BP203T BIOCHEMISTRY-THEORY

UNIT-ONE

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UNIT 1: Biomolecules- 08 Hours

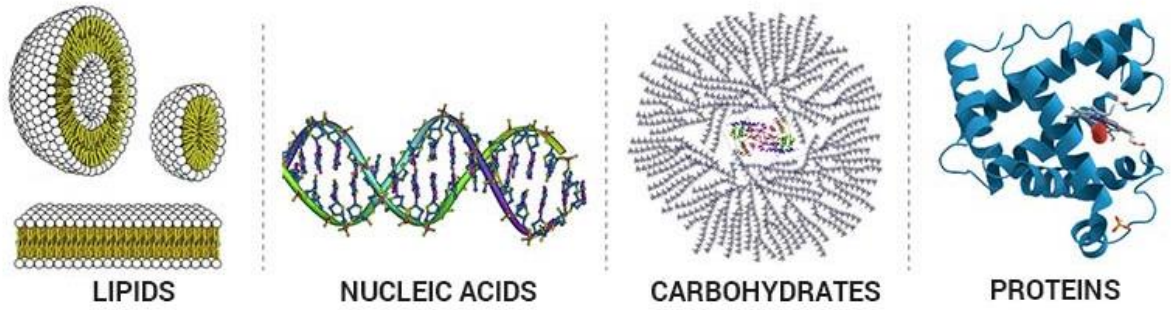
Introduction, classification, chemical nature and biological role of carbohydrate, lipids, nucleic acids, amino acids and proteins.

INTRODUCTION:

Biomolecules are the most essential organic molecules, which are involved in the maintenance and metabolic processes of living organisms. These non-living molecules are the actual foot-soldiers of the battle of sustenance of life. They range from small molecules such as primary and secondary metabolites and

hormones to large macromolecules like proteins, nucleic acids, carbohydrates, lipids etc.

TYPES OF BIOMOLECULES:



There are four major classes of Biomolecules – Carbohydrates, Proteins, Nucleic acids and Lipids. Each of them is discussed below.

CARBOHYDRATES:

- Carbohydrates are chemically defined as polyhydroxy aldehydes or ketones or compounds which produce them on hydrolysis.
- In layman's terms, we acknowledge carbohydrates as sugars or substances that taste sweet.
- They are collectively called as saccharides
- (Greek: sakcharon = sugar).
- Depending on the number of constituting sugar units obtained upon hydrolysis, they are classified as monosaccharides (1 unit), oligosaccharides (2-10 units) and polysaccharides (more than 10 units).
- They have multiple functions', they're the most abundant dietary source of energy; they are structurally very important for many living organisms as they form a major structural component, e.g. cellulose is an important structural fibre for plants.

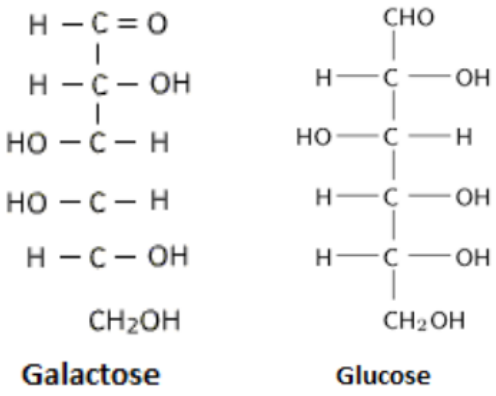
CLASSIFICATION OF CARBOHYDRATES :

- The carbohydrates are further classified into simple and complex which is mainly based on their chemical structure and degree of polymerization.

- Simple Carbohydrates (Monosaccharides, Disaccharides and Oligosaccharides)
- Simple carbohydrates have one or two sugar molecules. In simple carbohydrates, molecules are digested and converted quickly resulting in a rise in the blood sugar levels. They are abundantly found in milk products, beer, fruits, refined sugars, candies, etc. These carbohydrates are called empty calories, as they do not possess fiber, vitamins and minerals.
- Plants, being producers, synthesize glucose ($C_6H_{12}O_6$) using raw materials like carbon dioxide and water in the presence of sunlight. This process of photosynthesis converts solar energy to chemical energy. Consumers feed on plants and harvest energy stored in the bonds of the compounds synthesized by plants.

1. Monosaccharides

Glucose is an example of a carbohydrate monomer or monosaccharide. Other examples of monosaccharides include mannose, galactose, fructose, etc. The structural organization of monosaccharides is as follows:



Monosaccharides may be further classified depending on the number of carbon atoms:

(i)Trioses ($C_3H_6O_3$): These have three carbon atoms per molecule. Example: Glyceraldehyde

(ii)Tetroses ($C_4H_6O_4$): These monosaccharides have four carbon atoms per molecule. Example: Erythrose.

Similarly, we have-

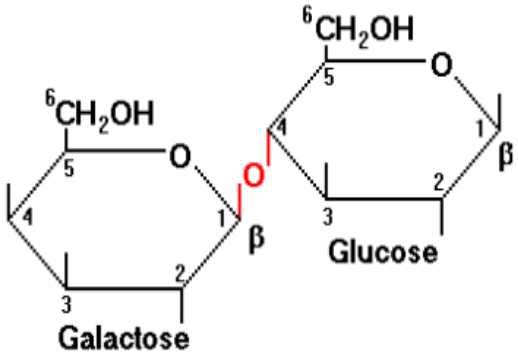
(iii) Pentoses,

(iv) Hexoses, and

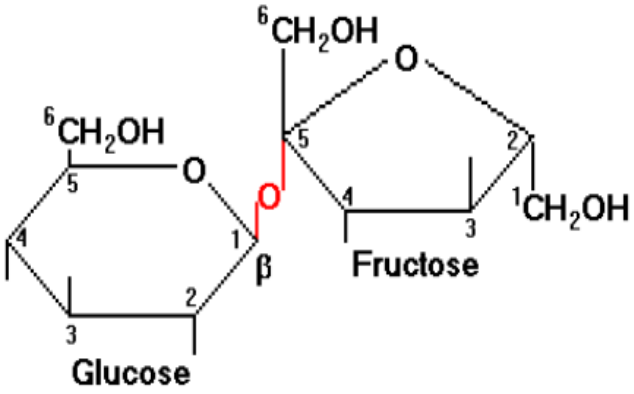
(v) Heptoses

2. Disaccharides

Two monosaccharides combine to form a disaccharide. Examples of carbohydrates having two monomers include- Sucrose, Lactose, Maltose, etc.



LACTOSE= GLUCOSE +GALACTOSE

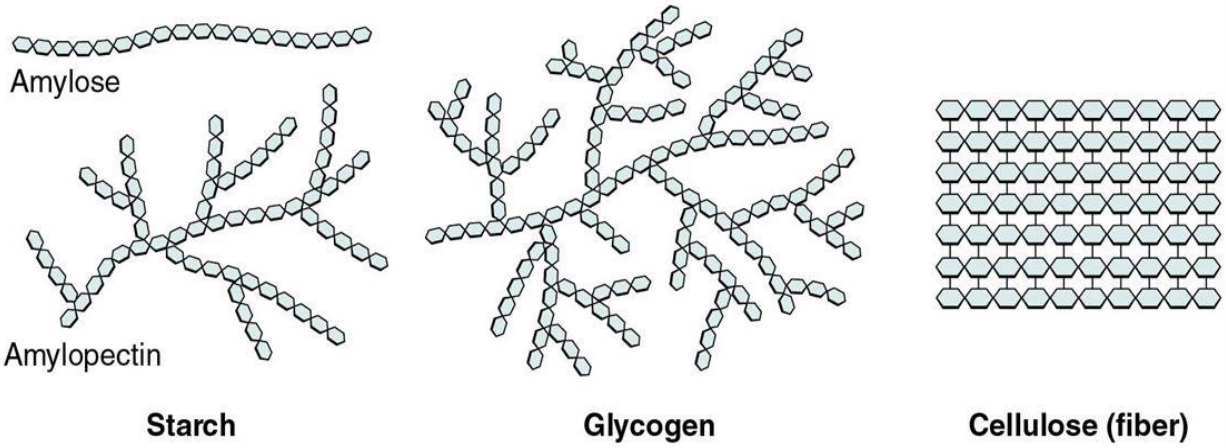


SUCROSE= GLUCOSE + FRUCTOSE

3. Oligosaccharides:

- Carbohydrates formed by the condensation of 2-9 monomers are called oligosaccharides. By this convention, trioses, pentoses, hexoses are all oligosaccharides.
- Complex Carbohydrates (Polysaccharides)
- Complex carbohydrates have two or more sugar molecules, hence they are referred to as starchy foods.
- In complex carbohydrates, molecules are digested and converted slowly compared to simple carbohydrates. They are abundantly found in lentils, beans, peanuts, potatoes, peas, corn, whole-grain bread, cereals, etc.
- Polysaccharides are complex carbohydrates formed by the polymerization of a large number of monomers.

- Examples of polysaccharides include starch, glycogen, cellulose, etc. which exhibit extensive branching and are homopolymers – made up of only glucose units.



1. Starch is composed of two components- amylose and amylopectin. Amylose forms the linear chain and amylopectin is a much-branched chain.

2. Glycogen is called animal starch. It has a structure similar to starch, but has more extensive branching.

3. Cellulose is a structural carbohydrate and is the main structural component of the plant cell wall. It is a fibrous polysaccharide with high tensile strength. In contrast to starch and glycogen, cellulose forms a linear polymer.

FUNCTIONS OF CARBOHYDRATES:

- The main function of carbohydrates is to provide energy and food to the body and to the nervous system.
- Carbohydrates are known as one of the basic components of food, including sugars, starch, and fibre which are abundantly found in grains, fruits and milk products.
- Carbohydrates are also known as starch, simple sugars, complex carbohydrates and so on.
- It is also involved in fat metabolism and prevents ketosis.
- Inhibits the breakdown of proteins for energy as they are the primary source of energy.

- An enzyme by name amylase assists in the breakdown of starch into glucose, finally to produce energy for metabolism.

SOURCES OF CARBOHYDRATES:

1. Simple sugars are found in the form of fructose in many fruits.
2. Galactose is present in all dairy products.
3. Lactose is abundantly found in milk and other dairy products.
4. Maltose is present in cereal, beer, potatoes, processed cheese, pasta, etc.
5. Sucrose is naturally obtained from sugar and honey containing small amounts of vitamins and minerals.

These simple sugars that consist of minerals and vitamins exist commonly in milk, fruits, and vegetables. Many refined and other processed foods like white flour, white rice, and sugar, lack important nutrients and hence, they are labelled "***enriched***." It is quite healthy to use vitamins, carbohydrates and all other organic nutrients in their normal forms.

Carbohydrate Foods

Eating too much sugar results in an abnormal increase in calories, which finally leads to obesity and in turn low calories leads to malnutrition. Therefore, a well-balanced diet needs to be maintained to have a healthy life. That is the reason a balanced diet is stressed so much by dietitians.

Let us look into the differences between the good and bad carbohydrates.

Good Carbohydrates	**Bad Carbohydrates**
High in Nutrients	Low in nutrients
Moderate in calories	High in calories

Low in sodium and saturated fats	High in sodium and saturated fats
Low in trans-fat and cholesterol	High in trans-fat and cholesterol
They are complex carbs. For instance: Legumes, vegetables, whole grains, fruits, and beans.	Foods considered bad carbs rarely have any nutritional value. Some of the foods include white flour, rice, pastries, sodas and processed foods.

EXAMPLES OF CARBOHYDRATES:

Following are the important examples of carbohydrates:

- Glucose
- Galactose
- Maltose
- Fructose
- Sucrose
- Lactose
- Starch
- Cellulose
- Chitin

PROTEINS:

Proteins are another class of indispensable biomolecules, which make up around 50per cent of the cellular dry weight. Proteins are polymers of amino acids arranged in the form of polypeptide chains.

The structure of proteins is classified as primary, secondary, tertiary and quaternary in some cases. These structures are based on the level of complexity

of the folding of a polypeptide chain. Proteins play both structural and dynamic roles. Myosin is the protein that allows movement by contraction of muscles. Most enzymes are proteinaceous in nature.

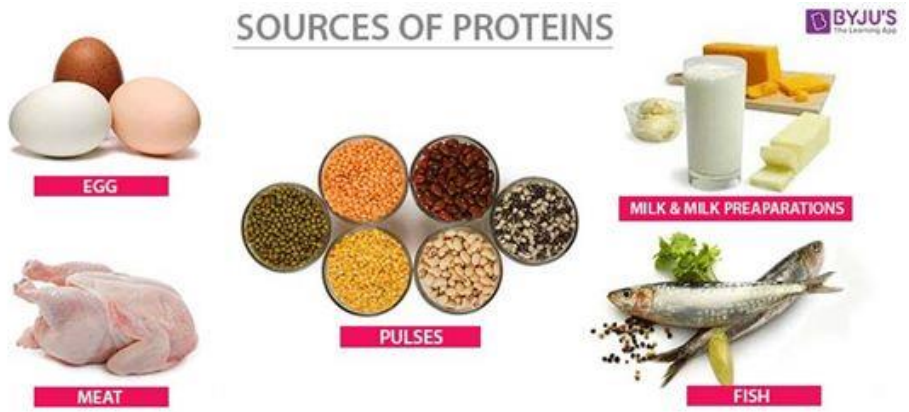
In general, they are two types of protein molecules fibrous proteins and globular proteins. Fibrous proteins are insoluble and elongated. Globular proteins are soluble and compact. Fibrous and Globular proteins may comprise one or four types of protein structures and they include primary, secondary, tertiary and quaternary structure.

- ✓ **Primary Structure:** It is a specific sequence of amino acids. The order of amino acids bonded together is detected by information stored in genes.
- ✓ **Secondary Structure:** It is a three-dimensional form of a local segment of proteins. They are formed by hydrogen bonds between the atoms along the backbone of the polypeptide chain.
- ✓ **Tertiary Structure:** It is determined by R-groups. It is a three-dimensional shape of a protein. Many numbers of tertiary structure fold to form Quaternary Structure.
- ✓ **Quaternary Structure:** It is the arrangement of multiple folded protein subunits in a multi-subunit complex.

PROTEIN SYNTHESIS:

- Protein synthesis takes place through a process called translation.
- This process occurs in the cytoplasm.
- It involves the rendering of genetic codes. Ribosomes of a cell help in translating genetic codes into a polypeptide chain.
- These polypeptide chains become functioning proteins only after undergoing certain modifications.

SOURCES OF PROTEIN:



Although there are debates about the intake of carbohydrates and fats in order to maintain a proper health, a minimum amount of daily protein intake is always a doctor's first recommendation. The most common food which has a higher amount of protein are eggs, almond, chicken, oats, fish and seafood, soy, beans and pulses, cottage cheese, Greek yogurt, milk, broccoli, and quinoa.

FUNCTIONS OF PROTEINS:

1. **Enzymes:** Enzymes mostly carry out all numerous chemical reactions which take place within a cell. They also help in regenerating and creating DNA molecules and carry out complex processes.
2. **Hormones:** Proteins are involved in the creation of various types of hormones which help in balancing the components of the body. For example hormones like insulin, which helps in regulating blood sugar and secretin. It is also involved in the digestion process and formation of digestive juices.
3. **Antibody:** Antibody also known as an immunoglobulin. It is a type of protein which is majorly used by the immune system to repair and heal the body from foreign bacteria. They often work together with other immune cells to identify and separate the antigens from increasing until the white blood cells destroy them completely.
4. **Energy:** Proteins are the major source of energy that helps in the movements of our body. It is important to have the right amount of protein in order to convert it into energy. Protein, when consumed in excess amounts, gets used to create fat and becomes part of the fat cells.

Listed below are few functions of Proteins.

Aspect	Functions	Examples
Storage	Legume Storage, albumin, and proteins.	Supplies food during the early stage of the seedling or embryo.
Hormone Signalling	Counterpart activities of different body parts.	Glucagon and Insulin.
Transport	It transport substances throughout the body through lump or blood cells.	Hemoglobin.
Contraction	To carry out muscle contraction.	Myosin.
Digestive Enzyme	Breaks down nutrients present in the food into smaller portions so that it can be easily absorbed	Pepsin, Amylase, and Lipase

NUCLEIC ACIDS:

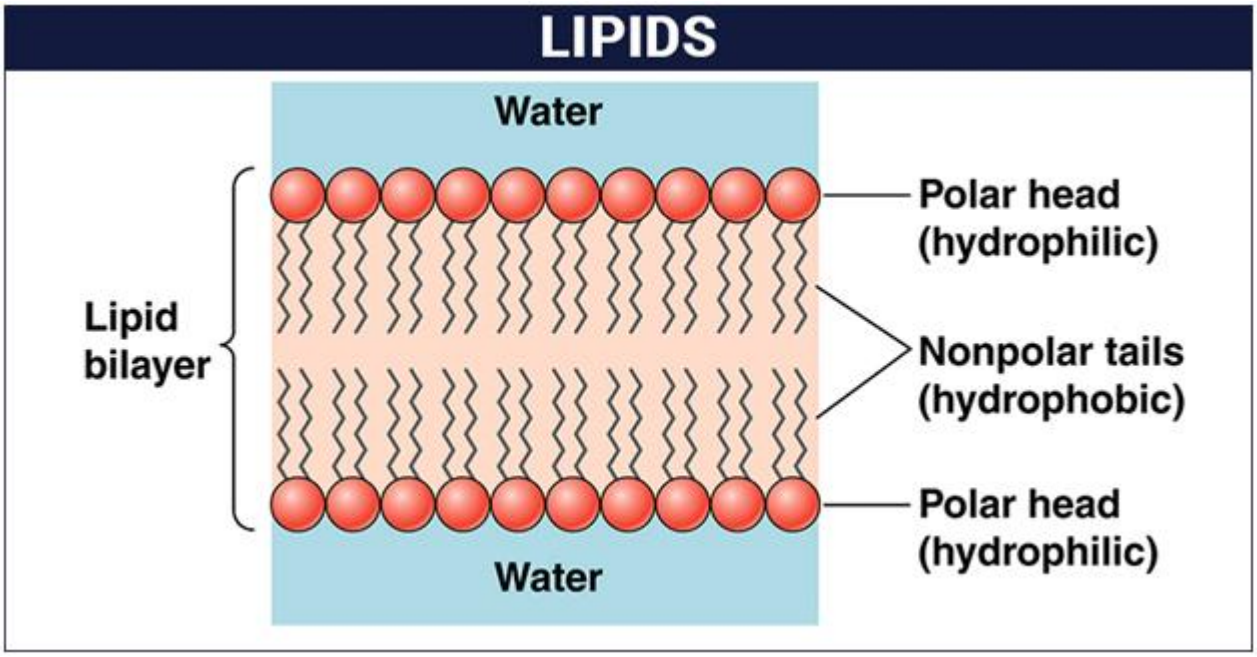
- Nucleic acids refer to the genetic material found in the cell that carries all the hereditary information from parents to progeny.
- There are two types of nucleic acids namely, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).
- The main function of nucleic acid is the transfer of genetic information and synthesis of proteins by processes known as translation and transcription.
- The monomeric unit of nucleic acids is known as nucleotide and is composed of a nitrogenous base, pentose sugar, and phosphate. The nucleotides are linked by a 3’ and 5’ phosphodiester bond.
- The nitrogen base attached to the pentose sugar makes the nucleotide distinct.

- There are 4 major nitrogenous bases found in DNA: adenine, guanine, cytosine, and thymine.
- In RNA, thymine is replaced by uracil.
- The DNA structure is described as a double-helix or double-helical structure which is formed by hydrogen bonding between the bases of two antiparallel polynucleotide chains.
- Overall, the DNA structure looks similar to a twisted ladder.

LIPIDS:

- Lipids are organic substances that are insoluble in water, soluble in organic solvents, are related to fatty acids and are utilized by the living cell. They include fats, waxes, sterols, fat-soluble vitamins, mono-, di- or triglycerides, phospholipids, etc. Unlike carbohydrates, proteins, and nucleic acids, lipids are not polymeric molecules. Lipids play a great role in the cellular structure and are the chief source of energy.

- These organic compounds are nonpolar molecules, which are soluble only in nonpolar solvents and insoluble in water because water is a polar molecule. In the human body, these molecules can be synthesized in the liver and are found in oil, butter, whole milk, cheese, fried foods and also in some red meats.



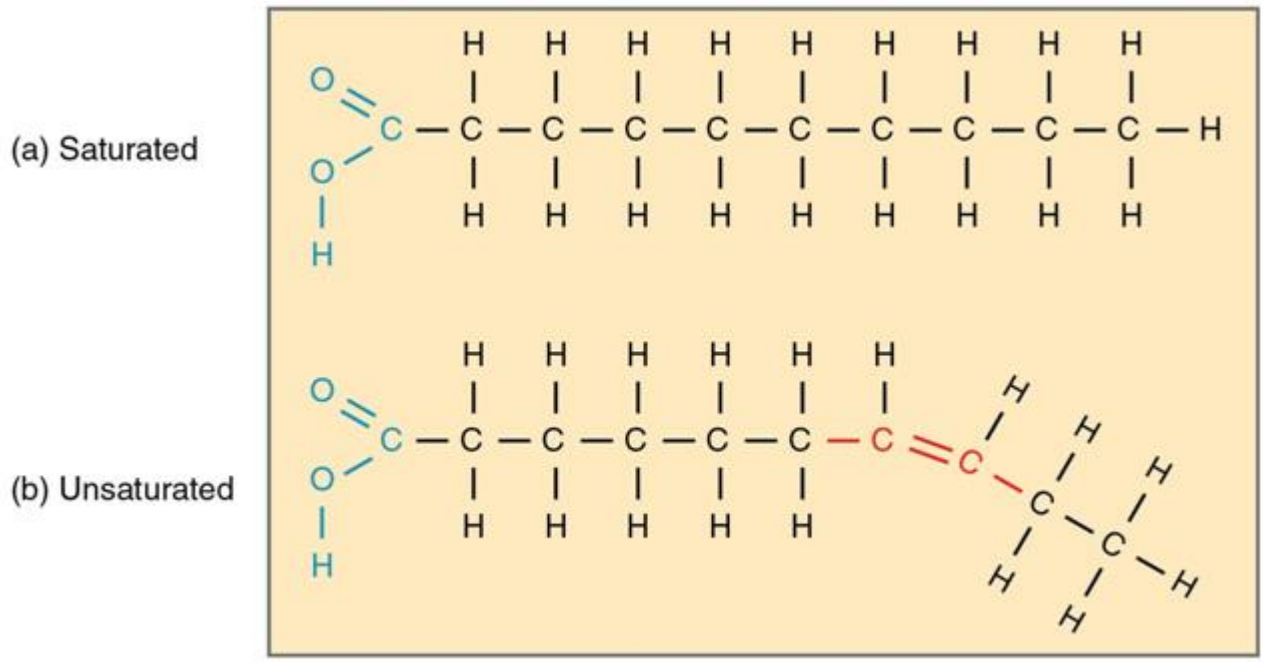
PROPERTIES OF LIPIDS:

Lipids are a family of organic compounds, composed of fats and oils. These molecules yield high energy and are responsible for different functions within the human body. Listed below are some important characteristics of Lipids.

1. Lipids are oily or greasy nonpolar molecules, stored in the adipose tissue of the body.
2. Lipids are a heterogeneous group of compounds, mainly composed of hydrocarbon chains.
3. Lipids are energy-rich organic molecules, which provide energy for different life processes.
4. Lipids are a class of compounds characterised by their solubility in nonpolar solvents and insolubility in water.
5. Lipids are significant in biological systems as they form a mechanical barrier dividing a cell from the external environment known as the cell membrane.

LIPID STRUCTURE:

Lipids are the polymers of fatty acids that contain a long, non-polar hydrocarbon chain with a small polar region containing oxygen. The lipid structure is explained in the diagram below:



Lipid Structure – Saturated and Unsaturated Fatty Acids

CLASSIFICATION OF LIPIDS:

Lipids can be classified into two main classes:

- Nonsaponifiable lipids
- Saponifiable lipids

Nonsaponifiable Lipids :

A nonsaponifiable lipid cannot be disintegrated into smaller molecules through hydrolysis. Nonsaponifiable lipids include cholesterol, prostaglandins, etc

Saponifiable Lipids :

- A saponifiable lipid comprises one or more ester groups, enabling it to undergo hydrolysis in the presence of a base, acid, or enzymes, including waxes, triglycerides, sphingolipids and phospholipids.
- Further, these categories can be divided into non-polar and polar lipids.
- Nonpolar lipids, namely triglycerides, are utilized as fuel and to store energy.
- Polar lipids, that could form a barrier with an external water environment, are utilized in membranes. Polar lipids comprise sphingolipids and glycerophospholipids.

- Fatty acids are pivotal components of all these lipids.

TYPES OF LIPIDS:

Within these two major classes of lipids, there are numerous specific types of lipids important to live, including fatty acids, triglycerides, glycerophospholipids, sphingolipids and steroids. These are broadly classified as simple lipids and complex lipids.

Simple Lipids :

Esters of fatty acids with various alcohols.

1. **Fats:** Esters of fatty acids with glycerol. Oils are fats in the liquid state
2. **Waxes**: Esters of fatty acids with higher molecular weight monohydric alcohols

Complex Lipids :

Esters of fatty acids containing groups in addition to alcohol and a fatty acid.

1. **Phospholipids**: These are lipids containing, in addition to fatty acids and alcohol, a phosphoric acid residue. They frequently have nitrogen-containing bases and other substituents, eg, in glycerophospholipids the alcohol is glycerol and in sphingophospholipids the alcohol is sphingosine.
2. **Glycolipids (glycosphingolipids)**: Lipids containing a fatty acid, sphingosine and carbohydrate.
3. **Other complex lipids**: Lipids such as sulfolipids and amino lipids. Lipoproteins may also be placed in this category.

Precursor and Derived Lipids :

These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid-soluble vitamins, and hormones. Because they are uncharged, acylglycerols (glycerides), cholesterol, and cholesteryl esters are termed neutral lipids. These compounds are produced by the hydrolysis of simple and complex lipids.

Some of the different types of lipids are described below in detail.

Fatty Acids:

Fatty acids are carboxylic acids (or organic acid), usually with long aliphatic tails (long chains), either unsaturated or saturated.

- **Saturated fatty acids**

Lack of carbon-carbon double bonds indicate that the fatty acid is saturated. The saturated fatty acids have higher melting points compared to unsaturated acids of the corresponding size due to their ability to pack their molecules together thus leading to a straight rod-like shape.

- **Unsaturated fatty acids**

Unsaturated fatty acid is indicated when a fatty acid has more than one double bond.

“Often, naturally occurring fatty acids possesses an even number of carbon atoms and are unbranched.”

On the other hand, unsaturated fatty acids contain a cis-double bond(s) which create a structural kink that disables them to group their molecules in straight rod-like shape.

ROLE OF FATS :

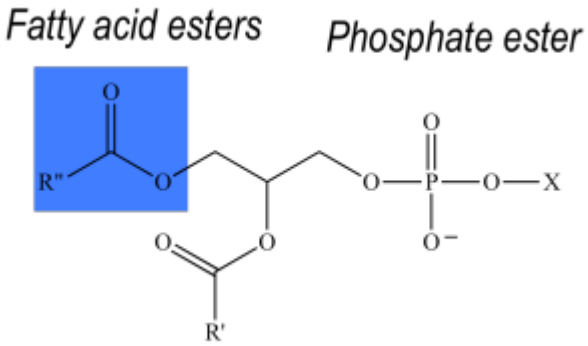
Fats play several major roles in our body. Some of the important roles of fats are mentioned below:

- Fats in the correct amounts are necessary for the proper functioning of our body.
- Many fat-soluble vitamins need to be associated with fats in order to be effectively absorbed by the body.
- They also provide insulation to the body.
- They are an efficient way to store energy for longer periods.

Waxes

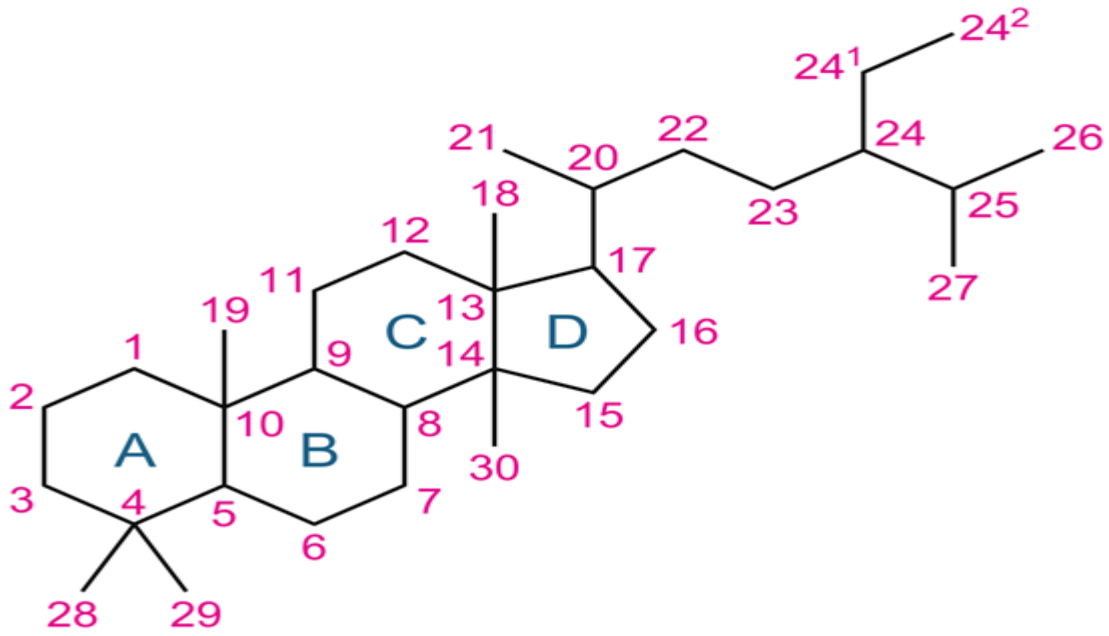
- Waxes are “esters” (an organic compound made by replacing the hydrogen with acid by an alkyl or another organic group) formed from long-alcohols and long-chain carboxylic acids.
- Waxes are found almost everywhere. Fruits and leaves of many plants possess waxy coatings, that can safeguard them from small predators and dehydration.
- Fur of a few animals and the feathers of birds possess same coatings serving as water repellants.
- Carnauba wax is known for its water resistance and toughness (significant for car wax).

Phospholipids



- Membranes are primarily composed of phospholipids that are Phosphoacylglycerols.
- Triacylglycerols and phosphoacylglycerols are the same, but, the terminal OH group of the phosphoacylglycerol is esterified with phosphoric acid in place of fatty acid which results in the formation of phosphatidic acid.
- The name phospholipid is derived from the fact that phosphoacylglycerols are lipids containing a phosphate group.

Steroids



- Our bodies possess chemical messengers known as hormones, that are basically organic compounds synthesized in glands and transported by the bloodstream to various tissues in order to trigger or hinder the desired process.
- Steroids are a kind of hormone that is typically recognized by their tetracyclic skeleton, composed of three fused six-membered and one five-membered ring, as seen above. The four rings are assigned as A, B, C & D as observed in the shade blue, while the numbers in red indicate the carbons.

Cholesterol

- Cholesterol is a wax-like substance, found only in animal source foods. Triglycerides, LDL, HDL, VLDL are different types of cholesterol found in the blood cells.
- Cholesterol is an important lipid found in the cell membrane. It is a sterol, which means that cholesterol is a combination of steroid and alcohol. In the human body, cholesterol is synthesized in the liver.
- These compounds are biosynthesized by all living cells and are essential for the structural component of the cell membrane.
- In the cell membrane, the steroid ring structure of cholesterol provides a rigid hydrophobic structure that helps boost the rigidity of the cell membrane. Without cholesterol, the cell membrane would be too fluid.

- It is an important component of cell membranes and is also the basis for the synthesis of other steroids, including the sex hormones estradiol and testosterone, as well as other steroids such as cortisone and vitamin D.

EXAMPLES OF LIPIDS :

There are different types of lipids. Some examples of lipids include butter, ghee, vegetable oil, cheese, cholesterol and other steroids, waxes, phospholipids, and fat-soluble vitamins. All these compounds have similar features, i.e. insoluble in water and soluble in organic solvents, etc.

UNIT II : Carbohydrate metabolism

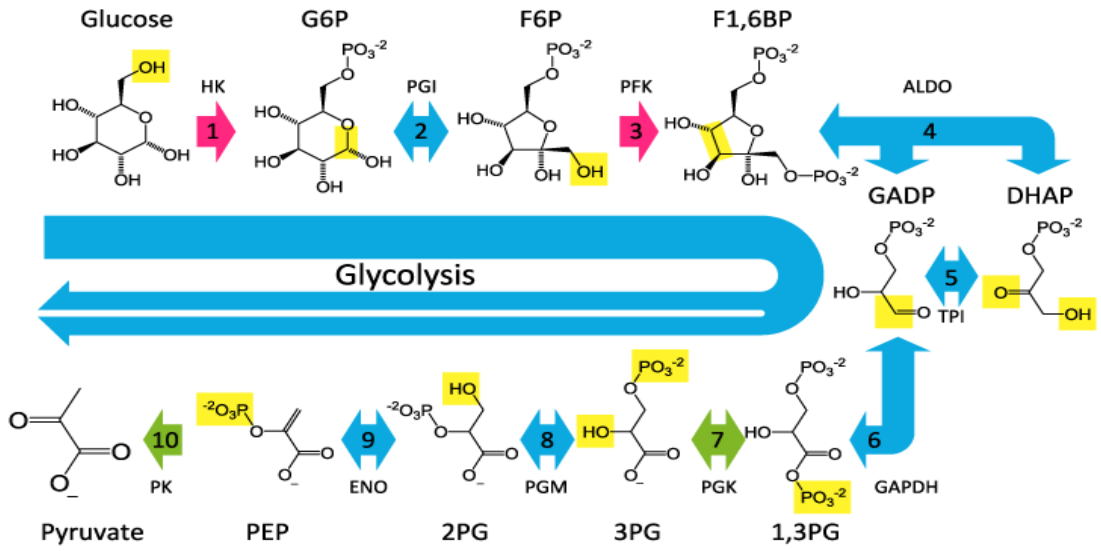
- **Glycolysis – Pathway, energetics and significance**
- **Citric acid cycle- Pathway, energetics and significance**
- **HMP shunt and its significance; Glucose-6-Phosphate dehydrogenase**
- **(G6PD) deficiency**
- **Glycogen metabolism Pathways and glycogen storage diseases (GSD)**
- **Gluconeogenesis- Pathway and its significance**
- **Hormonal regulation of blood glucose level and Diabetes mellitus**
    - **Biological oxidation**
- **Electron transport chain (ETC) and its mechanism.**
- **Oxidative phosphorylation & its mechanism and substrate**
- **phosphorylation**
- **Inhibitors ETC and oxidative phosphorylation/Uncouplers**

CARBOHYDRATE METABOLISM

Glycolysis is the metabolic process that converts glucose into pyruvic acid."

GLYCOLYSIS:

Glycolysis is the process in which glucose is broken down to produce energy. It produces two molecules of pyruvate, ATP, NADH and water. The process takes place in the cytosol of the cell cytoplasm, in the presence or absence of oxygen.

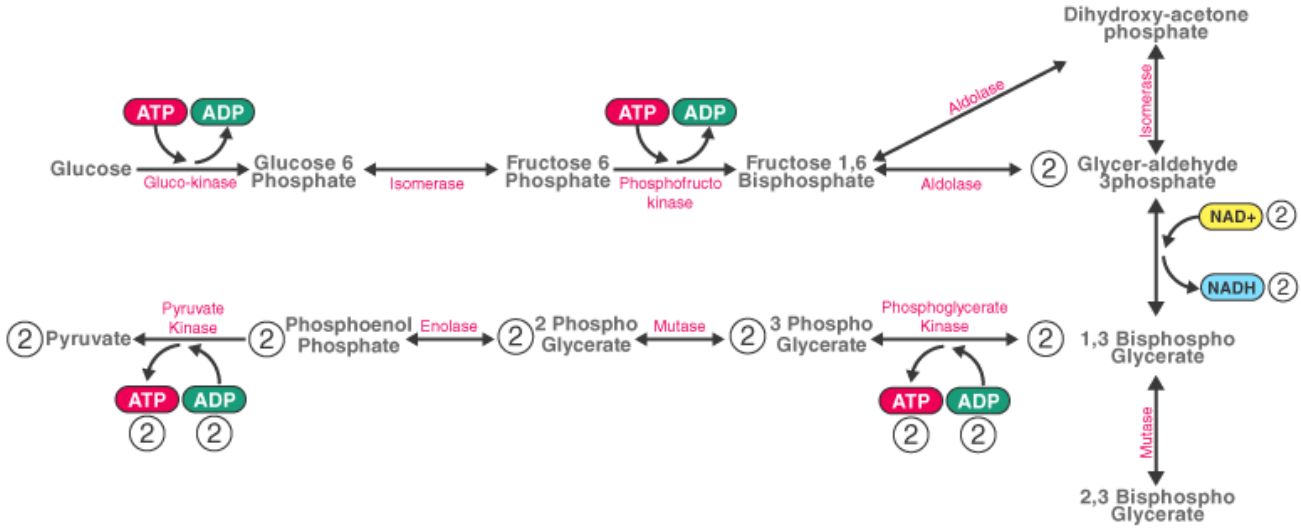


Glycolysis is the primary step of cellular respiration. In the absence of oxygen, the cells take small amounts of ATP through the process of fermentation.

This metabolic pathway was discovered by three German biochemists- Gustav Embden, Otto Meyerhof, and Jakub Karol Parnas in the early 19th century and is known as the EMP pathway (Embden–Meyerhof–Parnas).

Glycolysis Pathway

The glycolysis pathway occurs in the following stages:



Stage 1 :

- A phosphate group is added to glucose in the cell cytoplasm, by the action of enzyme hexokinase.
- In this, a phosphate group is transferred from ATP to glucose forming glucose,6-phosphate.

Stage 2 :

Glucose-6-phosphate is isomerized into fructose,6-phosphate by the enzyme phosphoglucomutase.

Stage 3 :

The other ATP molecule transfers a phosphate group to fructose 6-phosphate and converts it into fructose 1,6-bisphosphate by the action of enzyme phosphofructokinase.

Stage 4 :

The enzyme aldolase converts fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, which are isomers of each other.

Step 5 :

Triose-phosphate isomerase converts dihydroxyacetone phosphate into glyceraldehyde 3-phosphate which is the substrate in the successive step of glycolysis.

Step 6 :

This step undergoes two reactions:

- The enzyme glyceraldehyde 3-phosphate dehydrogenase transfers 1 hydrogen molecule from glyceraldehyde phosphate to nicotinamide adenine dinucleotide to form NADH + H^+.
- Glyceraldehyde 3-phosphate dehydrogenase adds a phosphate to the oxidized glyceraldehyde phosphate to form 1,3-bisphosphoglycerate.

Step 7 :

Phosphate is transferred from 1,3-bisphosphoglycerate to ADP to form ATP with the help of phosphoglycerokinase. Thus two molecules of phosphoglycerate and ATP are obtained at the end of this reaction.

Step 8 :

The phosphate of both the phosphoglycerate molecules is relocated from the third to the second carbon to yield two molecules of 2-phosphoglycerate by the enzyme phosphoglyceromutase.

Step 9 :

The enzyme enolase removes a water molecule from 2-phosphoglycerate to form phosphoenolpyruvate.

Step 10 :

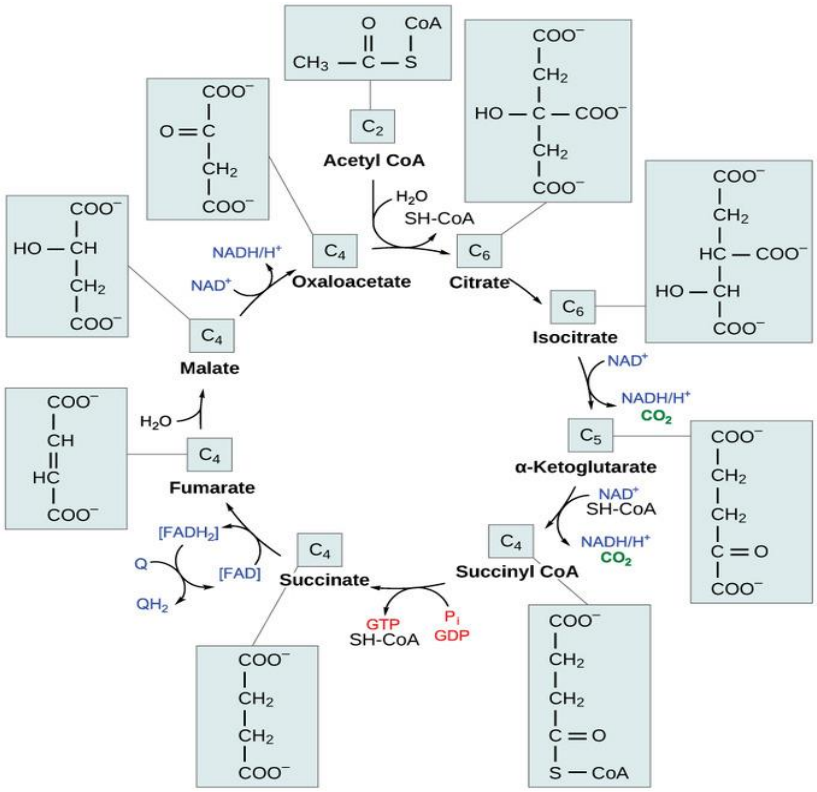
A phosphate from phosphoenolpyruvate is transferred to ADP to form pyruvate and ATP by the action of pyruvate kinase. Two molecules of pyruvate and ATP are obtained as the end products.

Key Points of Glycolysis

- It is the process in which a glucose molecule is broken down into two molecules of pyruvate.
- The process takes place in the cytoplasm of plant and animal cell.
- Six enzymes are involved in the process.
- The end products of the reaction include 2 pyruvate, 2 ATP and 2 NADH molecules.

CITRIC ACID CYCLE (KREBS CYCLE)

- The citric acid cycle takes place in the matrix of the mitochondria.
- Almost all of the enzymes of the citric acid cycle are soluble, with the single exception of the enzyme succinate dehydrogenase, which is embedded in the inner membrane of the mitochondrion.
- Unlike glycolysis, the citric acid cycle is a closed loop: the last part of the pathway regenerates the compound used in the first step.
- The eight steps of the cycle are a series of redox, dehydration, hydration, and decarboxylation reactions that produce two carbon dioxide molecules, one GTP/ATP, and reduced forms of NADH and FADH2.
- This is considered an aerobic pathway because the NADH and FADH2 produced must transfer their electrons to the next pathway in the system, which will use oxygen.
- If this transfer does not occur, the oxidation steps of the citric acid cycle also do not occur. Note that the citric acid cycle produces very little ATP directly and does not directly consume oxygen.



The citric acid cycle: In the citric acid cycle, the acetyl group from acetyl CoA is attached to a four-carbon oxaloacetate molecule to form a six-carbon citrate molecule. Through a series of steps, citrate is oxidized, releasing two carbon dioxide molecules for each acetyl group fed into the cycle. In the process, three NAD+ molecules are reduced to NADH, one FAD molecule is reduced to FADH2, and one ATP or GTP (depending on the cell type) is produced (by substrate-level phosphorylation). Because the final product of the citric acid cycle is also the first reactant, the cycle runs continuously in the presence of sufficient reactants.

Steps in the Citric Acid Cycle

Step 1. The first step is a condensation step, combining the two-carbon acetyl group (from acetyl CoA) with a four-carbon oxaloacetate molecule to form a six-carbon molecule of citrate. CoA is bound to a sulfhydryl group (-SH) and diffuses away to eventually combine with another acetyl group. This step is irreversible because it is highly exergonic. The rate of this reaction is controlled by negative feedback and the amount of ATP available. If ATP levels increase, the rate of this reaction decreases. If ATP is in short supply, the rate increases.

Step 2. Citrate loses one water molecule and gains another as citrate is converted into its isomer, isocitrate.

Steps 3 and 4. In step three, isocitrate is oxidized, producing a five-carbon molecule, α-ketoglutarate, together with a molecule of CO_2 and two electrons, which reduce NAD+ to NADH. This step is also regulated by negative feedback from ATP and NADH and by a positive effect of ADP. Steps three and four are both oxidation and decarboxylation steps, which release electrons that reduce NAD^+ to NADH and release carboxyl groups that form CO_2 molecules. α-Ketoglutarate is the product of step three, and a succinyl group is the product of step four. CoA binds the succinyl group to form succinyl CoA. The enzyme that catalyzes step four is regulated by feedback inhibition of ATP, succinyl CoA, and NADH.

Step 5. A phosphate group is substituted for coenzyme A, and a high- energy bond is formed. This energy is used in substrate-level phosphorylation (during the conversion of the succinyl group to succinate) to form either guanine triphosphate (GTP) or ATP. There are two forms of the enzyme, called isoenzymes, for this step, depending upon the type of animal tissue in which they are found. One form is found in tissues that use large amounts of ATP, such as heart and skeletal muscle. This form produces ATP. The second form of the enzyme is found in tissues that have a high number of anabolic pathways, such as liver. This form produces GTP. GTP is energetically equivalent to ATP; however, its use is more restricted. In particular, protein synthesis primarily uses GTP.

Step 6. Step six is a dehydration process that converts succinate into fumarate. Two hydrogen atoms are transferred to FAD, producing $FADH_2$. The energy contained in the electrons of these atoms is insufficient to reduce NAD^+ but adequate to reduce FAD. Unlike NADH, this carrier remains attached to the enzyme and transfers the electrons to the electron transport chain directly. This process is made possible by the localization of the enzyme catalyzing this step inside the inner membrane of the mitochondrion.

Step 7. Water is added to fumarate during step seven, and malate is produced. The last step in the citric acid cycle regenerates oxaloacetate by oxidizing malate. Another molecule of NADH is produced.

Products of the Citric Acid Cycle

Two carbon atoms come into the citric acid cycle from each acetyl group, representing four out of the six carbons of one glucose molecule. Two carbon dioxide molecules are released on each turn of the cycle; however, these do not

necessarily contain the most recently-added carbon atoms. The two acetyl carbon atoms will eventually be released on later turns of the cycle; thus, all six carbon atoms from the original glucose molecule are eventually incorporated into carbon dioxide. Each turn of the cycle forms three NADH molecules and one $FADH_2$ molecule. These carriers will connect with the last portion of aerobic respiration to produce ATP molecules. One GTP or ATP is also made in each cycle. Several of the intermediate compounds in the citric acid cycle can be used in synthesizing non-essential amino acids; therefore, the cycle is amphibolic (both catabolic and anabolic).

Breakdown of Pyruvate

After glycolysis, pyruvate is converted into acetyl CoA in order to enter the citric acid cycle.

Introduction

The hexose monophosphate shunt, also known as the pentose phosphate pathway, is a unique pathway used to create products essential in the body for many reasons. The HMP shunt is an alternative pathway to glycolysis and is used to produce ribose-5-phosphate and nicotinamide adenine dinucleotide phosphate (NADPH). This pathway occurs in the oxidative and non-oxidative phases, each comprising a series of reactions. The HMP shunt also has significance in the medical world, as enzyme or co-factor deficiencies can have potentially fatal implications on the affected patients.

Function

The HMP shunt is parallel to the glycolysis pathway and takes place in the cytoplasm. A 6-carbon sugar, glucose, may enter the glycolytic pathway or enter the alternative HMP shunt depending on the cell's individual needs at the time. Once the glucose enters the HMP shunt, it undergoes a series of reactions, broken down into the oxidative(irreversible) and non-oxidative phases (reversible). The oxidative phase is responsible for converting the intermediate glucose-6-phosphate to 6-phosphogluconate, using the glucose-6-phosphate dehydrogenase (G6PD) enzyme. The by-product of this reaction is the important molecule NADPH. 6-phosphogluconate then converts into ribulose-5-phosphate, and NADPH gets produced again as a by-product.[1][2][3]

The non-oxidative phase of the HMP shunt involves the conversion of ribulose-5-phosphate to ribose-5-phosphate (R-5-P) through a series of independent

reactions. It is important to note that no NADPH molecules get created in this part of the HMP shunt. R-5-P in this reaction can be returned to the glycolytic pathway as fructose-6-phosphate. This step requires the transketolase enzyme with the presence of the thiamine co-factor. Thiamine also participates in a plethora of other metabolic reactions throughout the body. It is used by enzyme alpha-ketoglutarate in the Krebs cycle, for the enzyme pyruvate dehydrogenase as well as branch-chained ketoacid dehydrogenase.[3]

The HMP shunt pathway is under the regulation of the demands of NADPH in the respective tissue. The rate-limiting enzyme is G6PD and has allosteric inhibition directed by the presence of NADPH and allosteric activation via the presence of NADP+. Consequently, the activity of G6PD activity also increases in a fed state with a high carbohydrate diet, and conversely, decreases in a starving or a diabetic state.[3][4]

Mechanism

The importance of the HMP shunt is R-5-P and NADPH molecules that generated in the reaction. R-5-P undergoes a series of reactions to create the different ribose sugars that comprise the DNA and RNA molecules, required to carry genetic information. Moreover, R-5-P also converts into erythrose-4-phosphate for the synthesis of aromatic amino acids. As such, this pathway is an essential source of these ribose sugars.[5]

NADPH is also an important molecule as it has a plethora of functions throughout the body. It takes part in anabolism processes such as that of synthesis steroids and fatty acids. NADPH is also important in the respiratory burst process, an essential component of the immune response within phagolysosomes. The NADPH is used to reduce oxygen into an oxygen radical that converts into hydrogen peroxide, which in turn, transforms into bleach. Bleach in the phagolysosomes is introduced to offending pathogens to cause death. NADPH is also required to reduce the molecule glutathione. Using glutathione reductase and NAPDH, oxidized glutathione is converted into its reduced form, and can detoxify free radicals and peroxides; this limits the possibility of any free radical injury in tissues with NADPH present, namely that the red blood cells.

Since this pathway creates NADPH, a reducing agent utilized in many anabolic processes, the HMP shunt is common in more tissues than others. These tissues are those with significant anabolic functions such as adrenal cortex (fatty acid

and steroid synthesis), mammary gland (milk production), liver, and red blood cells.

Clinical Significance

- Interruptions in the HMP shunt can have drastic effects on an individual. A pathology that can arise is the deficiency of the enzyme glucose-6-phosphate dehydrogenase, the enzyme required to produce NADPH in the initial step of the HMP shunt.
- The consequences are mostly seen in red blood cells as the cells can no longer defend against the constant introduction of oxidizing agents. This condition of glucose-6-phosphate dehydrogenase deficiency is an X-linked recessive disorder, most commonly seen in African Americans.
- The deficiency leads to hemolytic anemia in response to any of the offending agents such as fava beans, medications like nitrofurantoin, or anti-malarial drugs.
- Infections are the most likely offending agents to exacerbate this condition as the inflammation introduces free radicals into red blood cells that cannot detoxify those radicals, and oxidative damage ensues.
- The damage occurs as the injury causes protein and globin chains to denature within the red blood cells.
- The globin chains aggregate and form the characteristic Heinz bodies seen in G6PD deficient patients. Consequently, the spleen phagocytoses the aggregated portion of the RBC and leaves the remnant bite cells in circulation to create degmacytes, or bite cells, as seen in a peripheral smear of these patients.
- Although detrimental to the health, this condition does confer a degree of resistance to malaria as the parasites require the reduced form of glutathione to proliferate while these patients only have the oxidized form in the red blood cells.
- Thus, the evolutionary advantage maintains a deficiency in the African American population. Another use of the HMP shunt in medicine is its use in diagnosing patients with thiamine (vitamin B1) deficiency.
- Thiamine deficient patients present with a wide array of symptoms, ranging from Wernicke-Korsakoff syndrome to dry or wet beriberi. Wernicke-Korsakoff is characterized by a triad of ataxia, ophthalmoplegia, and confabulations. Wet beriberi can result in cardiac failure and dilated cardiomyopathies, while dry beriberi results in

sequelae of neurological symptoms. As thiamine deficiency can lead to so many complications and potentially become fatal, it is important to detect the deficiency promptly. The deficiency is tested by administering thiamine to a suspected patient and detecting the activity of the transketolase enzyme of HMP shunt within red blood cells. If transketolase activity increases, it confirms the diagnosis of thiamine deficiency. As such, thiamine, as well as dextrose, is administered since thiamine deficiency causes impaired glucose breakdown

GLYCOGEN: THE STORAGE FORM OF GLUCOSE

Significance and localization of glucose

- Carbohydrates are key components of our diet. They provide energy, they are precursors of lipids and amino acids, and they store energy in the form of **glycogen**.
- The stored form of glucose in plants is starch, which resembles the glycogen stored in human or animal cells. The special chemical structure of glycogen allows for **rapid synthesis and breakdown**, which means that the body can respond quickly when glucose is in short supply.
- Glycogen is present in cells in the form of **cytosolic granules**. Glycogen granules contain both the enzymes for synthesis and breakdown. Glycogen is present in all cells—except in erythrocytes. The quantities used for the energy requirements of the cells themselves are minimal.
- Significant amounts of glycogen are stored in only two organs: in the liver (approximately 150 g) and in skeletal muscles (approximately 300 g).

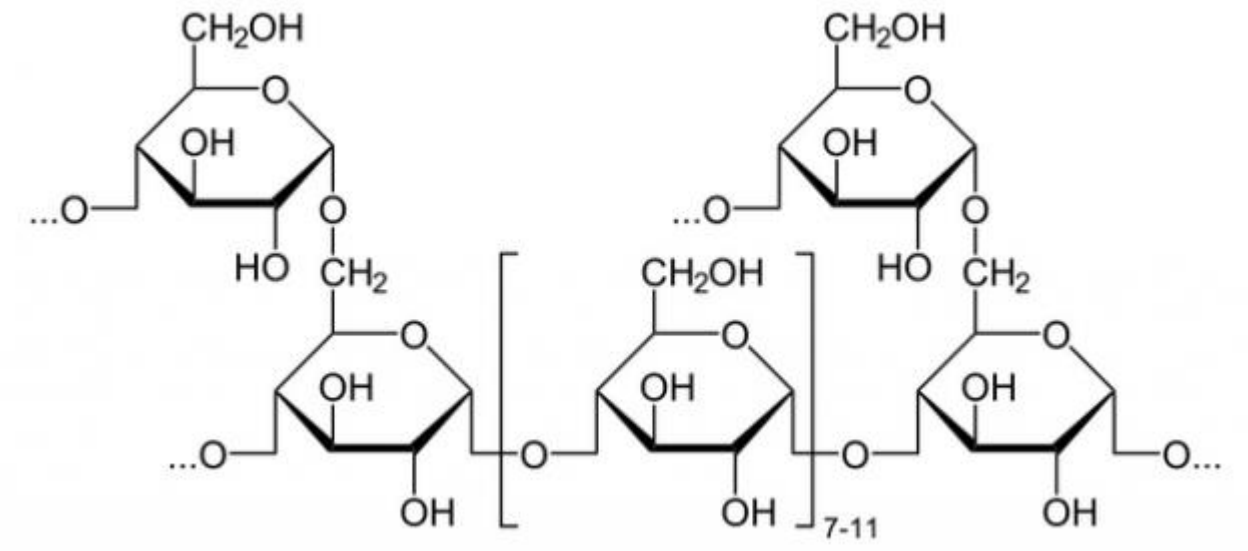
FUNCTION OF GLYCOGEN STORAGE :

The **function of glycogen** differs greatly between the two major sites of glycogen storage—in the liver and in the skeletal muscles:

- **Liver glycogen** is the first and immediate source of glucose for the maintenance of blood glucose levels to meet the needs of the organism as a whole, especially of the brain and the RBCs.

- The **glycogen in skeletal muscles** serves exclusively as an energy source for the muscle itself.

STRUCTURE OF GLYCOGEN :



- Glycogen is a branched polymer consisting of residues of glucose, which are linked by **α-1,4 O-glycosidic** bonds with **α-1,6 branches** every 8–10 residues.
- These linkages create a tree-like polymer consisting of up to 50,000 glucose monomers, which appear as cytosolic grains when examined with an electron microscope. Glycogen provides energy storage **with minimum effect on cellular osmolarity**. It has only minimal osmotic activity due to its small size. Free glucose cannot be stored due to its high osmotic activity.
- **Note:** Free glucose would cause each cell to burst due to its osmotic activity.
- Another advantage is the branched structure of glycogen. The resulting numerous non-reducing ends ensure rapid mobilization and maintenance of the blood glucose level.

GLYCOGENESIS :

The synthesis of glycogen does not usually involve the de novo formation of glycogen but instead lengthens existing glycogen molecules by adding glucosyl residues. Every glycogen molecule has, at its core, a **glycogenin** protein, which is a glycoprotein that remains attached to the reducing end of glycogen during its degradation.

Step 1 of glycogenesis

The first step corresponds to the first reaction of glycolysis. Glucose is phosphorylated to **glucose-6-phosphate**. In skeletal muscles, this reaction is catalyzed by the enzyme **hexokinase** and, in the liver, by the enzyme **glucokinase**.

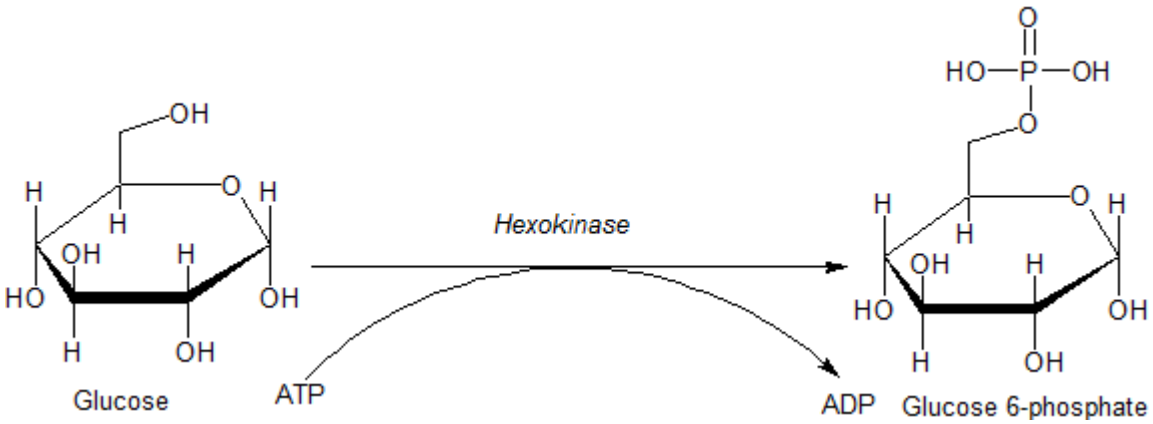


Image: Action of hexokinase with glucose as a substrate. By Jmun7616. License: Public Domain.

Step 2 of glycogenesis

The second step consists of the isomerization of **glucose-1-phosphate** by the enzyme **phosphoglucomutase**.

Step 3 of glycogenesis

In order to produce an O-glycosidic compound for the synthesis of glycogen, a high level of energy is required. For this reason, glucose-1-phosphate is initially activated by a reaction with **uridine triphosphate (UTP)**. This produces **uridine diphosphate (UDP)-glucose** and **pyrophosphate**, which is hydrolytically cleaved into two phosphates by the enzyme **pyrophosphatase**.

Step 4 of glycogenesis

Now, the glucose can be transferred to the **C4–OH group** on one of the non-reducing ends of glycogen to form an α-1,4 glycosidic bond. This reaction is catalyzed by the enzyme **glycogen synthase**, liberating UDP, which is then recycled by conversion to UTP with adenosine triphosphate (**ATP**).

De novo synthesis of a glycogen molecule

New glucose residues can attach only to an existing glycogen molecule if the straight chain of the existing glycogen molecule is at least four glucose units long. For de novo synthesis, a **primer** (starter substrate) is essential; in this case, it is the protein **glycogenin** with its activity as a glycosyltransferase.

The **glycosyltransferase** links the **tyrosine residue** of the protein with UDP-glucose. UDP is cleaved and a glucose molecule is attached. If eight glucose units attach to the tyrosine residue of the glycogenin, the glycogen synthase can elongate the chain.

Incorporation of branch points in glycogen

The characteristic α-1,6 branches of glycogen are the products of an enzyme called amylo-(1,4→1,6)-transglycosylase = **branching enzyme**.

The **amylo-(1,4→1,6)-transglycosylase** adds branches to the growing glycogen molecule by forming α-1,6 linkages by binding to a linear α-1,4 chain that consists of at least 11 **glucose monomers**. Of these, a chain of seven glucose monomers is removed and transferred to the OH group of the C6 of a glucose residue. Thus, located between two branch points are at least four glucose monomers.

Note: The high density of branches characteristic for glycogen gives rise to a great number of non-reducing ends, which determines the possible rates of synthesis and breakdown and enables a maximum speed of glucose release. It is thus possible to quickly access glycogen stores to lower or increase blood glucose levels when required.

THE BREAKDOWN OF GLYCOGEN: GLYCOGENOLYSIS :

- **Glycogenolysis** follows a different pathway than glycogenesis.

- **Glucose-1-phosphate**, a high-energy compound, is released. The steps of glycogenolysis are as follows:
- At the free non-reducing ends of glycogen, **glycogen phosphorylase** catalyzes phosphorolytic cleavage of the α-1,4-glycosidic linkages of glycogen, releasing glucose-1-phosphate as the reaction product. For this purpose, free inorganic phosphate is required.

- Glucose-1-phosphate can isomerize to glucose-6-phosphate and enter glycolysis.
- The liver enzyme **glucose-6-phosphatase** converts glucose-6-phosphate to glucose, which regulates and maintains blood glucose levels. The skeletal muscles lack this enzyme and can therefore not release glucose into the bloodstream.
- Glycogen phosphorylase is **dependent on pyridoxal phosphate (PLP)** and can only cleave α-1,4-glycosidic bonds. It stops cleaving α-1,4 linkages four glucose monomers away from an α-1,6 branch point.

DEGRADATION OF BRANCHING POINTS DURING GLYCOGENOLYSIS :

The **debranching enzyme** (**4-alpha-glucanotransferase**) is a bifunctional enzyme that is responsible for the degradation of branching points. It has the following functions:

- **Transferase activity:** The enzyme 4-alpha-glucanotransferase transfers a segment of three glucose units from α-1,6 branched four-unit chains (the result of glycogen phosphorylase activity) to an adjacent branch of the glycogen chain.
- **Glucosidase activity:** The enzyme 4-alpha-glucanotransferase hydrolytically cleaves the remaining α-1,6 linkage, producing glucose and a linear chain of glycogen.

Regulation of Glycogen Metabolism

- Glycogen metabolism is regulated by two enzymes: **glycogen phosphorylase** and **glycogen synthase**. The coordination is mainly dependent on hormone-mediated and, in part, allosteric regulatory effects.
- The **allosteric regulation** is a form of regulation of enzyme activity carried out by certain enzymes (**allosteric enzymes**) that are almost always composed of multiple subunits. These may occur in more than one stable conformation.
- A negative feedback mechanism leads to the inhibition of activity or synthesis of one or more enzymes through the end product. The inhibition of enzyme synthesis is called **enzyme expression**. The inhibition of enzyme activity is called an allosteric effect.

REGULATION OF THE BREAKDOWN OF GLYCOGEN (GLYCOGENOLYSIS) :

- Two distinct isoforms of glycogen phosphorylase exist; one is expressed in the liver and the other in the skeletal muscle. Because the process of glycogen metabolism is different in these two parts of the body, the **muscles and liver are regulated separately**.
- **Regulation of glycogen breakdown in skeletal muscle and all non-liver cells**
- Two forms of glycogen phosphorylase exist in the skeletal muscle: **phosphorylase a** (the active form) and **phosphorylase b** (the inactive form). The conversion of the inactive to the active form is catalyzed by phosphorylase **kinase**. This enzyme is activated by hormones. The enzyme **protein kinase A** (PKA) regulates the phosphorylase kinase by phosphorylation.
- **Calcium ions** also have an activating effect on the skeletal muscle. In a working muscle, the **sarcoplasmic reticulum** releases calcium ions, thus increasing the intracellular calcium concentration. The actual activation of glycogen phosphorylase is mediated by a **calcium-calmodulin complex**.
- **Phosphorylase b** is also subject to allosteric effects. The inactive enzyme can become partially active, and elevated levels of adenosine monophosphate (AMP) can activate phosphorylase b. Even before phosphorylase kinase becomes active in order to meet specific demands of the cell (hormonally controlled), ATP and glucose-6-phosphate inhibit the activation of phosphorylase b, which means that the inactive state is favored. This mechanism prevents unnecessary depletion of muscle glycogen stores when the metabolic demands have already been met.

Regulation of the breakdown of glycogen in the liver

In the liver, the enzyme phosphorylase kinase catalyzes the conversion of phosphorylase b to phosphorylase a. ATP and AMP are present in the liver; however, they are not relevant, because the liver does not degrade glycogen for its own use. Instead, **the liver meets its own energy requirements using fatty acids**.

Regulation of the synthesis of glycogen (glycogenesis)

Regulatory mechanisms of glycogen synthesis in the liver and skeletal muscles are the same. Glycogen synthase exists in an active dephosphorylated form, called **glycogen synthase a**. The inactive phosphorylated form is called **glycogen synthase b**. The conversion into the respective forms is mediated by protein kinase A, without further involvement of a kinase.

Also subject to allosteric regulation is the glycogen synthase b, which is activated by high concentrations of glucose-6-phosphate.

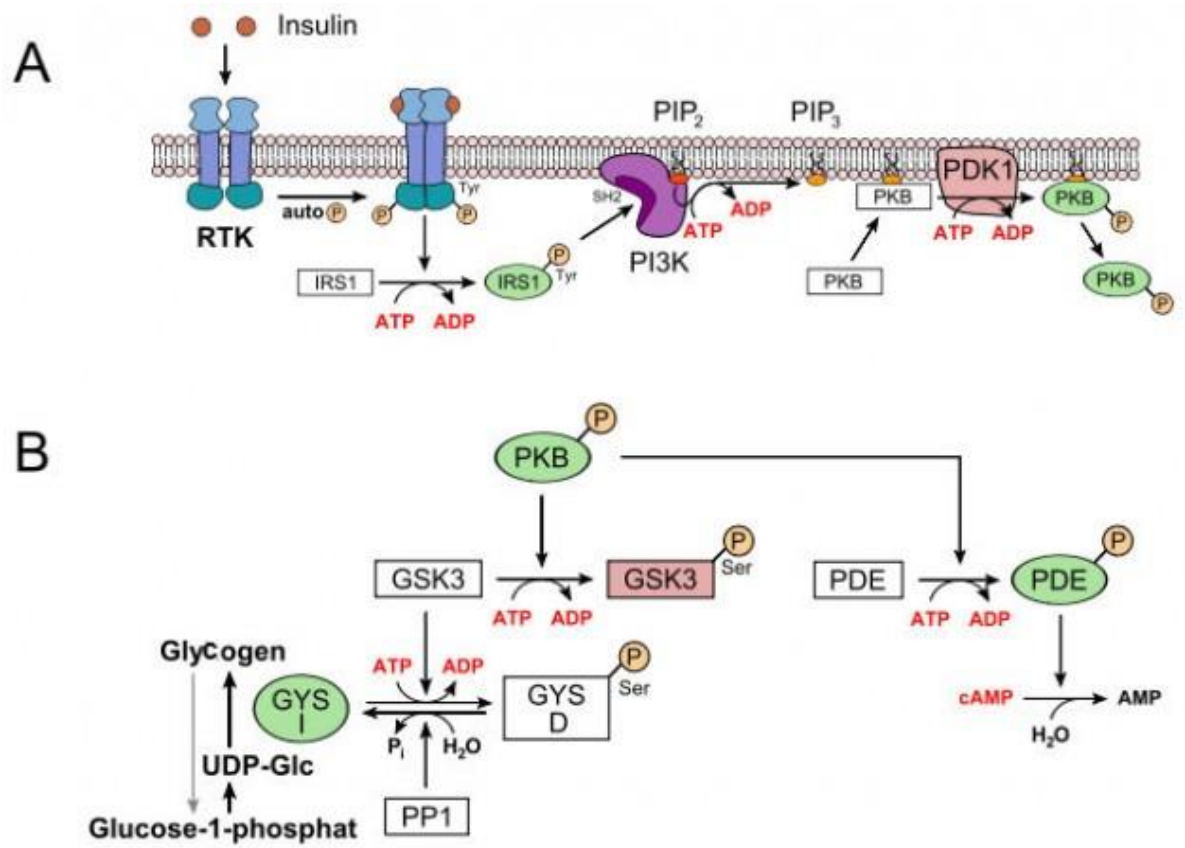
Note: Glycogen phosphorylase is activated by phosphorylation; glycogen synthase is activated by dephosphorylation.

HORMONAL CONTROL OF GLYCOGEN METABOLISM

- This important control mechanism prevents glycogen from being synthesized at the same time that it is being broken down. Three hormones play an important role here: **glucagon**, **adrenaline**, and **insulin**. Glucagon and adrenaline stimulate glycogen degradation, while insulin stimulates the synthesis of glycogen.
- Upon activation of the insulin receptor, the **phosphodiesterase** is activated, decreasing adenosine 3′,5′-cyclic monophosphate (**cAMP**) **levels** and inactivating **protein kinase** (PKA). An inactive PKA decreases the level of phosphorylation of the phosphorylase kinase, which has an inhibitory effect on this enzyme. This, in turn, decreases the rate of glycogen degradation.
- In addition, protein kinase B is activated, which reinforces the phosphorylation of **glycogen synthase kinase 3** (GSK3) and thereby inactivates it. As a result, GSK3 phosphorylates the glycogen synthase to a lesser extent, causing the latter to become more active, which amplifies glycogen synthesis.
- The phosphoprotein phosphatase 1 (PP1) catalyzes the key step, the **dephosphorylation of glycogen synthase**, which is responsible for glycogen synthesis. The latter can be inactivated by the **downstream metabolic effects** of adrenaline and glucagon (cAMP–PKA). Thus, adrenaline and glucagon contribute to the inactivation of glycogen synthesis.

Note: Adrenaline and glucagon have antagonistic effects on the mentioned signaling cascades, which are activated and inactivated by insulin.

The following illustrations show the different mechanisms at a glance:



Clinical Relevance: Glycogen Storage Diseases

Glycogenoses are a group of hereditary diseases affecting the metabolism of glycogen, resulting in the extensive accumulation of glycogen deposits in organs and in muscle tissue. Deficient enzymes involved in glycogen metabolism are responsible for glycogenoses. The most common disease is the autosomal recessive defect of glucose-6-phosphorylase wherein glycogen is synthesized, but cannot leave the cell.

The liver stores more and more glycogen, resulting in an enlarged liver (**hepatomegaly**) (up to 10 kg (22 lb)). Furthermore, glucose levels in the blood can no longer be maintained. This leads to severe **hypoglycemia** between meals.

So far, 11 distinct glycogen storage diseases and subforms have been identified. The typical symptoms and complications in addition to hepatomegaly include: hypoglycemia, nephromegaly, cirrhosis of the liver, and myasthenia.

These are the **most common types of glycogenoses**:

- Gierke’s disease (glycogen storage disease type 1)
- Pompe’s disease (glycogen storage disease type 2)
- Cori’s disease (glycogen storage disease type 3)

The treatment is aimed at maintaining a consistent blood glucose level in order to avoid severe hypoglycemia (especially at night).

Gluconeogenesis is the metabolic process by which organisms produce sugars (namely glucose) for catabolic reactions from non-carbohydrate precursors. Glucose is the only energy source used by the brain (with the exception of ketone bodies during times of fasting), testes, erythrocytes, and kidney medulla. In mammals this process occurs in the liver and kidneys.

Introduction

The need for energy is important to sustain life. Organisms have evolved ways of producing substrates required for the catabolic reactions necessary to sustain life when desired substrates are unavailable. The main source of energy for eukaryotes is glucose. When glucose is unavailable, organisms are capable of metabolizing glucose from other non-carbohydrate precursors. The process that coverts pyruvate into glucose is called gluconeogenesis. Another way organisms derive glucose is from energy stores like glycogen and starch.

Overview

Gluconeogenesis is much like glycolysis only the process occurs in reverse. However, there are exceptions. In glycolysis there are three highly exergonic steps (steps 1,3,10). These are also regulatory steps which include the enzymes hexokinase, phosphofructokinase, and pyruvate kinase. Biological reactions can occur in both the forward and reverse direction. If the reaction occurs in the reverse direction the energy normally released in that reaction is now required. If gluconeogenesis were to simply occur in reverse the reaction would require too much energy to be profitable to that particular organism. In order to overcome this problem, nature has evolved three other enzymes to replace the glycolysis enzymes hexokinase, phosphofructokinase, and pyruvate kinase when going through the process of gluconeogenesis:

1. The first step in gluconeogenesis is the conversion of pyruvate to phosphoenolpyruvic acid (PEP). In order to convert pyruvate to PEP there are several steps and several enzymes required. Pyruvate carboxylase, PEP carboxykinase and malate dehydrogenase are the three enzymes responsible for this conversion. Pyruvate carboxylase is found on the mitochondria and converts pyruvate into oxaloacetate. Because oxaloacetate cannot pass through the mitochondria membranes it must be first converted into malate by malate dehydrogenase. Malate can then cross the mitochondria membrane into the cytoplasm where it is then converted back into oxaloacetate with another malate dehydrogenase. Lastly, oxaloacetate is converted into PEP via PEP carboxykinase. The next several steps are exactly the same as glycolysis only the process is in reverse.
2. The second step that differs from glycolysis is the conversion of fructose-1,6-bP to fructose-6-P with the use of the enzyme fructose-1,6-phosphatase. The conversion of fructose-6-P to glucose-6-P uses the same enzyme as glycolysis, phosphoglucoisomerase.
3. The last step that differs from glycolysis is the conversion of glucose-6-P to glucose with the enzyme glucose-6-phosphatase. This enzyme is located in the endoplasmic reticulum.

Glycolysis

Regulation

Because it is important for organisms to conserve energy, they have derived ways to regulate those metabolic pathways that require and release the most energy. In glycolysis and gluconeogenesis seven of the ten steps occur at or near equilibrium. In gluconeogenesis the conversion of pyruvate to PEP, the conversion of fructose-1,6-bP, and the conversion of glucose-6-P to glucose all occur very spontaneously which is why these processes are highly regulated. It is important for the organism to conserve as much energy as possible. When there is an excess of energy available, gluconeogenesis is inhibited. When energy is required, gluconeogenesis is activated.

1. The conversion of pyruvate to PEP is regulated by acetyl-CoA. More specifically pyruvate carboxylase is activated by acetyl-CoA. Because acetyl-CoA is an important metabolite in the TCA cycle which produces a lot of energy, when concentrations of acetyl-CoA are high organisms use pyruvate carboxylase to channel pyruvate away from the TCA cycle. If the organism does not need more energy, then it is best to divert those metabolites towards storage or other necessary processes.
2. The conversion of fructose-1,6-bP to fructose-6-P with the use of fructose-1,6-phosphatase is negatively regulated and inhibited by the molecules AMP and fructose-2,6-bP. These are reciprocal regulators to glycolysis' phosphofructokinase. Phosphofructosekinase is positively regulated by AMP and fructose-2,6-bP. Once again, when the energy levels produced are higher than needed, i.e. a large ATP to AMP ratio, the organism increases gluconeogenesis and decreases glycolysis. The opposite also applies when energy levels are lower than needed, i.e. a low ATP to AMP ratio, the organism increases glycolysis and decreases gluconeogenesis.
3. The conversion of glucose-6-P to glucose with use of glucose-6-phosphatase is controlled by substrate level regulation. The metabolite responsible for this type of regulation is glucose-6-P. As levels of glucose-6-P increase, glucose-6-phosphatase increases activity and more glucose is produced. Thus glycolysis is unable to proceed.

Regulation of blood glucose is largely done through the endocrine hormones of the pancreas, a beautiful balance of hormones achieved through a negative feedback loop. The main hormones of the pancreas that affect blood glucose include insulin, glucagon, somatostatin, and amylin.

Insulin (formed in pancreatic beta cells) lowers BG levels, whereas glucagon (from pancreatic alpha cells) elevates BG levels.

Somatostatin is formed in the delta cells of the pancreas and acts as the "pancreatic policeman," balancing insulin and glucagon. It helps the pancreas alternate in turning on or turning off each opposing hormone.

Amylin is a hormone, made in a 1:100 ratio with insulin, that helps increase **satiety**, or satisfaction and state of fullness from a meal, to prevent

overeating. It also helps slow the stomach contents from emptying too quickly, to avoid a quick spike in BG levels.

As a meal containing carbohydrates is eaten and digested, BG levels rise, and the pancreas turns on insulin production and turns off glucagon production. Glucose from the bloodstream enters liver cells, stimulating the action of several enzymes that convert the glucose to chains of glycogen—so long as both insulin and glucose remain plentiful. In this postprandial or "fed" state, the liver takes in more glucose from the blood than it releases. After a meal has been digested and BG levels begin to fall, insulin secretion drops and glycogen synthesis stops. When it is needed for energy, the liver breaks down glycogen and converts it to glucose for easy transport through the bloodstream to the cells of the body (Wikipedia, 2012a).

In a healthy liver, up to 10% of its total volume is used for glycogen stores. Skeletal muscle cells store about 1% of glycogen. The liver converts glycogen back to glucose when it is needed for energy and regulates the amount of glucose circulating between meals. Your liver is amazing in that it knows how much to store and keep, or break down and release, to maintain ideal plasma glucose levels. Imitation of this process is the goal of insulin therapy when glucose levels are managed externally. Basal–bolus dosing is used as clinicians attempt to replicate this normal cycle.

While a healthy body requires a minimum concentration of circulating glucose (60–100 mg/dl), high chronic concentrations cause health problems and are toxic:

- **Acutely**: Hyperglycemia of >300 mg/dl causes polyuria, resulting in dehydration. Profound hyperglycemia (>500 mg/dl) leads to confusion, cerebral edema, coma, and, eventually, death (Ferrante, 2007).
- **Chronically**: Hyperglycemia that averages more than 120 to 130 mg/dl gradually damages tissues throughout the body and makes a person more susceptible to infections. The glucose becomes syrupy in the bloodstream, intoxicating cells and competing with life-giving oxygen.

The concentration of glucose in the blood is determined by the balance between the rate of glucose entering and the rate of glucose leaving the circulation. These signals are delivered throughout the body by two pancreatic hormones, insulin and glucagon (Maitra, 2009). Optimal health requires that:

- When blood glucose concentrations are low, the liver is signaled to add glucose to the circulation.
- When blood glucose concentrations are high, the liver and the skeletal muscles are signaled to remove glucose from the circulation.

The final stage of aerobic respiration is the **electron transport chain**, which is located on the inner mitochondrial membrane

- The inner membrane is arranged into folds (cristae), which increases the surface area available for the transport chain

The electron transport chain releases the energy stored within the reduced hydrogen carriers in order to synthesise ATP

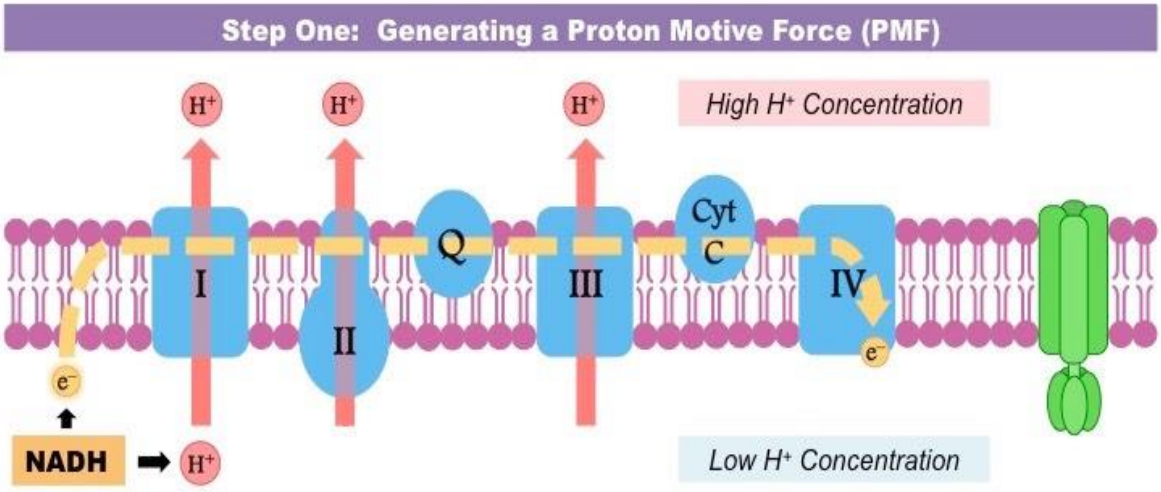
- This is called oxidative phosphorylation, as the energy to synthesise ATP is derived from the oxidation of hydrogen carriers

Oxidative phosphorylation occurs over a number of distinct steps:

- Proton pumps create an electrochemical gradient (proton motive force)
- ATP synthase uses the subsequent diffusion of protons (chemiosmosis) to synthesise ATP
- Oxygen accepts electrons and protons to form water

Step 1: Generating a Proton Motive Force

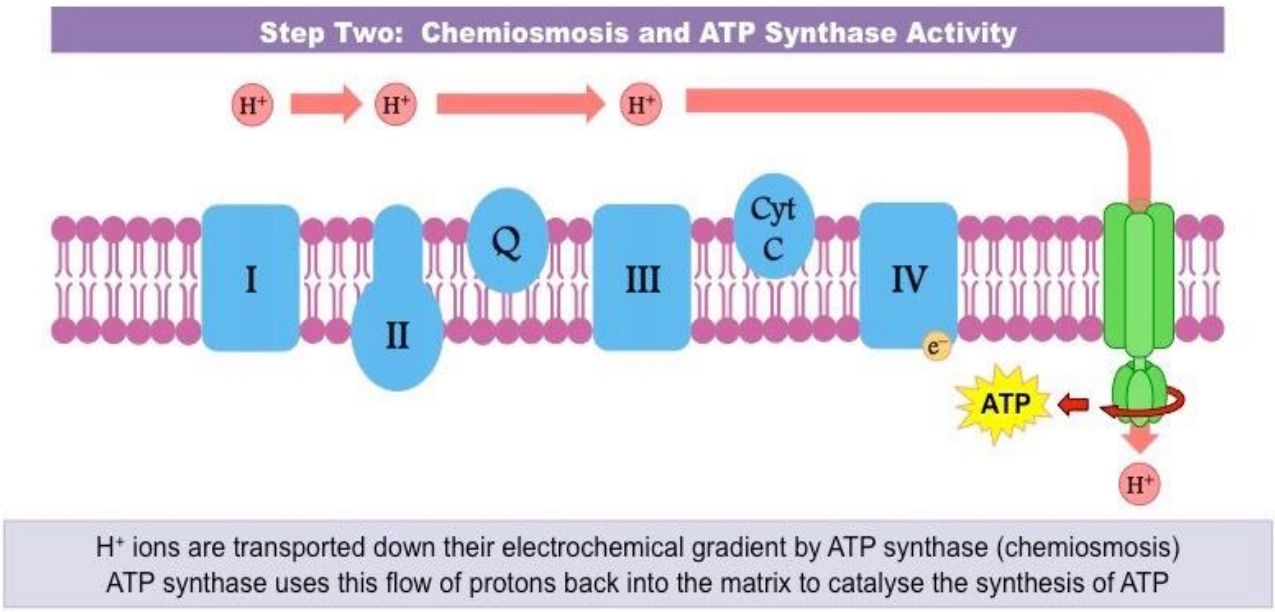
- The hydrogen carriers (NADH and $FADH_2$) are oxidised and release high energy electrons and protons
- The electrons are transferred to the electron transport chain, which consists of several transmembrane carrier proteins
- As electrons pass through the chain, they lose energy – which is used by the chain to pump protons (H^+ ions) from the matrix
- The accumulation of H^+ ions within the intermembrane space creates an electrochemical gradient (or a proton motive force)



High energy electrons released by hydrogen carriers are shuttled through the electron transport chain
The released energy is used to translocate H^+ ions from the matrix, creating an electrochemical gradient

Step Two: ATP Synthesis via Chemiosmosis

- The proton motive force will cause H^+ ions to move down their electrochemical gradient and diffuse back into matrix
- This diffusion of protons is called chemiosmosis and is facilitated by the transmembrane enzyme ATP synthase
- As the H^+ ions move through ATP synthase they trigger the molecular rotation of the enzyme, synthesising ATP

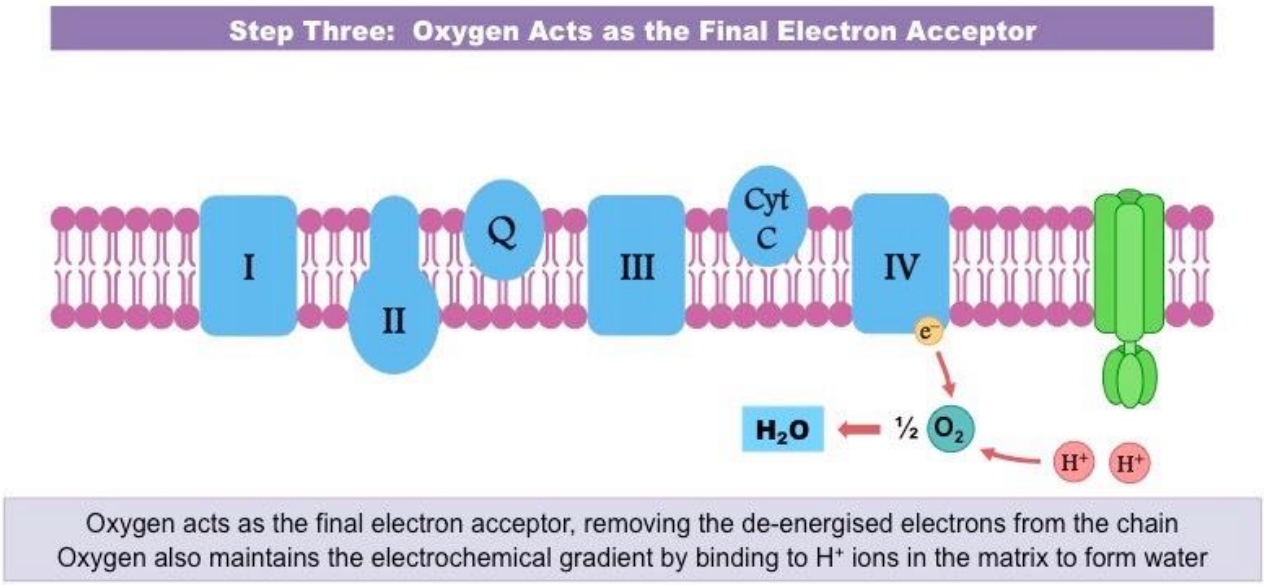


H⁺ ions are transported down their electrochemical gradient by ATP synthase (chemiosmosis)
ATP synthase uses this flow of protons back into the matrix to catalyse the synthesis of ATP

• Oxygen is needed to bind with the free protons to maintain the hydrogen gradient, resulting in the formation of water

Step Three: Reduction of Oxygen

- In order for the electron transport chain to continue functioning, the de-energised electrons must be removed
- Oxygen acts as the final electron acceptor, removing the de-energised electrons to prevent the chain from becoming blocked
- Oxygen also binds with free protons in the matrix to form water – removing matrix protons maintains the hydrogen gradient
- In the absence of oxygen, hydrogen carriers cannot transfer energised electrons to the chain and ATP production is halted



Oxidative Phosphorylation

- Hydrogen carriers donate high energy electrons to the electron transport chain (located on the cristae)
- As the electrons move through the chain they lose energy, which is transferred to the electron carriers within the chain
- The electron carriers use this energy to pump hydrogen ions from the matrix and into the intermembrane space
- The accumulation of H^+ ions in the intermembrane space creates an electrochemical gradient (or a proton motive force)
- H^+ ions return to the matrix via the transmembrane enzyme ATP synthase (this diffusion of ions is called chemiosmosis)
- As the ions pass through ATP synthase they trigger a phosphorylation reaction which produces ATP (from ADP + Pi)
- The de-energised electrons are removed from the chain by oxygen, allowing new high energy electrons to enter the chain
- Oxygen also binds matrix protons to form water – this maintains the hydrogen gradient by removing H^+ ions from the matrix

What is Oxidative Phosphorylation?

Oxidative phosphorylation, also known as electron transport-linked phosphorylation, refers to the metabolic pathway in which the energy released by nutrients during oxidation is utilized to generate ATP through electrical transport chain. And it is an important cellular energy conversion process and the final process of cell respiration in eukaryotes.

Oxidative phosphorylation occurs in the mitochondrial inner membrane of eukaryotic cells or the cytoplasm of prokaryotes.

Oxidative Phosphorylation & Substrate-Level Phosphorylation

When it comes to oxidative phosphorylation, we have to talk about its "good partner"--substrate-level phosphorylation.

Substrate-level phosphorylation is a metabolic reaction in which the energy-rich phosphorylated compound resulting from the coupled reaction transfers its phosphate group to ADP for ATP synthesis. Or GDP is recharged a phosphate group to generate GTP.

● The Similarities between Oxidative Phosphorylation and Substrate-level Phosphorylation

The main similarity between oxidative phosphorylation and substrate-level phosphorylation is that both their ultimate production is ATP.

● The Differences between Oxidative Phosphorylation and Substrate-Level Phosphorylation

The biggest difference between oxidative phosphorylation and substrate-level phosphorylation is the source of the energy needed to convert ADP to ATP. Substrate level phosphorylation directly phosphorylates ADP to ATP by using the energy from a coupled reaction. While oxidative phosphorylation involves two coupled reactions that are considered to simultaneously occur. In the period

of oxidative phosphorylation, the energy produced during the oxidative reaction is transferred to ADP to form ATP.

The Function of Oxidative Phosphorylation

Oxidative phosphorylation provides bulk ATP for living organisms, and the ATP is the main energy source for maintaining life activity. Oxidative phosphorylation also involves the formation of reactive oxygen species (ROS) and the regulation of apoptosis.

The Process of Oxidative Phosphorylation

When a hydroelectric dam works, it converts potential energy released from the falling water into kinetic energy, which turns into electrical energy. Similar to the steps of generating electricity from a hydroelectric dam, ADP makes ATP by a process called chemiosmosis during oxidative phosphorylation.

In eukaryotes, when catabolism such as glycolysis or citric acid cycle occurs, NADH is produced, which is a coenzyme containing a very high transfer electrical potential. When NADH is oxidized in the mitochondrial matrix, its electrons pass through the electron transport chain (ETC) to the electron receptor-oxygen, and simultaneously releases energy that pumps the resulting hydrogen ions through the inner mitochondrial membrane. It spontaneously forms an electrochemical concentration gradient across the inner mitochondrial membrane due to a higher concentration of hydrogen ions in the intermembrane space and a lower concentration in the matrix. When hydrogen ions pass through the inner mitochondrial membrane across electrochemical gradient, ATP synthase captures the proton-motive force for the production of ATP. This process is called chemiosmosis.

The electron transport chain is a series of proteins located on the inner membrane of the mitochondria.

● **NADH-Coenzyme Q Oxidoreductase**

The first enzyme in the electron transport chain is the NADH-CoQ oxidoreductase, also known as NADH dehydrogenase or complex I, which is the first entry of protons through the electron transport chain. It catalyzes the oxidation of NADH through coenzyme Q10. As two electrons pass through complex I, four protons are pumped from the mitochondrial matrix into the intermembrane space.

● Succinic-Coenzyme Q Oxidoreductase

The second enzyme that allows protons to passes through the electron transport chain is succinic-coenzyme Q oxidoreductase, also known as succinate dehydrogenase or complex II. It catalyzes the oxidation of succinic acid to form fumarate and the reduction of coenzyme Q10 to ubiquinone (QH2). This reaction does not involve the transfer of electrons, nor does it pump out protons, providing less energy to compare with the oxidation process of NADH. The third entry to the proton on the electron transport chain is electron transfer flavin-coenzyme Q oxidoreductase, also known as electron transfer flavin dehydrogenase, which reduces Q10 by using electrons from electron transfer flavin in the mitochondrial matrix.

● Coenzyme Q-cytochrome C Reductase

Coenzyme Q-cytochrome C reductase, also known as complex III, catalyzes the oxidation of QH2, and the reduction of cytochrome c and ferritin. In this reaction, cytochrome C carries an electron. Coenzyme Q is reduced to QH2 on one side of the mitochondrial membrane, while QH2 is oxidized to coenzyme Q10 on the other side, resulting in the transfer of protons on the membrane, which also contributes to the formation of proton gradients.

● Cytochrome c Oxidase

The last protein complex in the electron transport chain is cytochrome c oxidase, also called complex IV. It mediates the final reaction on the electron transport chain - transferring electrons to the final electron receptor oxygen -

oxygen reduces to water - pumping protons through the membrane. At the end of this reaction, protons that directly pumped out and that consumed by the reduction of oxygen to water increase the proton gradient.

Finally, the proton-motive force generated by the proton concentration gradient drives the ATP synthase to phosphorylate ADP to form ATP.

There is another electron-donating molecule - FADH2 in eukaryotes. FADH2 is also the intermediate metabolite during the earlier stage of cellular respiration such as glycolysis or citric acid cycle. In the FADH2 electrical transport chain, FADH2 bypasses the complex I and enters the electrical transport chain by the complex II because it contains less electrical potential than NADH. FADH2 is oxidized to FAD and coenzyme Q is reduced to QH2 in the reaction. And this reaction does not pump out protons either. The subsequent reactions are nearly the same as those in the NADH2 electron transport chain.

Prokaryotes such as bacteria and archaea have many electron transfer enzymes that can use a very wide range of chemicals as substrates. As the same with eukaryotes, electron transport in prokaryotic cells also uses the energy released by oxidation from the substrate to pump protons across the membrane to create an electrochemical gradient, which drives ATP synthase to generate ATP. The difference is that bacteria and archaea use many different substrates as electron donors or electron receptors. This also helps prokaryotes to survive and grow in different environments.

Factors Affecting Oxidative Phosphorylation

● Inhibitors

Under normal conditions, electron transfer and phosphorylation are tightly coupled. Some compounds can affect electron transport or interfere with phosphorylation reactions, all of which cause oxidative phosphorylation abnormalities. Here introduce four factors affecting oxidative phosphorylation.

Respiratory chain inhibitor: A substance that blocks electron transport at a certain part of the respiratory chain and inhibits the oxidation process. Some respiratory chain inhibitors bind to iron-sulfur proteins in NADH-Q reductase and block the transmission of electrons from NADH to CoQ, such as rotenone, phenoxymycin A, and barbital, ampicillin. Some substances inhibit the electron transfer between Cytb and Cytc1, such as antimycin A and dimercaptopropanol. Cyanide, azide, H2S, and C0 inhibit cytochrome oxidase, making electrons unable to pass to oxygen.

Oxidative phosphorylation inhibitors: These reagents directly interfere with the formation of ATP and also prevent electron transfer. The combination of oligomycin and dicyclohexylcarbonyldiimide with the F0 unit of ATP synthase prevents the hydrogen ions from flowing back from the proton channel, rendering the phosphorylation process incomplete, thus blocking the oxidative phosphorylation of intact mitochondria.

Uncoupling agent: The uncoupling agent separates the two coupling processes of electron transfer and ATP synthesis. Such compounds only inhibit the formation of ATP, but do not affect the electron transfer process. So the free energy generated by electron transfer is converted into heat energy, which excessively uses oxygen and fuel substrates. Such agents cause the electron transfer to lose normal control, resulting in excessive utilization of oxygen and fuel substrates, and energy is not stored. A typical uncoupler is 2,4-dinitrophenol (DNP). Because DNP is a fat-soluble substance, it can move freely in the mitochondrial membrane. When it enters the matrix, it can release H+. Return to the cytosol side. The H+ can be combined to eliminate the transmembrane gradient of H+, so that the energy released by the oxidation process cannot be used for the synthesis reaction of ATP, but referred to as a proton carrier.

● The Regulation of ADP

The rate of oxidative phosphorylation in normal organisms is mainly regulated by ADP. When the body uses ATP increase, the ADP concentration increases and the oxidative phosphorylation rate is increased after transporting into the mitochondria; otherwise, the ADP deficiency causes the oxidative phosphorylation rate to slow down. This regulation allows the rate of ATP production to adapt to physiological needs.

- **Thyroid Hormone**

Thyroid hormone can activate Na+-K+ATPase on the cell membrane of many tissues, accelerate the decomposition of ATP into ADP and Pi, and increase the number of ADP into mitochondria, thus decreasing the ATP/ADP ratio and accelerating the oxidative phosphorylation rate. As the synthesis and decomposition rate of ATP increases, the body's oxygen consumption and heat production increase, the basal metabolic rate increases, and the basal metabolic rate are one of the most important clinical indications for patients with hyperthyroidism.

- **Mitochondrial DNA Mutation**

Due to its naked circular double helix structure and the absence of protein protection and damage repair system of mitochondrial DNA (mtDNA), it is susceptible to mutate by oxidative phosphorylation. mtDNA encodes 13 proteins involved in oxidative phosphorylation. Therefore, mtDNA mutations can affect the oxidative phosphorylation process, resulting in a decrease in ATP yield and thus leading to many related diseases.

Diseases and Abnormal Oxidative Phosphorylation

Oxidative phosphorylation exerts a multiply role in the body. So once it is abnormal, it will cause diseases.

Many mitochondrial diseases are linked to defective oxidative phosphorylation. And tissues with high energy requirements are particularly susceptible to undergo oxidative phosphorylation defects, like brain, nerves, retina, bone and heart muscle. When there is impairment in oxidative phosphorylation in these

tissues, it could clinically manifest as seizures, hypotonia, ophthalmoplegia, convulsions, muscle weakness, and cardiomyopathy, etc.

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