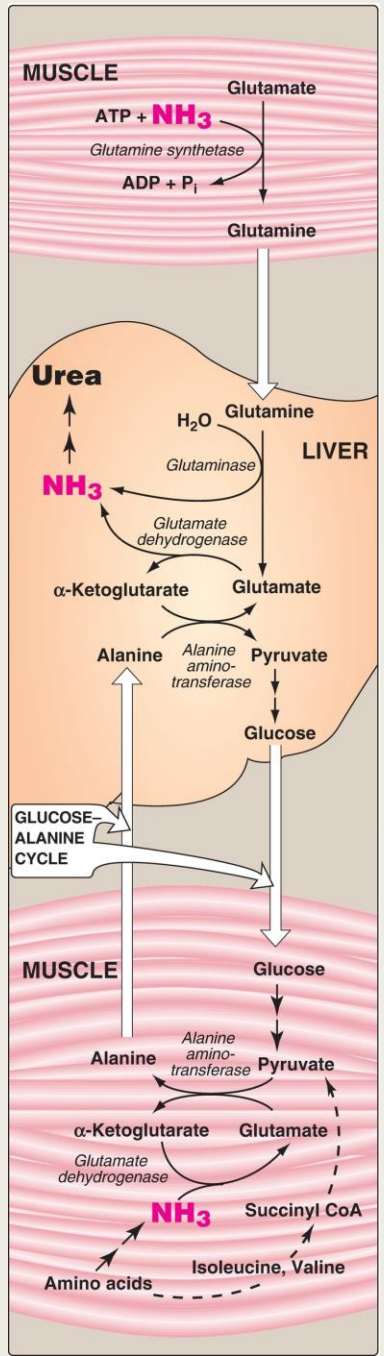


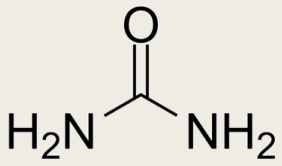
D-Amino acid oxidase

- Diet
- Efficiently metabolized to α-keto acids, ammonia, and hydrogen peroxide in the peroxisomes of liver and kidney cells by FAD–dependent *D-amino acid oxidase* (*DAO*)
- The α-keto acids can enter the general pathways of amino acid metabolism and be reaminated to L-isomers or catabolized for energy
- *DAO* degrades D-serine, the isomeric form of serine that modulates *N*-methyl-D-aspartate (NMDA)-type glutamate receptors. Increased *DAO* activity has been linked to increased susceptibility to schizophrenia
- *DAO* also converts glycine to glyoxylate
- *L-Amino acid oxidases* are found in snake venom

Ammonia transport to the liver

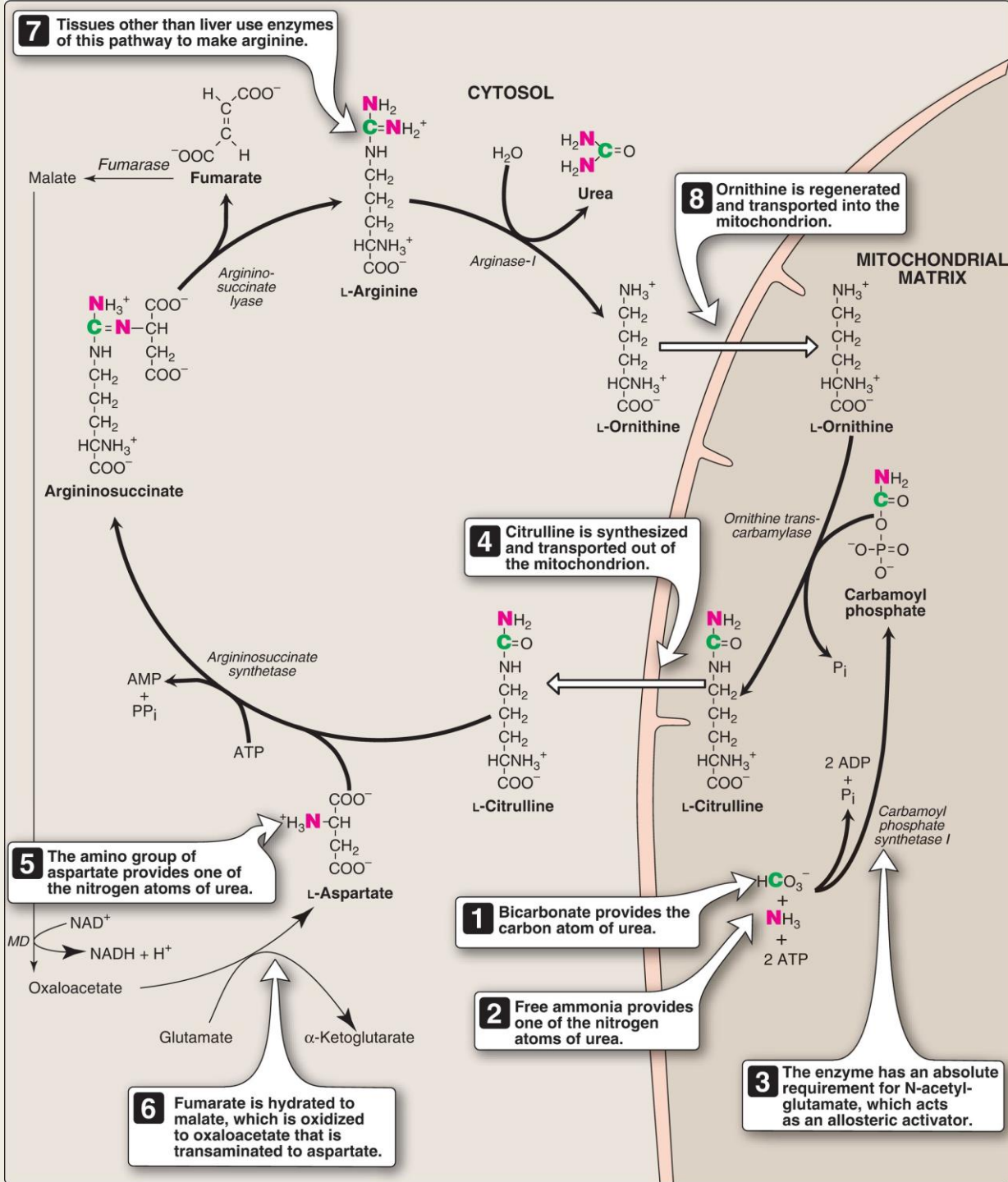
- Two mechanisms:
  - *Glutamine synthetase; glutaminase; GDH, urea*
  - *Glucose–alanine cycle; ALT; GDH; urea*





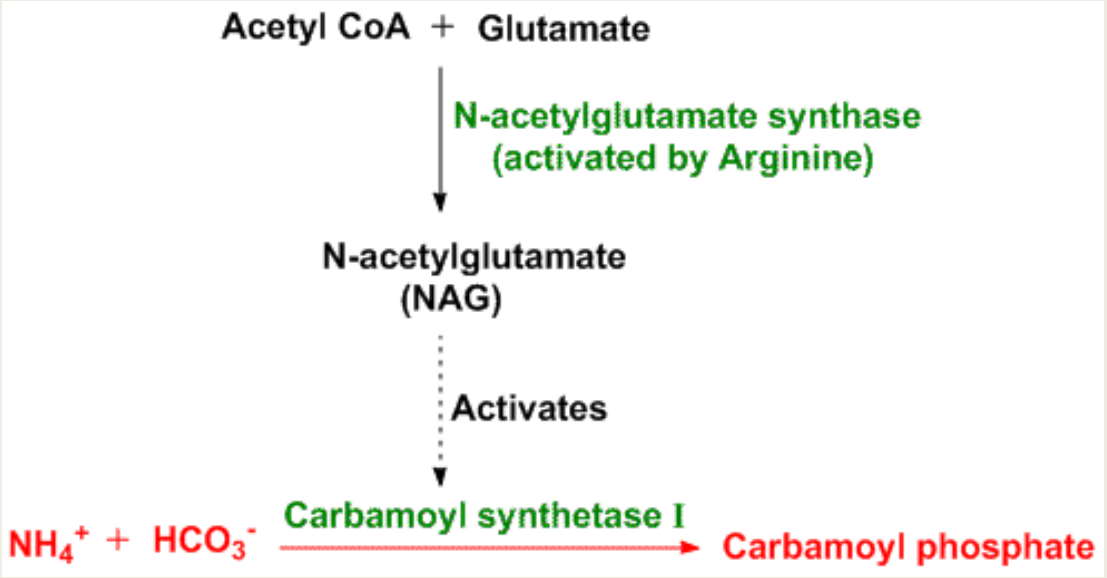
UREA CYCLE

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



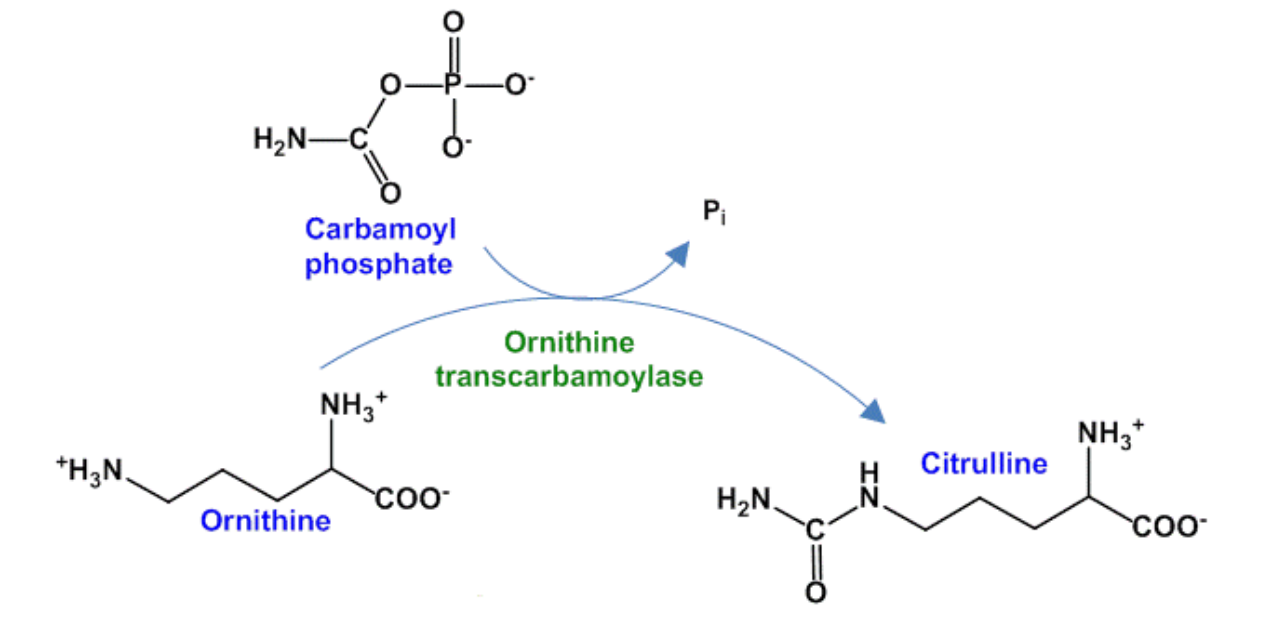
Carbamoyl phosphate formation

- *Carbamoyl phosphate synthetase I (CPS I)*
- *2 A*TP
- Oxidative deamination of glutamate (GDH)
- *CPS I* requires N-acetylglutamate (NAG) as a positive allosteric activator



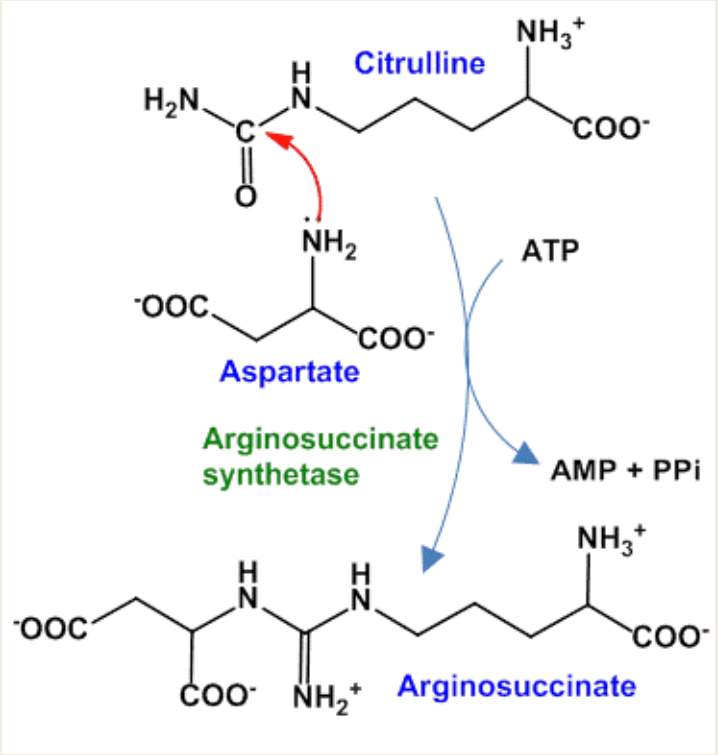
Citrulline formation

- *Ornithine transcarbamylase* (*OTC*)
- Cytosol
- Antiporter: Ornithine and citrulline
- Ornithine is regenerated

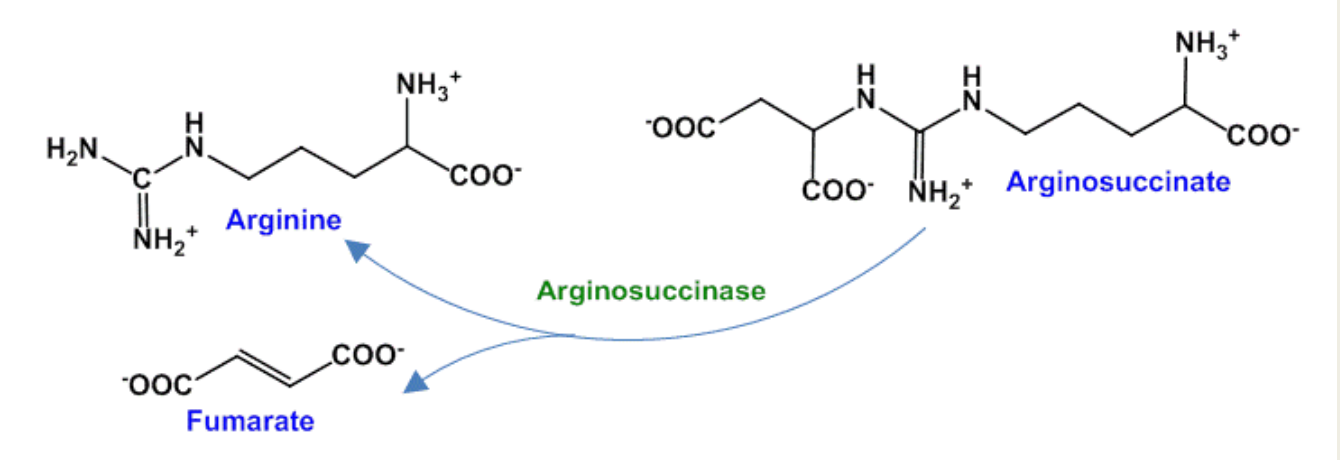


Argininosuccinate formation and cleavage

- *Argininosuccinate synthetase*
- Aspartate provides 2nd N
- ATP: AMP and pyrophosphate

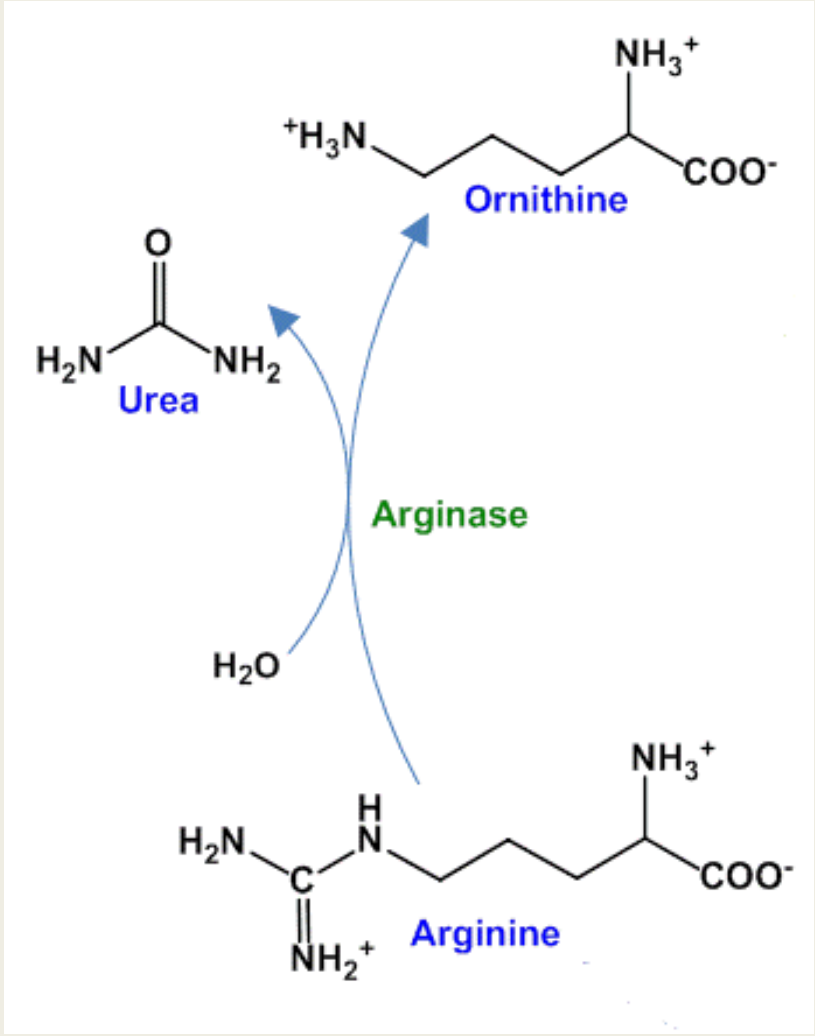


- *Argininosuccinate lyase*
- *Link to TCA (MDH); AST; malate aspartate shuttle; NADH production*



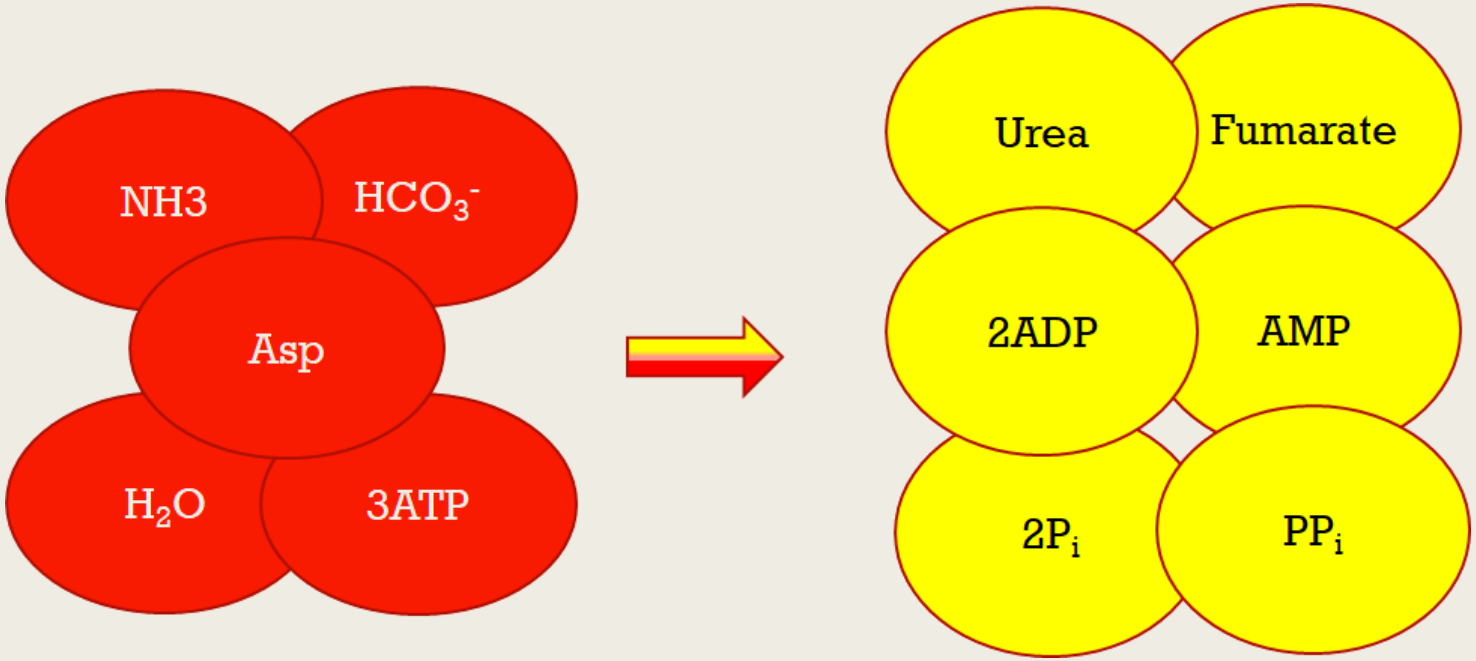
Arginine cleavage and Fate of urea

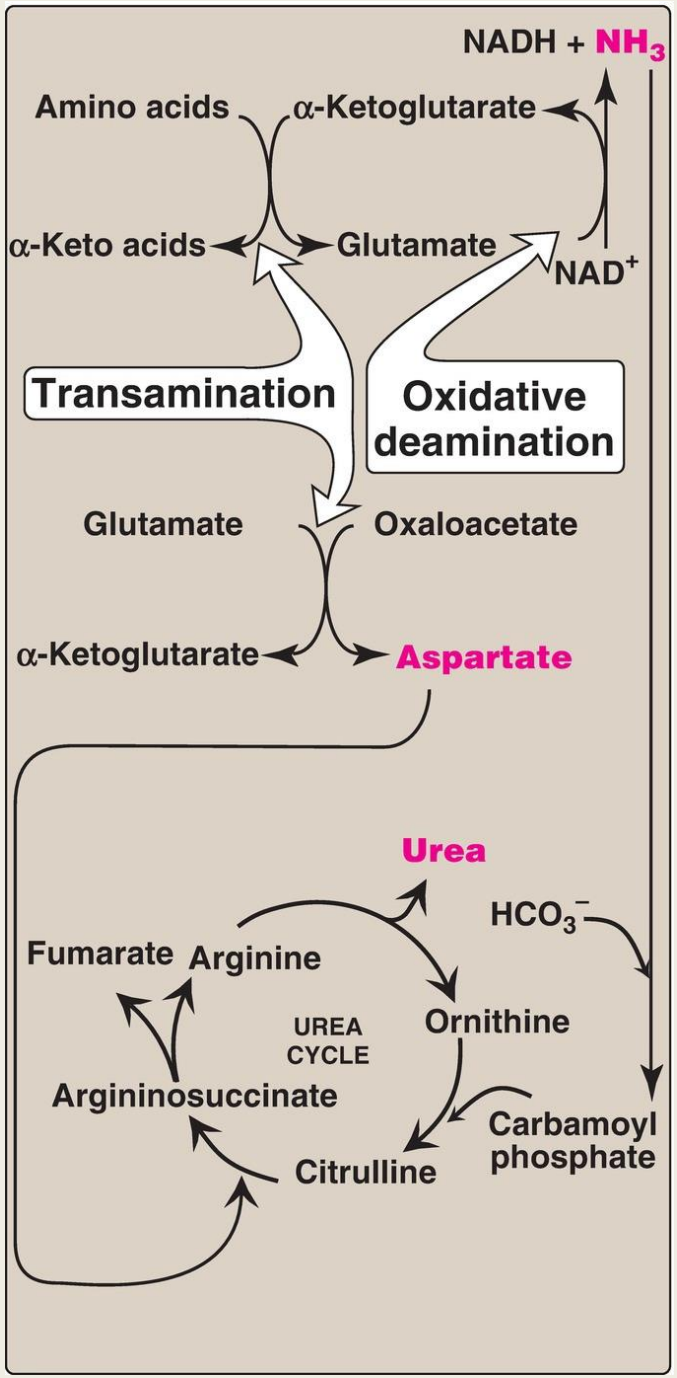
- *Arginase-I:* virtually exclusive to the liver
- Other tissues, such as the kidney, can synthesize arginine from citrulline
- Blood to urine; small intestine (urease); kidney failure (hyperammonemia and antibiotics)



Overall stoichiometry

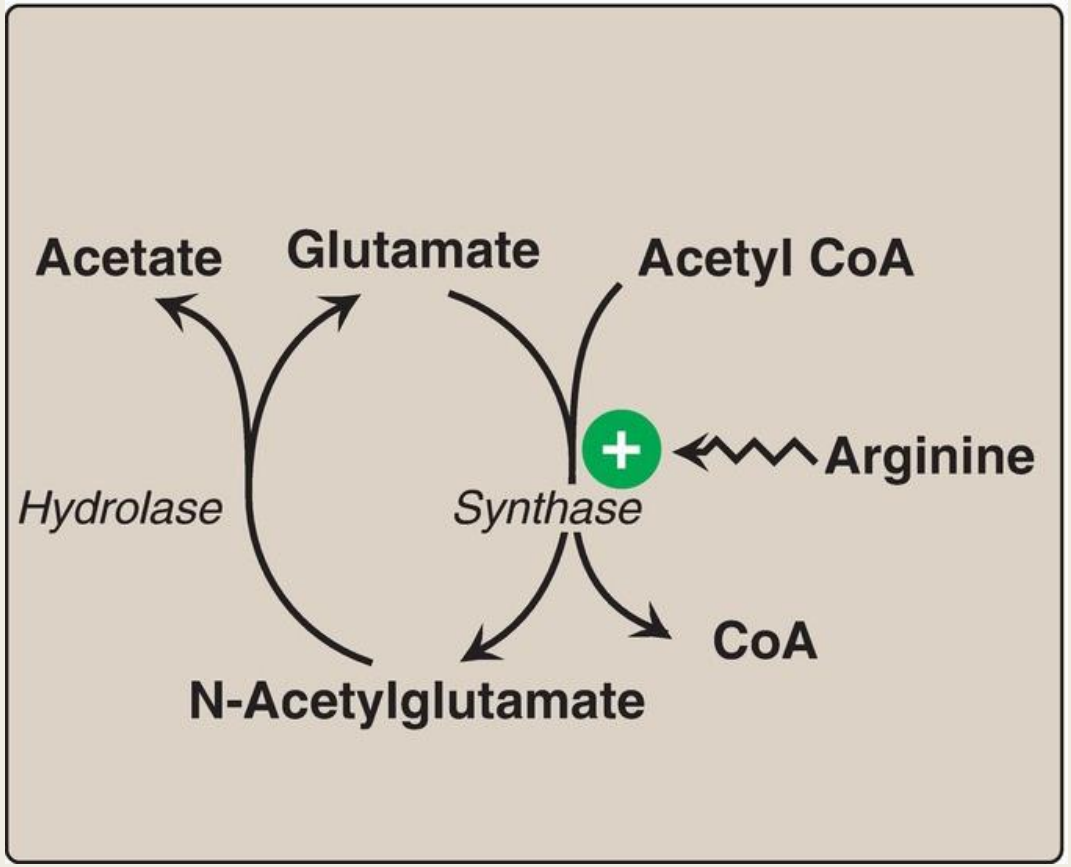
- Synthesis of urea is irreversible, why?
- Free ammonia and aspartate
- Glutamate is the immediate precursor of both ammonia

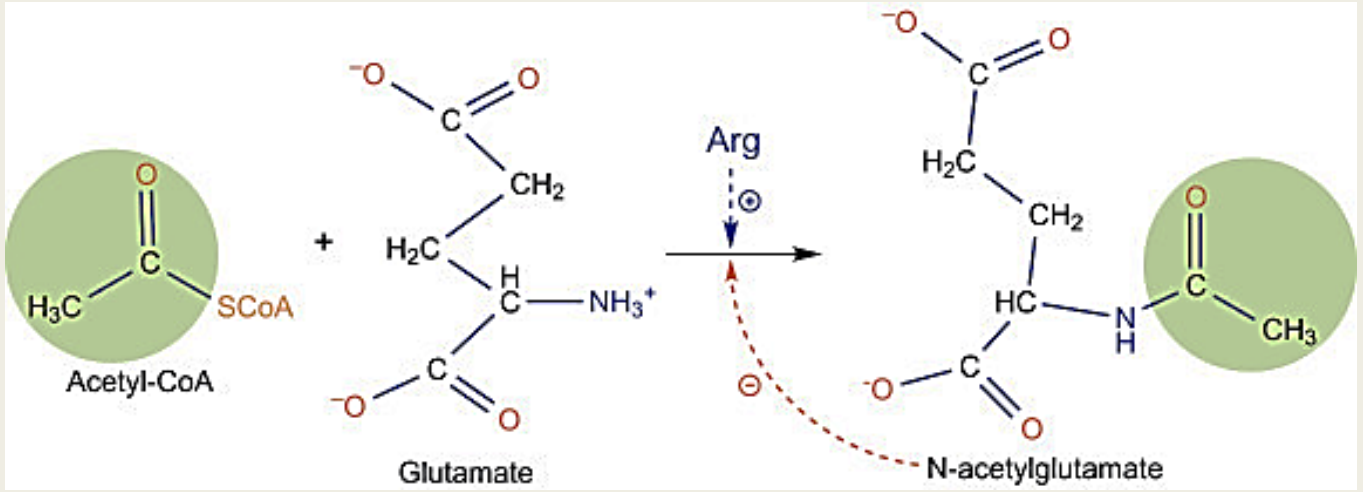




Regulation

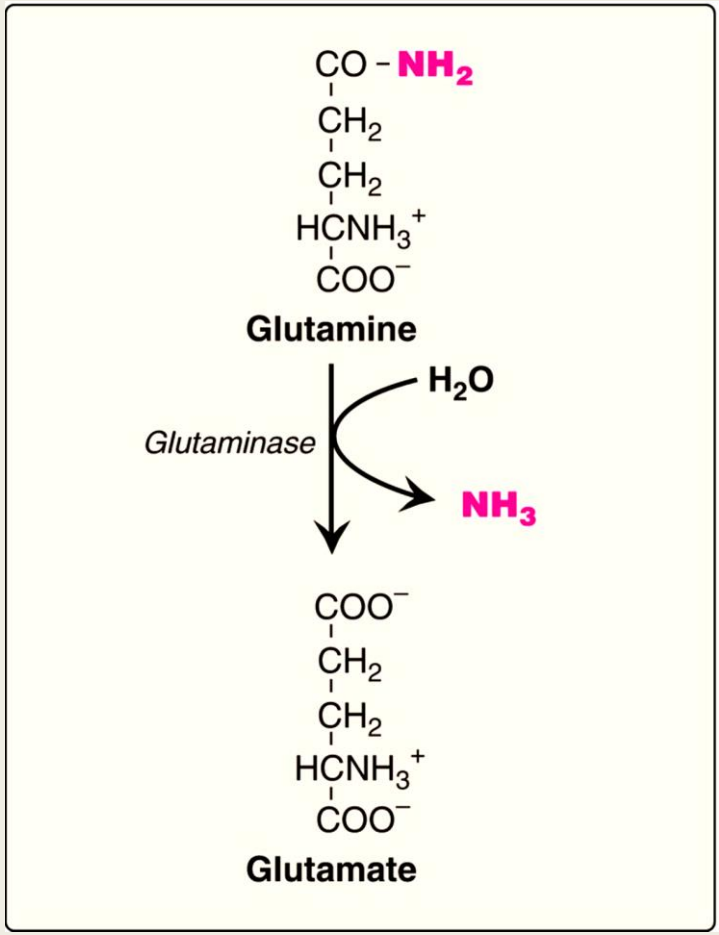
- Rate-limiting step (NAG): It increases the affinity of *CPS I* for ATP
- *N-acetylglutamate synthase* (*NAGS*)
- Arginine is an activator
- Substrate availability (short-term regulation) and enzyme induction (long term)





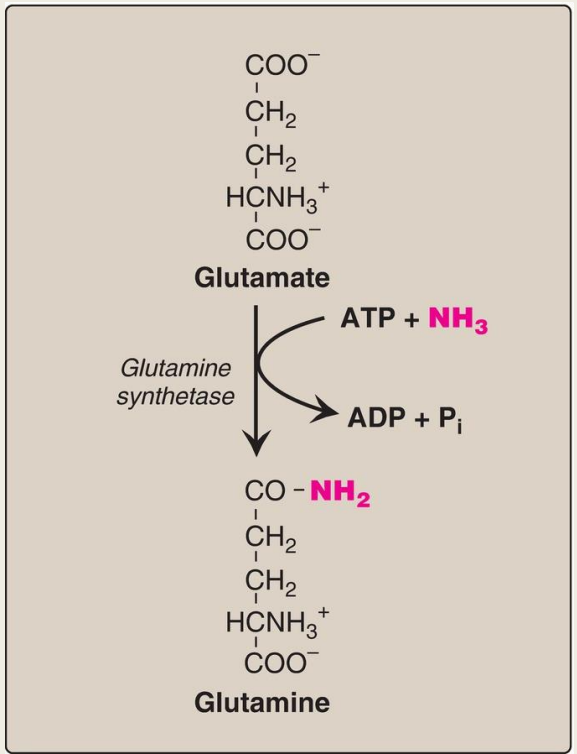
AMMONIA METABOLISM

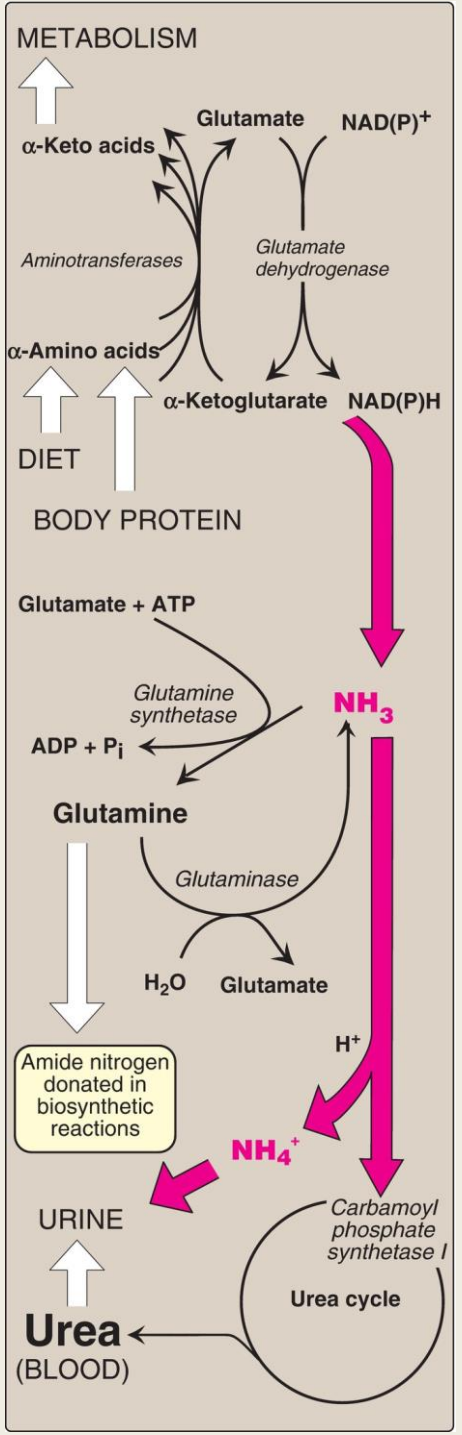
- Produced by all tissues
- Disposed of primarily by formation of urea
- Level must be kept very low (hyperammonemia, CNS)
- Sources:
  - *AAs (liver, STAA)*
  - *Glutamine (BCAA); Acid-base balance (kidneys); urea (liver); intestinal glutaminase (gluconeogenesis)*
  - *Intestinal bacteria: bacterial urease; portal vein*
  - *Amines: diet and hormones or (NTs); MAO*
  - *Purines and pyrimidines*



Transport in the circulation

- Rapid removal of blood ammonia
- Glutamine and alanine (primarily in skeletal muscle, also CNS)
- Urea is safe relatively



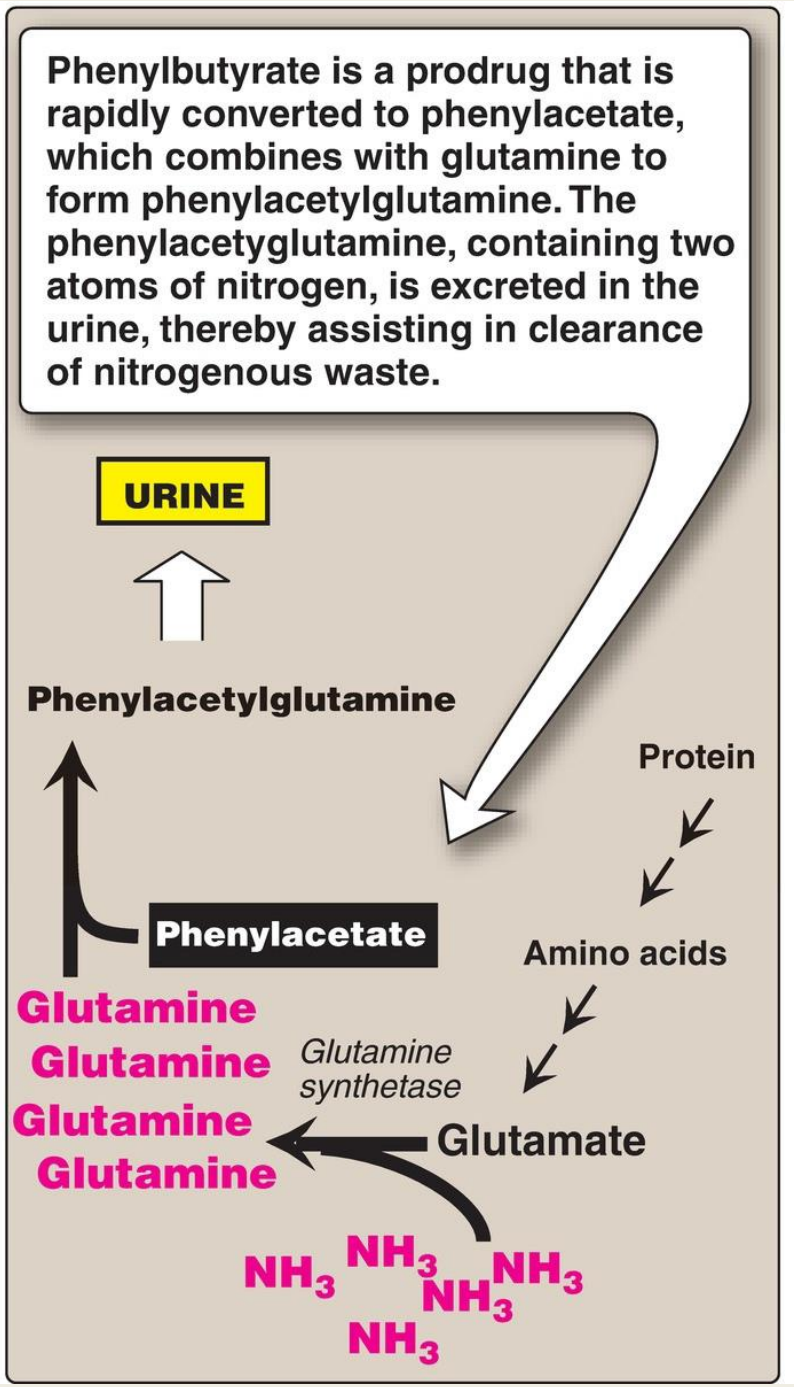


Hyperammonemia

- Levels are normally low (5–35 µmol/l)
- Can be >1,000 µmol/l; medical emergency (tremors, slurring of speech, somnolence (drowsiness), vomiting, cerebral edema, and 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 of the other urea cycle disorders follow an autosomal-recessive inheritance pattern*
  - *Arginase deficiency is less severe*

Management?


Phenylbutyrate is a prodrug that is rapidly converted to phenylacetate, which combines with glutamine to form phenylacetylglutamine. The phenylacetyglutamine, containing two atoms of nitrogen, is excreted in the urine, thereby assisting in clearance of nitrogenous waste.
URINE
Phenylacetylglutamine
Protein
Phenylacetate
Amino acids
Glutamine
Glutamine
Glutamine
Glutamine
Glutamine synthetase
Glutamate
NH3
NH3
NH3
NH3
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