31

Sheet Slides

DONE BY

Lujain Hamdan

CONTRIBUTED IN THE SCIENTIFIC CORRECTION

Faisal Nimri

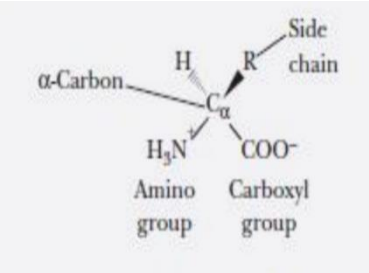
CONTRIBUTED IN THE GRAMMATICAL CORRECTION

...

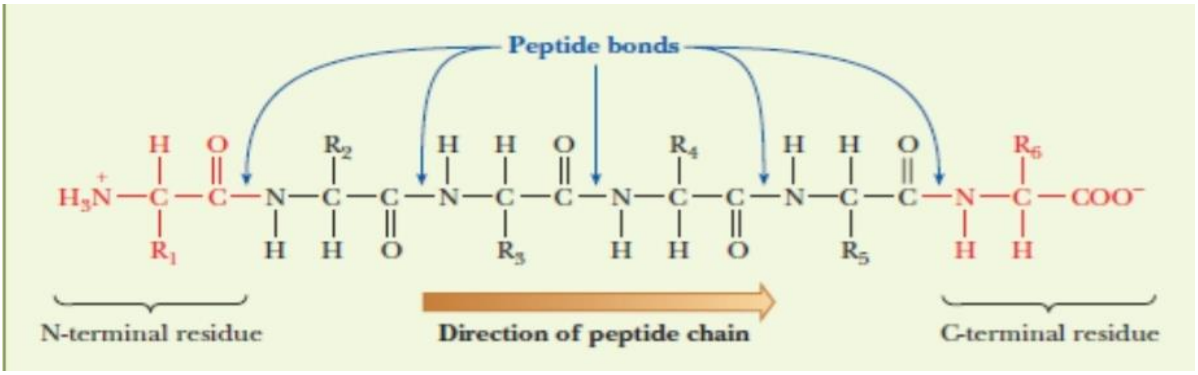
DOCTOR

Diala Abu-Hassan

Amino Acids Metabolism



- Amino acid has an alpha-carbon which is connected to Carboxyl group, Amino group, hydrogen atom and R group which differs from an amino acid to another.
- Some amino acids can share the same metabolic pathway, other amino acids can have specific pathways that are related to their R groups.
- Amino acids are not stored by the body, there are always inputs (sources of AA) or outputs (depletion routes) in the body to achieve balance.
- Peptides contain two to several dozens AA.
- Polypeptide chain contain many amino acids (usually more than a hundred).
- Human proteins are made from L-amino acids.



Amino Acids Catabolism : An overview

⇨ The first phase of catabolism involves the removal of the α-amino groups (usually by transamination and subsequent oxidative deamination), forming ammonia and the corresponding α-keto acid—the "carbon skeletons" of amino acids.

⇨ In the second phase of amino acid catabolism, the carbon skeletons of the α-ketoacids are converted to common intermediates of energy producing, metabolic pathways. These compounds can be metabolized to CO2 and water, glucose, fatty acids, or ketone bodies by the central pathways of metabolism. **Amino Acids** can also be involved in the synthesis of other Nitrogen-containing compounds like creatine, melanin pigment, heme group and hormones like melatonin, serotonin, epinephrine, or norepinephrine.

Amino acid pool

- Free amino acids are present throughout the body, in cells, blood, and the extracellular fluids.
- The AA pool is small, it's about 90–100 g of AAs, The amount of protein in the body is about 12 kg in a 70-kg man.
- In healthy, well-fed individuals, the input to the amino acid pool is balanced by the output, that is, the amount of amino acids contained in the pool is constant.
- The amino acid pool is said to be in a steady state, and the individual is said to be in nitrogen balance.

This pool is supplied by three sources:

1) Degradation of body proteins. To stop their function or to prevent their accumulation.
2) Proteins or amino acids digestion.
3) Synthesis of nonessential amino acids from simple intermediates of metabolism.

The amino pool is depleted by three routes:

1) Synthesis of body protein.
2) Amino acids consumed as precursors of essential nitrogen-containing small molecules.
3) Conversion of amino acids to glucose, glycogen, fatty acids, ketone bodies, or $CO_2 + H_2O$.

Protein turnover

- The process in which the rate of protein synthesis is sufficient to replace the degraded protein. Most proteins in the body are constantly being synthesized and then degraded, permitting the removal of abnormal or unneeded proteins.
- leads to the hydrolysis and resynthesis of 300–400 g of body protein each day.
- In healthy adults, the total amount of protein in the body remains constant.

Rate of turnover :

- The rate of protein turnover varies widely for individual proteins.

i. Short-lived proteins (for example, many regulatory proteins and misfolded proteins) are rapidly degraded, having half-lives measured in minutes or hours. If these proteins stay in the body, they can cause a disease or cancer.
ii. Long-lived proteins, with half-lives of days to weeks, constitute the majority of proteins in the cell.
iii. Structural proteins, such as collagen, are metabolically stable, and have half-lives measured in months or years.

- The half-life of a protein is influenced by the nature of the N-terminal residue. For example : (not for memorization)

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

- It is affected by the mode of expression : how and when this protein is synthesized.

a. Many proteins have induced expression mode, which need certain stimuli to be synthesized. Regulation of synthesis determines the concentration of protein in the cell, with protein degradation assuming a minor role.
b. Other proteins have constitutive expression mode, that the rate of synthesis is relatively constant, and cellular levels of the protein are controlled by selective degradation. "House-keeping genes always stay in the on mode".

Protein Degradation:

- Proteins are tagged for degradation by chemical alterations such as **oxidation** or **ubiquitin tagging.**
- There are two major enzyme systems responsible for degrading damaged or unneeded proteins :

1. The ATP-independent degradative enzyme system of the lysosomes.

- It degrades primarily extracellular proteins, such as plasma proteins that are taken into the cell by endocytosis, and membrane proteins. They are internalized by receptor-

mediated endocytosis. The resulting vesicle is fused with the lysosome to start degradation. It's a simple hydrolysis, doesn't require energy (ATP).

Lysosome: membrane-enclosed organelle, has internal low pH (around 4-5), whereas the cytosol has pH around 6-7 (varies according to the cell type). Lysosome enzymes have acidity resistance, they are called acid hydrolases.

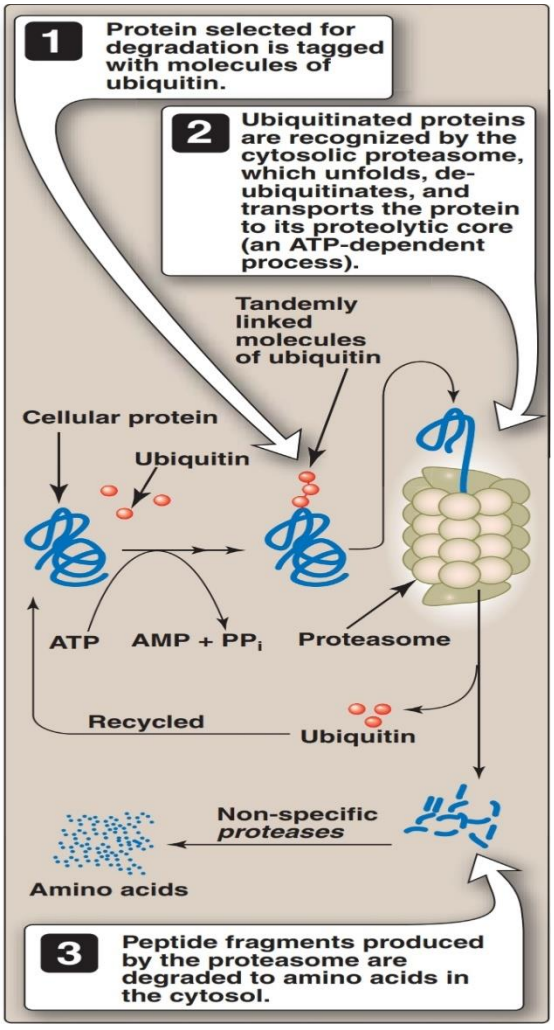
2. The ATP-dependent ubiquitin-proteasome system the cytosol.

- It degrades mainly **endogenous proteins**, that is, proteins that were synthesized within the cell.
- A proteasome is a multi-protein complex that forms a barrel shaped structure which is hollow from the inside (not an organelle).

The selective degradation of proteins by the ubiquitin-proteasome complex (unlike simple hydrolysis by proteolytic enzymes) requires energy in the form of ATP.

Ubiquitin-proteasome proteolytic pathway:

a) Proteins selected for degradation by the ubiquitin-proteasome system are first covalently attached to multiple molecules of ubiquitin, a small, globular, **non-enzymatic protein**.
b) Ubiquitination of the target substrate occurs through linkage of the α-carboxyl group of the C-terminal glycine of ubiquitin to the ε-amino group of a lysine on the protein substrate by a three-step, enzyme-catalyzed, ATP-dependent process.
c) The consecutive addition of ubiquitin moieties generates a polyubiquitin chain.
d) Proteins tagged with ubiquitin are then recognized by the proteasome (a large, barrel-shaped, macromolecular, proteolytic complex, which functions like a garbage disposal.)
e) The proteasome unfolds, deubiquitinates, and cuts the target protein into fragments that are then further degraded to amino acids, which



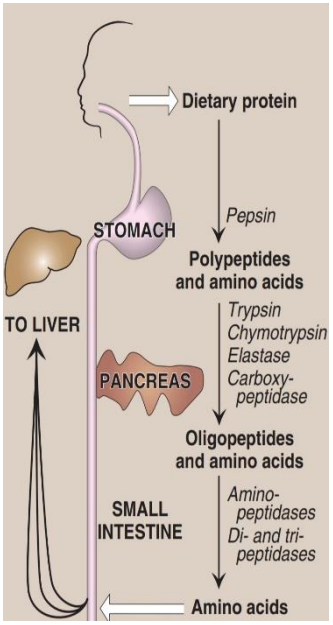
enter the amino acid pool. The ubiquitins are recycled.

DIGESTION OF DIETARY PROTEINS

Cervical cancer :

- In the females, most cases are caused by Human Papillomavirus (mainly strain 16 and 18). This virus is normally found in males and doesn't cause any disease, it's transferred to females by sexual relationships.
- The virus's genome encodes E3 protein, which increases the ubiquitination of P53 **protein** (P53 gene is a tumor suppressor gene)
- P53 inhibits cell division. By the continuous degradation of p53, the proliferation increases, resulting in cancer.
- It takes a long time (10-15 years) to cause cancer.
- This infection can be prevented by a vaccine

- Most of the nitrogen in the diet is consumed in the form of protein, typically amounting to 70–100 g/day in the American diet
- Proteins must be hydrolyzed to yield di- and tripeptides as well as individual amino acids, which can be absorbed.
- Proteolytic enzymes responsible for degrading proteins are produced by three different organs: the stomach, the pancreas, and the small intestine. No protein digestion in mouth or esophagus.



1) Digestion of proteins by gastric secretion

The digestion of proteins begins in the stomach, which secretes gastric juice—a unique solution containing hydrochloric acid and the proenzyme, pepsinogen.

1. Hydrochloric acid :

Stomach acid is too dilute (pH 2–3) to hydrolyze proteins. The acid, secreted by the *parietal cells*, functions instead to :

A. Kill some bacteria.

B. Denature proteins to make them more susceptible to subsequent hydrolysis by proteases.
C. Activation of proenzymes by proteolytic cleavage, in which certain sequence of amino acids is removed from the enzymes structure to open the active site.

2. Pepsin :

- An acid-stable endopeptidase is secreted by the *chief cells* of the stomach as an inactive zymogen (or proenzyme), pepsinogen.
- Zymogens contain extra amino acids in their sequences that prevent them from being catalytically active. Pepsinogen is activated to pepsin by HCl.
- Pepsin releases peptides and a few free amino acids from dietary proteins.

2) Digestion of proteins by pancreatic enzymes in small intestine :

- Small intestine has a higher pH, around 6, which functions to :
  ⇨ Inactivation of pepsin, Activation of pancreatic proenzymes (secreted as zymogens)

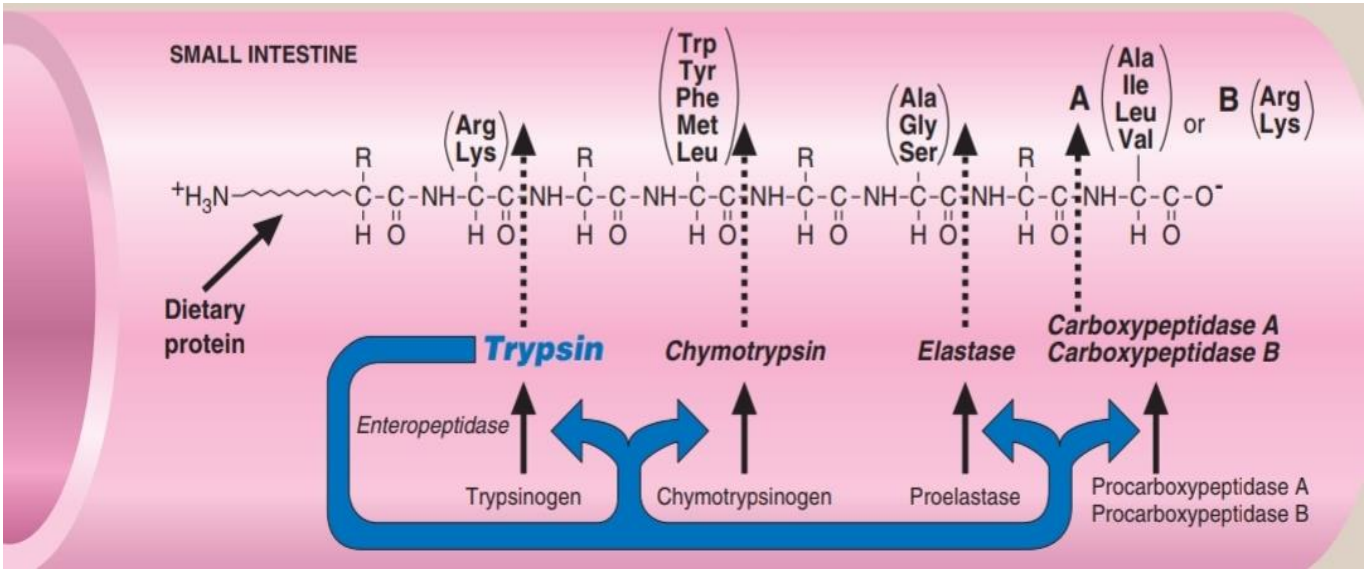
- On entering the small intestine large polypeptides produced in the stomach by the action of pepsin are further cleaved to oligopeptides and amino acids by a group of pancreatic proteases, which are secreted as zymogens :
  ⇨ Endopeptidases : Trypsinogen, Chymotrypsinogen, Proelastase.
  ⇨ Procarboxypeptidase A & B : to cut the peptide bond at the C-terminus.
- Each of these enzymes has a different specificity for the amino acid R-groups adjacent to the susceptible peptide bond. For example, trypsin cleaves only when the carbonyl group of the peptide bond is contributed by arginine or lysine. Elastase cuts after alanine, glycine, or serine.

These notes are mentioned in the slide only :

- 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 luminalsurface of intestinal mucosal cells converts the pancreatic zymogen trypsinogento 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

3) Digestion of oligopeptides by enzymes of the small intestine

The luminal surface of the intestine contains aminopeptidase—an exopeptidase that repeatedly cleaves the N-terminal residue from oligopeptides to produce even smaller peptides and free amino acids.

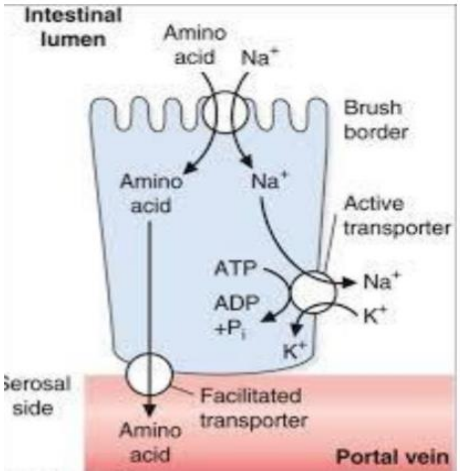


Absorption of amino acids and small peptides

I. Free amino acids are taken into the enterocytes by two mechanisms depending on amino acid type :

- Na+-independent transport.
- Na+-linked secondary transport system of the apical membrane.

II. Di- and tri - peptides, however, are taken up by a H+-linked transport system. The peptides are hydrolyzed in the cytosol to amino acids that are released into the portal system by facilitated diffusion. Thus, only free amino acids are found in the portal vein after a meal containing protein.



》》These amino acids are either metabolized by the liver or released into the general circulation. [Note: Branched-chain amino acids are important examples of amino acids that are not metabolized by the liver, but instead are sent from the liver primarily to muscle via the blood.]

Abnormalities in protein digestion :

1- In individuals with a deficiency in pancreatic secretion (for example, due to chronic pancreatitis, cystic fibrosis, pancreatic cancer, or surgical removal of the pancreas), the digestion and absorption of fat and protein are incomplete, because no lipases and proteases are secreted, So proteins and fats can't be absorbed. Patients suffer malnutrition. This results in the abnormal appearance of lipids (called steatorrhea) and undigested protein in the feces.

2- Celiac disease (celiac sprue) حساسية القمح :

It's an allergy against gluten, a protein found in wheat, barley and rye. The Ingestion of gluten will result in immune-mediated damage to the small intestine. The damaged portion will become flat and smooth, which causes malabsorption and diarrhea.

Patients must eat free-gluten food.

TRANSPORT OF AMINO ACIDS INTO CELLS

- The concentration of free amino acids in the extracellular fluids is significantly lower than that within the cells of the body. This concentration gradient is maintained because active transport systems, driven by the hydrolysis of ATP, are required for movement of amino acids from the extracellular space into cells. At least seven different transport systems are known that have overlapping specificities for different amino acids.
- The small intestine and the proximal tubule of the kidney have common transport systems for amino acid uptake; therefore, a defect in any one of these systems results in an inability to absorb particular amino acids into the gut and into the kidney tubules.
- Some amino acids can share the same transporter, For example, one system is responsible for the uptake of cystine and the dibasic amino acids, ornithine, arginine, and lysine (represented as "COAL").
- Some amino acids has their own transporter like : tryptophan.

In the inherited disorder cystinuria, this carrier system is defective, and all four amino acids appear in the urine. Cystinuria occurs at a frequency of 1 in 7,000 individuals, making it one of the most common inherited diseases, and the most common genetic error of amino acid transport. COAL transport will stop, these amino acids gather in the tubular fluid in the renal system, then they will be excreted in urea. High concentration of them in the tubular fluid leads to oversaturation which ends up with the precipitation of cystine to form kidney stones (calculi). Calculi (also called Urolithiasis) can block the urinary tract. Oral hydration is an important part of treatment for this disorder to dilute the concentration of cystine.

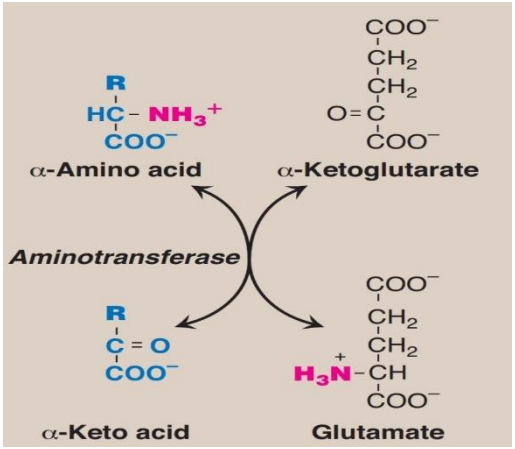
REMOVAL OF NITROGEN FROM AMINO ACIDS

The presence of the α-amino group keeps amino acids safely locked away from oxidative breakdown. Removing the α-amino group is essential for producing energy

from any amino acid, and is an obligatory step in the catabolism of all amino acids. Once removed, this nitrogen can be incorporated into other compounds or excreted, with the carbon skeletons being metabolized.

A. Transamination:

- ⇨ The funneling of amino groups to glutamate.
- ⇨ The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-Ketoglutarate, which becomes glutamate. The products are an α-keto acid (derived from the original amino acid) and glutamate.



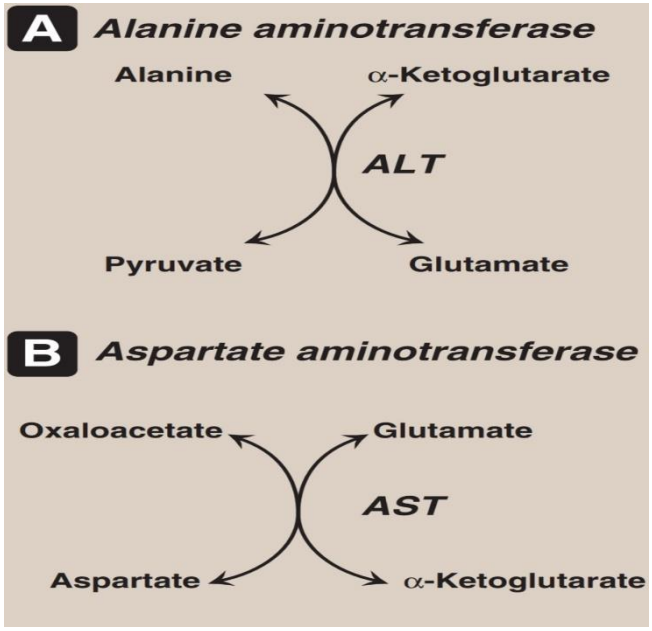
- ⇨ Glutamate produced by transamination can be oxidatively deaminated, or used as an amino group donor in the synthesis of nonessential amino acids.
- ⇨ Each aminotransferase is specific for one or, at most, a few amino group donors. Aminotransferases are named after the specific amino group donor, because the acceptor of the amino group is almost always α-Ketoglutarate.
- ⇨ Transamination needs pyridoxal phosphate as a coenzyme, which carries the amino group at certain steps.

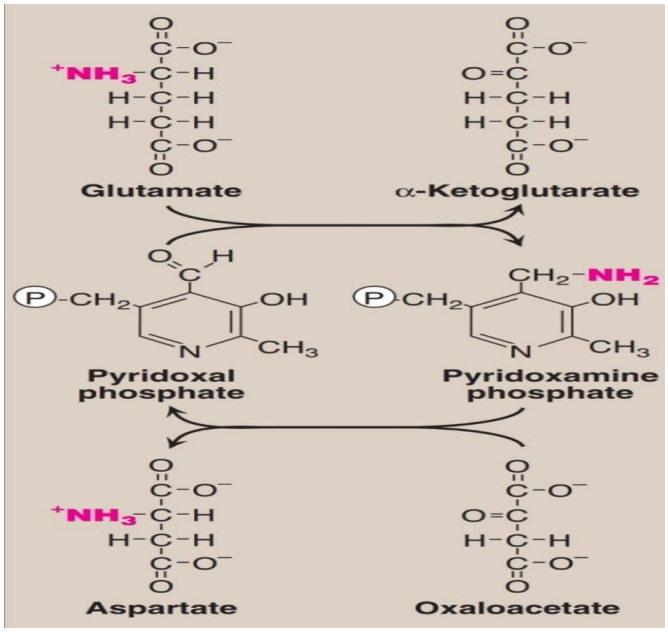
- Alanine aminotransferase (ALT) :

- ✓ It is present in many tissues, catalyzes the transfer of the amino group of alanine to α-Ketoglutarate, resulting in the formation of pyruvate and glutamate.
- ✓ The reaction is readily reversible, It functions in the direction of glutamate synthesis. Thus, glutamate, in effect, acts as a "collector" of nitrogen from alanine.

- Aspartate aminotransferase (AST):

- ✓ AST transfers amino groups from glutamate to oxaloacetate, forming aspartate, which is used as a source of nitrogen in the urea cycle. (It's an exception for aminotransferases' work).

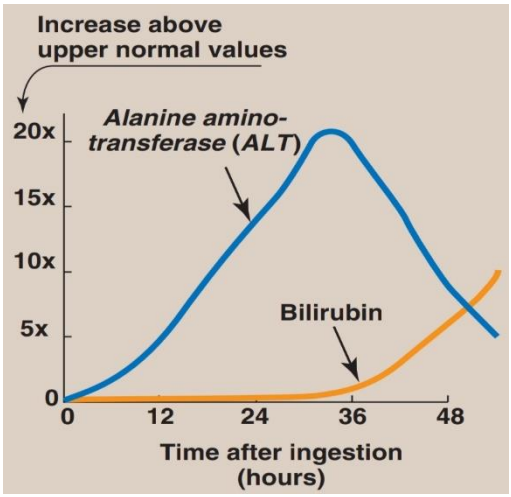




Equilibrium of transamination reactions :

For most transamination reactions, the equilibrium constant is near one. This allows the reaction to function in both amino acid degradation through removal of α-amino groups and biosynthesis through addition of amino groups to the carbon skeletons of α-keto acids. (Keq=1).

Diagnostic value of plasma aminotransferases :



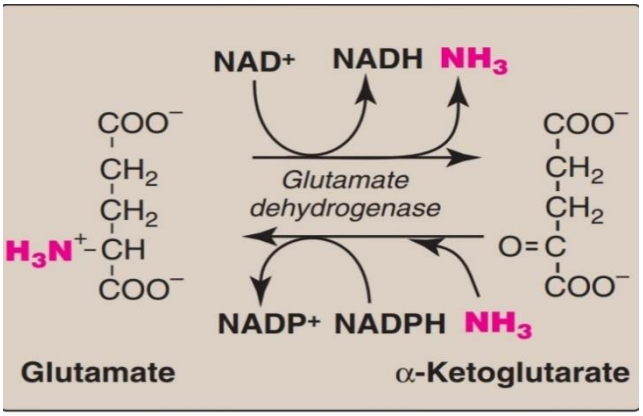
- Aminotransferases are normally intracellular enzymes, with the low levels found in the plasma representing the release of cellular contents during normal cell turnover. The presence of elevated plasma levels of aminotransferases indicates damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lysis, resulting in release of intracellular enzymes into the blood.
- Two aminotransferases—AST and ALT—are of particular diagnostic value when they are found in the plasma :

A. Nonhepatic disease: MI and muscle disorders.

B. Liver disease:

- ✓ Plasma AST, ALT and bilirubin are elevated in nearly all liver diseases, but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis, toxic injury, and prolonged circulatory collapse.
- ✓ ALT is more specific than AST for liver disease because AST is found in cardiac and skeletal muscles.
- ✓ AST is more sensitive because the liver contains larger amounts of AST. Serial enzyme measurements are often useful in determining the course of liver damage.

B. Oxidative Deamination :



Oxidative deamination by glutamate dehydrogenase results in the liberation of the amino group as free ammonia (NH3). These reactions occur primarily in the liver and kidney. They provide **α-keto acids** that can enter the central pathway of energy metabolism, and **ammonia**, which is a source of nitrogen in urea synthesis.

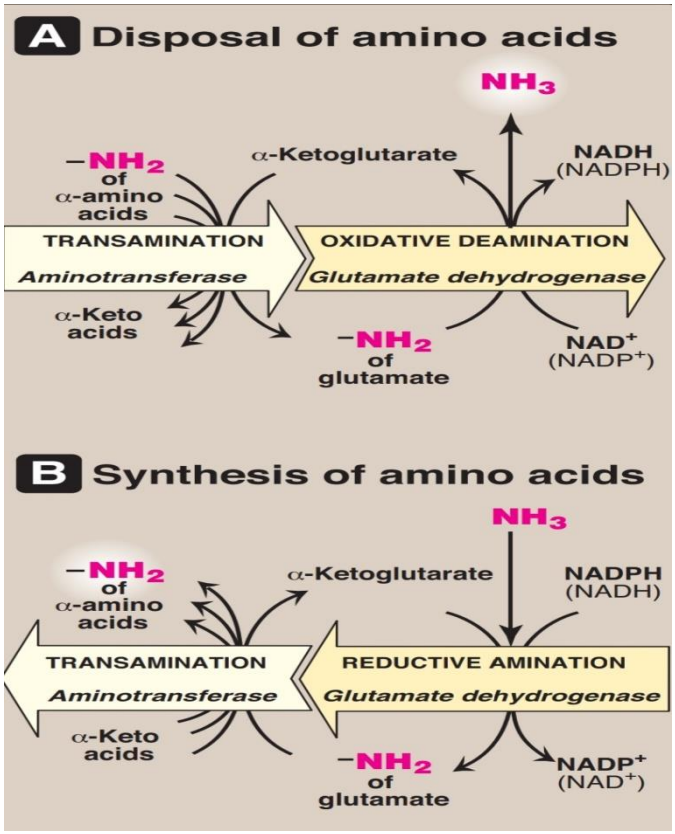
❖ Coenzymes :

Glutamate dehydrogenase can use either NAD+ or NADP+ as a coenzyme.

- NAD+ is used primarily in oxidative deamination (the simultaneous loss of ammonia coupled with the oxidation of the carbon skeleton).
- NADPH is used in reductive amination (the simultaneous gain of ammonia coupled with the reduction of the carbon skeleton).

❖ Direction of reactions :

The direction of the reaction depends on the relative concentrations of glutamate, α-Ketoglutarate, and ammonia, and the ratio of oxidized to reduced coenzymes. For example, after ingestion of a meal containing protein, glutamate levels in the liver are elevated, and the reaction proceeds in the direction of amino acid degradation and the formation of ammonia. [Note: the reaction can also be used to synthesize amino acids from the corresponding α-keto acids.]



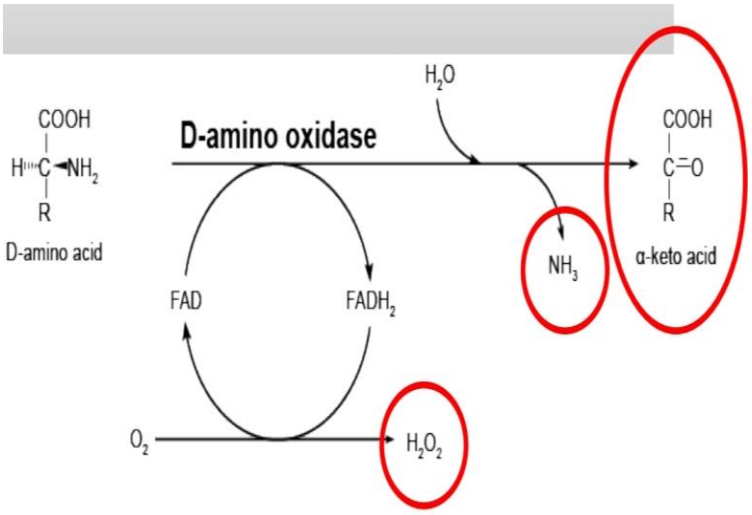
❖ Allosteric regulators :

➔ Guanosine triphosphate (GTP) is an allosteric inhibitor of glutamate dehydrogenase, because it's presence in high conc. means the energy levels are high in the cell.

➔ Adenosine diphosphate (ADP) is an activator. Thus, when energy levels are low in the cell, amino acid degradation by glutamate dehydrogenase is high, facilitating energy production from the carbon skeletons derived from amino acids.

D-Amino acid oxidase

- D-Amino acids are found in plants and in the cell walls of microorganisms, but are not used in the synthesis of mammalian proteins. D-Amino acids are, however, present in the diet, and are efficiently metabolized by the kidney and liver.
- D-Amino acid oxidase (DAO) is an FAD-dependent peroxisomal enzyme that catalyzes the oxidative deamination of these amino acid isomers, producing α-keto acids, ammonia, and hydrogen peroxide.



- The α-keto acids can enter the general pathways of amino acid metabolism, and be reanimated to L-isomers, or catabolized for energy.
- Increased DAO activity has been linked to increased susceptibility to schizophrenia.
- L-amino acid oxidases are components of several snake venoms.

C. 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).

✓ A portion of the free ammonia is excreted in the urine, but most is used in the synthesis of urea which is quantitatively the most important route for disposing of nitrogen from the body.

✓ Why not to dilute ammonia concentration in the plasma to become nontoxic ?

- Because our body doesn't have the enough liquid to do that.

✓ Sources of ammonia :

I. Glutamine: Most of this ammonia is excreted into the urine as NH_4^+ (acid –base balance)

II. 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.

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

IV. Purines and Pyrimidines : In the catabolism of purines and pyrimidines, amino groups attached to the rings are released as NH3.