Concept of metabolism

Introduction, anabolic and catabolic pathways, regulation of metabolism, role of regulatory enzymes; classification, nomenclature and importance of enzyme; concept of coenzyme, apoenzyme and prosthetic group.

The entire spectrum of chemical reactions occurring in the living system is collectively referred to as **metabolism**. Metabolism is the total amount of the biochemical reactions involved in maintaining the living conditions of the cells in an organism. Metabolism encompasses the various biochemical processes, reactions, and conversions that transform one form of energy to another.

All living organisms require energy for different essential processes and for producing new organic substances. Any molecule that is synthesized or utilized in metabolism is a physically-recognizable form of energy.

In Biology, the definition of metabolism goes by *"life-sustaining chemical reactions involving biologically-active chemical compounds and molecules"*. Therefore, metabolism is the biological way to conserve energy in some or the other form when different types of organisms produce or metabolize biologically-active chemical molecules.

The entire process of nutrition has two main parts- **ingestion** of food and **utilization** of food for energy. In every living organism, let it be a simple prokaryotic bacterial cell or a eukaryotic cell, the process of nutrition is the same. The food which we eat happens to be useless until and unless it undergoes metabolic changes.

During metabolism, **biomolecules** present in the food get utilized to extract the energy from the cell. In addition, conversion and formation of the biomolecules take place. In other words, the transformation of one compound results in the formation of another molecule. For example, the proteins we obtained from the food are metabolized into amino acids, which are later utilized to synthesize another protein required by the cell.

The concept of metabolic reactions concentrates on the utilization of food for energy. Ingested food needs to be utilized for the turnover. The nutrition is the key and energy extraction is the target of metabolism. The dynamic state of body constituents and the concept of **metabolism** are discussed below in detail.

When we refer to biologically-active molecules, we are actually referring to chemical molecules that have biological activity and play a pivotal role in sustaining essential biological pathways.

There are primarily 4 basic biochemicals: **carbohydrates**, **lipids**, **nucleic acids**, and **proteins**. Apart from these four, there are two more biochemicals that are generally studied. They are coenzymes and minerals. All these molecules play some of the most vital

roles without which the proper functioning, coordination, and efficiency of biological systems is not possible. It is therefore important to learn about each of them in more detail and gain some useful insights about their roles and purposes in metabolic activities.

Anabolism (making of bonds) **i**s the *constructive metabolism* involves <u>synthesis</u> and production of complex molecules from simpler <u>monomers</u> of biochemicals. The biosynthetic reactions involving the formation of complex molecules from simple precursors constitute anabolism. Thus anabolism is the sequence of enzyme catalyzed reactions in which nutrients are used to form comparatively complex molecules in the living cells with moderately simpler structures.

The process of anabolism is also referred to as biosynthesis. The process includes the production of components of cells such as proteins, carbohydrates, lipids which require energy in the form of ATP which are energy rich compounds. These compounds are synthesized during the breakdown processes such as catabolism. Anabolinc processes in growing cell control catabolic processes.

A clear demarcation between catabolism and anabolism is rather difficult. Since there are several intermediates common to both the processes. The term **amphibolism** is also in use for reactions which are both catabolic and anabolic in nature. For eg. Citric acid cycle (Krebs cycle) – it is amphibolic pathway because it functions in both the degradative and biosynthetic processes.

The anabolic and catabolic pathways are not reversible and operate independently.

Generally, the metabolic pathways occur in specific cellular locations (mitochondria, microsomes, etc.) are controlled by different regulatory signals. Intermediary metabolism refers to the entire range of catabolic and anabolic reactions, not involving nucleic acid. Energy metabolism deals with the metabolic pathways concerned with the storage and liberation of energy.

All metabolic changes take place in multiple reactions and follow a particular pathway called the metabolic pathway. The metabolic pathway includes a series of reactions. The metabolite flow, the rate, and direction at which metabolism takes place are called the dynamic state of body constituents. All metabolic reactions are catalyzed by a set of proteinaceous compounds called **enzymes**. Hence, metabolism is an enzyme-catalyzed reaction which provides biomolecules, needed by the cells for growth, maintenance, and repair etc. The purposes of metabolic pathways can be summarized in the below three points:

> ➤ To extract energy from the food for cellular activities.

> ➤ To convert food to building blocks, to synthesize biomolecules such as carbohydrates, proteins, lipids and nucleic acids.

> ➤ To eliminate waste and toxic products.
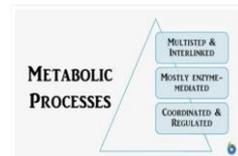
Metabolic processes are tightly-regulated, interlinked processes that aid in molecular conversions from one form to another

Regulation of metabolism

For life to proceed in orderly fashion, the flow of metabolites participating in anabolic and catabolic pathways must be regulated.

Regulation of metabolic processes is achieved in a number of ways. Since all cellular reactions are catalysed by specific enzymes, the rate of metabolic pathway depends on the concentrations (also called levels) and catalytic efficiency of enzymes of that pathway.

Not all the enzymes of any pathway are under regulatory control, nor it is necessary to do so from the cellular economy point of view. An overall regulation is achieved by modulation of the rates of only some reactions which occur at critical points along the pathway, e.g., the first step or a branching point where more than one pathway meet, etc.

As the environments of most organisms are constantly changing, the reactions of metabolism must be finely regulated to maintain a constant set of conditions within cells, a condition called homeostasis.

Metabolic regulation also allows organisms to respond to signals and interact actively with their environments.

Two closely linked concepts are important for understanding how metabolic pathways are controlled.

Firstly, the *regulation* of an enzyme in a pathway is how its activity is increased and decreased in response to signals.

Secondly, the *control* exerted by this enzyme is the effect that these changes in its activity have on the overall rate of the pathway (the flux through the pathway).

For example, an enzyme may show large changes in activity (*i.e.* it is highly regulated) but if these changes have little effect on the flux of a metabolic pathway, then this enzyme is not involved in the control of the pathway.

There are multiple levels of metabolic regulation - intrinsic and extrinsic regulation

In intrinsic regulation, the metabolic pathway self-regulates to respond to changes in the levels of substrates or products; for example, a decrease in the amount of product can increase the flux (the rate of turnover of molecules) through the pathway to compensate. This type of regulation often involves allosteric regulation of the activities of multiple enzymes in the pathway.

Extrinsic control involves a cell in a multicellular organism changing its metabolism in response to signals from other cells. These signals are usually in the form of water-soluble messengers such as hormones and growth factors and are detected by specific receptors on the cell surface. These signals are then transmitted inside the cell by second messenger systems that often involved the phosphorylation of proteins.

ENZYME

Enzymes are central to metabolism since these are the biocatalysts catalyzing almost all cellular reactions. These include slow but thermodynamically feasible reactions at ambient cellular conditions, which include biological pH, temperature, as well as the molar concentrations of the reactants and the products. The metabolic pathways need to be modified in response to the cellular needs. This occurs through regulation of the activity of various enzymes.

General Characteristics of Enzymes

An enzyme is usually a **protein, acting as catalyst in specific biochemical reaction**. Every reaction in the cell requires its own specific enzyme. Each cell in the human body contains 1,000s of different enzymes. Most enzymes are globular proteins, while a few enzymes are made of RNA. These catalyze biochemical reactions involving nucleic acids. Enzymes undergo all the reactions of proteins and denaturation of enzymes takes place due to pH or temperature change. For eg. A person suffering high fever runs the risk of denaturing certain enzymes.

Discovery:

Discovered in yeast for the first time, the biocatalysts were called "enzyme" by Wilhelm Kuhne in 1878 (Greek **en** - in; **zyme** – yeast) i.e. "in yeast". Earlier Louis Pasteur had called these vital factors present in intact yeast cells as "ferments," since these were thought to be responsible for carrying out fermentation. Chemical nature of enzymes was not established till the time Sumner crystallized *urease* from jack beans and established their proteinaceous

nature in 1926. Earlier enzymes were thought to be small biologically active molecules analogous to hormones. Sumner postulated that all enzymes were proteins. It was after John Northrop and Moses Kunitz had crystallized trypsin and pepsin in 1930 that Sumner's conclusions were widely accepted. Sumner was awarded the Nobel Prize in 1946.

Haldane postulated that the weak bonding between enzyme and substrate might be responsible for the reactions catalyzed by them. Since then thousands of enzymes have been isolated and characterized. A new science called "enzymology" developed, which dealt with study of enzymes. Barring ribozymes (catalytic RNA molecules), all enzymes are proteins. While some of the enzymes consist of proteins only (simple proteins), in others a non-protein part is also part of their structures (conjugated proteins).

Structure and composition of Enzymes

The enzymes which are wholly made up of proteins are called simple enzymes, eg. Urease, amylase, pepsin, trypsin, etc. Most of the enzymes are made up of two parts – the protein and non protein part. Such enzymes are called **conjugated enzymes**.

Major part of enzyme is a protein part called **apoenzyme**. The protein part is supplemented with non-protein part which is essential for enzymatic activity. The non protein part, when tightly bound to apoenzyme, is called **prosthetic group**, but if it is not tightly bound, it is known as **coenzyme** or **cofactor**. The complete enzyme including the prosthetic group and/or coenzyme is often called **holoenzyme**.
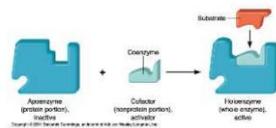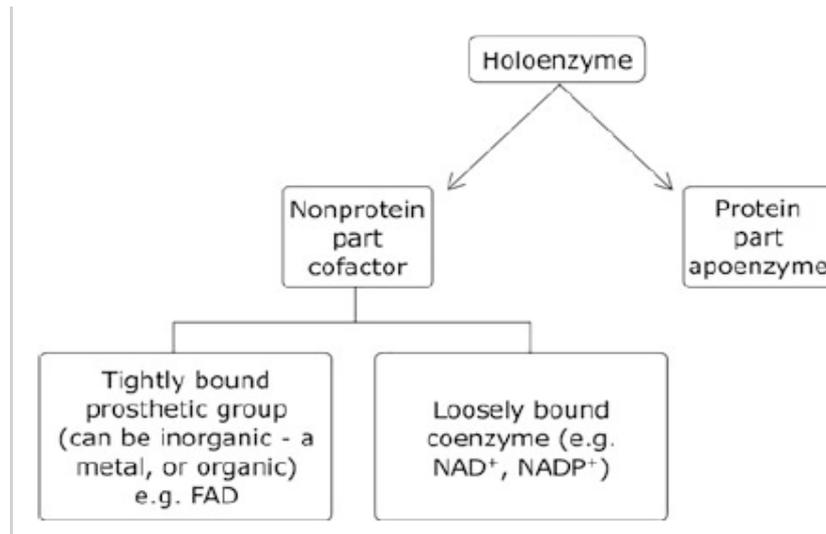
The enzymes consist of following structures

Protein part or Apoenzyme

Protein part of apoenzyme constitute the major portion of an enzyme. Different enzymes possess different sequences of amino acids and variable length of protein molecules. The specific sequence of amino acids in a protein molecule provides specific nature of enzymes which is important for the biological activity. The proteins are colloidal in nature and therefore provide a very large surface area per unit volume, which is characteristic of enzymes. Only a few enzymes are entirely made up of protein. Mostly they are associated with prosthetic group or coenzymes. Proteolytic enzymes for example, are entirely proteins. They do not possess non protein part.

Active site

Experimental finding demonstrate that the enzyme catalyses the reaction by combining with the substrate. The substrate physically comes in contact with the specific site in the protein molecule. The surface of folded protein possess specific binding site known as active site. The active site may thus be defined as that particular part of the protein which binds to the substrate to form an enzyme substrate complex. Some enzymes possess only one active site, while others have several such sites. The substrate binding site is termed as active site, while regulatory substances are bind with the regulatory site.

Prosthetic group

Many enzyme require the presence of an essential non-protein part for efficient performance of their function. The non protein part which is tightly bound to enzyme is termed as prosthetic group. The simple prosthetic groups are metalic ions, such as Cu, Zn, Mn, Mo, etc. Other prosthetic groups are organic compounds which include cytochrome, flavoproteins,

pyridoxal phosphate, heme iron or biotin etc. These groups are associated with the active sites of enzyme molecules and in some cases they undergo a reversible chemical change.

Cofactors and Coenzymes

The non-protein part of these enzyme (conjugated proteins) is called **cofactor**. The term cofactor is usually employed for **inorganic ions** such as chloride (Cl-) and magnesium (Mg^{2+}). In case where cofactor is **inorganic**, such as metals (Mg^{2+}, Zn^{2+}, Fe^{2+}), the enzymes are called as **metalloenzymes**.

The **organic** cofactors are called **coenzymes**. Cofactors may be loosely bound with the enzyme proteins or may be tightly associated through a covalent bond. Cofactors, which are tightly associated with the protein part of the enzymes, are called prosthetic group, which may be inorganic or organic in nature. *Coenzymes bind loosely to the active site of enzymes*.

Sometimes both metal and the organic molecules are required as the cofactors for enzyme activity. In case of cytochromes, the prosthetic group **heme**, along with a metal ion (Fe^{3+}), is bound to enzyme protein through hydrogen bonding, hydrophobic interactions, and the covalent bonding to a specific site of the enzyme protein.

The functionally active enzyme, in case of conjugated proteins, is called **holoenzyme**, and the protein portion of the enzyme is called **apoenzyme**. There is no clear cut distinction between 'prosthetic group' and coenzymes. It is conveniently defined that the prosthetic groups are tightly bound to enzymes whereas the coenzymes are loosely attached. Since these substances are intimately concerned with the overall reactions, they are termed as cofactors or coenzymes.

Apoenzyme (protein portion), inactive + Cofactor (nonprotein portion), activator → Holoenzyme (whole enzyme), active
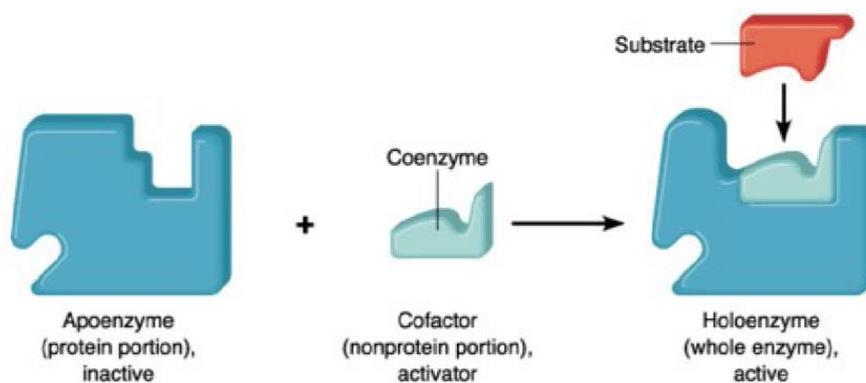
Nomenclature and Classification of Enzymes

After thousands of enzymes had been discovered, different strategies were adopted for nomenclature of enzymes -

(i) By adding suffix "-ase" to the name of the substrate: Substrate is the substance on which the enzyme acts upon. For example, enzymes acting upon proteins were called proteinases, those acting upon lipids were called as "lipases," and the ones acting on nucleic acid were named as "nucleases." Specific names were also given to enzymes acting on specific substrates, such as "urease," "lecithinase," or "maltase," for the enzymes acting on urea, lecithine, or maltose, respectively.

(ii) Another strategy adopted to name the enzymes was to add suffix "-ase" to the kind of reaction catalyzed by the enzymes, e.g., isomerases (which catalyze isomerization), hydrolases (catalyzing hydrolysis reactions), transaminases (catalyzing transamination), etc.

(iii) Both of the above systems of naming the enzymes appeared inadequate since the naming was either based on the type of molecules on which the enzyme acted upon or the type of reaction catalyzed by them. Another system was adopted in which some of the enzymes were named both on the basis of substrate utilized and the reaction catalyzed by them. For example, succinic acid dehydrogenase signifies both the name of the substrate succinic acid as well as the reaction catalyzed by them dehydrogenation.

To maintain uniformity in naming enzymes the International Union of Biochemistry and Molecular Biology (IUBMB) set up an Enzyme Commission (EC) on enzyme nomenclature, which gave its first recommendations in 1961. Some of the recommendations given by EC are as follows:

1. Each enzyme can have a trivial name, which is short and is easy to use. The systematic name of the enzyme however should be formed according to the definite rules showing the action of the enzyme as much as possible.

It should have two parts: the first name denotes the substrate, the second one with the suffix "-ase" which specifies the reaction catalyzed by them. Additional information, if there is any, is given in parenthesis. For example, malate dehydrogenase whichcatalyzes the following reaction:

$$L - Malate + NAD^+ \rightarrow Pyruvate + CO_2 + NADH + H^+$$
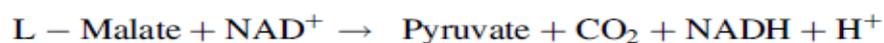
The enzyme can be called L-malate: NADH oxidoreductase (decarboxylating).

2. All enzymes were classified into six classes depending upon the type of reaction catalyzed by them. Each enzyme is given a classification number. The classification number is known as Enzyme Commission (EC) number assigned by the nomenclature committee of IUBMB.

The classification number has four digits, e.g., if the classification number of an enzyme is a, b, c, and d; here 'a' stands for the number of the class given in the classification

number, 'b' is the number of subclass, 'c' is the number of sub-subclass, while 'd' represents the number of sub-sub-subclass which specifies the actual substrate of the enzyme which distinguishes it from other enzymes catalyzing similar reactions.

In the following enzyme-catalyzed reaction:

$$ATP + D - glucose \rightarrow ADP + glucose\ 6 - phosphate$$
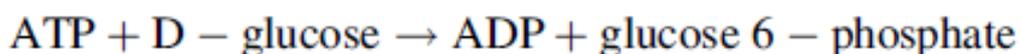
- The trivial name of the enzyme is hexokinase/glucokinase, which is commonly used. The systematic name of the enzyme catalyzing the reaction is ATP: glucose phosphotransferase which indicates that the enzyme catalyzes transfer of phosphoryl group from ATP to glucose.

- The classification number (Enzyme Commission number) is E.C 2.7.1.1. The first number 2 signifies class number (transferase); the second number 7 is about the phosphate group transferred; the third number 1 is about the number of the sub-subclass which signifies a phosphotransferase with a hydroxyl group as an acceptor, while the last digit 1 is the number of sub-subsubclass which includes D-glucose as the phosphoryl group acceptor.

Major classes of enzymes -

Class No.	Name of the class	Nature of the reaction catalyzed
1	Oxidoreductases	• Catalyze transfer of hydrogen or oxygen atoms or electrons from one substrate to another, also called oxidases, dehydrogenases, or reductases. • Substrate that is oxidized is electron donor. • Systematic name is based on *donor: acceptor group oxidoreductase*. • Common name will be dehydrogenase except where electron acceptor is oxygen, then called oxidases
2	Transferases	• Catalyze group transfer reactions. • Systematic names are formed according to the scheme *donor: acceptor group transferase*. • Common name according to acceptor group transferase or donor group transferase.
3	Hydrolases	• Catalyze hydrolytic cleavage of C-C, C-O, and C-N bonds and some other bonds including phosphoanhydride bonds. • Common name in many cases formed by the name of the substrate with suffix "-ase"
4	Lyases	• Catalyze cleavage of C-C, C-O, C-N, or other bonds by elimination, leaving double bonds or rings, or catalyze addition of groups to double bonds.

		• Systematic name is formed according to the pattern substrate group-lyase. Hyphen is important part of the name.
5	Isomerases	• Catalyze cleavage of C-C, C-O, C-N, or other bonds by elimination, leaving double bonds or rings, or catalyze addition of groups to double bonds. • Systematic name is formed according to the pattern substrate group-lyase. Hyphen is important part of the name.
6	Ligases	• Catalyze joining together two molecules forming C-C, C-O, C-S, and C-N bonds by condensation reactions coupled with hydrolysis of ATP or similar triphosphate.