Catabolism of Proteins & Amino Acid Nitrogen

University of Anbar/College of Pharmacy

Second semester 2020-2021 / Biochemistry II / 3rd stage

References :

1- Harper’s Illustrated Biochemistry

2- Lehninger Principles of Biochemistry

By

Dr. Muthanna Owaid Hussein

Dietary Protein Is Enzymatically Degraded to Amino Acids

In humans, the degradation of eaten proteins to their component amino acids occurs in the gastrointestinal tract. Entry of dietary protein into the stomach stimulates the gastric mucosa to **secrete** the hormone gastrin, which in turn stimulates the secretion of hydrochloric acid by the parietal cells and pepsinogen by the chief cells of the gastric glands . The acidic gastric juice (pH 1.0 to 2.5) is both an antiseptic, killing most bacteria and other foreign cells, and a denaturing agent, unfolding globular proteins and rendering their internal peptide bonds more accessible to enzymatic hydrolysis. Pepsinogen (Mr 40,554), an inactive precursor, or zymogen , is converted to active pepsin (Mr 34,614) by an autocatalytic cleavage (a cleavage mediated by the pepsinogen itself) that occurs only at low pH. In the stomach, pepsin hydrolyzes ingested proteins at peptide bonds on the amino-terminal side of Leu and the aromatic amino acid residues Phe, Trp, and Tyr, cleaving long polypeptide chains into a mixture of smaller peptides

In animals, amino acids undergo oxidative degradation in three different metabolic circumstances:

1. During the normal synthesis and degradation of cellular proteins (protein turnover; some amino acids that are released from protein breakdown and are not needed for new protein synthesis undergo oxidative degradation.
2. When a diet is rich in protein and the ingested amino acids exceed the body's needs for protein synthesis, the surplus is catabolized; amino acids cannot be stored.
3. During starvation or in uncontrolled diabetes, when carbohydrates are either unavailable or not properly utilized, cellular proteins are used as fuel.

Proteins are converted to amino acids by digestive enzymes.

Many of the digestive proteases are produced and secreted as inactive zymogens. They are converted to their active forms by the **removal** of a peptide fragment in the lumen of the digestive tract.

The digestion of proteins begins in the stomach, where pepsin converts dietary proteins into smaller polypeptides.

In the lumen of the small intestine, proteolytic enzymes produced by the pancreas (trypsin, chymotrypsin, elastase, and the carboxypeptidases) cleave the polypeptides into oligopeptides and amino acids.

The digestive enzymes produced by the **intestinal epithelial cells** (aminopeptidases, dipeptidases, and tripeptidases) cleave the small peptides to amino acids.

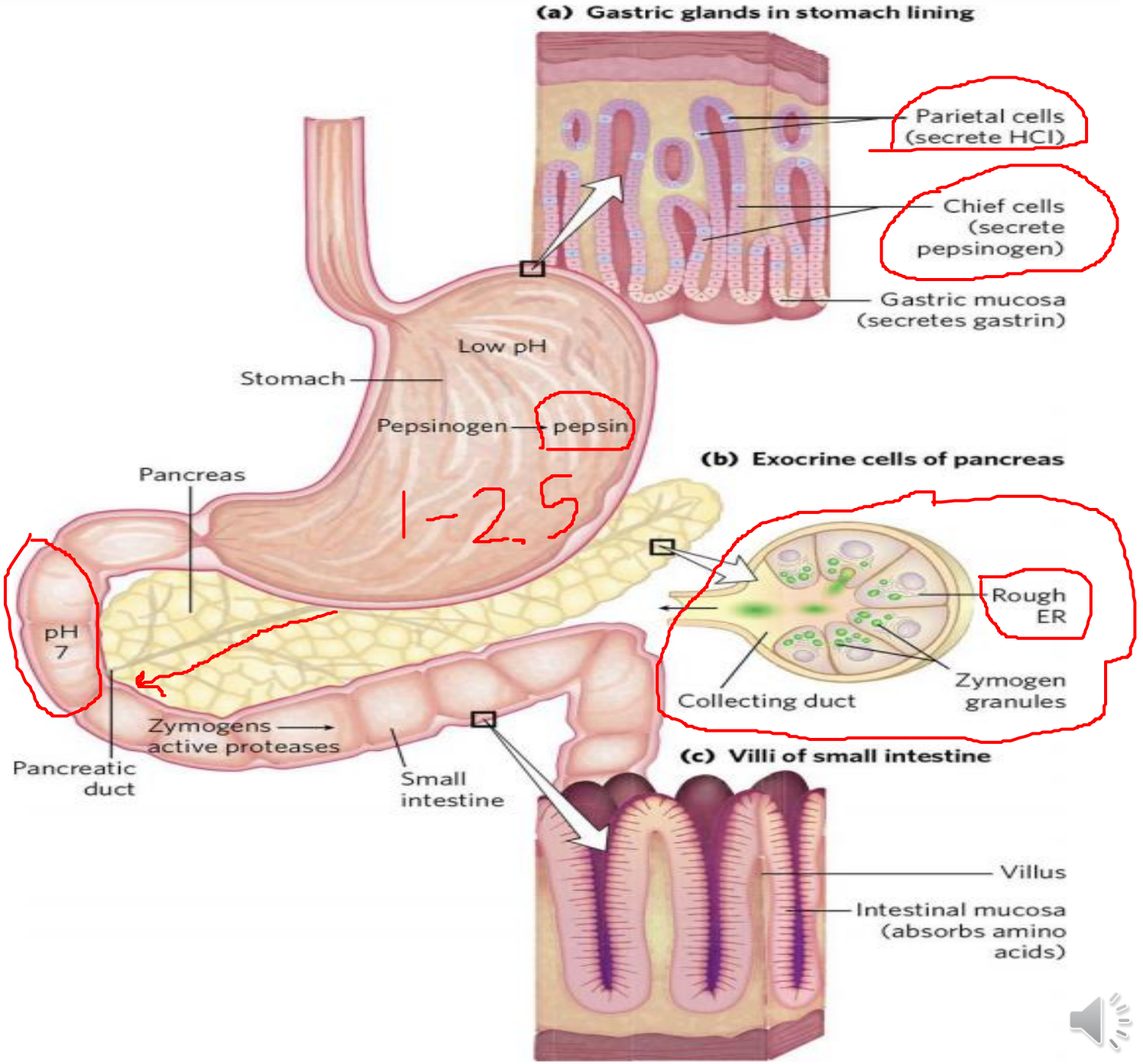
Amino acids, the final products of protein digestion, are absorbed through intestinal epithelial cells and enter the blood

Part of the human digestive (gastrointestinal) tract.

(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.

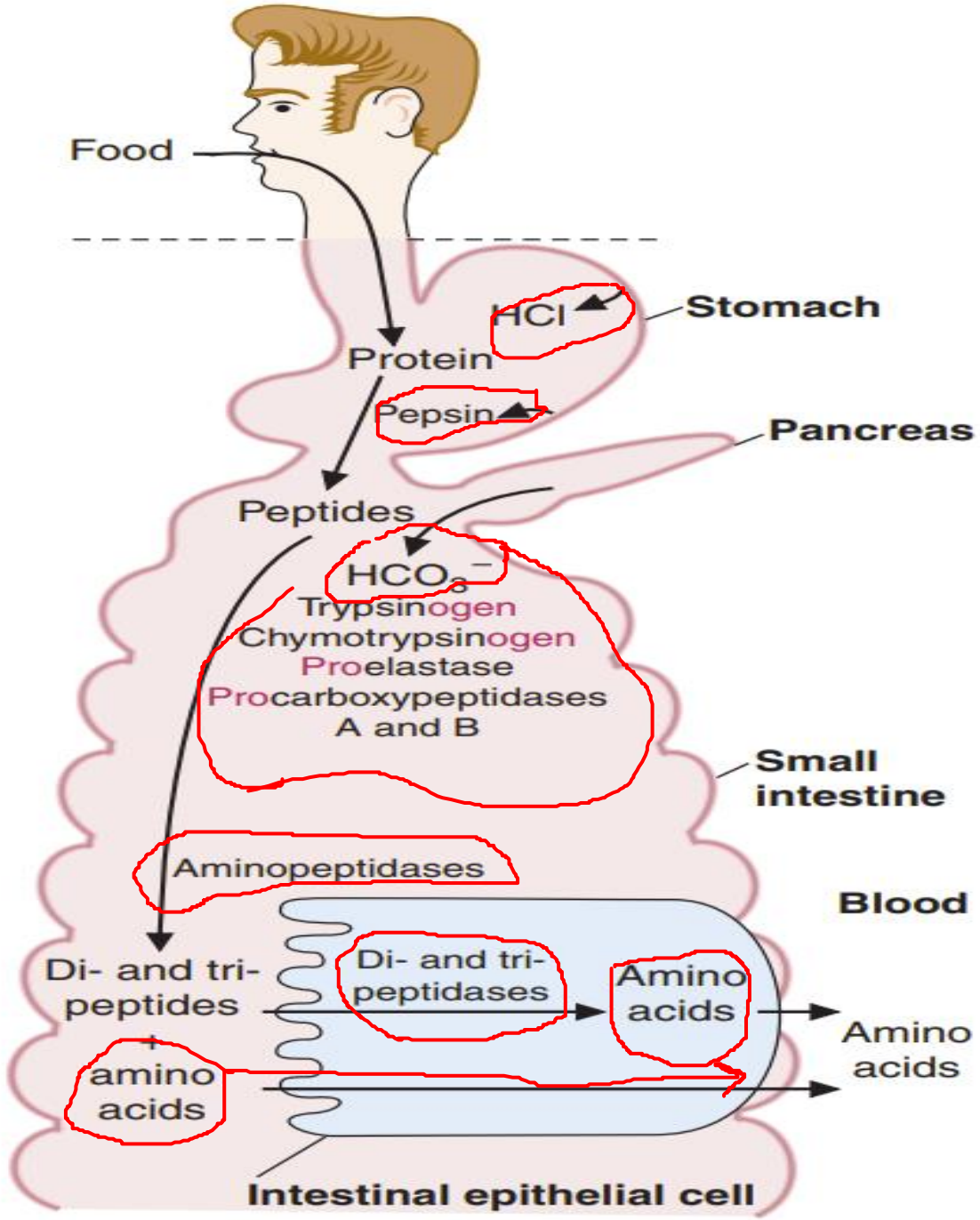
(b) The cytoplasm of exocrine cells of the pancreas is completely filled with rough endoplasmic reticulum, the site of synthesis of the zymogens of many digestive enzymes.

(c) In the small intestine, amino acids are absorbed through the epithelial cell layer (intestinal mucosa) of the villi and enter the capillaries.



The digestion of proteins

The proteolytic enzymes trypsin, chymotrypsin, elastase, and the carboxypeptidases are produced as zymogens (the [pro] and [ogen], in red, accompanying the enzyme name) that are activated by cleavage after they enter the gastrointestinal lumen.



Pepsin has a broad specificity but tends to **cleave** peptide bonds in which the amino group is contributed by the aromatic amino acids or by leucine.

Trypsin cleaves peptide bonds in which the carboxyl group is contributed by arginine or lysine

Chymotrypsin usually cleaves peptide bonds in which the carboxyl group is contributed by the aromatic amino acids or by leucine. Chymotrypsinogen, the inactive zymogen, is cleaved to form chymotrypsin by trypsin.

Elastase cleaves at the carboxyl end of amino acid residues with small, uncharged side chains such as alanine, glycine, or serine. Proelastase, the inactive zymogen, is cleaved to elastase by trypsin.

Carboxypeptidase A cleaves aromatic amino acids from the C-terminal end of peptides.

Carboxypeptidase B cleaves the basic amino acids, lysine and arginine, from the C-terminal end of peptides

Aminopeptidases are exopeptidases produced by the intestinal cells that **cleave** one amino acid at a time from the N-terminal end of peptides.

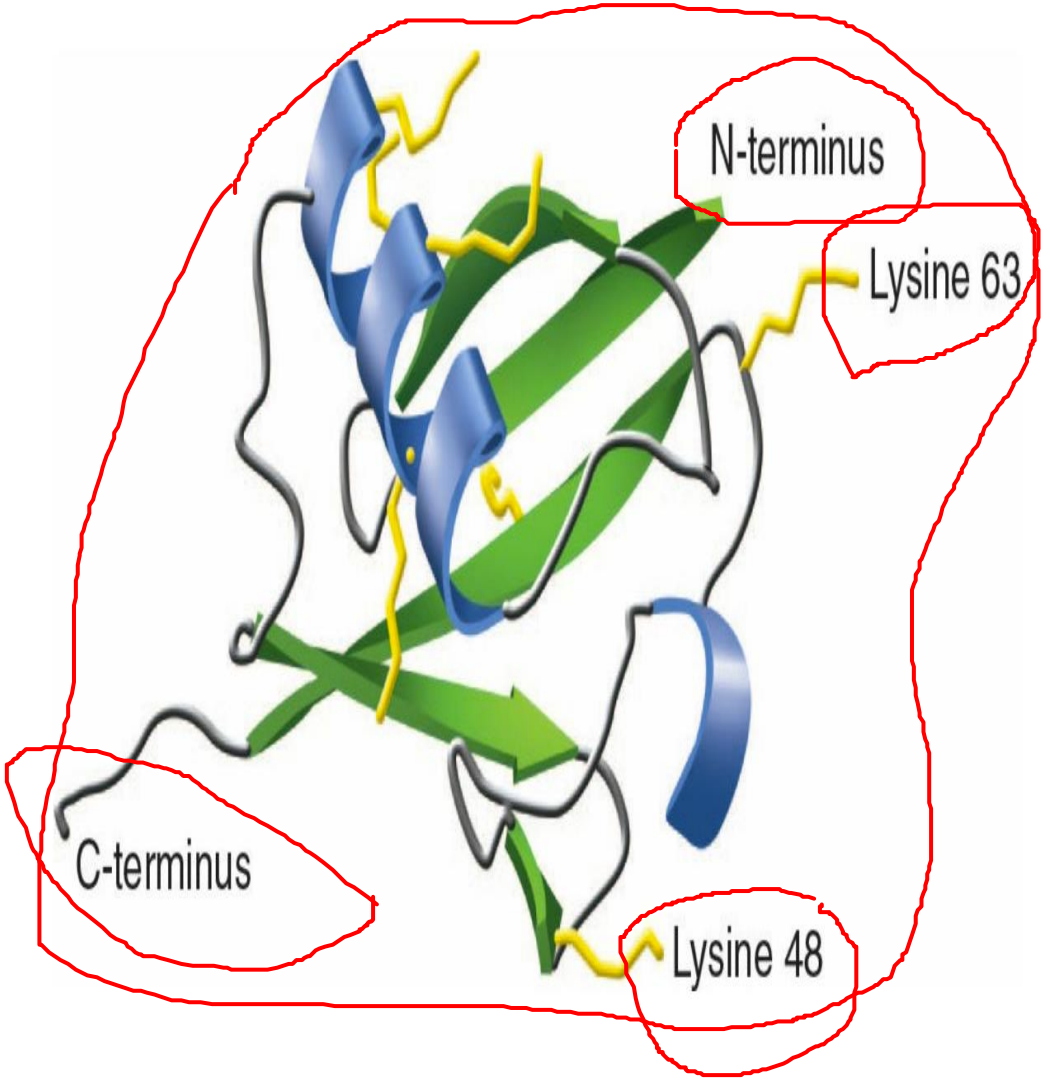
Dipeptidases and tripeptidases associated with the intestinal cells produce amino acids from dipeptides and tripeptides.

ATP & Ubiquitin-Dependent Degradation

Degradation of regulatory proteins with short half-lives and of abnormal or misfolded proteins occurs in the cytosol and requires ATP and ubiquitin. Named based on its presence in all eukaryotic cells, ubiquitin is a small.

Reactions involved in the attachment of ubiquitin (Ub) to proteins.

Three enzymes are involved. E1 is an activating enzyme, E2 a transferase, and E3 a ligase.



Ubiquitin-proteasome proteolytic pathway:

Proteins selected for degradation by this mechanism

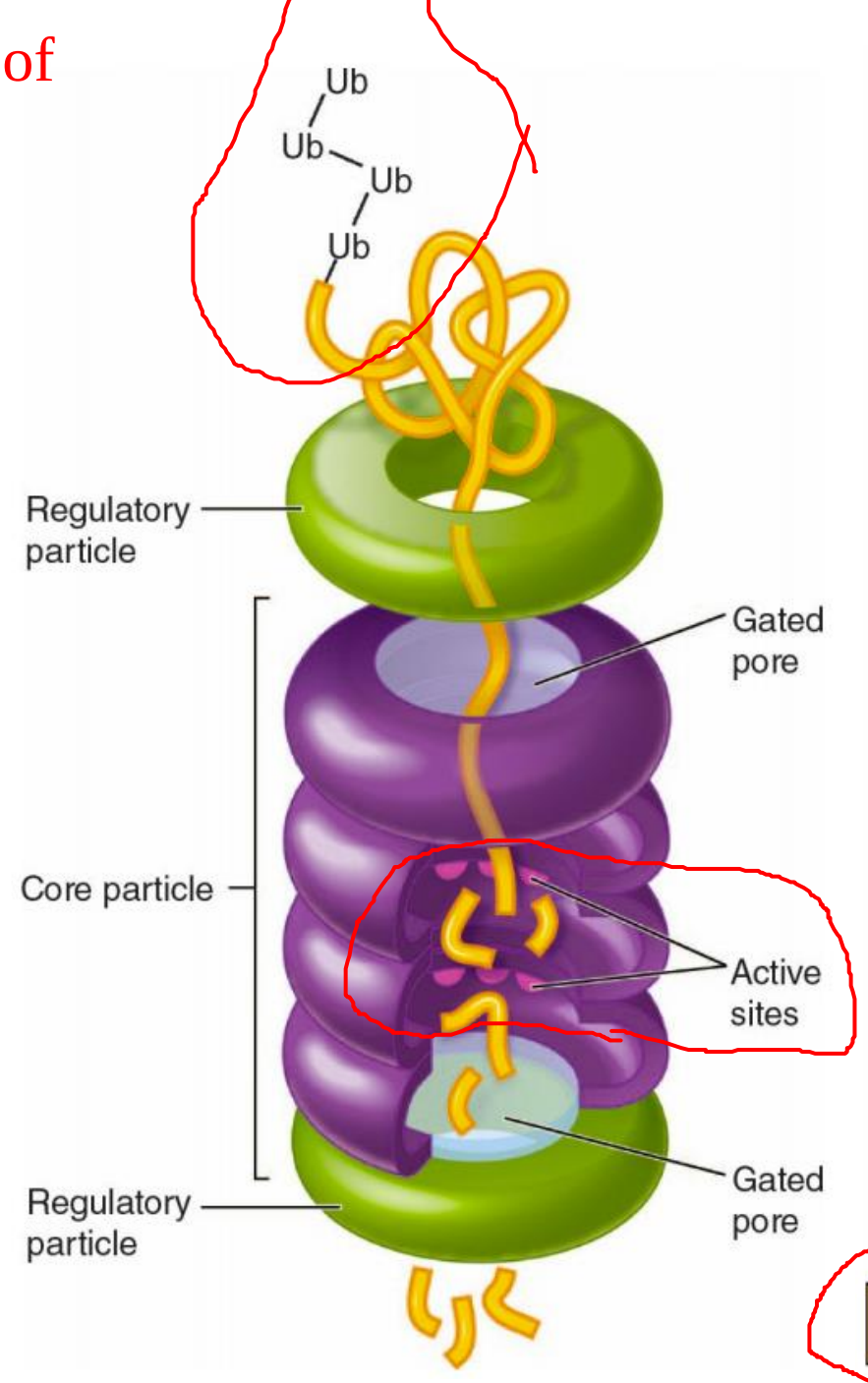
1. Ubiquitination: linkage of ubiquitin with target substrate (protein) take place in three step enzyme catalyzed process: The target protein first covalently attached to ubiquitin, the linkage of the α-carboxyl glycine of ubiquitin to a lysine$\in -amino\ group$ on protein.

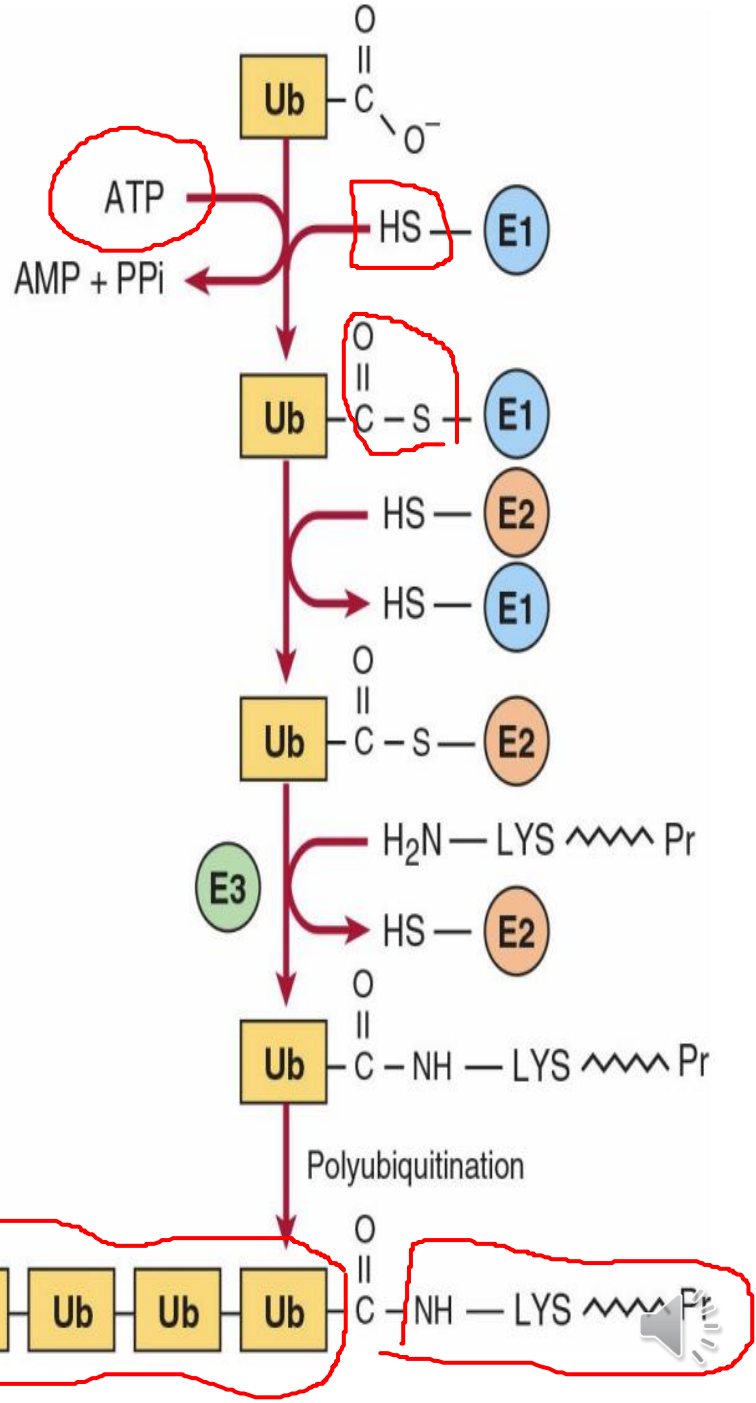
2. The following of addition of ubiquitin moieties generates a polyubiquitin chain.

3. Protein that labelled with ubiquitin are then recognized by a large barrel shaped macromolecular proteolytic complex called a proteasome which cuts the target protein into fragments that are then further degraded to amino acids, which enter the amino acid pool.

Representation of the structure of a proteasome.

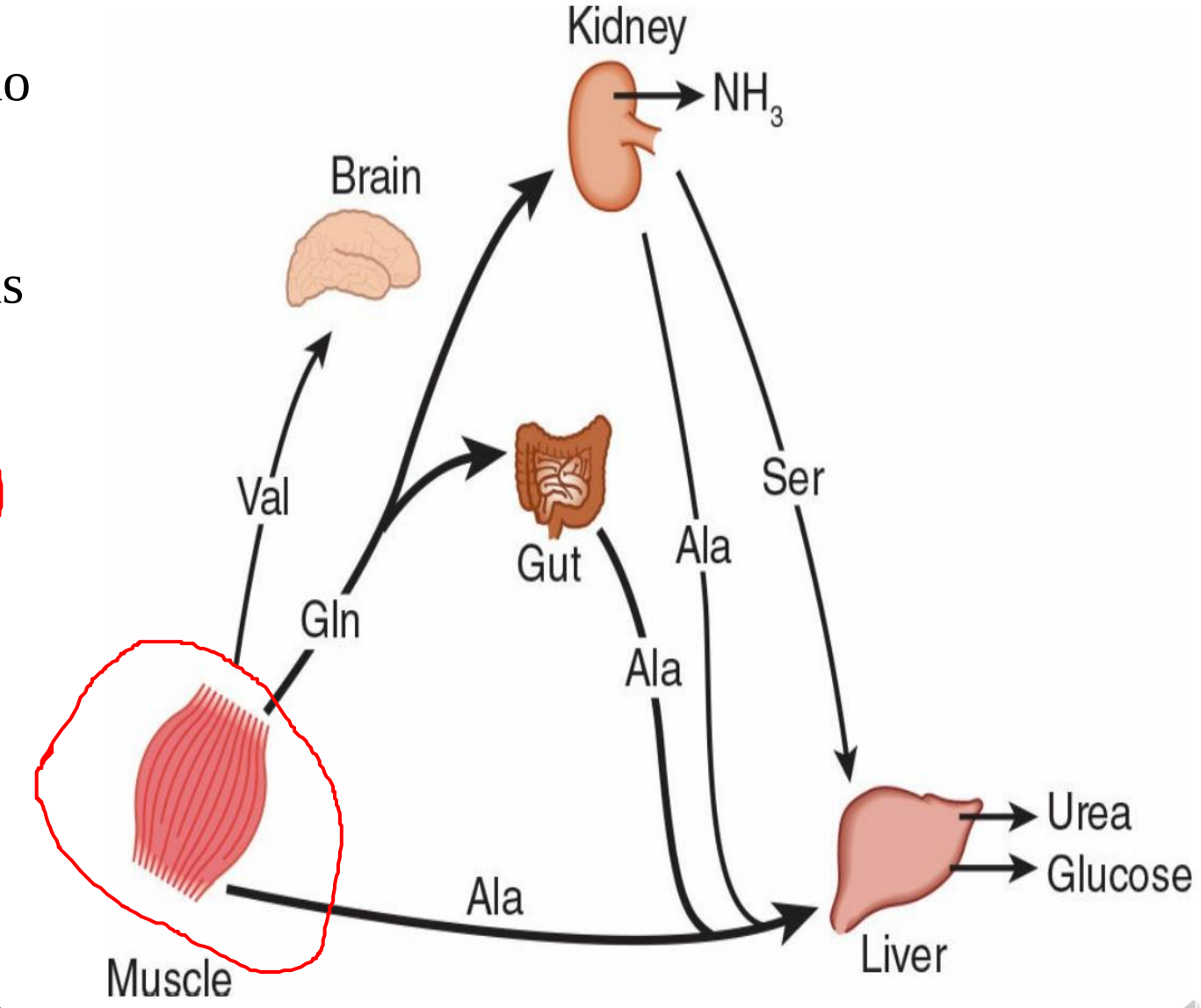
The upper ring is gated to permit only polyubiquitinated proteins to enter the proteosome, where immobilized internal proteases degrade them to peptides.



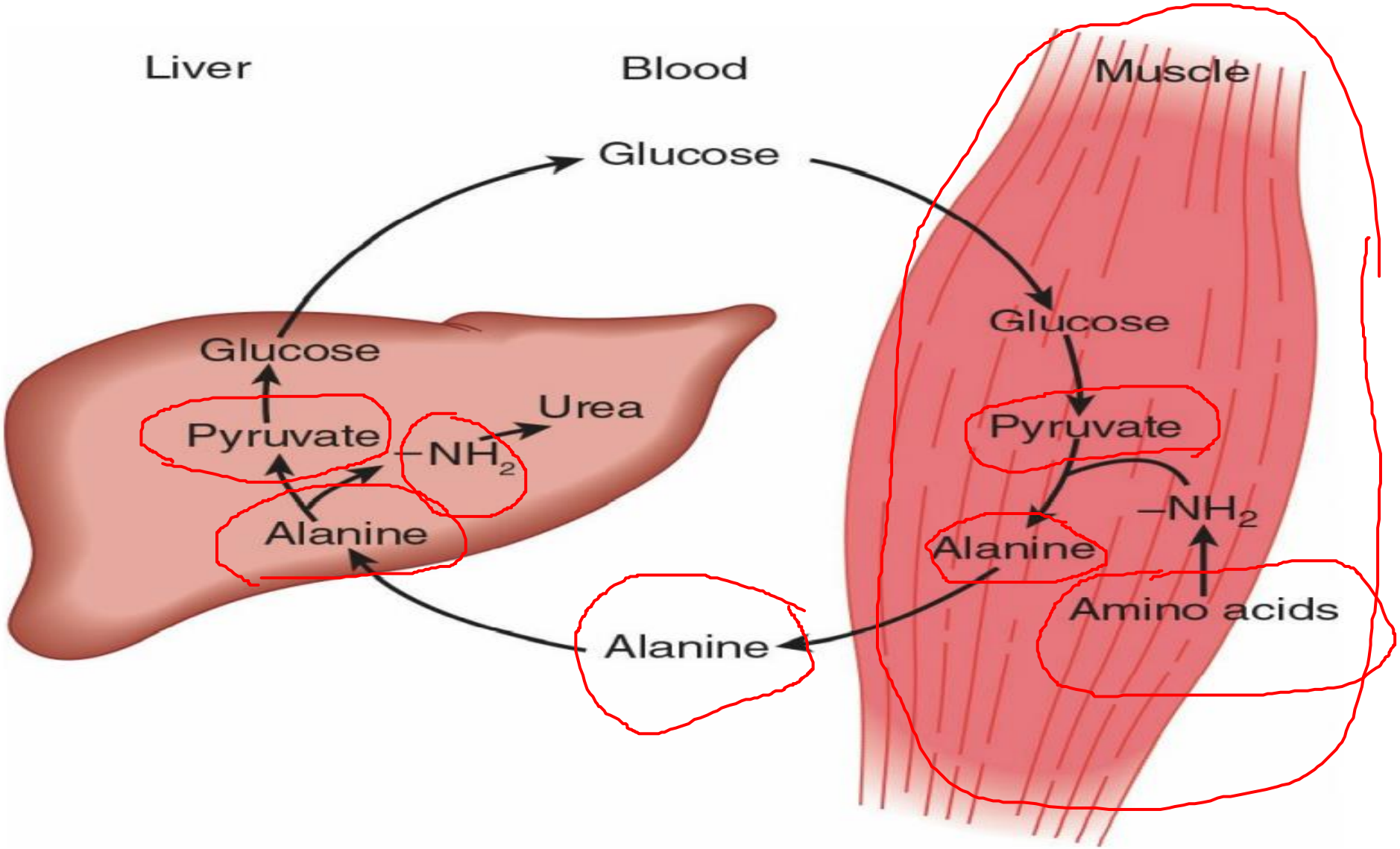


NTERORGAN EXCHANGE MAINTAINS CIRCULATING LEVELS OF AMINO ACIDS

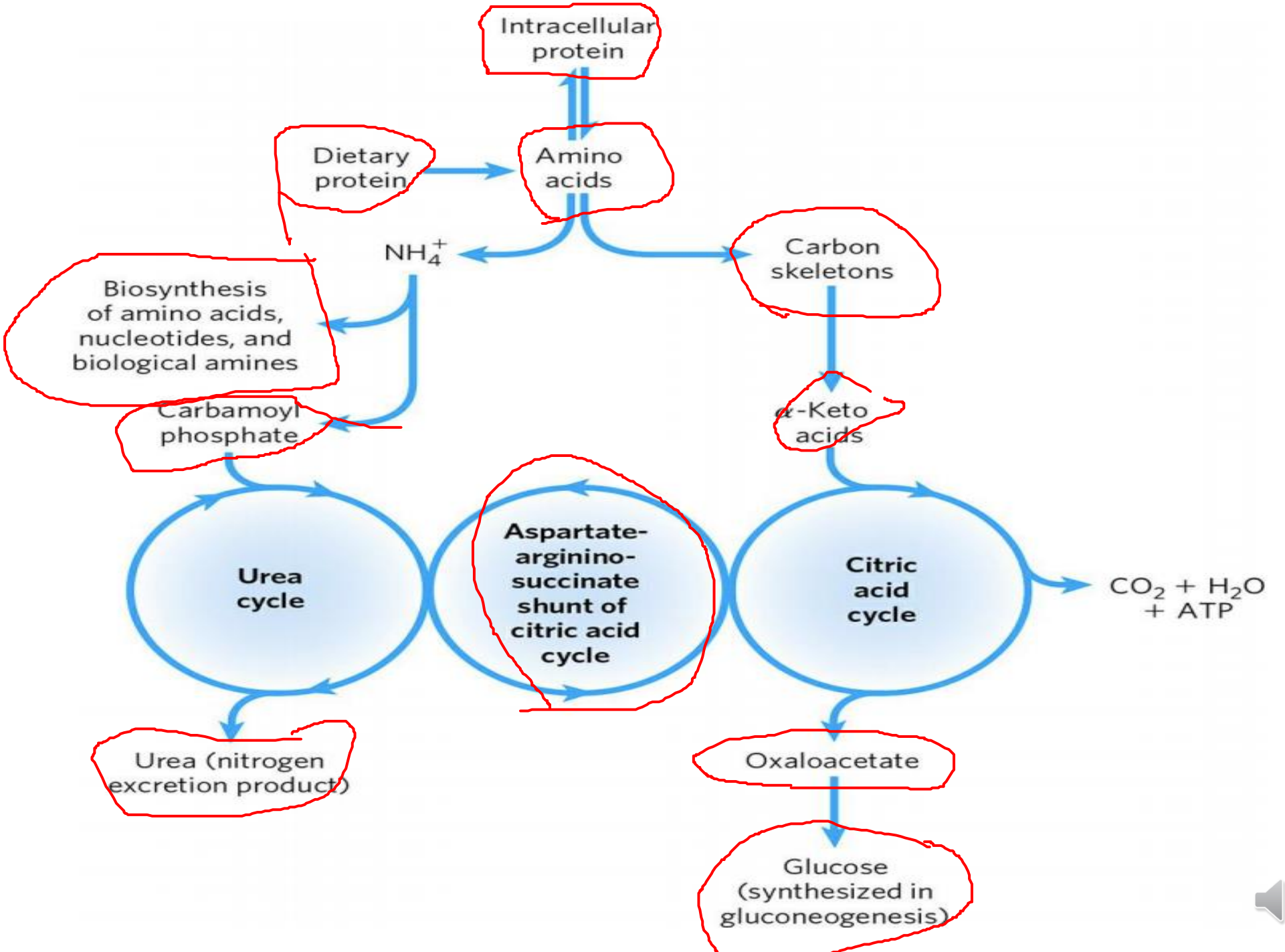
The maintenance of steady-state concentrations of circulating plasma amino acids between meals depends on the net balance between release from endogenous protein stores and utilization by various tissues. **Muscle** generates over half of the total body pool of free amino acids, and liver is the site of the urea cycle enzymes necessary for disposal of excess nitrogen. Muscle and liver thus play major roles in maintaining circulating amino acid levels.



Alanine is a key gluconeogenic amino acid. The rate of hepatic gluconeogenesis from alanine is far higher than from all other amino acids.



Overview of amino acid catabolism in mammals.



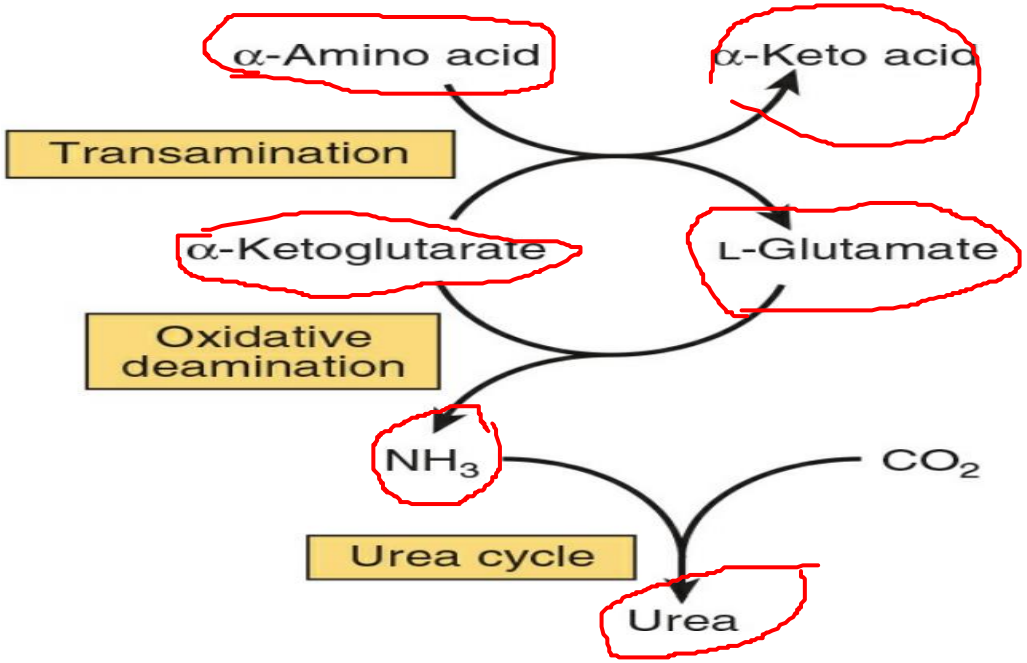
ANIMALS CONVERT α-AMINO NITROGEN TO VARIOUS END PRODUCTS

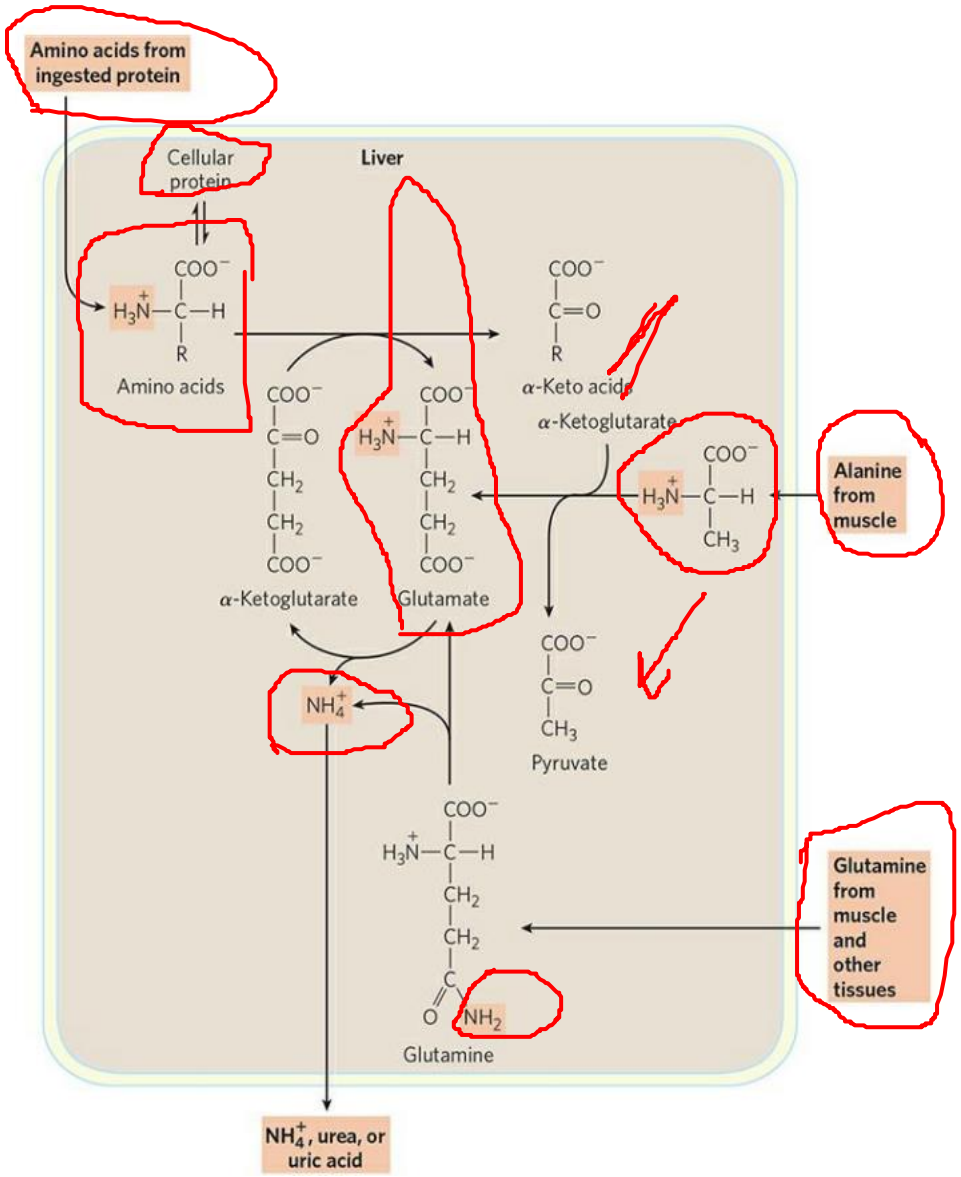
Depending on their ecological role and physiology, different animals excrete excess nitrogen as ammonia, uric acid, or urea.

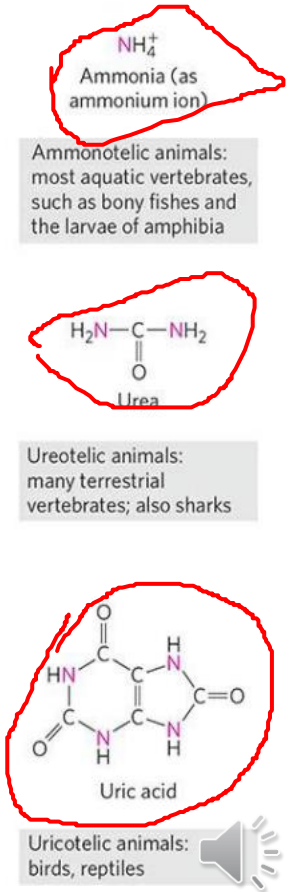
BIOSYNTHESIS OF UREA

Urea biosynthesis occurs in four stages

(1) Transamination
(2) oxidative deamination of glutamate
(3) ammonia transport
(4) reactions of the urea cycle

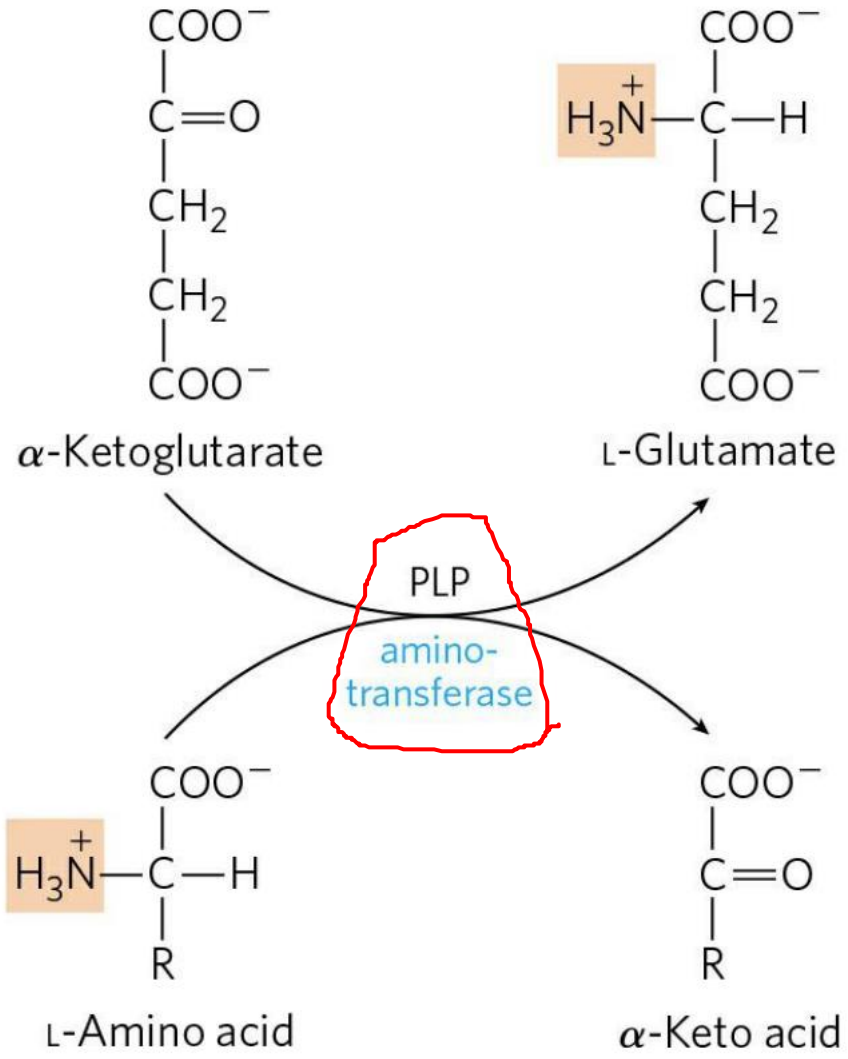


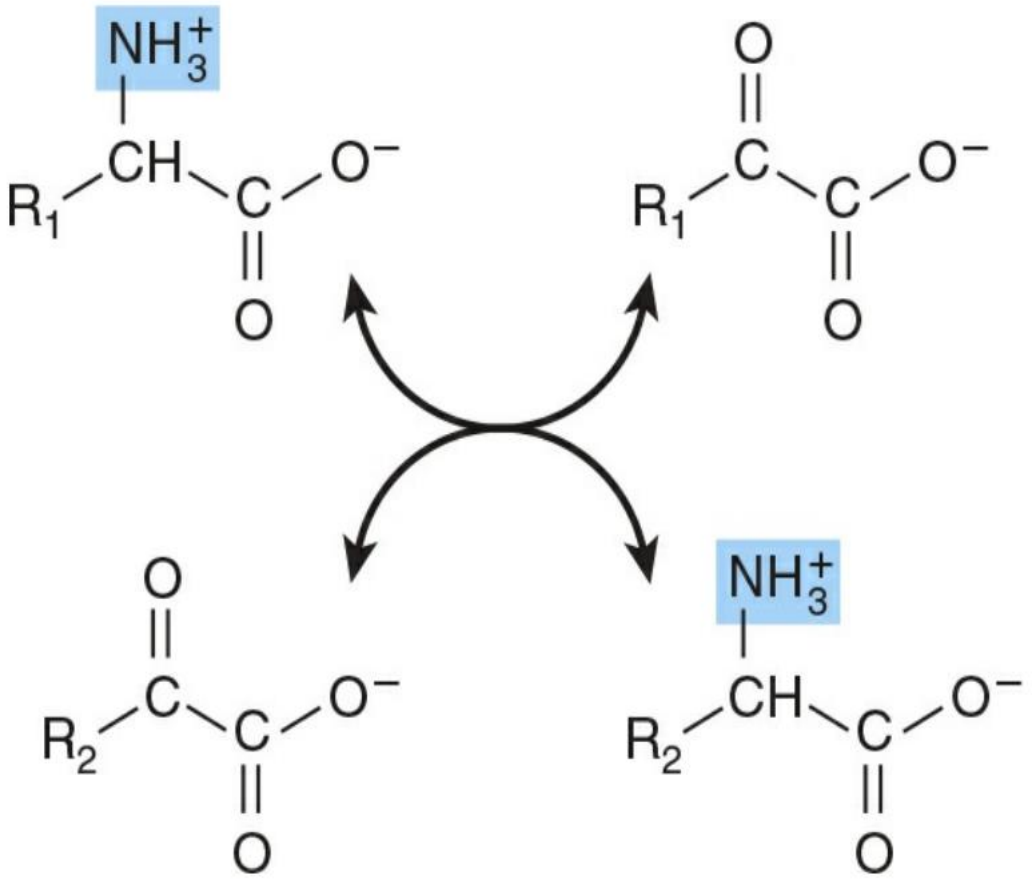




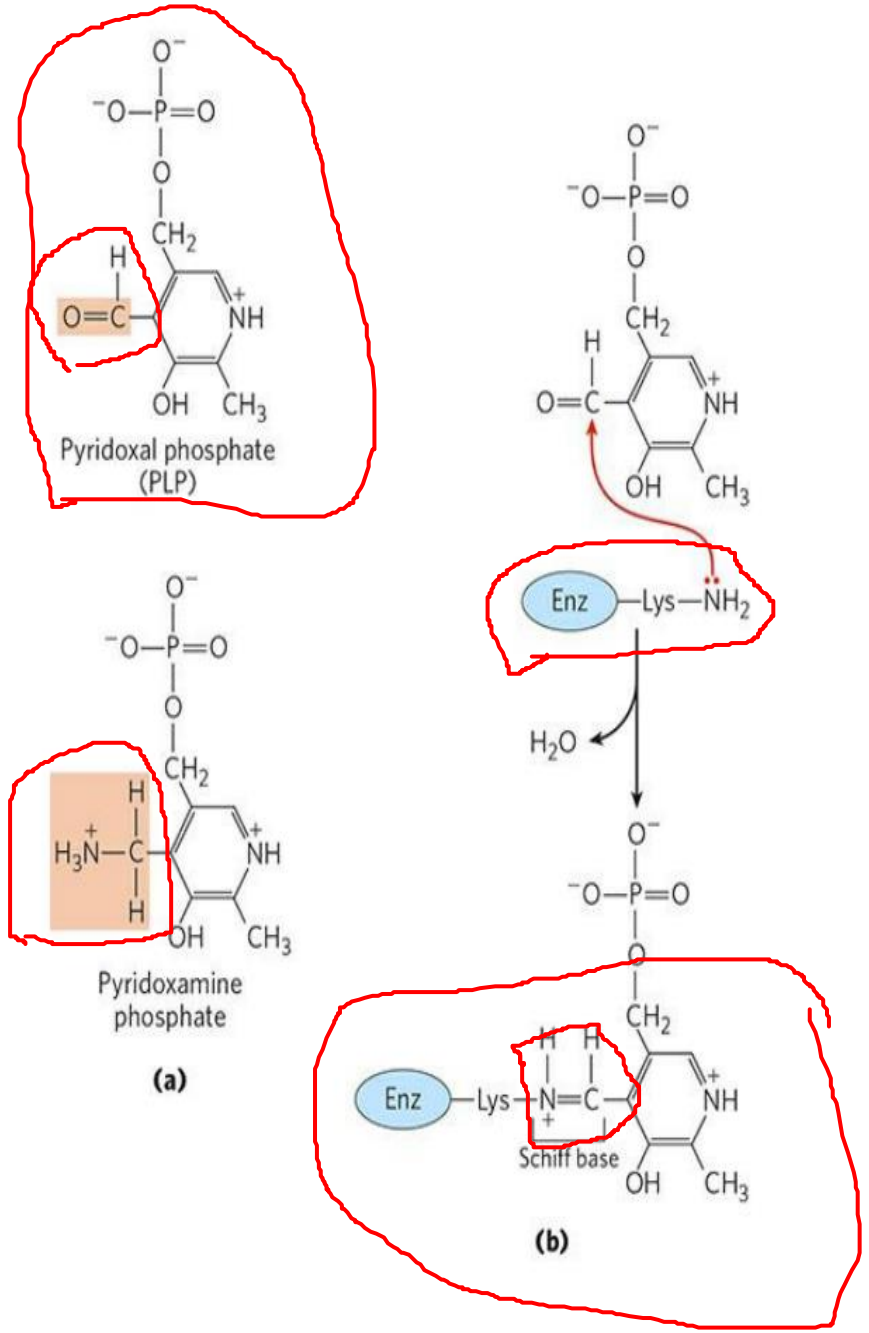
Transamination Transfers α-Amino Nitrogen to α-Ketoglutarate, Forming Glutamate

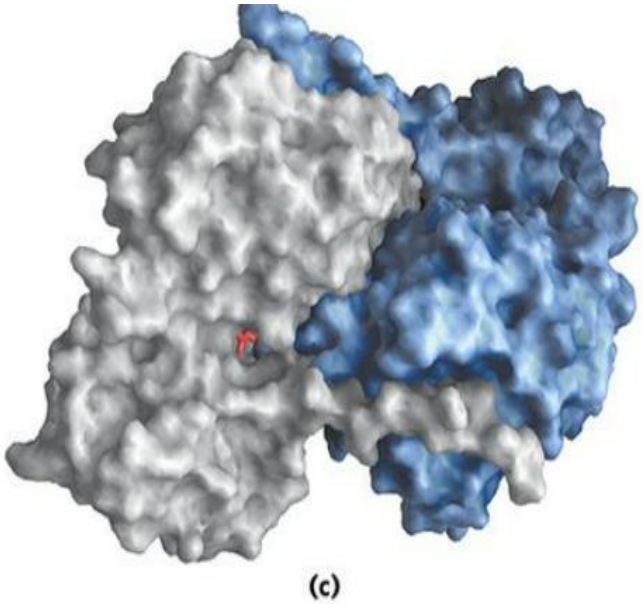
Transamination reactions interconvert pairs of α-amino acids and α-keto acids

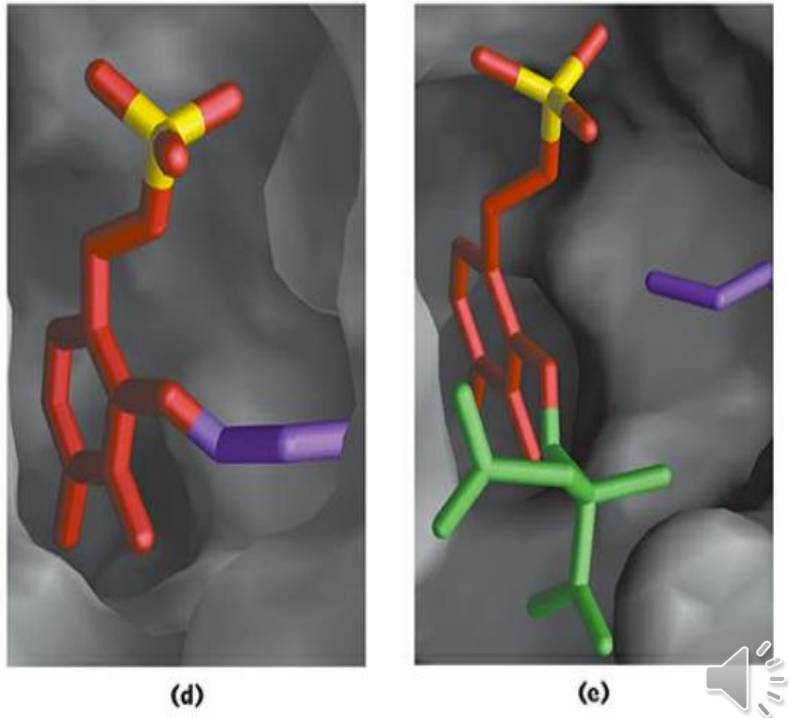




Pyridoxal phosphate, the prosthetic group of aminotransferases. (a) Pyridoxal phosphate (PLP) and its aminated form, pyridoxamine phosphate, are the tightly bound coenzymes of aminotransferases. The functional groups are shaded. (b) Pyridoxal phosphate is bound to the enzyme through noncovalent interactions and a Schiff-base (aldimine) linkage to a Lys residue at the active site.







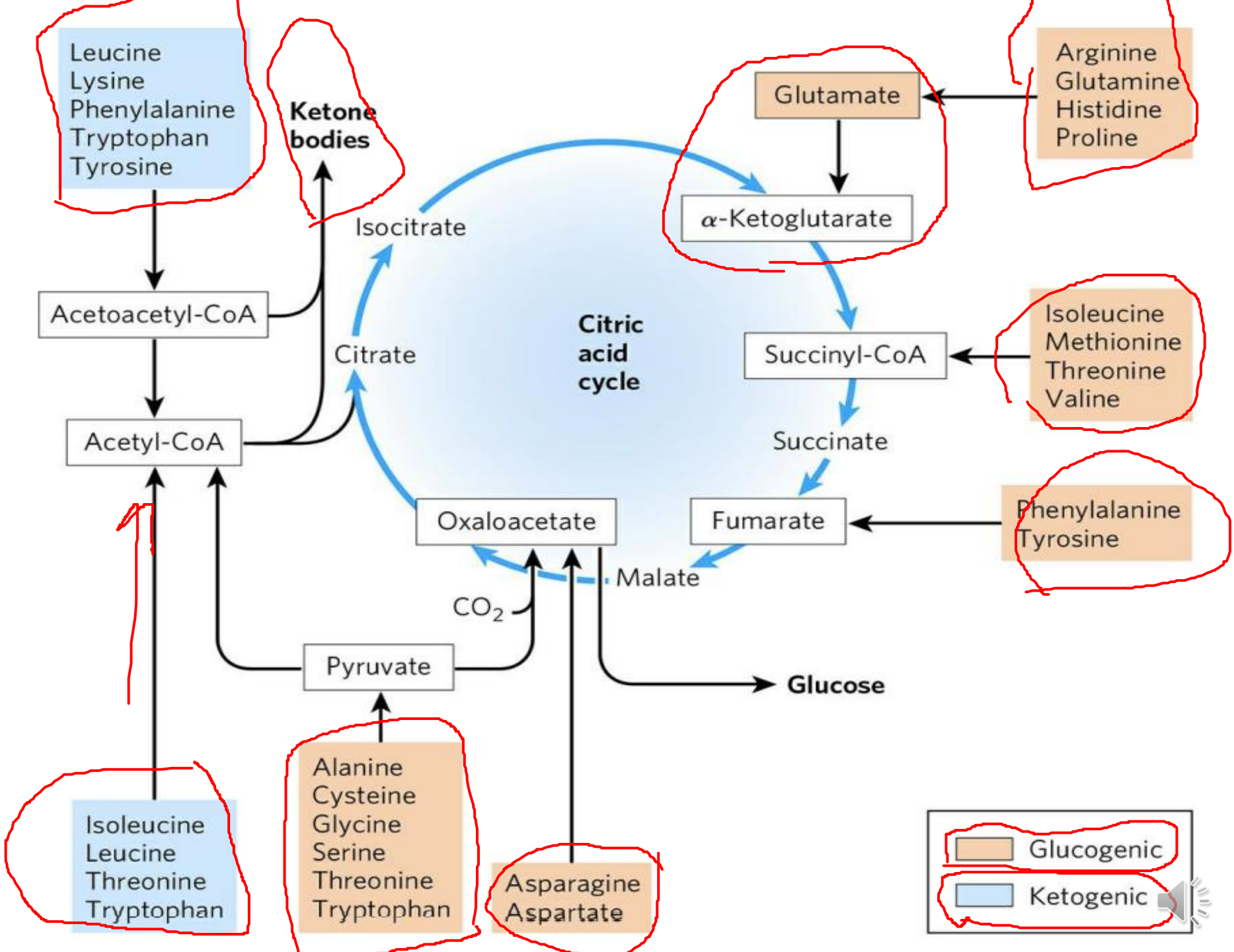
Mechanism action of aminotransferases:

- All aminotransferases require the coenzyme pyridoxal phosphate (a derivative of vitamin B), which is covalently linked to the ε-amino group of a specific lysine residue at the active site of the enzyme.

* Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino acid, at the same time regenerating the original aldehyde form of the coenzyme.

Pathways of Amino Acid Degradation

Summary of amino acid catabolism. Amino acids are grouped according to their major degradative end product.



Reactions of the urea cycle

NH4 + and aspartate provide the nitrogen that is used to produce urea, and CO_2 provides the carbon. Ornithine serves as a carrier that is regenerated by the cycle.

Carbamoyl phosphate is synthesized in the first reaction from NH4+, CO2, and two ATP. Inorganic phosphate and two ADP are also produced. Enzyme: carbamoyl phosphate synthetase I, which is located in mitochondria and is activated by N-acetylglutamate.

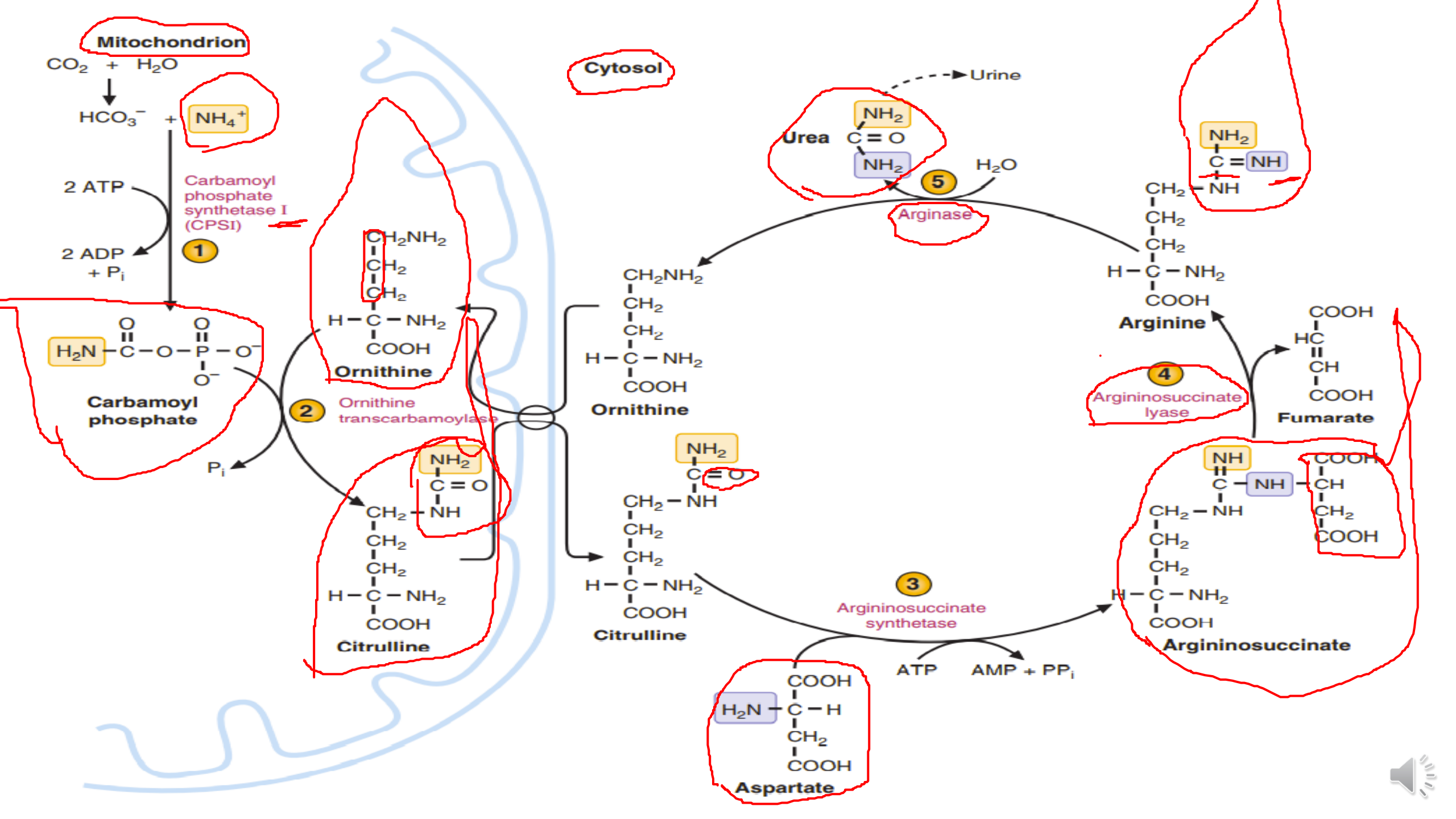
Ornithine reacts with carbamoyl phosphate to form citrulline. Inorganic phosphate is released. Enzyme: ornithine transcarbamoylase, which is found in mitochondria. The product, citrulline, is transported to the cytosol in exchange for cytoplasmic ornithine.

Citrulline combines with aspartate to form argininosuccinate in a reaction that is driven by the hydrolysis of ATP to AMP and inorganic pyrophosphate. Enzyme: argininosuccinate synthetase

Argininosuccinate is cleaved to form arginine and fumarate. Enzyme: argininosuccinate lyase. This reaction occurs in the cytosol.

Arginine is cleaved to form urea and regenerate ornithine. Enzyme: arginase, which is located primarily in the liver and is inhibited by ornithine.

Urea passes into the blood and is excreted by the kidneys. The urea excreted each day by a healthy adult (about 30 g) accounts for about 90% of the nitrogenous excretory products.


Mitochondrion
CO_2 + H_2O
HCO_3^- + NH_4^+
2 ATP
2 ADP + P_i
Carbamoyl phosphate synthetase I (CPSI)
1
Carbamoyl phosphate
2
Ornithine transcarbamoylase
P_i
Ornithine
Citrulline
Cytosol
Ornithine
Citrulline
3
Argininosuccinate synthetase
ATP
AMP + PP_i
Aspartate
Argininosuccinate
4
Argininosuccinate lyase
Fumarate
Arginine
5
Arginase
Urea
Urine
H_2O

THANK YOU