VNIVERSITAT DE VALÈNCIA

LESSON 27. METABOLISM OF AMINO ACIDS (I): ORIGIN OF AMINO ACIDS

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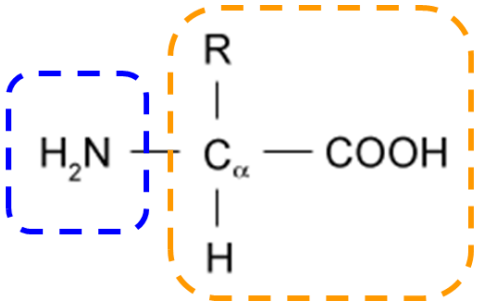
1. GENERAL ASPECTS: ORIGIN AND DESTINATION OF AMINO ACIDS



AMINO ACIDS

PROTEINS

Structural blocks (amino acids)
Plastic or structural function

Protein turnover

Lesson 27

BIOMOLECULES

Prosthetic groups

Neurotransmitters

Biogenic amines

Purines and pyrimidine bases

Lesson 30

DEGRADATION

Amino group loss

NH_3 + Carbon chain

Urea cycle

Lesson 29

Glucose
CO_2
Acetil-CoA
Ketone bodies

Lesson 28

Amino acids cannot be stored or secreted.
Site of tissue degradation: liver and, occasionally, skeletal muscle.

1. GENERAL ASPECTS: ORIGIN AND DESTINATION OF AMINO ACIDS

Source of amino acids:

- Protein digestion from the diet:
  - Essential amino acids
  - Non-essential amino acids
- Intracellular protein degradation due to protein turnover
- Body protein degradation in pathological situations:

  starvation, diabetes mellitus, etc.

Essential	Non-essential
Histidine (His)	Alanine (Ala)
Isoleucine (Ile)	Arginine (Arg)
Leucine (Leu)	Asparagine (Asn)
Lysine (Lys)	Aspartate (Asp)
Methionine (Met)	Cysteine (Cys)
Phenylalanine (Phe)	Glutamate (Glut)
Threonine (Thr)	Glutamine (Gln)
Tryptophan (Trp)	Glycine (Gly)
Valine (Val)	Proline (Pro)
	Serine (Ser)
	Tyrosine (Tyr)

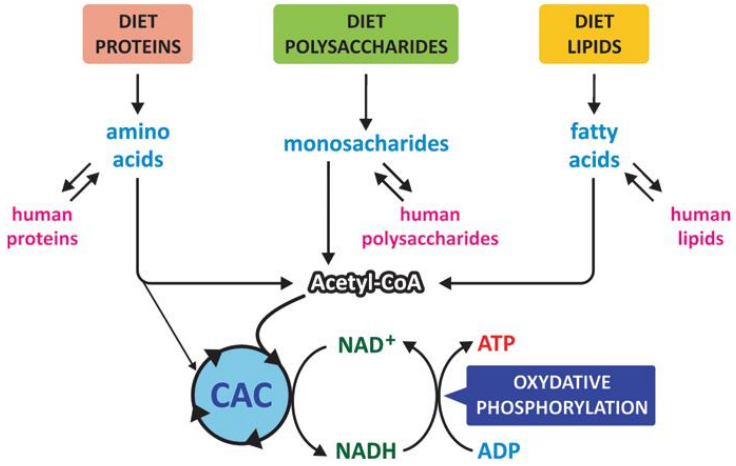
Classification of amino acids based on human nutritional requirements. Conditional amino acids are underlined.

Destination of amino acids:

- Precursor for the synthesis of new proteins and other nitrogen compounds:

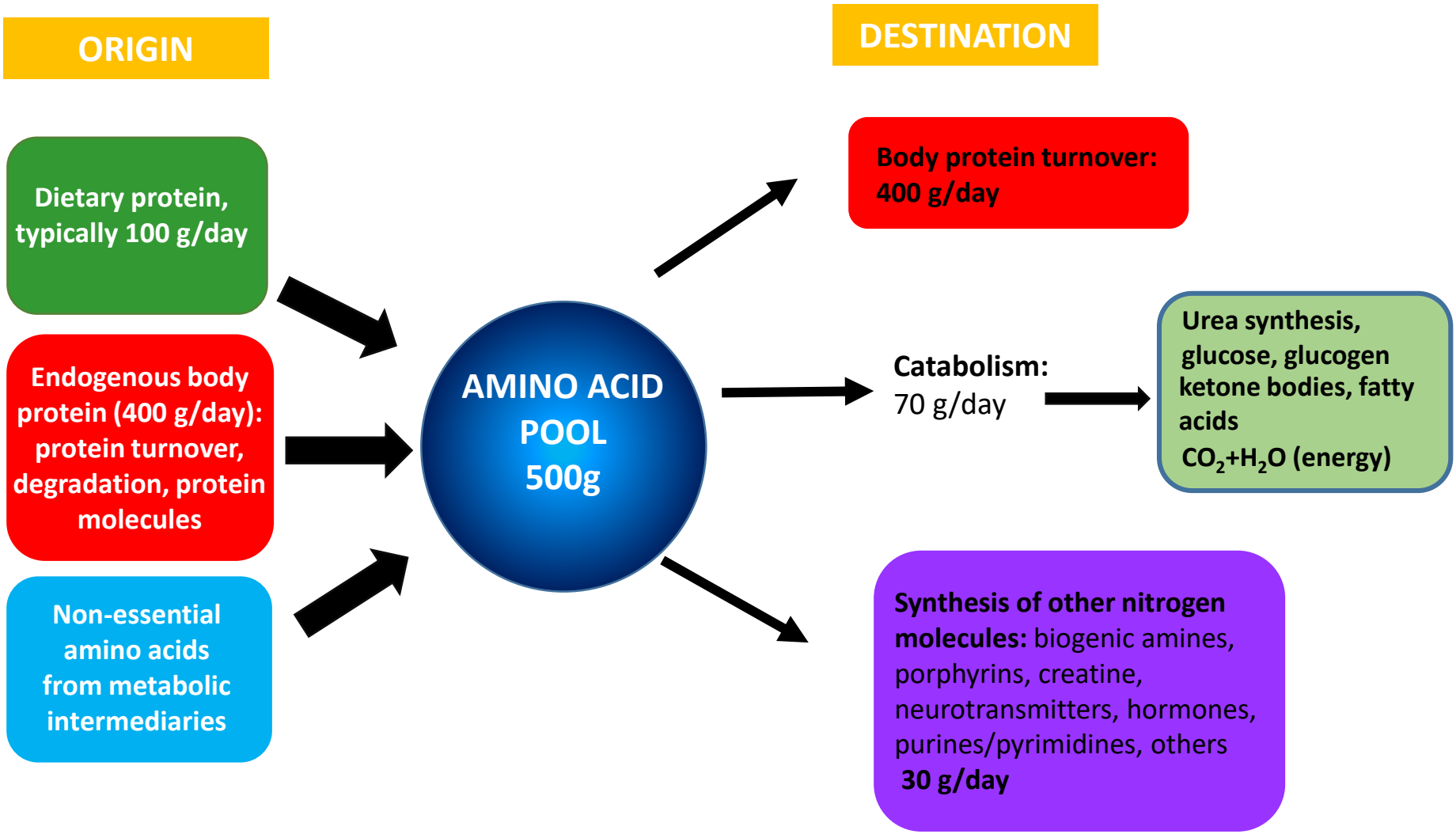
  Nucleotides, the heme group, glutathione
- Surplus is degraded for energetic purposes

  10-15 % of the energy



Da Poian et al., 2021

1. GENERAL ASPECTS: ORIGIN AND DESTINATION OF AMINO ACIDS



2. NITROGEN BALANCE

- Nitrogen in the body maintains a balance.
- Most organisms do not have nitrogen reservoirs.
- Nitrogen is necessary for the synthesis of amino acids and nitrogen bases.
- It is present in the biosphere as nitrate (NO_3^-) or dinitrogen (N_2), and must be reduced to ammonium ($NH4^+$) to be incorporated into proteins.
- The main source of nitrogen is our diet.
- Excess nitrogen in the organism is toxic.

Nitrogen balance: The difference between total nitrogen ingested and total nitrogen excreted

NITROGEN = INGESTED N_2 – EXCRETED N_2

Nitrogen balance:

INGESTED NITROGEN = EXCRETED NITROGEN

(Healthy adult and a balanced diet)

Nitrogen balance: nitrogen uptake and excretion is similar. Protein synthesis and protein degradation are equivalent.

Positive nitrogen balance:

INGESTED NITROGEN > EXCRETED NITROGEN

(Growth, pregnancy, adults recovering from illness)

Positive nitrogen balance → net protein synthesis

Negative nitrogen balance:

EXCRETED NITROGEN < INGESTED NITROGEN

(Post-surgery, prolonged fasting, deficiency of any amino acid)

Negative nitrogen balance → more nitrogen is excreted than is incorporated

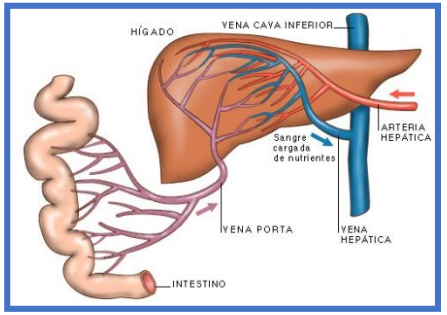
3. PROTEINS FROM THE DIET: DIGESTION, ABSORPTION AND TRANSPORT OF AMINO ACIDS

Diet: 70–100 g ingested hydrolyzed protein gives rise to di/tri-peptides and amino acids to be absorbed, which then leads to:

1. Degradation: proteolytic enzymes responsible for degradation come from: the stomach, the pancreas and the small intestine (duodenum) to obtain amino acids.

2. Absorption: amino acid transport from intestinal cells (enterocytes from the small intestine), from the jejunum, and through the portal vein to the liver (distribution and use).

3. Transport: from the liver to peripheric tissues the through hepatic vein.



- The quality or biological value of dietary proteins depends on their content of essential amino acids.
- Unlike lipids or carbohydrates, amino acids are not stored in the body.
-Blood levels depend on the balance between the biosynthesis and the degradation of body proteins; regardless of their origin, amino acids form a pool (reserve) that passes into the blood and is distributed to the tissues.
- The amino acid reserve is used for synthesizing new proteins and derivative compounds to maintain nitrogen balance.

3. PROTEINS FROM THE DIET: DIGESTION, ABSORPTION AND TRANSPORT OF AMINO ACIDS

FOOD

↓ **EXTRACTION** (chewing and mechanical activity in the stomach)

PROTEINS

↓ **DENATURALIZATION** (acid pH in the stomach)

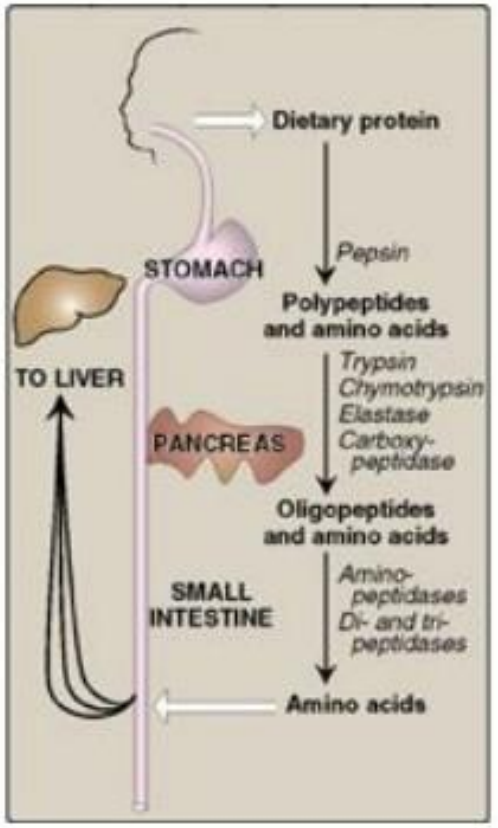
DENATURED PROTEINS

↓ **HYDROLYSIS**

- **ENDOPEPTIDASES**
  - **PEPSIN (stomach)**
  - **TRYPSIN (pancreas)**
  - **CHYMOTRIPSIN (pancreas)**
  - **ELASTASE (intestine)**
  - **ENTEROKINASE (intestine)**
- **EXOPEPTIDASES**
  - **CARBOXYPEPTIDASES**
  - **AMINOPEPTIDASES**

↓

AMINO ACIDS

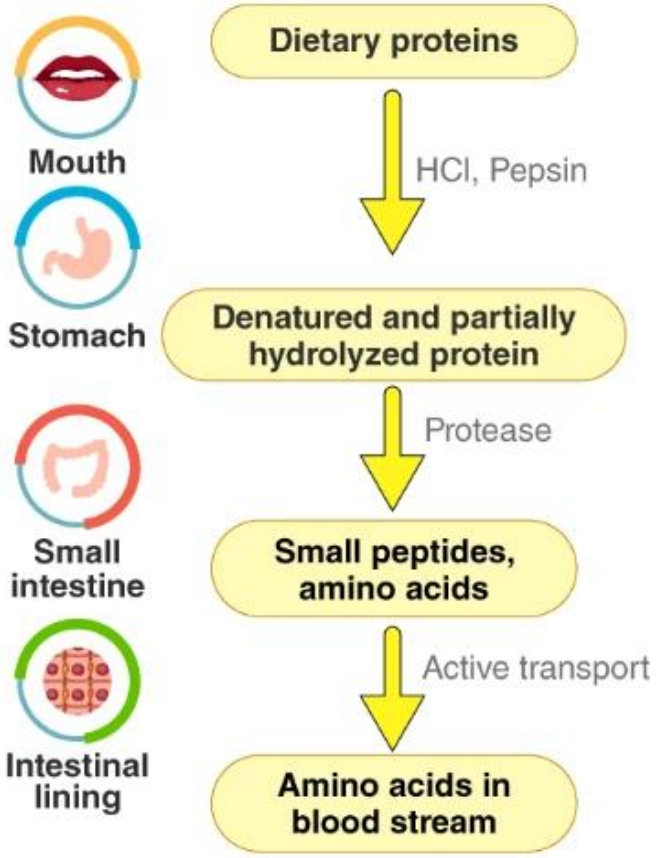


DIGESTION OF DIETARY PROTEINS

Digestion in the stomach: gastric juice, a unique solution containing hydrochloric acid (HCl), which denatures and presents antibacterial activity, and **pepsinogen, which** is a **zymogen, begins digestion in the stomach.**

In the presence of HCl, **pepsinogen** is auto-processed (autocatalysis), which gives rise to **pepsin**, which generates polypeptides and some free amino acids from the diet.

Digestion follows in the small intestine, where pancreatic juices and enzymes from the cells in the intestinal wall act through **endopeptidases** (which cut inside the polypeptide) and **exopeptidases** (which cut at the end of the polypeptides).

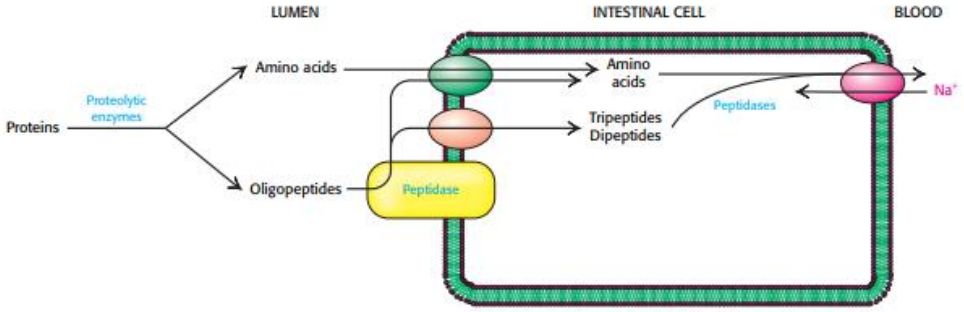


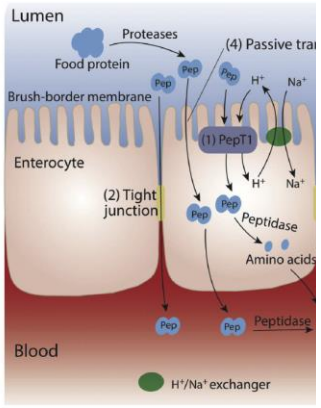
INTESTINAL ABSORPTION OF AMINO ACIDS AND SMALL POLYPEPTIDES

This is an active transport system in which:

Free amino acids are mainly absorbed through a sodium-dependent secondary active transport by solute transporter proteins (SLC) of the apical membranes.

Dipeptides and tripeptides are transported by a proton-linked peptide transporter (PepT1). The peptides are hydrolyzed to free amino acids in the enterocytes.





The portal vein transports amino acids to the liver.

Finally:

Amino acids are transported to the liver through the portal vein, where they are metabolized or released into the bloodstream. This occurs with all amino acids except branched-chain amino acids (BCAA), which are metabolized in the muscle.

PROTEIN TURNOVER

Protein turnover (degradation/synthesis): this is a continuous physiological process by which functional or structural proteins are hydrolyzed and replaced by new ones.
Endogenous proteins show a high protein turnover (300-400 g/day).

Constant quantity:

A balance between synthesis and degradation or between constitutive synthesis and selective degradation.

Characteristics:

- **A general process.**
- **Quantitatively important.**
- **Specific.**
- **A regulated process.**
- **An energy-consuming process.**

Physiological Functions:

- To regenerate "anomalous" proteins.
- To correct errors in protein synthesis, oxidative damage, etc.
- To replace protein losses by physiological processes: peeling secretion
- To regulate metabolism; to control the amount of enzyme.

The rate of protein turnover can vary:

The half-life of proteins is highly variable.
Turnover rate.

There are two main turnover systems:

- **Cytosolic systems: ubiquitin, proteasome/**ATP dependent
- **Lysosome-degrading enzymes,** acid hydrolases/ATP independent
  Autophagy: intracellular protein degradation
  Heterophagy: extracellular proteins incorporated by endocytosis degradation.

PROTEIN TURNOVER RATE

In healthy adults, the total amount of protein in the body remains constant because the rate of protein synthesis is just sufficient to replace the protein that is degraded. This process, called protein turnover, leads to the hydrolysis and re-synthesis of 300–400 g of body protein each day.

The rate of protein turnover varies widely for individual proteins, and protein half-life may vary greatly.

- Short-life proteins are rapidly degraded, with half-lives measured in minutes or hours, i.e. there are many regulatory proteins and misfolded proteins.
- Long-life proteins, with half-lives of days to weeks, constitute the majority of proteins in the cell.
- Structural proteins, such as collagen, are metabolically stable and have half-lives measured in months or years.

CHEMICAL SIGNALS FOR PROTEIN DEGRADATION:

PROTEIN TARGETING: TAGGING SEQUENCES

Protein degradation can be influenced by some structural aspect of the protein:

- Chemical alteration by oxidation
- Ubiquitin tagging

The half-life of a protein is also influenced by the amino (N)-terminal residue:

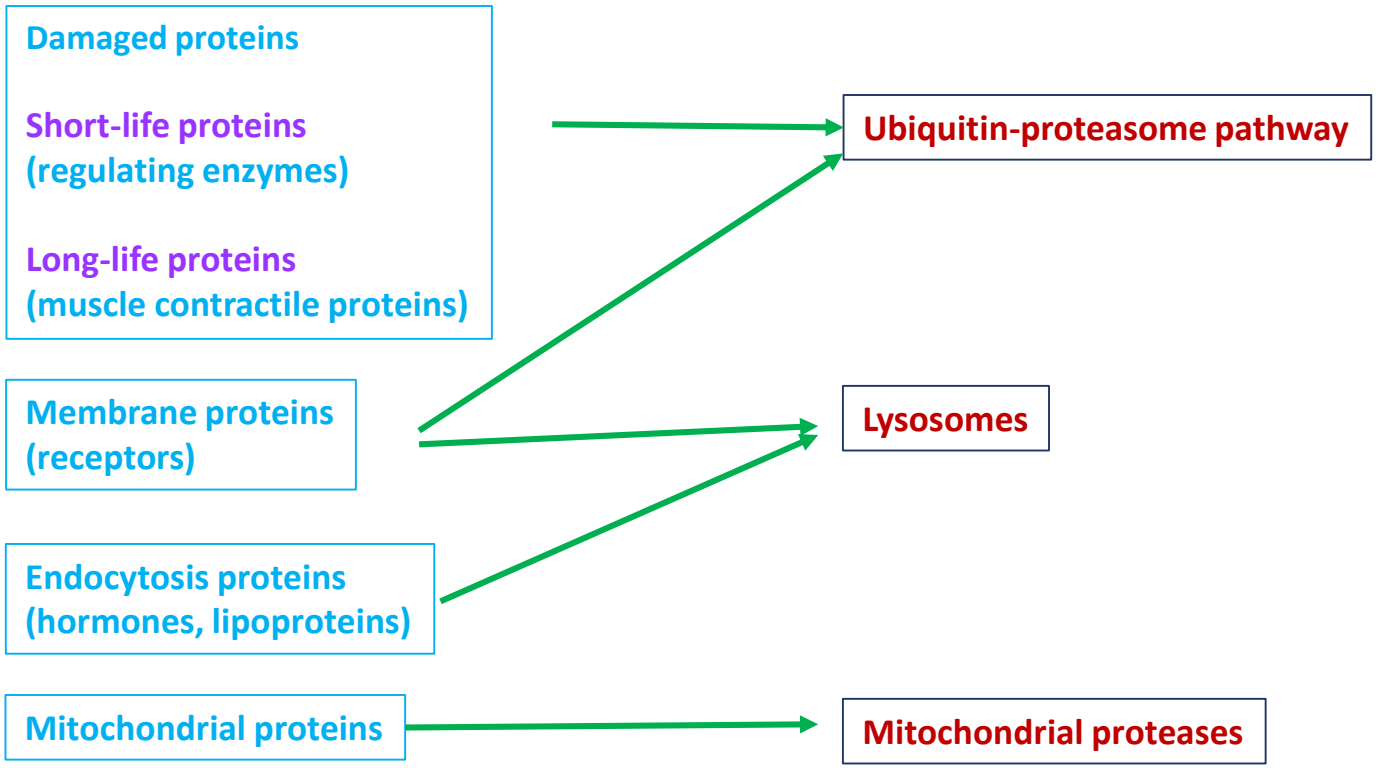
- Proteins that have serine as the N-terminal amino acid are long-lived, with a half-life of over 20 hours.
- Proteins with aspartate at their N-terminus have a half-life of only 3 minutes.

Proteins rich in sequences containing proline, glutamate, serine, and threonine (called PEST sequences) are rapidly degraded and therefore have short half-lives.

PROTEIN DIGESTION SYSTEMS

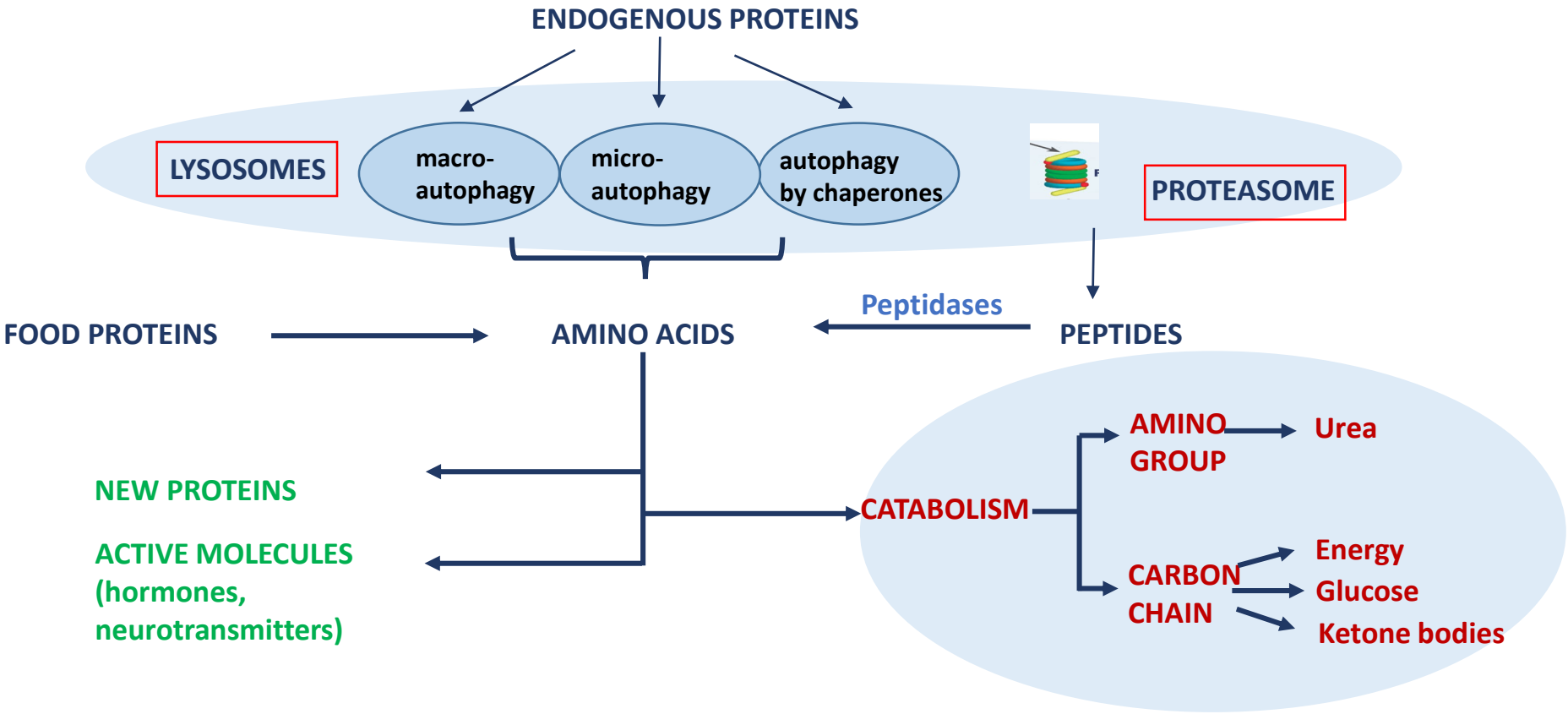
There are two main turnover systems:

- **Cytosolic systems: ubiquitin, proteasome/**ATP dependent
- **Lysosome-degrading enzymes,** acid hydrolases/ATP independent
  - **Autophagy**: intracellular protein degradation
  - **Heterophagy**: extracellular proteins incorporated by endocytosis degradation



PROTEIN DIGESTION SYSTEMS

- **Cytosolic systems: ubiquitin, proteasome/**ATP dependent
- **Lysosome-degrading enzymes,** acid hydrolases/ATP independent
  - **Autophagy**: intracellular protein degradations
  - **Heterophagy**: extracellular proteins incorporated by endocytosis degradation



PROTEIN DIGESTION SYSTEMS

Regulated process: intracellular proteolysis

There are two main turnover systems:

- **Cytosolic systems: ubiquitin, proteasome/**ATP dependent
- **Lysosome-degrading enzymes,** acid hydrolases/ATP independent

But also:

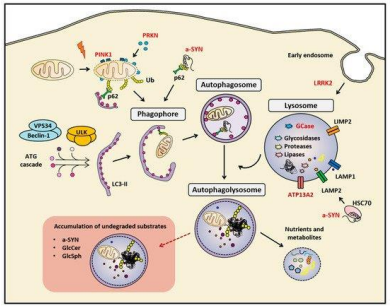
INTRACELLULAR PROTEOLYSIS

- **Cytosolic**
  - **Ubiquitin/proteasome (ATP dependent)**
  - **Calpains (Ca^{2+} dependent)**
  - **Apoptosis: Caspases**
- **Lysosomic**

- **Consisting of degrading enzymes, acid hydrolases (optimal pH =5)**
- **ATP independent**
- **Cathepsins: highly heterogeneous**
- **Involved in:**
  - **Autophagy: to degrade intracellular proteins**
  - **Heterophagy: to degrade extracellular proteins incorporated by endocytosis**

This process involves 'digesting' their content, which is carried out in endocytic vesicles.

The lysosome-dependent autophagy degradation pathway is one of the dysregulated pathways: Parkinson's Disease.



Cells 2019, 8(11), 1317

4. AMINO ACIDS FROM ENDOGEN PROTEINS. PROTEIN TURNOVER.

PROTEIN DIGESTION SYSTEMS

INTRACELLULAR PROTEASOME

UBIQUITIN/PROTEASOME SYSTEM (ATP dependent)

UBIQUITIN AND PROTEASOME

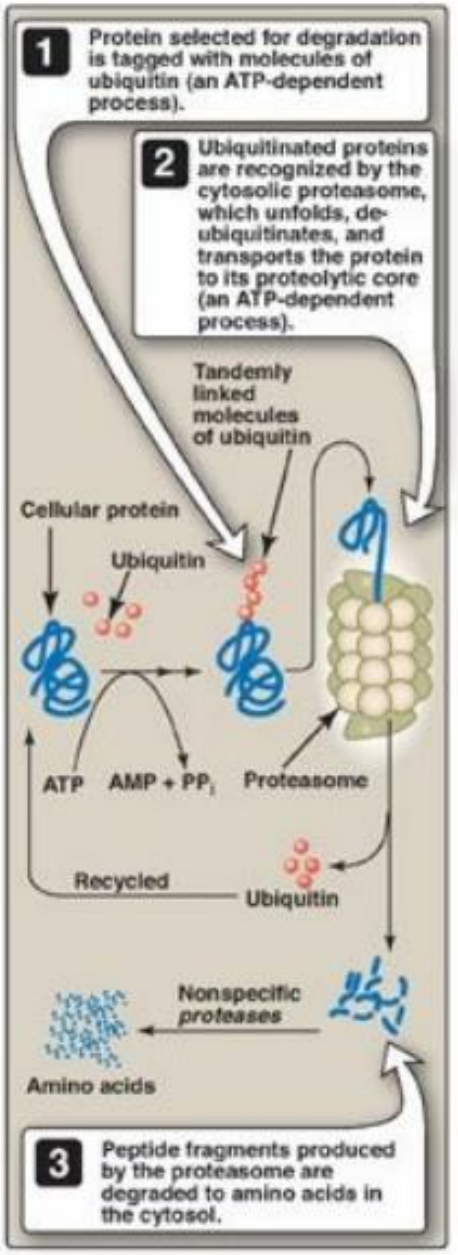
UBIQUITIN

- Small protein 8.5 kDa (76 amino acids)
- Present in all eukaryotic cells
- Highly conserved (3 different amino acids between yeast and mammals)
- Serves as a unifying protein degradation labelling/labelling system

and a prior step towards other modifications for proteolysis

PROTEASOME

Proteases complexes that digest tagged proteins with Ubiquitin (Ub)



Harvey and Ferrier

PROTEIN DIGESTION SYSTEMS

UBIQUITIN/PROTEASOME (ATP-dependent proteolysis)

PROTEIN UBIQUITINATION:

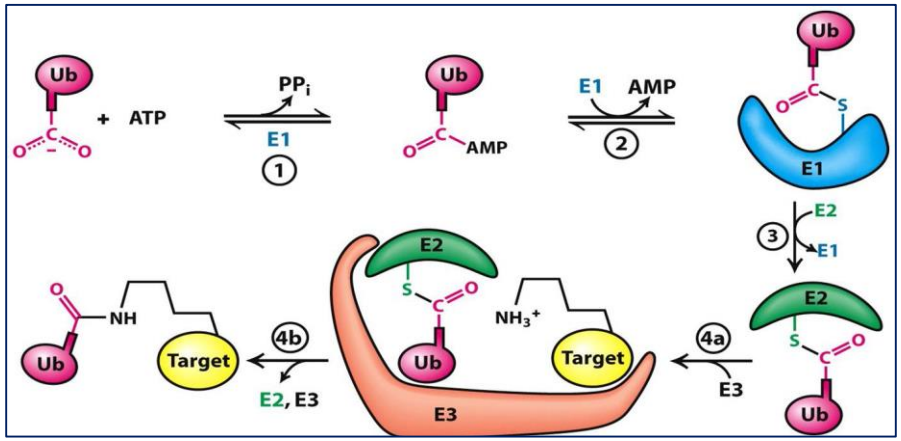
Ubiquitin binds to a lysine residue from the protein to be degraded.

This three-step process involves 3 enzymes:

Enzyme E1: Ubiquitin activation

Enzyme E2: Ubiquitin-transporter protein; Enzyme E2 transfers activated Ubiquitin from E1 to the substrate bonded to E3

Enzyme E3: Ubiquitin-protein ligase recognizes protein to be degraded (substrate)



- **Proteins to be degraded can be mono- or poly-ubiquitinated.**
- **The bonding of 4 Ubiquitins creates a ubiquitination chain.**
- **Proteins may have several ubiquitination chains.**

Stryer

PROTEIN DIGESTION SYSTEMS

UBIQUITIN/PROTEASOME (ATP-dependent proteolysis)

PROTEASOME 26S:

- Proteasome 26s is a multi-catalytic, barrel-shaped complex
- It is macromolecular and proteolytic
- It consumes ATP
- It is composed of:

Protein subunit 20S

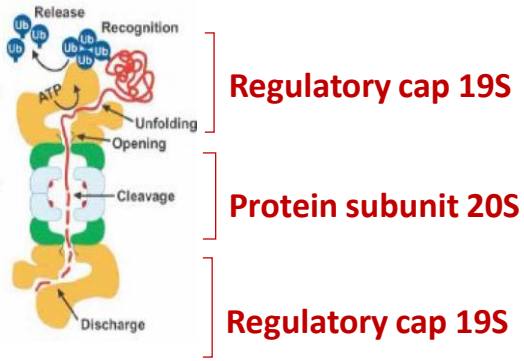
Core

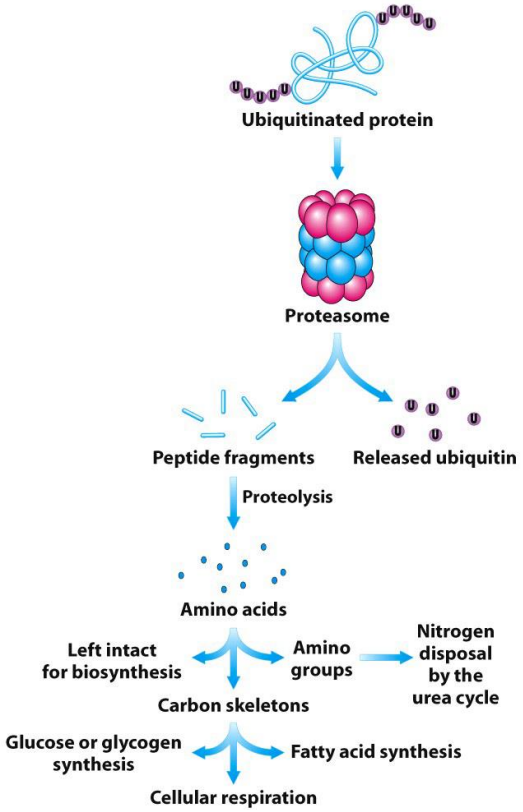
Catalytic body

4 rings, 2 of which are central and 2 are at each end.

Regulatory cap 19S

Multiple ATPases and Ubiquitin-binding sites





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The proteasomal degradation pathway is essential for many cellular processes:

- Cell proliferation and differentiation
- Metabolic regulation
- Cell-cycle control
- Stress response and the elimination of abnormal proteins

5. AMINO ACID BIOSYNTHESIS

- Bacteria and plants can synthesize all 20 amino acids

- Mammals can only synthesize non-essential amino acids (11)

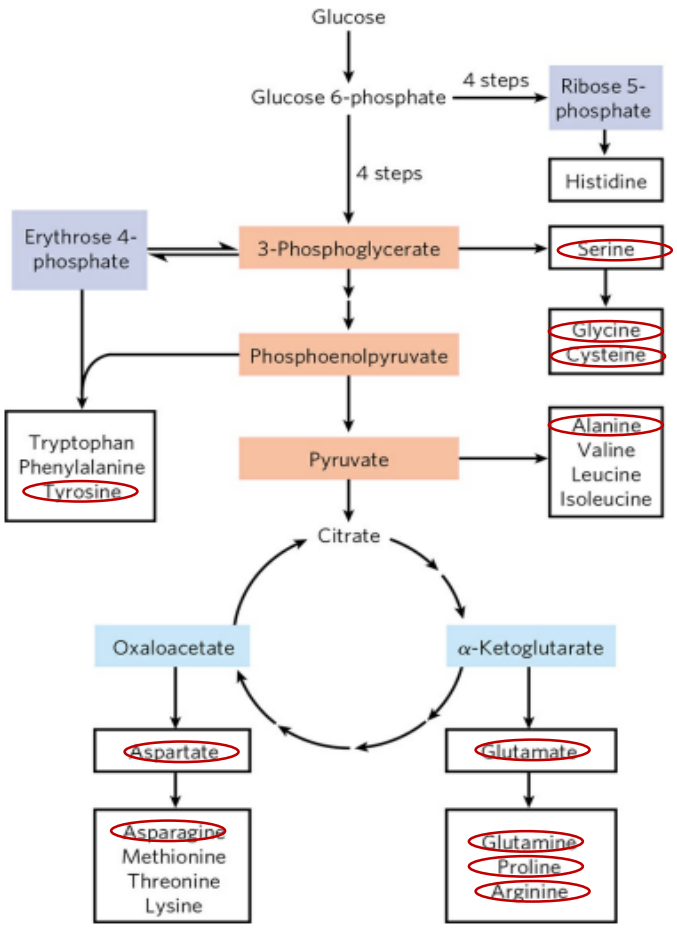
Alanine, Arginine, Aspartate, Asparagine, Cysteine, Glycine, Glutamate Glutamine, Proline, Serine, and Tyrosine

Essential amino acids: from the diet (9)

Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine

Conditional amino acids become essential in special circumstances (6)

Arginine, Cysteine, Glycine, Glutamine, Tyrosine, and Proline

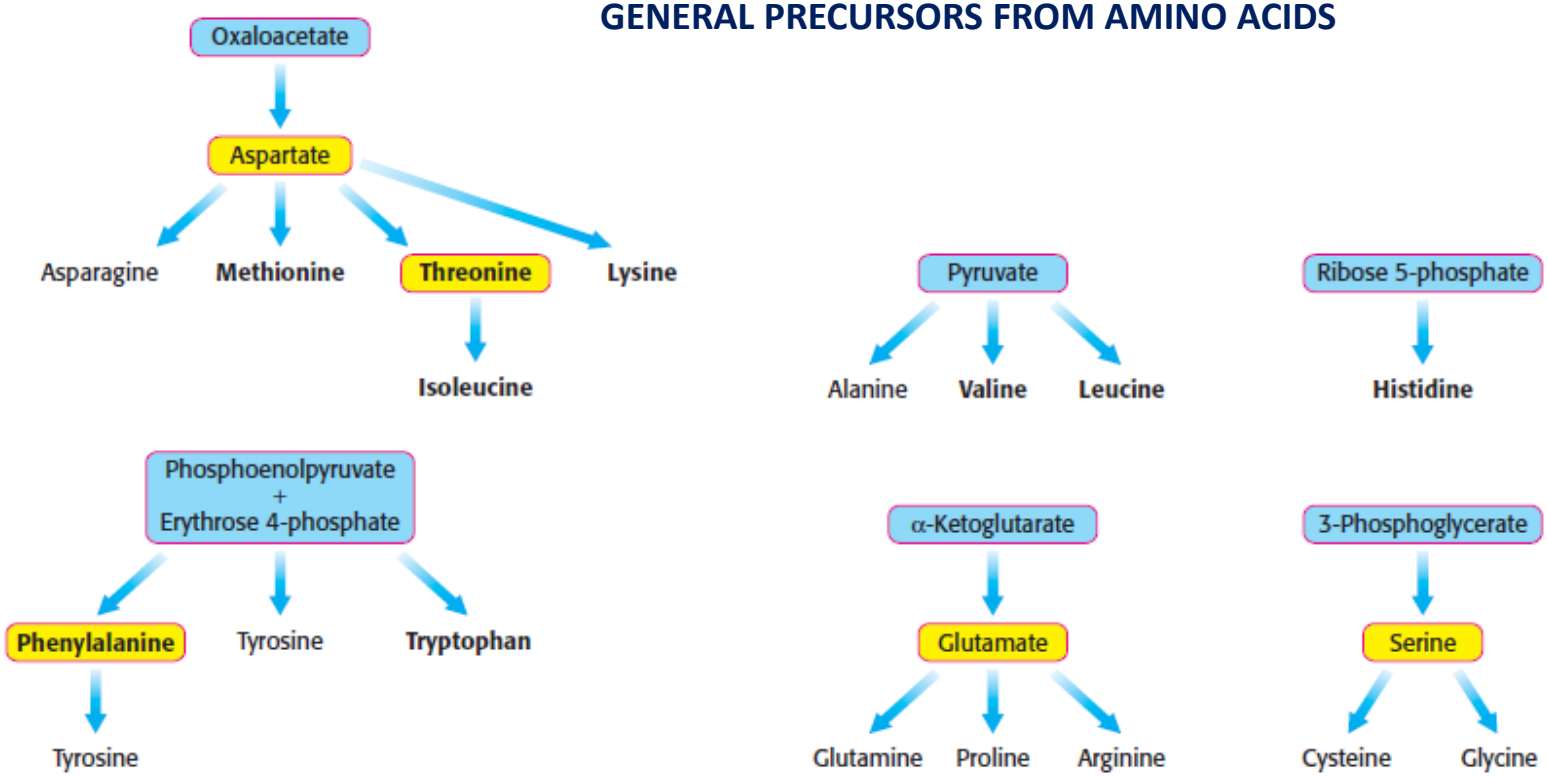


The carbon chain comes from:

- **Glycolysis** (3-phosphoglycerate, phosphoenolpyruvate, pyruvate)
- **Citric acid cycle** (oxaloacetate, α-ketoglutarate)
- **Pentose phosphate pathway** (ribose-5-phosphate)

- Nitrogen comes from Glutamate or Glutamine
- Aromatic amino acid biosynthesis pathways are more complex

GENERAL PRECURSORS FROM AMINO ACIDS



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Essential and non-essential amino acids:

Of the 20 protein amino acids, our organism can synthesize eleven, ten of which use glucose as the carbon chain donor.

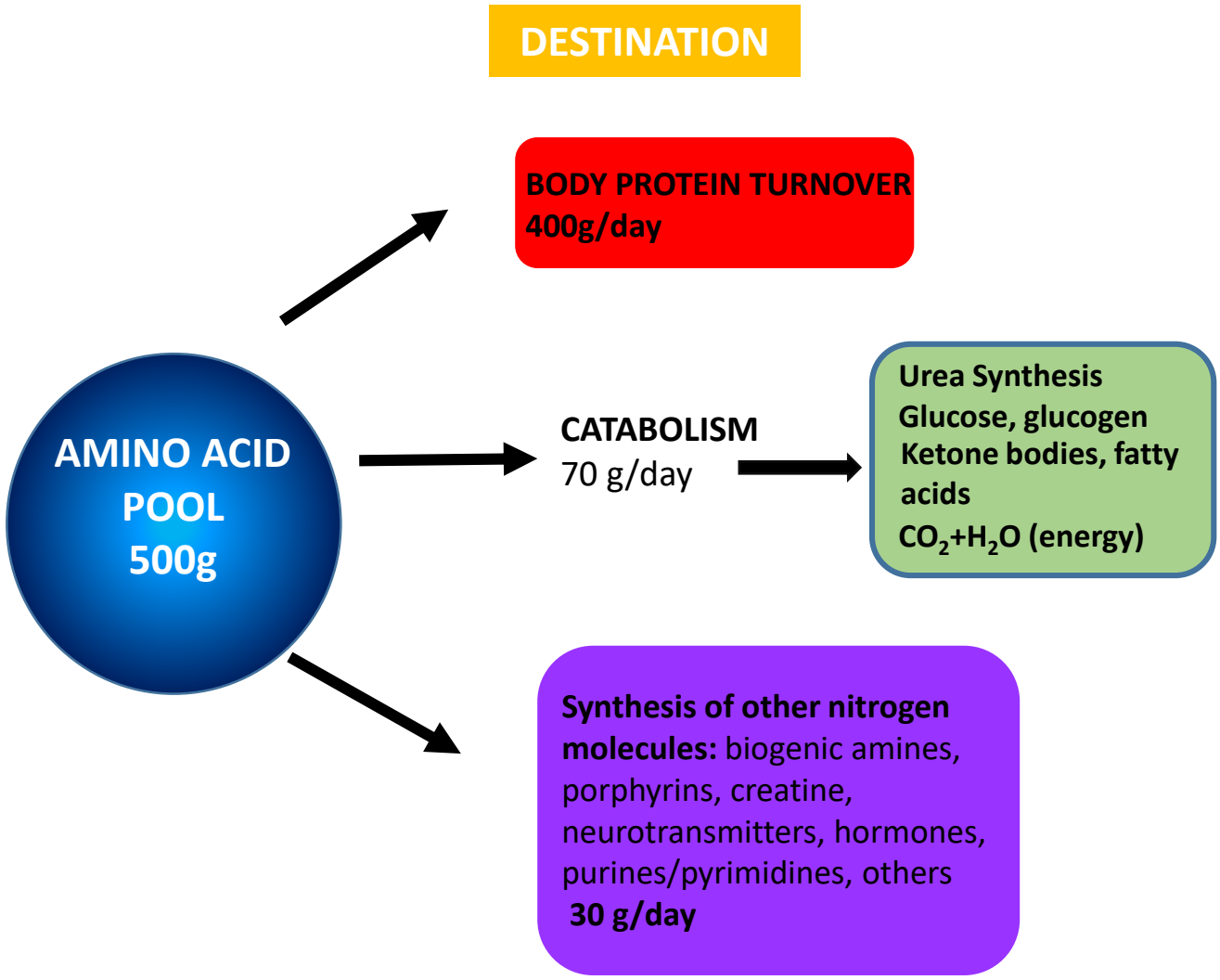
The amino group is formed from Glutamate or Glutamine.

Essential	Non-essential
Histidine (His)	Alanine (Ala)
Isoleucine (Ile)	Arginine (Arg)
Leucine (Leu)	Asparagine (Asn)
Lysine (Lys)	Aspartate (Asp)
Methionine (Met)	Cysteine (Cys)
Phenylalanine (Phe)	Glutamate (Glut)
Threonine (Thr)	Glutamine (Gln)
Tryptophan (Trp)	Glycine (Gly)
Valine (Val)	Proline (Pro)
	Serine (Ser)
	Tyrosine (Tyr)

Conditionally essential:
Arg, His, in growth
Cys, requires S from Met
In mammals, Tyr comes from Phe hydroxylation

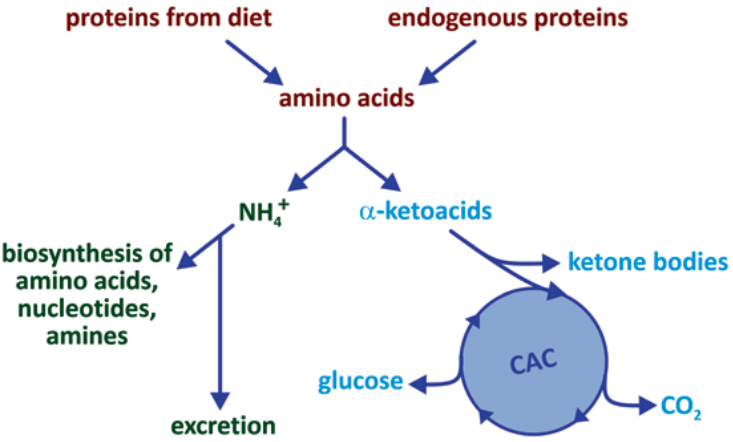
- **Essential** amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) must be incorporated through **diet**.
- **Arginine** and supplemental **histidine** must be added in **growth** periods.
- **Non-essential** amino acids can be synthesized by the organism. The carbon chain for 10 amino acids can be obtained from glucose.
- **Cysteine** requires **sulfur** from the essential amino acid **methionine**.
- **Tyrosine** is produced by **hydroxylation** of the essential amino acid **phenylalanine**.
- Amino acids are **precursors** for the synthesis of several other **nitrogen compounds**, such as purines and pyrimidines, the heme group, creatine, catecholamines and other biogenic amines.

6. BIOSYNTHESIS OF NEW NITROGEN MOLECULES



6. BIOSYNTHESIS OF NEW NITROGEN MOLECULES

In addition to their role in building structural protein blocks, amino acids are precursors to many specialized biomolecules, including hormones, coenzymes, nucleotides, alkaloids, cell wall polymers, porphyrins, antibiotics, pigments and neurotransmitters.



Amino acid	Biomolecule
Aspartate/Glycine/Glutamine	Purine and pyrimidine bases
Tryptophan	NAD^+ ring
Glycine	Porphyrins/Heme group
Arginine/Glycine/Methionine	SAM/Creatine/Creatine-P
Cysteine	CoA
Arginine	Nitric Oxide (NO_2)
	Hormones
Phenylalanine/Tyrosine	Thyroid hormones
Tryptophan	Melatonin
	Neurotransmitters
Tyrosine	Dopamine/Adrenaline/Noradrenaline
Glutamate	GABA
Histidine	Histamine
Tryptophan	Serotonin

Da Poian

Glycine ⟶ Porphyrins/Heme group

Porphyrins are cyclic compounds that bind easily to metal ions, usually **ferrous iron** (Fe^{2+}) or **ferric iron** (Fe^{3+}).

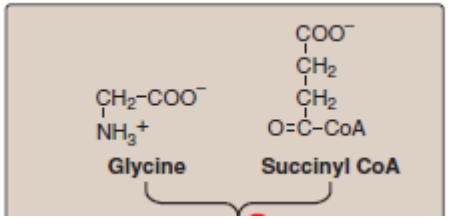
The most prevalent metalloporphyrin in humans is the **Heme Group.**

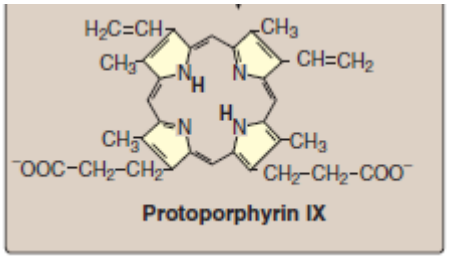
Prosthetic group of:

-Hemoglobin (Hb), myoglobin.

-Cytochromes, the Cytochrome P450 System (CYP), monooxygenase.

-Catalases, nitric oxide synthetase and peroxidase.





Protoporphyrin IX

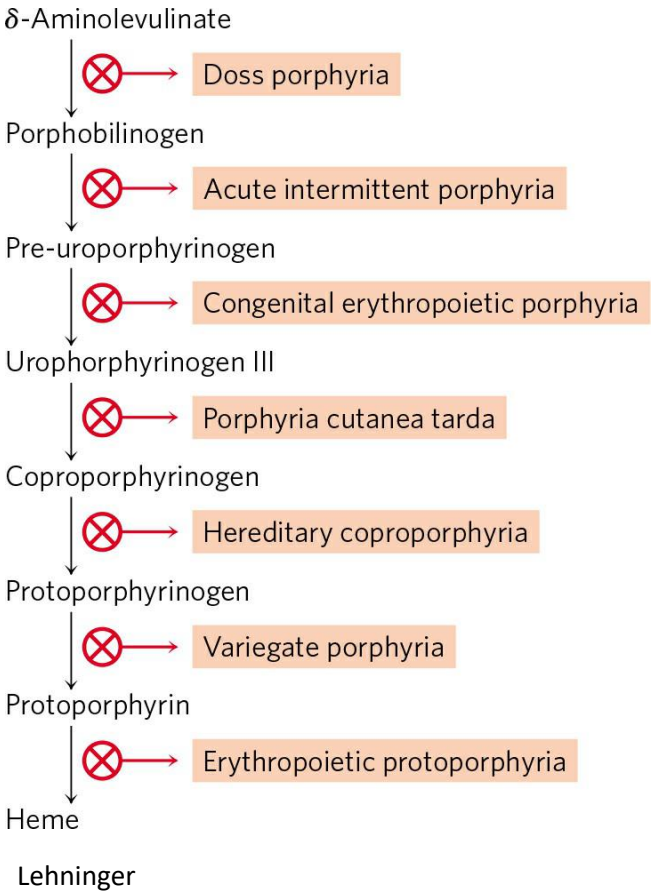
Glycine + Succinyl-CoA

Heme Group contains Fe^{2+} coordinated in the middle of a tetrapyrrolic ring of protoporphyrin IX

***Glycine: non-essential**

6. BIOSYNTHESIS OF NEW NITROGEN MOLECULES

Glycine ⟶ Porphyrins/Heme group



Lehninger

Porphyries are a group of genetic diseases that result from defects in enzymes of the biosynthetic pathway from glycine to porphyrins.

Specific porphyrin precursors accumulate in erythrocytes, body fluids and the liver.

The most common form is acute intermittent porphyria. Most individuals who inherit this condition are heterozygotes and are usually asymptomatic because the single copy of the normal gene provides a sufficient level of enzyme function. However, certain nutritional or environmental factors (which are still poorly understood) can cause a buildup of intermediates, leading to attacks of acute abdominal pain and neurological dysfunction.

One of the rarer porphyries results in an accumulation of uroporphyrinogen I, an abnormal isomer of a protoporphyrin precursor. This compound stains the urine red, causes the teeth to fluoresce strongly in ultraviolet light, and makes the skin abnormally sensitive to sunlight.

Many individuals with porphyria are anaemic because insufficient heme is synthesized.

The symptoms of most porphyries are now readily controlled by dietary changes or the administration of heme or heme derivatives.

***Glycine: non-essential**

6. BIOSYNTHESIS OF NEW NITROGEN MOLECULES

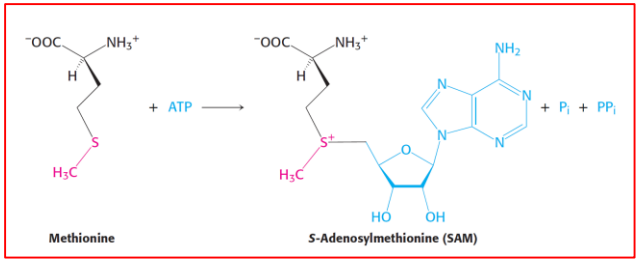
Arginine/Glycine/Methionine ⟶ SAM/creatine-P/ creatinine

Phosphocreatine, derived from **creatine**, is an important energy buffer in skeletal muscle. It provides intracellular ATP in muscle contraction.

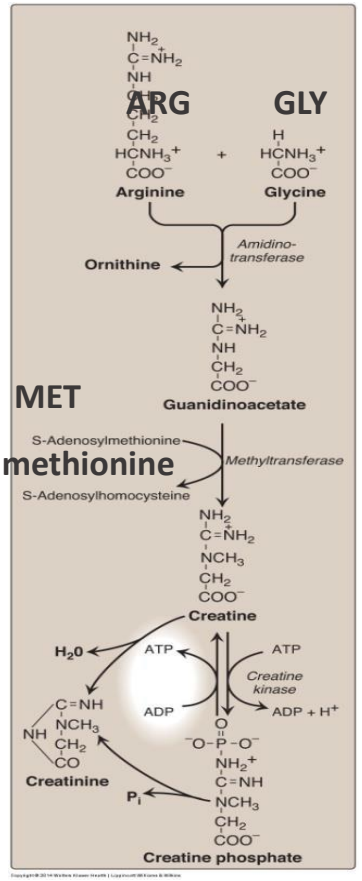
Creatine is synthesized from glycine and arginine and is also present in skeletal muscle, the myocardium and the brain.

Methionine, in the form of ***S*-Adenosylmethionine**, acts as a methyl group donor.

S-Adenosylmethionine (SAM): from Methionine



S-Adenosylmethionine (SAM)



Phosphocreatine and **creatine** are cycled to produce **creatinine**, which is finally excreted in the urine. **Creatinine** is an important marker of **renal function.**

***Arginine and Glycine: non-essential**

***Methionine: essential**

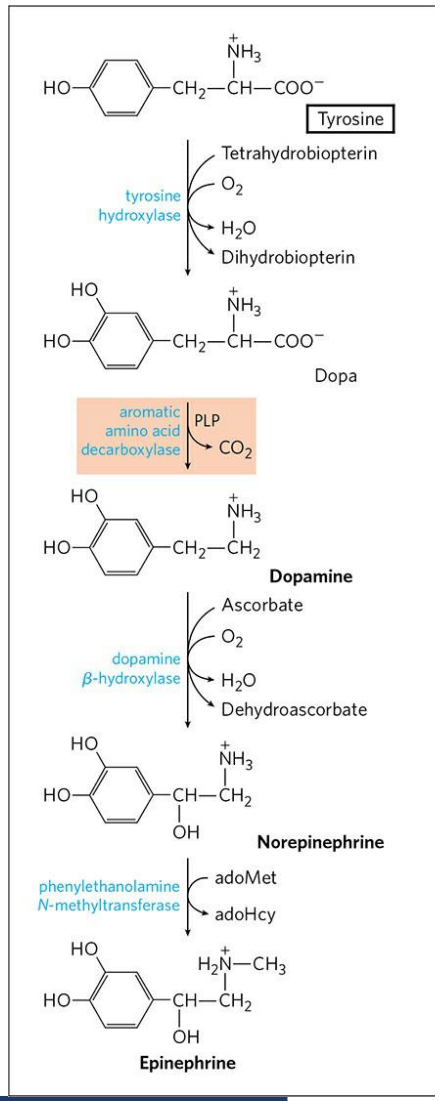
6. BIOSYNTHESIS OF NEW NITROGEN MOLECULES

Neurotransmitters

Tyrosine → Dopamine/adrenaline/noradrenaline

Catecholamines

- **Dopamine, norepinephrine (NE, noradrenaline) and epinephrine (or adrenaline) are biologically active amines. They are derived from tyrosine and are biogenic amines.**
- **Dopamine and noradrenaline are synthesized in the brain and work as neurotransmitters.**
- **Adrenaline is synthesized from noradrenaline in the adrenal medulla. It is considered a hormone and a neurotransmitter.**



BIOGENIC AMINES

Functions of catecholamines:

- They act as vasoconstrictors in some tissues and as vasodilators in others.
- They increase the heart rate and are bronchial muscle relaxants.
- They stimulate glycogenolysis in muscle and lipolysis in adipose tissue.

They are rapidly degraded and eliminated from the body.

*In mammals, tyrosine is obtained from phenylalanine hydroxylation.

Neurotransmitters

Histidine ⟶ Histamine

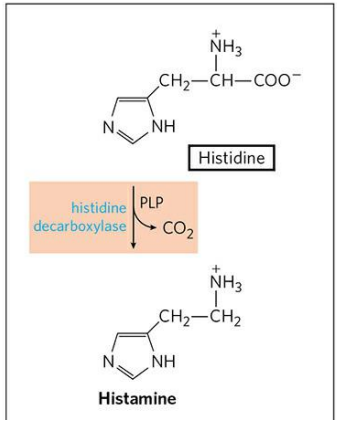
Histamine:

-Histamine has vasodilator action and lowers blood pressure.

-It collaborates in the constriction of the bronchioles.

-It stimulates the production of HCl and pepsin in the stomach.

-It is released abruptly in response to the entry of allergenic substances into the tissues.



Decarboxylation of Histidine
Loss of CO_2
Coenzyme:
Pyridoxal phosphate (PLP)

*Histidine: essential

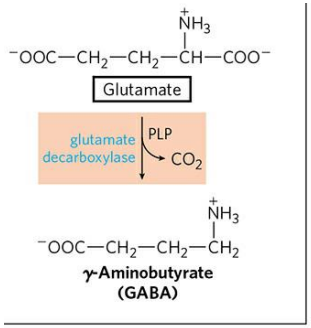
Glutamate ⟶ GABA

GABA:

-GABA is produced in the central nervous system.

-It is a chemical intermediate regulating neuronal activity.

-It is an inhibitor/depressant of nerve impulse transmission.



*Glutamate: non-essential

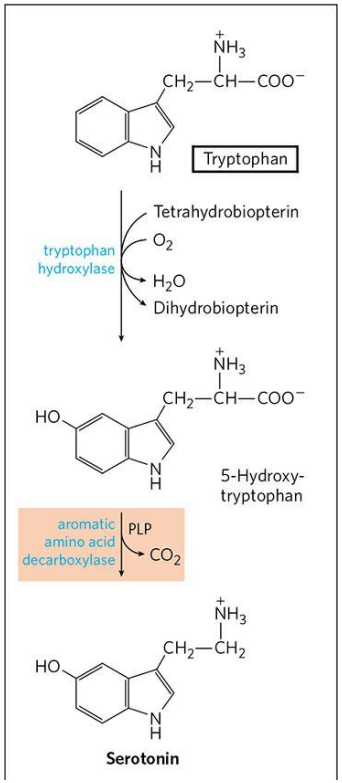
6. BIOSYNTHESIS OF NEW NITROGEN MOLECULES

Tryptophan ⟶ Serotonin

- Serotonin is a **neurotransmitter** that exerts multiple regulatory actions on the nervous system.
- The largest amount of serotonin is found in the **intestinal mucosa** and the smallest amount is found in the **central nervous system.**

- Serotonin has multiple **physiological roles:**
  - -It is involved in the perception of pain
  - -It is involved in the regulation of sleep, appetite, temperature, blood pressure, cognitive functions and mood (it causes a sense of well-being)
  - -It is the happiness hormone

It is of particular interest in the development of antidepressants as selective serotonin **inhibitors** reuptake serotonin and **maintain serotonin levels.**



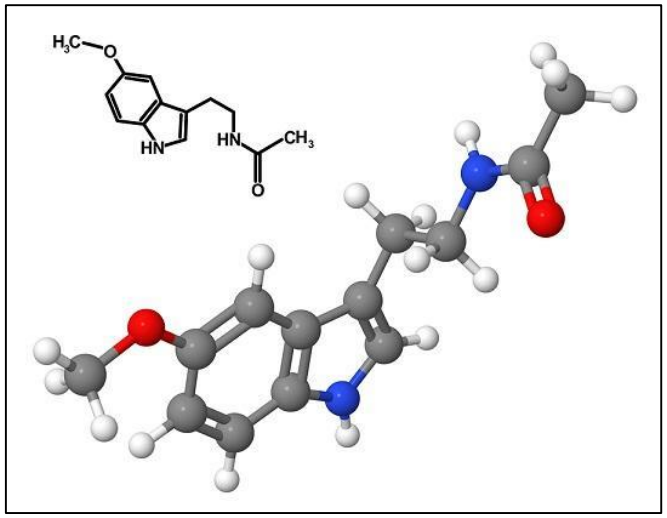
Pyridoxal phosphate (PLP)

***Tryptophan: essential**

Hormones

Tryptophan → Melatonin

- Melatonin or N-acetyl-5-methoxytryptamine is a **neurohormone**.
- It varies with the day/night cycle.
- It is produced in the pineal gland; it is inhibited by light and stimulated by darkness (it is the darkness hormone).
- Melatonin secretion peaks in the middle of the night and gradually falls during the second half of the night.



*Tryptophan: essential