BIOCHEMISTRY **1st Year, 2nd Semester**

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Lecture 8

IV. PROTEIN METABOLISM

The proteins metabolism is a major component of the general metabolism, since proteins are involved in life related processes.

Proteins make up the basic structure of the living matter (**plastic role**), they are the main molecules performing the biochemical and pHisiological processes (**functional role**), and secondarily they are used for an **energetic purpose**. About 15 - 20% of the daily energy needs is accomplished by proteins. The catabolism of the amino-acids produces 5,5 kcal/mole.

From a chemical point of view, the proteins are organic macromolecules resulting from the poly-condensation of a number of amino-acids within a polypeptide chain. Within the metabolic processes, the protein synthesis consists in condensation of amino-acids, while the constituent amino-acids are obtained by proteins hydrolysis.

The proteins needs of the body per time unit is established both quantitatively as well as qualitatively.

Quantitatively, it can be established by knowing the quantity of amino-acids which is lost within the time unit by biochemical processes of irreversible transformation, with loss of the nitrogen from the molecule. The nitrogen represents 16,5 % of the proteins mass.

This nitrogen is eliminated through urine (95% as urea), perspiration, and fecal matter. The food intake of nitrogen is estimated by measuring the contents of nitrogen of the food ingested, while by measuring the contents of nitrogen of the excreted compounds (urea and ammonia from urine and perspiration, undigested proteins from fecal matter), N excreted is obtained.

The difference between N food intake and N excreted = **NITROGEN BALANCE**
This can have the following values:

- 0 (zero) – healthy body, adult;
- + (positive) - growth, pregnancy, lactation, convalescence;
- - (negative) – consumptive diseases, bleeding, unbalanced nutrition.

Qualitatively, the protein needs depend on the type of amino-acids they contain, in terms of the possibility of the human body to synthesize them. Thus, of the 21 proteinogenic amino-acids:

- 11 are **bio-synthesizable** (can be produced in the body);

- **2 (Arg, His) are partially bio-synthesizable** (the exogenous contribution is needed only during the growth period; as an adult, the body is capable to synthesize all the required quantity of these amino acids);
- **8 (Val, Leu, Ile, Met, Lyz, Phe, Trp, Thr) are non-synthesizable (essential),** their exogenous contribution being mandatory throughout the entire lifetime.

If only one of the essential amino-acids is scarce, some proteins cannot be produced and, under such circumstances, the nitrogen balance becomes negative irrespective of the contents of the other amino-acids. During this type of scarcity, the hydrolysis of the muscle structure proteins occurs in order to release the essential scarce amino-acid, while the rest of amino-acids from the hydrolysis are catabolized with the excretion of nitrogen.

The biological value of food proteins is measured by comparing the proportion of essential amino-acids from the food with the proportion needed for a good nutrition. The biological value of proteins (B.V.) is an indicator of their quality and represents the quantity of protein nitrogen retained by the body from a certain ingested protein.

$$B.V. = (N_{retained}/N_{ingested}) \times 100$$

A biological value of 100 signifies that a certain protein ingested is completely used by the body, with no loss. The proteins biological value differs according to food and depends on some factors, such as calories input, strenuous activities, and quantity of proteins ingested.

The biological value of the main food proteins

Proteins source	Biological value
Eggs (egg white)	100
Milk	93
Fish, beef	75
Corn	72
Wheat	44

The quantity of protein ingested is in an inverse proportional relation with the biological value. Thus, a proteins input from the dairy products in intake of 0,2 g/kg is associated to a biological value of almost 100, while increasing its intake to 0,5 g/kg leads to a decrease of the B.V. to 70.

In order to appreciate the biological value of proteins, the nitrogen balance is used, calculated for subjects whose diet, for a certain period of time, does not contain any proteins and who are then administered different quantities of proteins.

The biological value is actually the relation between the nitrogen balance and the uptake of proteic N. Regular average nutritional habits, with a mixture of vegetal and animal proteins have a global biological value of 70, 1 g proteins/kg body/day is needed for an adult with a null nitrogen balance. If the protein biological value is smaller, the demand will be proportionally higher. In general, the animal proteins (except for the collagen) have a biological value higher than the vegetal proteins.

In the case of vegetarians, in order for the amino-acids availability to be optimum, it is necessary that the diet of these persons should comprise simultaneously both cereal,

as well as vegetables, since they have a different content of essential amino-acids. Both vegetables as well as cereal contain valine, threonine, phenylalanine, leucine. Cereals, unlike vegetables, have a low content of isoleucine and lysine, but they are excellent sources of methionine and tryptophan.

IV.1. Digestion and absorption of proteins

The food proteins are enzymatically hydrolyzed into the digestive tract up to the constituent amino-acids which are absorbed by the intestine.

The proteolytic enzymes (peptidases) act on the peptidic bonds within the proteins and can be divided into:

- **Endo-peptidase** – cut the interior of the polypeptide chain;
- **Exo-peptidase (amino-peptidase and carboxy-peptidase)** – cut off the N-terminal or C-terminal amino-acids.

Most of these enzymes are synthesized as inactive catalytic form, named **pro-enzyme** or **zymogene,** in order to prevent the hydrolysis of its own structures. For this reason, the activation process occurs only into the gastric cavity or the intestinal lumen, in order for the activated enzymes to act only on the food proteins. Activation of pro-enzymes is a process of removing certain blocking polypeptide ends, a process leading to the exposure of the active center of the proteolytic enzymes, which becomes catalytically active.

The proteolytic enzymes (the peptidase) can be found within the 3 digestive juices: gastric, pancreatic and intestinal.

I. Gastric Digestion

The first action from the proteins digestion process is represented by their contact with the hydrochloric acid from the gastric juice, which leads to their denaturation. A destruction of the tertiary structure, an unfolding of the polypeptide chains occur, the denatured proteins becoming accessible to the enzymatic attack of the endopeptidases from the stomach.

The following peptidases can be found within the gastric juice: pepsin, gastricsin and rennin.

a) **Pepsin** – is secreted by the gastric mucosa as the inactive form of pepsinogen, which upon the contact with the hydrochloric acid or in a self-catalytic manner, loses a polypeptidic fragment of 44 amino-acids (terminal N) activating itself into pepsin. The cataytic action of the pepsin consists in breaking the peptide bonds at the level of the amino group of the amino-acids Phe or Tyr.
b) **Gastricsin** – represents 1/3 – 1/2 of the gastric peptide action in the case of an adult, and almost completely in the case of infants. The enzyme acts at an optimum pH of 3.
c) **Rennin (lab ferment)** – is an enzyme specific to the infant, acts at a pH of 4-5 and has the role of coagulating the breast milk, retaining it in the stomach for about 2 hours. During this time, a partial hydrolysis of milk proteins occurs under the action of the gastricsin, while the resulted peptides may penetrate the stomach wall, reaching the circulatory system.

Being endopeptidases, the enzymes of the gastric juice hydrolyze about 1/10 – 1/5 of the total peptide bonds of food proteins, resulting short polypeptides.

The polypeptides, generated by the action of pepsin, stimulate the secretion of the cholecystokinine into the duodenum, a process which induces the intestinal and bile secretion.

II. Intestinal Digestion

Within the intestinal lumen, the products of the gastric digestion, the polypeptides, are subject to the action of the enzymes from the pancreatic juice and of the enzymes of the intestinal mucosa, the latter acting at the level of the intestinal villi or even within the cells of the intestinal mucosa.

The enzymes of the pancreatic juice

Trypsin – secreted into the pancreas as inactive trypsinogen, it is activated in the intestine by losing an N-terminal hexapeptide under the action of an intestinal enterokinase and then self-catalytically. As a catalytic action, it is an endopeptidase which acts at the level of the carboxyl groups of the basic amino-acids, Liz and Arg. In order to avoid the injuries which can be caused by a possible activation of the trypsinogen on the pancreas-intestine route, the pancreatic juice contains inhibitors (antitrypsin) which inactivate the accidentally activated trypsin into the pancreas or the pancreatic duct.

Chymotrypsin – secreted into the pancreas as chymotrypsinogen, is activated into the intestine under the action of the trypsin which removes 2 dipeptides (4 amino-acids) from the structure of the chymotrypsinogen. As a catalytic action, it is an endo-peptidase which acts at the level of the carboxyl groups of the amino-acids Phe, Tyr, Trp.

Elastase – secreted as pro-elastase, is activated by trypsin into the intestine as elastase. As a catalytic action, it is an endo-peptidase which acts on the elastin and on the proteins containing neutral hydrophobe amino-acids Gli, Ala.

Carboxypeptidase – is an exopeptidase produced by activating the inactive form, pro-carboxypeptidase at the intestine level by trypsin. As a catalytic action, it is an exo-peptidase which separates the amino-acid at the C- terminal end of peptides.

The end products of the action of the pancreatic juice are the amino-acids, di- and tri- peptides.

The intestinal juice enzymes

The amino-acids, the di- and tri- peptides can cross the intestinal wall by the amino-acids transport system and because of this, the action of intestinal peptidases occurs both at the surface as well as inside the cells of the intestinal mucosa. It has been established that a great part of the food proteins is absorbed under the form of some small peptides which, within the enterocytes are hydrolyzed at amino-acids.

The main peptidases acting at this level are:

Amino-peptidase – enzymes which hydrolyze the peptide bond of the N- terminal amino-acids, separating them from the peptide chain.

Di-peptidases - are peptidases which hydrolyze the peptide bond within the dipeptides by forming two amino-acids.

Absorption of amino-acids

It occurs at the level of the small intestine, only L-amino-acids being absorbed. The absorption process occurs by active transportation, dependent on energy and temperature (the transportation can also be made by a passive mechanism, through diffusion, but this is minor). Seven amino-acids transportation systems have been identified according to the nature of the amino-acids. Through absorption, the amino-acids enter the portal vein, liver, systemic circulation; the concentration of the free amino-acids within the blood varies between 20 - 30 mg%.

From the extracellular environment, the amino-acids enter cells by an active transportation process against the concentration gradient, as the intracellular concentration of the amino-acids is 10 times higher than within the circulation.

The amino-acids are reabsorbed from the primary urine into the renal tubule by an active process. In the case of newborns, peptides can be absorbed through the stomach wall as well, while through the intestinal wall (small intestine) intact proteins can be absorbed by pinocytosis. This process is important for the transfer of antibodies from the mother to the newborn, a process responsible for the immunity of the infant during the period 0 – 6 months.

IV.2. Metabolic transformations of amino-acids

The total reserve of amino-acids which can be freely found within the external and intracellular environment is of about 50g and represents ***the common pool of amino-acids.***

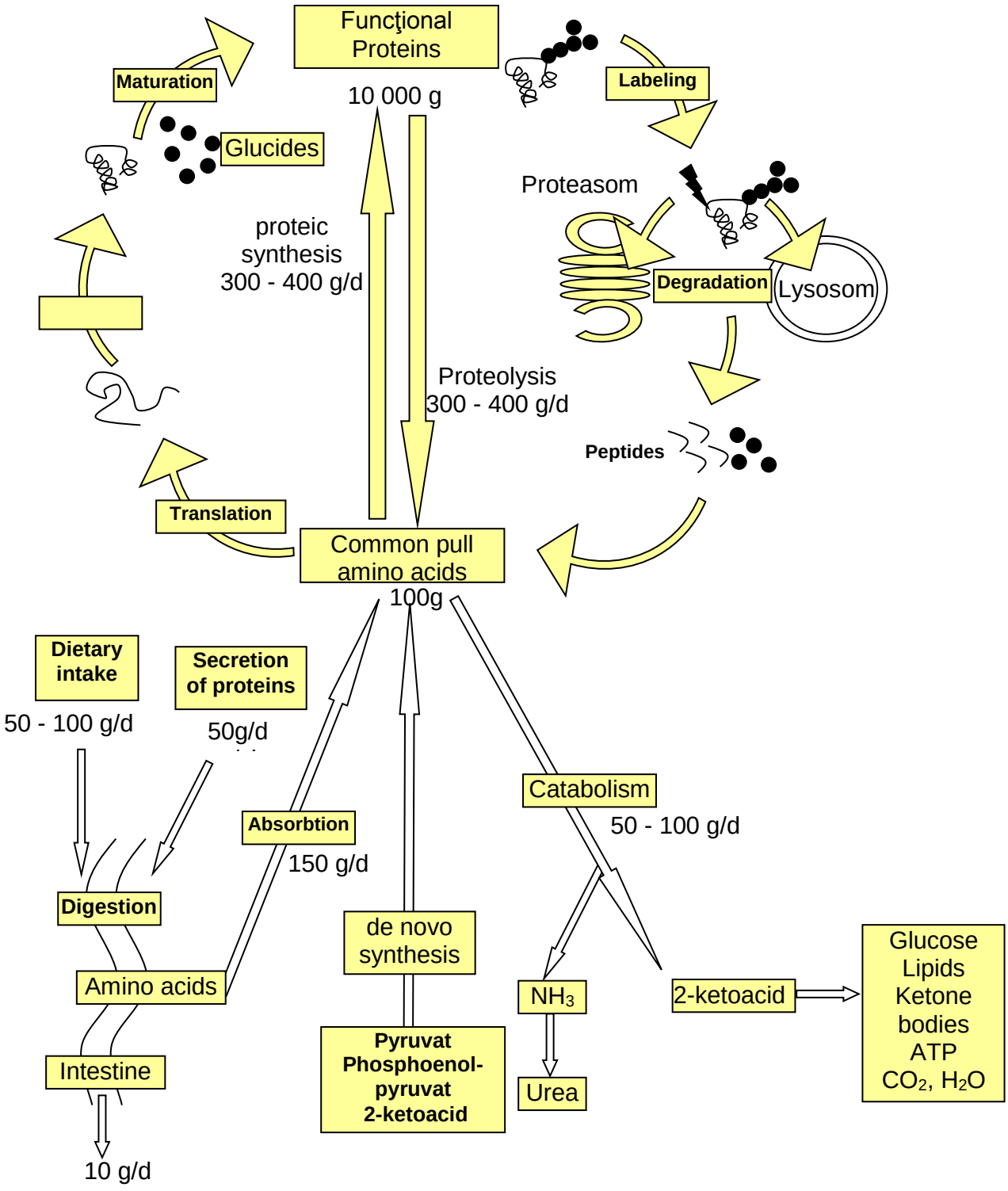
An individual amino-acid passes 5-6 times through the common pool until it is irreversibly degraded.

The main sources of amino-acids supply of the common pool are: food intake (intestinal absorption), endogenous biosynthesis and the catabolism of the endogenous proteins.

The main uses of the amino-acids are: proteins and peptides synthesis – a major and priority process, syntheses of important non-proteic compounds: heme, purine bases, pyrimidine bases, coenzymes, biogenic amines, etc. and secondarily as energetic substrate (caloric capacity - 5,5 kcal/g).

Thus, the daily proteins synthesis is of 200-400 g (and the same quantity is degraded), while the food intake is <100 g. The stress hormones (cortisol, adrenaline) or the cytokines, whose concentration increases in various stressful conditions, stimulate the protein catabolism and inhibits the synthesis.

The amino-acids are catabolized mainly by deamination and secondarily by decarboxylation.



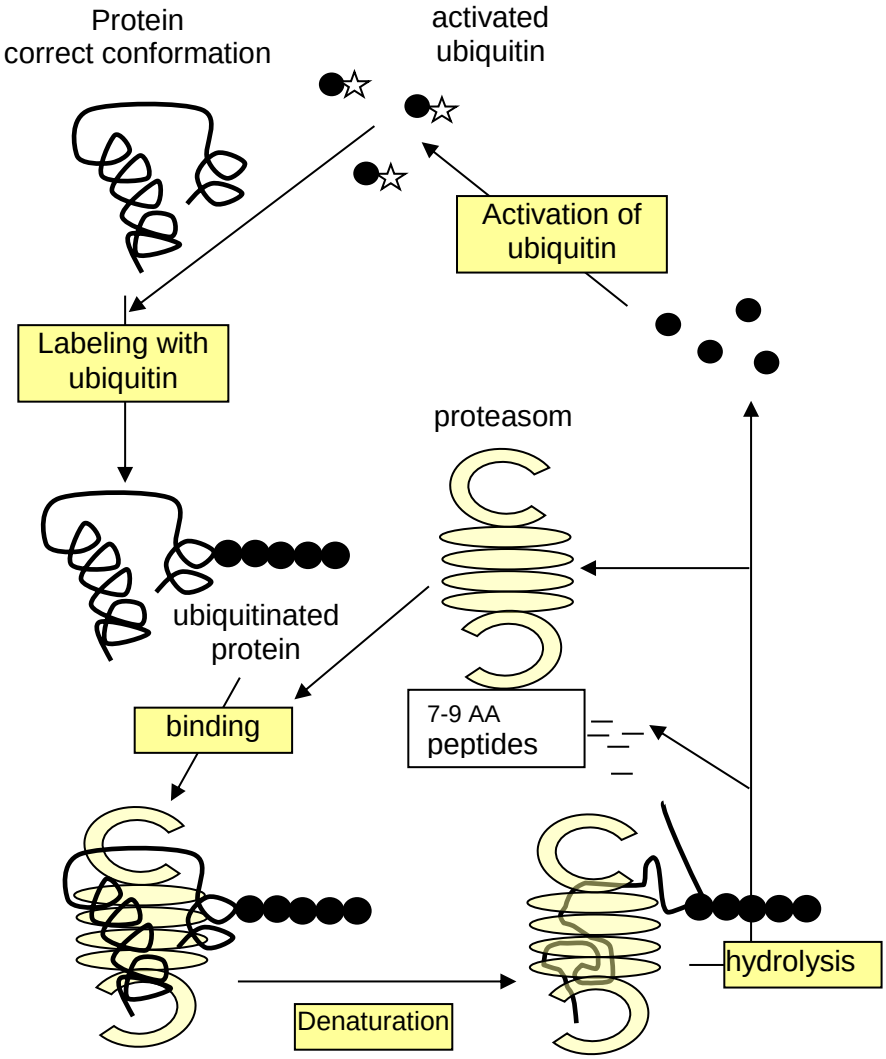
The common pool of amino-acids

IV.2.1. Regeneration of proteins

Within the general process of continuous regeneration of the cellular structures, cells disappear and are destroyed based on a programmed death process (apoptosis), and once with them, the component molecules including proteins, are also metabolized.

The protein molecules are continuously renewed, within the so-called ***proteins dynamic state***, determined using the isotope ^{15}N ***(N15 turnover)***. The renewal time is different (4 days – fibrinogen, 10 days – proteins from the liver, intestine, pancreas, plasma, 100 days – muscle proteins, Hb, cytochroms, 300 days – collagen).

Proteins regeneration is based on a quick proteins catabolism process meant to remove the proteins whose structure has been affected throughout their normal functioning, the proteins containing within their primary structure amino-acids inserted erroneously, or enzymes acting within the control points of the metabolic pathways. In the case of eukaryotic organisms, this process is based on a ATP-dependent mechanism which involves a small globular protein (8,5 kd), called **ubiquitin**.



Degradation of ubiquitin marked proteins in the proteasome

Ubiquitin is found within all eukaryotic cells (hence its name), and its structure is the same for most of the species. The isolated ubiquitin from yeast is different from the human one by only 3 amino-acids of the total of 76. Its role is marking the proteins to be catabolized, by binding with a covalent bond its C-terminal glycine to the ε-amino group of lysine residues of the protein to be categorized. The isopeptide bond thus formed needs an energetic contribution, supplied by ATP. Linking the ubiquitin to the target protein requires the intervention of three enzymes called E1 (ubiquitin activating enzyme), E2 (ubiquitin conjugation enzyme), E3 (ubiquitin - protein ligase) and is made throughout three stages:

1. Thioester linking the C-terminal carboxyl group of ubiquitin to the thiol group of a cysteinee residue of E1, hydrolyzing two macroergic bonds from ATP.
2. Transfer of activated ubiquitin on a thiol group from E2.
3. Transfer of ubiquitin from E2 to the ε-amino group of the target protein, catalyzed by E3.

The degradation of a protein involves four or more molecules of ubiquitin. These form real chains by attaching the ε-amino group of a lysine resifue from a ubiquitin molecule to the C-terminal carboxyl group of another one. The intervention of several ubiquitin molecules is more efficient for two reasons. On one hand, the interaction of ubiquitin molecules generates binding surfaces, and on the other hand, detaching one of the molecules is not followed by losing the signal indicating the protein degradation.

The degrading itself of proteins marked by ubiquitin is accomplished by a protease complex called **proteasome** or **proteasome 26S**. It is made up of two components: a component with catalytic activity with mass 20S and a component with regulating action with a mass of 19S. Component 20S weights 700 kd and is made up of 28 homologous subunits, disposed in 4 rings, each with 7 subunits, disposed so as to form a barrel structure. The two rings disposed at the ends are called α subunits and the two central rings are called β subunits. The active sites of the protease are located at the level of the N-terminal end of some β subunits, namely those containing threonine or serine. The hydroxyl groups of these amino-acids are, along with the amino groups, the nucleophile groups attacking the carboxyl groups involved in the peptide bonds of the degraded proteins, forming some acyl-enzyme intermediaries. Proteins are catabolized progressively, without releasing intermediary compounds, up to peptides formed by 7-9 amino-acids.

Component 19S has the role of controlling the access inside the structure. It weights 700 kd and is made up of 20 subunits. It can be found at both ends of component 20S and has the property of attaching to the polyubiquitin chains. The most important components of complex 19S are represented by 6 ATP-ases associated with regulating the cellular cycle and the biosynthesis of the cellular organelles. Although the role of ATP-ase is not exactly known, it seems that by the hydrolysis of ATP, the 19S complex induces conformational alterations of the proteasome 20S, followed by the protein entering inside the complex. At the same time, complex 19S also presents an isopeptidasic activity which makes the ubiquitin molecules able to be detached and reused, and the proteins catabolized up to amino-acids, under the action of other cellular proteases.

Although not all signals starting the proteins ubiquitination process have been identified, some of them have been however distinguished. To this end, it has been found out that the half-life ($t_{1/2}$) of cytosolic proteins is strongly influenced by the type of amino-

acids from the N-terminal end (the N-terminal rule). For instance, a protein containing methionine at the N-terminal end has a bisection time of over 20 hours, while an arginine protein from this position has a half-lifetime of about 2 minutes.

The half-life ($t_{1/2}$) of proteins according to the N-terminal amino-acids

N-terminal amino-acid	($t_{1/2}$) protein
Methionine, glycine, serine, valine, alanine, threonine	>20 hours
Isoleucine, glutamine	30 minutes
Tyrosine, glutamic acid	10 minutes
Prolyne	7 minutes
Leucine, fenylalanin, asparagine, lysine	3 minutes
Arginine	2 minutes

It was found that some amino-acids, called **destabilizing amino-acids**, such as arginine or lysine, favor a quick ubiquitination, while others, called **stabilizing amino-acids** (methionine, prolyne) expand the life of the protein.

There are some other types of signals which mark the proteins to be catabolized and which are represented by the so-called destruction boxes (N-terminal sequence of Arg-Thr-Ala-Leu-Gly-Asp-Ile-Gly-Asn type) within the structure of the mitotic cyclins or by the increased content of prolyne, glutamic acid, serine and threonine (PEST sequences) of some proteins.

Medical significance of protein regeneration

A series of normal and pathological processes are partially controlled by the catabolization of certain proteins: genes transcription, cellular cycle, organogenesis, inflammatory response, tumor suppression, cholesterol metabolism and antigens processing.

For instance, within the inflammatory response, the transcription factor named NF-kB initiates the expression of specific genes once activated. Its activity is accomplished by the catabolization of an inhibiting protein, named I-kB. This inhibiting protein attaches to cytoplasmic NF-kB and keeps it inactive. As a response to the inflammatory signals, I-kB is phosphorylated at the level of two serine residues, creating thus a binding site for E3. By attaching the E3 enzyme, I-kB ubiquitination and catabolization is initiated, which leads to the release of NF-kB and, implicitly, to the transcription of the target genes.

Another example is that of the human papilloma human virus which codes a protein activating the E3 enzyme. The enzyme initiates the ubiquitination of the tumor suppressor factor p53, as well as of other proteins controlling the DNA repairing, which are, this way, destroyed. Activating the E3 enzyme has been outlined in over 90% of the cervical cancer cases. Thus, inappropriately marking a protein which has an important regulatory role, followed by its destruction, can be the ground for the tumorigenesis. Not only the quick catabolism of some factors acting as tumor suppressors, but also the incapacity of catabolizing some proteins involved in activating cell division (oncogenes products) may initiate the tumorigenesis.

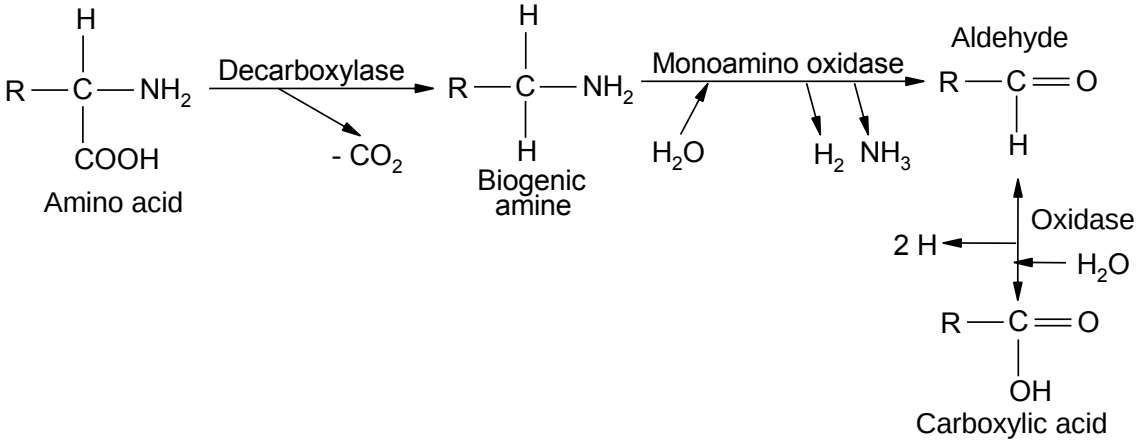
Besides the cancerous processes, there are also other diseases which involve the quick or inefficient destruction of some proteins: renal diseases, astm, neurodegenerative diseases (Alzheimer disease and Parkinson disease – associated with forming some

protein structures characteristic in neurones), cystic fibrosis (determined in some cases by the quick destruction of the chloride ion channels), Liddle syndrome (characterized by the lack of destroying the sodium channels in the kidneys, which leads to an increased absorption of Na^+ and the installation of HTA). Currently, developing some treatments that inhibit the effect on the proteosomes is being considered. One such medication is the bortezomib, used in the treatment of the refractory multiple myeloma, whose mechanism of action is based on inhibiting NF-kB by stabilizing I-kB.

IV.2.2. Amino-acids catabolism

IV.2.2.1. Decarboxylation

Although the process is reduced quantitatively, the end products are primary amines, very active compounds from a biological point of view. Only the carbon from the C_α position is decarboxylated. The enzyme is called ***amino-acids decarboxylase, piridoxal-phosphate dependent.*** The exception is the methionine decarboxylase, which has the pyruvate as co-factor, the methionine being first activated at S-adenosylmethionine. The biogenic amines formed are quickly degraded.



The biogenic amines resulting from various amino-acids are:

1. Tyr → Tyramine – tissue hormone
   → Catecholamines: Dopamine, Noradrenaline, Adrenaline (epinephrine)

2. Trp → Tryptamine – tissue hormone
   → Serotonine (5'-hydroxytryptamine) –neurotransmitter
   → Melatonine (5-methoxy-N-acetyltriptamine) – tissue hormone

3. His → Histamine – tissue hormone

4. Ser → Ethanolamine (Cholamine) → Phosphatidylcholamine (phosphatide) (cephaline)

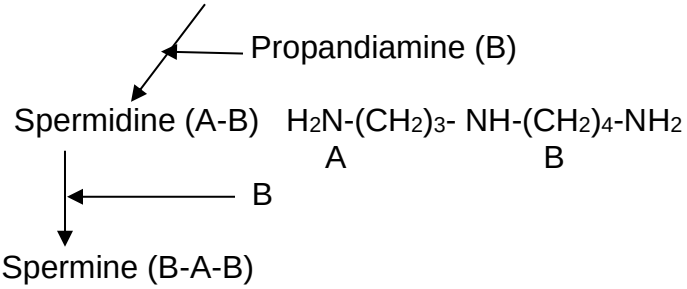
5. Cys → Cysteamine – active SH component of CoA
→ Taurine – conjugated bile acids

6. Asp → β - alanine → pantothenic acid → CoA
→ peptide component – carnosine, anserine

7. Glu → GABA (γ-aminobutiric acid) – neurotransmitter

8. Lys → Cadaverine

9. Ornithine —decarboxylase→ Putrescine (1,4-diaminobutane) (A)



The spermidine and the spermine act as transcription factors at a nuclear level, participating in cellular division processes.

IV.2.2.2. Deamination

It is the main way of amino-acids metabolization. Several oxidative deamination mechanisms have been identified, all of them forming ***ketoacids and ammonia.***

1. Flavinic D-Amino acid oxidase (FAD) acts within the renal tissue. They are specific to D-amino-acids, thus acting only upon glycine.

Amino-acid + Flavoprotein + $H_2O \rightarrow$ α-ketoacid + NH_3 + Flavoprotein H_2
Flavoprotein H_2 + X (O_2) $\rightarrow$ Flavoprotein + XH_2 (H_2O_2)

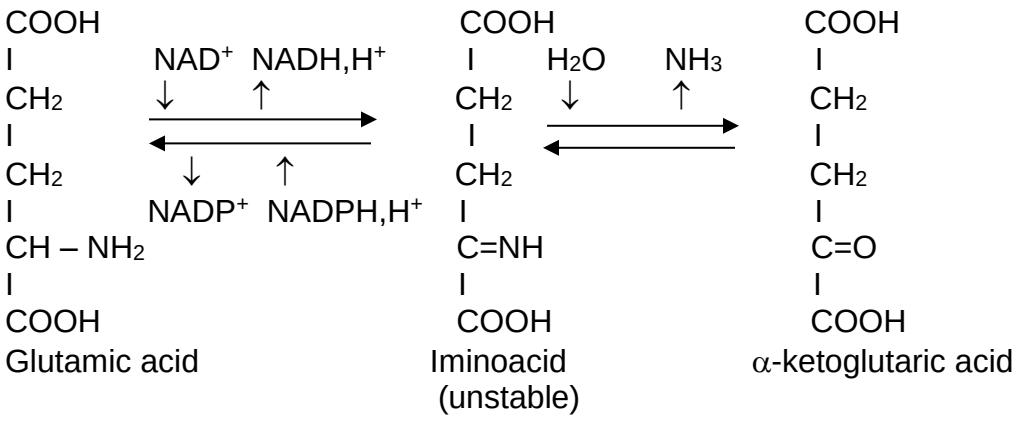
2. **Flavinic L-Amin oacid oxidase (FMN)** acts within the renal and hepatic tissue. It is linked to the cellular matrix; it has a weak activity.

- It does not act upon the amino-acids: Gli, β - hydroxilated (Ser, Thr), diacids (Glu, Asp), diamines (Lys).

3. **The L-Amino acid oxidases, NAD^+ dependent,** are very active, being active in all cells, except for the muscle cell. More important is the **the glutamate dehydrogenase, NAD^+ dependent**. It is allosterically regulated as follows:

- the inhibiting factors are: ATP, GTP, NADH
- the stimulating factors are: ADP.

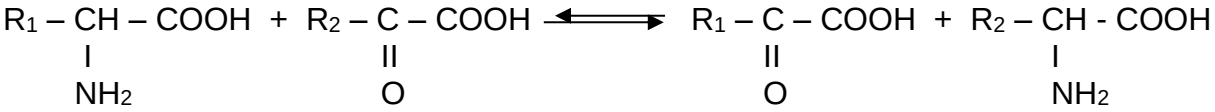
The enzyme catalyzes the following reaction:



Due to the fact that the glutamat dehydrogenase is the most active form, aabout 70% of the amino acids deamination is done through a coupled reaction of the transaminases with the L-glutamat dehydrogenase.

The transaminases catalyze the transfer of the amino group of an amino-acid on a α -ketoacid. The enzyme has the **pyridoxal phosphate as co-factor**.

The general reaction is:



The transaminases are found within all cells, the most common being:

a. alanin transaminase (ALAT, ALT, GPT, TGP), which catalyzes the reaction:

$$\text{Ala} + \alpha\text{–ketoacid} \leftrightarrow \text{Pyruvate} + \text{amino-acid}$$

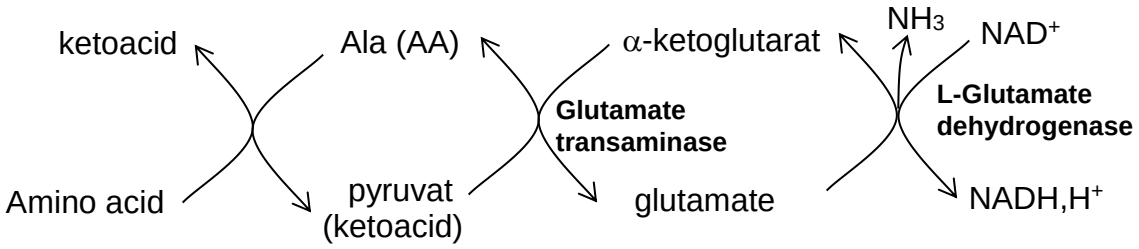
b. glutamat transaminase (ASAT, AST, GOT, TGO), catalyzes the reaction:

$$\alpha\text{ – cetoglutarate} + \text{aminoacid} \leftrightarrow \text{Glu} + \alpha\text{ – ketoacid}$$

The specificity is increased for the pairs: alanine + pyruvate, glutamic acid + α – cetoglutarate, and relative for other amino-acids.

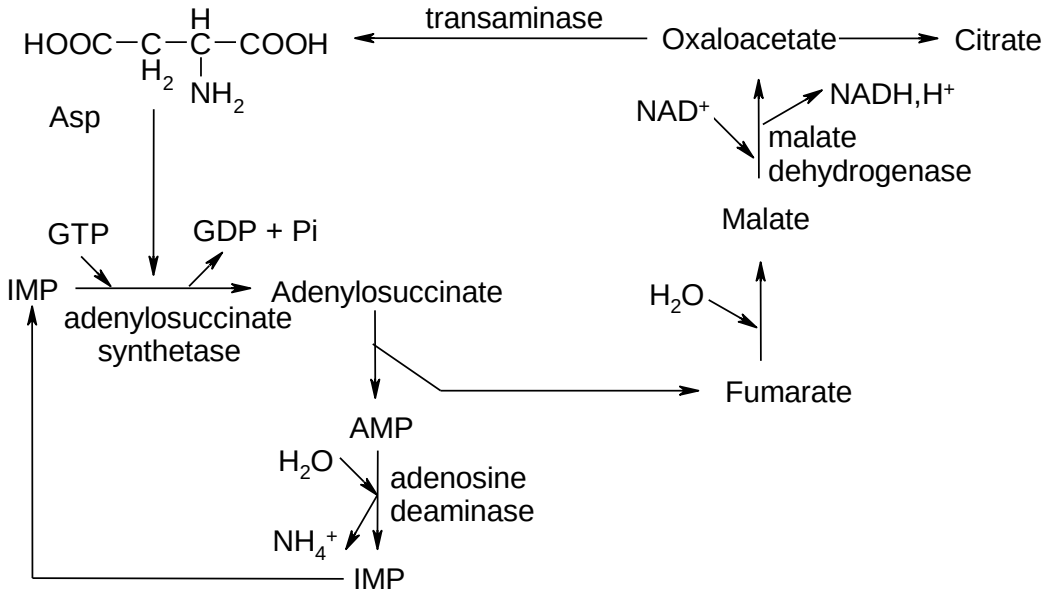
The affinity of amino-acids, except for glutamic acid, for glutamat transaminase is different. 14 amino-acids have a decreasing affinity in the order: Asp, Ala, Val, Leu, Ile, Tir, Phe, Met, Trp, Arg, Cis,Gli, His, Ser, and 4 amino-acids: Thr, Pro, Ho-Pro, Liz can be transaminized only very slowly.

Since the glutamate transaminase is the most prevalent and has the greatest reaction rate, the deamination of most of the amino-acids is carried out in a coupled process.



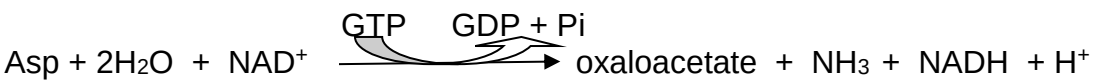
As the 14 amino-acids have an affinity for the glutamate dehydrogenase, 14/20 =70% of the amino-acids are deaminated by this mechanism. For the rest of them, there are particular mechanisms (with lower activity).

Within the muscle cell, where there is no glutamate dehydrogenase, the deamination is made by the “purine nucleotides” cycle. It must be stated that Asp is the first partner of the glutamate transaminase, in balance with Glu.



The purine nucleotides cycle
(IMP-inosine monophosphate, AMP-adenosine monophosphate)

Global reaction:



Medical significance of transaminases

In clinical practice, usually 2 transaminases are analyzed, in order of their affinity for the substrate, GOT (ASAT) and GPT (ALAT). The enzymes are linked to the cellular structures, having a concentration of 10000 times bigger within the cell than in the serum. Their occurrence in large concentration within the serum indicates:

A) **Cellular injuries** – either inflammatory or ischemic (necrosis)

B) **Location of the injured tissue** - GOT – preferentially in the miocardic muscle cell
- GPT – preferentially the hepatic cell.

Normal values: ASAT (GOT): 0 - 19 U/L
ALAT (GPT): 0 - 23 U/L

IV.2.2.3. Possibilities of catabolizing the hydrocarbon skeleton

Within the process of amino-acids catabolization, either by deamination, or by decarboxylation, ketoacids result (hydrocarbon skeleton). These are catabolized specifically for each amino-acid, forming different compounds. Research on experimental animals subjected to restrictive diets with only one type of amino-acid, has shown that the amino-acids thus investigated have produced 3 types of effects:

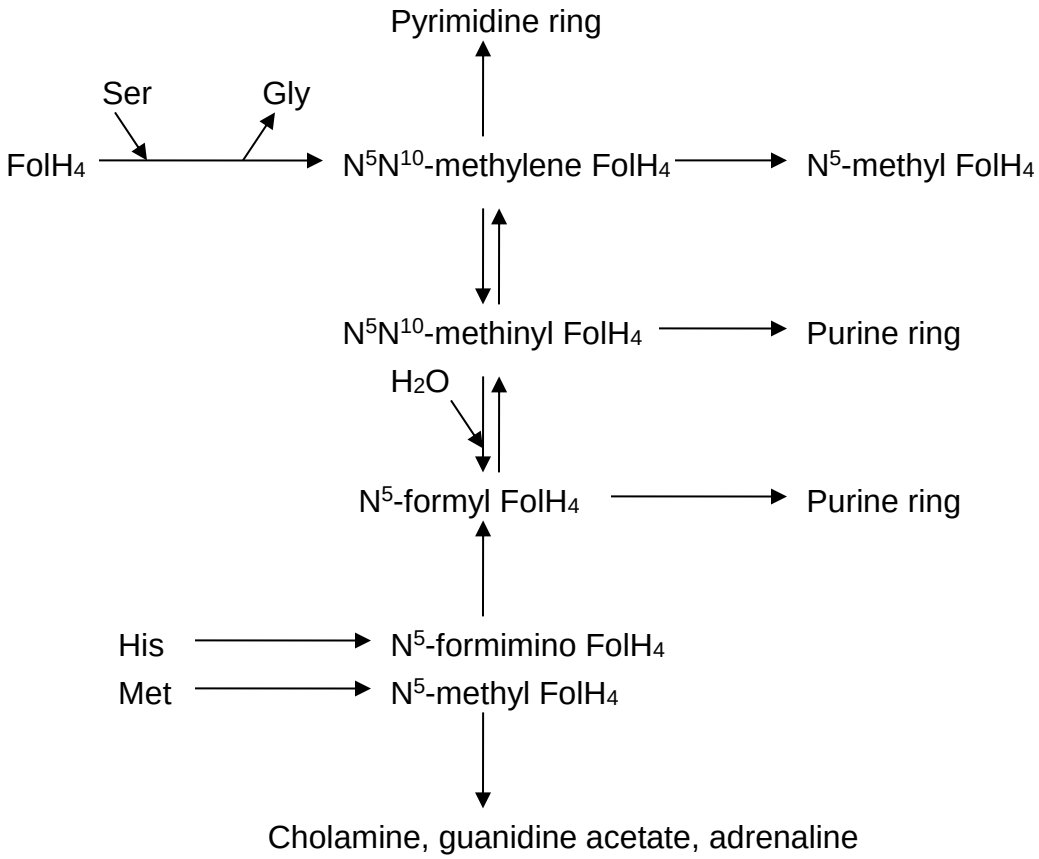
1. **Hyperglycemia** – this type of amino-acids has been included into the category of pure glucoplastic amino-acids and comprises Ala, Gly, Cys, Ser, His, Thr, Asp, Asn, Glu, Gln, Met, Val, Arg, Pro.
2. **Ketonemia** – this type of amino-acids has been included into the category of pure ketoplastic amino-acids and comprises Leu, Lys.
3. **Hyperglicemia and ketonemia –** this type of amino-acids has been included into the category of mixed amino-acids = glucoplastic + ketoplastic and comprises Trp, Ile, Phe, Tyr.

The subsequent discovery of the metabolic pathways has allowed the biochemical explanation of the phenomenon:

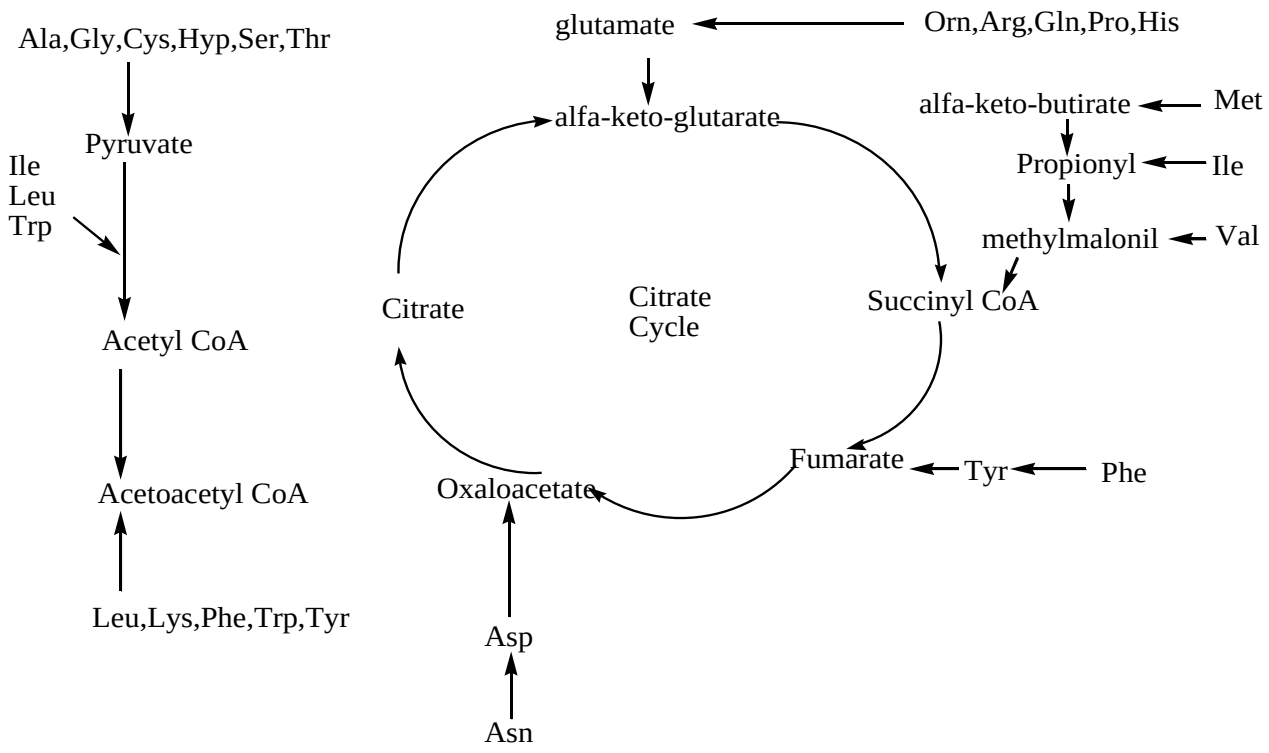
- the pure glucoplastic amino-acids produce intermediaries of gluconeogenesis by the catabolization of their hydrocarbon skeleton (pyruvic acid, alpha-ketoglutaric acid, fumaric acid, succinyl-CoA, oxaloacetic acid)
- the pure ketoplastic amino-acids produce by the catabolization of the hydrocarbon skeleton acetyl-CoA or acetoacetyl-CoA
- the mixed amino-acids produce by the catabolization of the hydrocarbon skeleton both intermediaries of gluconeogenesis as well as acetyl-CoA or acetoacetyl-CoA.

The hydrocarbon skeletons resulting from deamination can still release monocarbon units (formyl, formimino, methyl, methylene) throughout the catabolization process, units transferred by the coenzyme Fol H_4.

The monocarbon units have an essential role in the synthesis of the purine and pyrimidine bases, therefore of the nucleic acids, in the remaking of Met, within various methylation processes (cholamine, noradrenaline).



Monocarbon units (formyl, formimino, methyl, metylene) and their role in the synthesis of the purine and pyrimidine bases and some methylation reactions



Possibilities of catabolizing the hydrocarbon skeleton of amino-acids

IV.2.3. Ammonia metabolism

Ammonia is a **toxic substance (especially neurotoxic)**, even in small concentrations in blood; ammonia intoxication symptoms are tremor, slurring of speech, blurring of vision, and in serious cases coma and death.

Ammonia production is appreciable, considering that about 30 - 50 mmoles ammonia is eliminated within 24 hours through urine, but especially that a great part of the protein nitrogen is eliminated as urea (300-600 mmoles urea is eliminated daily).

Nevertheless, for humans, ammonia concentration in plasma is normally very low (10 – 80 μg/100 ml, 5 – 50 μmoli/liter), because due to its toxicity, ammonia is transported in blood as glutamine. Most of the ammonia is transported as glutamine which is obtained from **glutamic acid** and **ammonia** in the presence of **glutamine synthetase**, a ubiquitous enzyme (the reaction needs ATP).

The liver and the kidneys capture glutamine from the blood, they have specific **glutaminase**, which catalyzes the irreversible hydrolysis of the glutamine forming **ammonia** and **glutamic acid**. At renal level, the ammonia thus formed within the tubular renal cells diffuses through the membrane of these cells where, accepting protons, creates **ammonia ions** which are eliminated through urine. Thus, eliminating a part of the ammonia is important in relation to preserving the alkaline reserve of the plasma. The ammonia production increases in the metabolic acidosis and decreases in the metabolic alkalosis.

Ammonia sources of the body.

The main source of ammonia is the deamination biochemical processes of amino-acids. All amino-acids are deaminated by the system α-ketoglutarate – glutamate, except for the glycine which is deaminated by direct ammonia release under the action of the glycine oxidase enzyme (co-factor $FolH_4$). In addition, ammonia is also generated within the purine and pyrimidine nucleotides catabolism, by oxidation of amines (under the

action of mono-amine- and diamine-oxidase), as well as to a certain extent by the decomposition of the urea that may be present within the intestine lumen (under the action of the bacterial urease, releasing ammonia, which is absorbed through the portal vein; the blood of this vein normally has an ammonia concentration above the blood concentration). A secondary ammonia source is represented by glutamine (and asparagine), formed by glutamic acid and ammonia (respectively aspartic acid and ammonia) which, under the action of the glutaminase (respectively asparaginase) can be decomposed hydrolytically again to ammonia and GLU (respectively ASP).

Ureogenesis

It is the first biochemical cycle described by Krebs-Henseleit in 1932. It is the mechanism providing the permanent elimination of NH_3 from the body by turning it into urea, a soluble and actually non-toxic substance, that is eliminated through urine, perspiration, and digestive juices.

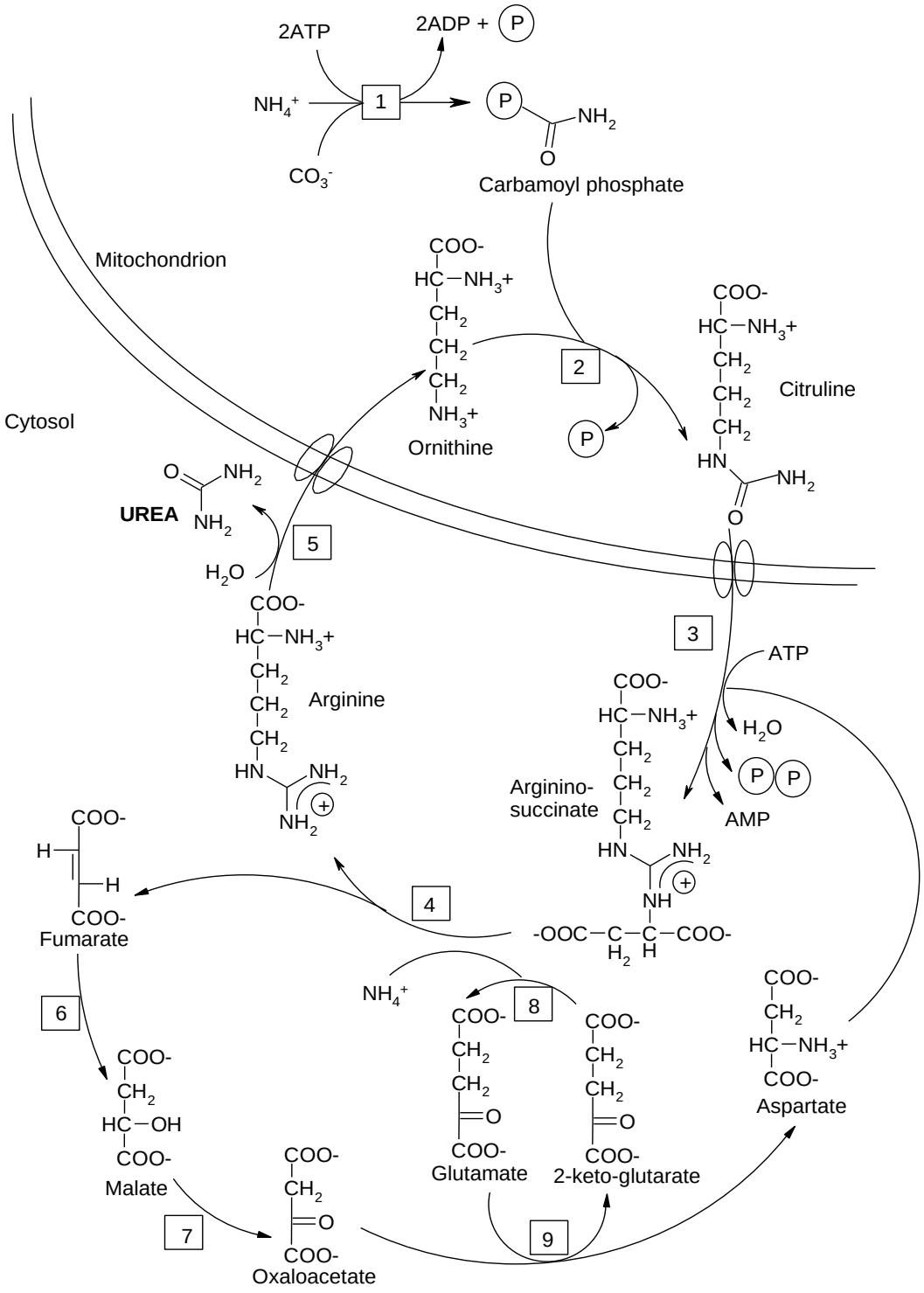
The ureogenesis occurs in the liver, producing 0,3-0,6 moles (20 - 40 g urea)/day. It comprises 5 reactions: the first two ones take place within the mitochondria, and the next three ones within the cytoplasm. For each ammonia molecule turned into urea, the energy of 4 macroergic bonds of ATP is used up. The ammonia source within mitochondria is provided by glutamine, which can release 2 molecules of NH_3, under the successive action of 2 enzymes: glutaminase and glutamate dehydrogenase.

$$Gln \longrightarrow NH_4^+ + Glu$$

$$Glu + NAD^+ + H_2O \longrightarrow NH_4^+ + NADH + \alpha\text{–}KG$$

The functional significance of the urea synthesis reaction $2NH_3 + CO_2 \longrightarrow UREA + H_2O$ is:

1. Transforming the toxic NH_3 into urea with urinary elimination. The two N atoms of urea come from Glu.
2. Obtaining arginine, a semi- essential amino-acid by the reactions 2,3,4.



The ureogenetic cycle

1. Carbamoylphosphate synthetase
2. Ornitin carbamoyl transferase
3. Argininosuccinate synthetase
4. Argininosuccinate lyase
5. Arginase
6. Fumarate dehydrogenase
7. Malate dehydrogenase
8. Glutamate dehydrogenase
9. Aspartate transaminase

Medical significance

The urea level in the blood depends on:

- hepatic production
- renal clearance.

The normal serum concentration is comprised between 15 - 45 mg/dL. The increase of urea concentration can have the following causes:

1. **Intensification of the protein catabolism**, due either to a **food protein surplus** (1 g urea corresponds to the catabolization of 6,25 g proteins), or to **hunger**, when a pronounced muscle proteolysis occurs.
2. **Digestive bleeding**, followed by intense intestinal absorption of amino-acids, their catabolism will intensify ureogenesis.
3. **Renal insufficiency**. In order to verify this diagnosis, the concentration of other metabolites from the blood must be determined, such as creatinine, and only if these have an increased level, the diagnosis of renal insufficiency is confirmed.

Pathology

The defective functioning of ureogenesis can have genetic causes or the general alteration of the hepatic function, such as in the case of cirrhosis for instance. In all these cases, the outcome is a severe hyperammonemia, generating encephalopathy. These metabolic diseases due to the abnormal functioning of ureogenesis enzymes are potentially fatal, causing coma in case of high ammonia concentrations. A typical symptom is loss of consciousness, a result of ATP depletion. High ammonia concentration uses up the α-cetoglutaric acid, thus reducing the activity of the Krebs cycle and implicitly the ATP production.

Enzymatic defects of genetic cause

Several enzymatic defects which affect one or more enzymes of the ureogeneric cycle have been described. Each enzymatic defect manifests itself by hyperammonemia and the increase of the deficient enzyme precursor.

- the ornithine-carbamoyl-P-transferase deficiency ⟶ hyperammonemia
- the carbamoyl- phosphate – synthetase deficiency ⟶ hyperammonemia
- the arginine-succinic synthetase deficiency ⟶ hyperammonemia + citrullinemia
- the arginine-succinase deficiency ⟶ hyperammonemia + argininosuccinatemia
- the arginase deficiency ⟶ hyperammonemia + hyperargininemia

Therapy

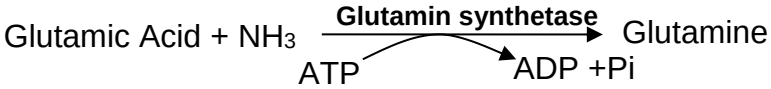
1. Limiting the protein intake and replacing the amino-acids with appropriate α-ketoacids.
2. Eliminating the surplus of NH_4^+ by blocking the activity of the bacteria from the colon either with antibiotics or by administration of levulose which induces acidic fermentation. This produces H^+, which combines with NH_3 and forms NH_4^+, thus preventing the portal absorption of NH_3.
3. Administration of deficient intermediaries of the citric cycle. For instance: α-ketoglutarate.

Infection with *Proteus mirabilis*

Urea hydrolysis with urease takes place in the human body only in the colon. In case of urinary infection with *Proteus mirabilis*, a micro-organism secreting urease, the forming of NH_3 occurs in the urine, leading to its alkalinization. The alkaline reaction of urine deposits the magnesium phosphate, forming renal calculi. The acute smell of ammonia from the public toilets is due to the activity of the microbial ureases.

IV.3. Transportation and metabolization of NH_3 in the body

The catabolism of amino-acids takes place in all tissues; however, the resulting NH_3 is turned into urea only within the liver or is eliminated as NH_4^+ only at the kidneys level. Knowing that the NH_3 is neurotoxic, the issue of NH_3 transportation from tissues to liver and kidneys arises. This transportation is accomplished through glutamine.

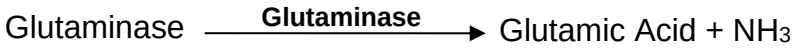


This reaction allows both the fixing of NH_3 from the cells (an essential process within the nervous tissue), as well as the transportation of NH_3 by the Gln in the blood. In this sense, the plasma concentration of Gln is by far the highest (8 mg%) of all amino-acids from the blood (0,2 - 0,3 mg %).

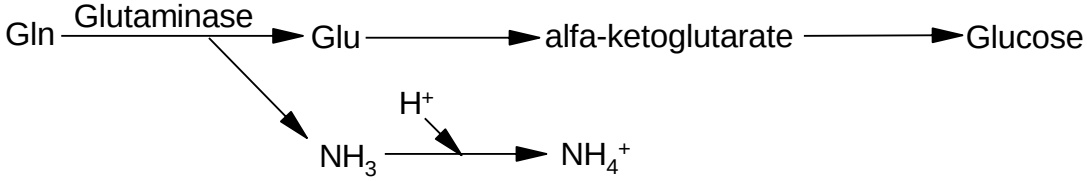
NH_3 transported by Gln will be provided to the tissues for:

- ureogenesis - within the liver
- ammonia-genesis – within the kidneys
- synthesis of purine and pyrimidine bases, synthesis of amino-sugars and amino-acids within all tissues.

Most of the NH_3 will be used within the liver and the kidneys. Gln is captured in the liver, which in the periportal area has concentrated the glutaminase enzyme and the ureogenesis enzymes.



The quantity of NH_3 to be transformed into urea within the liver depends on the acid-base balance of the body. In case of acidosis, a great part of NH_3 from the liver is fixed again as Gln (the perivenous zone of the liver is rich in glutamin synthetase) and it reaches the kidneys under this form. Within the kidneys, under the action of the glutaminase, Gln is turned into Glu and NH_3, which will fix H^+, turning into NH_4^+, eliminated through urine. Eliminating the NH_4^+ substitutes the elimination of Na^+ and K^+ thus protecting the alkaline reserve of the body.



In case of acidosis, more than 50% of the total NH_3 gets to kidneys. In acidosis, the ureogenesis thus decreases, and the consumption of HCO_3^- decreasing as well. Thus,

the increase of HCO_3^- will also reduce the acidosis. In conclusion, using the NH_3 in the body depends first of all on the acid-base balance of the body.

IV.4. Relations among tissues for amino-acids metabolization

As in the case of other metabolites, the amino-acids level from the blood is preserved at relatively constant values by the conjugated action of several tissues, of which the hepatic tissue and the muscle one play the main parts. The liver is the organ which accomplishes all the amino-acids processing operations. Of these, ureogenesis and gluconeogenesis are located exclusively within the liver. The muscles represent the amino-acids depositing tissue under the form of muscular proteins. Other tissues involved are: the renal tissue with a role within the ammonia-genesis and the Gln metabolism, and the intestine tissue, the entrance of amino-acids from food supplies. Amino-acids metabolization within the body follows, just as in the case of carbohydrates and lipids, the 2 important pHisiological stages: early postprandial and late postprandial.

A. Early postprandial

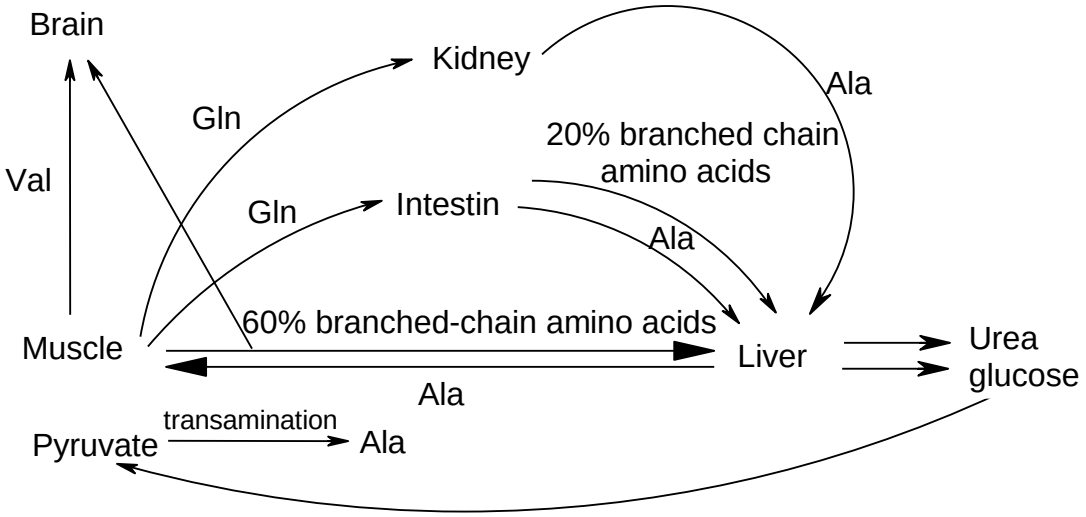
As a result of food intake, of protein digestion and of intestine absorption of amino-acids, the quantity of amino-acids increases within the portal vein, of which about 20% of the amino-acids have branched chains. This way they reach the liver, which acts as a filter, retaining most of the amino-acids and releasing into the circulating the surplus of amino-acids. The liver does not retain branched chain amino-acids, so that they will represent over 60% of the amino-acids released into circulation. From within the blood, the amino-acids are captured by tissues, of which the muscular tissue is the most active one. In a period of 1-3 hours from the food intake, the muscular tissue extracts the whole quantity of excess amino-acids from the blood. These are deaminated or processed for obtaining specific storage muscular proteins. Exceptions are the branched chain amino-acids, whose hydrocarbon skeleton will not be altered. The explanation consists in the fact that the branched chain amino-acids must have a constant concentration within the blood, since it is an important energy source of the brain tissue. For this reason, the muscular tissue will permanently provide for preserving this concentration within the blood, irrespective of the pHisiological status.

B. Late postprandial

Within the post-absorptive state, during starvation, metabolization of reserves occurs. Thus, within muscles, there takes place an intense proteolysis process, after which amino-acids result which will be released into the circulation. Most of them (over 50%) are represented by Gln and Ala.

Ala – is captured especially by the hepatic tissue, as it is a precursor for gluconeogenesis. The liver affinity for Ala is given by the fact that the saturation level of Ala from the liver is of 20-30 times bigger than the serum level of Ala.

Gln – is a transporter of the amino groups resulting from the muscular catabolism of other amino-acids. Gln is captured by the intestine and kidneys, where, after deamination, turns into Ala and Ser, amino-acids to be released into circulation. Hence, due to the special affinity of the liver for them, they will be captured by the liver and used for gluconeogenesis or ureogenesis.



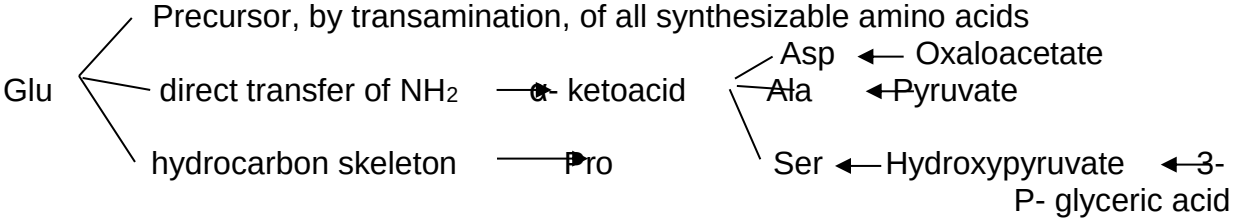
Early postprandial and late postprandial amino-acids metabolization

IV.5. Amino-acids biosynthesis

From the point of view of the possibility which the body has to synthesize them, amino-acids are divided into 3 categories:

1. Non-synthesizable (essential), a category comprising 8 amino-acids - Met, Thr, Phe, Lys, Trp, Val, Ileu, Leu.
2. Partially synthesizable (during the growing period, the body cannot synthesize the entire needs, the difference being supplemented by food supply), a category comprising 2 amino-acids - Arg, His.
3. Synthesizable, a category comprising 11 amino-acids - Tyr, Gly, Ser, Cys, Asp, Asn, Glu, Gln, Pro, Se-Cys.

The biosynthesis of the synthesizable amino-acids is accomplished to a great extent from the glutamic acid Glu.



Glu is synthesized by the reactions:

$$Gln + \alpha\text{-}KG + 2NADPH,H^+ \longrightarrow 2\ Glu + 2\ NADP^+$$

$$\alpha\text{-}KG + NH_3 \xrightarrow[\text{Glutamate dehydrogenase}]{NADPH,H^+ \quad NADP^+} Glu + H_2O$$

This reaction transforms ammonia nitrogen into amino acid nitrogen.

Forming the synthesizable amino-acids

1 Transamination of the corresponding α-ketoacid:

Glu ← α-KETOGLUTARATE (α-KG)
Ala ← PYRUVATE
Asp← OXALOACETATE
Gly ← GLYOXYLATE
Ser ←HYDROXYPYRUVATE ← 3P-Glyceric

NADH NAD^+

2 Amidification

Glu → Gln glutamin synthetase
Asp → Asn asparagin synthetase
} ATP is consumed

3. γ-semialdehyde —(Cycle formation C_2-C_5)→ pyrolidine carboxylic acid → Pro

Glu → Orn → Arg

4 Met → S-Adenosyl Met → homo Cys (+ Ser) → Cys + homo Ser

S-Adenosyl Met → CH_3

5 Phe —(Phe hydroxylase)→ Tyr

6 Pentose-phosphate → ribose 5-P → His