Amino acid metabolism: Disposal of Nitrogen

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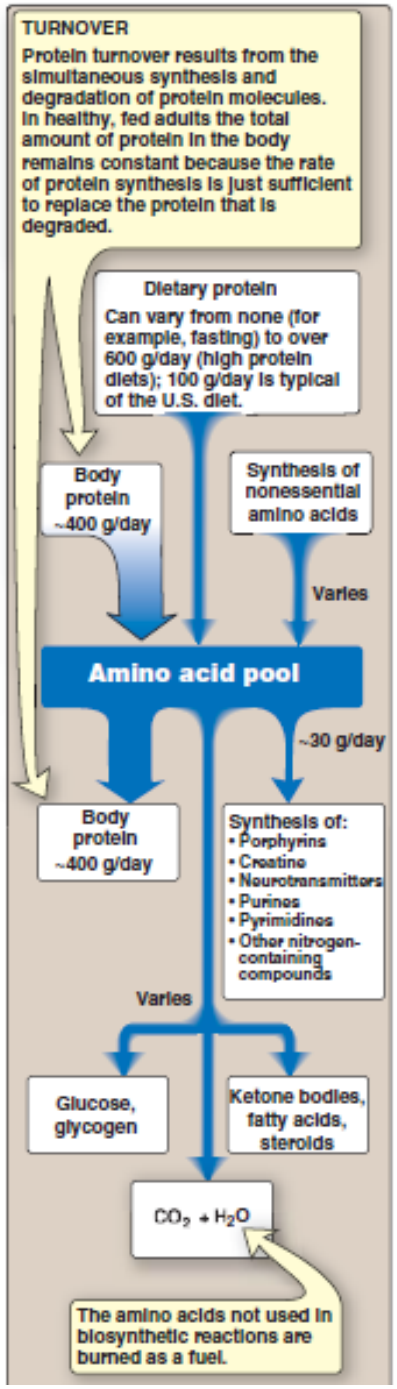
All images were taken from Lippincott's Biochemistry textbook except where noted

Amino acids (AAs)

- AAs are NOT stored in the body
- AAs sources are: diet, de novo synthesis or protein degradation
- AA metabolism overview:

1. α-amino group removal by transamination then oxidative deamination (N leaves the body as urea, ammonia or other compounds)
2. The resulting α-keto acids are converted to energy producing intemediates
3. Intermediate metabolism to CO_2, water, glucose, fatty acids, or ketone bodies

The metabolic processes have to keep harmony between amino acid pool and protein turn over



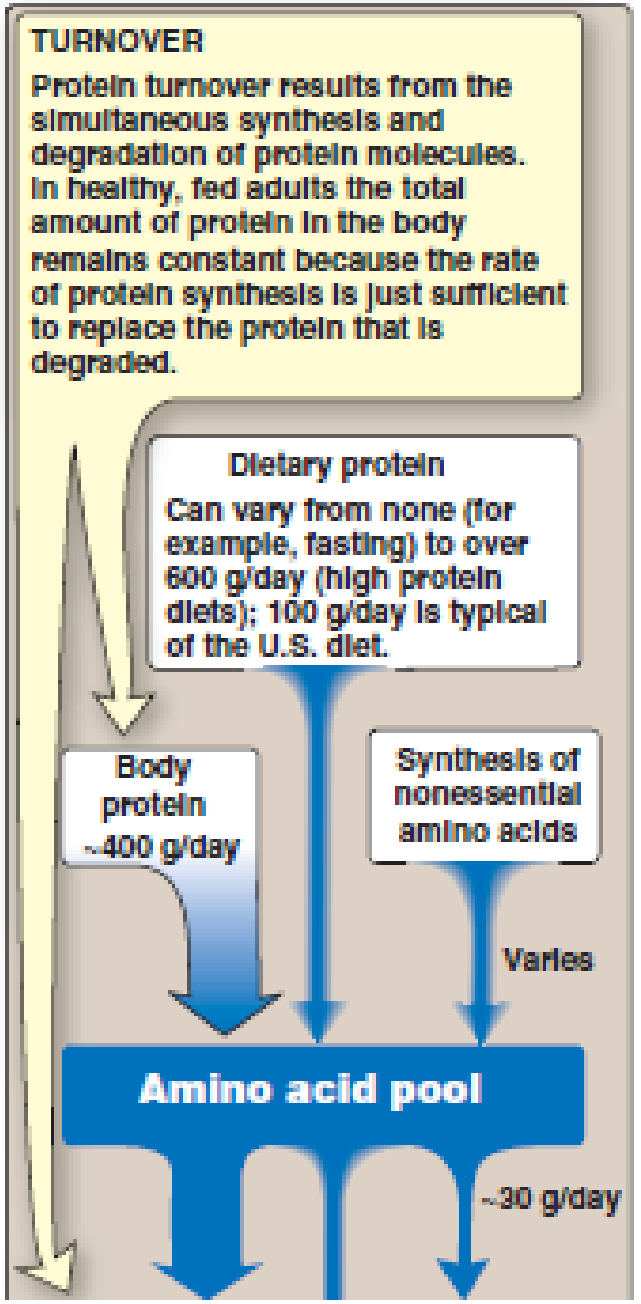
Sources and fates of amino acids

- The AA pool is small ~about 90–100 g of AAs
- The amount of protein in the body is about 12 kg in a 70-kg man.
- Normally, the amount of AAs in the AA pool is balanced by the output (constant amount)
- The amino acid pool is in a steady state, and the individual is in nitrogen balance.

Amino Acid Pool

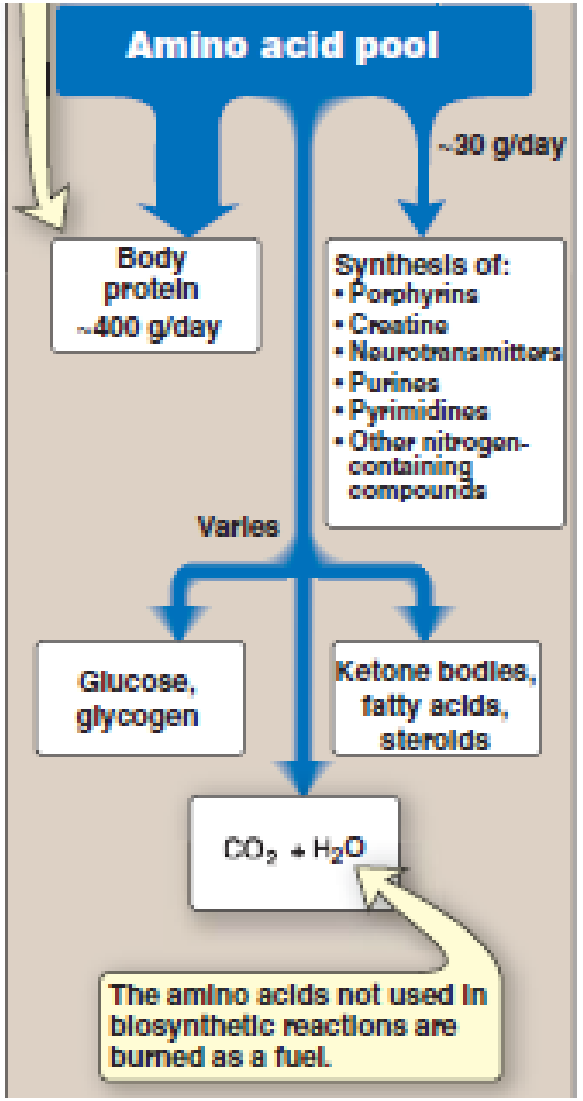
- AA sources:

1. Endogenous (body) protein degradation
2. Exogenous (dietary) protein digestion
3. Nonessential amino acids synthesized from metabolic intermediates



Amino Acid Pool Depletion Routes

- AAs are depleted by 3 routes:
- 1) Synthesis of body protein
- 2) AAs consumed as precursors of nitrogen-containing small molecules
- 3) Conversion of AAs to glucose, glycogen, fatty acids, ketone bodies, or $CO_2 + H_2O$



Protein Turnover

- Protein turnover is the process in which the rate of protein synthesis is sufficient to replace the degraded protein.
- Each day, 300–400 g of body protein is hydrolyzed and resynthesized
- In healthy adults, the total amount of protein in the body remains constant.

Rate of turnover

- Turnover varies widely for individual proteins.
- Short-lived proteins such as many regulatory proteins and misfolded proteins, are rapidly degraded, t1/2 is min-hours
- Most proteins are long-lived proteins (t1/2 days to weeks)
- Structural proteins, such as collagen, are metabolically stable (t1/2 months or years).

Rate of turnover

- For many proteins, regulation of synthesis determines the [protein in the cell] and protein degradation is minor

- For other proteins, the rate of synthesis is constitutive, or relatively constant, and [protein in the cells] is controlled by selective degradation.

Protein degradation

Two major enzyme systems are responsible for degrading damaged or unneeded proteins:

1. The ATP-dependent ubiquitin-proteasome system of the cytosol mainly endogenous proteins (proteins that were synthesized within the cell)

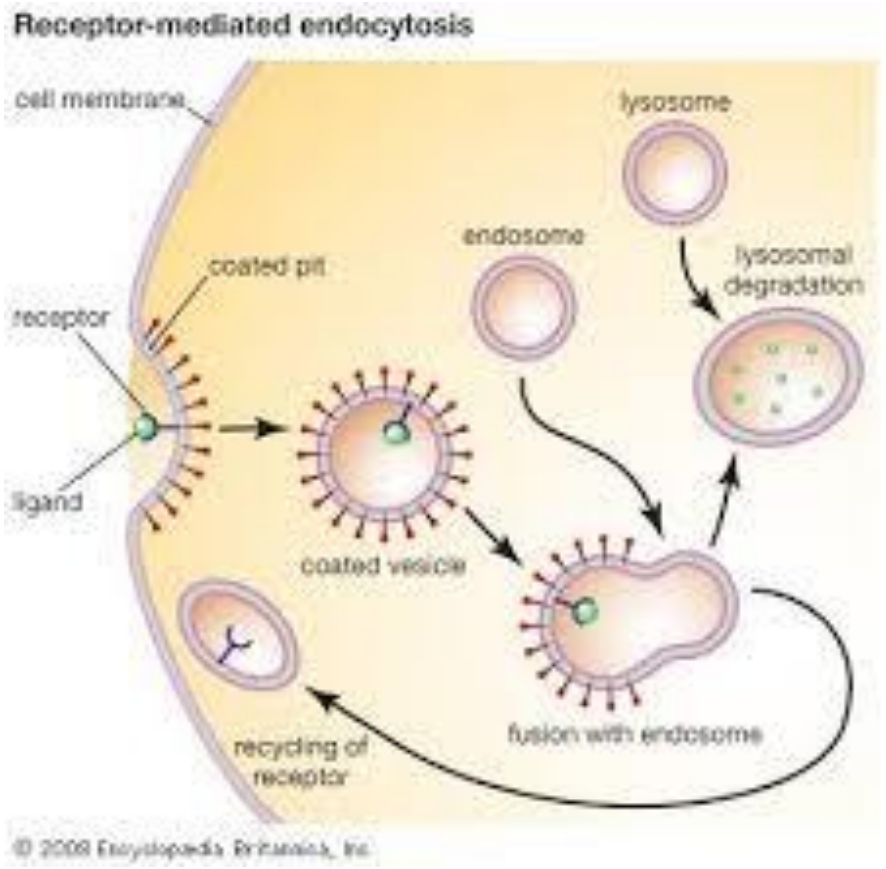
Protein degradation

2. The ATP-independent degradative enzyme system of the lysosome

Lysosomal enzymes (acid hydrolases) degrade primarily:

A. Extracellular proteins, such as plasma proteins, by endocytosis

A. Cell-surface membrane proteins by receptor-mediated endocytosis.



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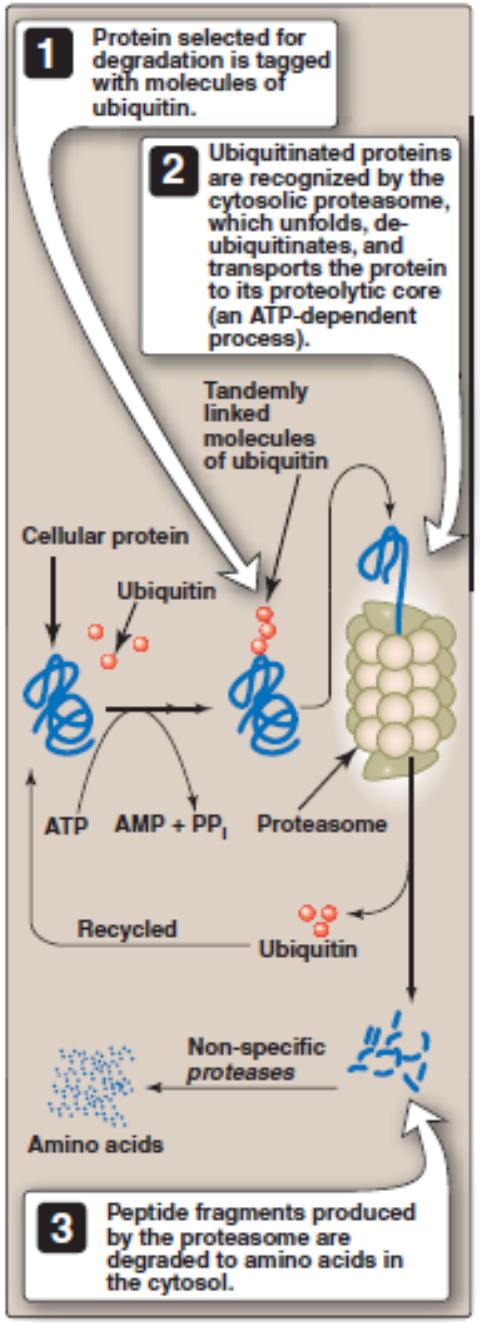
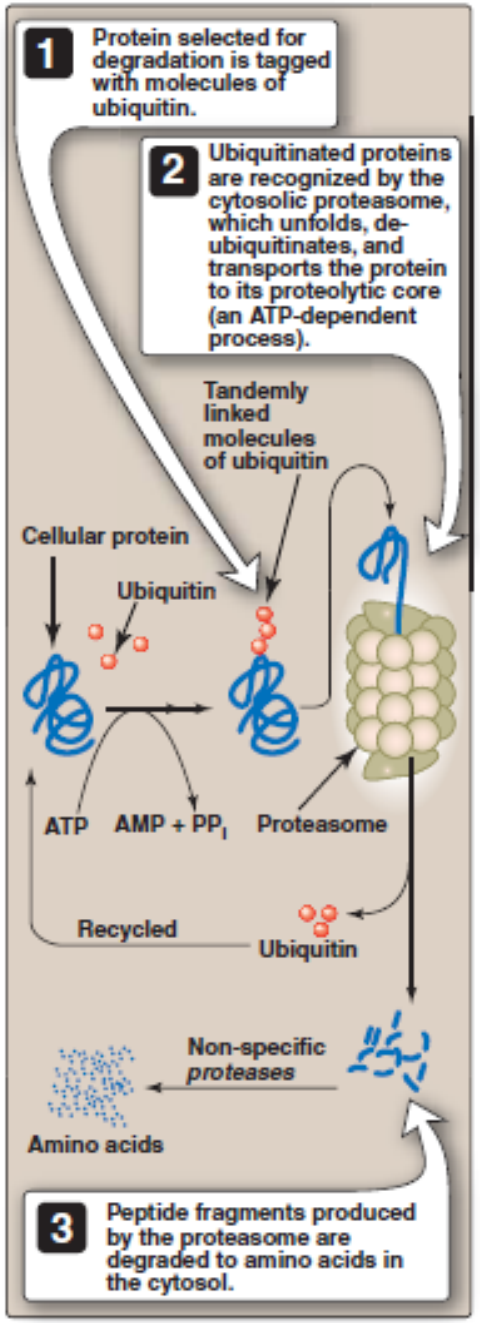


Ubiquitin-proteasome proteolytic pathway

Ubiquitin (Ub) is a small, globular, non-enzymic protein.

Ubiquitination of the target substrate is linking the α-carboxyl group of the C-terminal **Gly** of ubiquitin to the ε-amino group of a **Lys** on the protein substrate by a three-step, enzyme-catalyzed, ATP-dependent process.

Several Ub units are added to generate a **polyubiquitin chain**.



Ubiquitin-proteasome proteolytic pathway

A proteasome is a large, barrel-shaped, macromolecular, proteolytic complex that recognizes Ub-protein

The proteasome unfolds, deubiquitinates and cuts the target protein into fragments that are then further degraded to amino acids.

Protein degradation by the ubiquitin-proteasome complex requires energy (ATP)

Simple hydrolysis by proteolytic enzymes does not require energy

Chemical signals for protein degradation

How are proteins tagged for degradation?

By chemical alterations such as oxidation or ubiquitin tagging

Proteins have different half-lives

What affects the half life of a protein?

The nature of the N-terminal residue.

A. Proteins with Ser at the N-terminus are long-lived (t1/2 is > 20 hrs)
B. Proteins with Asp at the N-terminus have a t1/2 of only 3 minutes
C. Proteins rich in sequences containing Pro, Glu, Ser, and Thr (PEST sequences) are rapidly degraded (short t1/2).

DIGESTION OF DIETARY PROTEINS

70–100 g/day in the American diet

Proteins are too large to be absorbed by the intestine.

Protein digestion begins in the stomach

Stomach secretes the gastric juice that contains hydrochloric acid and the proenzyme, pepsinogen.

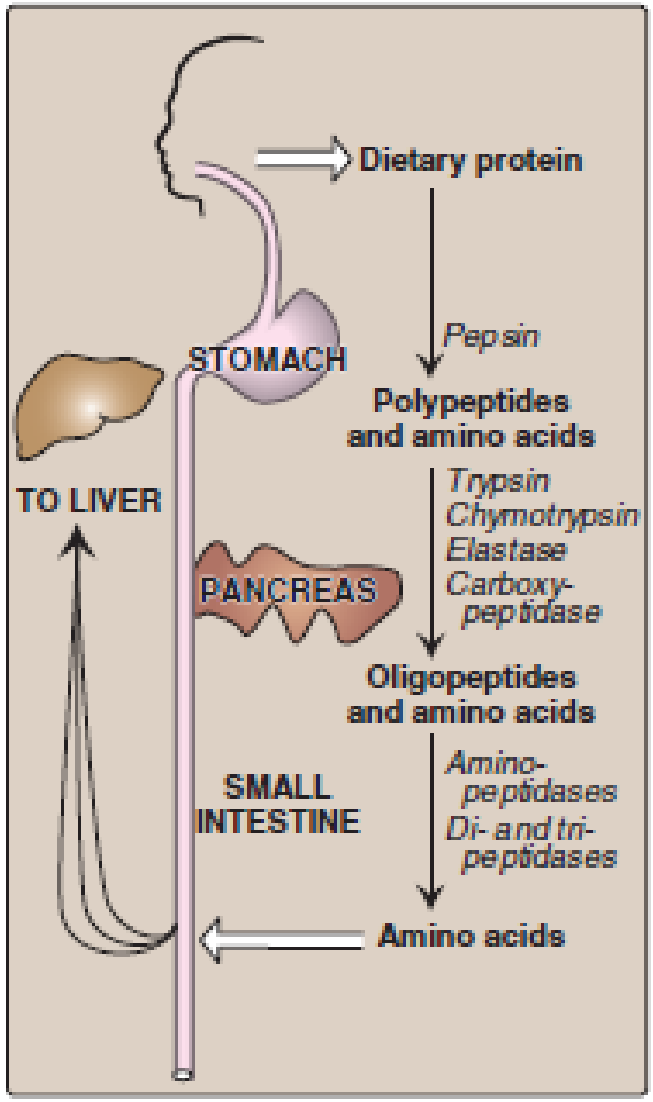


Figure 19.4
Digestion of dietary proteins by the proteolytic enzymes of the gastro-intestinal tract.

Digestion of proteins by gastric secretion

1. **Hydrochloric acid:** pH 2–3 to hydrolyze proteins.

HCl is secreted by the parietal cells

HCl functions:

A. kills some bacteria
B. denatures proteins to make them more susceptible to subsequent hydrolysis by proteases.

2. Pepsin: acid-stable endopeptidase

- Is secreted by the chief cells of the stomach as an inactive zymogen (or proenzyme), pepsinogen.

-Pepsinogen is activated to pepsin, either by HCl, or autocatalytically by other activated pepsin molecules.

-Pepsin releases peptides and a few free amino acids from dietary proteins.

Digestion of proteins by pancreatic enzymes in small intestine

Release of zymogens: The release and activation of the pancreatic zymogens is mediated by the secretion of cholecystokinin and secretin (two polypeptide hormones of the GIT)

Zymogen activation : Enteropeptidase (enterokinase)— the luminal surface of intestinal mucosal cells converts the pancreatic zymogen trypsinogen to trypsin (removal of a hexapeptide from the N-terminus of trypsinogen)

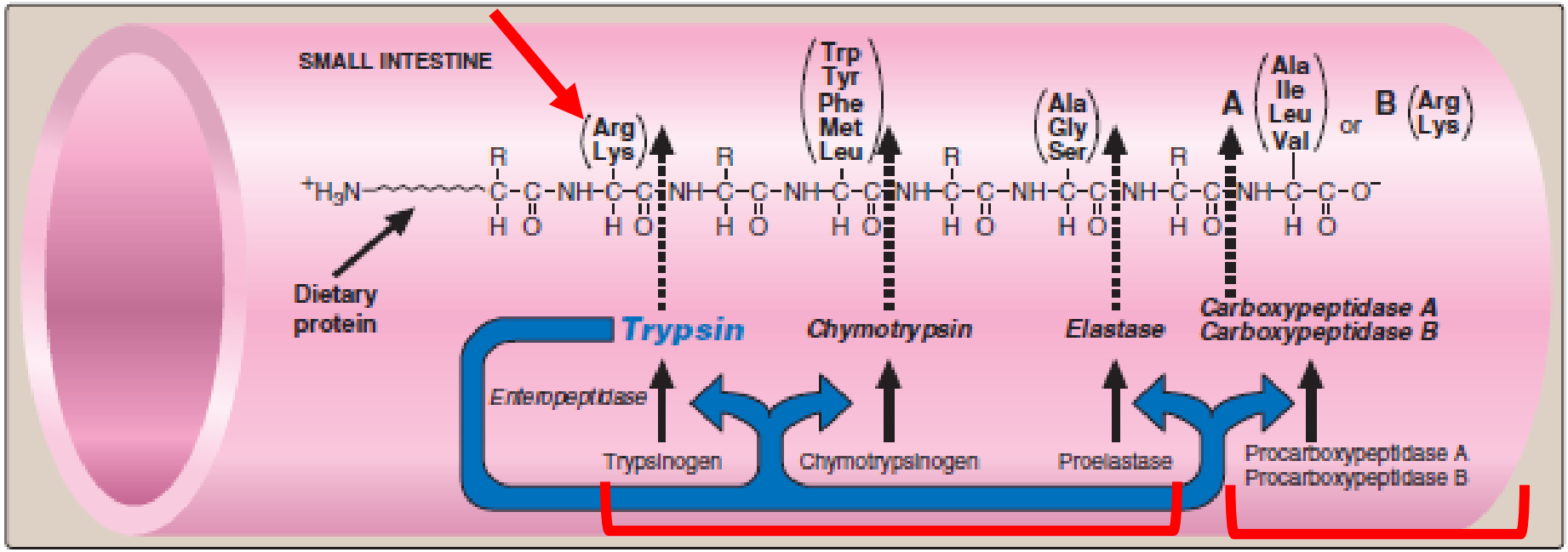
Trypsin subsequently converts other trypsinogen molecules to trypsin

Trypsin is the common activator of all pancreatic zymogens

Digestion of proteins by pancreatic enzymes in small intestine

Large polypeptides produced in the stomach are further cleaved to oligopeptides and amino acids by a group of pancreatic proteases.

Enzyme specificity: Each of these enzymes has a different specificity for the amino acid R-groups adjacent to the susceptible peptide bond



Serine endopeptidases Exopeptidases

Digestion of oligopeptides by enzymes of the small intestine

Aminopeptidase at the luminal surface of the intestine

Aminopeptidase is an exopeptidase that repeatedly cleaves the N-terminal residue from oligopeptides to produce smaller peptides and free AAs.

Absorption of amino acids and small peptides

Free AAs are absorbed into the enterocytes by a Na+-linked secondary transport system at the apical membrane.

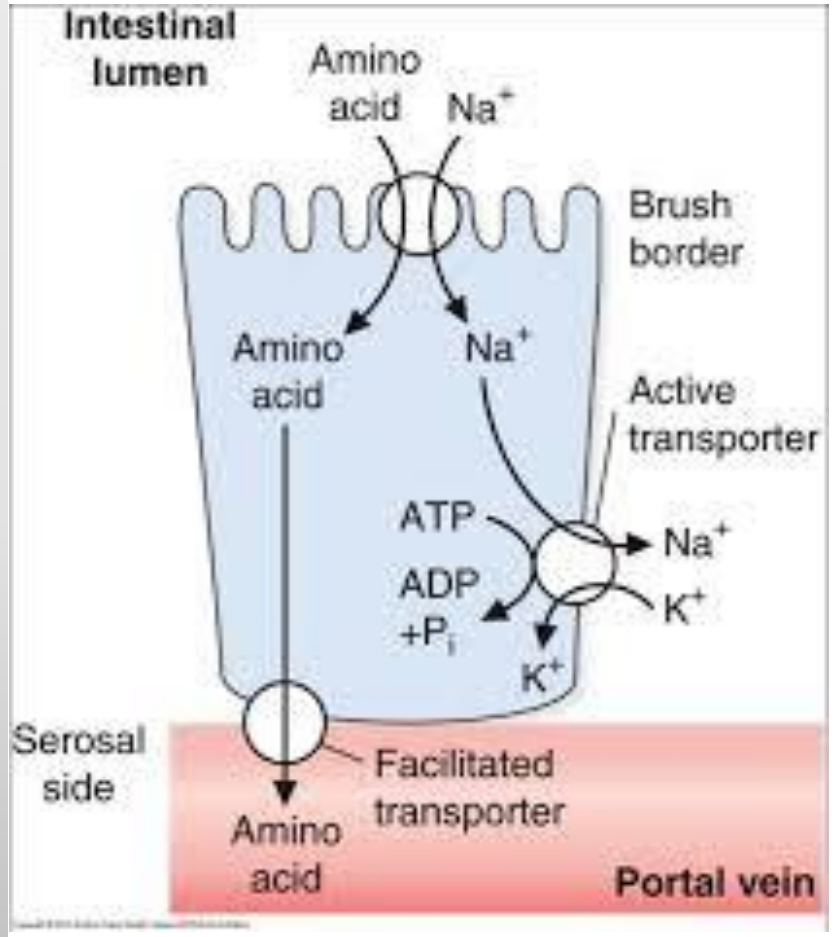
Di- and tri –peptides are absorbed by a H+-linked transport system.

The peptides are hydrolyzed in the cytosol to AAs

AAs are released into the portal system by facilitated diffusion.

AAs are either metabolized by the liver or released into the general circulation.

Branched-chain amino acids are not metabolized by the liver, but are sent from the liver to muscle via the blood



Clinical Hint: Abnormalities in protein digestion and Celiac disease

Pancreatic secretion deficiency due to chronic pancreatitis, cystic fibrosis, or surgical removal of the pancreas, results in incomplete fat and protein digestion.

Symptoms: abnormal appearance of lipids (**steatorrhea**), and **undigested protein** in the feces.

Celiac disease (celiac sprue) is a disease of **malabsorption** resulting from immune-mediated damage to the small intestine in response to ingestion of **gluten** (or gliadin produced from gluten), a protein found in wheat, barley and rye.

TRANSPORT OF AMINO ACIDS INTO CELLS

[Free AAs in the extracellular fluids] <<< [Free AAs within the cells]

Different transport systems have overlapping specificities for different AAs

AA transport systems:

The small intestine and the proximal tubule of the kidney

One system uptakes cystine and the dibasic amino acids, ornithine, arginine, and lysine (represented as “COAL”).

Defects in the transport of tryptophan (and other neutral amino acids) results in Hartnup disorder and pellagra-like dermatologic and neurologic symptoms.

Clinical Hint: Cystinuria

Inherited disorder

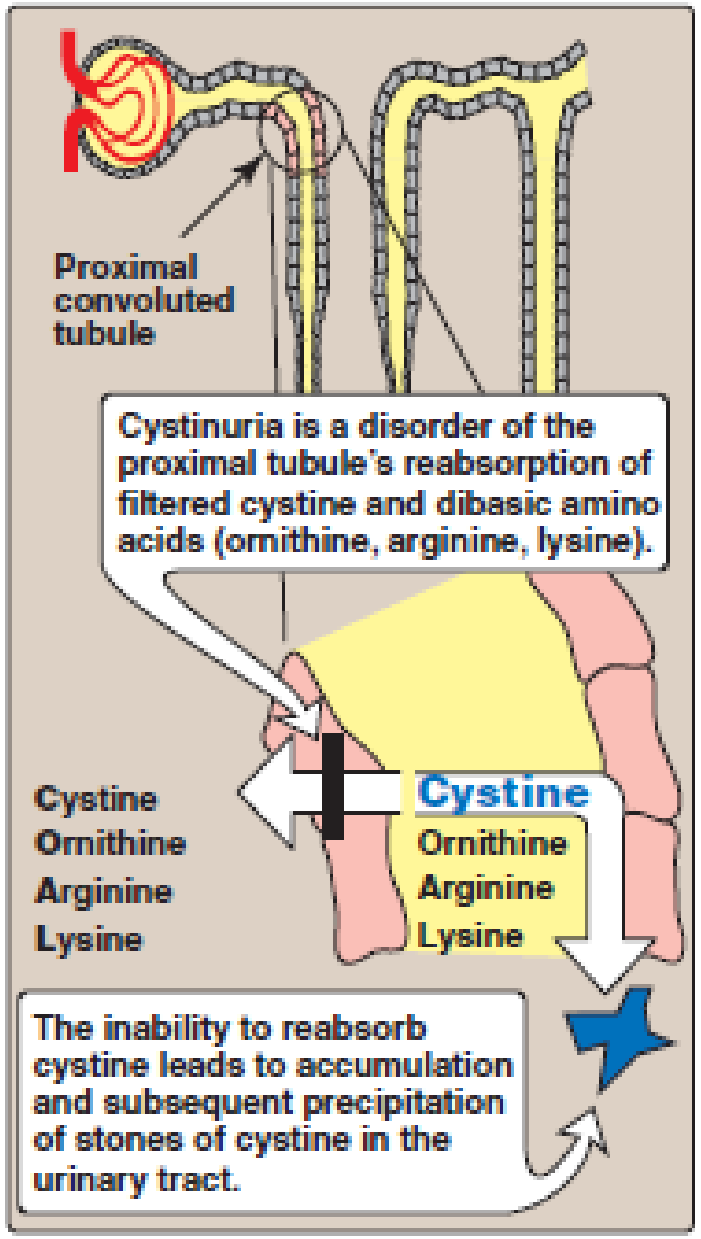
The most common genetic error of amino acid transport.

1 in 7,000 individuals

The COAL carrier system is defective, and all four amino acids appear in the urine.

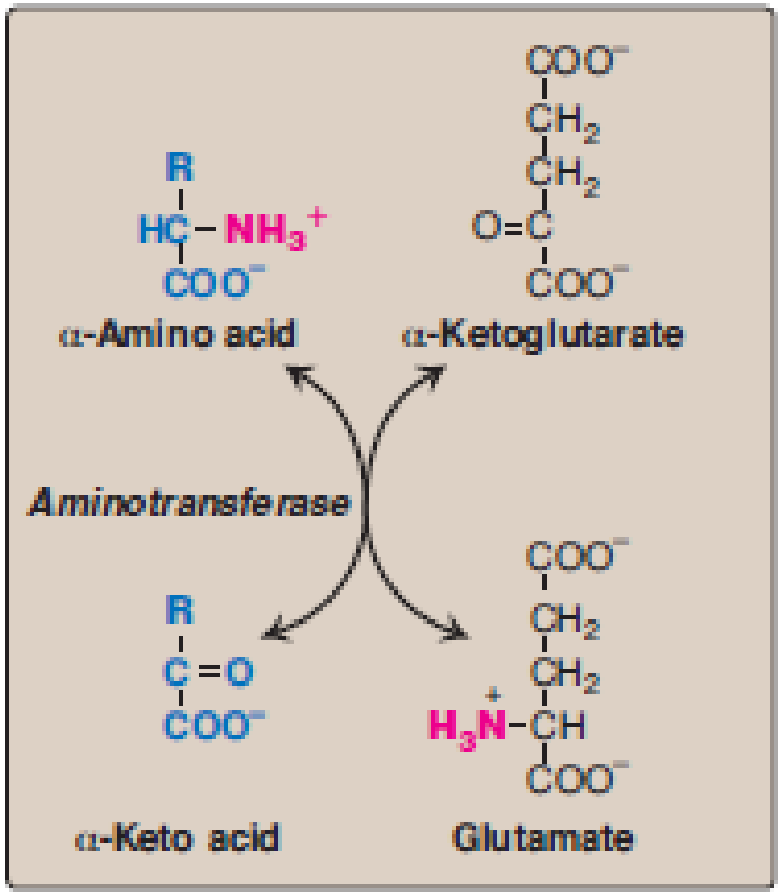
Clinical signs and symptoms:
- Precipitation of cystine to form kidney stones (calculi)

Oral hydration is important for the treatment of this disorder.



REMOVAL OF NITROGEN FROM AMINO ACIDS

Transamination: the funneling of amino groups to glutamate



Glutamate produced by transamination can be

1. oxidatively deaminated
2. or used as an amino group donor in the synthesis of nonessential AAs.

Transamination

Substrate specificity of aminotransferases:

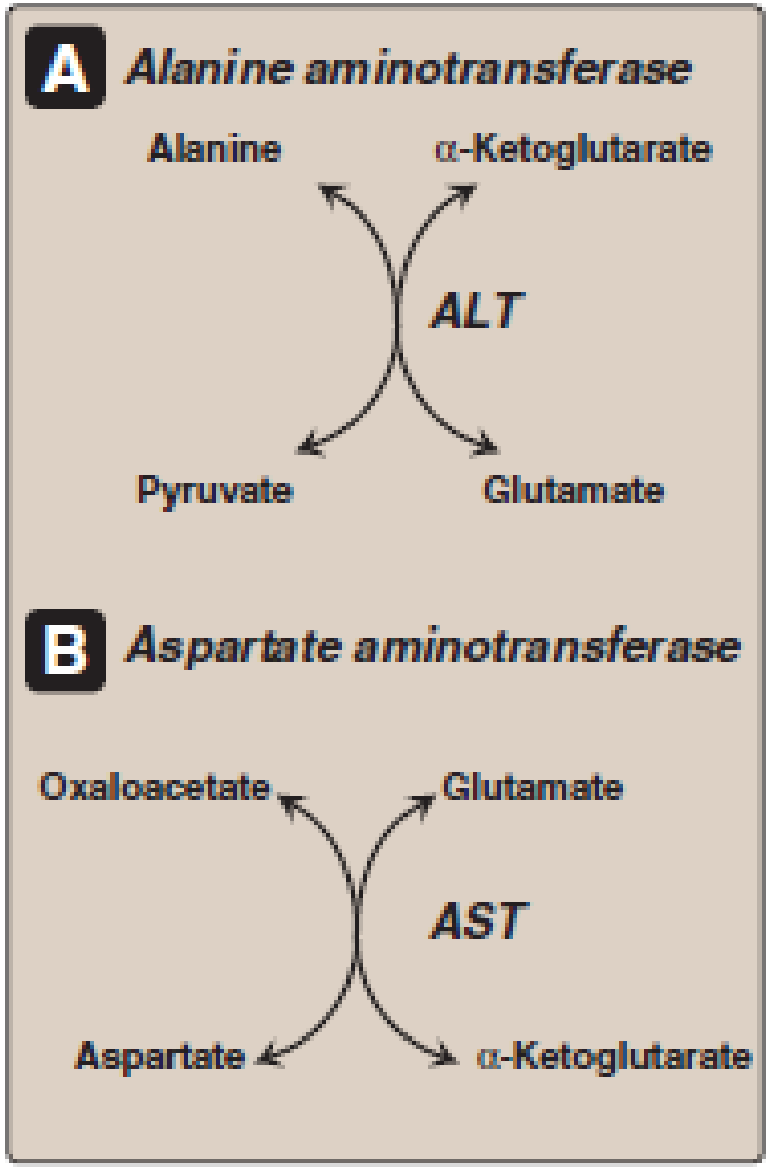
Each aminotransferase (AT) is specific for one or a few amino group donors.

The most important ATs are:

Alanine Aminotransferase (ALT)
Aspartate Aminotransferase (AST)

The equilibrium constant of transamination reactions is near one.

Keq=1 means the reaction functions in both amino acid degradation and biosynthesis according to the cellular needs



Alanine aminotransferase (ALT)

ALT is present in many tissues.

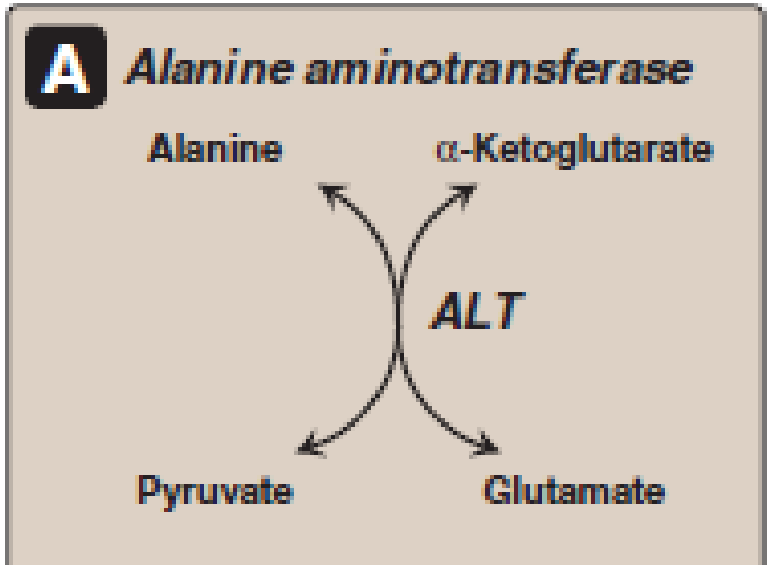
The enzyme catalyzes the transfer of the amino group of alanine to α-ketoglutarate

Reaction products: pyruvate and glutamate

The reaction is reversible.

During amino acid catabolism, ALT functions in the direction of glutamate synthesis.

Glu acts as a “collector” of nitrogen from Ala.



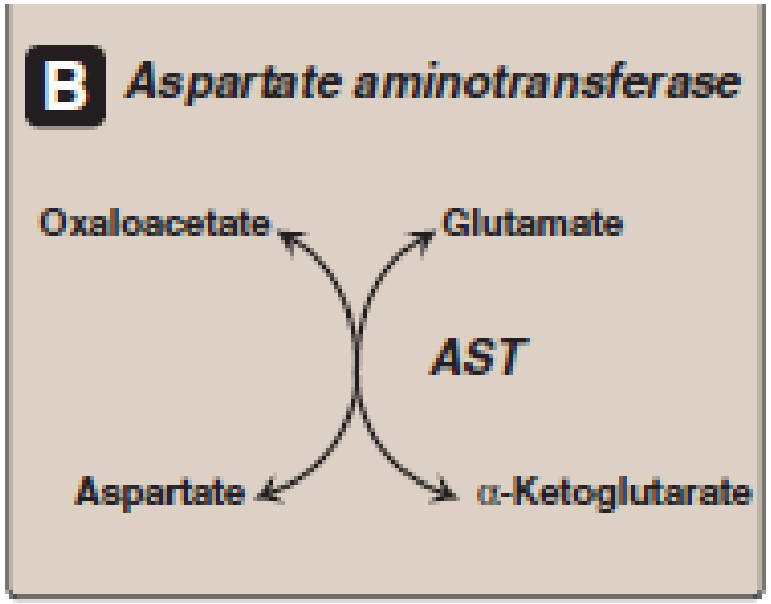
Aspartate aminotransferase (AST)

AST does not funnel amino groups to form Glu

During amino acid catabolism, AST transfers amino groups from glutamate to oxaloacetate, forming aspartate.

Aspartate is used as a source of nitrogen in the urea cycle

The AST reaction is reversible



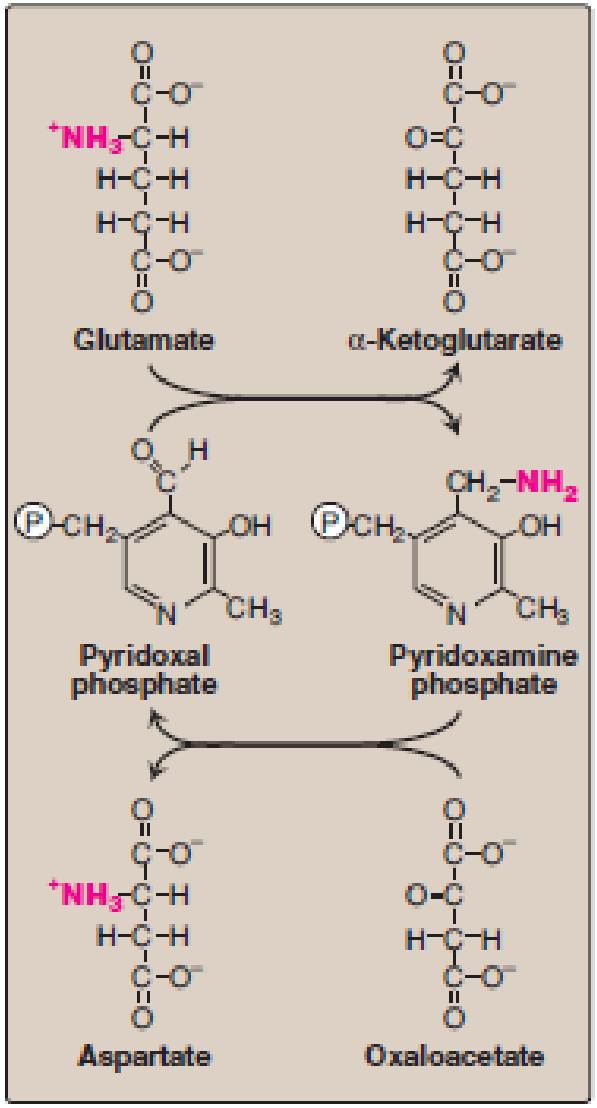
Mechanism of action of aminotransferases (ATs)

All ATs require pyridoxal phosphate (PP) as a coenzyme (vitamin B6 derivative)

PP is covalently linked to the -amino group of a specific lysine residue at the active site of the enzyme.

AT transfers the amino group of an AA to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate.

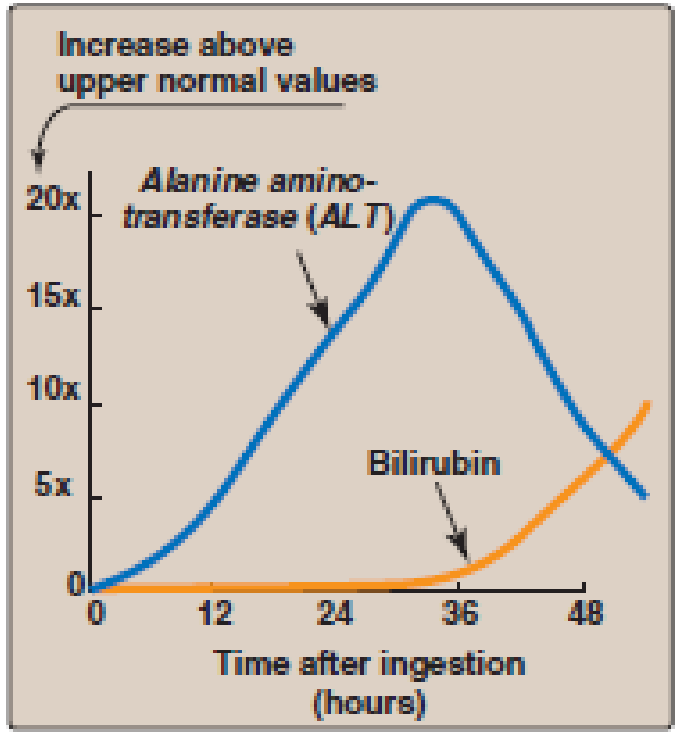
The pyridoxamine phosphate then reacts with an α-keto acid to form an amino acid and regenerate the original coenzyme.



Clinical hint: Diagnostic value of plasma aminotransferases

ATs are normally intracellular enzymes but low levels in the plasma represent the release of cellular contents during normal cell turnover.

AST and ALT have a diagnostic value when found in the plasma.



a. Liver disease: Plasma AST and ALT are elevated
Examples: severe viral hepatitis, toxic injury, and prolonged circulatory collapse.
ALT is more specific than AST for liver disease
AST is more sensitive because the liver contains larger amounts of AST.

b. Nonhepatic disease: MI and muscle disorders.

Oxidative deamination of amino acids

Oxidative deamination by glutamate dehydrogenase results in the liberation of the amino group as free ammonia (NH3)

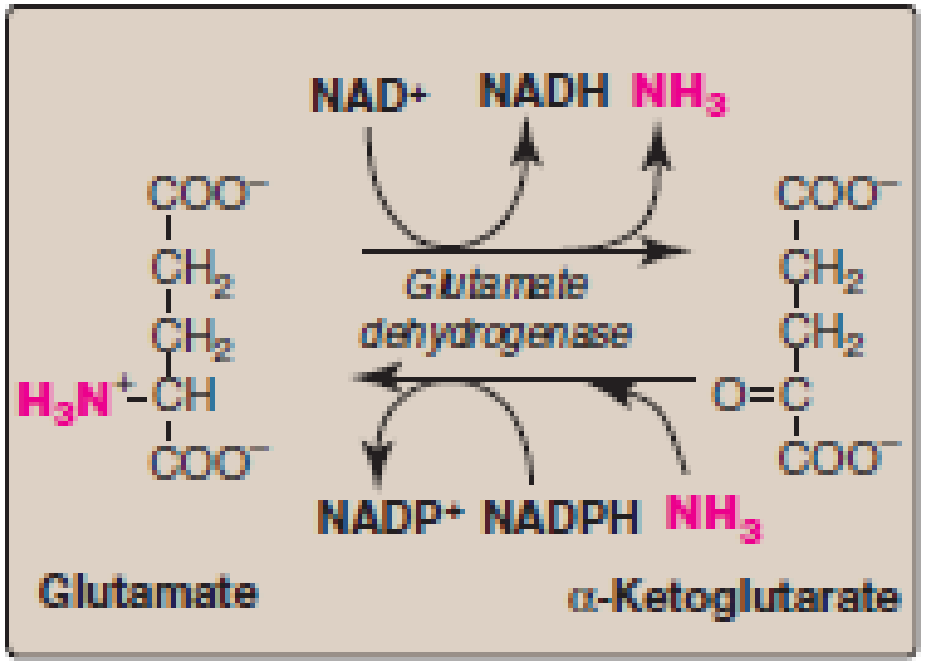
Glutamate is the only amino acid that undergoes rapid oxidative deamination

Reactions occur primarily in the liver and kidney.

Reaction products:

1. α-keto acids that can enter the central pathway of energy metabolism

2. ammonia, the source of nitrogen in urea synthesis.



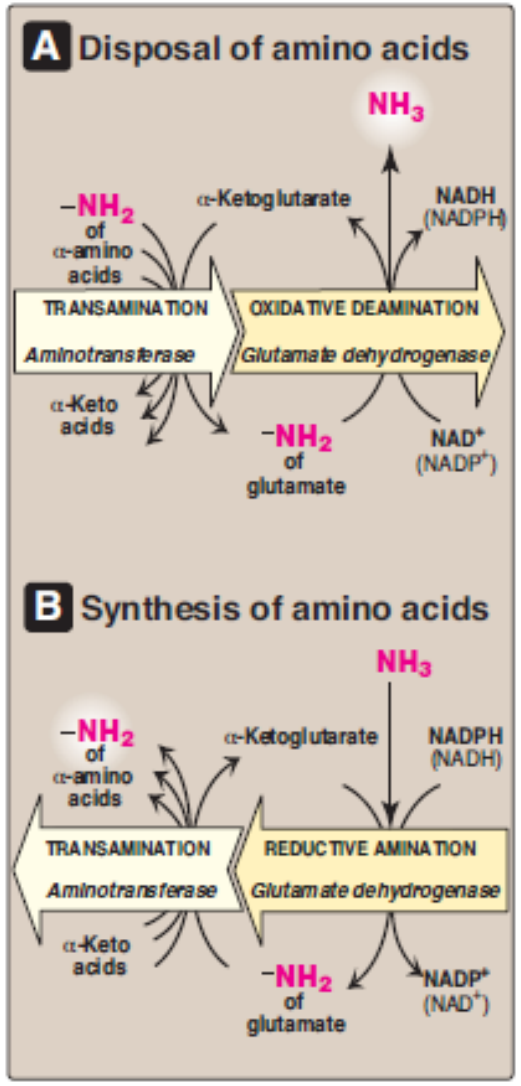
Oxidative deamination and reductive amination of amino acids

Direction of reactions depends on the relative concentrations of glutamate, α-ketoglutarate, and ammonia, and the ratio of oxidized to reduced co-enzymes.

After a meal that contains protein, glutamate levels in the liver are high, thus, more AA degradation and NH_3 formation

Allosteric regulators of glutamate dehydrogenase:

GTP is an allosteric inhibitor
ADP is an activator.



Main coenzyme

Oxidation of the carbon skeleton

Main coenzyme

Reduction of the carbon skeleton

D-Amino acid oxidase

D-Amino acids are found in plants and in the cell walls of microorganisms

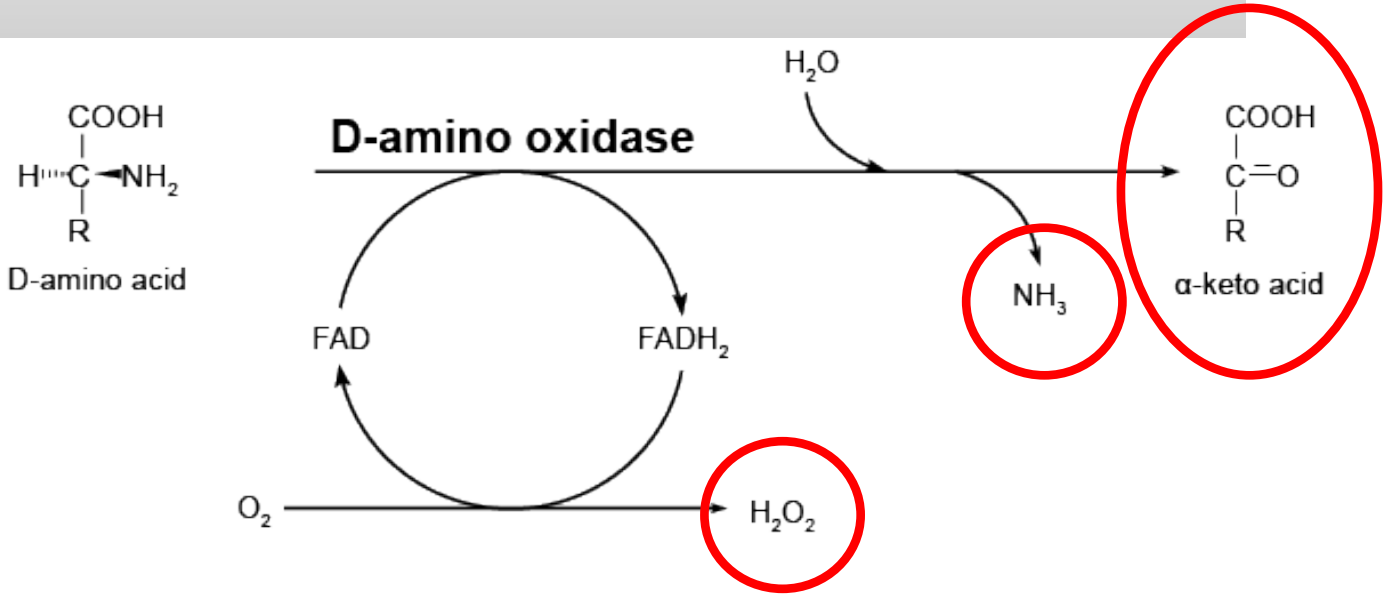
No D-amino acids in mammalian proteins

D-Amino acid metabolism by the kidney and liver.

D-Amino acid oxidase (DAO) is an FAD-dependent peroxisomal enzyme that catalyzes the oxidative deamination of D-amino acids

Increased DAO activity has been linked to increased susceptibility to schizophrenia.

L-amino acid oxidases are components of several snake venoms.



Metabolism of ammonia

Ammonia is produced by all tissues during the metabolism of different compounds

NH3 is disposed of primarily by formation of urea in the liver.

The level of ammonia in the blood must be kept very low, (hyperammonemia is toxic to the CNS)

Nitrogen should be moved from peripheral tissues to the liver for ultimate disposal as urea

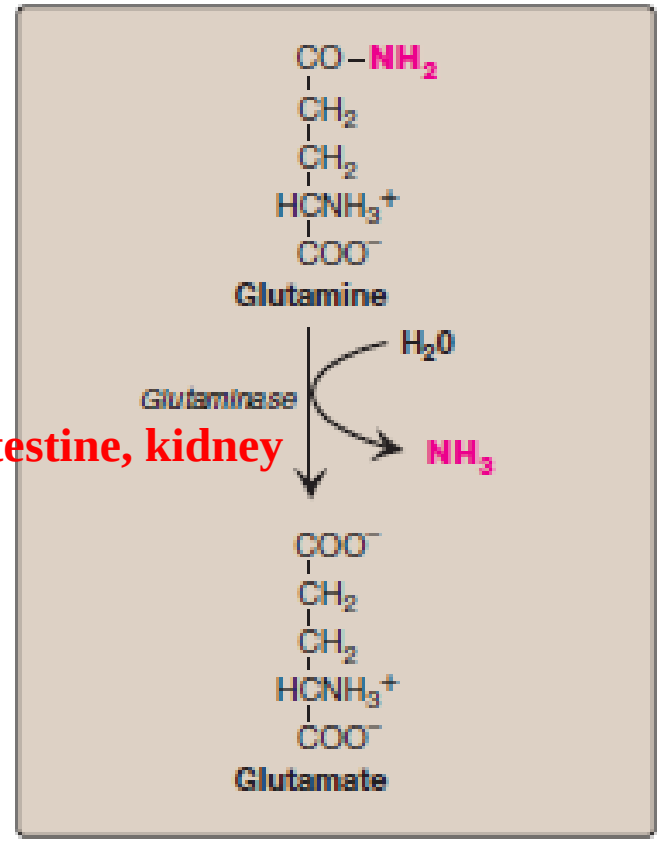
Sources of ammonia

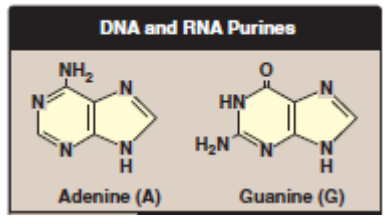
1. **From glutamine:** Most of this ammonia is excreted into the urine as NH_4^+ (acid –base balance)

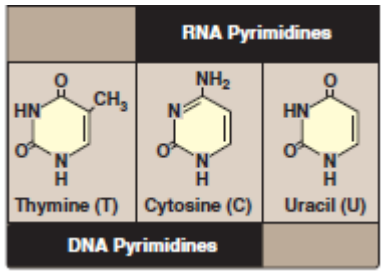
2. From bacterial action in the intestine: Ammonia is formed from urea in the intestinal lumen by the bacterial urease.
This NH3 is absorbed from the intestine by the portal vein and is converted to urea by the liver.

3. From amines: Amines in the diet, and monoamines that act as hormones or neurotransmitters, give rise to NH3 by amine oxidase

4. From purines and pyrimidines: In the catabolism of purines and pyrimidines, amino groups attached to the rings are released as NH3







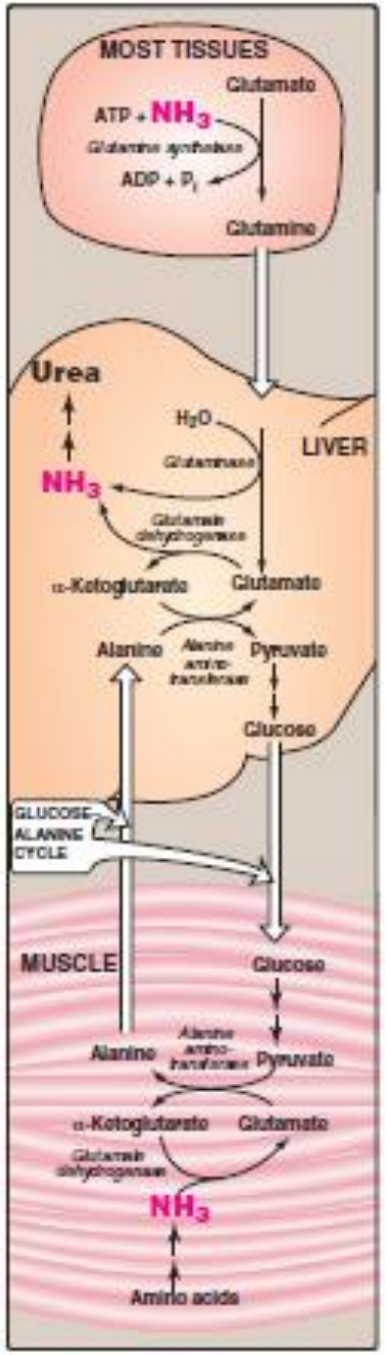
Transport of ammonia to the liver

NH_3 is transported from peripheral tissues to liver for conversion to urea.

Two mechanisms for ammonia transport:

1. By glutamine synthetase that combines NH_3 with Glu to form Gln
- Found in most tissues
- Requires energy
- A nontoxic transport form of ammonia
- The resulting glutamine is transported in the blood to the liver to be cleaved by glutaminase to produce glutamate and free ammonia

2. By transamination of pyruvate to form alanine
- Primarily in muscles
- Alanine is transported by the blood to the liver to be converted to pyruvate by transamination.
- Pyruvate can be used in gluconeogenesis (glucose-alanine cycle)



UREA CYCLE

Urea is a major disposal form of amino groups derived from AAs.

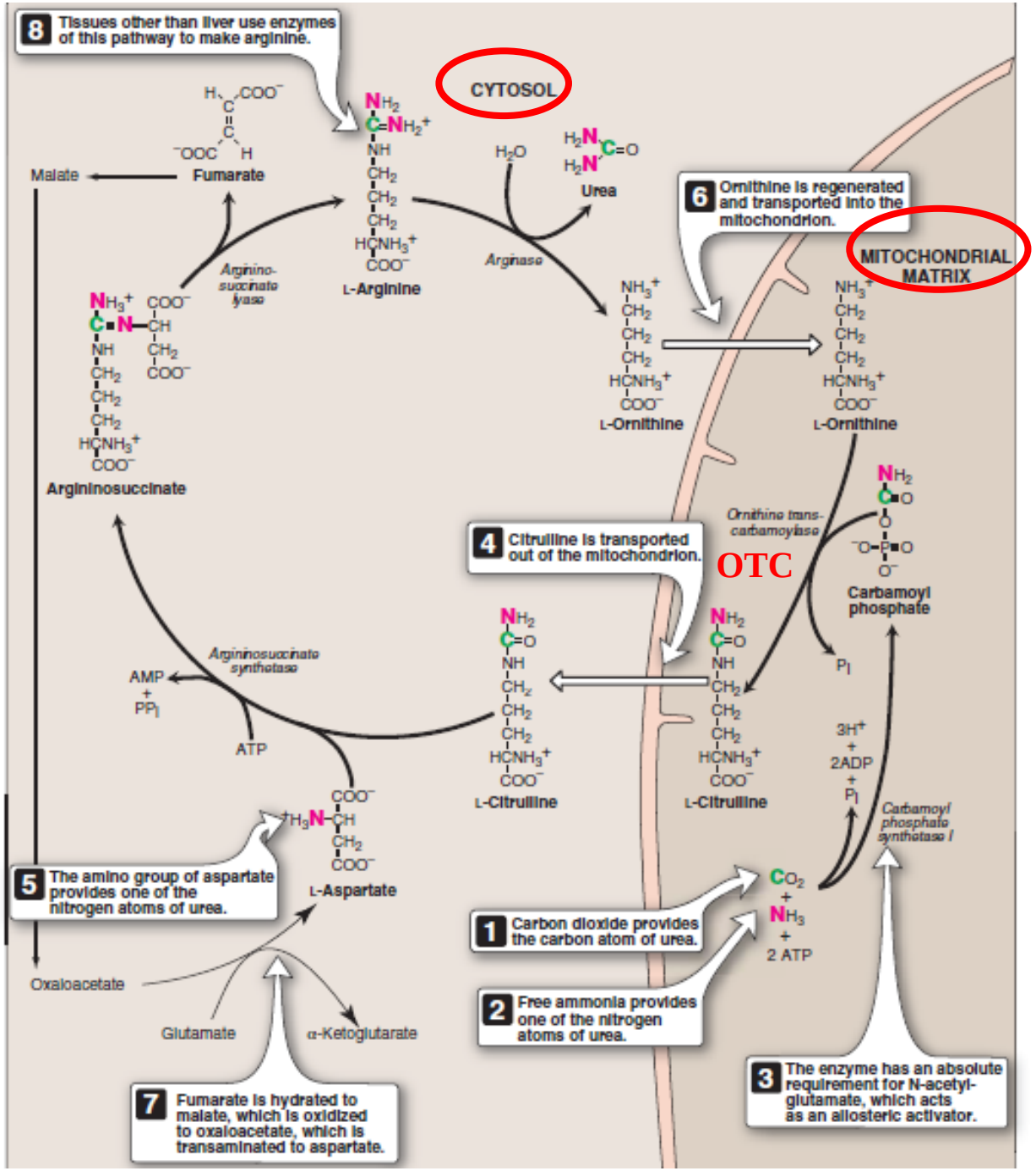
Urea accounts for about 90% of the N-containing components of urine.

One N of urea molecule is supplied by free ammonia (from oxidative deamination of Glu), and the other N by Asp.

The C and O of urea are derived from CO_2.

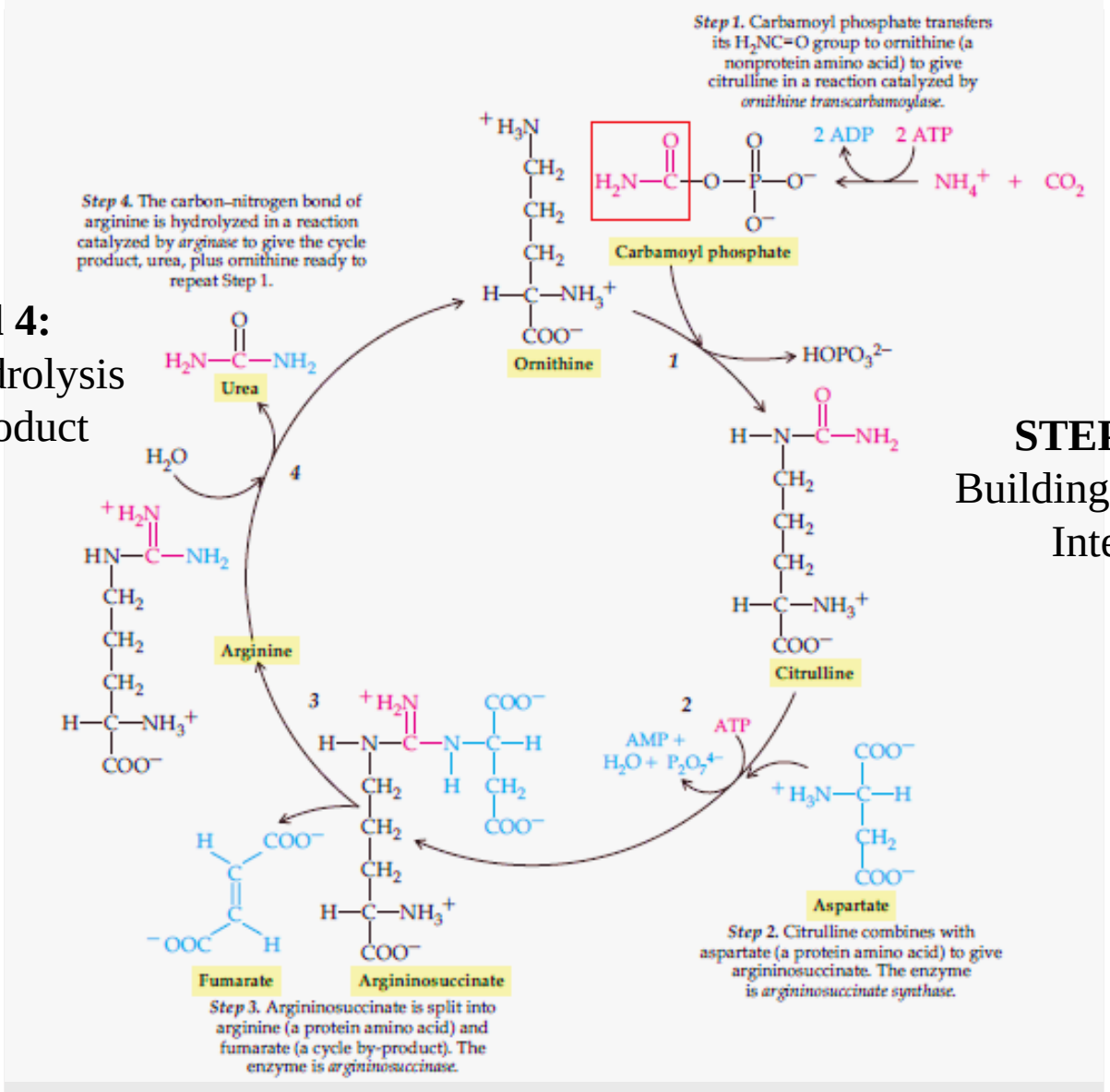
Urea is produced by the liver

Urea is transported in the blood to the kidneys for excretion in the urine.



The Urea Cycle

STEPS 3 and 4:
Cleavage and Hydrolysis of the Step 2 Product



STEPS 1 and 2:
Building Up a Reactive Intermediate

Overall stoichiometry of the urea cycle

$$\text{Aspartate} + NH_3 + CO_2 + 3\,ATP + H_2O \longrightarrow$$

$$\text{urea} + \text{fumarate} + 2\,ADP + AMP + 2\,P_I + PP_I$$

The synthesis of urea is irreversible, with a large, negative ΔG

For each urea molecule:

1. Four high-energy P-bonds
2. One nitrogen of the urea molecule is supplied by free NH3
3. The other nitrogen is supplied by aspartate.
4. Glutamate is the precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (through transamination of oxaloacetate by AST).

Regulation of the urea cycle

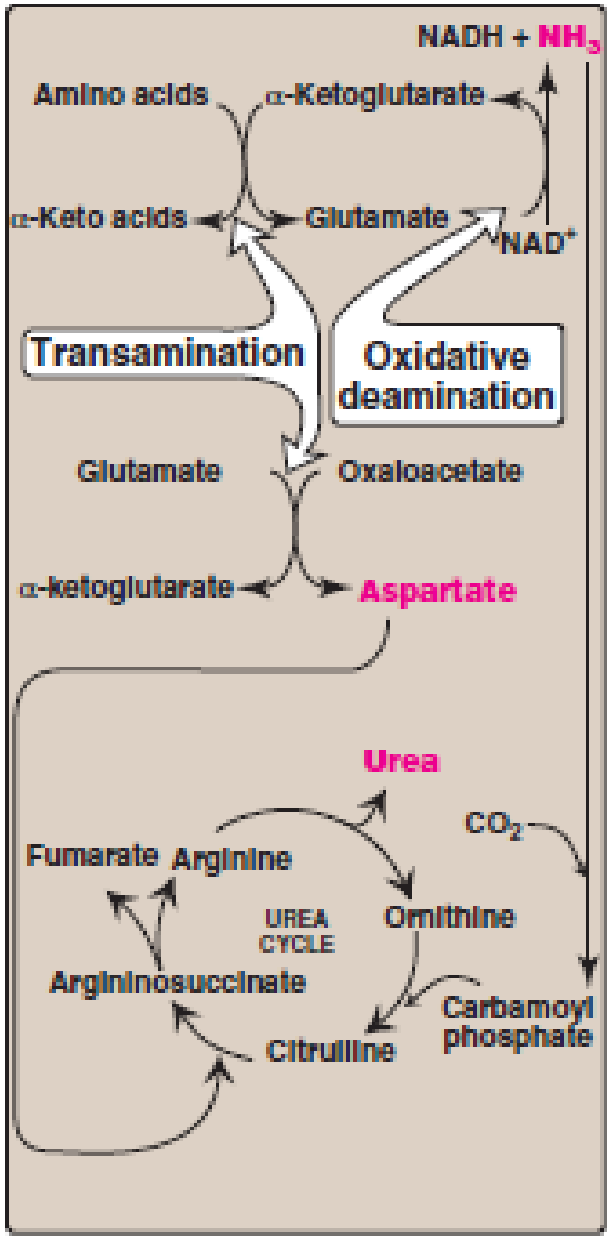
N-Acetylglutamate is an essential **activator** for carbamoyl phosphate synthetase I—the rate-limiting step in the urea cycle

N-Acetylglutamate is synthesized from acetyl coenzyme A and glutamate by N-acetylglutamate synthase

Arginine is an **activator** for N-Acetylglutamate synthesis

The intrahepatic concentration of N-acetylglutamate increases after a protein-rich meal (more glutamate and arginine are provided)

More protein in diet leads to increased urea synthesis rate



Clinical hint: Hyperammonemia

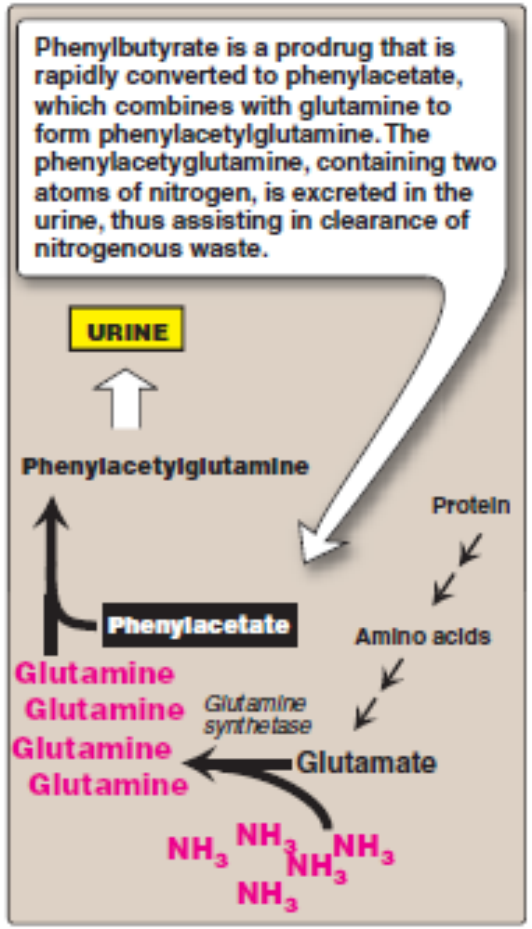
The levels of serum ammonia are normally low (5–35 μmol/L).

If liver function is compromised (genetic defects of the urea cycle or liver disease), NH3 blood levels can rise above 1,000 μmol/L (medical emergency)

NH3 has a neurotoxic effect on the CNS (tremors, slurring of speech, somnolence, vomiting, cerebral edema, and blurring of vision).

At high concentrations, ammonia can cause coma and death.

Clinical hint: Hyperammonemia



Types:

Acquired hyperammonemia: Liver disease due to viral hepatitis, or to hepatotoxins such as alcohol.

Congenital hyperammonemia: Genetic deficiencies of any of the five enzymes of the urea cycle leads to failure to synthesize urea

The overall prevalence estimated to be 1:25,000 live births.

Ornithine transcarbamoylase deficiency, which is X-linked (males), is the most common

All of the other urea cycle disorders follow an AR inheritance pattern.

Treatment: restriction of dietary protein, administration of compounds that bind covalently to AAs, producing nitrogen-containing molecules that are excreted in the urine