



Faculty of Nature and
Life Sciences

Faculty of Agronomy



Specialization:
Animal

Nutrition and Biochemistry

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Course for Master II students

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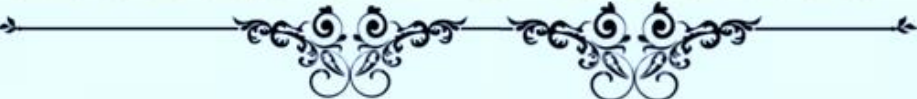
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INTRODUCTION



Nutrition and Biochemistry

Nutrition and biochemistry are two essential and complementary scientific fields that play a central role in understanding how the human body functions and how it interacts with food. Nutrition focuses on the study of food, its components such as carbohydrates, lipids, proteins, vitamins, minerals and how these nutrients influence health, growth, metabolism, and disease prevention. It also encompasses nutritional needs at different stages of life, eating habits, and the interactions between diet, environment, and society.

Biochemistry, on the other hand, examines the chemical and molecular processes that occur within living cells. It helps us understand how nutrients are digested, absorbed, transported, transformed, and utilized at the cellular and tissue levels. Through biochemistry, we can analyze the complex metabolic pathways that convert food into energy, building blocks for cells, and signaling molecules essential for the proper functioning of the body.

The connection between nutrition and biochemistry is therefore crucial. Together, they allow us to better understand how our dietary choices directly affect our health at the molecular level. This link is particularly important in today's context, marked by a rise in chronic diseases related to diet, such as type 2 diabetes, obesity, cardiovascular diseases, and certain cancers. Moreover, recent advances in nutritional biochemistry are paving the way for more personalized approaches to nutrition, taking into account individual metabolism, genetic background, and lifestyle.

Studying nutrition through the lens of biochemistry not only deepens our knowledge of the body's inner workings but also helps develop effective strategies to promote healthy eating, prevent nutritional imbalances, and improve overall quality of life.

**Subcellular Organization
And
Molecular Constituents**

I. Structure of Subcellular Organelles

The cell is the smallest structural, functional, and reproductive unit of all living organisms. "Every cell originates from a preexisting cell." A cell can either constitute a unicellular organism on its own (e.g., bacteria, protists, fungi) or combine with other cells to form a multicellular organism (animals, plants).

Cells are complex and highly organized units that contain various structures, each playing a specific role in maintaining cellular function. These structures, known as organelles, work together to carry out essential biochemical processes such as energy production, protein synthesis, and waste degradation. The table below summarizes the main organelles, their physical characteristics, and their biochemical roles.

Table I: Overview of Subcellular Structures and Their Functions

Cellular Structure	Physical Structure	Biochemical Role
Nucleus	Surrounded by a nuclear envelope (double membrane), contains chromatin (DNA + proteins)	Site of transcription (RNA synthesis) and DNA replication
Cytoplasm and Cytoskeleton	Aqueous solution with ions, organic molecules, and organelles; supported by filaments	Enables metabolic reactions (e.g., glycolysis, fatty acid synthesis) and intracellular transport
Mitochondria	Double membrane; internal matrix contains enzymes and mitochondrial DNA	Cellular respiration and ATP production via the Krebs cycle and the respiratory chain
Endoplasmic Reticulum (ER)	Network of interconnected membranes; rough (with ribosomes) or smooth	Rough ER: Protein synthesis and folding Smooth ER: Lipid synthesis and carbohydrate metabolism
Golgi Apparatus	Stack of flattened membrane sacs	Modifies, sorts, and packages proteins and lipids for transport
Lysosomes and Peroxisomes	Vesicles with hydrolytic enzymes (lysosomes) or oxidative enzymes (peroxisomes)	Lysosomes: Breakdown of macromolecules Peroxisomes: Metabolism of peroxides and lipids
Plasma Membrane	Lipid bilayer with proteins and carbohydrates	Regulates cellular exchanges and signal reception

II. Role of Water in Biochemical Processes

Water is an essential chemical compound for life. It consists of molecules, each made up of one oxygen atom and two hydrogen atoms, represented chemically as H₂O. The subscript "2" in the formula indicates that each water molecule contains two hydrogen atoms bonded to one oxygen atom. Water's unique chemical structure makes it a polar molecule, capable of forming hydrogen bonds. These properties allow water to act not only as a universal solvent but also as a key player in the structure, interactions, and biochemical reactions of living organisms. In biochemistry, water is far more than just a background medium; it actively participates in and regulates vital processes (table II).

Table II: Role of Water in Biochemical Processes

Function of Water	Description
Universal solvent	Dissolves polar and ionic substances, enabling chemical reactions.
Hydrogen bonding	Interacts with other molecules through hydrogen bonds (donor/acceptor).
Stabilization of biomolecules	Stabilizes the structure of proteins, nucleic acids, membranes, etc.
Hydrophobic effect	Repels nonpolar molecules, promoting protein folding and membrane formation
Support in enzymatic reactions	Acts as a reactant, product, or medium in biochemical reactions.
Molecular organization	Organizes molecules based on their affinity to water (hydrophilic/hydrophobic).
Thermal regulation	Its high heat capacity helps maintain stable temperatures in cells

III. Chemical Interactions in Biochemistry

Molecules are formed through the creation of chemical bonds, attractive forces between the atoms that compose them. These bonds determine the stability, shape, and reactivity of biomolecules. In biochemistry, several types of bonds play key roles in the structure and function of living systems.

III.1. Ionic Bond

An **ionic bond** forms between two atoms when one atom donates one or more electrons to the other. The atom that loses electrons becomes a **positively charged ion (cation)**, while the one that gains electrons becomes a **negatively charged ion (anion)**. These oppositely charged ions are strongly attracted to each other, creating a stable bond. This type of bond often occurs between highly electronegative and highly electropositive elements.

III.2. Covalent Bond

Covalent bonds occur when two atoms share one or more pairs of electrons. This is the most common type of bond in biological molecules. It allows the creation of stable and complex molecular structures, essential for the formation of proteins, carbohydrates, lipids, and nucleic acids.

III.3. Hydrogen Bond

Hydrogen bonds are weak but essential interactions that happen when a hydrogen atom, already involved in a covalent bond with an electronegative atom (such as oxygen or nitrogen), acquires a partial positive charge. This hydrogen atom can then be attracted to another nearby electronegative atom. Although hydrogen bonds are weaker than covalent or ionic bonds, they are numerous and crucial for maintaining the three-dimensional structure of proteins and DNA, as well as the unique properties of water.

III.4. Acid-Base Behavior

Beyond chemical bonds, the acid-base behavior of molecules is fundamental in biochemistry. An acid molecule can release H^+ ions (protons) into a solution, while a base can accept these H^+ ions. This behavior directly influences the pH of the environment, which regulates enzyme activity, protein stability, and overall chemical balance within cells. Acids and bases can be strong (fully dissociated) or weak (partially dissociated), and their interaction determines a system's ability to maintain a stable pH, often through buffer systems like the bicarbonate buffer in blood.

IV. Structure and Functions of Major Macromolecules

The main biochemical functions are organized around four major classes of biomolecules: **carbohydrates**, **lipids**, **proteins**, and **nucleic acids**. Each of these macromolecules plays a vital role in the structure, metabolism, and regulation of living organisms.

IV.1. Carbohydrates

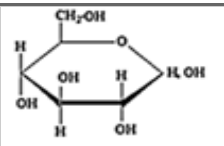
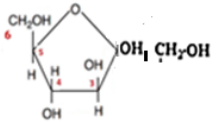
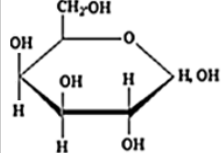
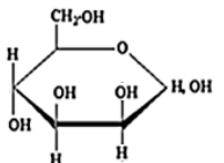
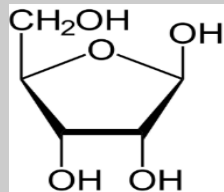
Carbohydrates are organic compounds made up of carbon (C), hydrogen (H), and oxygen (O). Also known as sugars or saccharides, they are among the most abundant and vital biomolecules in living organisms. Carbohydrates serve not only as a primary energy source for cellular activities but also play essential roles in energy storage, structural support, and cell-to-cell communication.

Carbohydrates can be classified into three main categories based on their size: Monosaccharides, Disaccharides and polysaccharides.

IV.1.1. Monosaccharides

Monosaccharides are the simplest carbohydrates and are essential to many biological processes. The most commonly found in living organisms are those made up of five or six carbon atoms, including glucose, fructose, galactose, mannose, and ribose. Each of these sugars has specific structures, functions, and sources, as shown in the table below.

Table III. Main Monosaccharides and Their Functions

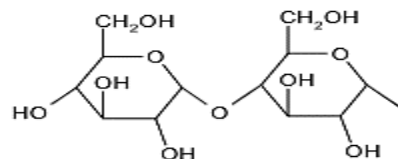
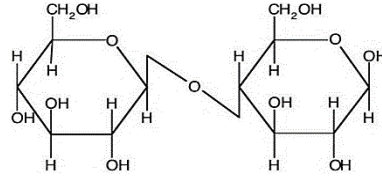
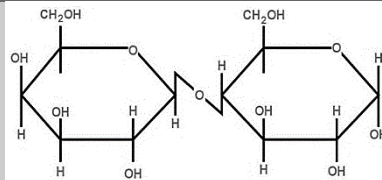
Monosaccharose	Structure	Role	Sources
Glucose		It is the sugar of the organism. It is transported by the blood and is the main sugar used by tissues.	It is found in fruit juices, as well as after the hydrolysis of starch, cane sugar, maltose, and lactose.
Fructose		It is converted into glucose in the liver and intestines, making it usable by the body.	Fructose is found in fruit juices, honey, dahlias, artichokes, and Jerusalem artichokes.
Galactose		It is converted into glucose in the liver and metabolized. It is synthesized in the mammary gland for the production of lactose in breast milk. Galactose is a component of glycolipids and glycoproteins.	The hydrolysis of lactose produces galactose.
Mannose		It is often involved in the glycosylation of proteins and lipids, a process essential for cell function, cell communication, and immune response.	A varied diet including fruits, vegetables, legumes, and mushrooms.
Ribose		• Ribose is a five-carbon sugar (pentose) essential for the structure of nucleic acids. • It forms the sugar backbone of RNA and is also a component of several important coenzymes such as ATP, NAD⁺, and FAD. • Ribose plays a crucial role in energy metabolism and protein synthesis through its involvement in RNA.	Ribose is not commonly found freely in the diet but is naturally produced by the body from glucose via the pentose phosphate pathway. It is present in small amounts in certain meats, organ meats (such as liver), and yeasts.

IV.1.2. Disaccharides

Diholositides are two sugars linked together by a glycosidic bond. Depending on the type of linkage between the two sugars, the diholositide can be non-reducing or reducing.

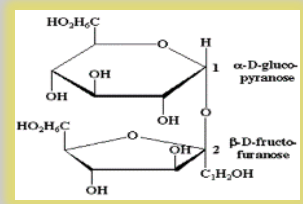
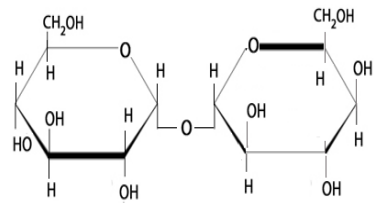
✚ **Reducing Diholositides** (Glycosidic Bond between Sugars): There is condensation of a hemiacetal function of one sugar with an alcoholic function of a second sugar via a glycosidic bond. Therefore, the diholositide retains a free hemiacetal -OH group responsible for the reducing power of the molecule (which exists in two anomeric forms). The main reducing diholositides are presented in Table IV.

Table IV: Main reducing diholosides

Common name	Official nomenclature	Structure
Maltose	α -D-glucopyranosyl (1-4) D-glucopyranose	
Cellobiose	β -D-glucopyranosyl (1-4) D-glucopyranose	
Lactose (milk sugar)	β -D-galactopyranosyl (1-4) D glucopyranose	

✚ **Non-reducing disaccharides** (glycosidic bond): The hemiacetal function of each sugar is condensed through a glycosidic bond. The main non-reducing disaccharides are shown in Table V.

Table V: Main non-reducing diholosides

Common name	Official nomenclature	Structure
Sucrose	α -D-glucopyranosyl (1-2) β -D- fructofuranoside	
Trehalose	α -D-glucopyranosyl (1-1) α -D-glucopyranoside	

IV.1.3. Polysaccharides

These are polymers with very high molar mass, resulting from the condensation of a large number of sugar molecules. In nature, they serve two main functions:

- They act as a form of carbohydrate energy storage (e.g., starch, glycogen).
- They play a structural role in certain cells (e.g., cellulose, chitin).

Table VI: Homogeneous polysaccharides

Name and role	Structure	Degrading enzymes
Starch (Plant reserve polysaccharide)	Starch is composed of two polysaccharides: ➤ Amylose: Amylose accounts for 15% to 30% of the starch mass. It is a linear polymer of glucose residues (ranging from 500 to 20,000 residues, with a molar mass of 10^5 to 10^6 Da) linked by α-(1,4)-D-glucosidic bonds. The branching occurs through α-(1,6)-D-glucosidic bonds, which make up only about 1%. ➤ Amylopectin: Amylopectin accounts for 70% to 85% of the starch mass. It differs from amylose in that it is a branched polymer.	1)- Amylases: There are two types: a- α-amylases (endoamylase) cut the α -1,4 bonds inside the chains by forming small oligosides (3 to 8 residues) that can contain 1 or 2 branch points since they do not cut the α -1,6 bonds. b- β-amylases (exoamylase) cut every other bond from the non-reducing end, thus releasing maltosyl units. During hydrolysis, they cause an inversion on C1 of maltose and it is the β form that is released. 2) Debranching enzymes: they cut the α -1,6 bonds of the branch points.
Glycogen (Reserve polysaccharide in animals)	Glycogen is very similar to amylopectin: it consists of glucose chains linked by α -(1,4) bonds with branches formed by α -(1,6) bonds. However, the chains in glycogen are much shorter, and the glycogen molecule is more densely packed, with a molecular mass that can reach up to 100 million Da.	1. α and β amylases 2. Debranching enzyme 3. Phosphorylases: they attack the chains from the non-reducing ends, through phosphorolysis of α -1,4 bonds, releasing D-glucose-1-phosphate.
Cellulose (Support polysaccharide (cell walls of plant cells))	It consists of long linear chains of glucose linked by β -(1,4) D-glucosidic bonds (300 to 1500 residues - MW = 5×10^4 - 2.5×10^6 Da). The repeating unit is cellobiose.	- Humans are unable to digest cellulose because they lack enzymes that act on β -glucosidic bonds. Its hydrolysis by acid produces cellobiose.
Chitin A supportive polysaccharide found in the exoskeleton of invertebrates (crustaceans, insects)."	It differs from cellulose by the C2 of glucose, where the hydroxyl group is replaced by an acetamide group. It is a polymer of N-acetyl-D-glucosamine residues linked by glycosidic bonds (β 2-1). → [N-acetyl- β -D-fructofuranosyl (2-1)] _n .	Chitinases

IV.2. Lipids

Lipids are a diverse and heterogeneous group of organic compounds that include fats, oils, steroids, and waxes. They are essential components of the human diet; not only because of their high energy content, but also due to their role as carriers of fat-soluble vitamins (A, D, E, and K) and essential fatty acids found in natural food sources.

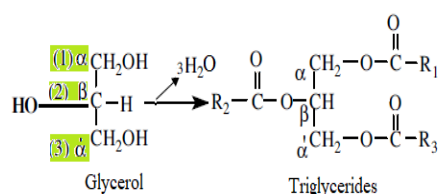
Beyond their nutritional value, lipids perform critical biological functions. Lipoproteins, which are complexes of lipids and proteins, are responsible for transporting lipids through the bloodstream, enabling the distribution of cholesterol and triglycerides to various tissues. Similarly, glycolipids, molecules composed of lipids bound to carbohydrates, are widely expressed on cell membranes, where they play important roles in cell recognition, signaling, and immune response. For example, glycolipids on the surface of red blood cells are responsible for blood group antigens, such as the ABO system.

IV.2.1. True lipids

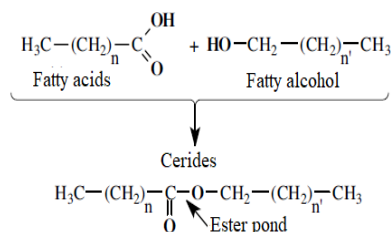
These result from the condensation of fatty acids (FAs) with alcohols through an ester or amide bond and are subdivided into:

a)- Simple lipids: Composed of three elements (C, H, O), they are neutral and classified according to the alcohol that esterifies the fatty acid:

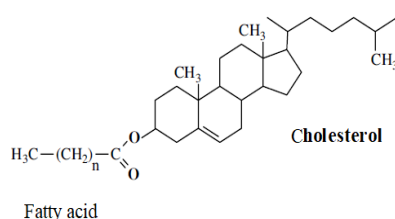
✚ **Glycerolipids:** The alcohol is glycerol.



✚ **Cerides:** The alcohols are long-chain (fatty) alcohols.



✚ **Sterides:** The alcohol is a sterol (polycyclic).



b)- Complex lipids: These heterolipids contain phosphate, sulfate, or carbohydrate groups. They are classified based on the molecule that binds the fatty acids:

✚ Either **glycerol**, which is distinguished from acylglycerols by the heterogroup and is subdivided into: Glycerophospholipids and Glyceroglycolipids (table VII).

Table VII: Complex lipids based on glycerol

Classes	Derivatives of the class	Esterification of the acid function	Structure	Common name
Glycerophospholipides	Phosphatidic Acids	/		/
		phosphatidyl serine		/
		Phosphatidyl ethanolamine		Cephalins
	Glycerophospholipides azotés	phosphatidyl choline		Lecithins
		phosphatidyl inositol		Inositides
	Non-nitrogenous glycerophospholipids	phosphatidyl glycerol		//
		Biphosphatidyl glycerol (cardiolipin)		Cardiolipins
Glycero glycolipides	/	/		1, 2-diacyl- β -D-galactopyranosyl-glycerol

- ✚ **Sphingoid base**, which defines sphingolipids. The latter are amides of sphingosine that form through the linkage of the carboxyl group of the fatty acid to the NH_2 group of sphingosine. The attachment of a fatty acid to the amino group results in a ceramide, which is the precursor molecule for the lipids in this group (table VIII).

Table VIII: Complex lipids based on sphingosine

Classes	Name	Lipid component	Structure
Acyl-Sphingosines	Ceramide	Sphingosine + fatty acid	
Sphingo-Phospholipide	Sphingo-myeline	Sphingosine + fatty acid + Phosphorylcholine	
Sphingo-Sidolipides	Cerebrosides	Sphingosine + fatty acid + sugar These sugars can be Gal (galactosylceramide) or Glu (glucosylceramide)	
	Gangliosides oligosylceramide	Sphingosine + fatty acid + chain of several sugars and sugar derivatives (NANA: N-acetylneuraminic acid, GalNAc: N-acetylgalactosamine)	

IV.2.2. Lipid-like compounds (lipoids)

- ❖ **Isoprenoids:** Derivatives of isoprene units, including the terpene compounds group and sterol derivatives.
- ❖ **Eicosanoids:** Mediators derived from fatty acids.

IV.2.3. Associations of lipids and conjugated lipids

These lipids participate in supramolecular non-covalent complexes that include proteins. In some cases, proteins may have a covalently bound lipid fraction.

Lipoproteins are responsible for the transport of lipids in a soluble form within the plasma. They are spherical macromolecular complexes consisting of a hydrophobic core and a peripheral monolayer. They differ from each other by:

- ✚ The importance and nature of their protein component.
- ✚ The composition of their lipid fraction: the larger the lipid core diameter, the more they contain apolar lipids, and the lower their density.

The different classes of lipoproteins are:

- ✚ **VLDL (Very Low Density Lipoproteins):** Endogenous triglycerides.
- ✚ **IDL (Intermediate Density Lipoproteins):** Provide triglycerides.
- ✚ **LDL (Low Density Lipoproteins):** Supply cholesterol to tissues that cannot synthesize enough for their needs. LDLs transport cholesterol, either free or esterified, in the blood and throughout the body to cells. LDLs are produced by the liver from very low density lipoproteins (VLDL).
- ✚ **HDL (High Density Lipoproteins):** Provide phospholipids.
- ✚ **Chylomicrons:** Triglycerides (during postprandial periods), transport dietary lipids. Chylomicrons, formed in the intestinal cells from dietary lipids, are very large lipid droplets, rich in triglycerides (about 90%). Upon exiting intestinal cells, triglycerides will provide fatty acids.

A deficiency in the uptake of LDL by target tissue cells increases cholesterol levels in blood vessels, leading to deposition and resulting in atherosclerosis. For this reason, LDL is often referred to as "bad cholesterol," in contrast to HDL, which is known as "good cholesterol."

IV.3. Proteins

Proteins are fundamental biological macromolecules composed of **linear chains of amino acids** linked by **peptide bonds**, formed through condensation between the carboxyl group of one amino acid and the amino group of the next. There are **20 standard proteinogenic amino acids**: alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. Among these, **8 to 9 are considered essential** (histidine being semi-essential under certain physiological conditions) because the human body cannot synthesize them in sufficient quantities and must obtain them through diet. From a nutritional perspective, proteins are supplied by a variety of **dietary sources**: **animal-based foods** such as meat, fish, eggs, and dairy provide **complete proteins** (containing all essential amino acids), whereas **plant-based sources** like legumes, cereals, nuts, and seeds generally offer **incomplete proteins** but can complement each other when combined properly in the diet.

Proteins exhibit a hierarchical structural organization: a **primary structure** defined by the amino acid sequence; a **secondary structure** consisting of regular motifs stabilized by hydrogen

bonds (α -helices and β -sheets); a **tertiary structure** representing the three-dimensional folding of the polypeptide chain; and sometimes a **quaternary structure** when multiple polypeptide subunits assemble (Fig.1). Protein biosynthesis involves two main cellular processes: **transcription**, during which genetic information encoded in DNA is transcribed into messenger RNA in the nucleus, followed by **translation**, which occurs in the cytoplasm at the **ribosomes**, where the mRNA is decoded to build a specific polypeptide chain. The final three-dimensional conformation, critical for biological function, is stabilized by various interactions (hydrogen bonds, disulfide bridges, hydrophobic interactions, Van der Waals forces); alteration of this structure, called **denaturation**, can lead to loss of function.

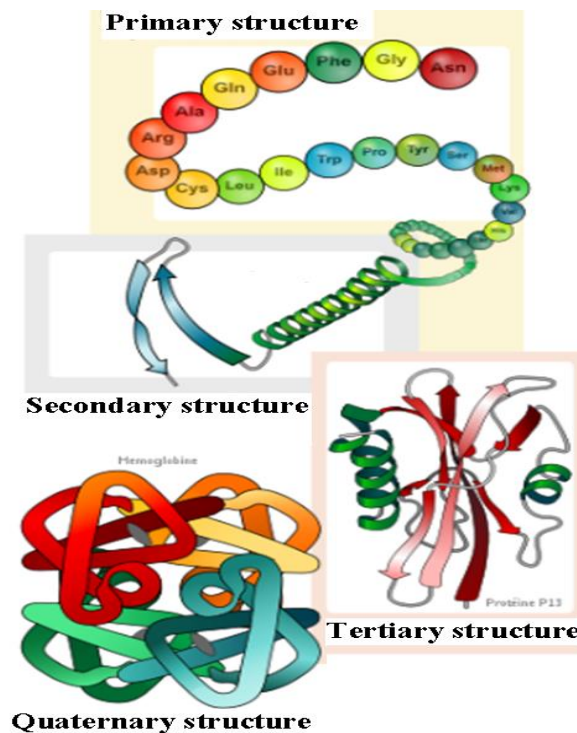


Fig.1: Structure of proteins

Proteins are essential for nutrition because they provide the necessary elements for building, repairing, and maintaining body tissues, including muscles, skin, organs, and bones. They also supply essential amino acids that the body cannot synthesize on its own and must obtain through the diet. When needed, proteins can also serve as an energy source. **In biochemistry**, proteins are fundamental because they are involved in nearly all chemical reactions and cellular functions: they act as enzymes to catalyze reactions, as hormones to regulate physiological processes, as antibodies for immune defense, and as transporters of vital molecules. They also play a key role in cell signaling, gene expression regulation, cell division, and DNA repair. Thus, proteins serve both as building materials and as dynamic agents of biological function.

IV.4. Enzymes

Enzymes are biological polymers that catalyze chemical reactions necessary for life. The presence and maintenance of a complete and balanced set of enzymes are crucial for the catabolism of nutrients that provide energy and basic chemical constituents. Enzymes allow the assembly of these basic constituents into proteins, DNA, membranes, cells, and tissues; they help control energy for cellular mobility, neural functioning, and muscle contraction.

The absolute stereospecificity of enzymes is particularly interesting for their use as catalysts in solution or immobilized on a support, to perform specific reactions in the synthesis of a drug or antibiotic. Enzymes play an important role in the production and enhancement of the nutritional value of food products. Chymosin is a protease used in cheese production.

IV.4.1. Functioning of Enzymes and Their Essential Components

Enzymes are biological catalysts produced by cells that accelerate biochemical reactions without being consumed or altered in the process, acting effectively even at very low concentrations. As genetically coded proteins (with the exception of ribozymes, which are catalytic RNAs), enzymes can be categorized into two types: **homogeneous enzymes**, made solely of amino acids, and **heteroenzymes**, which consist of a protein part (apoenzyme) and a non-protein part (cofactor).

The cofactor, essential for enzymatic activity, can be weakly bound acting as a co-substrate or *coenzyme* or covalently attached as a prosthetic group. Cofactors may include metal ions like Zn, Mg, or Cu, or organic molecules such as vitamins. *Coenzymes*, a specific type of cofactor, are biologically derived and can be either free (loosely bound and dissociating after each reaction) or bound (tightly linked and remaining with the enzyme).

Enzymes act on *substrates* the molecules modified during the reaction via weak interactions such as hydrogen bonds or van der Waals forces, transforming them into *products*, which are the new molecules formed as a result of the catalysis. Together, the enzyme, substrate, cofactors, and products form a dynamic system central to all cellular biochemical processes.

IV.4.2. Enzyme Kinetics

Enzyme kinetics is the study of the rates of enzymatic reactions and how they change in response to various conditions such as substrate concentration, enzyme concentration, pH, temperature, and the presence of inhibitors. It provides crucial insights into how enzymes function, their efficiency, and their mechanisms of action.

The **initial reaction rate (V_0)** refers to the speed of the reaction at the moment the enzyme first encounters the substrate, measured before any significant change in substrate concentration occurs.

The **maximum velocity (V_{max})** represents the highest possible reaction rate when all enzyme active sites are saturated with substrate beyond this point, adding more substrate does not increase the rate.

The **Michaelis constant (K_m)** is the substrate concentration at which the reaction reaches half of V_{max} and serves as an indicator of the enzyme's affinity for its substrate: a low K_m means high affinity, while a high K_m indicates lower affinity.

The **turnover number (kcat)** is the number of substrate molecules an enzyme converts into product per second under saturation conditions, providing a measure of catalytic speed.

Catalytic efficiency, expressed as $kcat/K_m$, reflects both how quickly and how effectively an enzyme binds and converts a substrate, and is useful for comparing different enzymes. These relationships are mathematically described by the **Michaelis-Menten equation**:

$$V_0 = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

Where:

- ✚ V_0 is the initial rate,
- ✚ V_{max} the maximum rate,
- ✚ $[S]$ is the substrate concentration,
- ✚ K_m is the Michaelis constant.

Experimentally, it is not possible to determine V_{max} ; only an approximate value is known. The two important values, V_{max} and K_m , can be obtained through various mathematical treatments, among which the double reciprocal plot (Lineweaver-Burk Plot) is one method. **Lineweaver and Burk** established a rate equation derived from the Michaelis-Menten equation, which allows for the determination of K_m and V_{max} values. Double reciprocal plot:

$$1/V = f(1/[S]) \rightarrow$$

$$\frac{1}{v} = \frac{K_M}{V_M} \left(\frac{1}{[S]} \right) + \frac{1}{V_M}$$

This is the equation of a line in the form: $y = ax + b$. The kinetic parameters K_m and V_{max} are determined graphically by direct reading from the x-axis (abscissa) and the y-axis (ordinate) (Fig. 2).

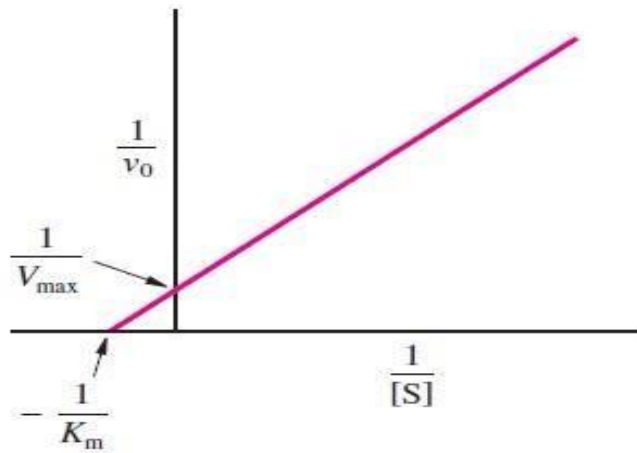


Fig. 2: Graphical representation by Lineweaver and Burk.

Several factors influence enzyme kinetics, including substrate concentration (which increases reaction rate until saturation), enzyme concentration, temperature (which raises the rate up to an optimum before denaturation occurs), and pH (each enzyme has an optimal range). Additionally, **inhibitors** can reduce enzyme activity: **competitive inhibitors** bind to the active site, **non-competitive inhibitors** bind elsewhere and change the enzyme's shape, and **uncompetitive inhibitors** bind only to the enzyme-substrate complex. Overall, enzyme kinetics provides essential insights into how enzymes function and how their activity can be regulated, which is vital for understanding biochemical processes and for applications in medicine, drug development, and biotechnology.

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Mitochondrial Electron Transport Chain



I. Generality

The electron transport chain is a fundamental process that takes place within the mitochondrion and enables the production of energy essential for the cell. Through a series of oxidation-reduction reactions, electrons are transferred across multiple protein complexes, resulting in the synthesis of ATP. This chain plays a key role in cellular respiration and in maintaining the organism's energy balance.

I.1. Mitochondria

I.1.1. Mitochondrial structure

Mitochondria are bacteria-sized organelles (about $1 \times 2 \mu\text{m}$ in size), which are found in large numbers in almost all eukaryotic cells. Typically, there are about 2000 mitochondria per cell, representing around 25% of the cell volume. Mitochondria are enclosed by two membranes, a smooth outer membrane and a markedly folded or tubular inner mitochondrial membrane, which has a large surface and encloses the matrix space. The folds of the inner membrane are known as cristae, and tube-like protrusions are called tubules. The intermembrane space is located between the inner and the outer membranes (Fig.1).

Both mitochondrial membranes are very rich in proteins. Porins (Fig.1) in the outer membrane allow small molecules ($< 10 \text{ kDa}$) to be exchanged between the cytoplasm and the intermembrane space. By contrast, the inner mitochondrial membrane is completely impermeable even to small molecules (with the exception of O_2 , CO_2 , and H_2O). Numerous transporters in the inner membrane ensure the import and export of important metabolites. The inner membrane also transports respiratory chain complexes, ATP synthase, and other enzymes. The matrix is also rich in enzymes (Fig. 1).

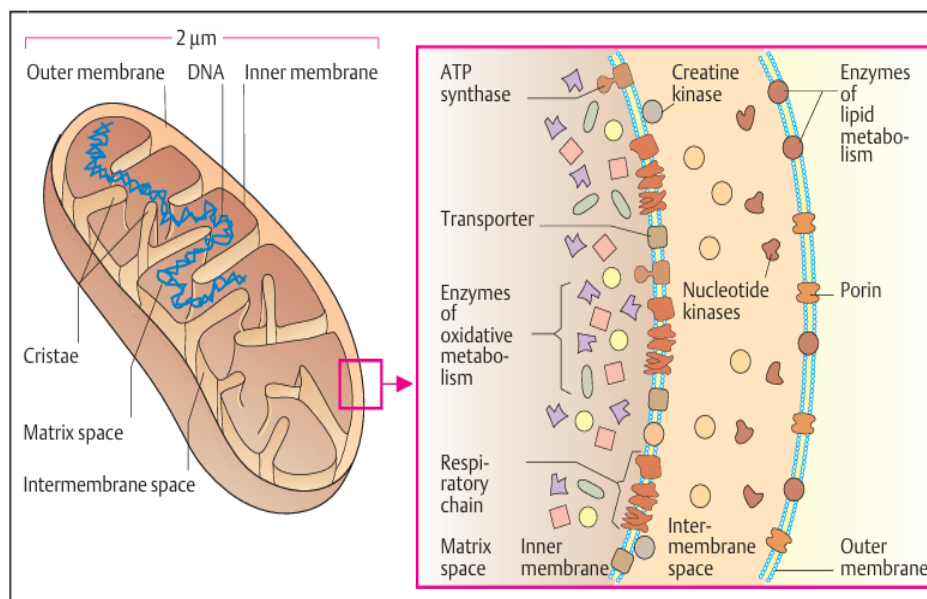


Fig.1. Mitochondrial structure.

The number and shape of the mitochondria, as well as the numbers of cristae they have, can differ widely from cell type to cell type. Tissues with intensive oxidative metabolism; e.g., heart muscle have mitochondria with particularly large numbers of cristae. Even within one type of tissue, the shape of the mitochondria can vary depending on their functional status. Mitochondria are mobile organelles.

I.1.2. Metabolic functions

Mitochondria are also described as being the cell's biochemical powerhouse, since through oxidative phosphorylation, they produce the majority of cellular ATP. Pyruvate dehydrogenase (PDH), the tricarboxylic acid cycle, β -oxidation of fatty acids, and parts of the urea cycle are located in the matrix. The respiratory chain, ATP synthesis, and enzymes involved in heme biosynthesis are associated with the inner membrane (Fig. 2).

The inner membrane itself plays an important part in oxidative phosphorylation. As it is impermeable to protons, the respiratory chain which pumps protons from the matrix into the intermembrane space via complexes I, III, and IV establishes a proton gradient across the inner membrane, in which the chemical energy released during NADH oxidation is conserved. ATP synthase then uses the energy stored in the gradient to form ATP from ADP and inorganic phosphate. Several of the transport systems are also dependent on the H^+ gradient.

In addition to the endoplasmic reticulum, the mitochondria also function as an intracellular calcium reservoir. The mitochondria also play an important role in “programmed cell death” apoptosis.

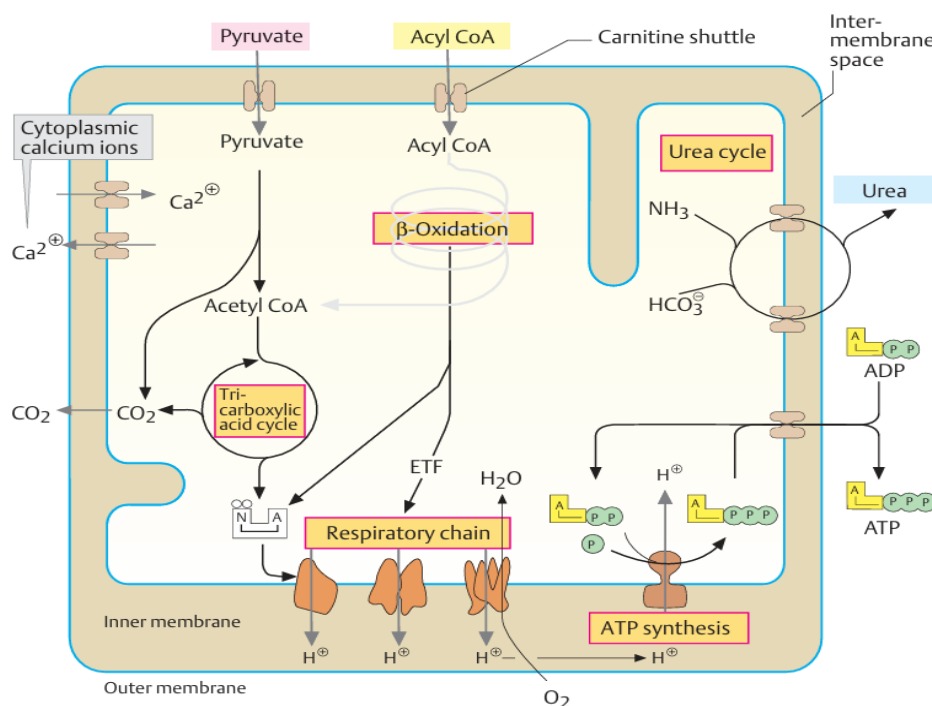


Fig. 2. Metabolic functions

I.2. Change in Free Energy and the Role of ATP in Energy

Free energy, often called Gibbs free energy (denoted as G), represents the energy available to do work in a chemical reaction. When a reaction occurs, free energy can decrease (exergonic reaction) or increase (endergonic reaction).

In cells, exergonic reactions release free energy that can be used to carry out vital functions. This energy is often temporarily stored in the form of ATP (adenosine triphosphate), the cell's "energy currency."

ATP conserves energy by binding high-energy phosphate groups. When the cell needs energy, ATP is hydrolyzed into ADP (adenosine diphosphate) and inorganic phosphate (P_i), releasing free energy that can be used for cellular processes such as muscle contraction, active transport, or molecular synthesis.

Thus, ATP acts as a crucial intermediate that captures energy released during exergonic reactions and redistributes it to endergonic reactions, enabling efficient and controlled energy balance within the cell.

II. Oxidative phosphorylation

Oxidative phosphorylation is the process by which **ATP is synthesized** using energy derived from the **electron transport chain**. This mechanism relies on several key components: electron donors such as NADH or FAD(2H), molecular oxygen (O_2) as the final electron acceptor, an intact inner mitochondrial membrane impermeable to protons, the full complement of electron transport chain complexes, and ATP synthase. The process is driven by a **proton electrochemical gradient** across the inner mitochondrial membrane, in accordance with the chemiosmotic hypothesis. As electrons are transferred through the complexes of the electron transport chain, protons are pumped from the mitochondrial matrix into the intermembrane space, generating the gradient. The return flow of protons through ATP synthase provides the energy needed for ATP production. This process is tightly regulated by the cell's rate of ATP utilization.

II.1. Electron transfer from NADH to O_2

In the electron transport chain, electrons donated by NADH or FAD(2H) are passed sequentially through a series of electron carriers embedded in the **inner mitochondrial** membrane. Each of the components of the electron transfer chain is oxidized as it accepts an electron, and then reduced as it passes the electrons to the next member of the chain. From NADH, electrons are transferred sequentially through NADH dehydrogenase (complex I), CoQ (coenzyme Q), the cytochrome b-c1 complex (complex III), cytochrome c, and finally cytochrome c oxidase (complex IV) (Fig. 3). NADH dehydrogenase, the cytochrome b-c1 complex and cytochrome c

oxidase are each multisubunit protein complexes that span the inner mitochondrial membrane. CoQ is a lipid soluble quinone that is not protein-bound and is free to diffuse in the lipid membrane. It transports electrons from complex I to complex III and is an intrinsic part of the proton pumps for each of these complexes. Cytochrome c is a small protein in the inner membrane space that transfers electrons from the b-c1 complex to cytochrome oxidase. The terminal complex, cytochrome c oxidase, contains the binding site for O_2 . As O_2 accepts electrons from the chain, it is reduced to H_2O (Fig.3).

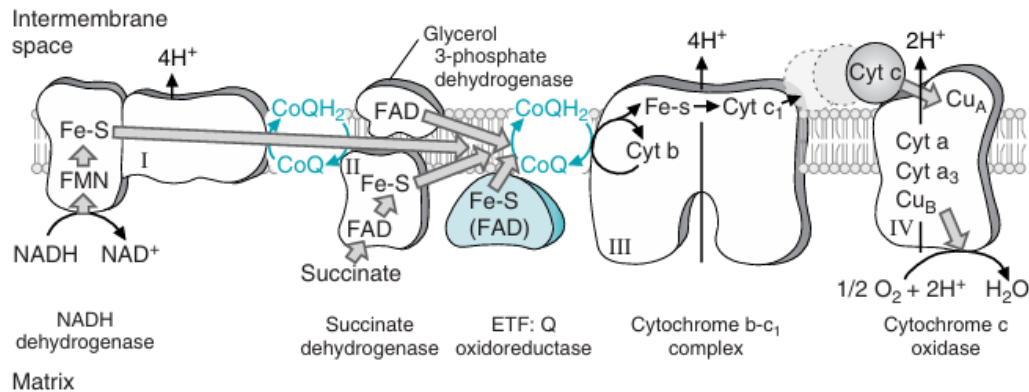


Fig. 3: Electron Transport Chain and Oxidative Phosphorylation

II.1.1. NADH dehydrogenase

NADH dehydrogenase is an enormous 42-subunit complex that contains a binding site for NADH, several FMN and iron-sulfur (Fe-S) center binding proteins, and binding sites for CoQ (Fig 3). An FMN **accepts two** electrons from NADH and is able to pass single electrons to the FeS centers. Fe-S centers, which are able to delocalize single electrons into large orbitals, transfer electrons to and from CoQ. Fe-S centers are also present in other enzyme systems, such as other proteins, which transfer electrons to CoQ, in the cytochrome b-c1 complex, and in aconitase in the TCA cycle.

II.1.2. Succinate dehydrogenase and other flavoproteins

In addition to NADH dehydrogenase, succinic dehydrogenase and other flavoproteins in the inner mitochondrial membrane also pass electrons to CoQ (Fig. 3). Succinate dehydrogenase is part of the TCA cycle. ETF-CoQ oxidoreductase accepts electrons from ETF (electron transferring flavoprotein), which acquires them from fatty acid oxidation and other pathways. Both of these flavoproteins have Fe-S centers. -Glycerophosphate dehydrogenase is a flavoprotein that is part of a shuttle for reoxidizing cytosolic NADH.

The free energy drop between NADH and CoQ of approximately 13 to 14 kcal is able to support movement of four protons. However, the FAD in succinate dehydrogenase (as well as ETF-CoQ oxidoreductase and -glycerophosphate dehydrogenase) is at roughly the same energy

level as CoQ, and there is no energy released as they transfer electrons to CoQ. These proteins do not span the membrane and consequently do not have a proton pumping mechanism.

II.1.3. Coenzyme Q

CoQ is the only component of the electron transport chain that is **not protein** bound. The large hydrophobic side chain of 10 isoprenoid units (50 carbons) confers lipid solubility, and CoQ is able to diffuse through the lipids of the inner mitochondrial membrane. When the oxidized quinone form accepts a single electron, it forms a free radical (a compound with a single electron in an orbital). The transfer of single electrons makes it the major site for generation of toxic oxygen free radicals in the body.

The semiquinone can accept a second electron and two protons from the matrix side of the membrane to form the fully reduced quinone. The mobility of CoQ in the membrane, its ability to accept one or two electrons, and its ability to accept and donate protons enable it to participate in the proton pumps for both complexes I and III as it shuttles electrons between them.

II.1.4. Cytochromes

The remainder of the components in the electron transport chain are cytochromes. Each cytochrome is a protein that contains a bound heme (i.e., an Fe atom bound to a porphyrin nucleus similar in structure to the heme in hemoglobin).

Because of differences in the protein component of the cytochromes and small differences in the heme structure, each heme has a different reduction potential. The cytochromes of the b-c1 complex have a higher energy level than those of cytochrome oxidase (a and a3). Thus, energy is released by electron transfer between complexes III and IV. The iron atoms in the cytochromes are in the Fe^{3+} state. As they accept an electron, they are reduced to Fe^{2+} . As they are reoxidized to Fe^{3+} , the electrons pass to the next component of the electron transport chain.

II.1.5. Copper and the reduction of oxygen

The last cytochrome complex is cytochrome oxidase, which passes electrons from cytochrome c to O_2 . It contains cytochromes a and a3 and the oxygen binding site. A whole oxygen molecule, O_2 , must accept four electrons to be reduced to $2 \text{H}_2\text{O}$. Bound copper (Cu) ions in the cytochrome oxidase complex facilitate the collection of the four electrons and the reduction of O_2 . Cytochrome oxidase has a much lower K_m for O_2 than myoglobin (the heme containing intracellular oxygen carrier) or hemoglobin (the heme-containing oxygen transporter in the blood). Thus, O_2 is “pulled” from the erythrocyte to myoglobin, and from myoglobin to cytochrome oxidase, where it is reduced to H_2O .

II.2. Electrochemical potential gradient

The chemiosmotic theory proposes that the energy released from redox reactions in the respiratory chain is used to pump protons from the **matrix** to the **intermembrane space** through three membrane-spanning complexes. These complexes capture electrons and protons on one side of the membrane and release protons on the other side while transferring electrons to the next component.

The Q cycle of the cytochrome b-c1 complex illustrates this coupling: coenzyme Q accepts two electrons and two protons in the matrix, then releases the protons into the intermembrane space while redistributing the electrons (Fig.4). The proton pumping mechanism at the NADH dehydrogenase complex remains partially understood, whereas the cytochrome c oxidase complex likely uses a “proton wire” for direct proton transfer.

This coupling is strict: **electron transfer** and **proton movement** are inseparable. When protons are not used for ATP synthesis, the proton gradient builds up, creating back-pressure that regulates proton pumping, electron transport, and oxygen consumption.

This electrochemical gradient, composed of a pH gradient and an electrical potential, constitutes the proton motive force that drives protons back into the matrix via ATP synthase, thus enabling ATP production.

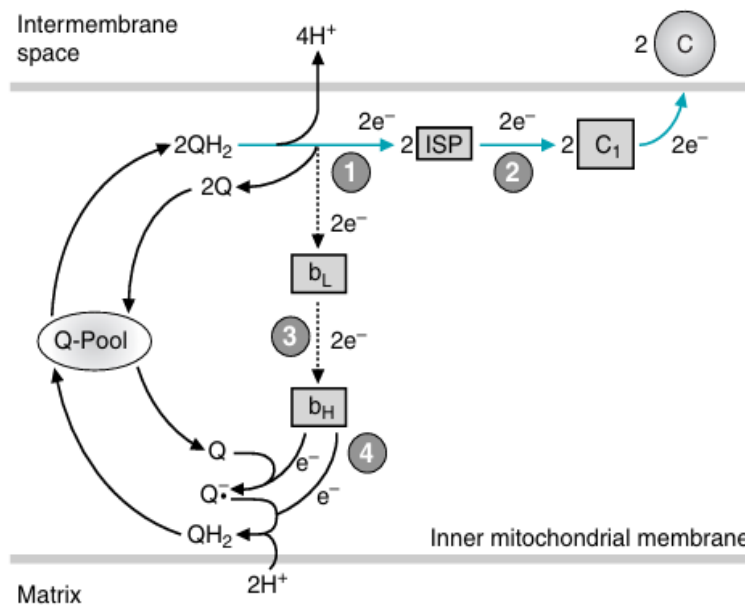


Fig. 4: Proton motive Q cycle for the b-c1 complex.

II.3. ATP Synthase

ATP synthase (F_0F_1 ATPase), the enzyme that generates ATP, is a multisubunit enzyme containing an inner membrane portion (F_0) and a stalk and headpiece (F_1) that project into the matrix (Fig. 5). The 12 c subunits in the membrane form a rotor that is attached to a central asymmetric shaft composed of the ϵ and γ subunits. The headpiece is composed of three $\alpha\beta$ subunit pairs. Each β subunit contains a catalytic site for ATP synthesis. The headpiece is held stationary by a δ subunit attached to a long **b** subunit connected to subunit **a** in the membrane.

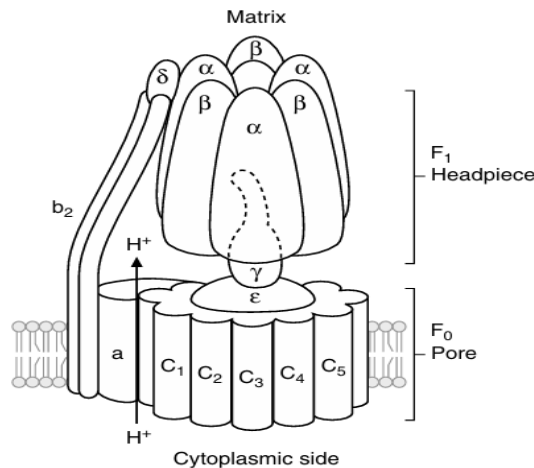


Fig. 5: ATP synthase (F_0F_1 ATPase).

The influx of protons through the proton channel turns the rotor. The proton channel is formed by the c subunits on one side and the a subunit on the other side. Although continuous, it has two offset portions, one portion directly open to the intermembrane space and one portion directly open to the matrix. In the current model, each c subunit contains a glutamyl carboxyl group that extends into the proton channel. As this carboxyl group accepts a proton from the intermembrane space, the c subunit rotates into the hydrophobic lipid membrane. The rotation exposes a different proton-containing c subunit to the portion of the channel directly open to the matrix side. Because the matrix has a lower proton concentration, the glutamyl carboxylic acid group releases a proton into the matrix portion of the channel. Rotation is completed by an attraction between the negatively charged glutamyl residue and a positively charged arginyl group on the a subunit.

According to the binding change mechanism, as the asymmetric shaft rotates to a new position, it forms different binding associations with the $\alpha\beta$ subunits (Fig. 6). The new position of the shaft alters the conformation of one β subunit so that it releases a molecule of ATP and another subunit spontaneously catalyzes synthesis of ATP from inorganic phosphate, one proton, and ADP. Thus, energy from the electrochemical gradient is used to change the conformation of the

ATP synthase subunits so that the newly synthesized ATP is released. Twelve c subunits are hypothesized, and it takes 12 protons to complete one turn of the rotor and synthesize three ATP.

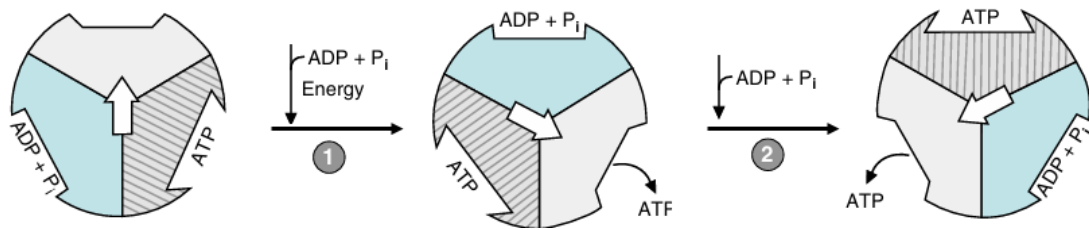


Fig. 6: Binding change mechanism for ATP synthesis.

III. Energy Yield from Electrons of NADH and FADH₂

Electrons from NADH enter the electron transport chain at Complex I, which pumps protons and helps create a strong electrochemical gradient. This process allows the production of about **3 ATP** molecules per NADH.

In contrast, electrons from FADH₂ enter at Complex II, which does not pump protons. As a result, the gradient formed is weaker, leading to a lower ATP yield of about **2 ATP** molecules per FADH₂.

IV. Respiratory Chain Inhibition and Sequential Transfer

In the cell, electron flow in the electron transport chain must be sequential from NADH or a flavoprotein all the way to O₂ to generate ATP. In the absence of O₂, there is no ATP generated from oxidative phosphorylation because electrons back up in the chain. Even complex I cannot pump protons to generate the electrochemical gradient, because every molecule of CoQ already has electrons that it cannot pass down the chain without an O₂ to accept them at the end. The action of the respiratory chain inhibitor cyanide, which binds to cytochrome oxidase, is similar to that of anoxia; it prevents proton pumping by all three complexes. Complete inhibition of the b-c1 complex prevents pumping at cytochrome oxidase because there is no donor of electrons; it prevents pumping at complex I because there is no electron acceptor. Although complete inhibition of any one complex inhibits proton pumping at all of the complexes, partial inhibition of proton pumping can occur when only a fraction of the molecules of a complex contain bound inhibitor. The partial inhibition results in a partial decrease of the maximal rate of ATP synthesis.

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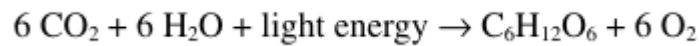


Photosynthesis



I. Overall Process

The best-understood reaction for the synthesis of glucose, and probably the most important quantitatively, is photosynthesis. Photosynthesis converts carbon from carbon dioxide to glucose with reducing equivalents supplied from water and energy supplied from light. This entire process takes place inside chloroplasts, specialized organelles in plant and algal cells that contain the necessary pigments and molecular machinery for capturing and converting light energy.



The energy in light is dependent on its wavelength, and is given by the following relationship

$$E = h\nu = \frac{hc}{\lambda}$$

- ✚ ν stands for the frequency of the light,
- ✚ h is a constant called Planck's constant,
- ✚ c is the speed of light,
- ✚ λ is the wavelength.

In other words, the energy of light is inversely proportional to its wavelength. The longer the wavelength, the less energy it contains. In the visible spectrum, the highest-energy light is toward the blue or violet end, while the lowest-energy is to the red.

I.1. Chloroplast

The chloroplast is an organelle found in the cells of **plants and algae**, containing **chlorophyll**, a green pigment essential for **photosynthesis**. Surrounded by a double membrane, the chloroplast houses internal membrane structures called thylakoids, which are stacked into units known as grana (Fig.1). Within the grana, the thylakoid membranes are arranged in **disk-like stacks**, where **light is captured** and used to convert **carbon dioxide and water into glucose**, releasing **oxygen** as a by-product. Each region of the chloroplast is **functionally specialized**: the **light reactions** of photosynthesis take place in the **grana**, where solar energy is converted into chemical energy in the form of **ATP and NADPH**, while the **dark reactions** (Calvin cycle) occur in the **stroma**, the surrounding fluid-filled region, where this energy is used to **fix carbon** and synthesize **glucose**. In this way, the chloroplast enables plants to **produce their own food** using the **energy of sunlight**.

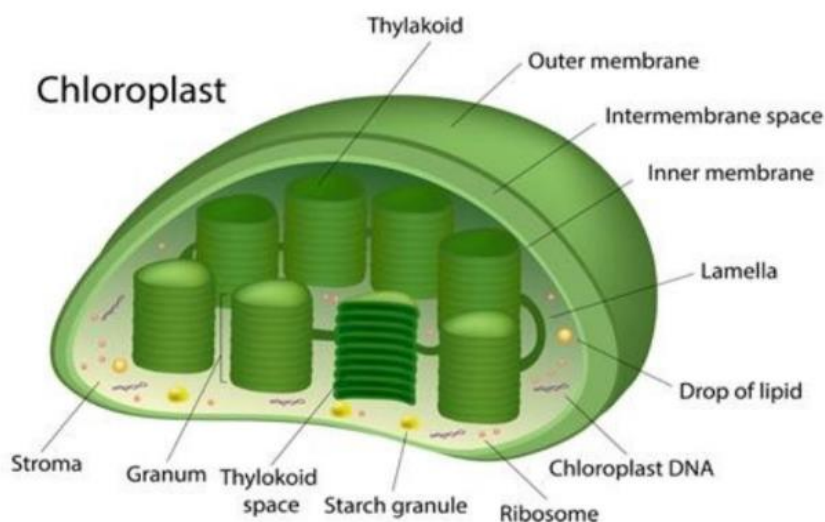


Fig.1: Chloroplast structure

I.2. Chlorophyll and the action spectrum of photosynthesis

The green color of the chloroplast (and therefore plants) comes from the chlorophyll that is stored in them. Chlorophyll is a tetrapyrrole ring system with a Mg^{2+} ion in the center, coordinated to the nitrogen of each pyrrole ring (Fig.2). The tetrapyrrole ring system is found as a bound cofactor (a prosthetic group) in many electron-carrying proteins, enzymes, and oxygen transporters. For example, tetrapyrroles are essential to the functioning of cytochrome c, various mixed function oxidases, and hemoglobin. Chlorophylls differ from other tetrapyrroles by possessing a long, branched phytol joined to the tetrapyrrole in an ether linkage. The phytol is an “anchor” to keep the chlorophyll inside the thylakoid membrane.

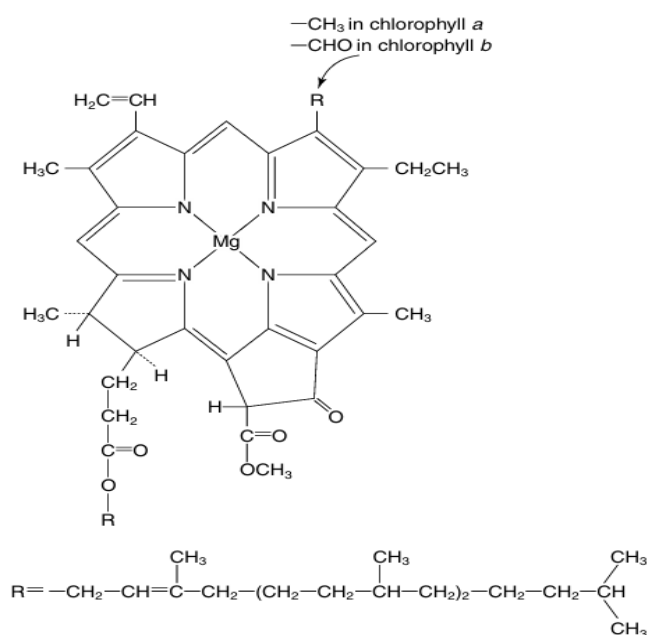


Fig. 2: Chlorophyll structure

Photosynthesis begins with the absorption of light in the thylakoid membrane. The energy of the light makes a difference in its effect on photosynthesis.

- The energy of a single photon of light is inversely proportional to its wavelength, with the visible region of the spectrum having less energy per photon than the ultraviolet region, and more than the infrared region. The energy of the visible spectrum increases from the red wavelengths through the blue and violet, according to the mnemonic ROY G. BIV (red, orange, yellow, green, blue, indigo, violet).
- Ultraviolet light, which has more energy than blue light, does not support photosynthesis. If it did reach the earth's surface, ultraviolet light would be energetic enough to break carbon carbon bonds. The bond-breaking process would lead to a net loss of fixed carbon as biomolecules were broken apart. Fortunately, the ozone layer in the atmosphere absorbs enough UV radiation to prevent this from occurring.
- Chlorophyll comes in two varieties, chlorophyll a and chlorophyll b. Although the wavelengths at which they absorb light differ slightly, both absorb red and blue light. The chlorophyll reflects the other colors of light; the human eye sees these colors as green, the color of plants.
- The antenna pigments and most chlorophyll molecules don't participate in the direct light reactions of photosynthesis. Instead, they are part of the light-harvesting complex, which "funnels" the photons they capture to a reaction center, where the actual reactions of photosynthesis occur. Altogether, the light-harvesting complex is over 90 percent efficient almost all the photons that fall on the chloroplast are absorbed and can provide energy for synthesis.
- Chlorophyll a and chlorophyll b participate in aspects of the light reaction; each must absorb a photon for the reaction to occur.

II. Photosynthesis reactions

Photosynthesis involves two sets of chemical events, termed the light and dark reactions. This terminology is somewhat misleading, because the entire process of photosynthesis is regulated to take place when an organism absorbs visible light. The light reactions refer to the set of reactions in which the energy of absorbed light is used to generate ATP and reducing power (NADPH). The dark reactions use this reducing power and energy to fix carbon, that is, to convert carbon dioxide to glucose. Biochemically, converting CO₂ to glucose without light is possible if a supply of reducing equivalents and ATP are available.

II.1. Light Reactions

The photosynthetic scheme begins when the pigments of the light harvesting complex absorb a photon of visible light. The absorption of light excites the pigment to a higher energy state. The pigments transfer their excitation energy to the reaction center chlorophyll molecule. Two types of reaction centers exist, generally called Photosystem I (PSI) and Photosystem II (PSII). Thus, two types of overall reactions make up photosynthesis the light and dark reactions, while two more types of reactions compose the light reaction those carried out by PSI and PSII. Absorption of a photon causes either type of chlorophyll to become more easily oxidized, that is, to give up an electron. The electron given up by either chlorophyll molecule flows through an electron transport chain, just as the electron given up by a mitochondrial cytochrome flows through the mitochondrial electron transport chain. Just as in mitochondrial electron transport, the flow of electrons leads to a proton gradient, which is used to synthesize ATP. Unlike mitochondrial electron transport, the terminal electron acceptor isn't oxygen but rather NADP.

II.1.1. Photosystem II

The photosystem pigment of PSII is a form of chlorophyll termed P680, because it is a pigment that absorbs light with a wavelength of 680 nanometers. Absorption of a photon by P680 leads to the excited form of the pigment, called P680*. P680* but not ground-state P680 gives up an electron to another molecule, plastoquinone (Fig. 3).

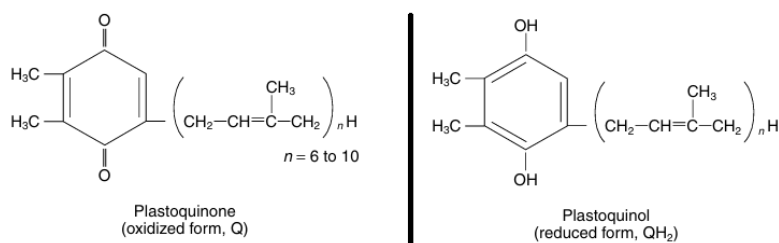


Fig. 3: Redox transformation of plastoquinone (Q) into plastoquinol (QH₂)

Another way of stating this relationship is that P680* has a more negative reduction potential than does P680. The following equation gives the free energy of the reduction reaction.

$$\Delta G^{\circ'} = -nFE^{\circ'}$$

The conversion of the P680 to P680* means that the free energy of its accepting an electron becomes more positive (the reaction is unfavored). A molecule that has a more positive free energy of reduction has a more negative free energy of oxidation. In other words, the P680* molecule is more easily oxidized than is P680.

The electron from P680* is transferred to a series of plastoquinone (PQ) derivatives, leaving behind an oxidized P680 molecule as shown in Figure 4. The reduction of plastoquinone is similar to that of Coenzyme Q in mitochondrial oxidation/reduction, in that PQ can accept either one or two electrons at a time. Plastoquinone molecules accept a proton (H^+) from the stroma for each electron they accept. This leaves the stroma more basic than it was before, creating part of the gradient that will be used for ATP synthesis.

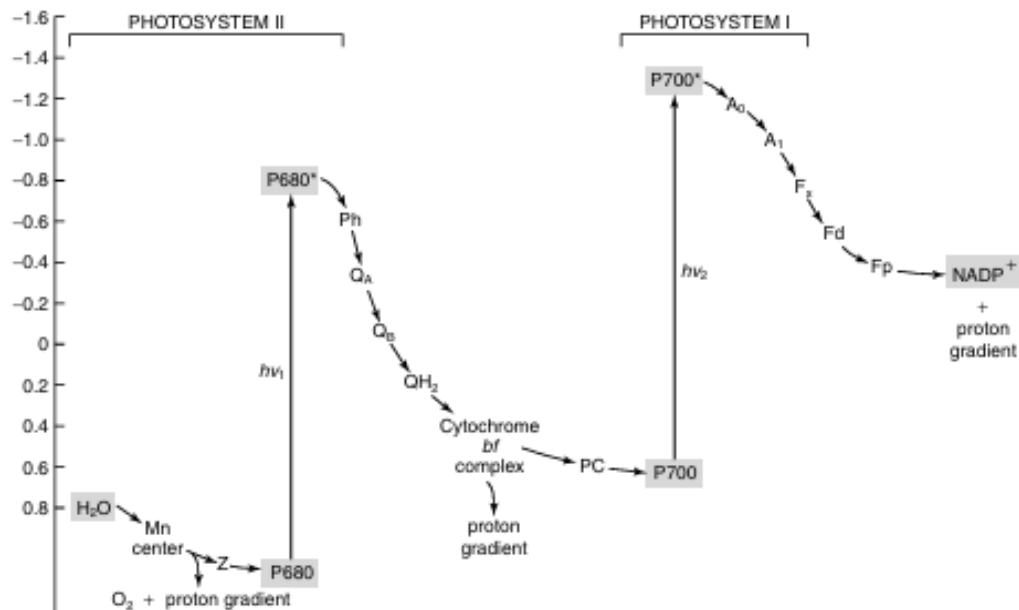
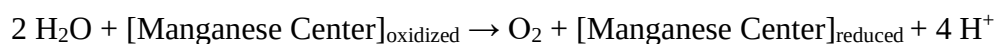


Fig. 4: Photosynthetic Electron Transport Chain: From Photosystems II and I to NADP Reduction.

From the quinones, the electron is transferred to plastocyanin and then to cytochrome bf. The two H⁺ ions (protons) left behind remain in the thylakoid lumen. As the electrons move down this electron transport chain, protons are pumped into the thylakoid lumen. Eventually the transported electron is given up to the oxidized P700 chlorophyll of Photosystem I.

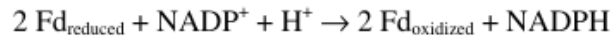
This movement of an electron to PSI from P680* leaves P680 in a non-excited, oxidized state. Oxidized P680 must be reduced to give up another electron. Hydrogen atoms derived from H₂O reduce it according to the reaction:



This oxidation transfers four electrons to the Manganese Center, a complex metalloprotein, which then donates the electrons through an intermediate to oxidized P680. The protons derived from water are transported into the thylakoid lumen. The protons pumped into the thylakoid lumen by PSII are used to make ATP through the action of coupling factor, in a mechanism similar to that of mitochondrial ATP synthesis.

II.1.2. Photosystem I

Just as the absorption of a photon of light converts P680 to P680*, which is a better reductant, so too does the absorption of light convert the chlorophyll of PSI to a species that gives up an electron more easily. P700* donates an electron to a series of mobile quinones and then to a ferredoxin protein (Fd). The ferredoxin reduces NADP to NADPH. This provides reducing power for conversion of CO₂ to carbohydrate.



After P700* donates the electron to the quinones, oxidized P700 accepts an electron from the cytochrome bf, which is the acceptor of the electron from Photosystem II. The reduced P700 is now poised to absorb a photon's energy and the cycle can start over.

II.1.3. Z-Scheme of Photosynthesis

The “Z-scheme” describes the oxidation/reduction changes during the light reactions of photosynthesis. The vertical axis in the figure represents the reduction potential of a particular species the higher the position of a molecular species, the more negative its reduction potential, and the more easily it donates electrons (Fig. 5).

In the Z-scheme, electrons are removed from water (to the left) and then donated to the lower (non-excited) oxidized form of P680. Absorption of a photon excites P680 to P680*, which “jumps” to a more actively reducing species. P680* donates its electron to the quinone-cytochrome bf chain, with proton pumping. The electron from cytochrome bf is donated to PSI, converting P700 to P700*. This electron, along with others, is transferred to NADP, forming NADPH. Alternatively, this electron can go back to cytochrome bf in cyclic electron flow.

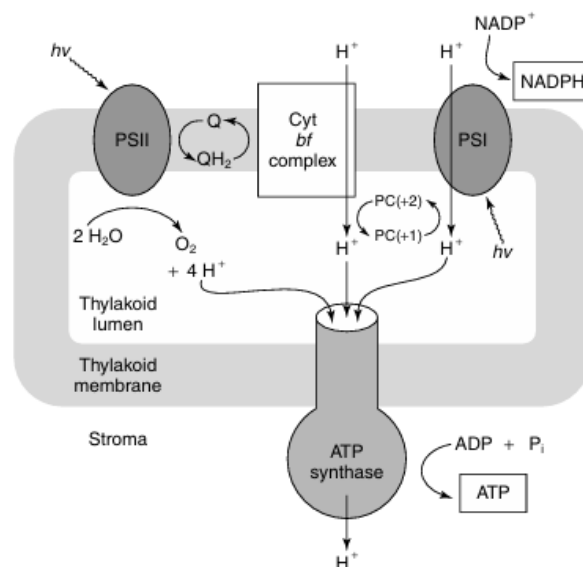


Fig. 5: Light-dependent phase of photosynthesis (non-cyclic photophosphorylation and electron transport chain).

II.1.4. ATP Synthesis

The protein complex that carries out this reaction is called coupling factor or, more accurately, ATP synthase. ATP synthase is a complex of several proteins, shaped like a mushroom, with the cap on the stromal side of the thylakoid disc and the stalk going through the thylakoid membrane. The actions of PSII and PSI pump protons into the lumen of the thylakoid. When chloroplasts are actively illuminated, the pH gradient produced by the two photosystems can be as much as four pH units, or a 10,000-fold difference in hydrogen ion concentration. These are the relative acidities of vinegar and tap water.

The membrane potential in chloroplasts is almost entirely composed of the ΔpH component. The thylakoid membrane is permeable to Mg^{2+} and Cl^- ions, so electrical neutrality is maintained. This differs from mitochondrial ATP synthesis where both a pH and an electrochemical potential exist. Because the chloroplast gradient is primarily ΔpH in nature, three protons must move across the membrane to synthesize an ATP, rather than the two that move during mitochondrial synthesis of a single ATP. The ATP and NADPH from synthesis are both formed on the stromal side of the thylakoid membrane. They are available for the fixation of CO_2 , which occurs in the stroma (Fig. 5).

II.2. Dark reactions

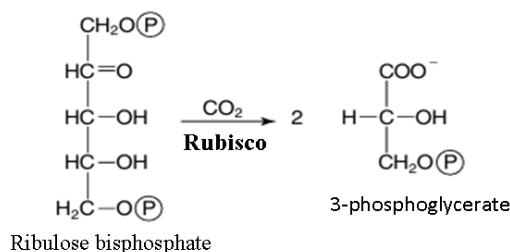
The dark reactions (Carbohydrate Synthesis) use ATP and NADPH to convert CO_2 into carbohydrate. The first step is fixing CO_2 into organic carbon. The basic reaction is addition of CO_2 to a phosphorylated acceptor. This step requires no direct input of energy. Two types of plants exist, which use different acceptor molecules.

- ✚ **In so-called C-3 plants**, the acceptor is a 5-carbon, doubly phosphorylated acceptor, and two 3-carbon phosphorylated compounds are formed.
- ✚ **In C-4 plants**, the acceptor is phosphoenolpyruvate, and the carboxylation makes the 4-carbon acid oxaloacetic acid and releases inorganic phosphate.

The ATP and NADPH from the light reactions are used for making the acceptors and converting the first products into glucose.

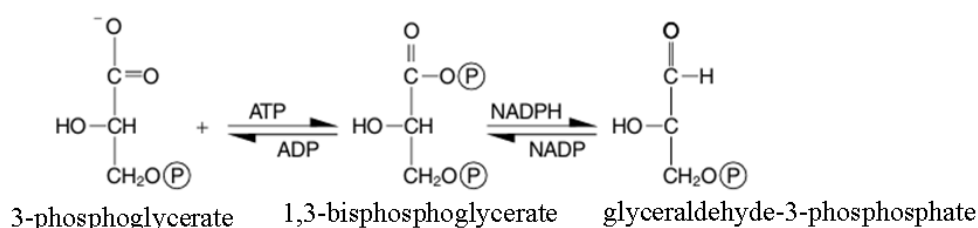
II.2.1. C-3 Photosynthesis

This pathway is sometimes called the **Calvin-Benson cycle**, after the biochemists who elucidated it. The 5-carbon, doubly phosphorylated carbohydrate, ribulose biphosphate is the acceptor for CO₂; the enzyme is called ribulose-bisphosphate carboxylase/oxygenase (called **Rubisco**).

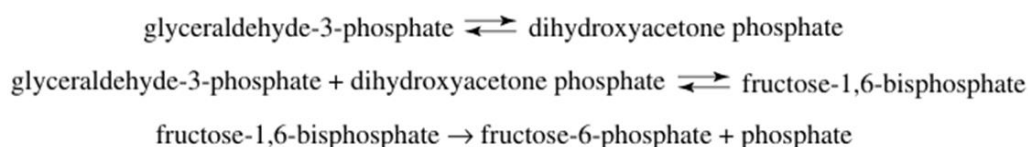


The initial product of the reaction is unstable and quickly falls apart to yield two molecules of 3-phosphoglycerate

The rest of the Calvin cycle is involved in interconversion of carbohydrates to make glucose (or starch) and the regeneration of the ribulose-bisphosphate acceptor. The reactions are also found in the pathways for gluconeogenesis and the pentose phosphate shunt. The first step is the phosphorylation of 3-phosphoglycerate by the same reactions involved in gluconeogenesis



The glyceraldehyde-3-phosphate can be converted into the 6-carbon sugar phosphate called fructose-6-phosphate by the reactions of triose phosphate isomerase, aldolase, and fructose biphosphase.

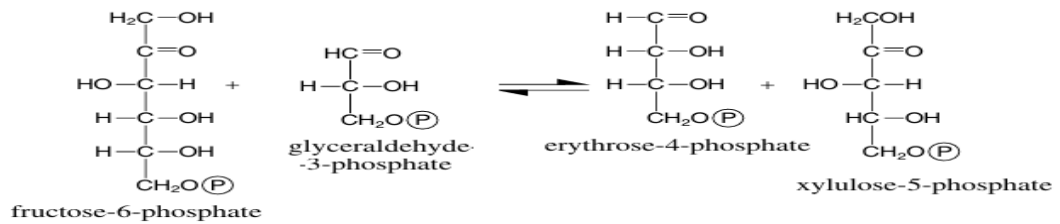


Note that the last step is irreversible, just as it is in gluconeogenesis. In shorthand, the reactions to this point are: 5 + 1 = 3 + 3 = 6

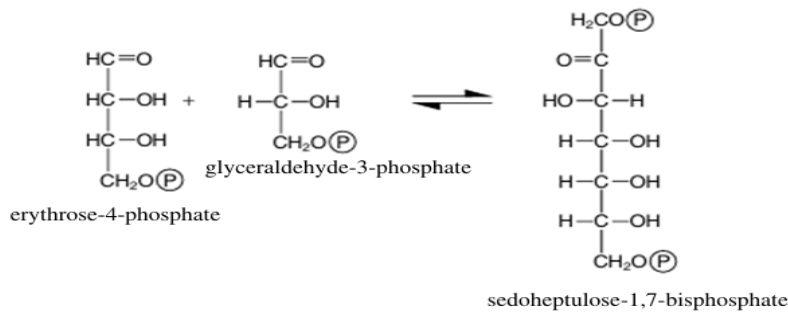
Regeneration of ribulose bisphosphate occurs by the same reactions that occur in the hexose-monophosphate shunt. The hexose monophosphate shunt interconverts 3, 4, 5, 6 and 7 carbon sugar phosphates. In the formation of one molecule of glucose from CO₂, carrying out the previous

reactions **six** times is necessary. Making the **six** molecules of ribulose-bisphosphate required for fixing six CO₂ s'occurs by the following sets of reactions:

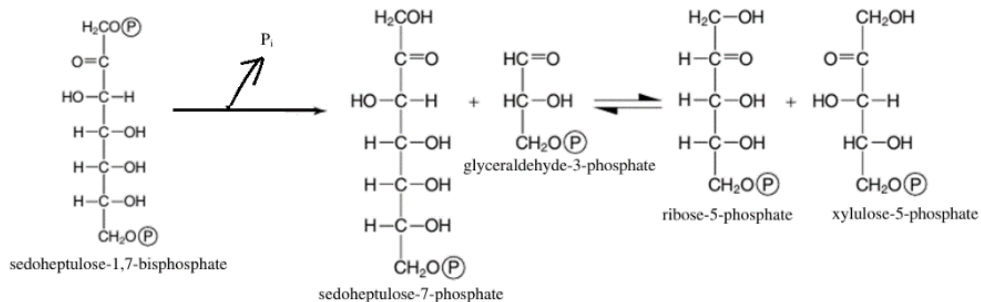
Reaction 1: Transketolase transfers a 2-carbon unit



Reaction 2: Transaldolase transfers a 3-carbon unit



Reaction 3: Transketolase transfers a 2-carbon unit



The 5-carbon sugar phosphates are interconverted by the action of epimerase and isomerase to yield ribulose-5-phosphate, which is phosphorylated by the enzyme ribulose phosphate kinase to make ribulose bisphosphate, the acceptor of CO₂.

Ribulose phosphate kinase is active only when a cystine disulfide on the enzyme is reduced to two cysteines. An electron carrier, thioredoxin, carries out this reduction, and is then itself reduced by electrons from NADPH. Because the action of Photosystems I and II forms NADPH, this reduction ensures that ribulose bisphosphate is made only when enough light exists to support Photosynthesis. In other words, the light and dark reactions are coupled.

The following figure (6) summarizes all the reactions of the Calvin-Benson cycle, which take place in the stroma of chloroplasts. This cycle consists of 13 enzymatic reactions, catalyzed by eleven distinct enzymes, and is divided into three phases: Carboxylation, Reduction and Regeneration of ribulose-1,5-bisphosphate.

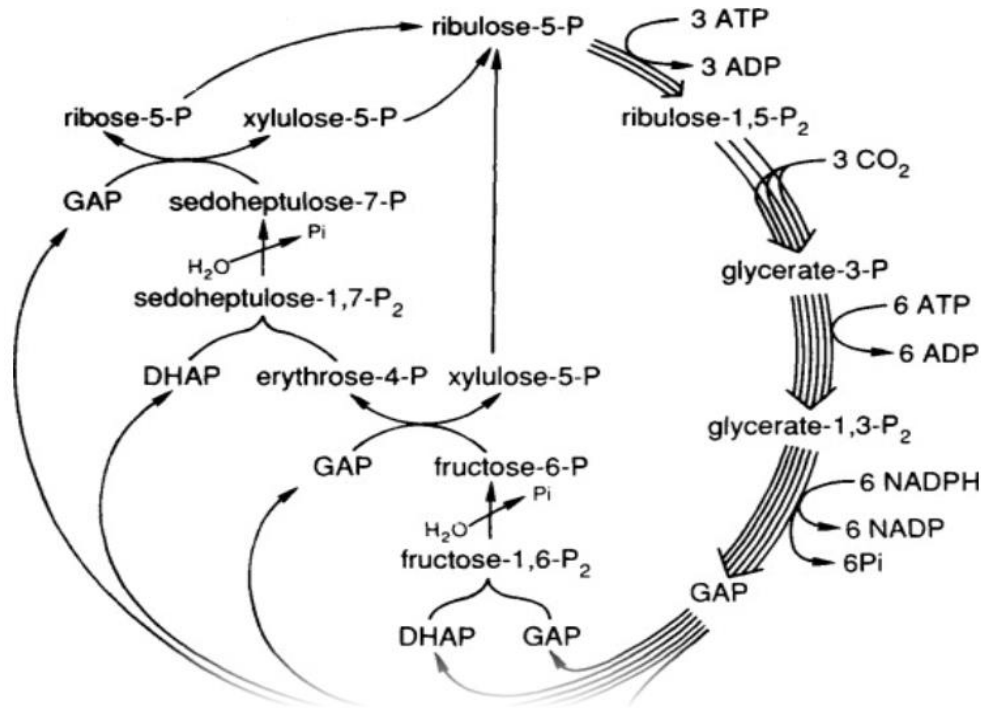
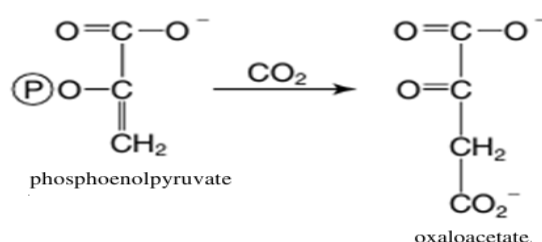


Fig. 6: Benson–Calvin cycle

II.2.2. C-4 photosynthesis

C4 photosynthesis is an evolutionary adaptation developed by certain plant species to enhance survival in hot, dry, and high-light environments. The name refers to the fact that the first stable compound formed after CO₂ fixation is a **four-carbon** acid, typically **oxaloacetate**. The key advantage of this pathway lies in its ability to minimize **photorespiration**, an energetically wasteful process that occurs in C3 photosynthesis when the enzyme RuBisCO fixes oxygen instead of carbon dioxide. By concentrating CO₂ around RuBisCO, the C4 pathway significantly improves photosynthetic efficiency under environmental stress conditions, such as high temperatures and limited water availability.

✚ In these C4 plants, the enzyme phosphoenolpyruvate carboxy lase first converts CO₂ to oxaloacetate.



PEP carboxylase has a lower K_m for CO_2 than does Rubisco. Further, O_2 is a very poor substrate for this enzyme. This means that, at relatively low concentrations of CO_2 , the delivery of carbon into photosynthesis products is more efficient than in C_3 plants and oxygenation doesn't occur.

- PEP carboxylase is concentrated in special mesophyll cells in the outer part of the leaf. This means that the cells most exposed to the atmosphere are the most efficient at converting CO_2 into organic products. Photosynthesis involving Rubisco is more prominent in the bundle sheath cells located in the inner part of the leaf around the veins that carry compounds between different parts of the plant.
- After PEP carboxylase makes the **oxaloacetate**, it is transported to the bundle sheath cells. First, NADPH reduces it to **malate**, and it is then transported to the bundle sheath cells. In the bundle sheath cells, malic enzyme cleaves the malate to pyruvate and CO_2 for Rubisco. This generates NADPH as well, so the C_4 cycle consumes no reducing equivalents. Pyruvate is transported from the bundle sheath back to the mesophyll cells where it is rephosphorylated to phosphoenolpyruvate, expending the equivalent of two ATP “high energy phosphates (Fig. 7).

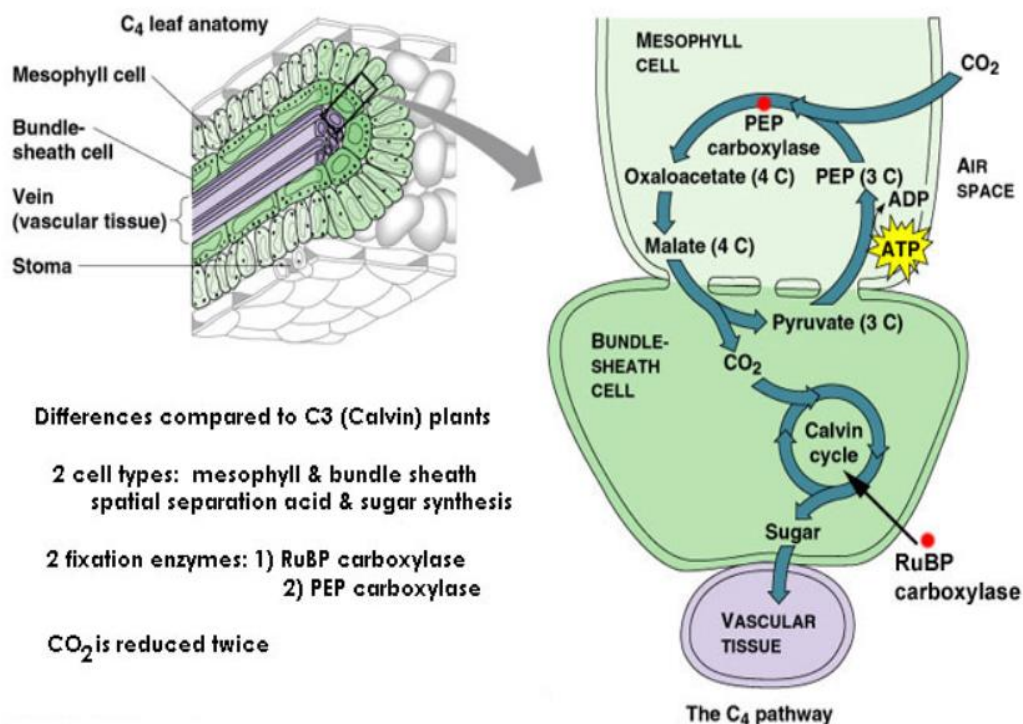
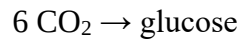


Fig. 7: C-4 photosynthesis

Overall, the C_4 cycle consumes **two** ATP equivalents to deliver a CO_2 to Rubisco. During active photosynthesis, this is not a problem, plenty of ATP exists from the action of Photosystems I and II.

III. Energetics of Photosynthesis

The light and dark reactions are coupled, this coupling is necessary because photosynthesis consumes a large amount of energy. To carry out the synthesis of a single molecule of glucose, the previous set of reactions must be carried out six times at a minimum.



This requires the generation of six separate molecules of ribulose biphosphate from ribulose phosphate, at the cost of one ATP each. Furthermore, two molecules of 1,3-bisphosphoglycerate must be made from the two 3-phosphoglycerates that are the initial product of each CO₂ fixation reaction. Conversion of each 1,3-bisphosphoglycerate requires an NADPH as well; therefore, two NADPH equivalents are consumed for each CO₂ fixed. Another way of saying this is that carbon is reduced from an oxidation number of -4 in CO₂ to an oxidation number of zero in carbohydrate (CH₂O). Therefore, synthesis of **one mole of glucose** requires the input of 18 ATPs and 12 NADPHs (Fig. 8).

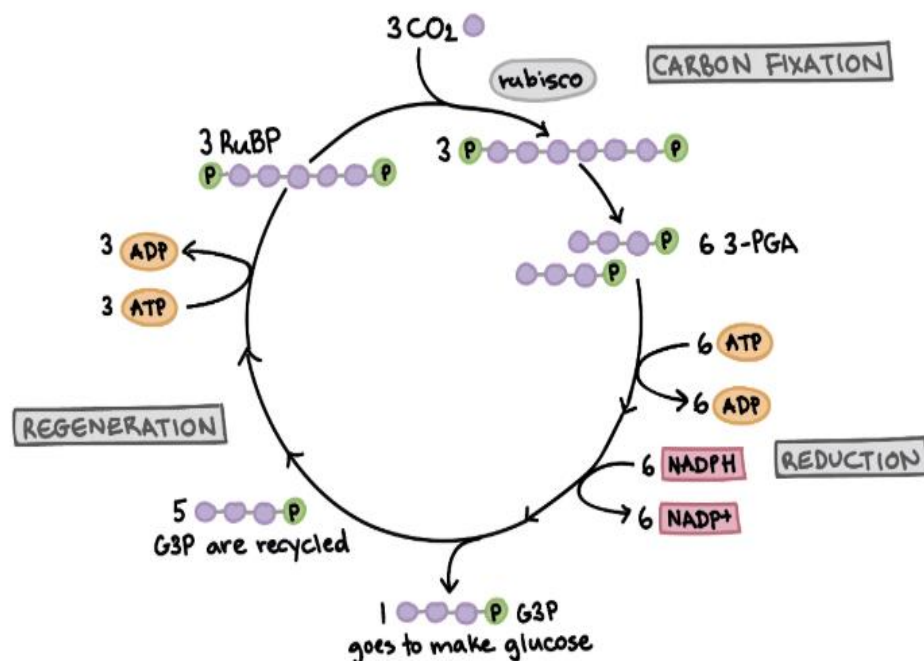
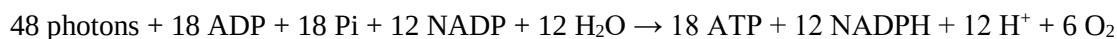


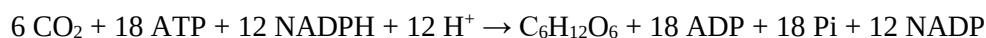
Fig. 10: Energy balance of the Calvin cycle

Generating one NADPH from NADP requires the transfer of two electrons through PSI and PSII. Transfer of one electron requires that a photon be absorbed by both PSI and PSII, so that the generation of 12 ATPs requires the absorption of $12 \times 2 \times 2 = 48$ photons. These 48 photons allow the synthesis of (just) 18 ATPs by coupling factor. Thus, the equation for photosynthesis can be written in two steps:

✚ Light reactions



✚ Dark reactions

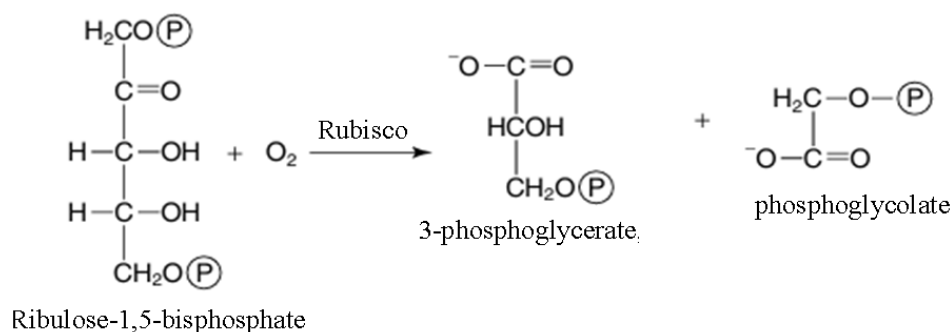


The energy in 18 moles of photons of blue light is about 8000 kJ and the energy required to synthesize a mole of glucose from CO₂ and water is 2870 kJ (684 kcal). This means that the maximal efficiency of photosynthesis is about 35 percent. Roughly one third of the available energy from sunlight is converted into glucose under optimal conditions.

IV. Photorespiration

The difficulty is in the protein that carries out the first step of photosynthesis. Molecular oxygen, O₂, competes with CO₂ for the active site of ribulose biphosphate carboxylase, leading to an oxidation and loss of the ribulose biphosphate acceptor. This competition is apparently intrinsic to the enzyme, because attempts to increase the discrimination for CO₂ by genetic engineering have resulted in a less-active enzyme, which fixes CO₂ very poorly.

Oxidation of ribulose-1,5-bisphosphate by Rubisco produces a 3-carbon compound, 3-phosphoglycerate, and a 2-carbon compound, phosphoglycolate. Because carbon is oxidized, the process is termed photorespiration.



Photorespiration reduces the efficiency of photosynthesis for a couple of reasons. First, oxygen is added to carbon. In other words, the carbon is oxidized, which is the reverse of photosynthesis the reduction of carbon to carbohydrate. Secondly, it is now necessary to resynthesize the ribulose biphosphate and to reduce the phosphoglycolate.

The 3-phosphoglycerate from photorespiration can reenter the Calvin-Benson pathway, but the phosphoglycolate must be recycled to make a useful compound. This recycling takes place in a specialized organelle termed the peroxisome. Peroxisomes lie between chloroplasts and mitochondria in the plant cell and serve to pass the 2-carbon products of oxygenation on for further

metabolism. In the chloroplast, the phosphoglycolate is dephosphorylated. Glycolate is transported to the peroxisome where molecular oxygen further oxidizes it to glyoxylate. The product is hydrogen peroxide, H_2O_2 , (the term peroxisome comes from this product) which is rapidly broken down by catalase to water and oxygen.

The glyoxylate is amidated to the amino acid glycine in the peroxisome.

Glycine is then transported to the mitochondrial matrix where the conversion of two glycines to one serine occurs with the loss of CO_2 and NH_3 from the pool of fixed molecules. The serine is transported into the peroxisome, where it is deaminated to glycerate. The glycerate is transported back to the chloroplast, where it is phosphorylated to 3-phosphoglycerate for the Calvin-Benson cycle (Fig. 9).

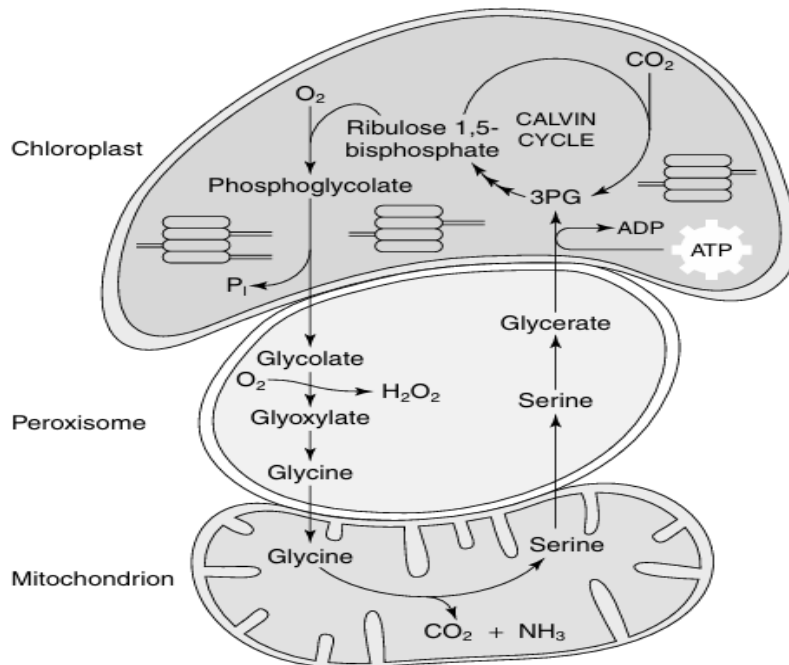


Fig. 9: Photorespiration.

This set of reactions is very detrimental to the efficiency of photosynthesis. Oxygen is added to carbon, CO_2 is lost, energy is consumed, and ribulose bisphosphate is destroyed. For a plant to be able to increase the discrimination of Rubisco for CO_2 would obviously be advantageous, but that hasn't happened, either naturally or through the efforts of scientists. An increased concentration of CO_2 in the atmosphere may lead to increased photosynthesis and decreased photorespiration, but high CO_2 concentrations would also contribute to global warming (and the increased photosynthetic carbon fixation would not likely reduce the amount of CO_2 in any event).

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Metabolic Biochemistry



General Overview and Biological Importance

Metabolism is the set of molecular transformations and energy transfers that occur within a cell or a living organism. It consists of two opposing mechanisms:

- ✚ **Catabolism:** This is the phase of metabolism during which relatively large and complex molecules are broken down into smaller and simpler molecules. This degradation process generates energy in the form of heat and ATP. One of the most common catabolic processes is digestion, during which complex sugars such as starch and proteins are broken down into simple sugars (glucose, fructose, ribose...) and amino acids, respectively, which can be directly used by the body. Catabolism also produces waste products, called catabolites (urea, CO₂...).
- ✚ **Anabolism:** This is the phase of metabolism during which large and complex molecules (proteins, glycogen, nucleic acids...) are synthesized from relatively simple precursors (amino acids, glucose...). Anabolism serves to build muscle mass, create body tissues, and store energy in the form of glycogen or triglycerides. This process requires (consumes) energy.

A good understanding of normal metabolism is essential for identifying the abnormalities underlying various diseases. Normal metabolism includes the body's ability to adapt to periods of fasting, physical exercise, pregnancy, and lactation. Abnormal metabolism may result from dietary or enzymatic deficiencies, abnormal hormone secretion, or the action of drugs or toxins.

The need for metabolic fuels remains relatively constant throughout the day, as average physical activity does not significantly increase metabolic demand. When dietary intake of metabolic fuels significantly exceeds energy expenditure, the surplus is stored primarily as triacylglycerol (TAG) in adipose tissue leading to obesity and its associated health problems. Conversely, when food intake is consistently lower than energy needs, fat and carbohydrate reserves become depleted, and amino acids from protein breakdown are used for energy instead of for protein synthesis. This results in weight loss and tissue wasting, which can ultimately lead to death.

The formation and utilization of glycogen and TAG stores are largely controlled by two hormones: insulin and glucagon. In diabetes, there is either a defect in insulin synthesis and secretion (Type I) or a reduced sensitivity of tissues to insulin action (Type II), leading to severe metabolic disturbances.



Carbohydrate Metabolism



I. General Overview

A constant source of blood glucose is absolutely essential for human life. Glucose is the preferred energy substrate for the brain and a fundamental source of energy for certain cells that lack mitochondria, such as red blood cells. Rapidly contracting skeletal muscles require a significant supply of glucose, which, through glycolysis, alone provides the necessary energy. Blood glucose comes from two sources:

- **Exogenous carbohydrates:** These are derived from food intake during meals (disaccharides, starch from plants, and glycogen from animals).
- **Endogenous carbohydrates:** Derived from glycogenolysis (breakdown of liver glycogen).

II. Glycogen Metabolism

Glycogen is the main form of carbohydrate storage in animals; it is the equivalent of starch in plants. It is a branched polymer of α -D-glucose. Glycogen is primarily found in the liver (to maintain blood glucose levels) and in muscles (as an immediately available source of glucose). Although the proportion of glycogen in the liver is higher than in muscle, the total body muscle mass is much greater than liver mass, so approximately three-quarters of the total glycogen is stored in muscles.

Glycogen storage diseases form a group of inherited disorders characterized by a defect in glycogen mobilization or the accumulation of abnormal forms of glycogen, leading to muscle weakness and, in some types, even premature death.

II.1. Glycogen Catabolism (Glycogenolysis)

II.1.1. Exogenous Glycogen (Dietary Origin)

In humans, starch and glycogen are the main sources of carbohydrates. The hydrolysis of these two polysaccharides into shorter fragments, then into oligosaccharides, is first carried out by **salivary α -amylase**, followed by **pancreatic α -amylase**, which continues the breakdown process in the intestine. This results in the formation of dextrins and maltose, which are then converted into glucose by the action of **dextrinase** and **maltase**, respectively (Fig.1). There is also a **debranching enzyme** in the intestinal mucosa capable of breaking down α (1 $\rightarrow$ 6) linkages.



Fig. 1: Catabolism of dietary glycogen

In the cytosol of higher plants and animals, there are three major fates of the glucose that is formed:

- It can be **oxidized through glycolysis** into pyruvate, serving as a source of ATP and intermediates for the citric acid cycle.
- It can be **oxidized via the pentose phosphate pathway**, leading to the production of **NADPH**, a reducing power essential for biosynthetic reactions, and **pentoses**, which are components of nucleic acids.
- It can be **stored as starch** (in plants) or **glycogen** (in animals), which are polysaccharides that, under certain physiological conditions, can be broken down to release readily usable glucose.

II.1.2. Endogenous Glycogen (Liver/Muscle)

Glycogenolysis (the breakdown of glycogen) enables the use of carbohydrate reserves by releasing stored glucose for further degradation. It is active during fasting periods. The main enzyme involved in the breakdown of endogenous glycogen (from the liver and muscles) is glycogen phosphorylase. This enzyme cleaves the α (1→4) glycosidic bond starting from the non-reducing end, and adds a phosphate group (provided by ATP) to the carbon 1 of the released glucose, forming glucose-1-phosphate.

Phosphorylase acts sequentially on glycogen chains until it reaches four glucose residues before an α (1→6) branch point. The remaining structure is called a limit dextrin (Fig. 2). A second enzyme, glucan transferase, acts on the limit dextrin by removing an oligosaccharide of three glucose residues from one branch and transferring it to another linear chain. This rearrangement enables glycogen phosphorylase to resume its activity on the extended chain (Fig. 2). After this action, a single glucose unit remains at the branch point, linked by an α (1→6) bond. Finally, an enzyme called α -glucosidase hydrolyzes this α (1→6) linkage, releasing free glucose.

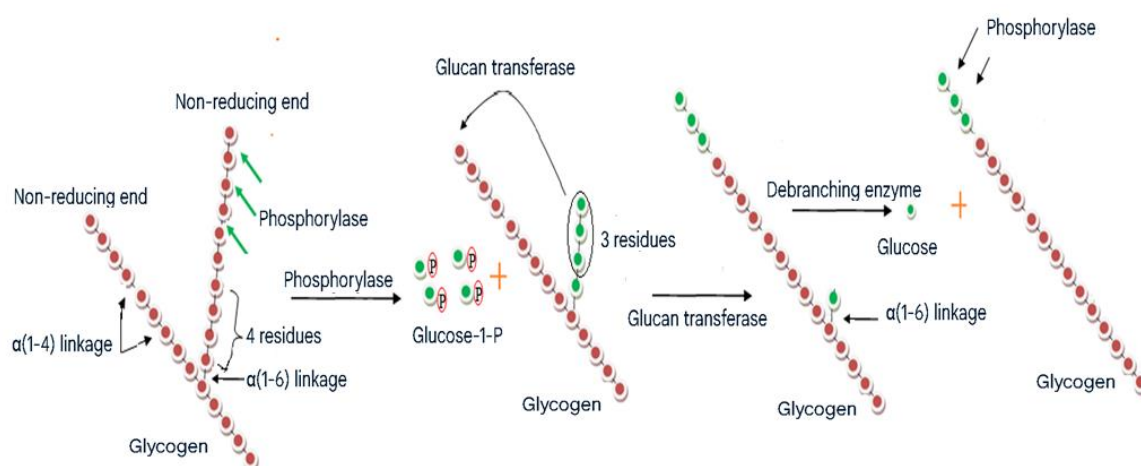


Fig. 2: Stages of hepatic glycogenolysis

Glucose-1-phosphate is **isomerized into glucose-6-phosphate** by the enzyme **phosphoglucomutase** (Fig. 3). Glucose-6-phosphate can then enter **glycolysis** in both the **liver and muscle**. However, the **primary goal** of hepatic glycogen breakdown is the **maintenance of blood glucose levels**. Only the **liver** possesses the enzyme **glucose-6-phosphatase** (Fig. 3), which allows the **hydrolysis of glucose-6-phosphate into free glucose**, enabling its **release into the bloodstream**.

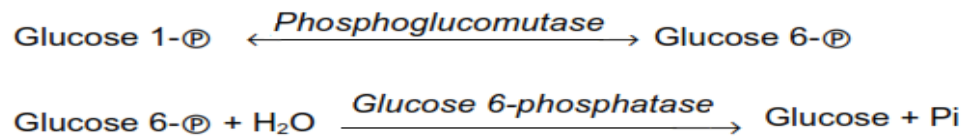


Fig. 3: Phosphorylation and dephosphorylation of glucose

II.2. Glycogen Anabolism (Glycogenesis)

Glycogenesis refers to the **storage of glucose** in the form of a polysaccharide (a glucose polymer) called **glycogen**. The **synthesis of glycogen** takes place in the **cytosol**. The **biosynthetic pathway of glycogen** involves a **special nucleotide derivative of glucose** (Fig. 4).

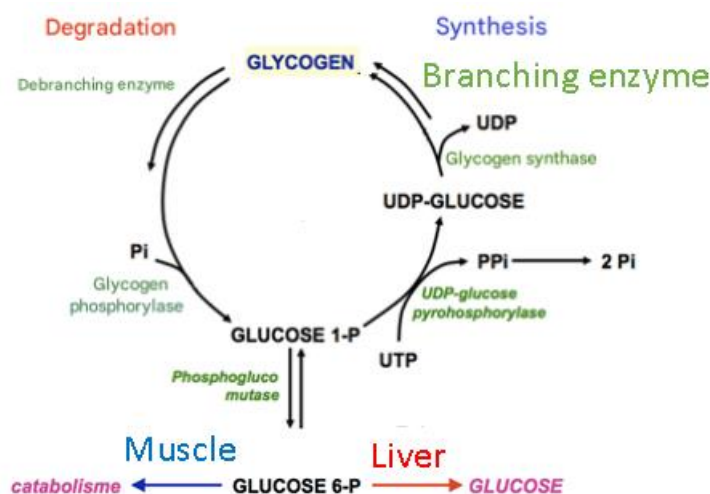


Fig. 4: Pathways of hepatic glycogenogenesis and glycogenolysis

- **Glucose is first phosphorylated** to form **glucose-6-phosphate**, a reaction catalyzed by **hexokinase** in muscle and **glucokinase** in the liver.
- **Glucose-6-phosphate is isomerized** into **glucose-1-phosphate** by **phosphoglucomutase**. This enzyme is itself phosphorylated, and the phosphate group participates in a **reversible reaction** in which **glucose-1,6-bisphosphate** acts as an intermediate.
- **Glucose-1-phosphate** then reacts with **uridine triphosphate (UTP)** to form an activated nucleotide, **uridine diphosphate glucose (UDP-Glc)** and **pyrophosphate (PPi)**; these last two steps **consume the equivalent of 2 ATP molecules**.

- **Glycogen synthase** catalyzes the formation of a **glycosidic bond** between the **C-1 of the activated glucose** from UDP-Glc and the **C-4 of a terminal glucose residue** (non-reducing end) of a glycogen primer (which must already be present to initiate the reaction). This reaction **releases uridine diphosphate (UDP)**.
- Once the chain has been extended by at least **11 glucose residues**, a second enzyme, the **branching enzyme**, transfers a segment of the **$\alpha(1\rightarrow4)$ chain** (at least 6 glucose residues) to a neighboring chain, creating an **$\alpha(1\rightarrow6)$ linkage** (Fig. 5). The new branches then grow through further **$\alpha(1\rightarrow4)$ glycosyl additions** and **additional branching**.

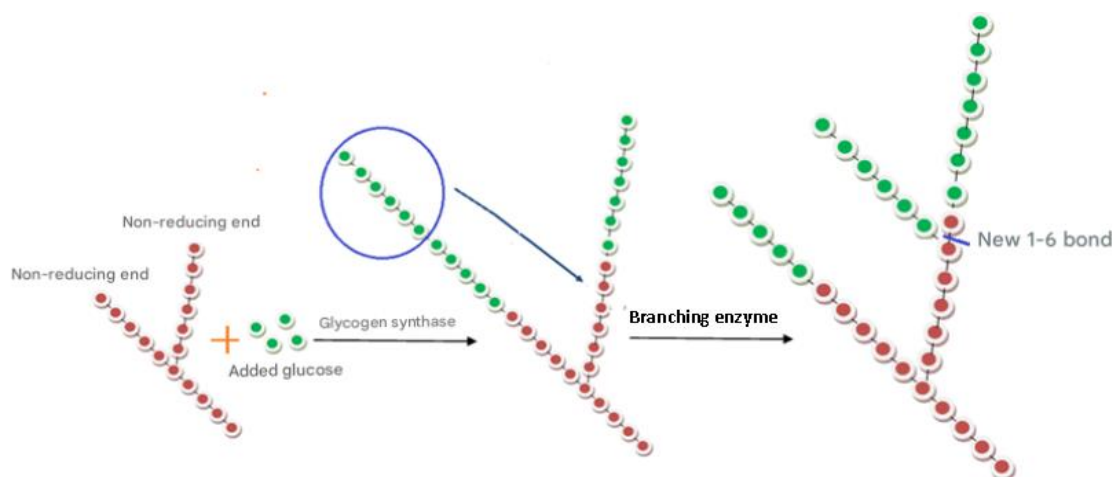


Fig. 5: Biosynthesis and branching mechanism of glycogen

II.3. Reciprocal Regulation of Glycogen Breakdown and Synthesis

The regulation of **glycogen degradation** and **glycogen synthesis** is **reciprocally coordinated** by **hormones**. This process is controlled by two key enzymes:

- **Protein kinase A (PKA)**, which is **cAMP-dependent** and activated by **glucagon** or **adrenaline**.
- **Protein phosphatase**, which is activated by **insulin**.

When **protein kinase A** is activated, it **phosphorylates glycogen phosphorylase kinase**, initiating a sequence of reactions that leads to **glycogen breakdown**. At the same time, it **phosphorylates glycogen synthase**, thereby **inactivating it**, which results in the **inhibition of glycogen synthesis** (Fig. 6).

Conversely, when the **insulin-dependent protein phosphatase** is activated, it **dephosphorylates** the phosphorylated glycogen synthase, thereby **restoring its activity**. It also dephosphorylates both **glycogen phosphorylase kinase** and **glycogen phosphorylase**, which **inhibits glycogen degradation**.

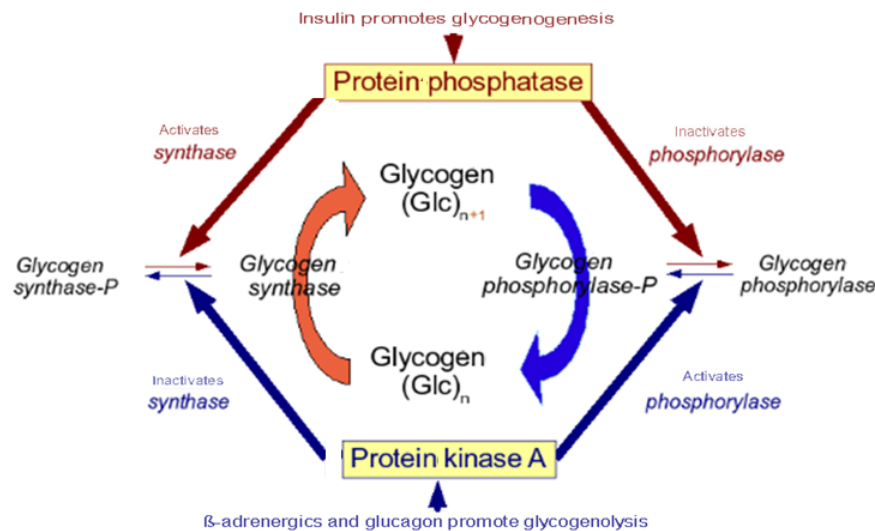


Fig.6: Regulation of glycogen metabolism

II.4. Disorders Related to Glycogen Metabolism

There are numerous genetic disorders related to glycogen metabolism, which can affect its degradation, synthesis, structure, or storage. The specific enzymes involved must be examined in the relevant tissues. Some of these conditions can be very severe, leading to premature death in childhood, while others have milder effects that are not life-threatening. A few examples include:

- ✚ **Glycogen Phosphorylase Deficiency in Skeletal Muscle (McArdle's Disease):** In McArdle's disease, muscle tissue lacks glycogen phosphorylase. The structure of glycogen in striated muscle is normal. Although mental development is unaffected, the following symptoms are observed:
 - Muscle weakness and cramps after exercise
 - No rise in lactate levels in muscles after intense exercise
 - Elevated muscle glycogen content
- ✚ **Glucose-6-Phosphatase Deficiency in the Liver (Von Gierke's Disease):** In Von Gierke's disease, the structure of glycogen is normal, but the disease affects the liver, kidneys, and intestine. Glucose-6-phosphate, produced from glycogen breakdown, can no longer be hydrolyzed into free glucose, which is a crucial reaction for maintaining blood glucose levels.

III. Metabolism of the Main Monosaccharide (Glucose)

III.1. Glucose Catabolism

Carbohydrate **catabolism** refers to the breakdown of glucose molecules, allowing the formation of **energy-rich molecules**.

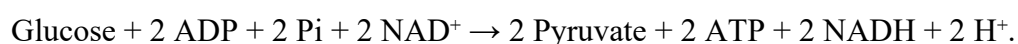
III.1.1. Glycolysis (Embden-Meyerhof Pathway)

Glycolysis is the first pathway in glucose catabolism. It takes place in the **cytosol**, is carried out by soluble enzymes, and occurs **anaerobically** (without oxygen input). Its primary role is the **synthesis of ATP** (energy-rich molecules), as well as the production of **pyruvate**, which can follow several metabolic fates. The process occurs in **10 enzymatic steps** (Fig. 7), divided into **two phases**:

- **Phase I: Energy Investment (Steps 1–5):** This is the preparatory phase of glycolysis. Here, the **six-carbon sugar** (glucose) is phosphorylated and split into **two three-carbon** molecules (glyceraldehyde-3-phosphate), with the **consumption of 2 ATP molecules**.
- **Phase II: Energy Payoff (Steps 6–10):** The two molecules of glyceraldehyde-3-phosphate are converted into **pyruvate**, producing **4 molecules of ATP**. In addition, **2 molecules of NADH** are formed, which can later be used to generate ATP through mitochondrial respiration.

a) Energy Yield of Glycolysis

During glycolysis, for each molecule of glucose, 2 ATP are consumed during the formation of glucose-6-phosphate and fructose-1,6-bisphosphate. Each glucose molecule produces 2 molecules of glyceraldehyde-3-phosphate. At the level of each triose phosphate, 1 NADH, H⁺, 2 ATP, and 1 pyruvate are formed. The final balance results in the production of 4 ATP and the consumption of 2 ATP. Therefore, the breakdown of one molecule of glucose through glycolysis leads to a net synthesis of 2 ATP, 2 NADH, H⁺, and 2 pyruvate molecules. The overall reaction is:



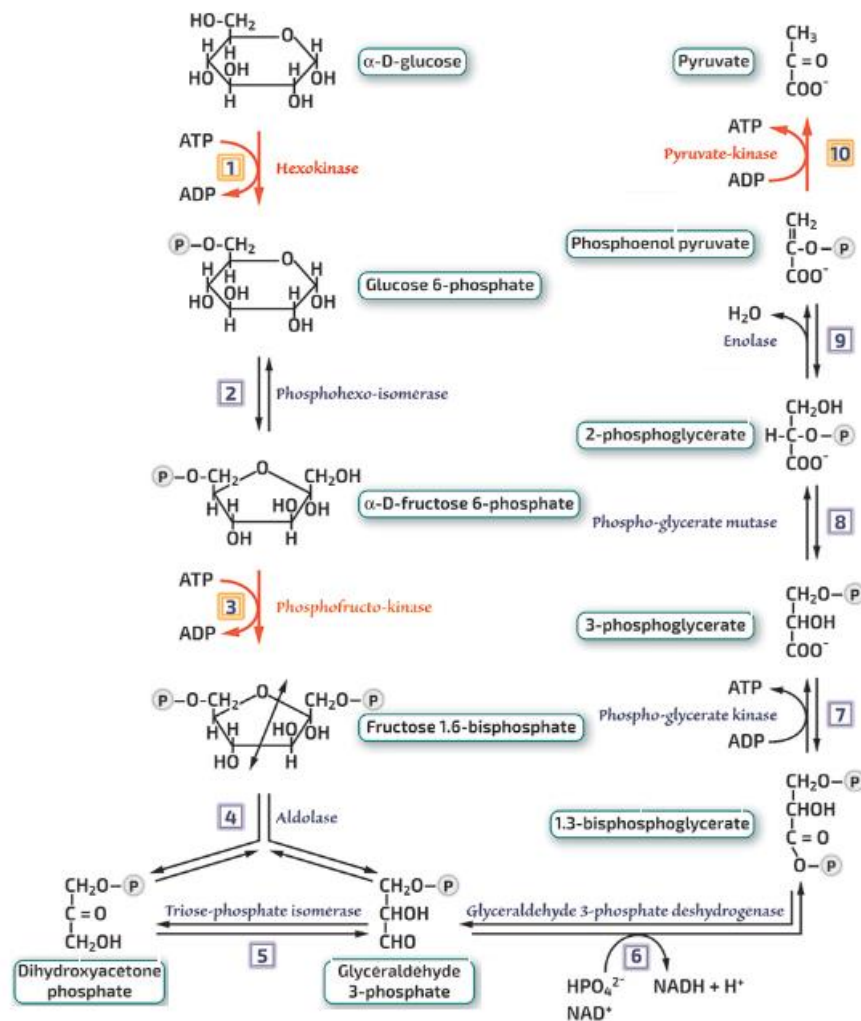


Fig. 7: Glycolysis (Embden-Meyerhof pathway)

b) Regulation of Glycolysis

In metabolic pathways, enzymes that catalyze **irreversible reactions** are potential control points. In glycolysis, enzymes are regulated through **three main mechanisms**: regulation by **allosteric effectors**, regulation by **phosphorylation/dephosphorylation**, and regulation by **gene expression** of the corresponding enzymes. Within glycolysis, three key irreversible reactions serve as major regulatory sites:

- The phosphorylation of glucose to glucose-6-phosphate, catalyzed by **glucokinase** or **hexokinase**. Hexokinase is **inhibited** by glucose-6-phosphate.
- The phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate, catalyzed by **phosphofructokinase-1 (PFK-1)**. This enzyme is **inhibited** by ATP, citrate, glucagon (in the liver), and **adrenaline** (in muscle), and is **activated** by **insulin** and **AMP**.
- The **conversion of phosphoenolpyruvate to pyruvate**, catalyzed by **pyruvate kinase**. This enzyme is **inhibited** by pyruvate, alanine, ATP, and **NADH, H^+** .

III.1.2. Fate of Pyruvate

Following glycolysis, the two molecules of pyruvate formed from one molecule of glucose can follow several metabolic fates depending on the needs of the organism.

III.1.2.1. Under Aerobic Conditions (in the presence of O₂)

Pyruvate can participate in **amino acid synthesis**, or it can enter the mitochondrion, where it is converted (via oxidative decarboxylation) into **acetyl-CoA** (acetyl-coenzyme A). This is an irreversible reaction catalyzed by the pyruvate dehydrogenase complex, a multienzyme complex located in the mitochondrial matrix (Fig. 8). This step also leads to the production of one NADH, H⁺.

Acetyl-CoA then has several possible fates:

- ✚ It can enter the **Krebs cycle** (also known as the tricarboxylic acid cycle or the citric acid cycle), which is the cell's main energy-producing pathway. This cycle takes place in the mitochondrial matrix and occurs exclusively under aerobic conditions. It consists of 9 major steps involving 8 enzymes (Fig. 9).
- ✚ It can also serve as a precursor for **lipid biosynthesis** reactions.

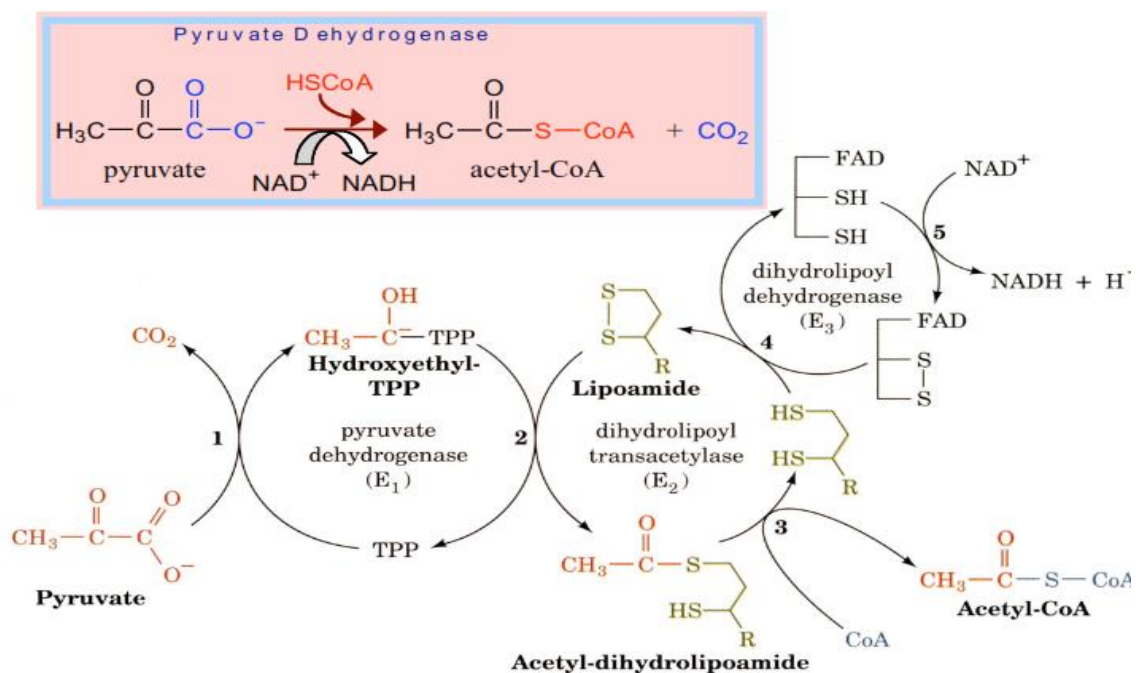


Fig. 8: Mechanism of action of Pyruvate dehydrogenase

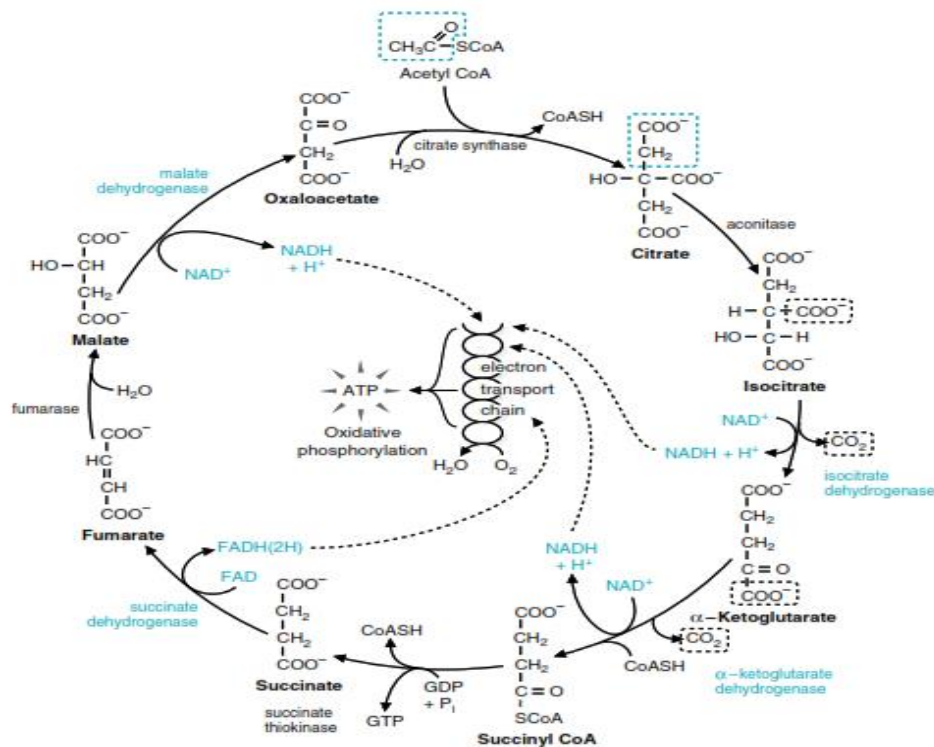


Fig. 9: Krebs cycle (Citric Acid Cycle)

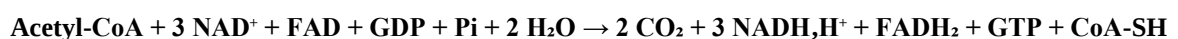
a) Krebs Cycle Yield

Under aerobic conditions, **acetyl-CoA** enters the **Krebs cycle**. One turn of the cycle (the use one molecule of acetyl-CoA) results in the production of:

- **3 NADH, H⁺**, each of which can theoretically produce **3 ATP** via the electron transport chain, for a total of **9 ATP**.
- **1 FADH₂**, which can theoretically yield **2 ATP** via the electron transport chain.
- **1 GTP**, which is energetically equivalent to **1 ATP**.

Therefore, the **complete oxidation of one acetyl-CoA** molecule via the Krebs cycle leads to the formation of **12 ATP equivalents**.

Overall reaction



b) Regulation of the Krebs Cycle

Three reactions in the Krebs cycle are **irreversible**, and they are catalyzed by enzymes that are **allosterically regulated**. For both energetic and catalytic reasons, these three reactions serve as key **control points** for the regulation of the overall flux through the Krebs cycle. The enzymes involved are:

- **Citrate synthase**, which catalyzes the first reaction of the Krebs cycle. Citrate acts as a competitive inhibitor of oxaloacetate.

- **Isocitrate dehydrogenase**, which is inhibited by high levels of ATP and NADH, and activated by NAD^+ and FAD^+ .
- The **α -ketoglutarate dehydrogenase complex**, which catalyzes a reaction similar to that of the pyruvate dehydrogenase complex. This enzyme is activated by calcium, and inhibited by succinyl-CoA and NADH.

III.1.2.2. Under Anaerobic Conditions (in the absence of O_2)

a) Homolactic Fermentation

In humans, **pyruvate** is converted into **lactic acid (lactate)** by the enzyme **lactate dehydrogenase** (Fig. 10), with the consumption of one NADH, H^+ (produced during glycolysis). This reaction serves to **regenerate NAD^+** , which is essential for glycolysis to continue when **ATP is needed**, but the rate of oxidative respiration is insufficient. This process occurs, for example, in muscle tissue during anaerobic activity. A sufficient level of lactate dehydrogenase activity to clear lactate exists only in the liver; a portion of the lactate is also excreted in the urine.

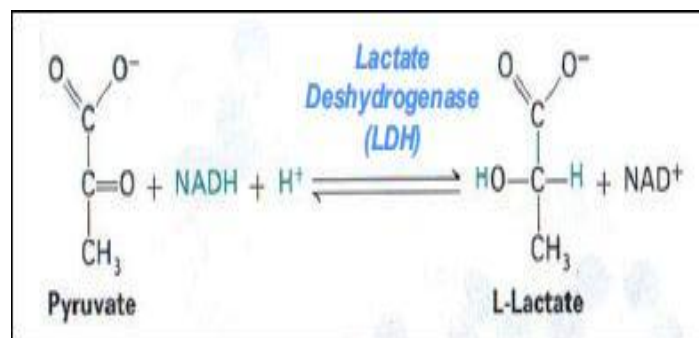


Fig.10: Homolactic fermentation

b) Alcoholic Fermentation

In yeast, pyruvate is converted into ethanol, with the consumption of one NADH, H^+ and the release of CO_2 , through the action of pyruvate decarboxylase and alcohol dehydrogenase (Fig. 11). This reaction also serves to regenerate NAD^+ , allowing glycolysis to continue.

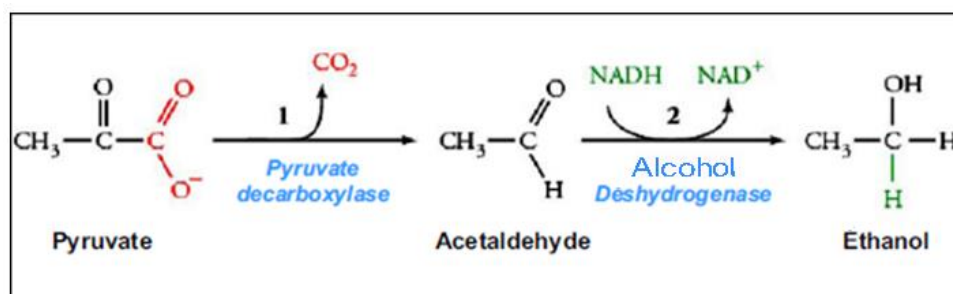


Fig. 11: Alcoholic fermentation

III.1.3. Energy Balance of Glucose Catabolism

✚ Glycolysis Yield

- **Under anaerobic conditions:** glycolysis yields **2 ATP**, since the **2 NADH,H⁺** produced are used in the formation of **lactate** or **ethanol**.
- **Under aerobic conditions:** glycolysis yields **8 ATP** (since each NADH,H⁺ can generate **3 ATP**, and 2 NADH = 6 ATP, plus 2 ATP directly formed).

✚ Pyruvate Decarboxylation Yield

Each pyruvate yields **1 NADH,H⁺** (equivalent to **3 ATP**), so **6 ATP** per glucose molecule (since glucose produces 2 pyruvate).

✚ Krebs Cycle Yield

Each acetyl-CoA yields **12 ATP**, and since one glucose gives **2 acetyl-CoA**, the total is **24 ATP** per glucose molecule.

Total Aerobic Yield from **One Glucose** Molecule: 8ATP (glycolysis) + 6ATP (pyruvate decarboxylation) + 24 ATP (Krebs cycle) = **38 ATP**

III.1.4. Pentose Phosphate Pathway (Phosphogluconate Pathway)

The pentose phosphate pathway (PPP) is an alternative glucose degradation pathway that runs **parallel to glycolysis**, and its main role is to produce **NADPH + H⁺** for **biosynthetic reactions** (especially for the synthesis of fatty acids and steroids) and for the reduction of glutathione. This pathway occurs primarily in the **cytosol** of cells in the mammary glands, adipose tissue, liver, and adrenal cortex. In addition to **NADPH**, the PPP also produces:

- **Ribose-5-phosphate**, a precursor for the synthesis of **nucleotides, nucleic acids** (DNA and RNA), and **coenzymes**.
- **Erythrose-4-phosphate**, a precursor of **aromatic amino acids**.

Glucose-6-phosphate serves as the starting substrate for both glycolysis and the pentose phosphate pathway. The cell's metabolic needs for **ATP** and **biosynthetic precursors** determine the relative flux between these two pathways. The pentose phosphate pathway is divided into two segments:

a) Irreversible Oxidative Phase

In this phase, **glucose-6-phosphate** is converted into **ribulose-5-phosphate**, producing **2 NADPH** and **1 CO₂** in the process.

- ❖ The first step is a **redox reaction** catalyzed by glucose-6-phosphate dehydrogenase.
- ❖ The second step is a **hydrolysis**, catalyzed by gluconolactonase.
- ❖ The third step is a **redox reaction** followed by spontaneous decarboxylation, catalyzed by 6-phosphogluconate dehydrogenase (Fig. 12).

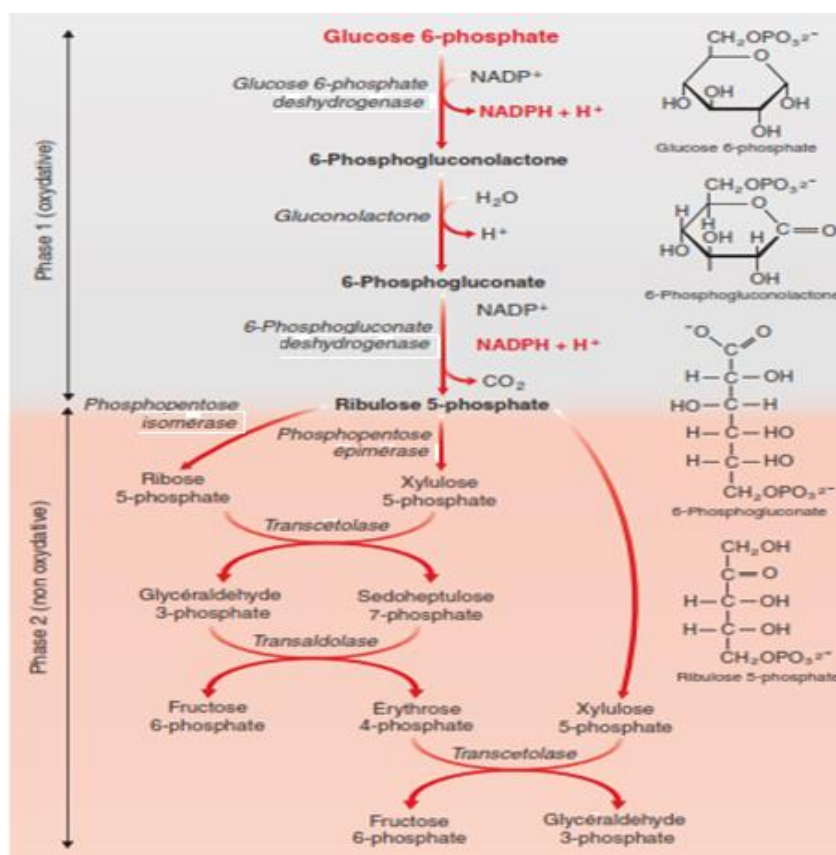


Fig. 12: Pentose phosphate pathway

b) Reversible Non-Oxidative Phase

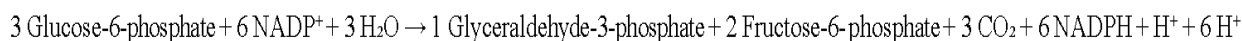
This phase begins with **interconversion** (isomerization and epimerization) of the pentose phosphates (Fig. 12):

- ❖ **Epimerization** of ribulose-5-phosphate into xylulose-5-phosphate: From 3 ribulose-5-P molecules, two are epimerized by phosphopentose epimerase.
- ❖ **Isomerization** of the remaining ribulose-5-P into ribose-5-phosphate by phosphopentose isomerase. These three pentose phosphates exist in equilibrium. Then follow several carbon transfer reactions:
- ❖ **Transketolase reaction:** This enzyme cleaves two carbons from xylulose-5-P and transfers the glycolaldehyde unit ($\text{CH}_2\text{OH}-\text{CHO}$) to ribose-5-P. Transketolase requires TPP (thiamine pyrophosphate) as a coenzyme.
- ❖ **Transaldolase reaction:** Transaldolase cleaves the first 3 carbon atoms from sedoheptulose to form dihydroxyacetone, transferring it to glyceraldehyde-3-phosphate (G3P), which is then converted into fructose-6-phosphate.
- ❖ **Second transketolase reaction:** Transketolase transfers a glycolaldehyde unit from xylulose-5-phosphate to erythrose-4-phosphate, forming fructose-6-phosphate and glyceraldehyde-3-phosphate.

- ❖ **Isomerization of fructose-6-phosphate into glucose-6-phosphate:** Phosphohexose isomerase converts both fructose-6-P molecules into 2 glucose-6-P. Thus, out of the 3 original ribulose-5-P molecules, 2 glucose-6-P are regenerated, and only one molecule is lost in the process.

a) Pentose Phosphate Pathway Energy Balance

The net energy yield is zero: this pathway does not produce or consume ATP. The overall reaction is:



- ❖ Notably, the 2 molecules of fructose-6-phosphate can be isomerized back into glucose-6-phosphate, so after one complete cycle, it is as if half a molecule of glucose has been oxidized to CO₂, meaning 3 glucose-6-P molecules are decarboxylated and 2 are regenerated.
- ❖ If we start from 6 molecules of glucose, processed by the enzymes of this pathway, in addition to CO₂ and NADPH, we also obtain 2 molecules of glyceraldehyde-3-phosphate, one of which can be converted by a phosphate isomerase into dihydroxyacetone phosphate (DHAP). DHAP and glyceraldehyde-3-P can then recombine to form one molecule of glucose-6-phosphate.
- ❖ Thus, by the end of the cycle, 4 glucose-6-phosphate molecules are regenerated, and only one molecule is effectively consumed. Therefore, in two complete cycles, the equivalent of one glucose molecule is fully oxidized, yielding 12 NADPH and 6 CO₂.

b) Regulation of the Pentose Phosphate Pathway

Regulation of the pentose phosphate pathway occurs mainly in the irreversible oxidative phase. The cytoplasmic concentration of NADP⁺ serves as a key regulatory factor. The reversible reactions in the non-oxidative phase are governed by chemical equilibria, depending on the metabolic needs.

- Glucose-6-phosphate dehydrogenase is inhibited by NADPH.
- 6-phosphogluconate dehydrogenase is inhibited by acetyl-CoA.

III.2. Carbohydrate Anabolism (Gluconeogenesis)

Gluconeogenesis is a series of biochemical reactions aimed at synthesizing glucose from non-carbohydrate compounds. The main gluconeogenic substrates are lactate, pyruvate, glycerol, and certain amino acids. It occurs in the cytosol, primarily in the liver, but also in the kidneys (mainly from amino acids). Gluconeogenesis is essentially the reverse of glycolysis, and the

reactions are similar across animals, plants, fungi, and microorganisms. Since the brain and red blood cells are strictly dependent on a sufficient glucose supply, gluconeogenesis begins to operate as early as eight hours after sleep.

The various precursors are first converted into glycolytic intermediates:

- ✚ Pyruvate for lactate, amino acids, and ketone bodies.
- ✚ Dihydroxyacetone phosphate for glycerol.

III.2.1. Gluconeogenesis Reactions from Pyruvate

Most of the steps that convert pyruvate into glucose are catalyzed by glycolytic enzymes (Fig. 13) operating in the reverse direction (reversible reactions). However, the three irreversible steps of glycolysis are bypassed by alternative reactions with more favorable thermodynamic equilibrium, catalyzed by enzymes specific to gluconeogenesis. The initiation of gluconeogenesis requires the conversion of two molecules of pyruvate into two molecules of phosphoenolpyruvate (PEP).

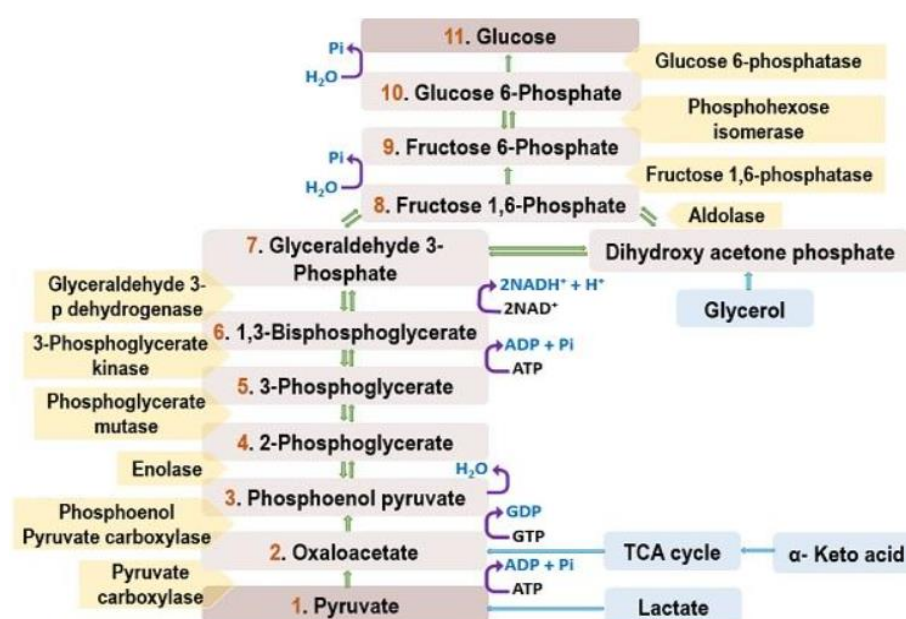


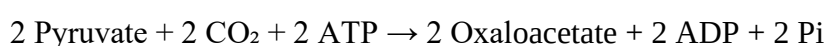
Fig. 13: Gluconeogenesis pathway

a - Conversion of Pyruvate to Phosphoenolpyruvate

To achieve the phosphorylation of pyruvate, **mitochondrial and cytosolic cooperation** is required (Fig. 14).

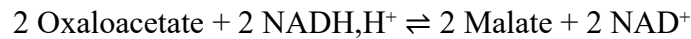
✚ Mitochondrial phase

Pyruvate, transported into the mitochondria, is first carboxylated by pyruvate carboxylase, located in the mitochondrial matrix. This enzyme is a biotin-dependent ligase, and ATP is required. Pyruvate carboxylase is found in the mitochondria of the liver and kidneys, but not in muscle.



The **oxaloacetate** formed is **reduced to malate** by mitochondrial malate dehydrogenase.

The **malate is then transported** to the cytosol:

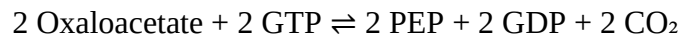


✚ Cytosolic phase

Malate is **re-oxidized** to oxaloacetate by cytosolic malate dehydrogenase:



Finally, oxaloacetate is **converted into phosphoenolpyruvate (PEP)** in a **reversible reaction** using **GTP**, catalyzed by **phosphoenolpyruvate carboxykinase (PEPCK)**, a key enzyme of gluconeogenesis:



Overall reaction for the conversion of pyruvate to PEP:

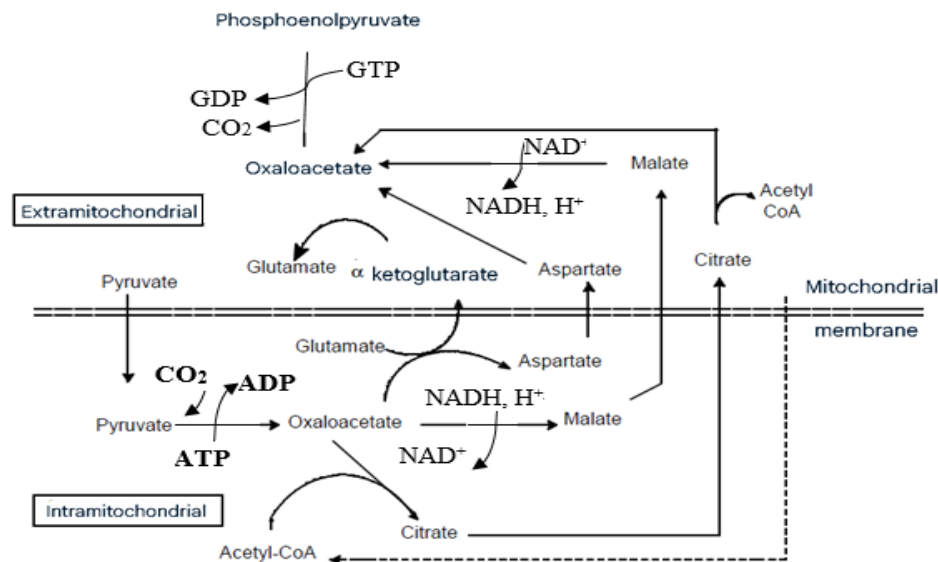
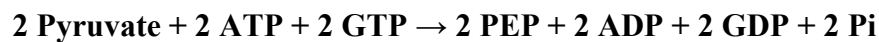


Fig. 14: Transformation of pyruvate into phosphoenolpyruvate

b) Hydrolysis of Fructose 1,6-Bisphosphate to Fructose 6-Phosphate

The conversion of **fructose 1,6-bisphosphate** into **fructose 6-phosphate** is the **second irreversible step** in gluconeogenesis. It is catalyzed by the enzyme **fructose 1,6-bisphosphatase** (Fig. 13), an **allosteric enzyme** that is **activated by citrate** and **inhibited by fructose 2,6-bisphosphate and AMP**.

c) Conversion of Glucose 6-Phosphate to Glucose

In most tissues, **gluconeogenesis stops at the level of glucose 6-phosphate**. However, in tissues responsible for maintaining **blood glucose homeostasis**, mainly the **liver** and to a lesser extent the **kidney**, **free glucose** is produced from glucose 6-phosphate through a **simple hydrolysis reaction** catalyzed by **glucose 6-phosphatase**, an enzyme located in the **endoplasmic reticulum** (Fig. 13).

III.2.2. Gluconeogenesis from glycerol

During fasting or energy deficiency, gluconeogenesis from glycerol becomes important because glycerol is released from lipid reserves. Glycerol is first activated into glycerol-3-phosphate by glycerol kinase using ATP. Then, glycerol-3-phosphate is oxidized into dihydroxyacetone phosphate (Fig. 15), which reacts with glyceraldehyde-3-phosphate to form fructose-1,6-bisphosphate, which can eventually be converted into glucose.

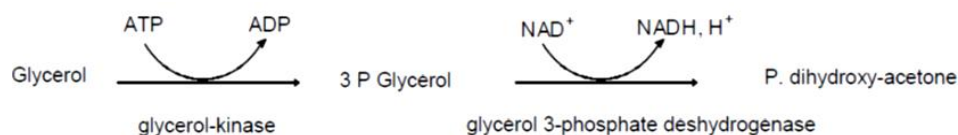


Fig. 15: Transformation of glycerol into dihydroxyacetone phosphate

III.2.3. Gluconeogenesis from Glucogenic Amino Acids

After transamination or deamination, glucogenic amino acids form either pyruvate or intermediates of the Krebs cycle (α -ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate) (Fig. 16). The glucose-producing potential varies from one amino acid to another: alanine, glutamate, aspartate, and glycine are among the most effective, whereas lysine and leucine are the only amino acids with no gluconeogenic potential.

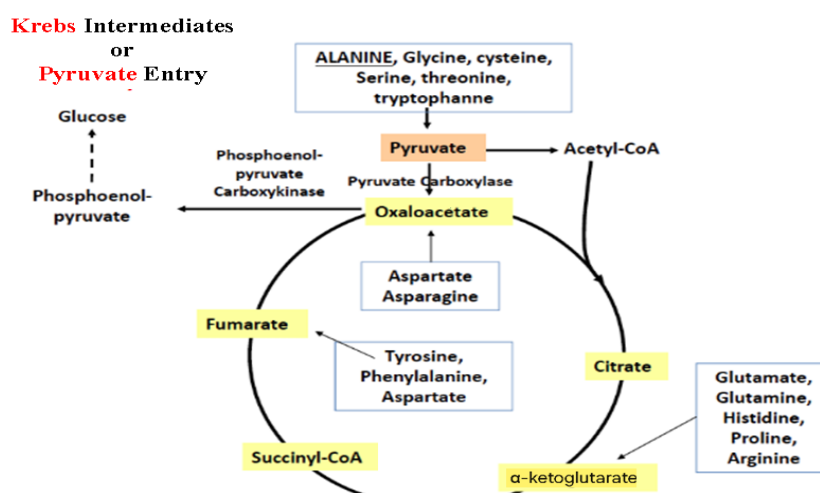


Fig. 16: Transformation of glucoforming AA into pyruvate or Krebs cycle intermediates.

III.2.4. Assessment of gluconeogenesis

Gluconeogenesis is coupled with the hydrolysis of 4 ATP and 2 GTP molecules: thus, six molecules of nucleoside triphosphates (Fig.17) are required to synthesize one molecule of glucose from pyruvate.

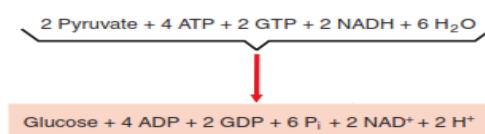


Fig. 17: Overall reaction of gluconeogenesis from pyruvate

III.2.5. Regulation

Glycolysis and gluconeogenesis are reciprocally regulated in such a way that one pathway is relatively inactive while the other is fully active.

IV. Metabolism of Other Monosaccharides

Monosaccharides are components of our diet that can be used as energy sources. Moreover, the body needs these sugars for the biosynthesis of glycoproteins. Hepatic metabolism allows the conversion of these hexoses into glucose.

It is observed that galactose and fructose are not stored in the body; they come from the diet and enter carbohydrate metabolism either through the storage pathway or glycolysis, depending on the tissue. It is also noted that galactose and fructose are not subject to hormonal regulation.

✚ **Fructose** can be phosphorylated on carbon 6 by hexokinase, but this enzyme is saturated by glucose. Fructose is mainly processed by hepatic fructokinase, which phosphorylates it on carbon 1. The sequence of reactions leading to glyceraldehyde-3-phosphate involves, in order: fructokinase, fructose-1-phosphate aldolase B, glyceraldehyde kinase, and triose phosphate isomerase (Fig. 18).

The overall reaction is: **Fructose + 2 ATP → 2 Glyceraldehyde-3-P + 2 ADP**

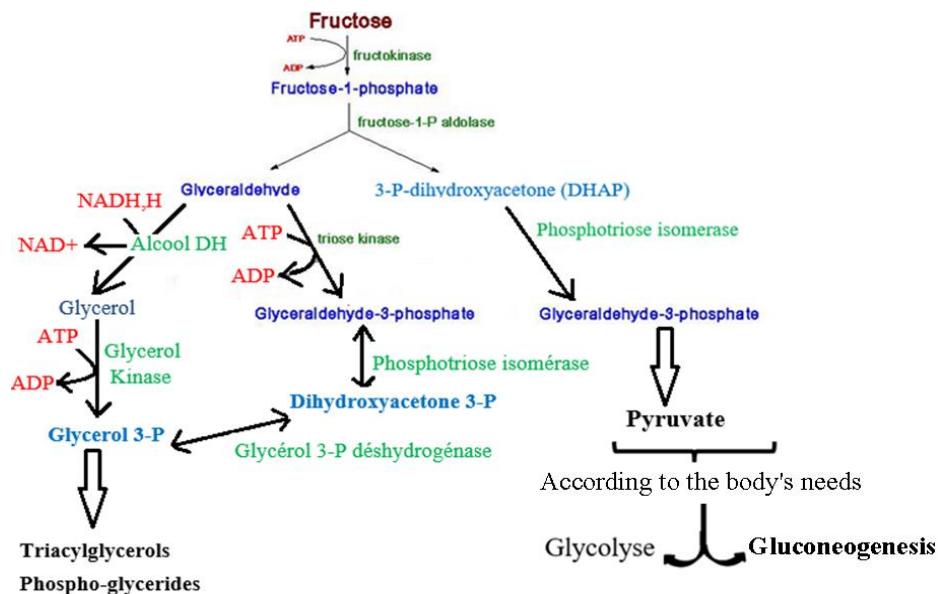


Fig. 18: Fructose metabolism

✚ **Galactose** can also be phosphorylated on carbon 6 by hexokinase, but for the same reasons as fructose, it is processed by hepatic galactokinase, which phosphorylates it on carbon 1. It is then involved in a secondary interconversion pathway from galactose to glucose before entering glycolysis. The sequence involves, in order: galactokinase, uridylyltransferase, UDP-hexose 4'-epimerase, and finally phosphoglucomutase (Fig. 19).

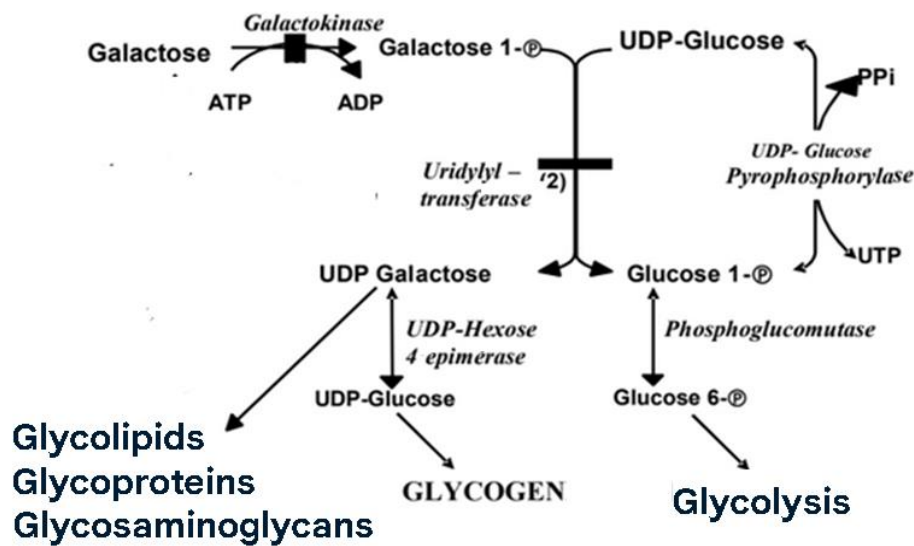


Fig. 19: Galactose metabolism

- ✚ **Mannose** is the C2 epimer of glucose and is found in foods rich in glycoproteins. It is primarily intended for the synthesis of glycoproteins. When mannose enters the cell, it is phosphorylated into mannose-6-phosphate by hexokinase in the presence of ATP. If mannose-6-phosphate is not used for glycoprotein biosynthesis, it can be isomerized into fructose-6-phosphate by mannose-phosphate isomerase. The resulting fructose-6-phosphate can then enter the glycolysis pathway (Fig. 20).

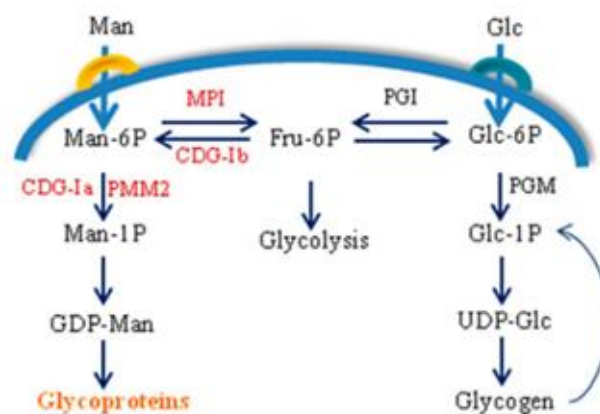


Fig. 20: Mannose metabolism

V. Metabolism of Disaccharides

Disaccharides ingested in the form of sucrose, maltose, and lactose are first hydrolyzed into their constituent monosaccharides (Fig. 21). Specific enzymes are secreted by the intestinal mucosa and remain attached to it.

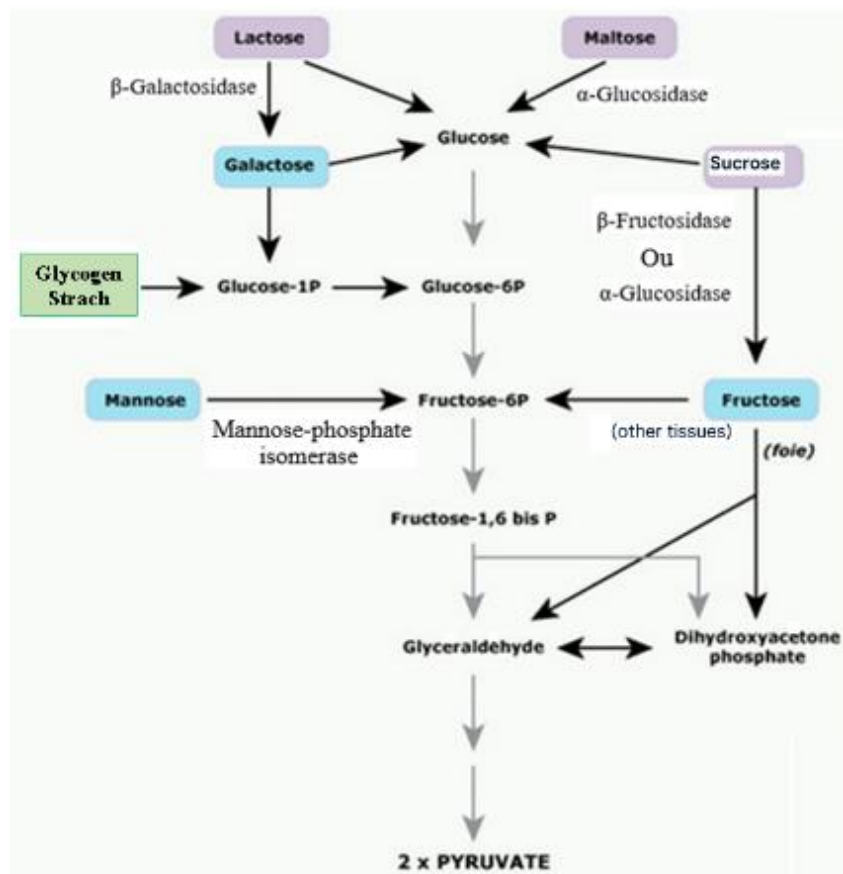


Fig. 21: Disaccharide mechanism

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Lipids Metabolism



I. General Overview

Lipids are complex compounds whose various components play energetic, structural, and functional roles, either directly or indirectly, and either immediately or over time (Fig. 1). The diet is the only source of so-called essential fatty acids (linoleic acid and linolenic acid), and is also the primary source of non-essential fatty acids, as the body's ability to synthesize them endogenously is quantitatively limited.

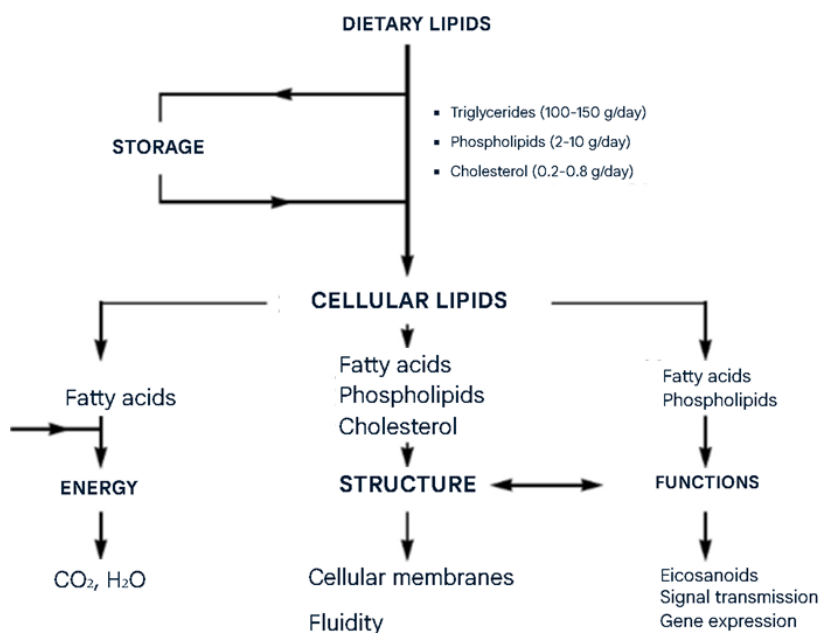


Fig. 1: Main pathways of lipid utilization

II. Lipid Metabolism

II.1. Lipid Catabolism

II.1.1. Dietary Lipids

The main lipids found in human and animal diets consist primarily of triglycerides (triacylglycerols), phospholipids, and sterols. The digestion of these lipids depends on pancreatic enzymes and bile salts.

a) Enzymes

The enzymes responsible for lipid hydrolysis are lipases and phospholipases. Their activity takes place in the small intestine.

- ✚ **Pancreatic triglyceride lipase** acts to hydrolyze triglycerides, ultimately releasing two fatty acids and one β -monoacylglycerol (Fig. 2). Only the ester bonds at the primary alcohol positions of the triglyceride are hydrolyzed.

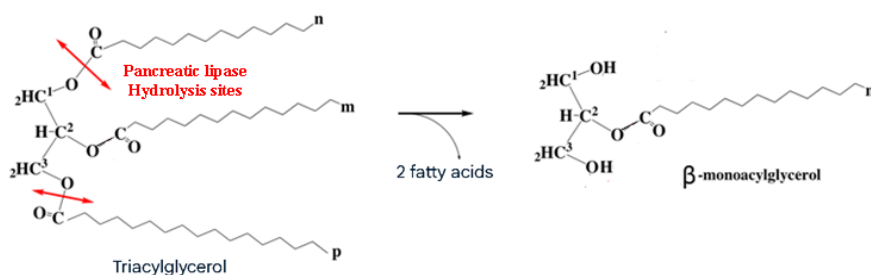


Fig. 2: Effect of pancreatic lipase

✚ Phospholipases present in the liver, pancreas, intestine, spleen, brain, red blood cells, and other tissues are enzymes that hydrolyze phospholipids. They are classified into four types: A1, A2, C, and D. Phospholipases A1 and A2 (also referred to as phospholipase B) cleave the fatty acids esterified to the primary and secondary hydroxyl groups of glycerol, respectively. The resulting molecules, which have lost one fatty acid, are known as lysophospholipids. Phospholipase C breaks the ester bond between glycerol and the phosphate group, while phospholipase D releases the alcohol that characterizes the specific phospholipid (Fig. 3).

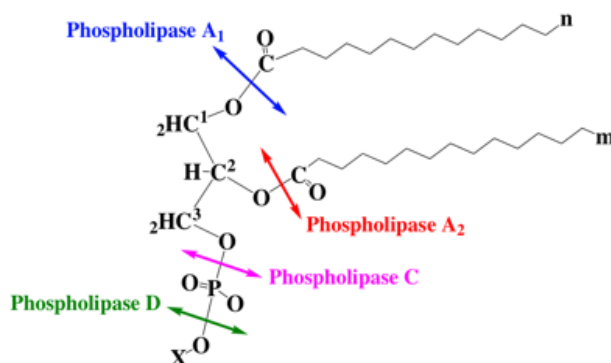


Fig. 3: Effect of phospholipases

These hydrolytic enzymes act only at the water lipid interface. As such, they bind to the surface of large fat droplets. The initial products of lipase and phospholipase activity, fatty acids and lysophospholipids, act as potent detergents, accelerating the process by breaking large fat droplets into smaller ones. Bile salts further assist in this emulsification and in the formation of micelles from triglycerides.

b) Absorption

Mixed micelles, formed after the complete action of lipases, contain fatty acids and β monoacylglycerols. These are absorbed by enterocytes, the absorptive cells of the small intestine. Once inside the enterocyte, fatty acids are taken up by a specific transporter and directed to the smooth endoplasmic reticulum. β monoacylglycerols, which can passively diffuse across membranes, also reach the endoplasmic reticulum. There, fatty acids and β monoacylglycerols are re-esterified into triacylglycerols by specific enzymes.

c) Lipoproteins

Lipoproteins are the transport forms of hydrophobic lipids in the bloodstream. They have a globular structure composed of a hydrophobic core of triglycerides, surrounded by proteins, cholesterol esters, and phospholipids. The major classes of lipoproteins are:

- **Chylomicrons:** synthesized in enterocytes (intestinal cells), transport dietary triglycerides to peripheral tissues and adipose tissue.
- **VLDL (Very Low Density Lipoproteins):** synthesized in the liver, transport endogenously produced triglycerides.
- **LDL (Low Density Lipoproteins):** carry cholesterol, often referred to as “bad” cholesterol due to their association with cardiovascular disease.
- **HDL (High Density Lipoproteins):** synthesized in the blood, return excess cholesterol from peripheral tissues back to the liver; considered “good” cholesterol.
- **IDL (Intermediate Density Lipoproteins):** metabolic intermediates, typically absent from circulation except in cases of hereditary hypolipidemia, a disorder of lipid metabolism

Lipids are transported to adipose tissues in the form of triglycerides, and it is also in this form that they are delivered to peripheral tissues. At the capillary level, chylomicrons and VLDL gradually attach to the walls where lipoprotein lipase hydrolyzes their triglycerides into fatty acids and β monoacylglycerol. The protein remnants of the chylomicrons return to the bloodstream and are cleared by the liver. Fatty acids and monoglycerides enter adjacent cells muscle or adipose cells by diffusion, driven by the concentration gradient between the two compartments. These compounds are either directly used in beta oxidation or reassembled into triglycerides for storage.

VLDL are converted into LDL in tissues. LDL particles are abundant in the circulation and serve as a source of exogenous cholesterol for tissues. During their circulation in the bloodstream, LDL particles bind to specific receptors located in certain regions of the plasma membrane called coated vesicles. When the number of receptor-ligand complexes is sufficient, the vesicle invaginates and pinches off, forming an endocytic vesicle (receptosome) within the cytoplasm. These receptosomes are transported through tubules to lysosomes, with which they fuse. Lysosomal enzymes then release fatty acids, cholesterol, and protein receptors, which are hydrolyzed into amino acids. Cholesterol is incorporated into the endoplasmic reticulum.

HDL particles continuously circulate and contain an enzyme called phosphatidylcholine cholesterol acyltransferase, which esterifies free cholesterol. They are taken up by hepatocytes and are eventually found in bile salts.

II.1.2. Storage Dietary Lipids

Storage triglycerides serve as an energy source usable by all cells. They are mobilized in the absence of glucose. Prolonged fasting, physical exercise, and stress promote their mobilization. These triglycerides are hydrolyzed by a hormone-sensitive triglyceride lipase (responsive to adrenaline, glucagon, noradrenaline, corticosteroids, pituitary hormones, etc.). Their hydrolysis in adipocytes releases fatty acids and β monoacylglycerol. The latter is further hydrolyzed inside cells by an intracellular lipase that is not hormone-sensitive.

II.2. Lipid Anabolism

This process occurs in the endoplasmic reticulum. Triglycerides are actively synthesized in the liver, adipose cells (adipocytes), and intestinal cells. In higher plants and animals, lipids have two precursors: L-glycerol and acetyl-CoA.

II.2.1. Triglyceride Synthesis

L-glycerol is derived from the reduction of 3-phosphodihydroxyacetone formed during glycolysis. This reaction is catalyzed by 3-phosphoglycerol dehydrogenase. Triglyceride synthesis involves three steps: formation of phosphatidic acid, dephosphorylation of phosphatidic acid into diglyceride, and esterification of the final hydroxyl group of glycerol (Fig. 4).

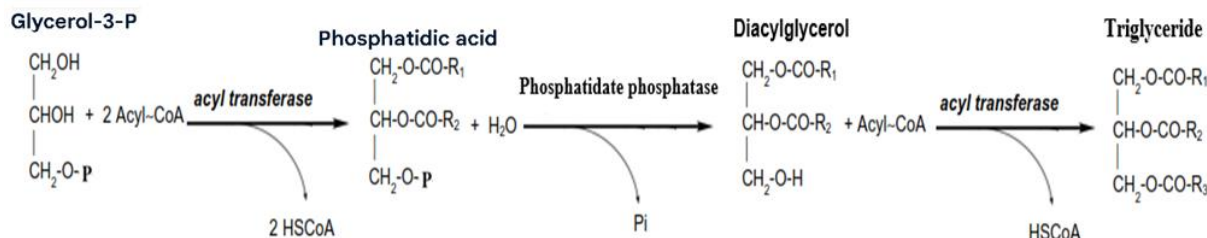


Fig. 4: Triglyceride synthesis

II.2.2. Phospholipid Synthesis

Triglyceride and phospholipid synthesis share the same enzymatic steps up to the formation of diacylglycerol. From that point, specific reactions enable the attachment of an alcohol group (such as choline, ethanolamine, inositol, etc.), which determines the specific type of phospholipid produced (Fig. 5).

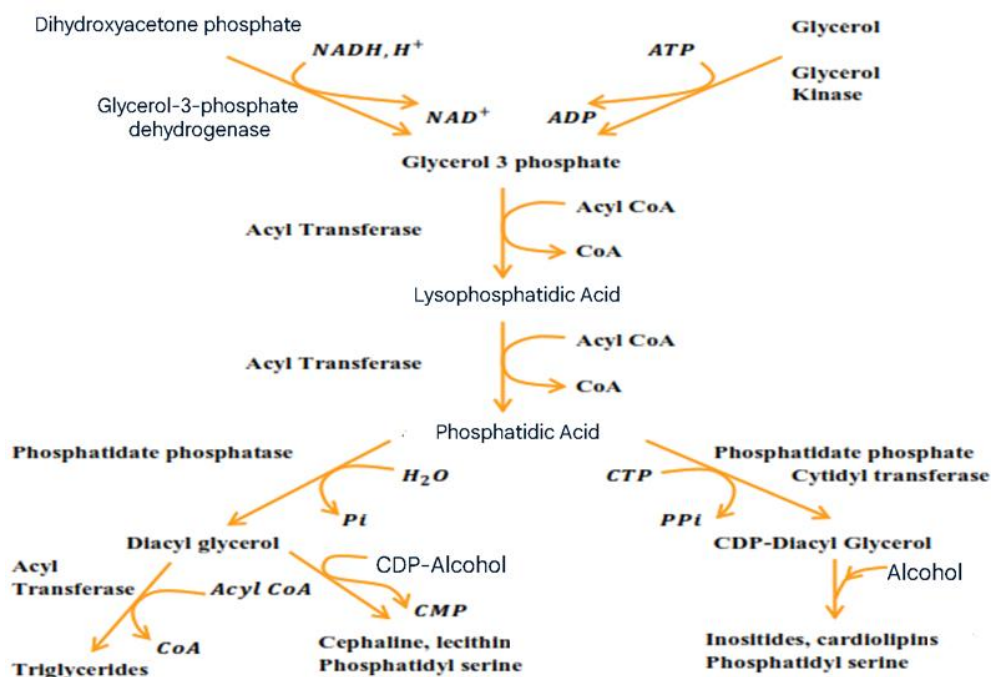


Fig. 5: Phospholipid synthesis

II.2.3. Sphingolipid Synthesis

All sphingolipids are derived from ceramides, which are based on sphingosine, an amino alcohol synthesized from palmitoyl-CoA and a hydroxylated amino acid, serine (Fig. 6).

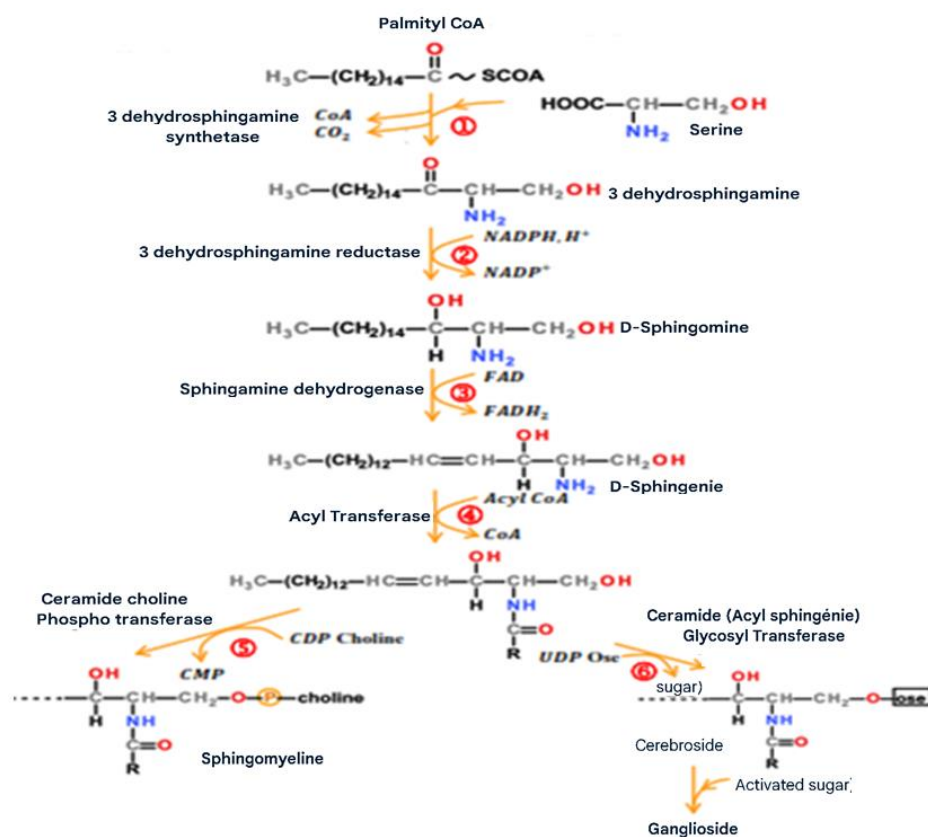


Fig. 6: Sphingolipid synthesis

III. Fatty Acid Metabolism

Fatty acids play a crucial role in important structural processes such as the biosynthesis of phospholipids and glycolipids, which make up cellular membranes, or in the targeting of numerous proteins to membranes through covalent attachment. They also participate in the biosynthesis of hormones and intracellular messengers, and are involved in energy production in the form of ATP.

III.1. Fatty Acid Catabolism

III.1.1. Fatty Acid Activation

Fatty acids can only enter metabolic pathways after being activated into acyl CoA. Cytosolic fatty acids are first converted into acyl CoA through a reaction catalyzed by acyl CoA synthetase (also known as thiokinase), in which ATP is required (Fig. 7).

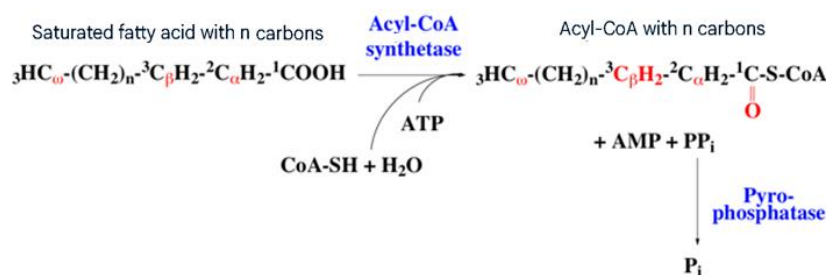


Fig. 7: Activation of cytosolic fatty acids

III.1.2. Transport of Acyl CoA into the Mitochondria

Since the inner mitochondrial membrane is impermeable to acyl CoA, it must be transported into the matrix using a specific carrier: the carnitine shuttle. At the level of the outer mitochondrial membrane, in a reaction catalyzed by carnitine acyltransferase I, the acyl group is transferred from the sulfur atom of CoA to the hydroxyl group of carnitine, a zwitterion, forming acyl carnitine. In this form, fatty acids cross the mitochondrial membranes with the help of a translocase and reach the matrix, where they are reconstituted with CoA in a reaction catalyzed by carnitine acyltransferase II (Fig. 8).

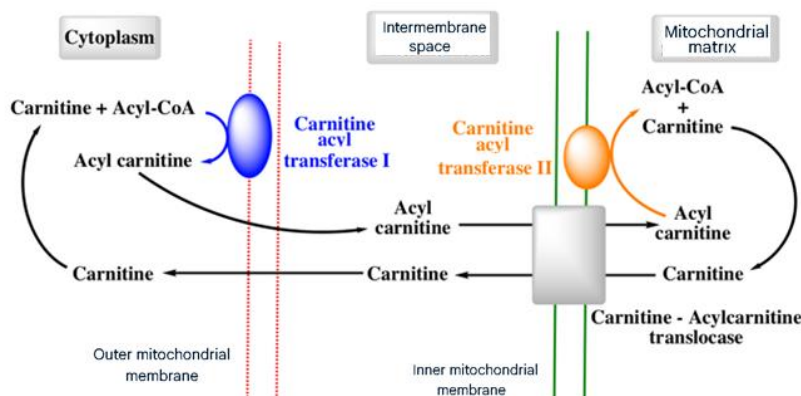


Fig. 8: Transfer of Acyl-CoA into the Mitochondrion

III.1.3. Mitochondrial Oxidation of Fatty Acids (β -Oxidation)

III.1.3.1. Saturated Fatty Acids

The β -oxidation pathway (also known as the Lynen spiral) consists of four recurring reactions that oxidize the β -carbon of acyl CoA and release acetyl CoA. This pathway is cyclic: each set of four reactions oxidation, hydration, a second oxidation, and thiolysis starts from an acyl CoA and results in the formation of a new acyl CoA shortened by two carbon atoms (C_n-2) (Fig. 9).

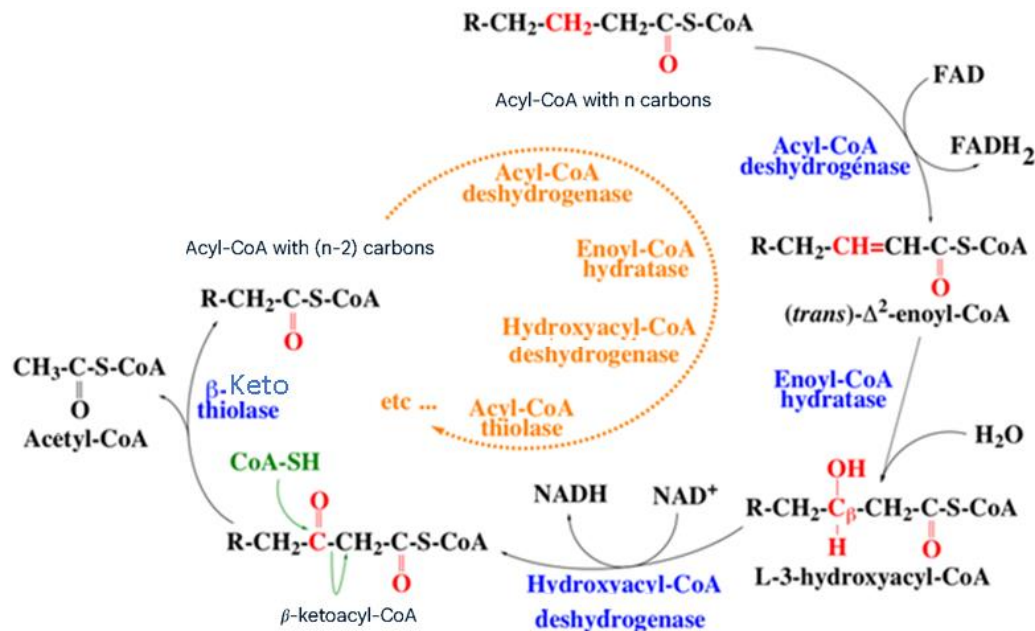
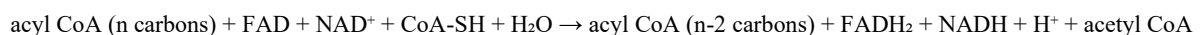


Fig. 9: β -oxidation of saturated fatty acids with an even number of carbon atoms

Subsequent turns of the spiral: The resulting $(n-2)$ carbon acyl CoA becomes the new substrate for another round of the four reactions, and the process continues until the entire fatty acid molecule is converted into acetyl CoA. As the chain shortens, different isoenzymes of acyl CoA deshydrogénases act depending on the chain length.

a) Yield of β -Oxidation of Saturated Fatty Acids

- The metabolic reaction for one turn of the spiral is:



🔗 **Energy yield per turn:** 5 ATP [1 $FADH_2$ (2 ATP) + 1 $NADH$ (3 ATP)]

- The cycle is repeated n times, releasing one acetyl-CoA and one acyl-CoA shortened by two carbon atoms each time, until the carbon chain is fully consumed. At the end of the cycles, the metabolic balance for a fatty acid with n carbon atoms is as follows:

$(\frac{n}{2} - 1)$ rounds of the Lynen spiral

$(\frac{n}{2})$ molecules of acetyl CoA

✚ Energy balance of complete oxidation:

- ❖ **2 ATP consumed** for the initial activation of the fatty acid.
- ❖ **Acetyl CoA yield:** Number of acetyl-CoA released ($\frac{\text{Nb carbons}}{2}$), which enter the Krebs cycle and release 12 ATP per turn ($12 \times \text{number of acetyl-CoA}$).
- ❖ **Number of cycle turns:** ($\frac{\text{Nb carbons}}{2} - 1$), releasing 5 ATP per turn, so the total number is $5 \times (\frac{\text{Nb carbons}}{2})$.

III.1.3.2. Odd-Chain Fatty Acids

Most natural fatty acids contain an even number of carbon atoms. However, some odd-chain fatty acids are produced, for example, by bacteria in the stomachs of ruminants. Their oxidation follows the same steps as even-chain fatty acids, with one key difference: the final round of β -oxidation produces one molecule of acetyl CoA and one molecule of propionyl CoA (which has three carbon atoms). Propionyl CoA is then converted into succinyl CoA (Fig. 10), an intermediate of the Krebs cycle.

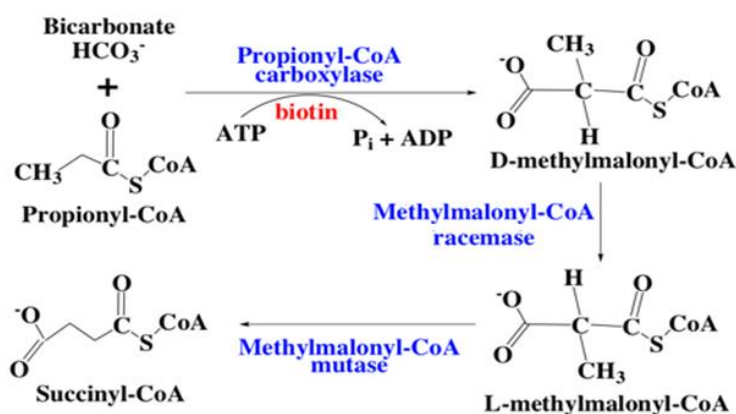


Fig. 10: Conversion of Propionyl-CoA to Succinyl-CoA

III.1.3.3. Unsaturated Fatty Acids

Unsaturated fatty acids are degraded in the same way as saturated fatty acids after their activation and attachment to coenzyme A. However, two enzymes, an isomerase and an epimerase, are necessary for the complete oxidation of these acids.

The action of the isomerase can be illustrated by the degradation of oleic acid (C18: ω 9), which has a cis double bond between carbons 9 and 10. The first three cycles remove 6 carbons in the form of 3 acetyl-CoA molecules. The remaining molecule has a double bond between C3 and C4 in the cis configuration, which prevents the formation of the β -oxidation double bond between C2 and C3. The isomerase converts the cis bond to trans and shifts it between C2 and C3, allowing β -oxidation to continue (Fig. 11).

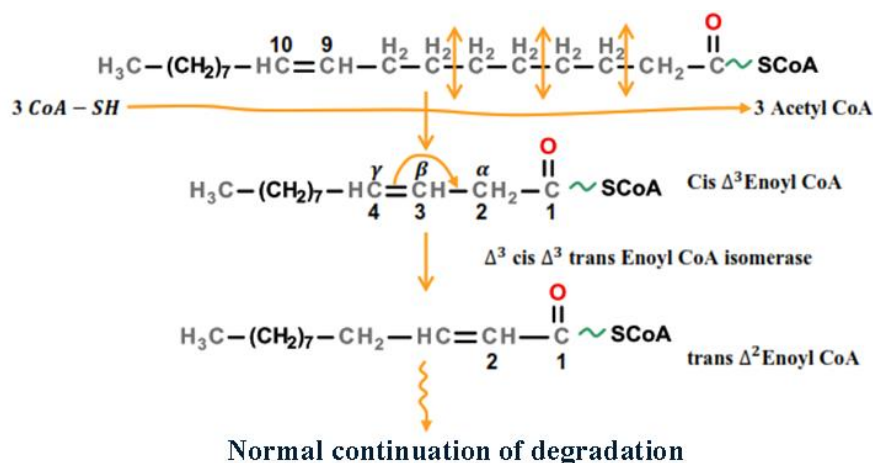


Fig. 11: β -oxidation of Unsaturated Fatty Acids

III.2. Fatty Acid Anabolism

In humans, the majority of fatty acids are exogenous; dietary intake largely meets the needs. However, most tissues (liver, mammary glands, adipose tissue) are capable of synthesizing fatty acids from acetyl-CoA or remodeling fatty acids (exogenous or endogenous) through elongation and/or desaturation. The biosynthesis of endogenous fatty acids involves two different mechanisms:

- Mitochondrial biosynthesis according to Lynen.
- Extramitochondrial biosynthesis according to Wakil.

III.2.1. Mitochondrial Biosynthesis by Lynen

This mechanism is based on the reversibility of the enzymatic reactions of β -oxidation of fatty acids, the only irreversible reaction being the one catalyzed by acyl-CoA dehydrogenase with FAD (the first oxidation reaction). The enzyme that replaces it is an acyl-CoA dehydrogenase reductase with NADPH₂. It is quantitatively minor and participates more in the elongation of pre-existing fatty acids.

III.2.2. Extramitochondrial Biosynthesis by Wakil

This biosynthesis takes place in the cytosol and requires the presence of NADPH₂ and ATP, acetyl-CoA, and a multi-enzyme complex called fatty acid synthase. A preliminary step is essential: the extramitochondrial transfer of acetyl groups. Acetyl-CoA cannot cross the inner mitochondrial membrane, so it is transported via the citrate shuttle (Fig. 12).

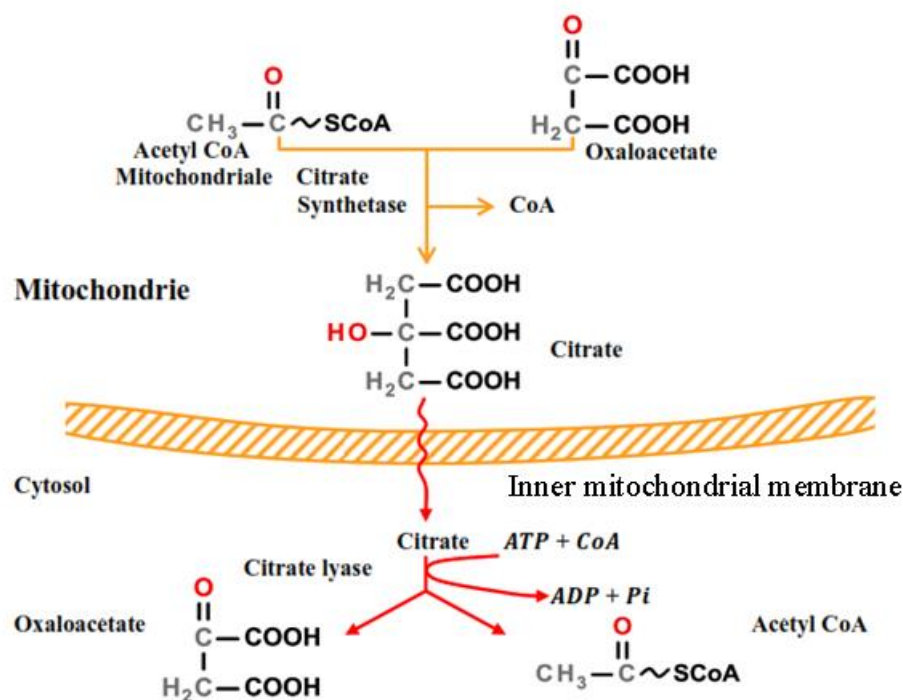


Fig. 12: Extramitochondrial Transfer of Acetyl Groups via the Citrate Shuttle

Fatty acid biosynthesis begins with the carboxylation of an acetyl-CoA to malonyl-CoA, catalyzed by acetyl-CoA carboxylase, an enzyme containing a biotin prosthetic group. The elongation cycle starts with the formation of acetyl-ACP (acyl carrier protein) and malonyl-ACP from acetyl-CoA and malonyl-CoA, catalyzed by acetyl transacylase and malonyl transacylase, respectively. Acetyl-ACP and malonyl-ACP form acetoacetyl-ACP in a reaction catalyzed by the acyl-malonyl-ACP condensation enzyme. A four-carbon unit is formed, and CO_2 is released. The decarboxylation of malonyl-ACP drives the reaction. The next three steps involve the reduction of the keto carbonyl group at C-3 to a methylene group; since this is a biosynthesis process, the reducing agent is NADPH. The elongation cycles continue until the formation of a C16-acyl-ACP. This molecule is then hydrolyzed into palmitate and ACP by a thioesterase, which acts as a regulator of fatty acid chain length (Fig. 13).

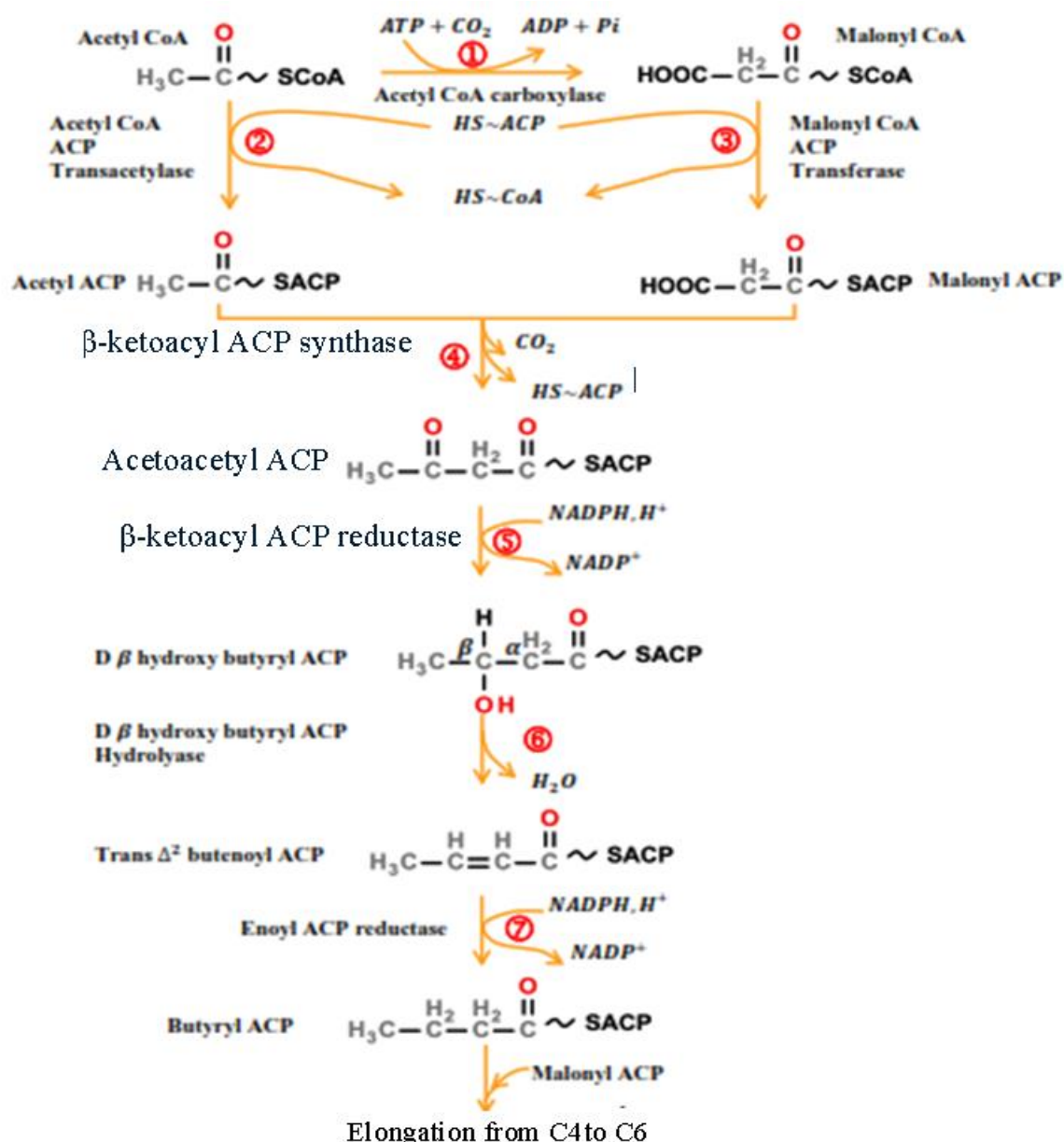
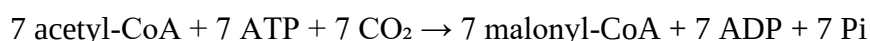


Fig. 13: Fatty acid biosynthesis

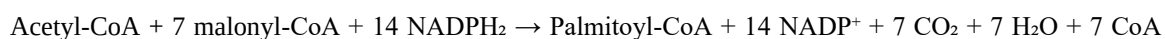
a) General Overview

The entire reaction for the synthesis of palmitic acid from acetyl-CoA can be divided into two parts:

- First, the formation of 7 malonyl-CoA:



- Then, 7 cycles of condensation and reduction:



III.3. Control of Fatty Acid Metabolism

To meet physiological needs, fatty acid metabolism is subject to very precise control, with acetyl-CoA carboxylase playing a key role. Insulin stimulates fatty acid synthesis by activating carboxylase, while glucagon and adrenaline have the opposite effect. Citrate, which signals that the modules necessary for energy production and cellular syntheses are abundant, activates carboxylase, whereas acyl-CoA and AMP inhibit it.

- ❖ Regulation of acetyl-CoA carboxylase occurs through reversible phosphorylation at a serine residue: phosphorylation by AMP-dependent protein kinase (AMPK) inhibits it, while dephosphorylation by a protein phosphatase activates it; AMPK itself is activated by AMP and inhibited by ATP. Thus, carboxylase is activated when the energy charge is low.
- ❖ Insulin stimulates dephosphorylation of carboxylase. Glucagon and adrenaline activate protein kinase A, which in turn inhibits phosphatase by phosphorylating it; thus, these catabolic hormones stop fatty acid synthesis.
- ❖ Citrate allosterically stimulates carboxylase. A high citrate level indicates that dicarbon units and ATP are available for fatty acid biosynthesis.
- ❖ Fatty acid synthesis and degradation are reciprocally regulated so they are not active simultaneously. Thus, during fasting, glucagon and adrenaline activate lipolysis, while insulin inhibits it.

IV. Formation and Utilization of Ketone Bodies

For acetyl-CoA to enter the citric acid cycle, oxaloacetate must be available to form citrate. For this condition to be met, carbohydrate degradation must proceed properly; otherwise, oxaloacetate concentration decreases because it is normally formed from pyruvate, a glycolysis product, by pyruvate carboxylase. "Fats burn in the flame of carbohydrates."

During fasting or diabetes, oxaloacetate is used by gluconeogenesis to form glucose and is therefore less available for condensation with acetyl-CoA. Under these conditions, acetyl-CoA is directed towards the formation, in hepatic mitochondria, of ketone bodies: acetoacetate, D-3-hydroxybutyrate, and acetone (Fig. 14).

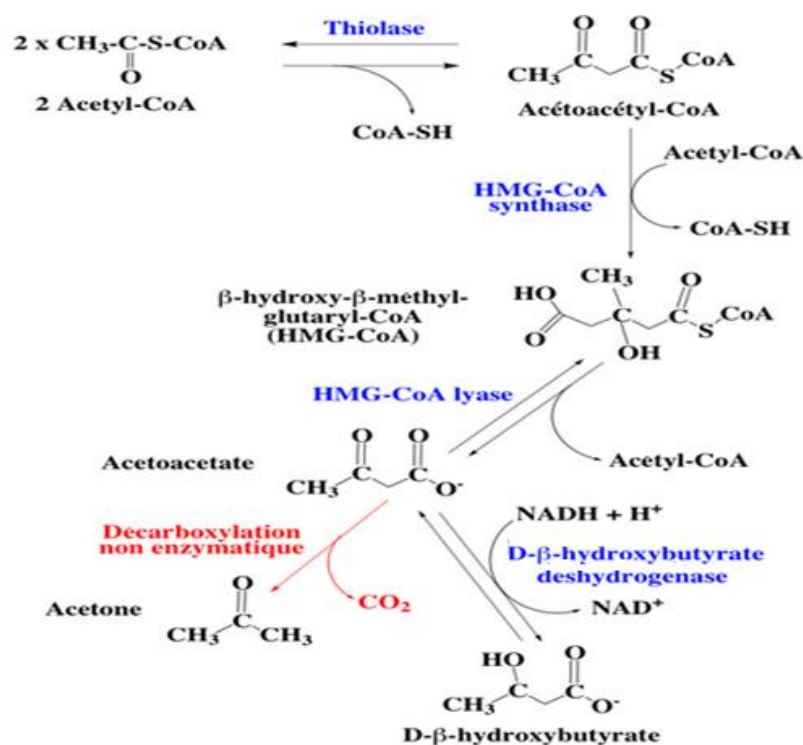


Fig. 14: Formation of Ketone bodies

Ketone bodies are important molecules in energy metabolism. The myocardium and renal cortex use acetoacetate as an energy source, while red blood cells and the brain typically rely on glucose as their primary energy substrate. However, during fasting or in diabetes, the brain can adapt to using acetoacetate as an alternative fuel. In addition, ketone bodies represent a water-soluble, transportable form of acetyl units, and they play a regulatory role in lipolysis. Elevated blood levels of acetoacetate lead to a decrease in lipolysis within adipocytes.

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Proteins Metabolism



I. General Overview

In animals, the source of amino acids is primarily dietary. Unlike carbohydrates and lipids, excess amino acids cannot be stored. They are rapidly degraded through transamination or oxidation to produce an ammonium ion and a carbon skeleton. The ammonium ion is either excreted, used in ureogenesis, or recycled for the synthesis of another amino acid. The carbon skeleton can also be reused to reform the corresponding amino acid or serve as a precursor either for the synthesis of carbohydrates (in the case of glucogenic amino acid skeletons) or for the synthesis of fatty acids (in the case of ketogenic amino acid skeletons).

The metabolism of amino acids in animals serves the following purposes:

- ❖ Maintaining the amino acid pool
- ❖ Ensuring protein turnover
- ❖ Serving as a nitrogen source for the biosynthesis of other nitrogen-containing molecules, such as purine or pyrimidine nucleotide bases.

II. Origin of Amino Acids

In humans, amino acids come from the breakdown of proteins in the digestive tract. When proteins enter the stomach, they stimulate the gastric mucosa to secrete a hormone called **gastrin**. Gastrin triggers the secretion of **hydrochloric acid** by the parietal cells and **pepsinogen** by the chief cells. The acidity of the gastric juice denatures the proteins, making them easier to hydrolyze, and converts pepsinogen into **pepsin**, its enzymatically active form. Pepsin hydrolyzes peptide bonds on the **N-terminal side** of aromatic residues such as **Phe** (phenylalanine), **Trp** (tryptophan), and **Tyr** (tyrosine), producing a mixture of shorter peptides.

When the acidic stomach contents enter the **duodenum**, a hormone called **secretin** is released, stimulating the pancreas to secrete **bicarbonate**, which raises the pH to around 7. The duodenum also releases another hormone, **cholecystokinin**, which stimulates the exocrine cells of the pancreas to secrete several **zymogens**: **trypsinogen**, **chymotrypsinogen**, and **procarboxypeptidases A and B**.

Trypsinogen is activated into **trypsin** by **enteropeptidase**, an enzyme secreted by intestinal cells. Trypsin then activates the other zymogens (including additional trypsinogen), converting them into **trypsin**, **chymotrypsin**, and **carboxypeptidases**, enzymes that specifically hydrolyze certain peptide bonds. The **free** amino acids resulting from this protein hydrolysis are absorbed by the **epithelial cells** lining the **intestine** and then directed to the **liver** (Fig. 1).

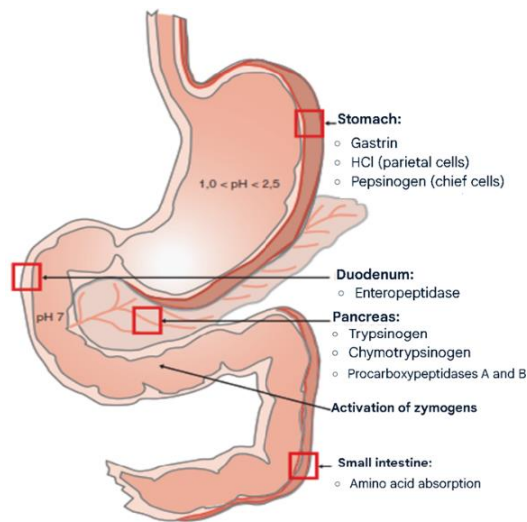


Fig. 1: Pathway of proteins in the digestive tract

III. Amino Acid Metabolism

III.1. Catabolism of Amino Acids

The degradation of amino acids leads to major metabolic intermediates. These intermediates can be fully oxidized into CO_2 and H_2O through the **citric acid cycle**. They may also be converted into **ketone bodies** and **fatty acids**, or into **glucose**. The amino acids that give rise to them are thus classified as **ketogenic** or **glucogenic**, respectively.

All 20 amino acids are ultimately broken down into seven major metabolic intermediates: acetoacetyl-CoA, acetyl-CoA, pyruvate, and four intermediates of the citric acid cycle: α -ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate (Fig. 2).

- **Six amino acids:** Leucine, Isoleucine, Phenylalanine, Tyrosine, Lysine, and Tryptophan are degraded into acetoacetyl-CoA or acetyl-CoA. These can be converted into ketone bodies or fatty acids, and the amino acids that give rise to them are thus considered **ketogenic**.
 - ✚ Only **Leucine** and **Lysine** are *exclusively ketogenic*, the breakdown of Leucine, which is abundant in most proteins, contributes significantly to ketosis during fasting.
 - ✚ Isoleucine, Phenylalanine, Tryptophan, and Tyrosine are both ketogenic and glucogenic, as some of their carbon atoms end up in acetyl-CoA or acetoacetyl-CoA, while others are converted into glucose precursors.
- The other **fourteen amino acids** are broken down into pyruvate, α -ketoglutarate, succinyl-CoA, fumarate, or oxaloacetate. These compounds can be converted into glucose via phosphoenolpyruvate, so these amino acids are considered **glucogenic**. However, those that

produce pyruvate are also ketogenic, since pyruvate can be further metabolized into acetyl-CoA.

In humans, **amino acid catabolism pathways** normally account for only **10 to 15%** of the total **energy production** required by the body. Therefore, they are **much less active** than glycolysis or fatty acid oxidation.

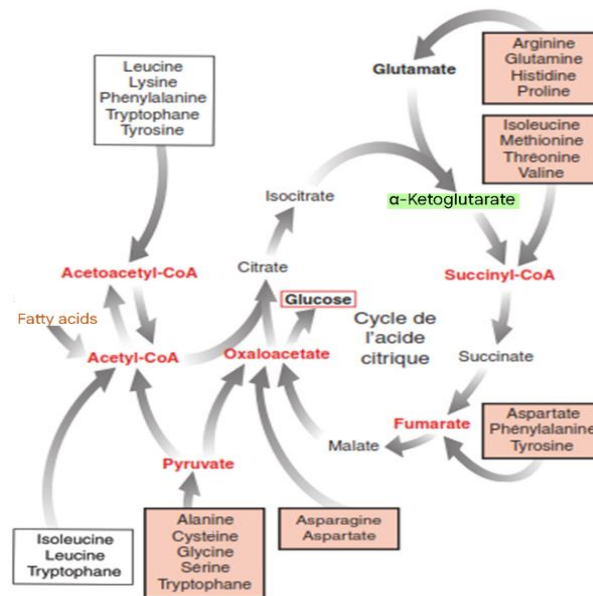


Fig. 2: Metabolic intermediates of amino acids

III.1.1. Amino Acid Degradation Reactions

III.1.1.1. Transamination

Transamination is a fundamental reaction in amino acid metabolism, as it plays a role in both their catabolism and biosynthesis. It involves the **transfer of an α -amino group** from an amino acid to an α -keto acid. The enzymes that catalyze these reactions are known as aminotransferases (or transaminases).

Major aminotransferases are present in all tissues, and the reactions they catalyze are reversible. The cofactor involved is pyridoxal phosphate, which is the prosthetic group of all aminotransferases and is derived from vitamin B6. In transamination reactions oriented toward amino acid degradation, the universal acceptor of the α -amino group is α -ketoglutarate, leading to the formation of glutamate.

Different aminotransferases exhibit specificity for particular amino acids. For example:

- **Aspartate aminotransferase (ASAT)**, also known as glutamate-oxaloacetate transaminase (GOT), catalyzes the transfer of the amino group from aspartate to α -ketoglutarate, forming glutamate and oxaloacetate (Fig. 3).

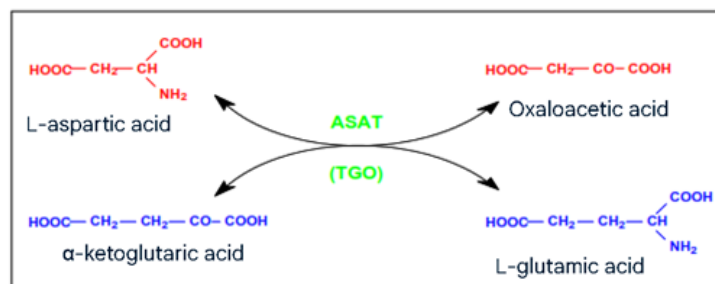


Fig. 3: Aspartate Aminotransferase

- **Alanine aminotransferase (ALAT)**, also known as glutamate-pyruvate transaminase (GPT), transfers the amino group from alanine to α-ketoglutarate (Fig. 4).

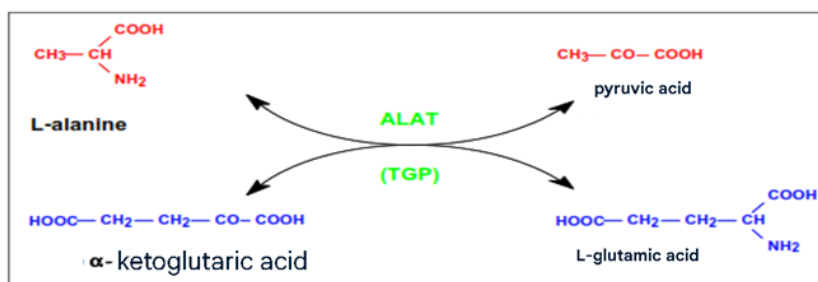


Fig. 4: Alanine Aminotransferase

III.1.1.2. Oxidative Deamination

In contrast to transamination, which involves the transfer of the α-amino group oxidative deamination releases the α-amino group as free ammonia (NH_3), while forming the corresponding α-keto acid skeleton. This process is highly active in the liver and kidneys. Two main enzymes are involved in this reaction: Glutamate dehydrogenase and Amino acid oxidases.

a) Glutamate Dehydrogenase

Glutamate dehydrogenase is a mitochondrial enzyme that deaminates L-glutamate, produced through transamination reactions into α-ketoglutaric acid, while releasing the α-amino group as ammonium ion (NH_4^+). This enzyme is allosterically regulated, being inhibited by ATP and GTP, and activated by ADP and GDP (Fig. 5).

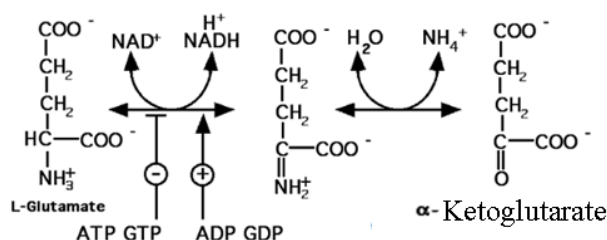


Fig. 5: Glutamate Dehydrogenase

b) Amino Acid Oxidases

There are two **flavoproteins** involved in the direct oxidation of amino acids: one contains FMN and the other FAD.

- The enzyme **L-amino acid oxidase**, which is found in the **liver**, uses FMN as a prosthetic group. It oxidizes amino acids by directly transferring the electrons captured by the coenzyme to molecular oxygen. This reaction results in the production of the corresponding α -keto acid, the release of ammonia (NH_3), and the formation of hydrogen peroxide (H_2O_2), which is subsequently broken down by catalases (Fig. 6).

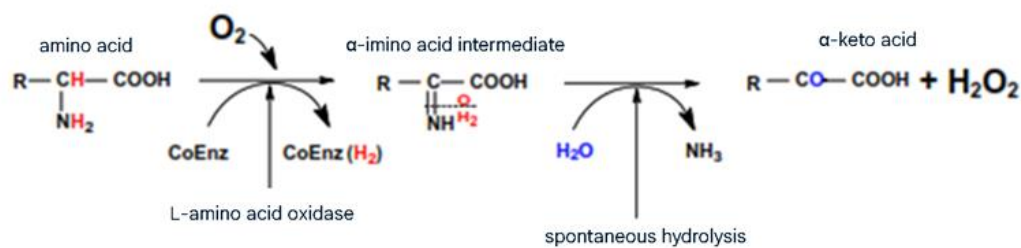


Fig. 6: Amino acid oxidases

- D-amino acid oxidase** is an enzyme found in high activity in the **liver and kidneys**. It specifically oxidizes D-amino acids, which originate from the hydrolysis of plant proteins and the cell membranes of microorganisms. Since these D-amino acids are not utilized by the animal body, they are degraded through a similar mechanism as previously described, but by D-amino acid oxidase, which uses FAD as its prosthetic group.

c) Other Deamination Mechanisms (Cytosolic)

Some amino acids can undergo direct deamination without the need for transamination or oxidative steps. This is the case for Serine, Threonine, and Histidine. The enzyme responsible in these reactions is a dehydratase.

- **Deamination by desaturation:** This mechanism applies to **histidine**, which undergoes deamination through a desaturation reaction, leading to the formation of urocanate (Fig.7).

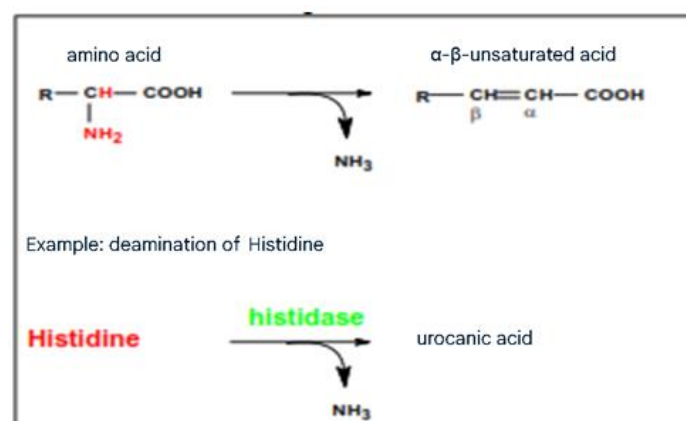


Fig. 7: Deamination of Histidine

- **Deamination via dehydration:** Serine and threonine are deaminated through dehydration reactions, catalyzed by specific dehydratases, producing the corresponding α -keto acids and ammonia (Fig. 8).

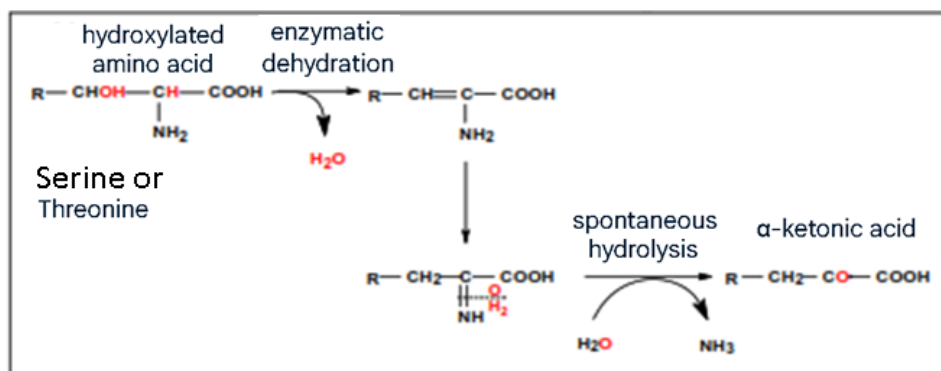


Fig. 8: Deamination of Serine and Threonine

III.1.1.3. Decarboxylation of Amino Acids

The decarboxylation reaction occurs in living organisms to produce various bioactive derivatives from amino acids. This reaction is catalyzed by enzymes known as decarboxylases (Fig. 9).

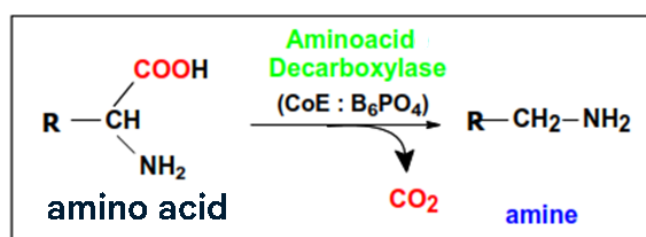
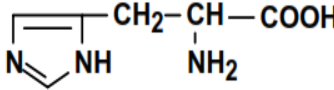
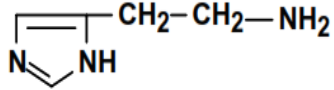
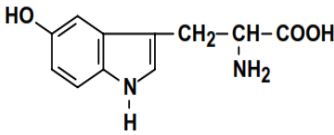
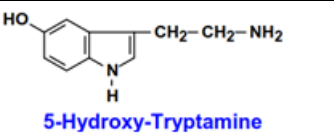
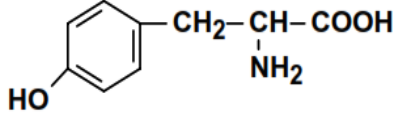
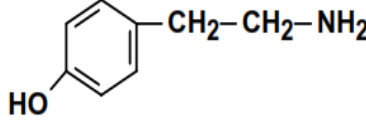


Fig. 9: Decarboxylation of amino acids

Some of the amines produced through decarboxylation exhibit physiological or pharmacodynamic activity. Many of these amines also play important roles in biochemical synthesis (Table I). An example is ethanolamine, which results from the decarboxylation of serine.

Table I: Selected products of amino acid decarboxylation

Amino Acid	Corresponding Amine	Function
 L-Histidine	 Histamine	Neuromediators Immune mediator
$\text{HOOC}-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COOH}$ L-Glutamic Acid	$\text{HOOC}-\overset{\alpha}{\text{CH}_2}-\overset{\beta}{\text{CH}_2}-\overset{\gamma}{\text{CH}_2}-\text{NH}_2$ γ-Aminobutyric acid (GABA)	Neuromediators
 5-Hydroxy-Tryptophane	 5-Hydroxy-Tryptamine (serotonin)	Neuromediators
 L-Tyrosine	 Tyramine	Causes release of histamine stored in mast cells.
$\text{HO}-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COOH}$ L-Serine	$\text{HO}-\text{CH}_2-\text{CH}_2-\text{NH}_2$ Ethanolamine	Component of phospholipids
$\text{HOOC}-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COOH}$ L-Aspartic Acid	$\text{HOOC}-\overset{\alpha}{\text{CH}_2}-\overset{\beta}{\text{CH}_2}-\text{NH}_2$ β-alanine	Component of coenzyme A
$\text{HS}-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COOH}$ L-Cysteine	$\text{HS}-\text{CH}_2-\text{CH}_2-\text{NH}_2$ Cysteinamine	Component of coenzyme A

III.1.2. Entry Points into Metabolic Flux

III.1.2.1. Pyruvate

Pyruvate serves as the entry point into metabolic flux for the three-carbon amino acids: alanine, serine, and cysteine (Fig. 10).

- ✚ **Alanine** is directly transaminated into pyruvate by Alanine aminotransferase.
- ✚ **Serine** is deaminated into pyruvate by Serine dehydratase.
- ✚ **Cysteine** can be converted into pyruvate through multiple pathways, with its sulfur atom ending up as H_2S , SCN^- , or SO_3^{2-} .

Glycine, which can be cleaved into CO_2 , NH_4^+ , and an active one-carbon unit, may also enter the Serine metabolism pathway after the addition of a hydroxymethyl group. Additionally, two carbon atoms from Tryptophan are converted into Alanine (Fig. 10).

The **pyruvate** formed from these amino acids can act as either a glucogenic or ketogenic precursor, depending on whether it is converted into oxaloacetate or acetyl-CoA, respectively.

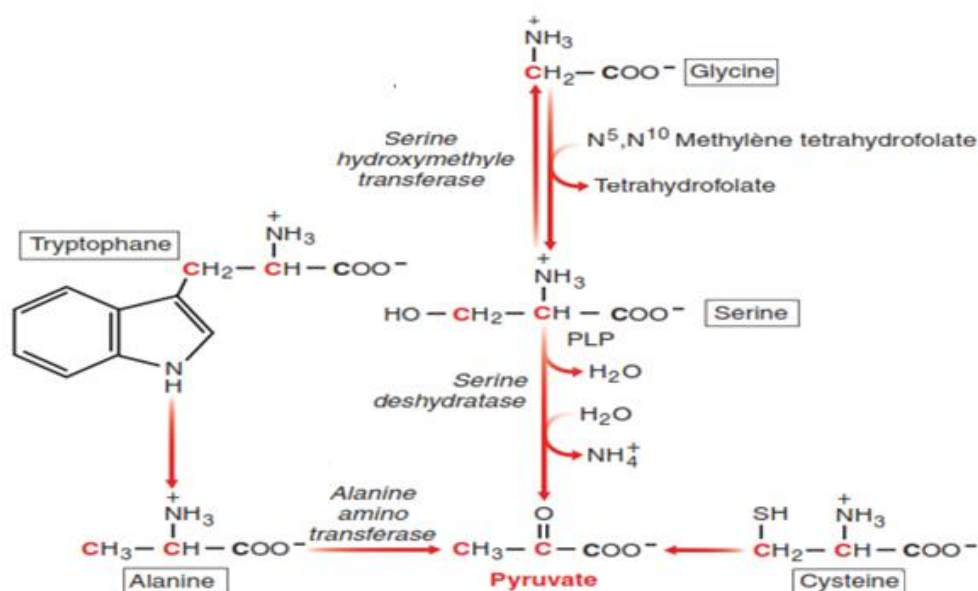


Fig. 10: Pyruvate as an Entry Point into Metabolic Flux

III.1.2.2. Oxaloacetate and α -Ketoglutarate

Aspartate and **Glutamate** undergo reversible transamination to form oxaloacetate and α -ketoglutarate, respectively. The catabolism of these amino acids therefore proceeds via the entry of their corresponding α -keto acid carbon skeletons into the metabolic flux (Fig. 11). Since oxaloacetate and α -ketoglutarate are intermediates of the citric acid cycle, both Aspartate and Glutamate are considered glucogenic. The degradation of Asparagine and Glutamine begins with their hydrolysis into Aspartate and Glutamate, respectively, catalyzed by asparaginase and glutaminase. Therefore, Asparagine and Glutamine are also glucogenic.

The carbon skeletons of five-carbon amino acids such as Proline, Arginine, and Histidine are ultimately converted into Glutamate (Fig. 11). Before being converted into glutamate:

- **Histidine** is first transformed into 4-imidazole-5-propionate,
- While **Proline** and **Arginine** are converted into glutamate γ -semialdehyde.

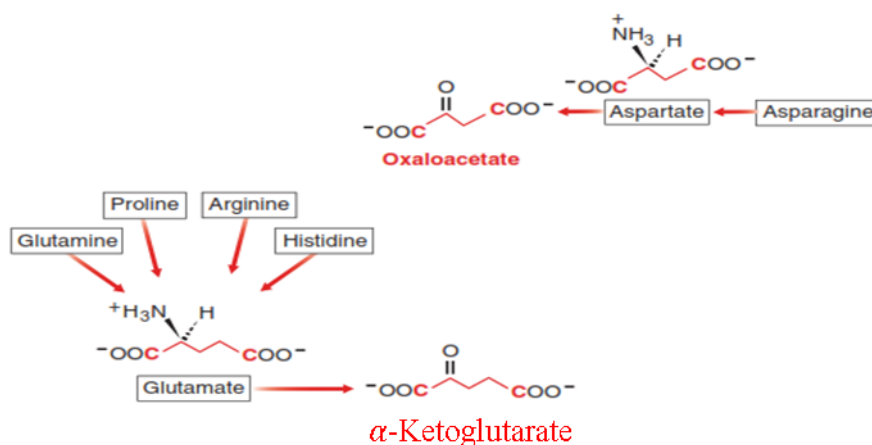


Fig. 11: Oxaloacetate and α -Ketoglutarate as entry points into metabolic flux

III.1.2.3. Succinyl-CoA

Following the degradation of the amino acids Methionine, Isoleucine, Valine, and Threonine, their carbon skeletons are funneled into the citric acid cycle via the intermediate succinyl-CoA. These four amino acids are first transaminated, then decarboxylated, and eventually converted into propionyl-CoA, which is the direct precursor of succinyl-CoA (Fig. 12).

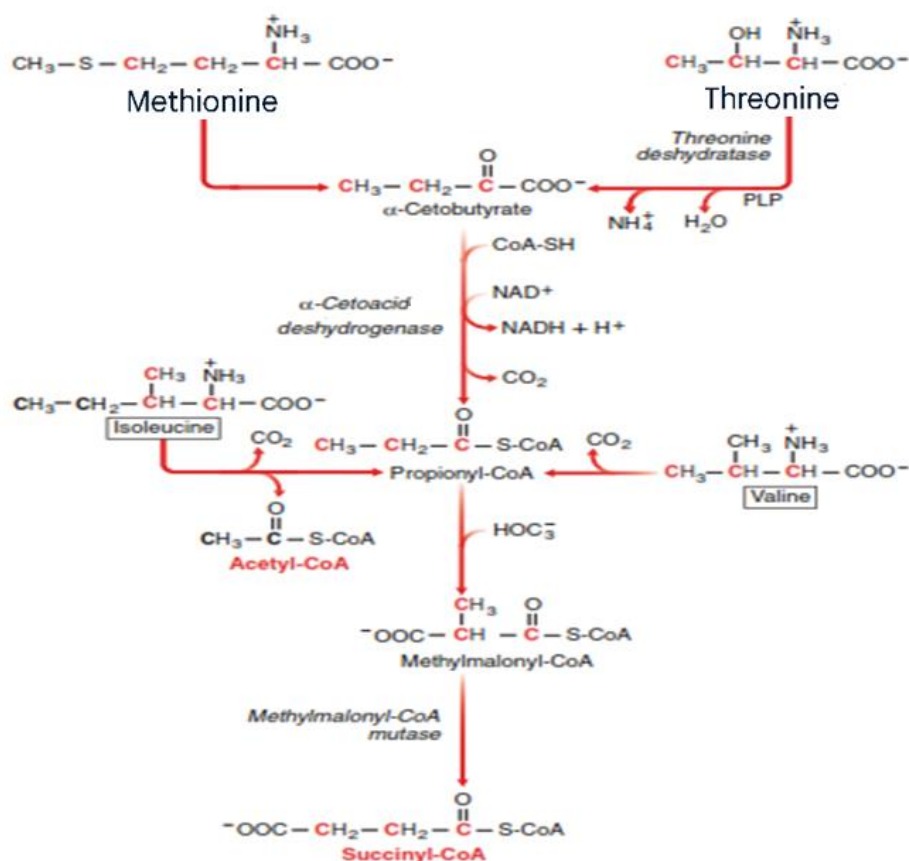


Fig. 12: Succinyl-CoA as an entry point into metabolic flux

III.1.2.4. Fumarate

Aspartate can also be metabolized into **fumarate** through the **urea cycle**. In addition, fumarate serves as the entry point for half of the carbon atoms from the degradation of phenylalanine and Tyrosine.

III.1.3. Degradation of Aromatic Amino Acids

The catabolism of the three aromatic amino acids phenylalanine, tyrosine, and tryptophan follows similar mechanisms: initial oxidation, nitrogen removal via transamination or hydrolysis and finally, opening of the aromatic ring coupled with oxidation.

Phenylalanine metabolism begins with its hydroxylation into tyrosine. This complex reaction involves molecular oxygen (O_2) and a reductant, tetrahydrobiopterin, and is catalyzed by

a monooxygenase, phenylalanine hydroxylase (Fig. 13-A). This step not only contributes to tyrosine biosynthesis but also marks the first step in phenylalanine catabolism.

Next, **Tyrosine** undergoes transamination to form p-hydroxyphenylpyruvate, a keto acid that then reacts with O₂ in a reaction catalyzed by the dioxygenase p-hydroxyphenylpyruvate hydroxylase, yielding homogentisate.

The **aromatic ring** of homogentisate is subsequently cleaved by a second dioxygenase, homogentisate oxidase, producing 4-maleylacetoacetate, which is isomerized into 4-fumarylacetoacetate. This compound is hydrolyzed into fumarate and acetoacetate.

The degradation of tryptophan also involves several oxygenases. Tryptophan 2,3-dioxygenase cleaves the pyrrole ring, while kynurenine 3-monooxygenase hydroxylates the remaining benzene ring. Alanine is then eliminated, and a third dioxygenase cleaves the remaining 3-hydroxyanthranilate, leading to the formation of acetoacetyl-CoA (Fig. 13-B).

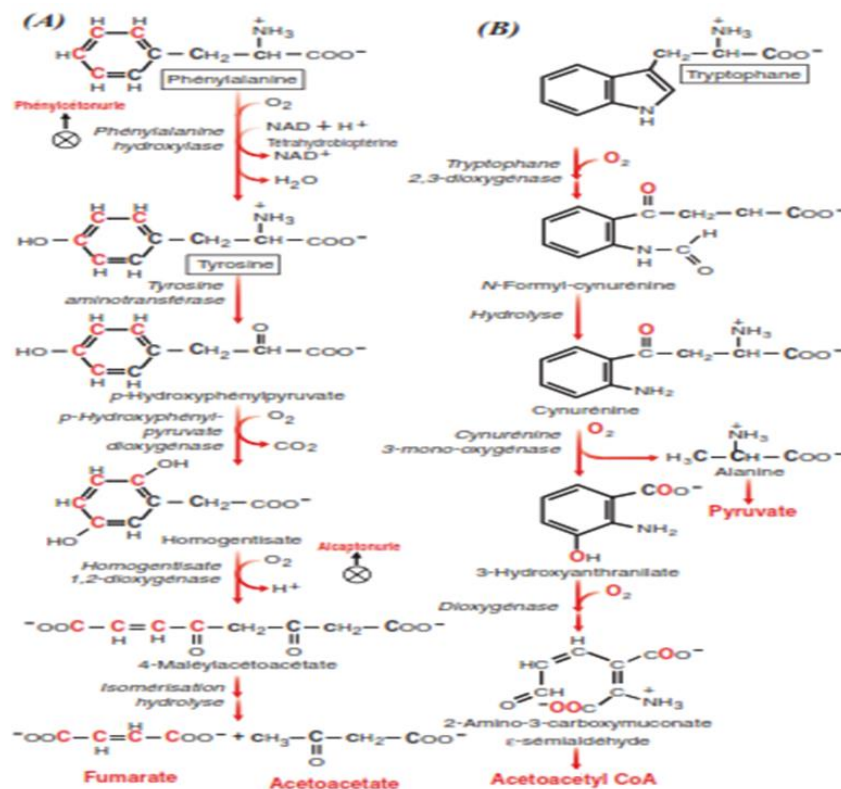


Fig. 13: Degradation of Aromatic Amino Acids

III.2. Anabolism of Amino Acids

Amino acids are molecules characterized by the presence of an amino group in the α -position relative to a carboxyl group. Each amino acid is distinguished from the others by the structure of its side chain (radical). There are two groups of amino acids depending on whether or not they can be synthesized by the human body.

Eight amino acids (Leucine, Isoleucine, Valine, Threonine, Phenylalanine, Tryptophan, Lysine, Methionine) cannot be synthesized by the body and are therefore called **essential** or **indispensable**. There is no general process for the synthesis of these amino acids. Among the essential AAs, two sub-groups can be distinguished: those whose synthesis is completely impossible (Lysine and Threonine), and those for which only the carbon skeleton cannot be synthesized. For the latter, the reversibility of transamination processes allows their synthesis, provided that the corresponding α -keto acids are supplied. There are situations in which an external (exogenous) supply becomes necessary because endogenous synthesis is insufficient to meet the body's needs; such as in the case of **Arginine** in growing **children** or in patients under stress. These amino acids are sometimes referred to as semi-essential or conditionally essential.

Cells have adopted a strategy based on mechanisms common to several amino acids. In many cases, pathways branch off from a common intermediate. Additionally, amino acid precursors are metabolites derived from key metabolic pathways such as glycolysis, the Krebs cycle (e.g., acetyl-CoA, α -ketoglutarate, succinyl-CoA, fumarate, oxaloacetate), or the pentose phosphate pathway.

The precursors of amino acids are α -keto acids, which can be directly used in transamination reactions or serve to synthesize amino acids. These precursors are generated in degradation processes primarily glycolysis and the tricarboxylic acid cycle. Carbohydrates are the main carbon donors found in amino acids. A given carbon skeleton can give rise to several amino acids, forming what is known as a family (Fig. 14):

- ✚ **α -Ketoglutarate** gives rise to the Glutamate family: Glutamate, Glutamine, Proline, Arginine, and Lysine.
- ✚ **Oxaloacetate** gives the Aspartate family: Aspartate, Asparagine, Methionine, Threonine, and Isoleucine.
- ✚ **3-phosphoglycerate** leads to the Serine family: Serine, Glycine, and Cysteine.
- ✚ **Pyruvate** gives rise to the Alanine family: Alanine, Valine, and Leucine.
- ✚ **Phosphoenolpyruvate** and **erythrose-4-phosphate** are the starting points for Phenylalanine, Tyrosine, and Tryptophan.
- ✚ **Ribose-5-phosphate** is the precursor for Histidine.

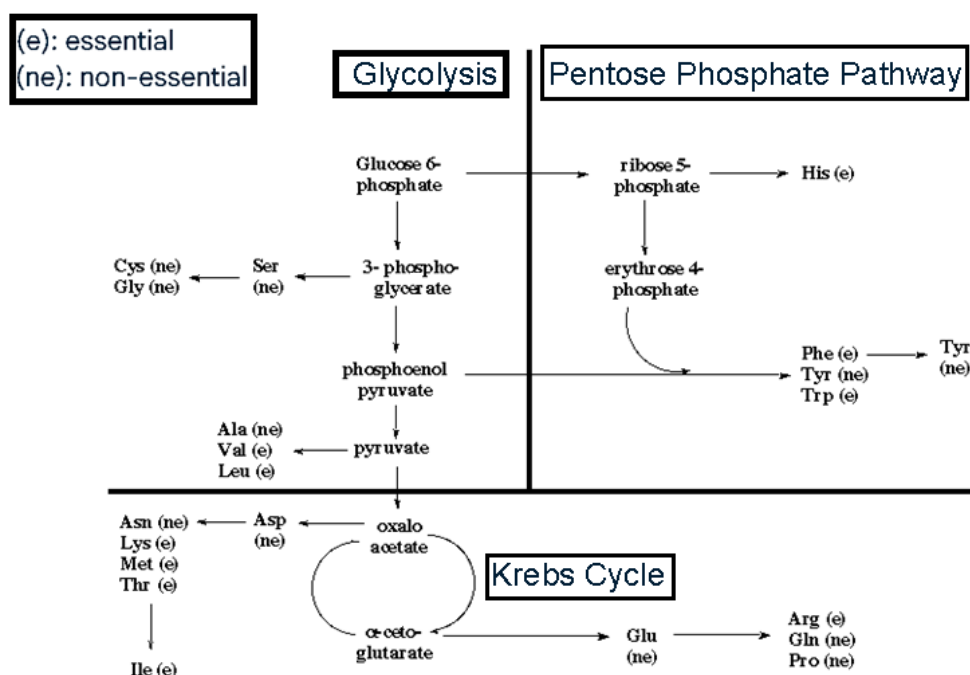


Fig. 14: Origins of amino acid synthesis

III.2.1. Synthesis of Amino Acids

III.2.1.1. Glutamate Family

a)- Glutamate and Glutamine

The first step in amino acid synthesis is the assimilation of NH_4^+ . One of the main entry points used by the cell to incorporate ammonia into amino acid metabolism is the reductive amination of α -ketoglutarate (which is derived from isocitrate in the Krebs cycle) into glutamate, catalyzed by glutamate dehydrogenase (Fig. 15).

The formation of Glutamine from Glutamate is catalyzed by glutamine synthetase (Fig. 15). In this reaction, the carboxyl group of glutamate is first phosphorylated to form γ -glutamyl phosphate, a highly reactive intermediate. This phosphate group is then attacked by a strong nucleophile such as the nitrogen atom from ammonia, leading to the formation of Glutamine. Glutamine, which is the most abundant amino acid in the blood (with concentrations ranging from 450 to 600 μM), is the main carrier of ammonium ions in the circulatory system. As such, glutamine plays a crucial role in reducing the level of free ammonium, a highly toxic compound.

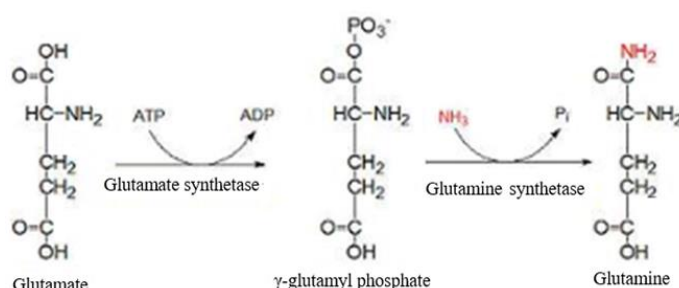
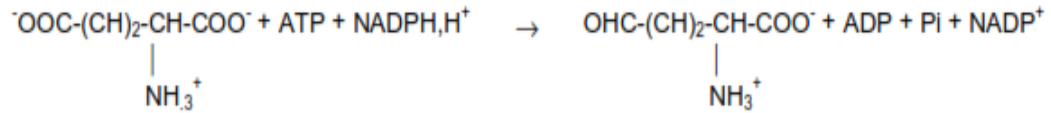


Fig. 15: Glutamine Synthesis

b)- Proline

Proline is synthesized from glutamate in two main steps:

- The carboxylic group on carbon 5 of glutamate is first reduced to an aldehyde. This step requires energy in the form of ATP and is catalyzed by glutamyl phosphate dehydrogenase.



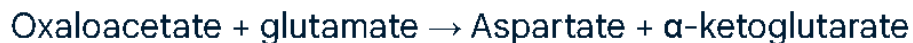
- The resulting glutamate semi-aldehyde then undergoes cyclization followed by a reduction to form Proline.

c)- Arginine

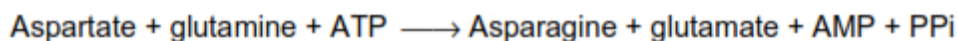
Arginine is an essential amino acid; however, it can be synthesized during hepatic ureagenesis.

III.2.1.2. Aspartate Family**a)- Aspartate**

Aspartate is formed by transamination of oxaloacetate. The α -amino group is donated by **Glutamate**. The reaction is catalyzed by aspartate aminotransferase.

**b)- Asparagine**

Asparagine synthesis is catalyzed by asparagine synthetase.

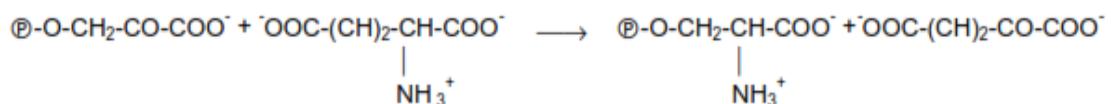
**III.2.1.3. Serine Family****a)- Serine**

Serine is synthesized from 3-phosphoglycerate via the following sequence:

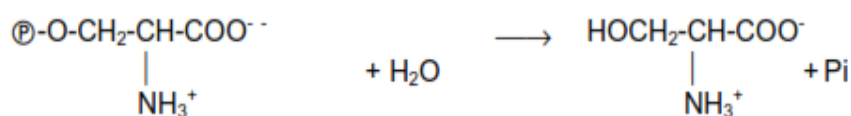
- A dehydrogenation step converts 3-phosphoglycerate to 3-phosphohydroxypyruvate, catalyzed by 3-phosphoglycerate dehydrogenase.



- This is followed by a transamination in the presence of Glutamate, catalyzed by phosphoserine transaminase.

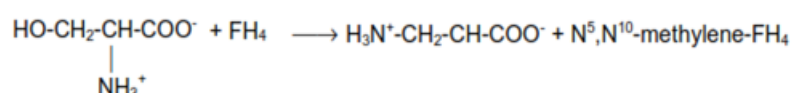


- Finally, under the action of a phosphatase, the phosphate group is hydrolyzed, yielding serine.



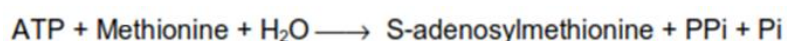
b)- Glycine

Glycine (also called glycol) is synthesized from **Serine** by the removal of the $\text{-CH}_2\text{OH}$ side chain. The reaction is catalyzed by serine hydroxymethyltransferase, using tetrahydrofolate as a coenzyme.



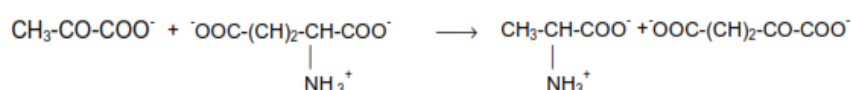
c)- Cysteine

Cysteine is synthesized from **Methionine** through a complex sequence of reactions involving the formation of S-adenosylmethionine. This is the only known case in which ATP loses all three of its phosphate groups in a single reaction.



III.2.1.4. Alanine Family

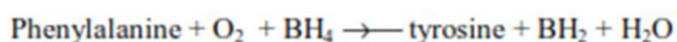
In this family, **Alanine** is the only **non-essential** amino acid. It is produced by transamination of pyruvate in the presence of Glutamate. The reaction is catalyzed by glutamate-pyruvate aminotransferase.



II.2.1.5. Aromatic Amino Acid Family

The aromatic amino acids Phenylalanine, Tyrosine, and Tryptophan are biosynthesized via complex pathways that only microorganisms and plants are capable of performing. In humans, these are essential amino acids that must be obtained from the diet.

Tyrosine is a non-essential amino acid that is unique in that it is derived from phenylalanine (an essential amino acid). It is considered a degradation product of phenylalanine. Tyrosine, also known as para-hydroxyphenylalanine, is formed through a reaction catalyzed by Phenylalanine hydroxylase. The reducing power is provided by tetrahydrobiopterin (BH_4), and the oxygen comes from the air.



III.2.2. Regulation of Ammoniogenesis

Ammoniogenesis is primarily regulated by the products of the reactions glutamate and ammonia which inhibit glutaminases and glutamate dehydrogenase, respectively.

IV. Fate of Ammonia

The complete oxidation of amino acids derived from proteins leads to the near equimolar production of NH_4^+ and HCO_3^- ions. In humans, the main pathway for nitrogen detoxification is ureagenesis, or urea synthesis a process that produces a non-toxic molecule synthesized by the liver and excreted in the urine.

However, it is important that not all ammonia be converted into urea, because ammonia plays several physiological roles, including a key function in acid-base balance at the level of the kidneys.

The transport of excess nitrogen to the liver is mainly ensured by Glutamine and Alanine. Certain aspects of these metabolic pathways, in particular the intestine/liver cooperation during the postprandial period, are especially important.

IV.1. Ureagenesis

Ureagenesis occurs in the liver. The urea cycle consists of five reactions, four of which constitute the actual cycle, culminating in the regeneration of ornithine and the release of a urea molecule. The cycle is partially mitochondrial and partially cytoplasmic.

IV.1.1. Mitochondrial Phases

a)- Synthesis of Carbamoyl Phosphate

In the mitochondria, carbamoyl phosphate synthetase uses CO_2 , NH_3 , and 2 ATP molecules as substrates to form carbamoyl phosphate. This reaction consumes two high-energy phosphate bonds (Fig. 16-A).

b)- Synthesis of Citrulline

Once carbamoyl phosphate is formed, it reacts with ornithine, which has been transported from the cytosol. Under the action of ornithine carbamoyltransferase (also called transcarbamylase), the carbamoyl group is transferred to ornithine, forming citrulline (Fig. 16-B).

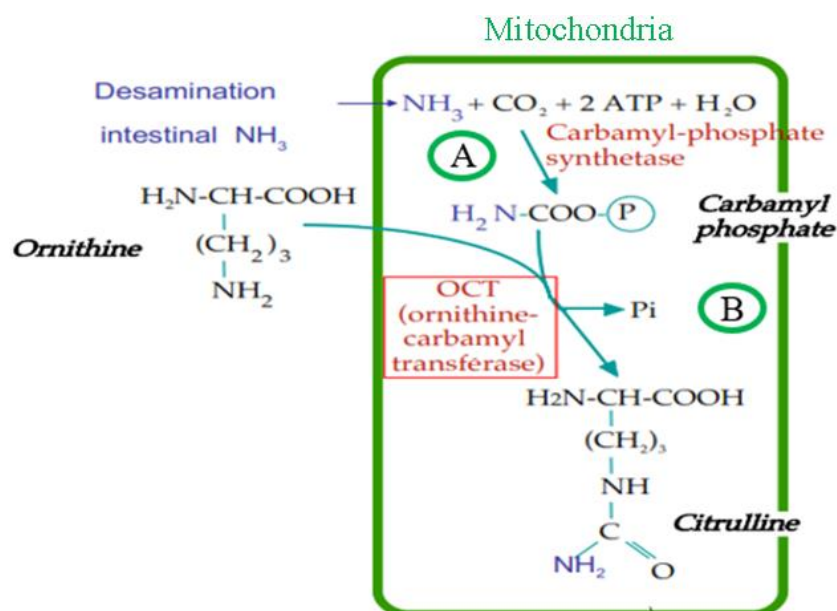


Fig. 16: Mitochondrial Phases of the Urea Cycle

IV.1.2. Cytosolic Phase

a)- Formation of Argininosuccinate

The citrulline formed in the mitochondria is transported into the cytosol. Under the action of argininosuccinate synthetase, citrulline condenses with Aspartate (which donates the second amino group) to form argininosuccinate, consuming two high-energy phosphate bonds from one molecule of ATP (Fig. 17-A).

b)- Formation of Arginine

This reaction is catalyzed by argininosuccinate lyase, which cleaves argininosuccinate into L-arginine and fumarate. This step is also part of the biosynthesis of arginine (Fig. 17-B). Fumarate is transported back into the mitochondria, where it is taken up by the Krebs cycle and oxidized into oxaloacetate. Oxaloacetate is then transaminated into Aspartate-by-aspartate aminotransferase, thereby creating a link between the Krebs cycle and the urea cycle.

c)- Hydrolysis of Arginine

The hydrolysis of arginine concludes the cycle. This reaction produces urea and ornithine, and is catalyzed by arginase. While urea is excreted via the urine, ornithine is transported back into the mitochondria to restart the cycle (Fig. 17-C).

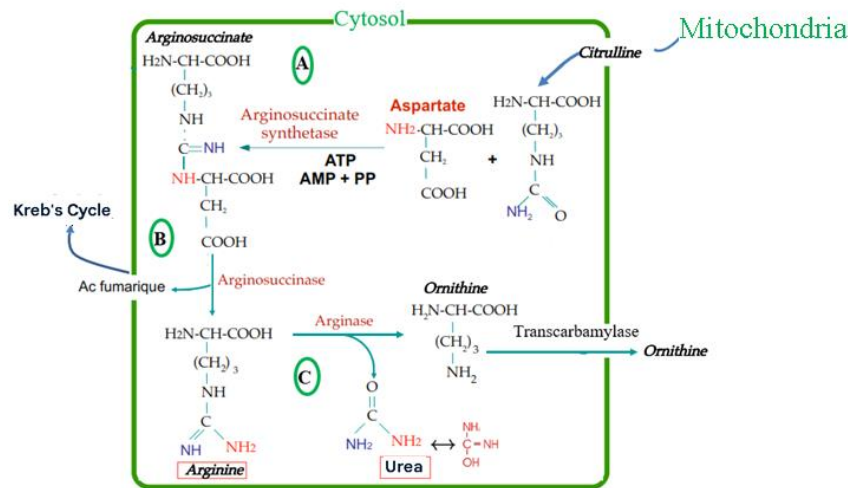
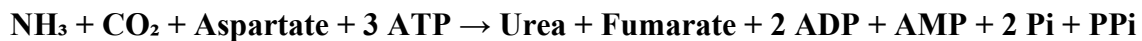


Fig. 17: Cytosolic phases of the Urea cycle

IV.2. Summary of the Urea Cycle

The overall equation for the urea cycle is:



During the formation of one molecule of urea, four high-energy phosphate bonds are consumed:

- ❖ $2 \text{ ATP} \rightarrow 2 \text{ ADP} + 2 \text{ Pi}$ during carbamoyl phosphate synthesis, and
- ❖ $1 \text{ ATP} \rightarrow \text{AMP} + \text{PPi}$ during argininosuccinate synthesis.

When fumarate is converted into oxaloacetate (via the Krebs cycle) and used to regenerate aspartate through transamination, one $\text{NADH} + \text{H}^+$ is produced, which corresponds to the generation of approximately 3 ATP. Conclusion, the elimination of one free ammonium ion and the amino group from aspartate as a molecule of urea results in the net consumption of only one high-energy phosphate bond.

IV.3. Regulation of Ureogenesis

Hormonal regulation of ureogenesis can occur **indirectly**, by modulating substrate availability. For example:

- ✚ **Cortisol** increases proteolysis and the muscle efflux of amino acids, thereby enhancing ureogenesis.
- ✚ **Glucagon** promotes hepatic uptake of amino acids, also stimulating ureogenesis.
- ✚ **Insulin**, on the other hand, reduces ureogenesis by directing amino acids toward protein synthesis.

Regulation can also occur **directly**, by influencing the **synthesis of enzymes** involved in the urea cycle.

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