METABOLISM OF NITROGEN COMPOUNDS

Oxidative degradation of biological macromolecules contributes to the generation of metabolic energy.

The fraction of metabolic energy obtained from amino acids, derived from the diet or from tissue proteins, varies depending on the tissue and the general metabolic conditions.

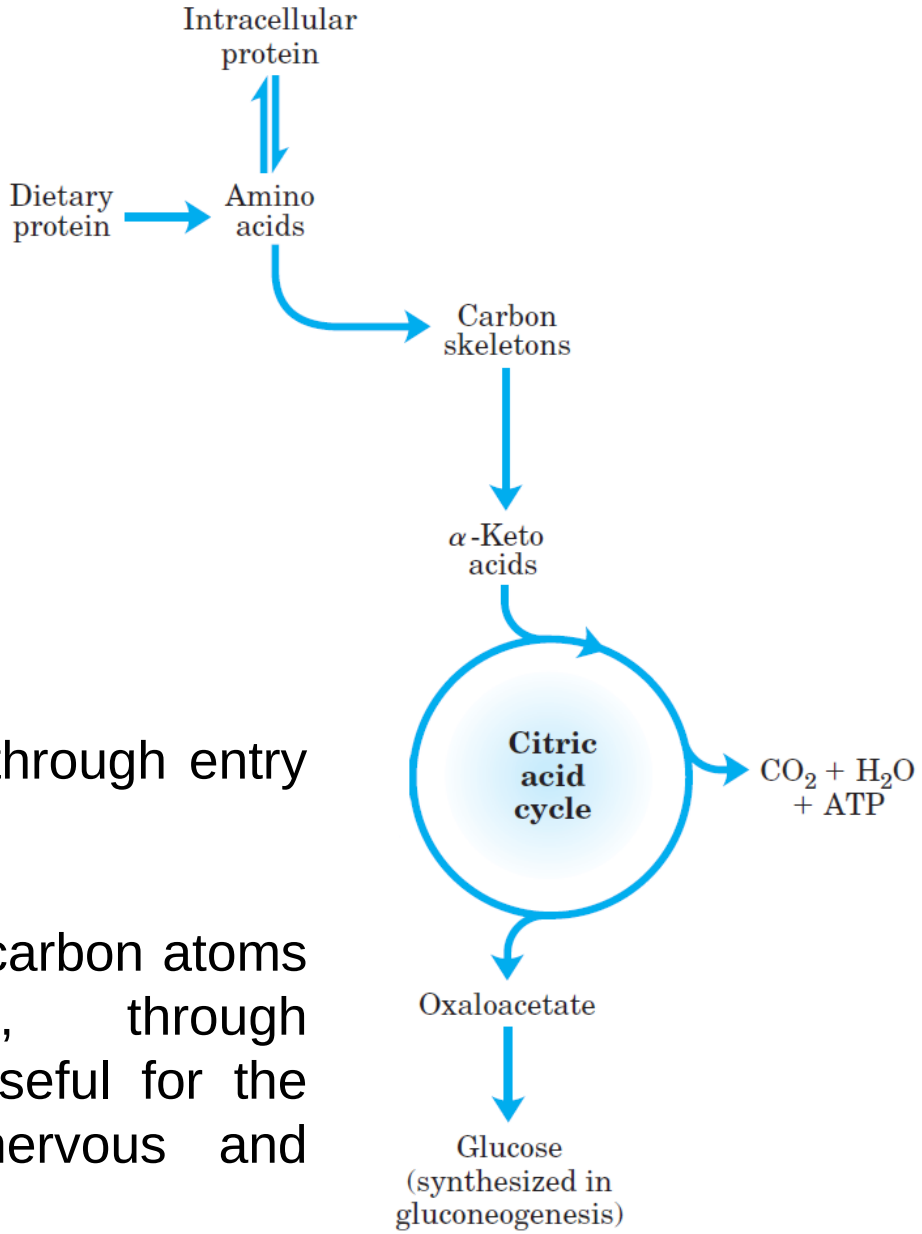
In humans, amino acids undergo oxidative degradation in three different contexts:

1. During protein turnover, amino acids that are not useful for the synthesis of new proteins are degraded;

2. If the diet is rich in proteins, therefore there is an excess of amino acid intake compared to the requirement for protein synthesis, this excess is catabolized (the amino acids are not stored).

3. In fasting and diabetes, in conditions of unavailability of carbohydrates for cells, the amino acids of cellular proteins are catabolized.

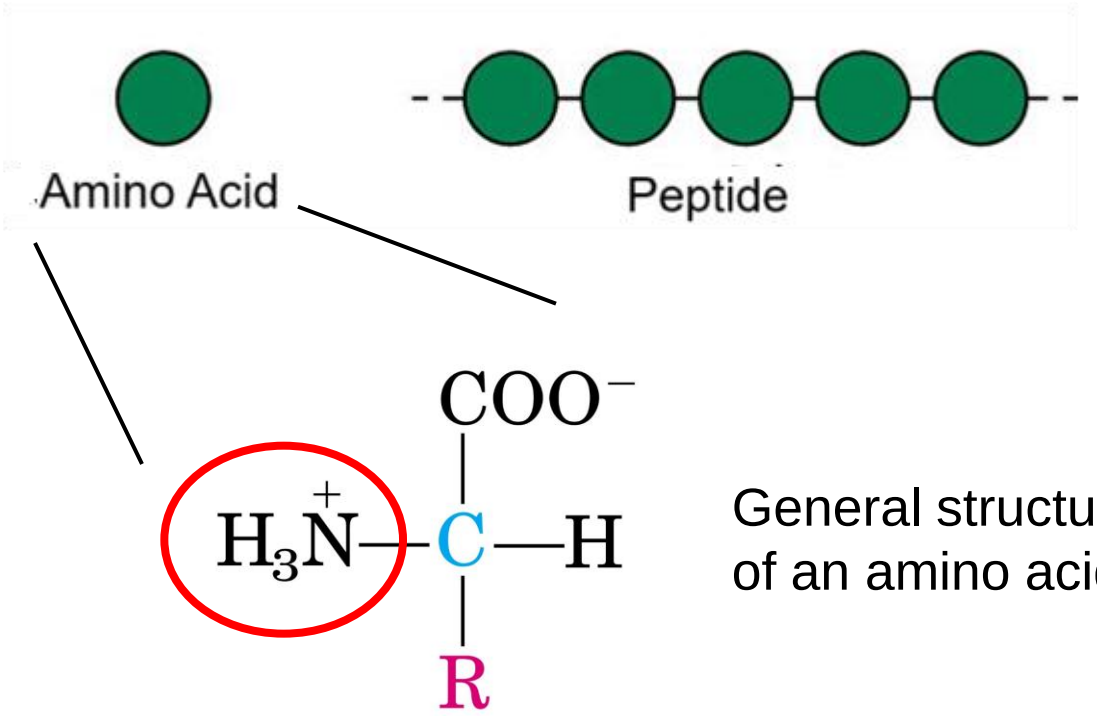
In the previous metabolic conditions, amino acids lose their amino groups and are transformed into the corresponding α-ketoacids, which:



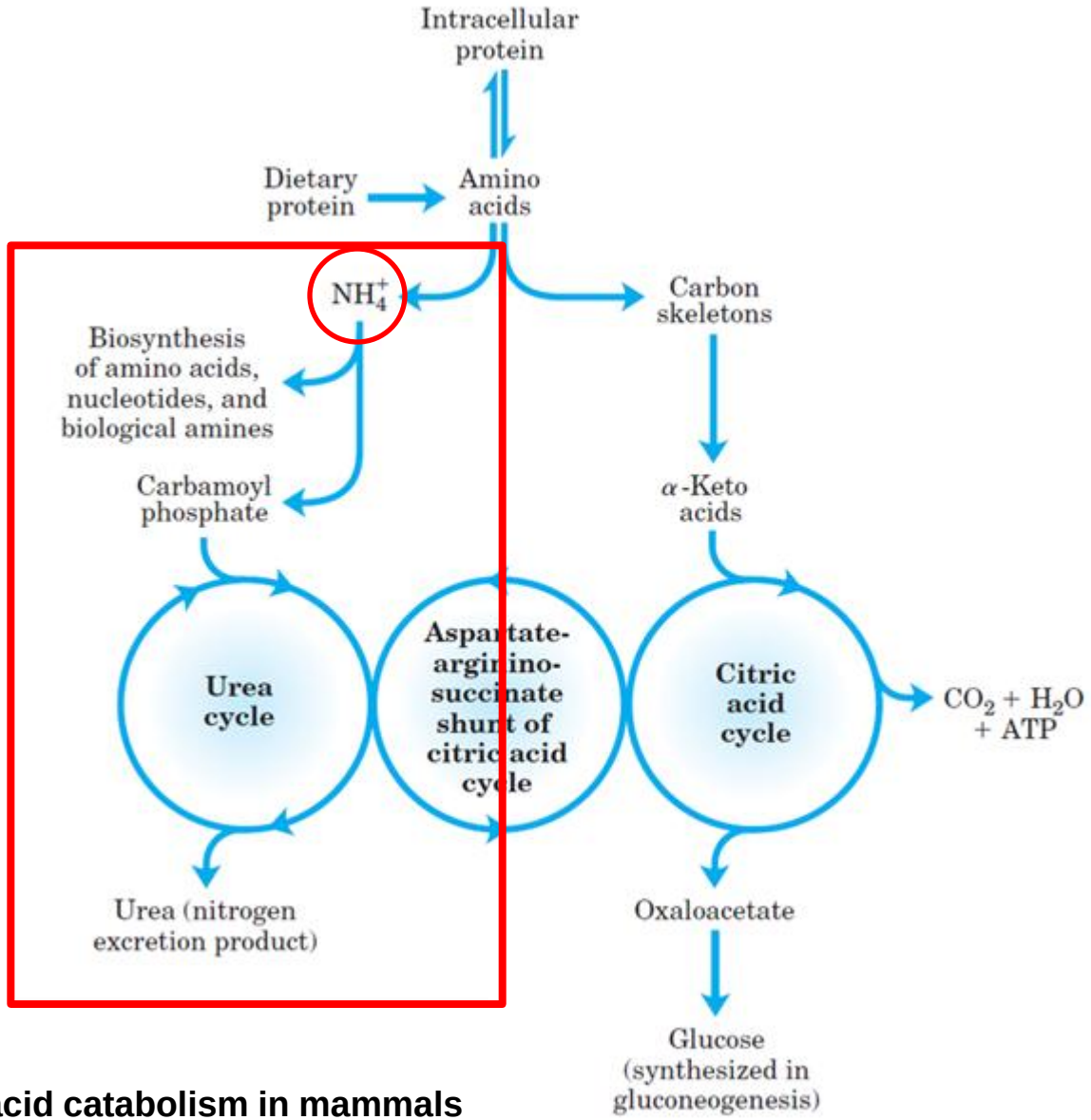
- are oxidized to CO_2 and H_2O (through entry into the citric acid cycle...).

- provide units with three or four carbon atoms that can be converted, through gluconeogenesis, into glucose useful for the needs of tissues such as nervous and muscular tissue.

A peculiarity of the oxidative processes of amino acids resides in the presence of their amino group $-NH_2$, which must first be detached from the carbon skeleton and treated through a specific metabolism.

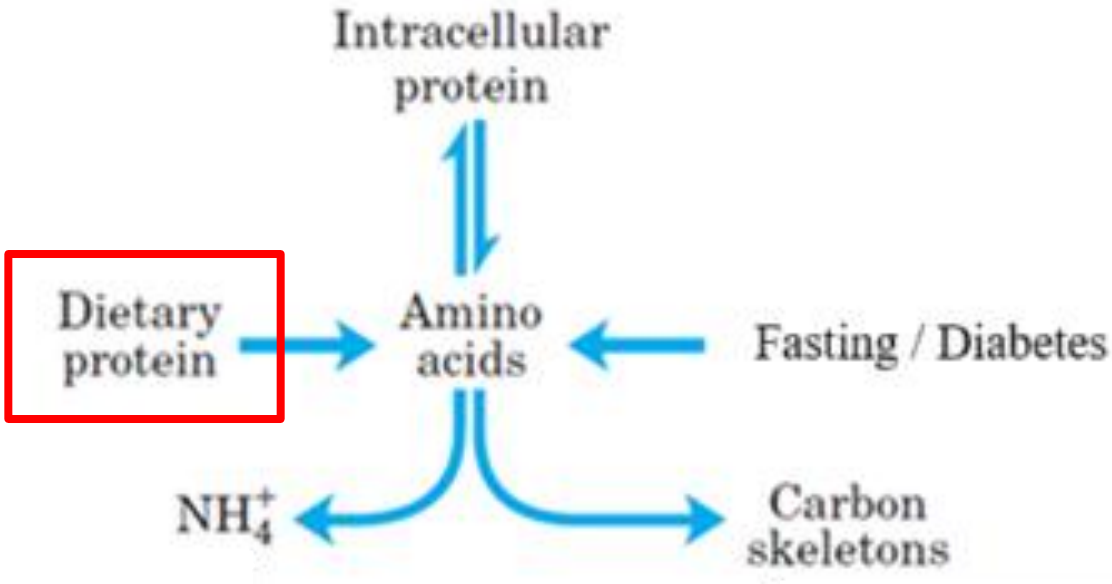


General structure of an amino acid



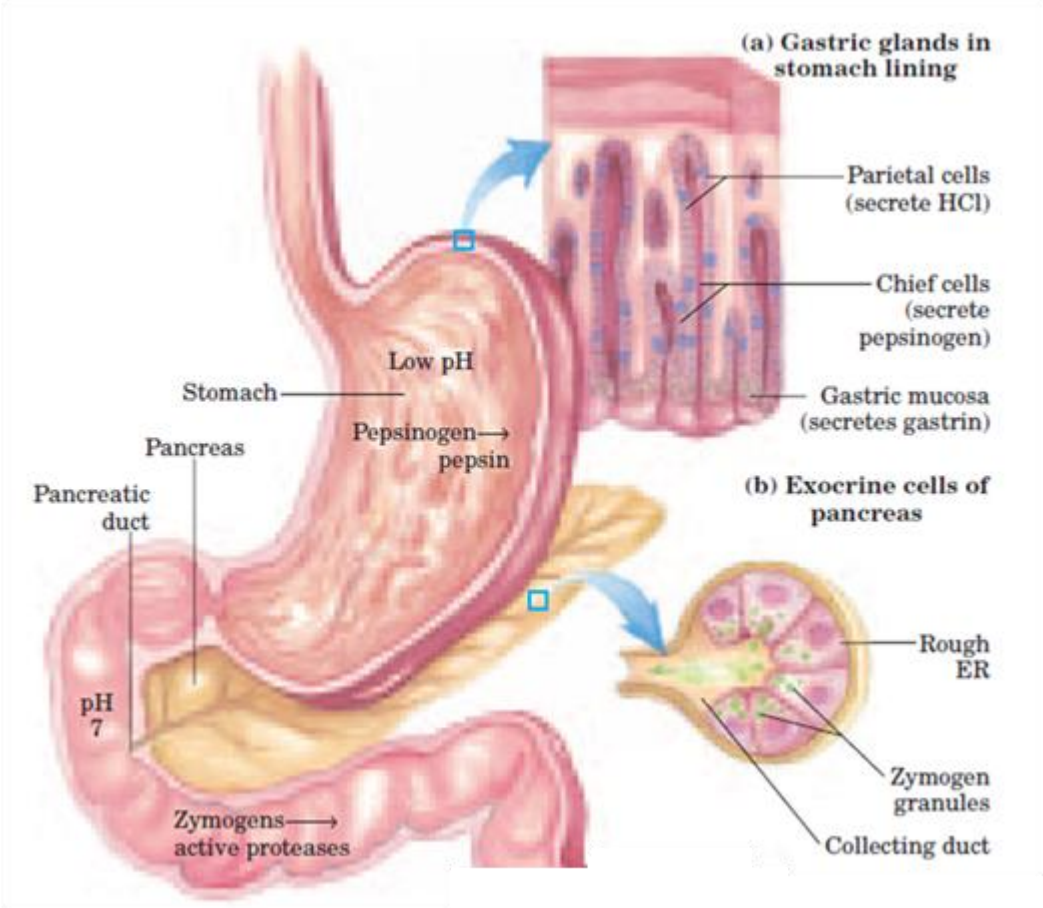
Overview of amino acid catabolism in mammals

The amino groups and the carbon skeleton take separate but interconnected pathways.



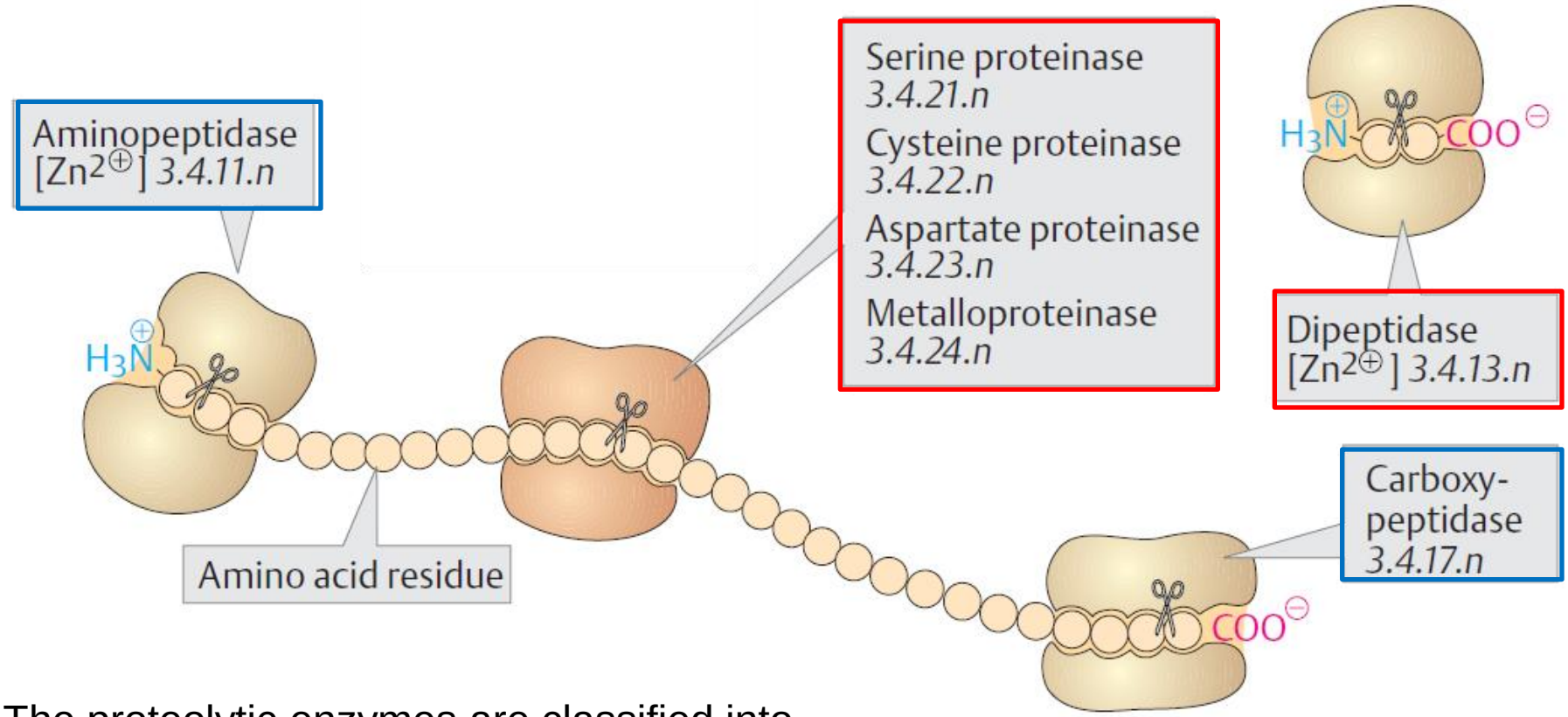
(a) The parietal cells and chief cells of the gastric glands secrete their products in response to the hormone gastrin. Pepsin begins the process of protein degradation in the stomach.

(b) The cytoplasm of exocrine cells is completely filled with rough endoplasmic reticulum, the site of synthesis of the zymogens of many digestive enzymes. The zymogens are concentrated in membrane-enclosed transport particles called zymogen granules. When an exocrine cell is stimulated, its plasma membrane fuses with the zymogen granule membrane and zymogens are released into the lumen of the collecting duct by exocytosis. The collecting ducts ultimately lead to the pancreatic duct and thence to the small intestine.



Proteolytic enzymes

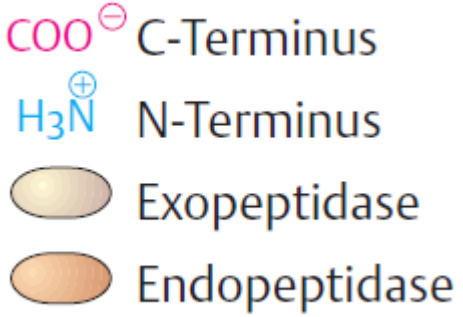
Combinations of several enzymes with different specificities are required for complete degradation of proteins into free amino acids. **Proteinases** and **peptidases** are found not only in the gastrointestinal tract, but also inside the cell.



The proteolytic enzymes are classified into **endopeptidases** and **exopeptidases**, according to their site of attack in the substrate molecule.

Proteolytic enzymes

The *endopeptidases* or *proteinases* cleave peptide bonds *inside* peptide chains. They “recognize” and bind to short sections of the substrate’s sequence, and then hydrolyze bonds between particular amino acid residues in a relatively specific way. The **proteinases** are classified according to their reaction mechanism. In *aspartate proteinases (e.g. pepsin),* for example, an aspartate residue in the enzyme is important for catalysis, while in *serine proteinases (e.g. trypsin),* it is a serine residue - and so on.



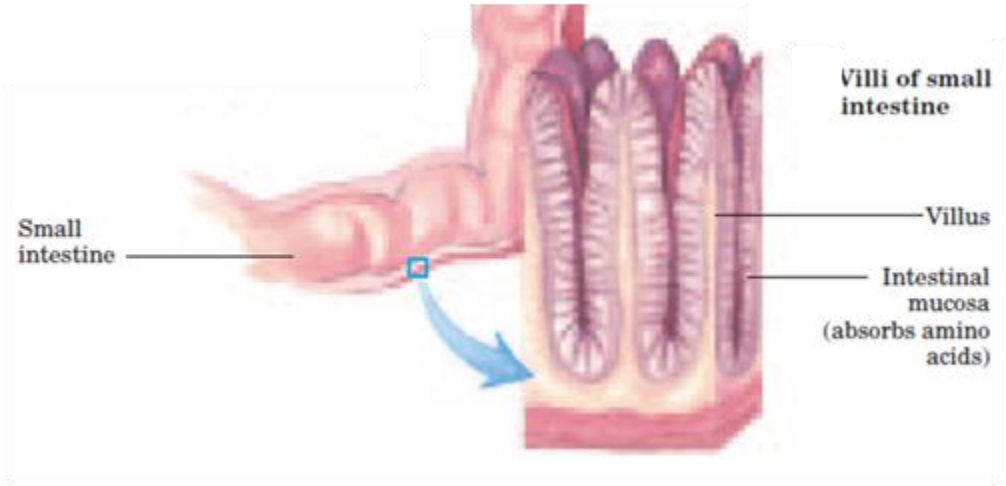
The exopeptidases attack peptides from their termini. Peptidases that act at the N-terminus are known as **aminopeptidases**, while those that recognize the C-terminus are called **carboxypeptidases**.

The **dipeptidases** only hydrolyze dipeptides.

Digestive proteolytic enzymes

Active form	Precursor	Site of action
Pepsin	Pepsinogen	Stomach
Trypsin	Trypsinogen	Pancreas
Chymotrypsin	Chymotrypsinogen	Pancreas
Carboxypeptidase	Pro-carboxypeptidase	Pancreas and intestine

Amino acids are absorbed through the epithelial cell layer (intestinal mucosa) of the villi and enter the capillaries. Recall that the products of lipid hydrolysis in the small intestine enter the lymphatic system after their absorption by the intestinal mucosa.

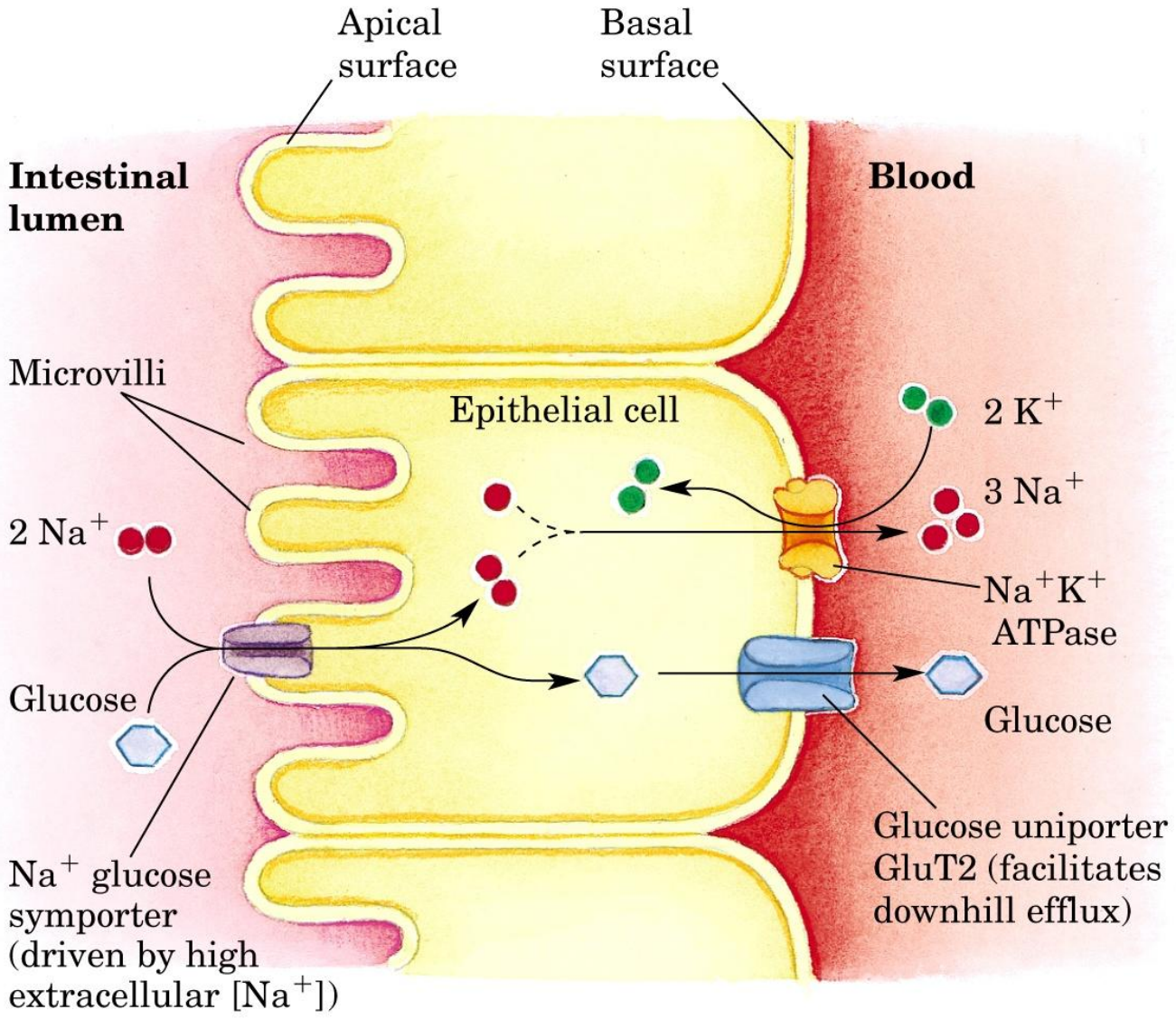


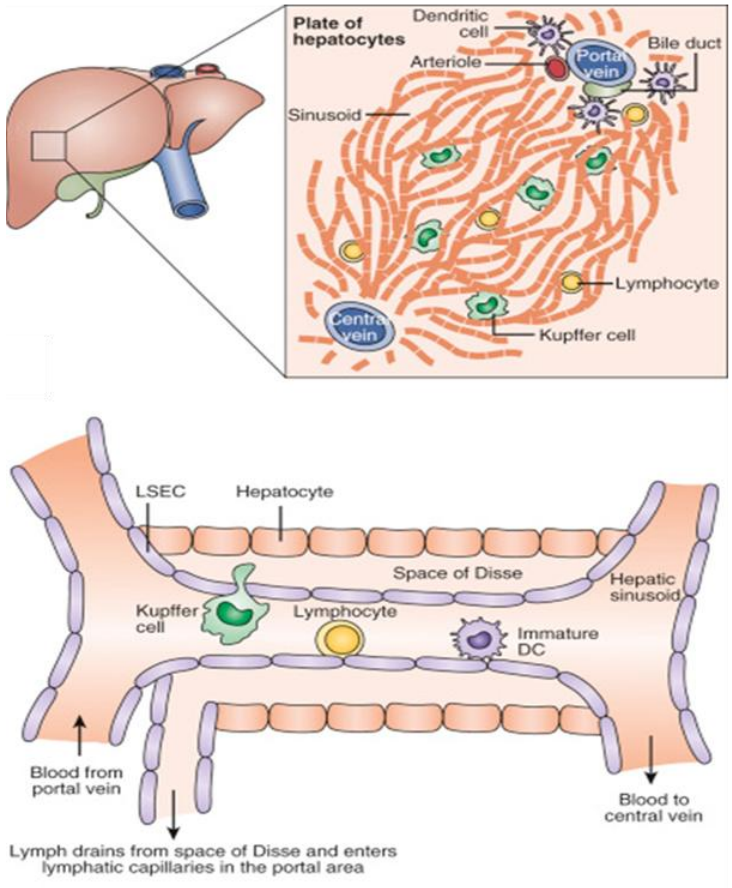
Amino acid absorption takes place in the intestine microvilli by secondary active transport (as for glucose)

Co-transport (symport) Na^+ / aminoacids:

a. Neutral aminoacids
b. Acidic aminoacids
c. Basic aminoacids
d. Glycine and proline

Na^+ gradient is maintained by the Na^+ pump (Na/K-ATPase)





Blood transport of amino acids

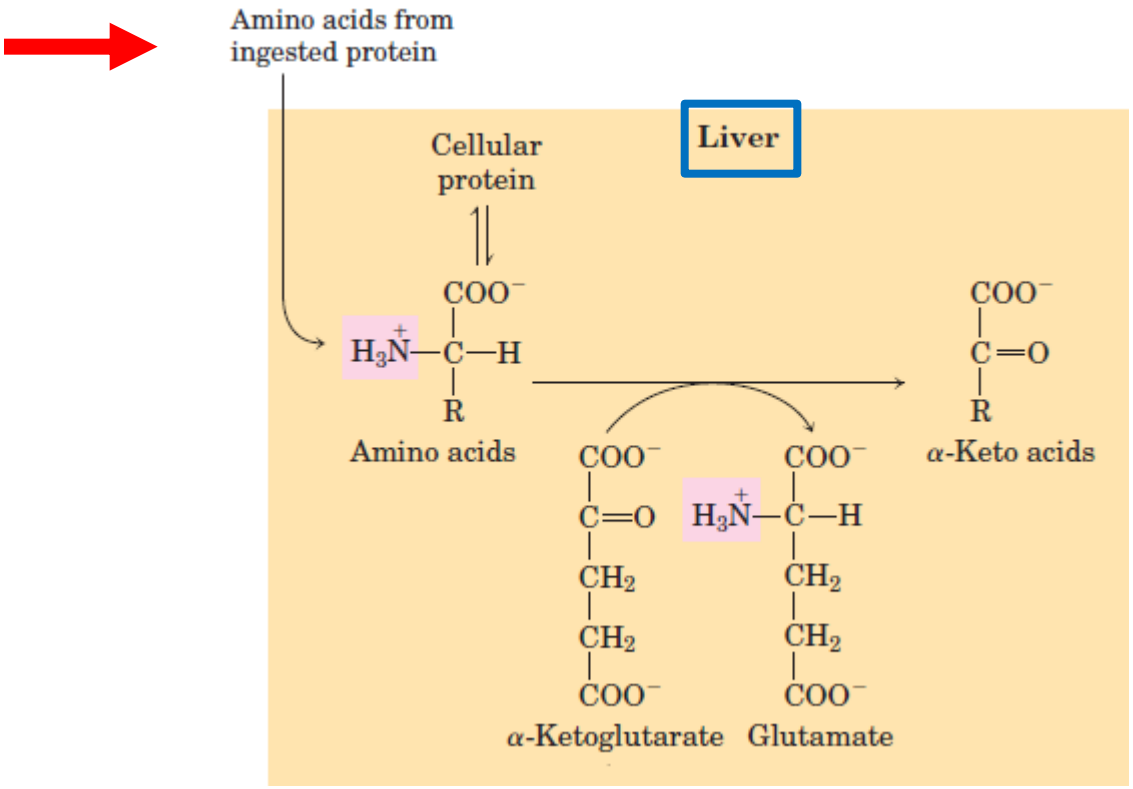
Intestinal villi

Blood of portal circulation

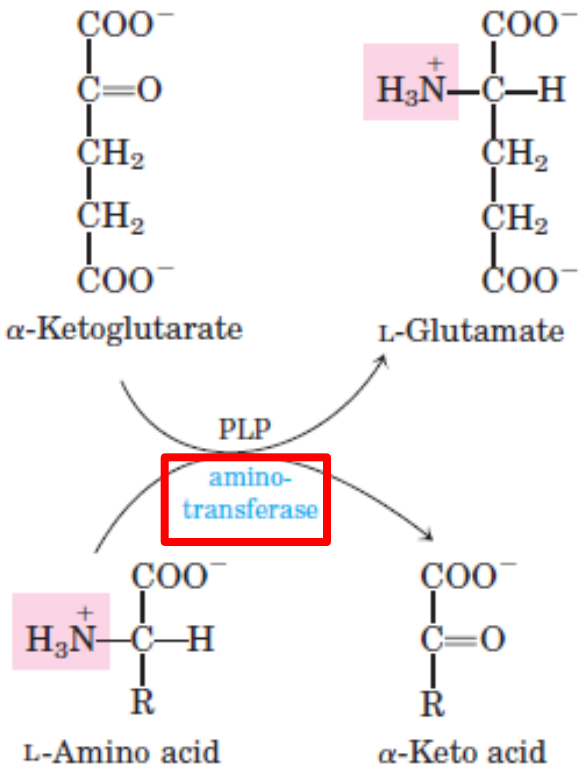
Hepatic lobule: sinusoids: here aminoacids enter hepatocytes after a meal and leave during fasting

Centrolobular vein.

General circulation



The degradation of most amino acids begins with a transamination, that is, with the transfer of the α-amino group to the α-ketoglutarate, which is thus transformed into glutamate, while the amino acid originates another oxyacid which is further degraded.



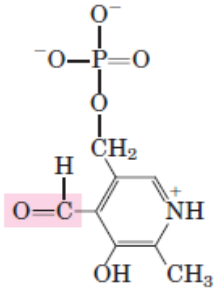
Enzyme-catalyzed transaminations

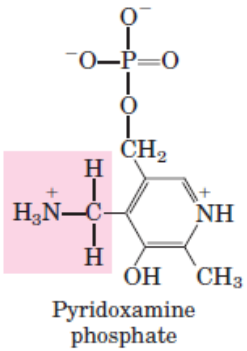
In many aminotransferase reactions, α-ketoglutarate is the amino group acceptor. All aminotransferases have pyridoxal phosphate (PLP) as cofactor. Although the reaction is shown here in the direction of transfer of the amino group to α-ketoglutarate, it is readily reversible.

Pyridoxal phosphate, the prosthetic group of aminotransferases

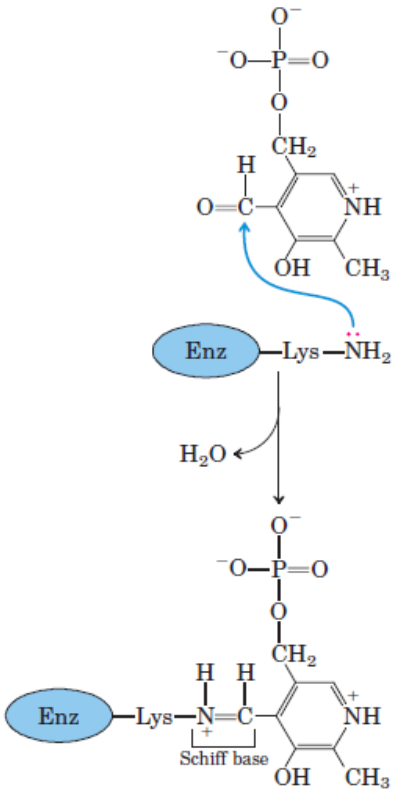
Pyridoxal phosphate (PLP; the active form of vitamin B6, pirydoxine) and its aminated form, pyridoxamine phosphate, are the tightly bound coenzymes of aminotransferases.

The functional groups are shaded.





Pyridoxal phosphate is bound to the enzyme through noncovalent interactions and a Schiff base linkage to a Lys residue at the active site. The steps in the formation of a Schiff base from a primary amine and a carbonyl group.

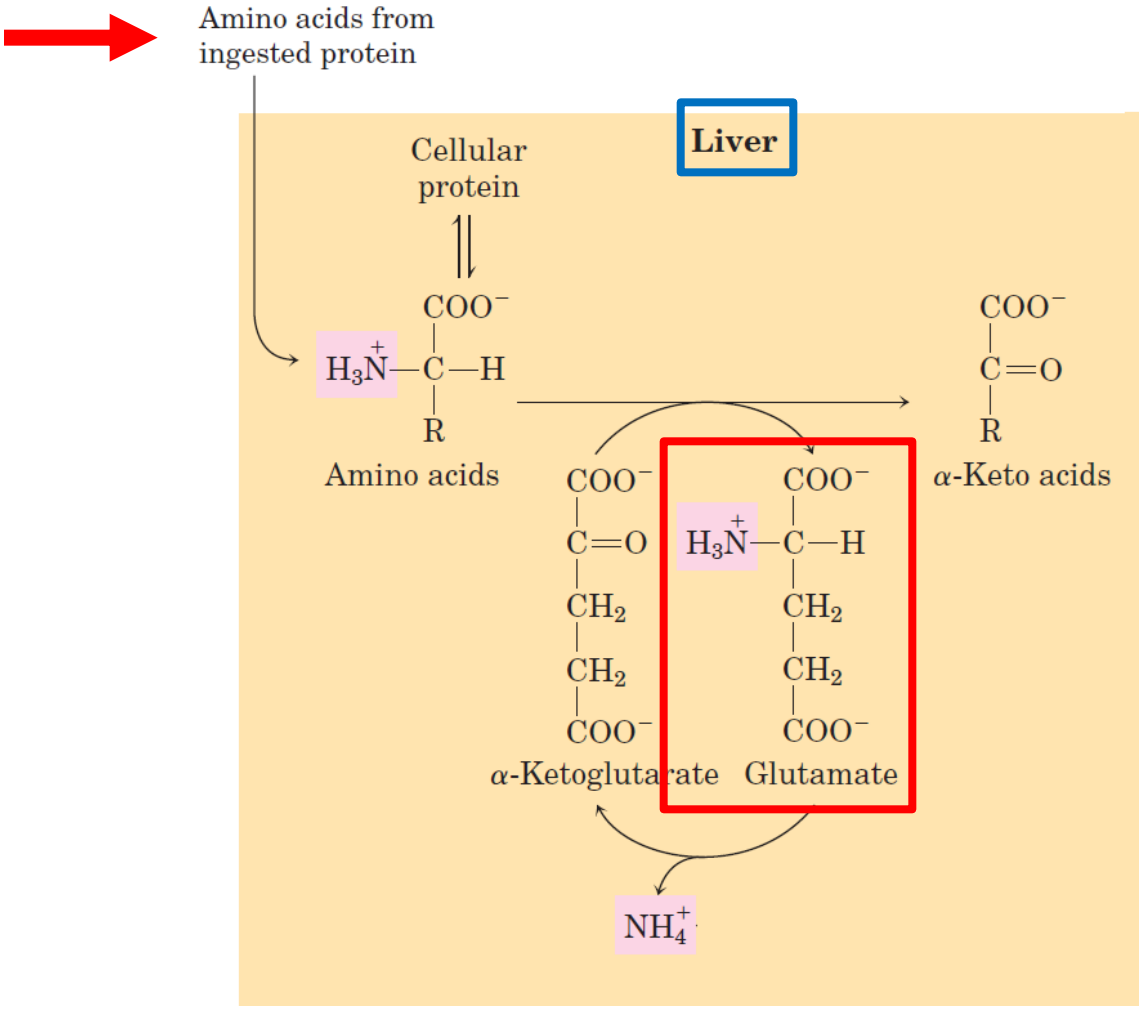


Transaminases are a group of enzymes involved in the metabolism of amino acids, present in various tissues but concentrated above all in liver cells. They intervene in transamination, that is, in the transformation of one amino acid into another, and reflect the health of the liver, but can be altered in diseases of the heart and striated skeletal muscles.

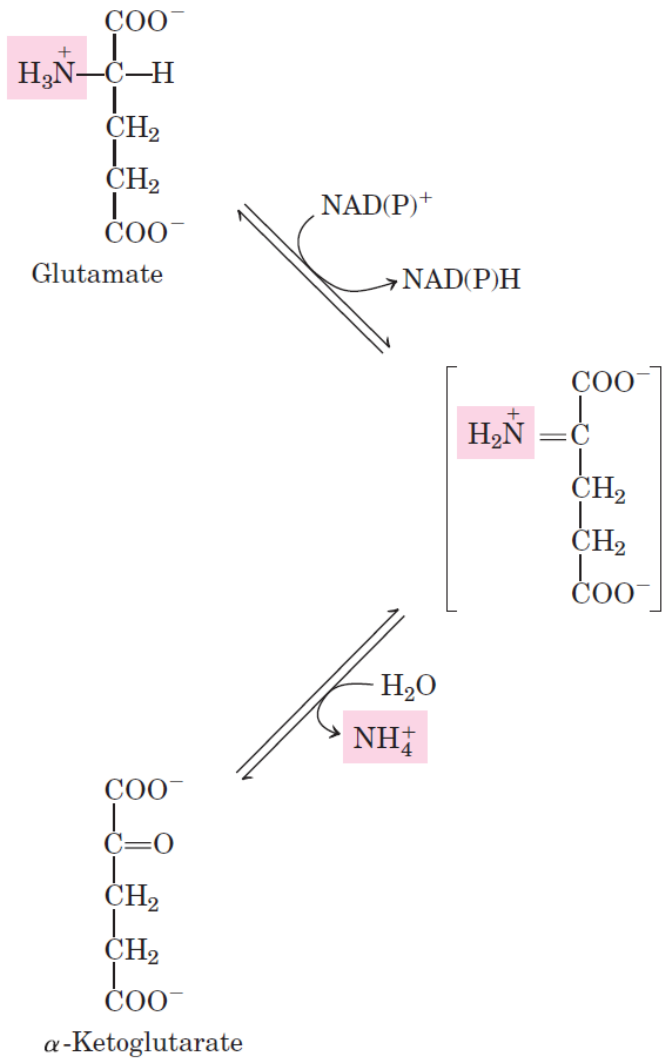
In routine tests, to indicate the health of the liver, the following are measured:

- **ALT** transaminase (Alanine aminotransferase or Glutamate-pyruvate transaminase GPT), which mainly affects the liver;

- **AST** transaminase (Aspartate aminotransferase or Glutamate-oxaloacetate transaminase GOT), which can also affect the heart and other striated muscles.



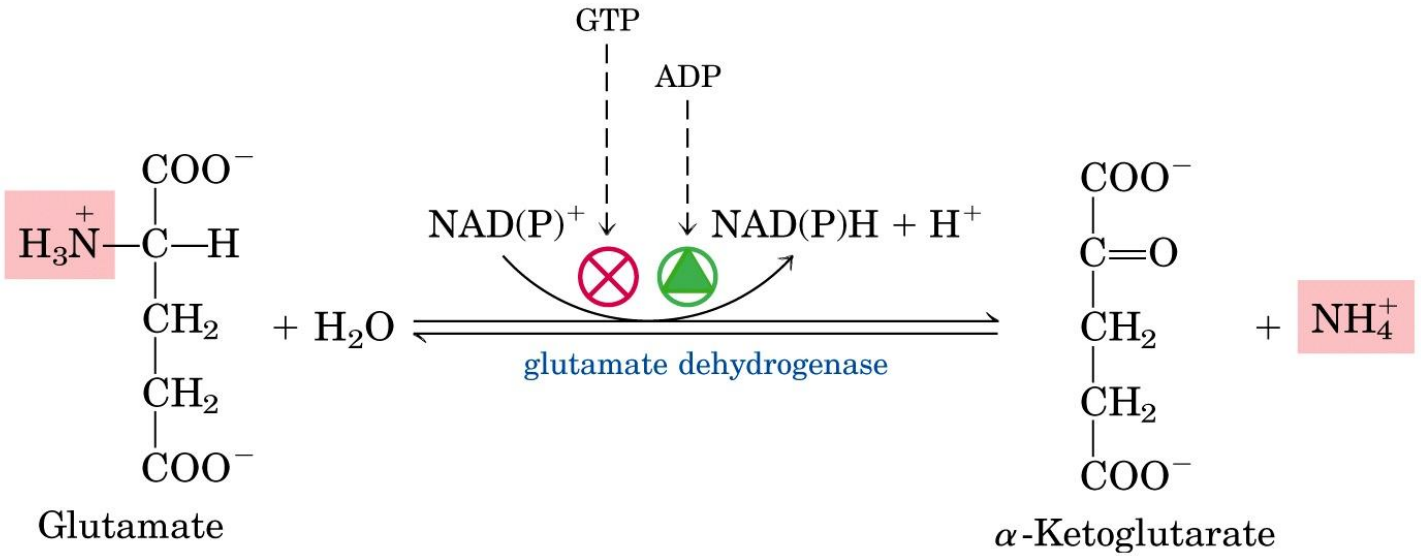
The release of ammonium ions (NH_4^+) from glutamate and the regeneration of α-ketoglutarate take place by oxidative deamination.



Reaction catalyzed by glutamate dehydrogenase

The glutamate dehydrogenase of mammalian liver has the unusual capacity to use either NAD^+ or $NADP^+$ as cofactor.

The mammalian enzyme is allosterically regulated by GTP and ADP.



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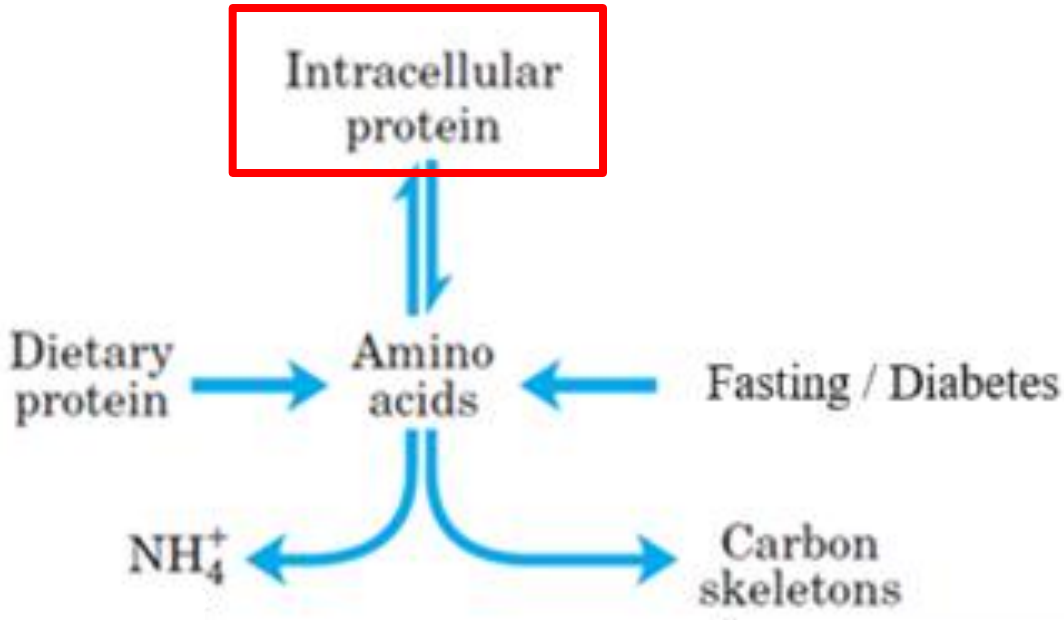
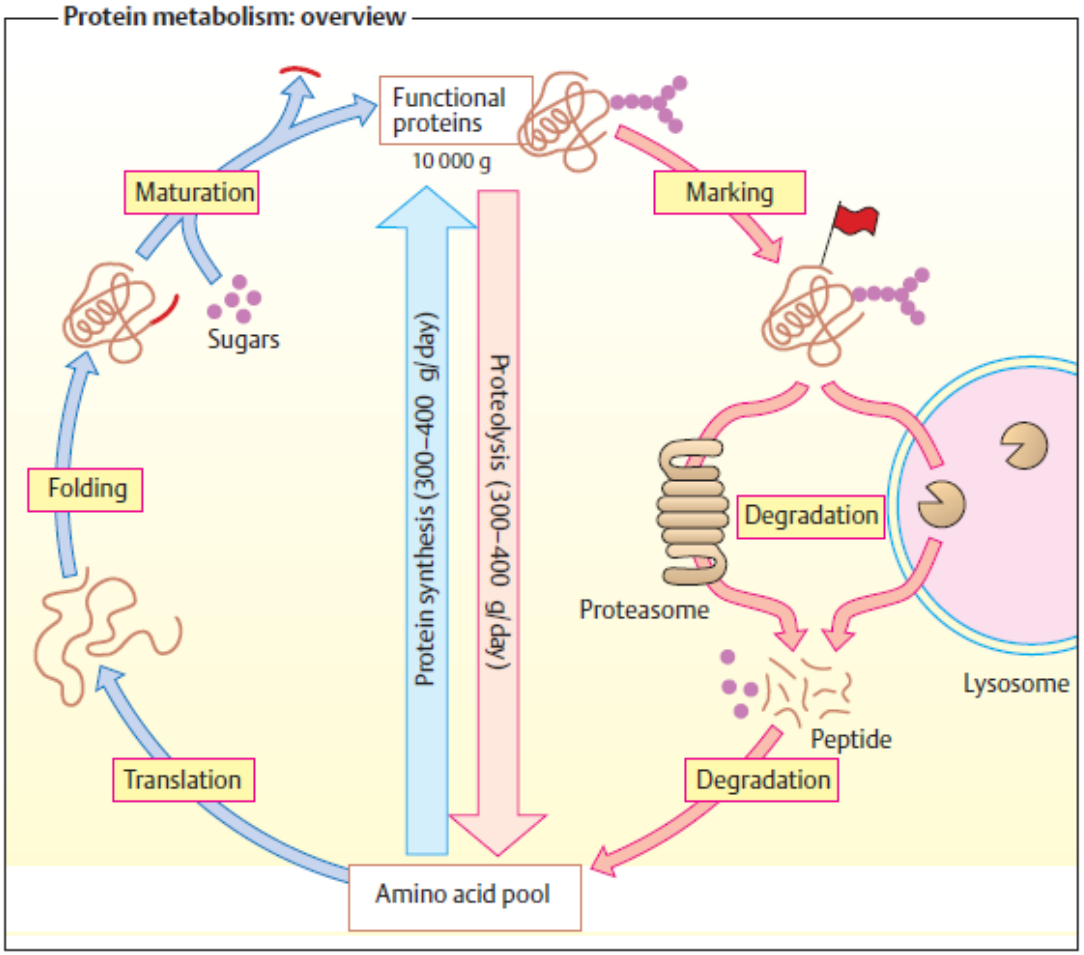


In adults, the **nitrogen balance** is generally in *equilibrium* — i. e., the quantities of protein nitrogen taken in and excreted per day are approximately equal. If only some of the nitrogen taken in is excreted again, then the balance is *positive.* This is the case during growth, for example. *Negative* balances are rare and usually occur due to disease.



J. Koolman, K.-H. Roehm *Color atlas of biochemistry* © Thieme

Individual protein have different half-lives

Relationship between Protein Half-Life and Amino-Terminal Amino Acid Residue

Amino-terminal residue	Half-life*
Stabilizing	
Met, Gly, Ala, Ser, Thr, Val	>20 h
Destabilizing	
Ile, Gln	~30 min
Tyr, Glu	~10 min
Pro	~7 min
Leu, Phe, Asp, Lys	~3 min
Arg	~2 min

Constitutive proteins have longer half-lives

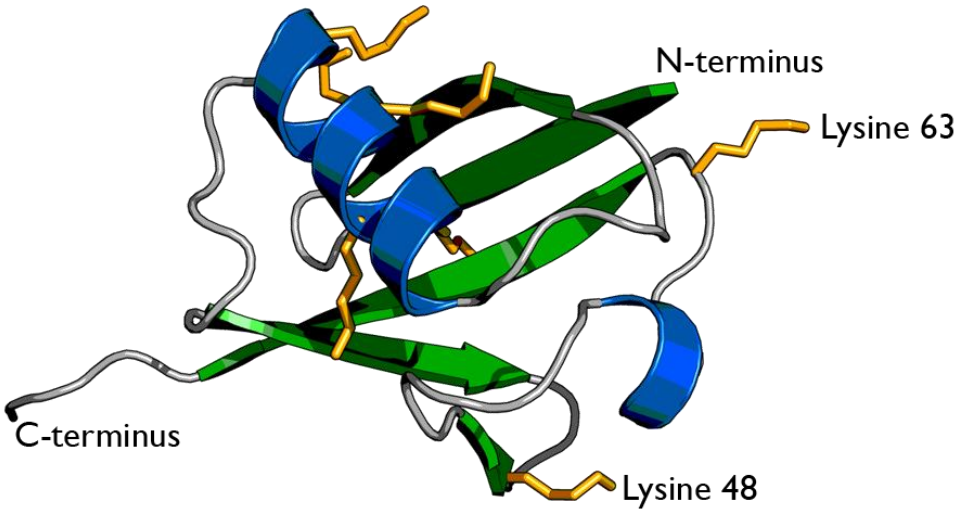
Inducible proteins have shorter half-lives

Specific protein degradation is a way for enzyme activity control

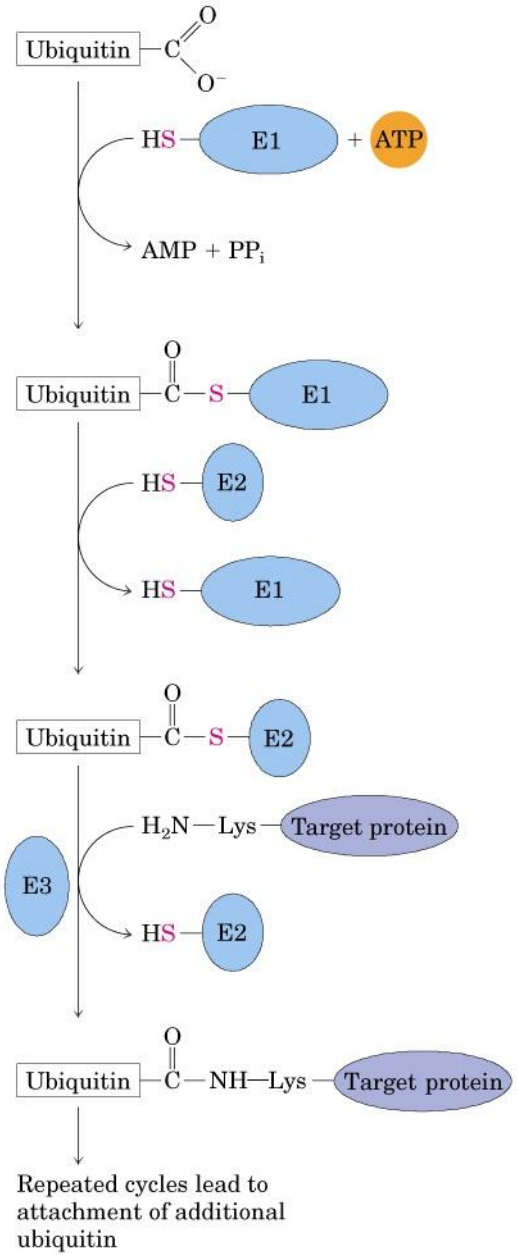
Damaged proteins are quickly removed

Intracellular protein degradation

Ubiquitin-dependent proteolysis (soluble proteins): ubiquitinated proteines are digested in the proteasome



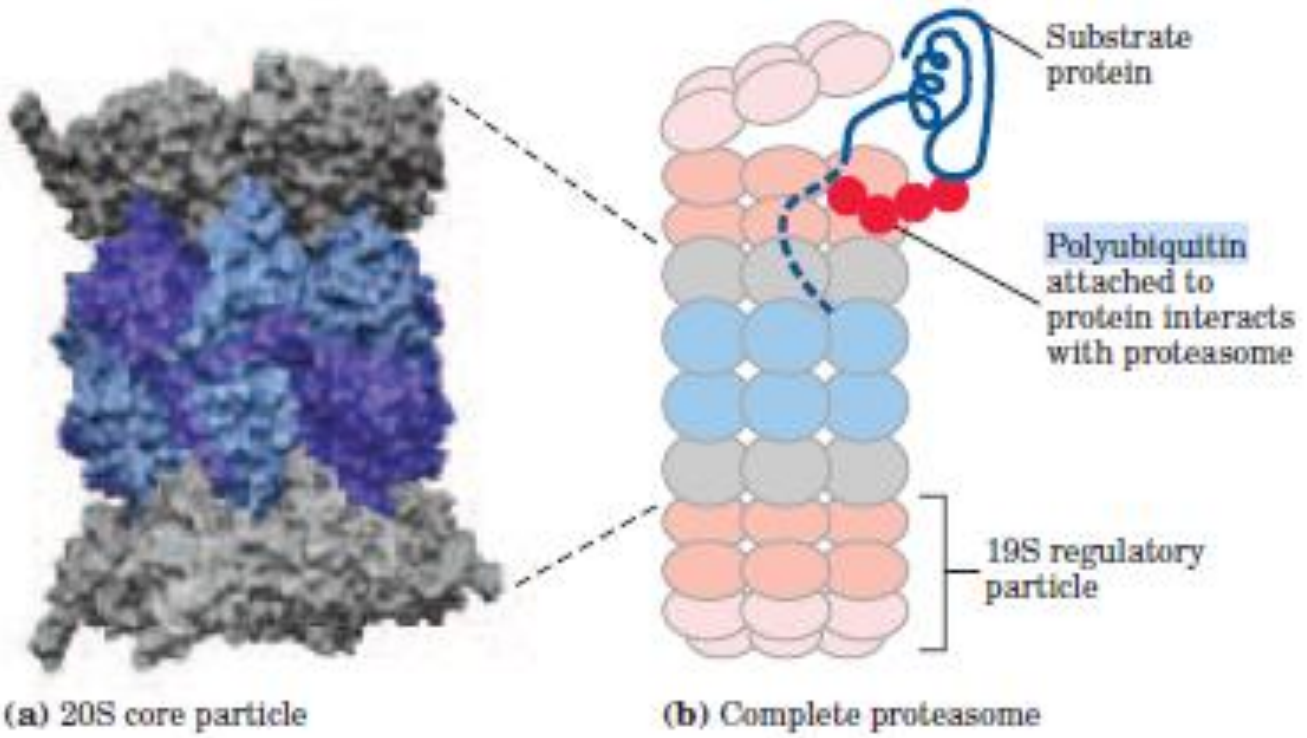
Ubiquitin



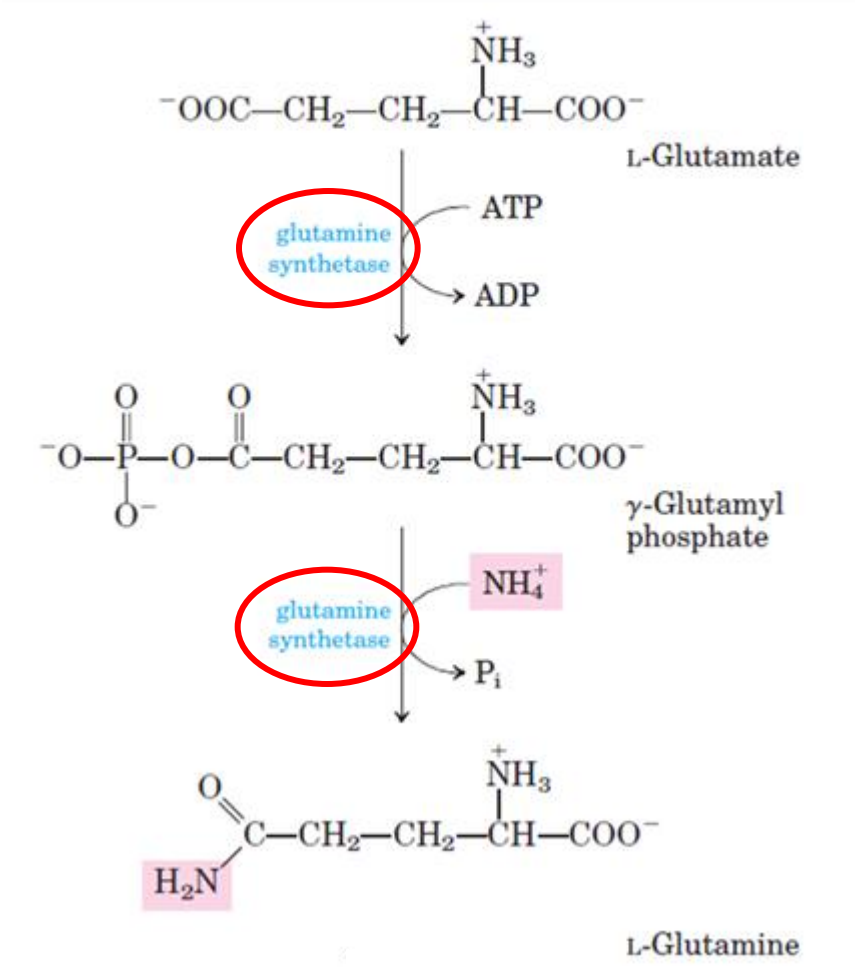
Three-step cascade pathway by which ubiquitin is attached to a protein

Two different enzyme-ubiquitin intermediates are involved. The free carboxyl group of ubiquitin's carboxyl-terminal Gly residue is ultimately linked through an amide (isopeptide) bond to an -amino group of a Lys residue of the target protein. Additional cycles produce polyubiquitin, a covalent polymer of ubiquitin subunits that targets the attached protein for destruction in eukaryotes.

Three-dimensional structure of the eukaryotic proteasome

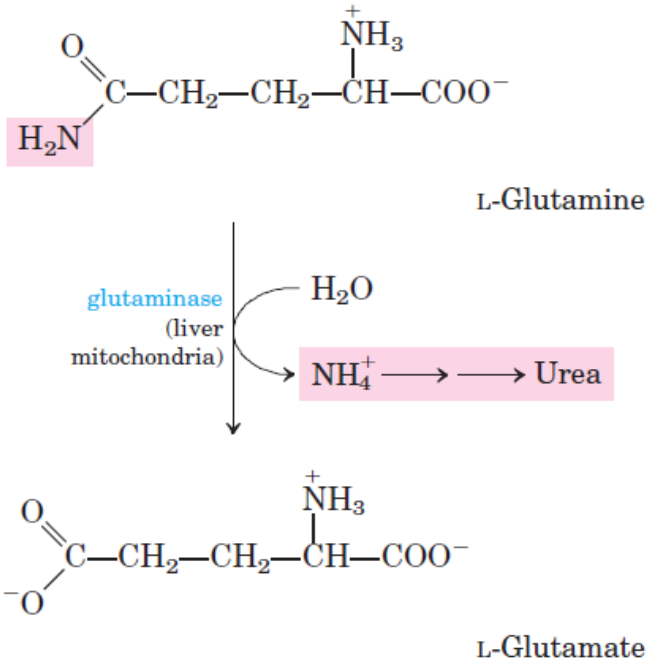


The 26S proteasome is highly conserved in all eukaryotes. The two subassemblies are the 20S core particle and the 19S regulatory particle. **(a)** The core particle consists of four rings arranged to form a barrel-like structure. Each of the inner rings has seven different subunits (light blue), three of which have protease activities (dark blue). The outer rings each have seven different subunits (gray). **(b)** A regulatory particle forms a cap on each end of the core particle. The core particle is colored as in **(a)**. The base and lid segments of each regulatory particle are presented in different shades of red. The regulatory particle unfolds ubiquitinated proteins (blue) and translocates them into the core particle.

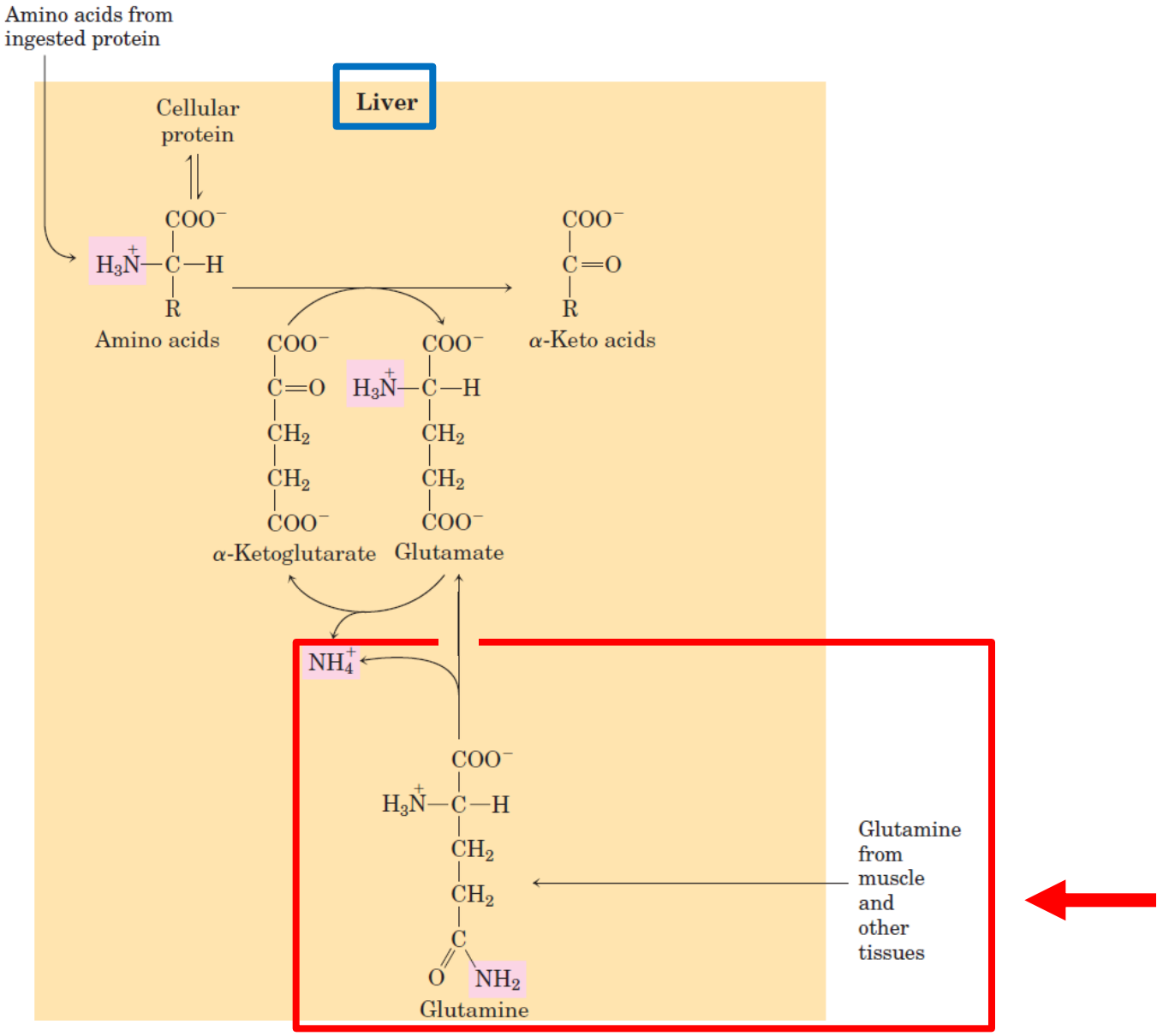


Ammonia transport in the form of glutamine

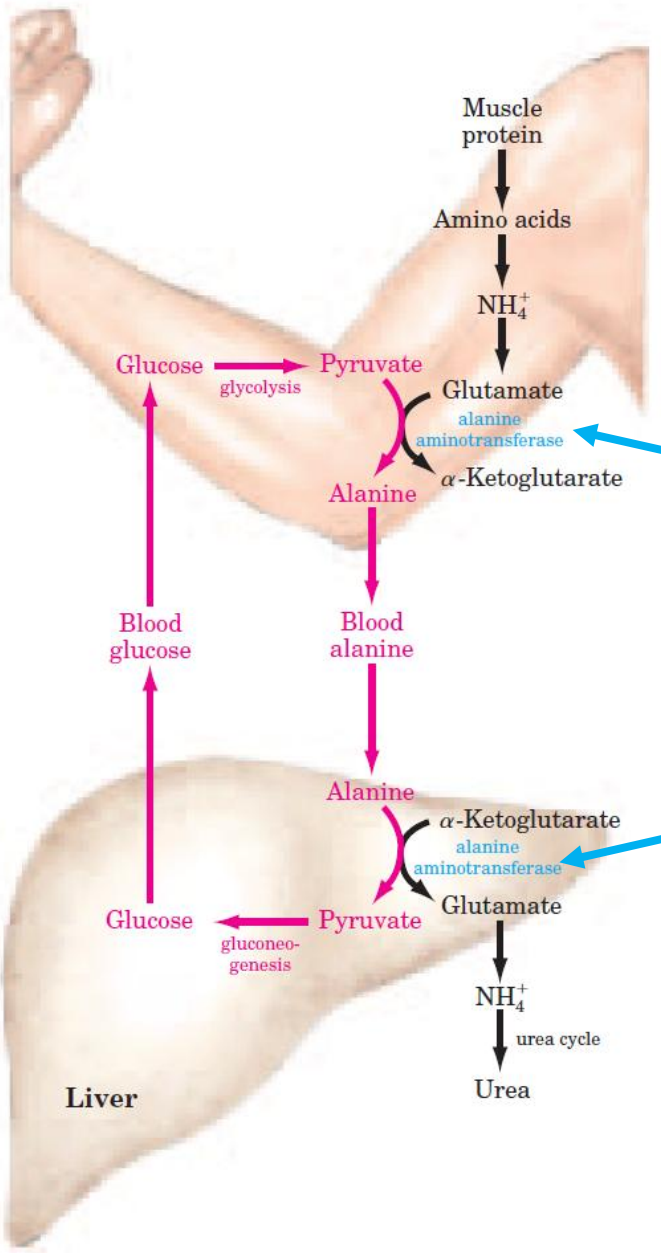
Excess ammonia in tissues is added to glutamate to form glutamine, a process catalyzed by glutamine synthetase. After transport in the bloodstream, the non-toxic glutamine enters the liver.



Within the liver NH_4^+ is liberated from glutamine by the enzyme glutaminase, in mitochondria.



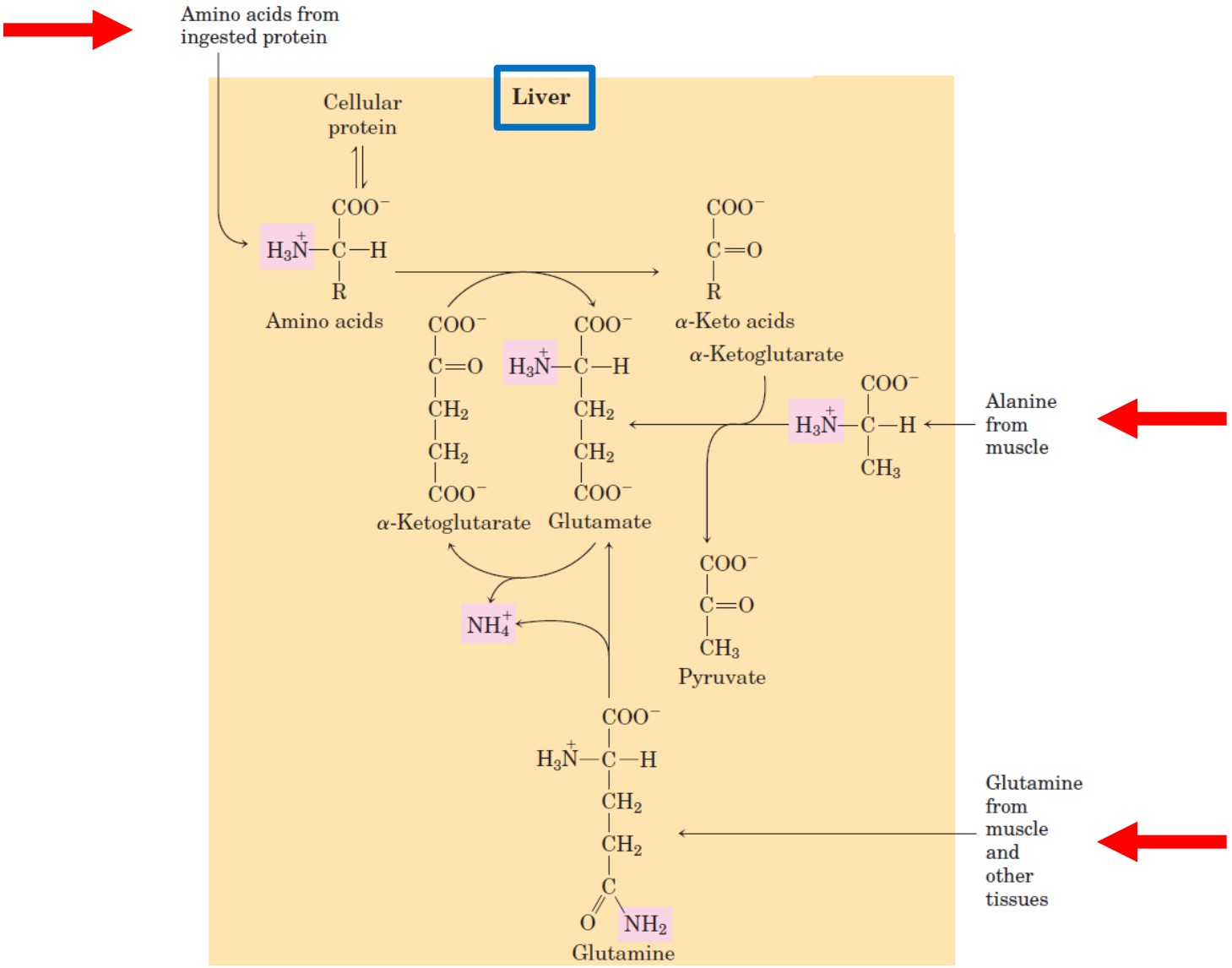
Alanine transports ammonia from skeletal muscles to the liver



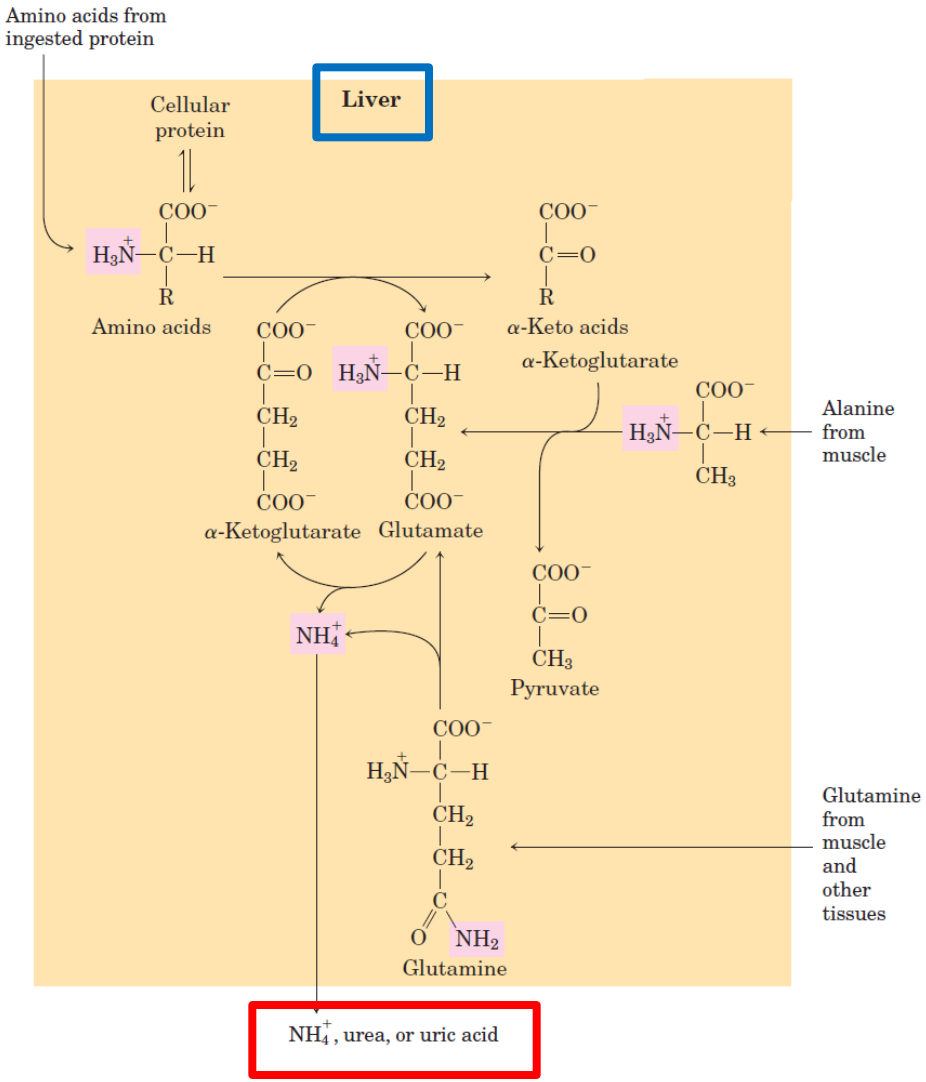
Alanine plays a special role in transporting amino groups to the liver in a nontoxic form, via a pathway called the **glucose-alanine cycle**.

When muscles degrade amino acids for fuel, amino groups are collected in the form of glutamate by transamination. Glutamate can be converted to glutamine for transport to the liver, as described above, or it can transfer its -amino group to pyruvate, a readily available product of muscle glycolysis, by the action of **alanine aminotransferase**.

The alanine so formed passes into the blood and travels to the liver. In the cytosol of hepatocytes, alanine aminotransferase transfers the amino group from alanine to -ketoglutarate, forming pyruvate and glutamate. Glutamate can then enter mitochondria, where the glutamate dehydrogenase reaction releases NH_4^+, or can undergo transamination with oxaloacetate to form aspartate, another nitrogen donor in urea synthesis.

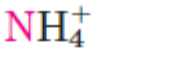


Excretory forms of nitrogen



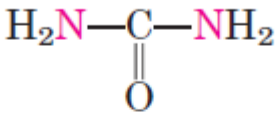
Excess NH_4^+ is excreted as ammonia (microbes, bony fishes), urea (most terrestrial vertebrates), or uric acid (birds and terrestrial reptiles).

Excretory forms of nitrogen



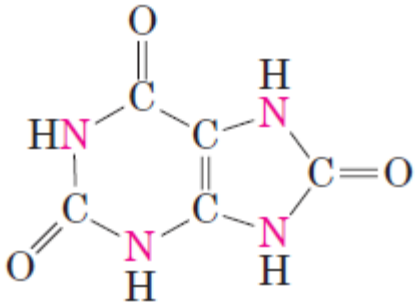
Ammonia (as ammonium ion)

Ammonotelic animals: most aquatic vertebrates, such as bony fishes and the larvae of amphibia



Urea

Ureotelic animals: many terrestrial vertebrates; also sharks

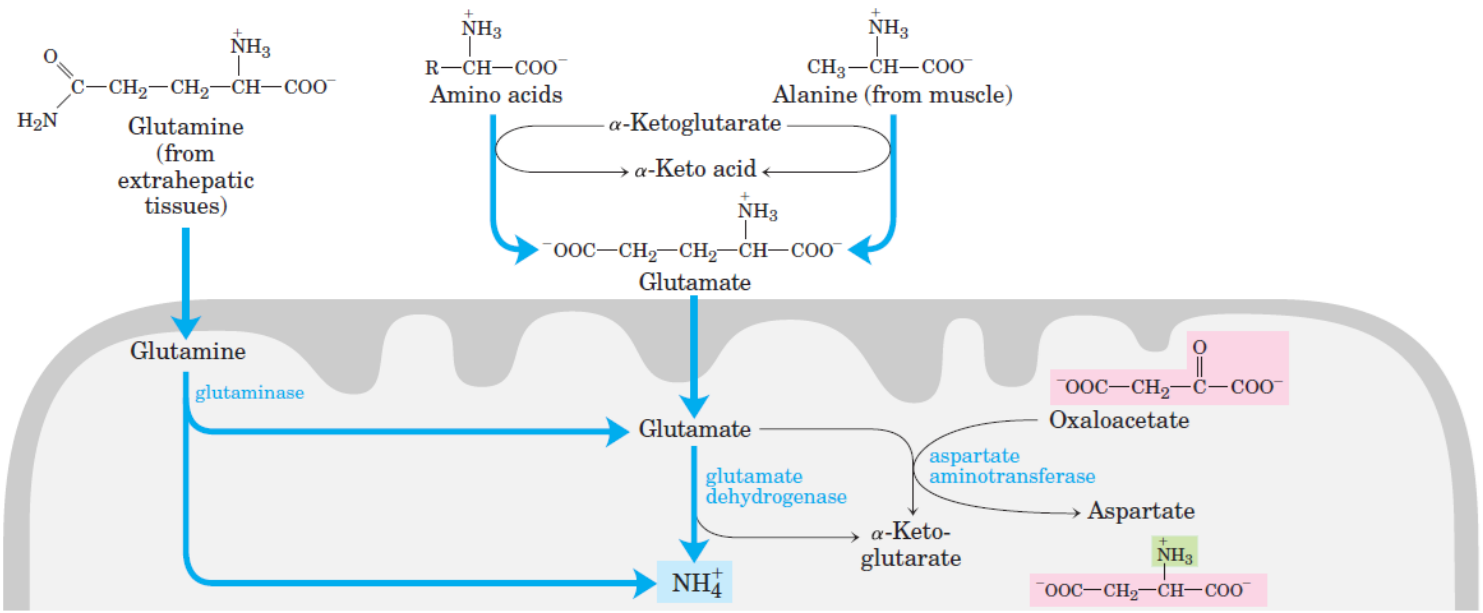


Uric acid

Uricotelic animals: birds, reptiles

Carbon atoms of urea and uric acid are highly oxidized; the organism discards carbon only after extracting most of its available energy of oxidation.

Liver



Liver mitochondria

NH_4^+

Ammonia (as ammonium ion)

Ammonotelic animals: most aquatic vertebrates, such as bony fishes and the larvae of amphibia

Ammonia is toxic and must be quickly excreted or transformed into a non-toxic compound as soon as it is formed.

1. Heart: direct toxic action of ammonium ion + hyperkalemia (tachyarrhythmias, fibrillation, arrest).

2. Central Nervous System: direct action of ammonium ion on motor neurons (reduction of postsynaptic inhibition due to alteration of ionic exchanges) with convulsive activity.

3. Lungs: pulmonary edema (increased capillary permeability following autonomic reflex stimuli) + polypnea (edema and acidosis)

Urea cycle and reactions that feed amino groups