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COLLEGE OF EDUCATION

BAGONG PILIPINAS

Biochemistry

Chapter 2: Proteins &
Chapter 3: Enzymes

SCI-M 227: Biochemistry Lecture
Schedule: TTh 8:30 am -10:00 am
Section: BSED-SCIENCE 2-2 and 2-B

Organized Content

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Submitted to:
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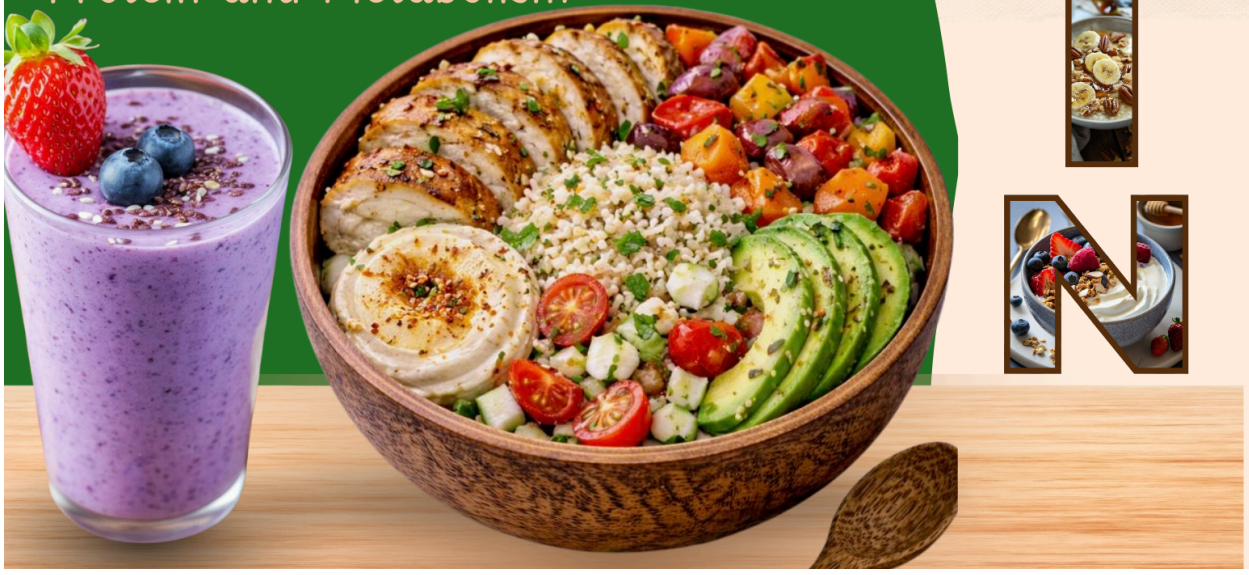
LEARNING OUTLINE

CHAPTER 2: PROTEINS

2.1. Amphoteric Property of Amino Acids
2.2 Properties of proteins
2.3 Structure of proteins
2.4 Peptide formation
2.5. Levels of Protein Organization
2.6 Classification Biological Functions and Tests for Proteins
2.7 Definition, types, stages, and Biological Importance of metabolism
2.8 Digestion and Absorption of Carbohydrates
2.9 Disease related to Protein
2.10 Recent Developments Concerning Protein and Metabolism



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PROTEINS

INTRODUCTION

Proteins are one of the most essential and versatile biomolecules in living organisms. They are large, complex molecules made up of amino acids linked together in specific sequences, forming unique three-dimensional structures that determine their functions. Proteins are often described as the “building blocks of life” because they play a role in nearly every biological process—from forming body structures and transporting substances to regulating metabolism and defending the body against disease.

At the cellular level, proteins serve a wide variety of functions. They act as enzymes that speed up chemical reactions, hormones that regulate body processes, antibodies that protect against infections, and structural components that give cells and tissues their shape and strength. Proteins are also involved in movement, communication between cells, and the repair and maintenance of body tissues. Because of their diverse roles, proper protein structure and function are vital for normal growth, development, and overall health.

Proteins are formed from amino acids, which possess unique chemical properties that influence how proteins behave and function. The way amino acids bond together and fold into specific shapes determines the stability, activity, and biological role of each protein. These structures can range from simple chains to highly organized, multi-level arrangements, allowing proteins to perform specialized and highly efficient tasks. However, when protein structure or metabolism is disrupted, it can lead to various health problems and diseases.

This discussion will provide a comprehensive overview of proteins and their importance in biological systems. It will begin by examining the amphoteric properties of amino acids and the general properties of proteins. It will then explore how proteins are structured, how peptide bonds are formed, and the different levels of protein organization. The classification of proteins, their biological functions, and the methods used to test them will also be covered. In addition, the concept of metabolism—including its definition, types, stages, and biological importance—will be discussed, along with the digestion and absorption of carbohydrates as part of energy processing. Finally, the discussion will address diseases related to protein dysfunction and highlight recent developments in protein research and metabolism, demonstrating how scientific advances continue to improve our understanding of health and disease.

2.1. AMPHOTERIC PROPERTY OF PROTEINS

What is the amphoteric property of amino acids?

Amino acids are amphoteric molecules because they contain both an acidic group (α-carboxyl, –COOH) and a basic group (α-amino, $–NH_2$) within the same structure. This dual nature allows them to act as proton donors or acceptors depending on the pH of their environment.

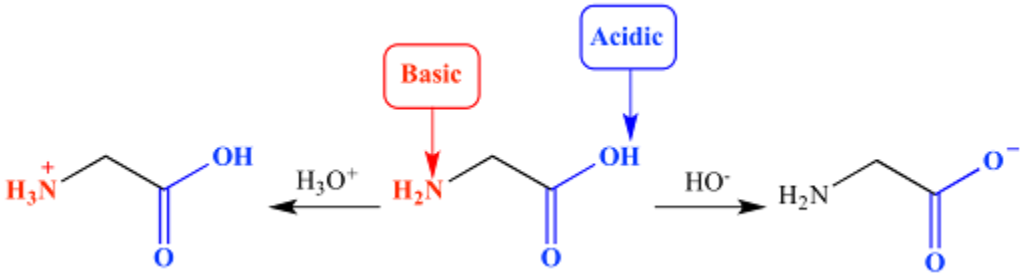


Figure 1. *Amphoteric behavior of glycine showing proton donation (acidic) and acceptance (basic) depending on pH.*

In aqueous solution near neutral pH, amino acids exist predominantly as zwitterions (dipolar ions). The carboxyl group loses a proton to become negatively charged ($–COO^-$), while the amino group gains a proton to become positively charged ($–NH_3^+$). Although the molecule carries both positive and negative charges, its net charge is zero. This zwitterionic form explains why amino acids have high melting points (often above 200°C) and are soluble in water but insoluble in nonpolar solvents.

The isoelectric point (pI) is the specific pH at which an amino acid carries zero net charge. At pH values below the pI, the molecule has a net positive charge and migrates toward the cathode in an electric field. At pH values above the pI, it has a net negative charge and migrates toward the anode. The pI can be calculated from the pKa values of ionizable groups:

- For neutral amino acids (e.g., glycine, alanine): $pI = (pKa_1 + pKa_2)/2$
  Example: Glycine ($pKa_1 = 2.34$, $pKa_2 = 9.60$) → $pI = 5.97$

- For acidic amino acids (aspartic acid, glutamic acid) with an extra carboxyl group: $pI = (pKa_1 + pKa\_R)/2$
  Example: Aspartic acid ($pKa_1 = 1.88$, $pKa\_R = 3.65$) → $pI = 2.77$

- For basic amino acids (lysine, arginine, histidine) with an extra basic group: pI = (pKa_R + pKa_2)/2

  Example: Lysine (pKa_R = 10.53, pKa_2 = 8.95) → pI = 9.74

Proteins and amino acids show minimum solubility at their pI because the absence of net charge eliminates electrostatic repulsion between molecules, allowing aggregation and precipitation. This principle is used in protein purification techniques such as isoelectric precipitation.

Histidine has special biochemical importance because its imidazole side chain has a pKa near 6.0. When incorporated into proteins, this pKa can shift to 6.5–7.5, making histidine an effective buffer at physiological pH. Consequently, histidine residues frequently appear in enzyme active sites where proton transfer is required (e.g., in carbonic anhydrase and chymotrypsin).

The amphoteric nature of proteins underlies several key separation techniques:

• **Ion-exchange chromatography**: Proteins bind to charged resins based on their net charge at a given pH. Cation exchangers bind positively charged proteins (pH < pI); anion exchangers bind negatively charged proteins (pH > pI).

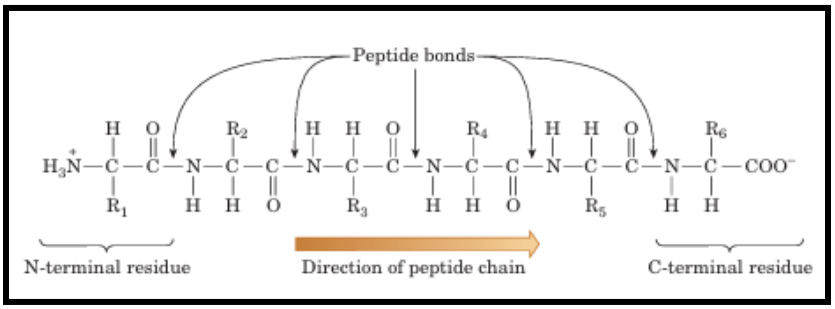
• **Isoelectric focusing**: Proteins migrate through a pH gradient until reaching the pH region matching their pI, where they stop moving. This technique can resolve proteins differing by as little as 0.01 pH units and is used as the first dimension in 2D gel electrophoresis.

2.2 PROPERTIES OF PROTEINS

What are the properties of Proteins?

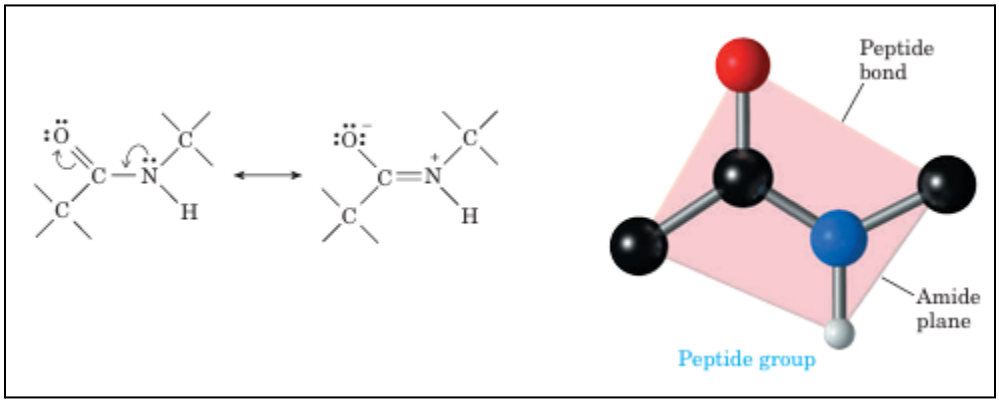
- The properties of proteins are based on properties of the peptide backbone and properties of the side chains. The peptide backbone consists of the repeating structure shown by the horizontal line of atoms in Figure 1.
- The atoms along the backbone are linked N-C-C-N-C-C- and so on. By convention, peptides are shown with the N-terminus on the left. As it turns out, much of the structure of a protein is due to the interactions of the atoms in the backbone without taking into account the nature of the R groups on the side chains.

Figure 2. A small peptide showing the direction of the peptide chain (N-terminal to C-terminal)



- Although the peptide bond is typically written as a carbonyl group bonded to an N-H group, such bonds can exhibit keto enol tautomerism. The carbon–nitrogen bond actually has around 40% double bond character. As a result, the peptide group that forms the link between the two amino acids is actually planar.

Figure 3. The resonance structures of the peptide bond lead to a planar group.

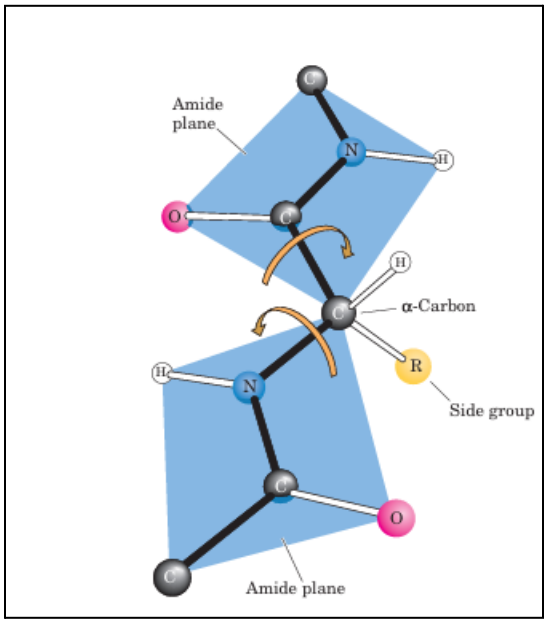


- This grouping is called the amide plane, and it has a tremendous influence on protein structure. There is freedom of rotation about the two bonds from the alpha carbon, but there is no rotation of the carbon–nitrogen bonds.
- A chain of amino acids linked via peptide bonds can be thought of as a series of playing cards linked by a swivel at their corners. The rigidity of the amide plane

  limits the possible orientation of the peptide.

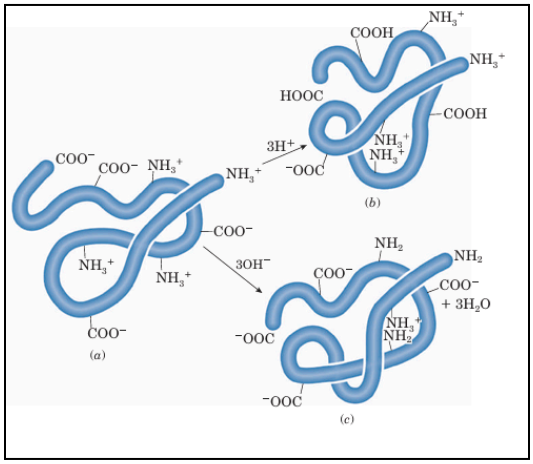
- The 20 different amino acid side chains supply variety and determine the rest of the physical and chemical properties of proteins. Among these properties, acid–base behavior is one of the most important.
- Like amino acids, proteins behave as zwitterions. The side chains of glutamic and aspartic acids provide acidic groups, whereas lysine and arginine provide basic groups (histidine does as well, but this side chain is less basic than the other two.

Figure 4. Planar nature of peptide bond. The rigid planar peptide groups (called "playing cards" in the text) are shaded.



- The isoelectric point of a protein occurs at the pH at which there are an equal number of positive and negative charges (the protein has no net charge). At any pH above the isoelectric point, the protein molecules have a net negative charge; at any pH below the isoelectric point, they have a net positive charge.
- Some proteins, such as hemoglobin, have an almost equal number of acidic and basic groups; the isoelectric point of hemoglobin is at pH 6.8. Others, such as serum albumin, have more acidic groups than basic groups; the isoelectric point of this protein is at pH 4.9. In each case, however, because proteins behave like zwitterions, they act as buffers—for example, in the blood (Figure 4).

Figure 5. Schematic diagram of a protein *(a)* at its isoelectric point and its buffering action when *(b)* H or *(c)* OH ions are added.



- The water solubility of large molecules such as proteins often depends on the repulsive forces between charges on their surfaces. When protein molecules are at a pH at which they have a net positive or negative charge, the presence of these like charges causes the protein molecules to repel one another. These repulsive forces are smallest at the isoelectric point, when the net charges are zero.
- When there are no repulsive forces, the protein molecules tend to clump together to form aggregates of two or more molecules, reducing their solubility. As a consequence, proteins are least soluble in water at their isoelectric points and can be precipitated from their solutions.
- To understand these functions, we must look at four levels of organization in their structures.
- The primary structure describes the linear sequence of amino acids in the polypeptide chain.
- The secondary structure refers to certain repeating patterns, such as the a-helix conformation or the pleated sheet or the absence of a repeating pattern, as with the random coil.
- The tertiary structure describes the overall 3D conformation of the polypeptide chain.
- The quaternary structure applies mainly to proteins containing more than one poly peptide chain (subunit) and deals with how the different chains are spatially related to one another.

2.3 STRUCTURE OF PROTEINS

Proteins are the final products of gene expression originating from DNA. They are produced through transcription (DNA → mRNA) and translation (mRNA → protein). Proteins function as structural components, enzymes, transporters, and regulators of cellular processes.

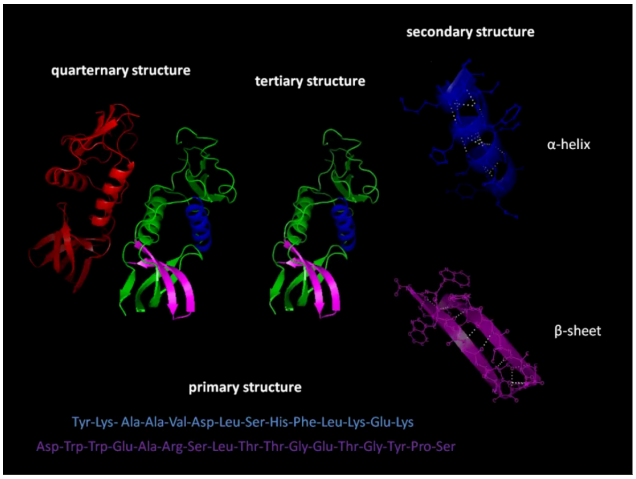
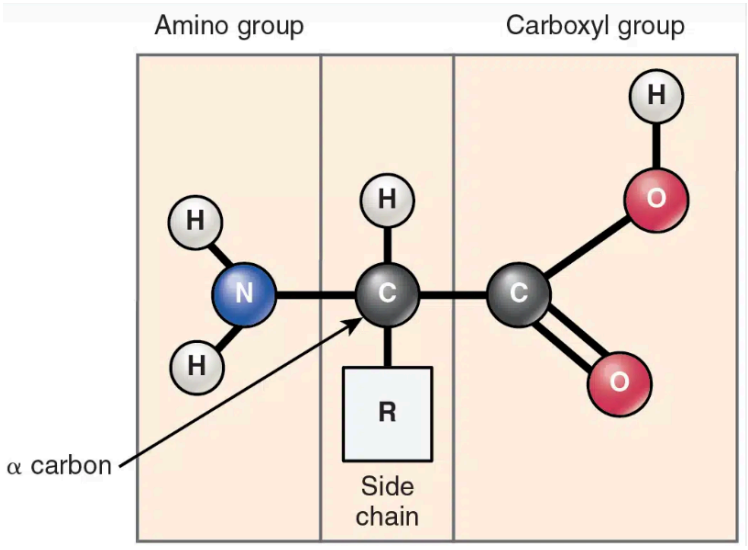


Figure 6: *Structures of proteins*

1. **Basic Structure of Amino Acids**

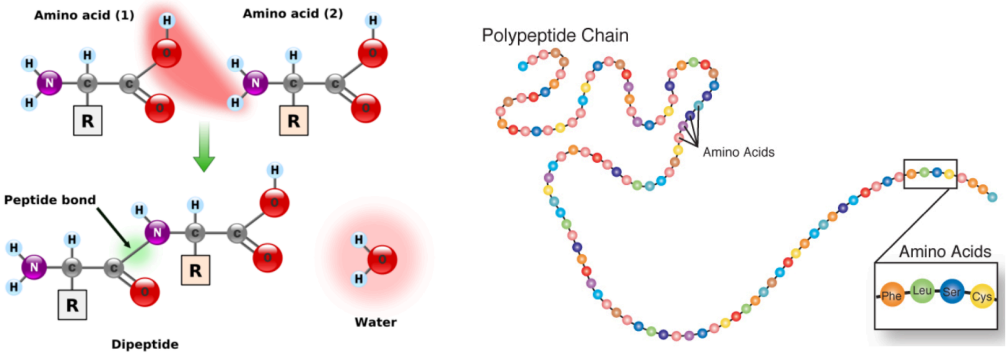


Proteins are composed of smaller units called amino acids. Each amino acid has a common structure consisting of:

- An alpha (central) carbon
- An amino group ($-NH_2$)
- A carboxyl group (-COOH)
- A hydrogen atom

- A side chain (R-group) -it determines each amino acid's chemical properties and behavior.

2. **Formation of Polypeptide Chain**



Amino acids are linked by peptide bonds formed via dehydration synthesis. Peptide bonds join the carboxyl group of one amino acid to the amino group of another. The resulting chain is called a polypeptide. The linear sequence of amino acids defines the protein's primary structure.

3. **Types of Amino Acids**

There are 20 standard amino acids, each with unique characteristics based on their R-groups. They can be classified as:

- **Nonpolar (hydrophobic)** – tend to avoid water
- **Polar, uncharged** – interact well with water
- **Charged (acidic or basic)** – participate in ionic interaction

4. **Forces That Shape Protein Structure**

The final shape of a protein is maintained by several chemical interactions:

- **Ionic bonds** – attractions between oppositely charged side chains.
- **Hydrogen bonds** – help stabilize protein folding.

- **Van der Waals interactions** – weak attractions between closely packed atoms.
- **Covalent bonds (Disulfide bridges)** – strong bonds formed between cysteine residues that add stability.

2.4 PEPTIDE FORMATION

Peptides are short chains made up of small building blocks called amino acids. These amino acids are connected to each other by special links called peptide bonds.

When the chain is longer, it is called a polypeptide.

If a polypeptide is very large—usually with a molecular mass of 10,000 Daltons (Da) or more—it is considered a protein. So, proteins are basically very large polypeptides.

There are also very short peptides:

- **Dipeptide** – 2 amino acids
- **Tripeptide** – 3 amino acids
- **Tetrapeptide** – 4 amino acids
- **Oligopeptide** – usually fewer than 20 amino acids

In a peptide chain, each amino acid becomes a residue once it joins the chain.

Most peptides are linear, meaning they have two ends:

- The N-terminal (the end with an amine group)
- The C-terminal (the end with a carboxyl group)

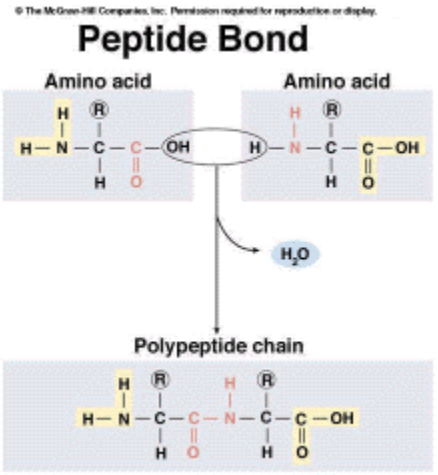
However, some peptides are different. Instead of having two open ends, they form a closed loop. These are called cyclic peptides.

Basically, Amino acids join together → form peptides → longer chains form polypeptides → very long polypeptides become proteins.

A peptide bond is the special bond that connects two amino acids together.

Each amino acid has:

- An amine group ($–NH_2$)
- A carboxyl group (–COOH)



When two amino acids join, the amine group of one amino acid reacts with the carboxyl group of another. During this reaction:

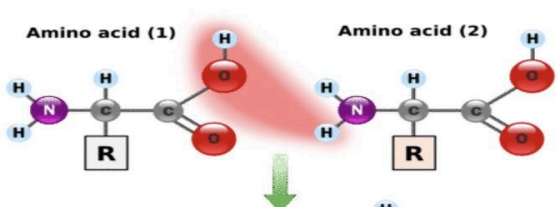
- A water molecule (H_2O) is removed.
- This type of reaction is called a dehydration reaction (because water is removed) or a condensation reaction (because two molecules combine into one).

After the water is removed:

- A new bond forms between the carbon (C) of the carboxyl group and the nitrogen (N) of the amine group.
- This new bond is called a peptide bond.
- Chemically, it forms a structure called an amide group (–CO–NH–).

Because one water molecule is removed, the two amino acids become connected into one larger molecule.

Two amino acids join → water is released → a peptide bond forms → a dipeptide is created.



Amino acids join together to form a molecule called a dipeptide. The C-N bond is called a peptide bond. The order of amino acids is by convention shown with the free amino group on the left and the free carboxyl group on the right.

2.5. LEVELS OF PROTEIN ORGANIZATION

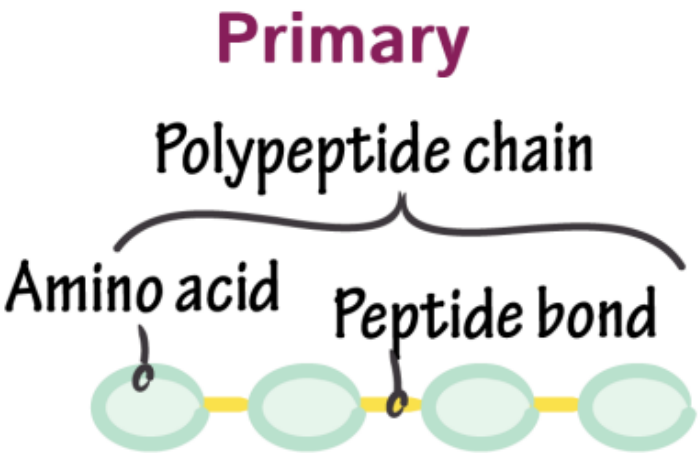
Proteins have a hierarchical structure, categorized into four levels:

1. Primary Structure

The linear sequence of amino acids in a polypeptide chain.

● Amino acids are linked by peptide bonds.

● Determines the protein's ultimate shape and function.



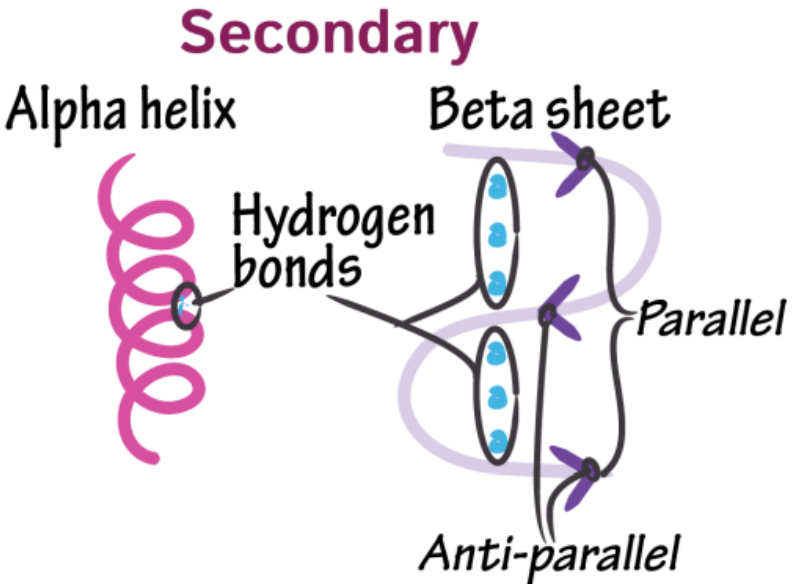
2. Secondary Structure

Local folding of the polypeptide chain due to hydrogen bonding.

● Two main types:

● Alpha (α) helix (🌀): Coiled, spiral structure.

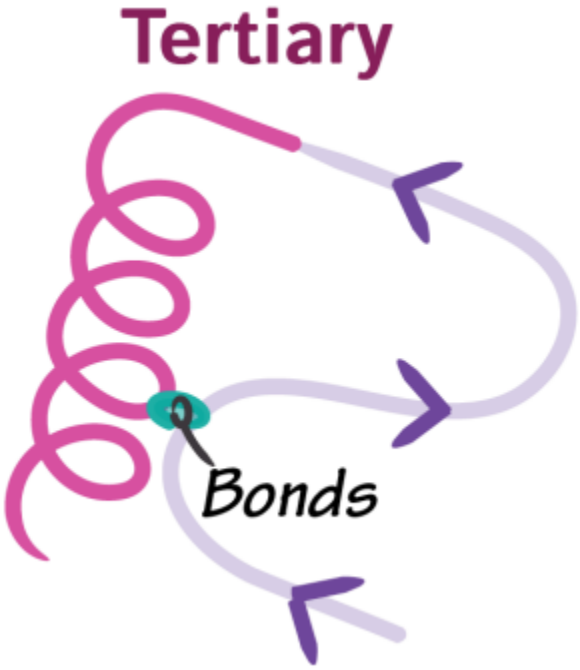
● Beta (β)-pleated sheet (📄): Folded, sheet-like structure.



3. Tertiary Structure

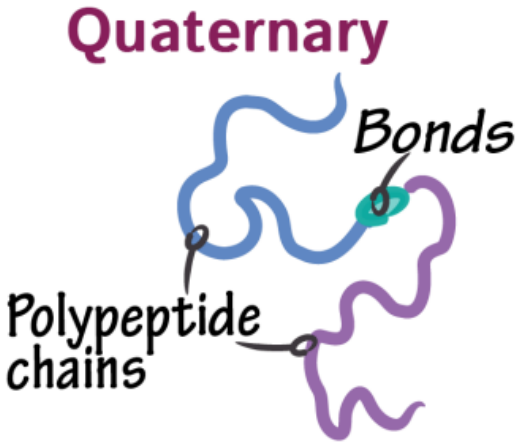
The three-dimensional (3D) folding of a single polypeptide chain.

- Shape is stabilized by R-group interactions, including:
- Hydrogen bonds
- Ionic bonds
- Disulfide bridges (strong covalent bonds between cysteine residues)
- Hydrophobic interactions (nonpolar side chains clustering away from water)



4. Quaternary Structure

- Formed when two or more polypeptide chains combine.
- Example: Hemoglobin, which consists of four subunits working together



2.6 CLASSIFICATION, BIOLOGICAL FUNCTIONS AND TESTS FOR PROTEINS

Classification of Proteins

A. Based on Chemical Composition

1. **Simple proteins** yield only amino acids upon complete hydrolysis.

- Albumins: Water-soluble (e.g., serum albumin, ovalbumin)
- Globulins: Soluble in dilute salt solutions (e.g., antibodies, fibrinogen)
- Glutelins: Soluble in dilute acids or bases (e.g., glutenin in wheat)
- Prolamins: Soluble in 70% ethanol (e.g., gliadin in wheat, zein in corn)
- Scleroproteins: Insoluble fibrous proteins (e.g., keratin in hair, collagen in connective tissue)

2**. Conjugated proteins** contain a non-protein prosthetic group attached to the polypeptide chain.

- Glycoproteins: Carbohydrate attached (e.g., antibodies, mucins)
- Lipoproteins: Lipid component (e.g., HDL, LDL for lipid transport in blood)
- Nucleoproteins: Nucleic acid bound (e.g., chromosomes, ribosomes)
- Phosphoproteins: Phosphate groups on serine/threonine (e.g., casein in milk)
- Hemoproteins: Heme group containing iron (e.g., hemoglobin, cytochromes)
- Metalloproteins: Metal ions essential for function (e.g., carbonic anhydrase with Zn^{2+}, ferritin storing Fe^{3+})

3**. Derived proteins** are products of partial hydrolysis or denaturation of native proteins, forming a continuum: proteoses → peptones → polypeptides → peptides → free amino acids.

B. Based on Structure

- **Fibrous proteins**: Elongated, insoluble structures with repetitive sequences providing mechanical strength. Examples include collagen (triple helix in connective tissue), keratin (coiled-coil in hair and nails), and elastin (providing elasticity to blood vessels).
- **Globular proteins**: Compact, spherical, water-soluble molecules with mixed secondary structures forming specific binding or catalytic sites. Most enzymes,

transport proteins, and regulatory proteins belong to this class (e.g., hemoglobin, myoglobin, insulin).

- **Membrane proteins**: Embedded in lipid bilayers with hydrophobic regions interacting with fatty acid chains and hydrophilic regions exposed to aqueous compartments. They function as transporters (e.g., GLUT4), receptors (e.g., G-protein coupled receptors), and ion channels.

Biological Functions of Proteins

1. **Enzymatic catalysis**: Accelerate biochemical reactions (e.g., hexokinase in glycolysis, DNA polymerase in replication).
2. **Transport**: Carry molecules within organisms (hemoglobin transports O_2; serum albumin transports fatty acids and hormones; membrane transporters move ions and nutrients across cell membranes).
3. **Structural support**: Form frameworks at cellular and tissue levels (collagen in bone and skin, keratin in epithelial cells, actin and tubulin in the cytoskeleton).
4. **Regulation**: Control metabolic pathways and gene expression (insulin and glucagon regulate blood glucose; transcription factors like p53 control gene activity).
5. **Defense**: Protect against pathogens and injury (immunoglobulins neutralize antigens; fibrinogen forms blood clots; complement proteins destroy microbes).
6. **Contractile function**: Enable movement (actin and myosin in muscle contraction; dynein and kinesin transport vesicles along microtubules).
7. **Storage**: Reserve nutrients for later use (ferritin stores iron; casein stores amino acids in milk; ovalbumin in egg white nourishes the embryo).
8. **Receptor function**: Receive and transmit signals (cell surface receptors like insulin receptors; intracellular receptors like steroid hormone receptors).

Tests for Proteins

General Tests

1. **Biuret test:**
   Principle: Cu^{2+} ions form a violet complex with peptide bonds in alkaline solution.
   Procedure: Add NaOH followed by dilute $CuSO_4$ to protein solution.
   Result: Violet color indicates proteins or polypeptides with two or more peptide bonds. Free amino acids do not react.
   Significance: Basis for quantitative protein estimation; color intensity proportional to peptide bond concentration.

2. **Ninhydrin test**:
   Principle: Ninhydrin reacts with free α-amino groups to form Ruhemann's purple (yellow for proline).
   Procedure: Heat protein or amino acid solution with 0.2% ninhydrin reagent.
   Result: Blue-purple color for most amino acids; yellow for proline and hydroxyproline.
   Significance: Detects free amino acids and proteins with exposed N-termini; used to visualize amino acids in chromatography.

Specific Test for Amino acids chains

1. **Xanthoproteic test**: Detects aromatic amino acids (tyrosine, tryptophan, phenylalanine). Concentrated HNO_3 nitrates aromatic rings, producing yellow color that turns orange upon alkalinization with NaOH.

2**. Millon's test**: Specific for tyrosine. Millon's reagent (Hg in HNO_3) reacts with phenolic group to give red color or precipitate.

3. **Hopkins-Cole test**: Specific for tryptophan. Glyoxylic acid in concentrated H_2SO_4 produces a violet ring at the interface with protein solution.

4. **Sakaguchi test**: Detects arginine. Alkaline α-naphthol with hypochlorite oxidizes the guanidinium group to give red color.

5. **Nitroprusside test**: Detects cysteine. Sodium nitroprusside reacts with free sulfhydryl groups (–SH) in alkaline medium to give red color. Disulfide bonds must first be reduced to give a positive test.

Quantitative Methods

1. **Bradford assay**: Coomassie Brilliant Blue dye binds to basic residues (arginine, lysine); measured at 595 nm. Fast and sensitive (1–20 µg).
2. **Lowry method**: Combines Biuret reaction with reduction of Folin-Ciocalteu reagent by tyrosine and tryptophan; measured at 750 nm. Sensitive but susceptible to interference.
3. **UV absorbance at 280 nm**: Aromatic residues (tryptophan, tyrosine) absorb UV light. Quick method for pure protein solutions; requires known extinction coefficient.

2.7 DEFINITION, TYPES, STAGES AND BIOLOGICAL IMPORTANCE OF PROTEINS

NATURE OF PROTEINS

The term “protein” comes from the Greek word *“proteios,”* which means “of first importance.” Proteins are the most versatile macromolecules and play essential roles in nearly all biological processes. They act as catalysts in chemical reactions, transport and store substances like oxygen, provide structural support and immune defense, enable movement, transmit nerve signals, and regulate growth and cell differentiation.

What Makes Up a Protein?

Proteins are linear polymers built of monomer units called amino acids. They are a chain of amino acids, a total 20 amino acids can be found in protein.

A typical protein consists of 300 or more amino acids, and the exact number and order of these amino acids differ for each protein. Amino acids can be arranged in countless combinations to create a wide variety of proteins. The specific sequence of amino acids determines how the protein folds into a unique three-dimensional shape. This shape is essential because it dictates the protein’s function, such as forming muscle tissue or acting as an enzyme. Each species, including humans, possesses its own distinct set of proteins.

MAJOR TYPES OF PROTEINS AND ITS FUNCTIONS

Proteins are important molecules in the body that perform many different functions. They can be grouped into major types based on their structure and role. The two main types are fibrous proteins and globular proteins. The table below discusses the function and differences of the types of proteins.

Major Types of Proteins	Description	Examples
Fibrous Protein	These are the proteins that are insoluble in water, have a long and narrow shape, and whose main function is to provide structure.	Collagen and Keratin
Globular Protein	These are the proteins that are insoluble in water, have a long and narrow shape, and whose main function is to provide structure.	Hemoglobin, insulin and amylase.

Table 1: *Types of Proteins*

BIOLOGICAL IMPORTANCE AND FUNCTION OF PROTEIN

Proteins are very versatile macromolecules. They play a critical role in regulation by forming hormones and antibodies for immune defense, they also ensure that the body stays balanced and protected. Proteins are also the ones that facilitate the transport of molecules across cell membranes and can even be utilized as an emergency energy source when carbohydrates and fats are unavailable.

Function/ Roles of Proteins	Description	Biological importance and examples
Defensive	These are specialized proteins that protect the body from antigens, or foreign substances.	Antibodies help neutralize these invaders by restricting their movement, making it easier for white blood cells to identify and eliminate them.

Contractile Proteins	These are the proteins responsible for contractions, especially in our muscles.	Myosin and actin are the two motor proteins responsible for muscle contraction and movement.
Enzymes	Enzymes are proteins that act as catalysts, used in speeding up chemical reactions. Without enzymes, the reactions would take place so slowly as to be useless, it is impossible for the human body to function without enzymes.	Amylase is a protein found in saliva and is responsible for breaking down starch into sugar.
Hormonal Proteins	These are chemical messengers that regulate and coordinate functions throughout the body. They transmit signals between cells, tissues, and organs to ensure that various biological processes work together properly.	Insulin is a protein responsible for managing your sugar level and this protein can be found in the pancreas.
Structural Proteins	These are the proteins that provide an internal structure to the cell (and are sometimes involved in cell movement). Structural proteins are	Collagen is a protein responsible for making connective tissues of the skin, bone, and ligaments.

	especially important in larger cells.	
Storage Proteins	These are the proteins that store nutrients for later use.	Ferritin is a protein that stores iron and can be found in the liver. .
Transport Protein	Transport proteins are specialized carrier molecules that transfer substances from one location to another within the body	Hemoglobin is a protein in the blood that transports oxygen from the lungs to body cells.

Table 2: *Functions of Proteins*

PROTEIN METABOLISM

Protein metabolism is the body's way of managing proteins and amino acids by constantly breaking them down or building them back up. This process is super important because it helps us maintain muscle, create the enzymes and hormones we need to function, and fix any damaged tissue. If we're ever running low on fuel, our body can actually use this process to get some extra energy.

STAGES OF PROTEIN

1. **Protein Digestion and Absorption**

   This process takes place in the stomach and small intestine. Proteins from the food we eat are broken down into smaller units called amino acids. After digestion, absorption happens when these amino acids enter the bloodstream. They are then transported to different cells in the body where they are used for various functions.

2. **Amino Acid Utilization**

   This process mainly happens in the liver and muscles. The body uses amino acids to make new proteins like enzymes, hormones, and muscle tissue. Some amino acids can also be changed into glucose or fat to give the body energy when needed.

3. **Amino Acid Degradation and Excretion**

   This process takes place in the liver and kidneys. In the liver, deamination happens, which means the amino group (nitrogen part) is removed from amino acids. The nitrogen forms ammonia, which is toxic, so the liver converts it into urea. The urea is then transported to the kidneys and removed from the body through urine.

2.8 DIGESTION AND ABSORPTION OF PROTEINS

Introduction

Protein digestion begins in the stomach and continues in the small intestine. Because proteins are large and complex molecules, the body must break them down into smaller units called amino acids before they can be absorbed and used. This process depends greatly on the action of digestive enzymes.

Process of Digestion and Absorption

1. Digestion in the Stomach

- The stomach releases gastric juice, which contains hydrochloric acid (HCl) and the enzyme pepsin.
- HCl denatures proteins, meaning it unfolds their structure so enzymes can work on them more easily.
- Pepsin starts breaking the proteins into smaller chains called peptides.

2. Digestion in the Small Intestine

- The partially digested proteins move into the duodenum, the first part of the small intestine.

- The pancreas releases important protease enzymes such as trypsin, chymotrypsin, and carboxypeptidase. These enzymes continue breaking peptides into even smaller pieces.
- The lining of the small intestine produces peptidase enzymes that complete the process, turning peptides into individual amino acids.

3. Absorption in the Small Intestine

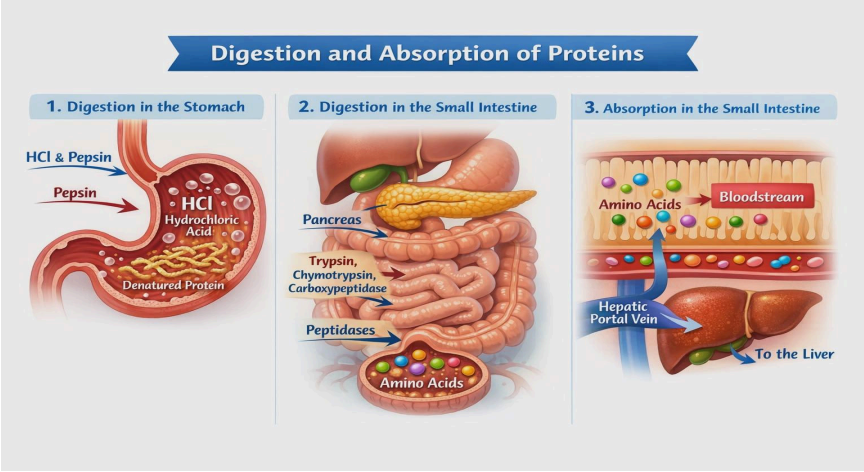
- The amino acids are absorbed through the walls of the small intestine into the bloodstream by active transport.
- They enter the hepatic portal vein and are carried to the liver, where they are processed and distributed to different parts of the body.

Summary and Importance

Proteins must be fully broken down into amino acids for the body to use them properly. The stomach and small intestine work together with enzymes like **pepsin, trypsin, chymotrypsin, and peptidase** to make this possible.

Once absorbed, amino acids are used for:

- Building and repairing muscles and tissues
- Producing enzymes and hormones
- Supporting the immune system
- Providing energy when needed
- Maintaining overall body functions and health



2.9 DISEASE RELATED TO PROTEINS

Moderation involves consuming balanced amounts of nutrients. Proteins are vital for body structure and function, and both protein deficiency and protein dysfunction can lead to serious health problems and disease.

1. Kwashiorkor

General Description

- **Malnutrition** is caused by too little, too much, or unbalanced nutrients.
- Includes **undernutrition** (wasting, stunting, underweight, vitamin deficiencies).
- Includes **overweight and obesity.**
- Includes **diet-related diseases** (heart disease, diabetes).
- Affects all ages, especially children.
- Linked to many deaths in children under 5.

Cause:

- Not enough **protein** in the diet
- Lack of important vitamins and minerals
- Eating mostly carbohydrates (like rice) but little protein (meat, eggs, milk)
- Common in poor communities and young children

Symptoms:

- **Swollen belly** and feet (edema)
- **Thin arms and legs** (muscle loss)
- Slow growth
- Dry, brittle hair
- Skin problems
- Weak body and gets sick easily
- Sad or irritable behavior

Treatment:

- Slowly give **protein-rich foods**
- **Special therapeutic food (RUTF)**
- **Give vitamins and minerals**
- **Treat infections**
- **Regular check-up and nutrition monitoring**

2. Marasmus

General Description:

- **Marasmus** is a severe form of malnutrition caused by a lack of both protein and calories. It happens when a person does not eat enough food for a long time. It mostly affects infants and young children and leads to extreme thinness, muscle wasting, and weakness.

Causes:

- **Severe lack of protein and calories**
- **Not eating enough food for a long time**
- **Poverty or food shortage**
- **Poor feeding practices (especially in infants and young children)**
- **Frequent infections (like diarrhea) that reduce nutrient absorption**

Symptoms:

- **Extreme thinness** (very low body weight)
- Very little body fat (“skin and bones” appearance)
- Weakness and low energy
- Dry, thin skin
- **Stunted growth** (slow height development)
- Weak immune system (gets sick easily)

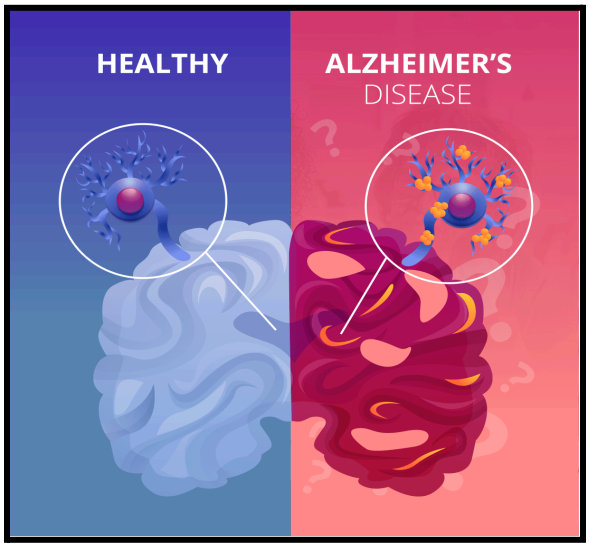
Treatment

- **Slow and careful refeeding with enough calories and protein**
- **Special therapeutic foods (RUTF or high-energy milk formulas)**
- **Vitamin and mineral supplements**
- **Treatment of infections and dehydration**
- **Regular growth monitoring**
- **Nutrition education for parents or caregivers**

3. Alzheimer

General Description:

- **Alzheimer's disease (AD):** most common type of dementia (60–80% of cases)
- **Progressive symptoms:**
  - Early: trouble remembering new information
  - Later: disorientation, mood/behavior changes, confusion, memory loss, difficulty speaking/swallowing/walking
- **Risk factor:** mainly age (65+), but early-onset possible under 65



Cause:

- Damage and death of **brain nerve cells**
- Build-up of abnormal proteins in the brain (beta-amyloid plaques and tau tangles)
- Main risk factor is **old age (65+)**
- Family history and genetics may increase risk
- Early-onset Alzheimer's can happen before age 65 (rare)

Symptoms:

Early Stage:

- Trouble remembering new information
- Forgetting names, dates, or recent events

Middle Stage:

- Confusion and disorientation
- Mood and behavior changes
- Difficulty speaking and understanding
- Problems with daily tasks

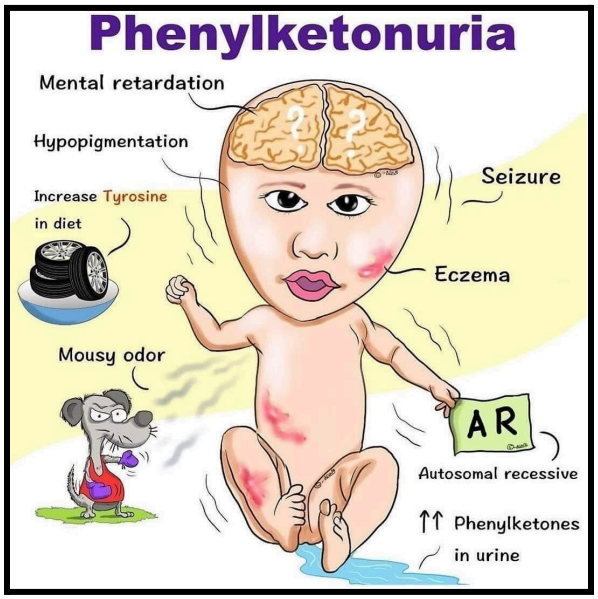
Late Stage:

- **Severe memory loss**
- **Difficulty walking, swallowing, and communicating**
- **Needs full-time care**

Treatment:

- **No cure yet**
- Medicines to **slow memory decline** (Donanemab, Lecanemab – for early stages)
- Other medicines to manage symptoms (memory, mood, behavior)
- Brain exercises and memory activities
- Support for caregivers and family
- Early diagnosis helps with planning and care

4. Phenylketonuria (PKU)



General Description:

- **Phenylketonuria (PKU) is an inherited disorder that causes high levels of phenylalanine, an amino acid, in the blood.**
- Phenylalanine is found in **protein-rich foods (**meat, eggs, milk, nuts) and some artificial sweeteners (like aspartame).

Cause:

- A **genetic (inherited) disorder**
- The body cannot properly break down **phenylalanine** (an amino acid)

- Caused by lack of the enzyme needed to process phenylalanine
- Phenylalanine builds up in the blood and becomes harmful to the brain

Symptoms

If not treated early

- Developmental delay
- Intellectual disability
- Seizures
- Behavioral problems
- Lighter skin and hair
- Skin problems like eczema

Babies look normal at birth but develop symptoms after a few months if untreated.

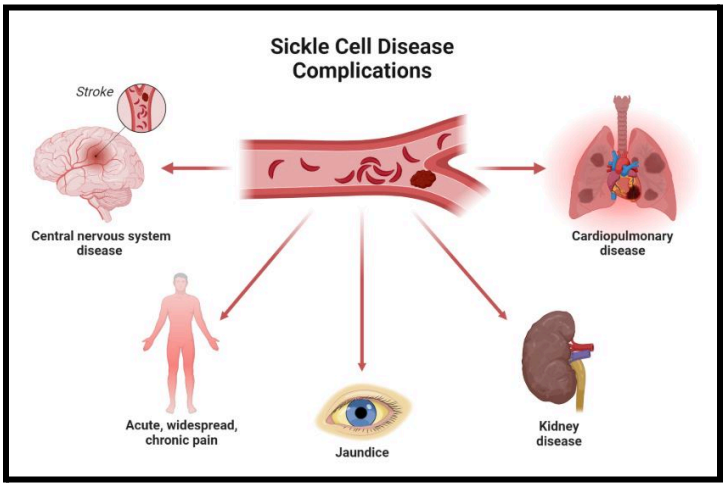
Treatment:

- **Strict low-phenylalanine diet**
- **Avoid high-protein foods (meat, milk, eggs, nuts, tofu)**
- **Special low-protein formula for babies**
- **Regular blood tests to monitor phenylalanine levels**
- **Lifelong diet management**
- **Pregnant women with PKU must follow a strict diet to protect the baby**

5. Sickle Cell Disease

General Description:

- Sickle cell disease (SCD) is an inherited disorder affecting hemoglobin, the oxygen-carrying part of red blood cells.



Cause:

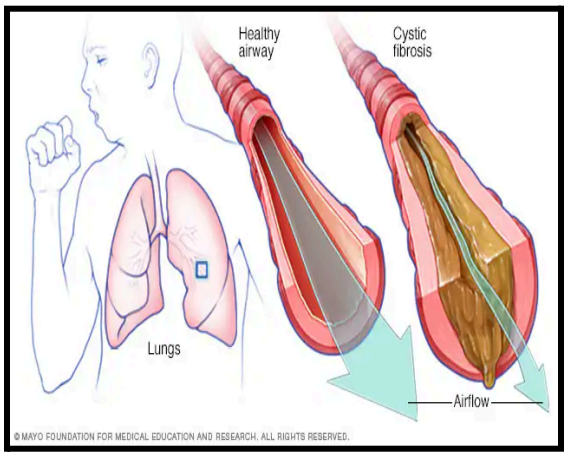
- An **inherited (genetic) disorder**
- Caused by mutation in the **HBB gene**
- Produces abnormal hemoglobin called **Hemoglobin S (HbS)**

- Red blood cells become **sickle-shaped (crescent-shaped)**
- Inherited in an **autosomal recessive pattern** (both parents are usually carriers)

Symptoms

- **Anemia** (low red blood cells) → fatigue and weakness
- **Shortness of breath**
- **Painful episodes (pain crises)**
- Frequent infections
- Delayed growth in children
- **Jaundice** (yellow skin and eyes)
- Swelling in hands and feet
- Organ damage (lungs, kidneys, spleen, brain)

6. Cystic Fibrosis



General Description:

- **Cystic fibrosis (CF)** is an inherited disease causing thick, sticky mucus that damages multiple organs.

Cause:

- An **inherited (genetic) disease**
- Caused by mutation in the **CFTR gene**
- **The CFTR gene controls salt and water movement in cells**
- Mutation causes **thick, sticky mucus** to build up in organs
- Inherited in an **autosomal recessive pattern** (both parents are usually carriers)

Symptoms:
Lungs:

- Difficulty breathing
- Frequent lung infections
- Persistent coughing with thick mucus

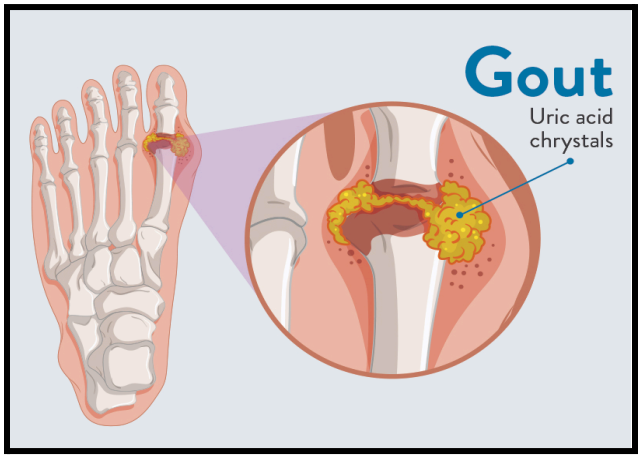
Digestive System:

- **Poor digestion**
- **Weight loss or difficulty gaining weight**
- **Malnutrition**

Treatment

- Airway clearance therapy (to remove mucus)
- Medicines to treat and prevent infections
- Anti-inflammatory drugs
- Proper nutrition and high-calorie diet

7. Gout (High Protein Metabolism Byproduct)



General Description:

- Gout is an **inflammatory rheumatic disease** characterized by:
  - Arthritis (joint inflammation and pain)
  - Abnormal **uric acid metabolism** → urate buildup in joints

Cause

- Build-up of **uric acid** in the blood
- Uric acid forms **crystals in joints**, causing inflammation
- Linked to **high purine intake** (found in meat, seafood, alcohol)
- Obesity, poor diet, genetics, and other health conditions can increase risk

Symptoms

- **Severe joint pain**, often in the big toe
- **Swelling, redness, and warmth** in affected joints
- Pain attacks (flares) that come suddenly
- Stiffness and limited movement in joints
- Chronic gout can cause joint damage over time

Treatment:

- **Diet changes**: reduce purine-rich foods and alcohol
- **Medications** to lower uric acid and reduce inflammation
- Maintain healthy weight and lifestyle
- Manage metabolic risk factors (like high blood pressure or diabetes)
- Regular monitoring of uric acid levels

2.10 RECENT DEVELOPMENTS CONCERNING PROTEIN AND METABOLISM

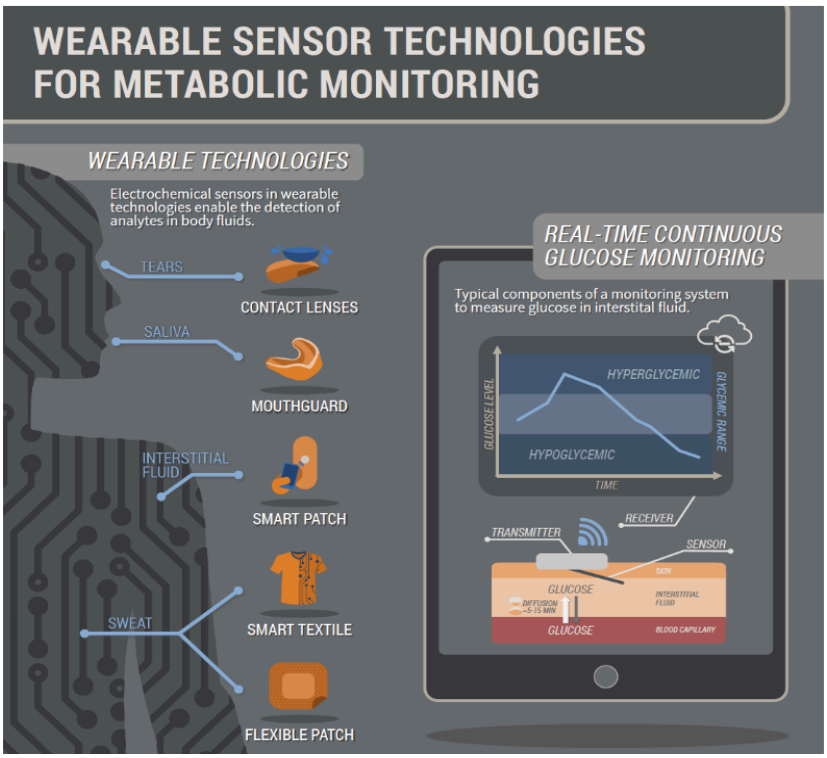
1. **CRISPR-Cas9**

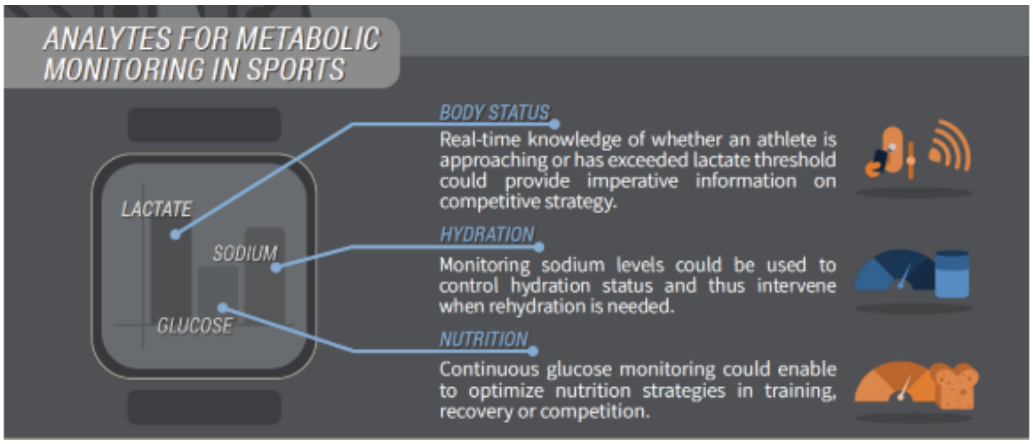
- CRISPR-Cas9 is a gene-editing technology that allows scientists to precisely modify DNA. Since genes control protein production, this tool can directly influence metabolic enzymes and pathways.
- CRISPR-Cas9 was developed as a gene-editing tool in 2012. It is widely used in research and medicine in countries such as the United States, China, and those in Europe.
- This technology is important because it allows correction of genetic mutations that cause metabolic disorders. It offers potential long-term or permanent treatment by fixing protein-related metabolic defects.
- CRISPR-based therapies are already approved for some genetic diseases. Research is ongoing for its use in metabolic liver disorders.

2. Xanthine Oxidase Inhibitors

- Xanthine oxidase inhibitors are drugs that block xanthine oxidase, an enzyme involved in purine metabolism. This reduces uric acid production in the body.
- The first xanthine oxidase inhibitor, allopurinol, was introduced in the 1960s. These drugs are commonly used worldwide, especially in countries with high gout prevalence.
- They are important in managing gout and related metabolic conditions. This shows how targeting a single protein enzyme can control metabolic imbalance.
- Newer inhibitors have improved safety profiles. Studies are exploring their role in cardiovascular and metabolic health.

3. Wearable Technology for Metabolic Monitoring

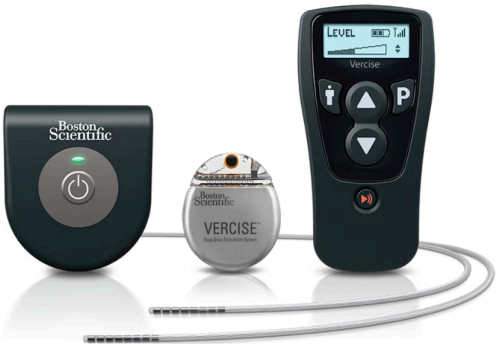


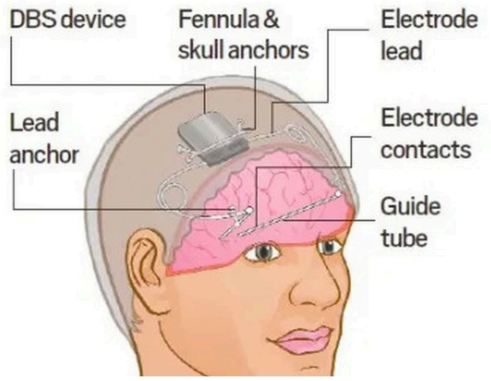


- Wearable technology includes devices that monitor metabolic indicators such as glucose levels, heart rate, and energy expenditure. These measurements reflect how the body processes nutrients and proteins.

- Wearable health devices became popular in the early 2010s. They are widely used in countries such as the United States, Japan, and South Korea.
- Wearables help in early detection and management of metabolic diseases like diabetes. They support personalized monitoring of metabolic health.
- Some wearables now include continuous glucose monitoring. Research is expanding toward sensors for amino acids and other metabolites.

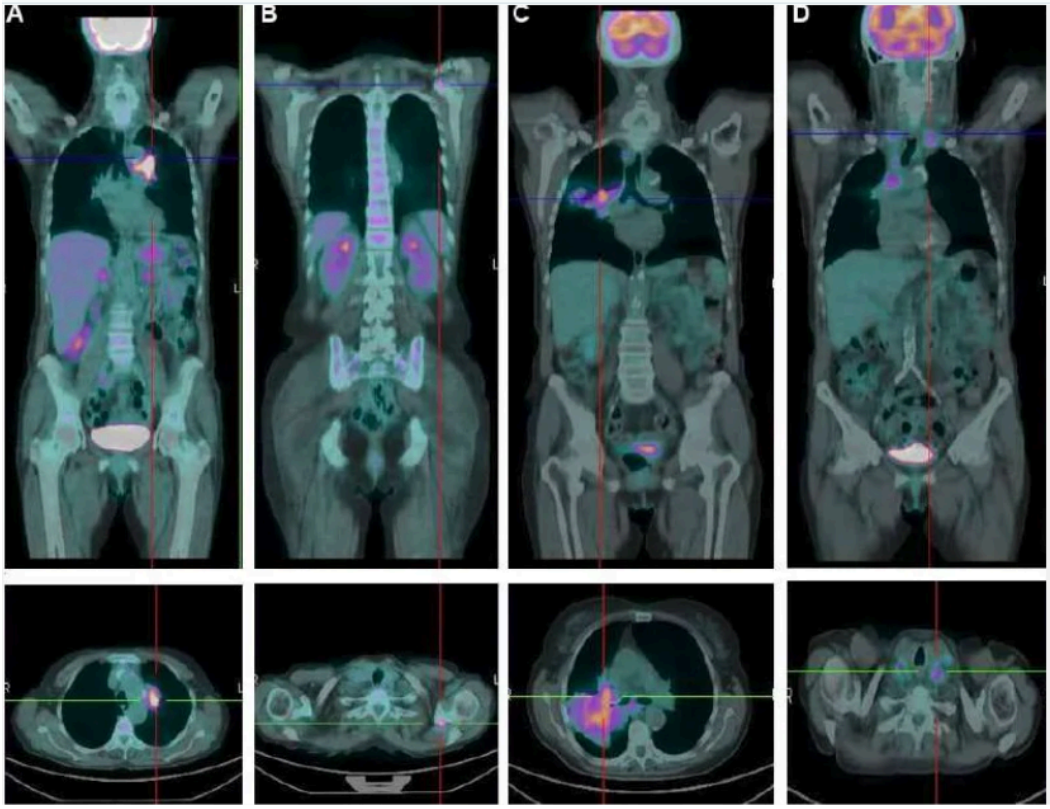
4. Deep Brain Stimulation (DBS)

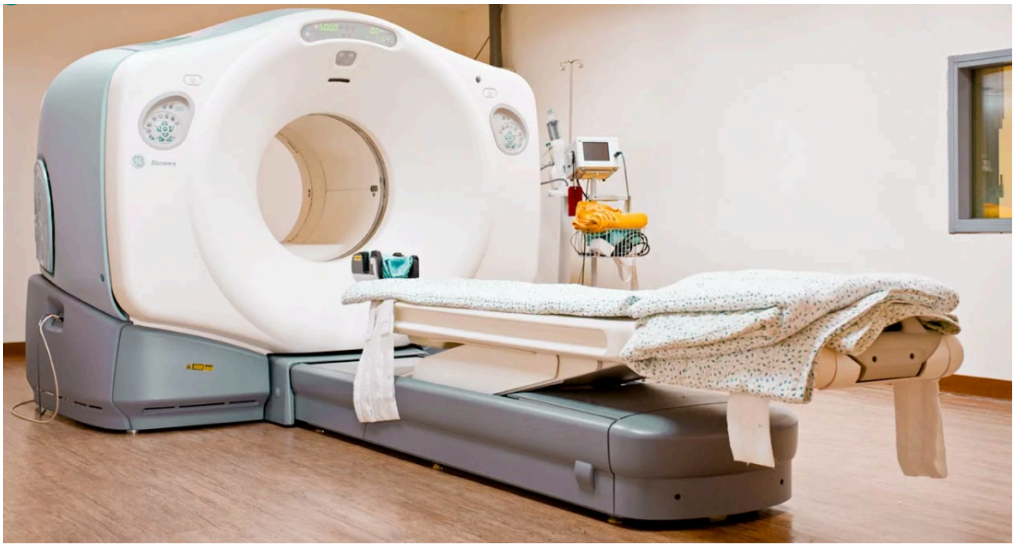




- Deep Brain Stimulation is a medical procedure that uses electrical signals to regulate specific brain regions. These brain areas influence appetite, hormone release, and energy balance.
- DBS was developed in the late 1980s and became widely used in the 1990s. It is commonly used in the United States and Europe.
- DBS is important because the brain plays a key role in controlling metabolic processes. By regulating neural signals, DBS can indirectly affect metabolism.
- DBS is mainly used for neurological disorders like Parkinson's disease. Research is ongoing on its effects on obesity and metabolic regulation.

5. Positron Emission Tomography (PET)

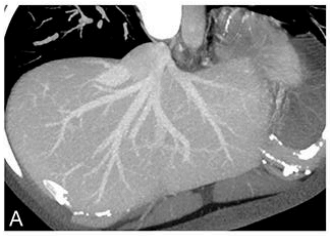




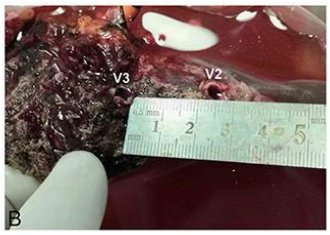
- PET is an imaging technique that visualizes metabolic activity in living tissues. It tracks how cells use glucose and other molecules involved in metabolism.
- PET was developed in the 1950s and became widely used in hospitals in the 1970s. It is commonly used worldwide in medical and research centers.

- PET is important for studying metabolic activity in diseases such as cancer and diabetes. It helps scientists understand how proteins function in real time.
- PET scans are often combined with CT or MRI. Different tracers can be used to study specific metabolic pathways.

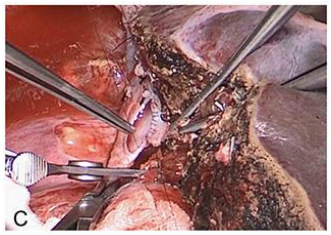
6. Liver Transplantation



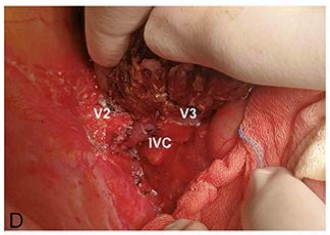
Donor's CTA before transplantation



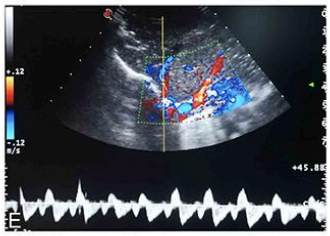
HV before dual anastomosis



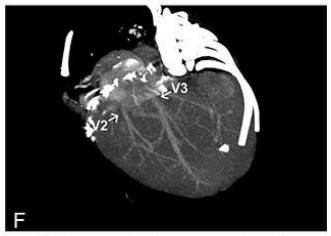
HV during dual anastomosis



HV after dual anastomosis



Doppler after dual anastomosis



CTA six months after transplantation

- Liver transplantation is a surgical procedure that replaces a diseased liver with a healthy one. The liver is central to protein, carbohydrate, and lipid metabolism.
- The first successful liver transplant was performed in 1967. It is commonly done in countries with advanced healthcare systems.
- This procedure restores normal metabolic and enzymatic functions. It is life-saving for patients with severe metabolic liver disorders.
- Some inherited metabolic diseases can be cured through liver transplantation. Advances in immunosuppressive drugs improve transplant success.

7. GLP-1 Receptor Agonists

- GLP-1 receptor agonists are medications that mimic glucagon-like peptide-1, a protein hormone that helps regulate blood sugar and appetite. They improve glucose metabolism and reduce food intake.
- GLP-1–based therapies were developed in the early 2000s. They are now widely used around the world, including in the United States, Europe, and Asia.
- This treatment is important because it improves metabolic control in people with diabetes and obesity. It shows how protein hormones play a key role in regulating metabolism.
- GLP-1 receptor agonists can help with weight loss and reduce the risk of cardiovascular disease. They are usually given as injections or weekly doses.

8. **Sephience**

- Sephience is an oral medication designed to treat phenylketonuria (PKU), a rare metabolic disorder caused by a mutation in the enzyme phenylalanine hydroxylase (PAH). The drug works by enhancing the activity and stability of PAH, helping the body break down the amino acid phenylalanine—a protein building block—more effectively.
- Sephience was approved by the U.S. Food and Drug Administration (FDA) in July 2025 and was already approved in Europe prior to that. It is now under review or being introduced in other global markets, including Asia and Latin America.
- This treatment is important because it helps manage a metabolic disorder at its protein core—the inability to break down an amino acid properly—which, if left untreated, can lead to severe neurological damage. Sephience offers a medical option that can reduce dietary restrictions and improve metabolic control for people with PKU.
- Without treatment, phenylalanine levels can reach toxic levels in the brain. Besides strict low-protein diets, Sephience broadens therapeutic choices and improves dietary flexibility for patients.

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LEARNING OUTLINE

CHAPTER 3: ENZYMES

3.1 Chemical Nature of Enzymes
3.2 Concept of Chemical catalysts
3.3 Classification and Nomenclature of Enzymes
3.4. Factors that Affect Enzyme Activity
3.5 Properties of Enzyme
3.6. Diseases related to enzymes
3.7 Recent developments and applications concerning enzymes

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ENZYMES

INTRODUCTION

Enzymes are specialized biological molecules that function as catalysts, meaning they speed up chemical reactions without being consumed or permanently changed in the process. They are essential for life because almost every biochemical reaction in living organisms depends on enzyme activity. From breaking down food during digestion to building complex molecules, generating energy, repairing cells, and regulating metabolism, enzymes make it possible for these processes to occur quickly and efficiently under normal body conditions. Without enzymes, many reactions necessary for survival would happen far too slowly to sustain life.

Most enzymes are proteins with complex three-dimensional structures that allow them to interact with specific molecules called substrates. This specificity is one of the most remarkable features of enzymes, each enzyme typically works on only one type of reaction or a closely related group of reactions. Enzymes operate by lowering the activation energy required for a chemical reaction, making it easier for molecules to react. Their activity can be influenced by environmental conditions such as temperature, pH, and the presence of other molecules, which is why maintaining balance within the body is so important for proper enzyme function.

Enzymes are not only vital in natural biological processes but also play an important role in medicine, industry, and biotechnology. They are used in diagnostic tests, drug development, food production, and even environmental management. However, when enzymes do not function properly due to genetic mutations, deficiencies, or other disruptions, various diseases and metabolic disorders can occur. Because of their importance, enzymes continue to be a major focus of scientific research, leading to new medical treatments and technological innovations.

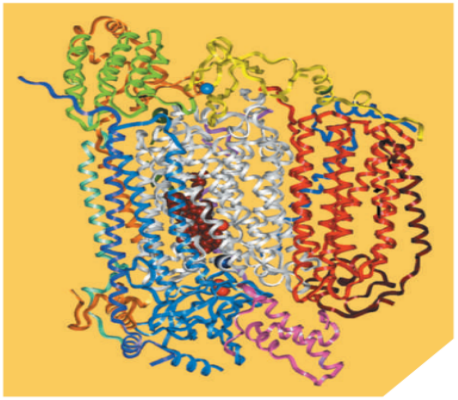
This discussion will provide a comprehensive understanding of enzymes by exploring their key concepts and functions. It will begin with their chemical nature and their role as chemical catalysts, explaining how their structure allows them to accelerate biochemical reactions. It will then examine how enzymes are classified and named based on the reactions they catalyze. The factors that affect enzyme activity such as temperature, pH, and inhibitors will also be discussed, along with the fundamental properties that make enzymes efficient and highly specific. In addition, the connection between enzyme malfunction and disease will be explored to highlight their importance

in human health. Finally, recent developments and modern applications of enzymes in science, medicine, and industry will be presented to show how enzyme research continues to shape advancements in technology and healthcare.

3.1 CHEMICAL NATURE OF ENZYMES

What are enzymes?

Enzymes act as biological catalysts. These are large molecules that increase the rates of chemical reactions without themselves undergoing any change.



Ribbon diagram of cytochrome *c* oxidase, the enzyme that directly uses oxygen during respiration.

What are they made of?

- **Globular Proteins:** The vast majority of enzymes are large molecules made of proteins folded into a "glob" shape.

A few biological catalysts are not proteins at all; they are made of ribonucleic acids (RNA).

- **Ribozymes** - They catalyze the self-cleavage of certain portions of their own molecules and have been implicated in the reaction that generates peptide bonds.

Many biochemists believe that during evolution, RNA catalysts emerged first, with protein enzymes arriving on the scene later

Enzymes are high molecular weight compounds made up principally of chains of amino acids linked together by peptide bonds (Figure 1). Enzymes can be denatured and precipitated with salts, solvents and other reagents. They have molecular weights ranging from 10,000 to 2,000,000 Da.

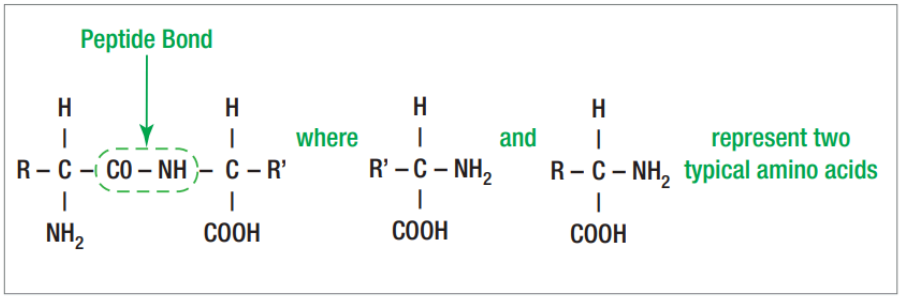


Figure 1: Typical protein structure – two amino acids joined by a peptide bond.

Other enzymes are not just **simple** proteins; they are **conjugated**, meaning they have multiple chemical parts working together.

- ☐ **Simple enzyme** is an enzyme composed only of protein(amino acid chains)
- ☐ **Conjugated enzyme** is an enzyme that has a nonprotein part in addition to a protein part.

- **Apoenzyme** - The protein (polypeptide) portion of the enzyme.
- **Cofactor** - The nonprotein part of an enzyme necessary for its catalytic function

Apoenzyme + cofactor = holoenzyme

This entire active complex is referred to as the **holoenzyme.**

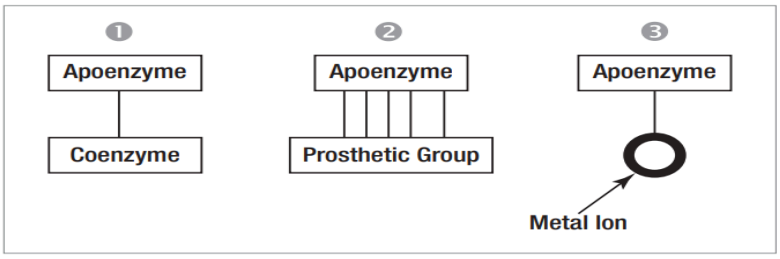


Figure 2: Holoenzymes plus various types of cofactors.

According to Holum, the cofactor may be:
1. A **coenzyme** – a non-protein organic substance which is dialyzable, thermostable and loosely attached to the protein part (e.g., B vitamins).
2. A **prosthetic group** – an organic substance which is dialyzable and thermostable which is firmly attached to the protein or apoenzyme portion (e.g., Heme).
3. A **metal-ion-activator** – these include K+, Fe2+, Fe3+, Cu2+, Co2+, Zn2+, Mn2+, Mg2+, Ca2+, and Mo3+.

The Catalytic Site and Reaction

- **Substrate:** The compound on which the enzyme works, and whose reaction it speeds up.
- **Active Site:** The substrate binds to a specific portion of the enzyme during the reaction.
    - During a reaction, the substrate is simultaneously surrounded by the apoenzyme, coenzyme, and any metal ion cofactors, forming an **enzyme-substrate complex**.

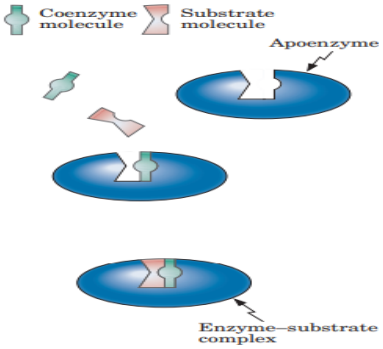


FIGURE 23.2 Schematic diagram of the active site of an enzyme and the participating components.

3.2 CONCEPT OF CHEMICAL CATALYSTS

A **CATALYST** is a chemical substance that speeds up the rate of a chemical reaction by lowering the activation energy needed for the reaction to occur. The catalyst itself is unchanged chemically by the end of the reaction.

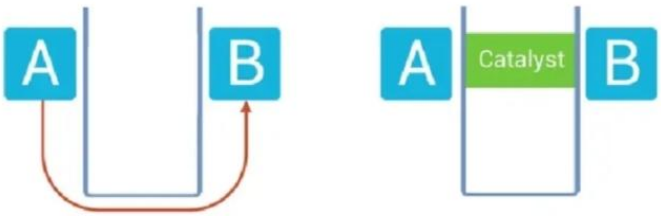
EXAMPLE:

- Consider the points A and B separated by a cliff

- The way to get from points A to B is to go around the cliff following the red path. It would take a lot of time and energy to reach point B.

- For uncatalyzed reactions (red path), the time it takes for the reaction to be completed is relatively longer. However, for catalyzed reactions, the catalysts (bridge) speed up the reaction by providing an alternate path with less required energy for reactants to turn into products

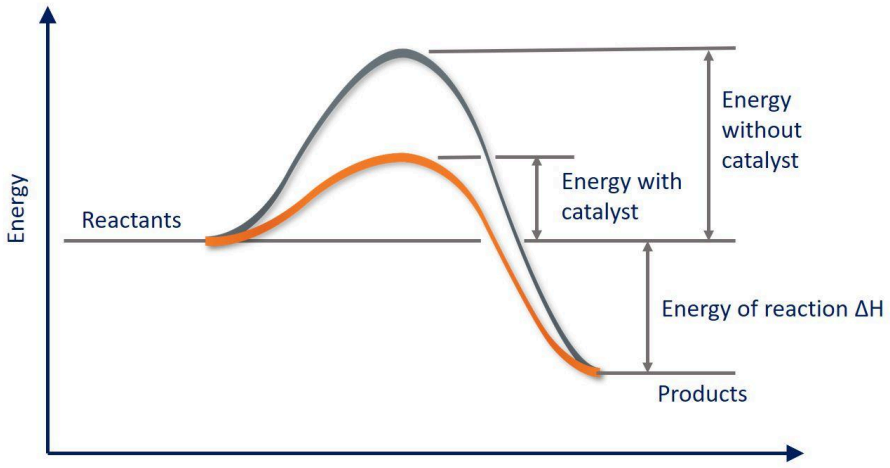
Mechanism of Catalysis



A catalyzed reaction often involves a series of steps.

1. **Bonding**- The catalyst forms a bond with the reacting molecules.
2. **Reaction**- The reacting molecules combine or rearrange to form the product.
3. **Separation**- The product separates from the catalyst.

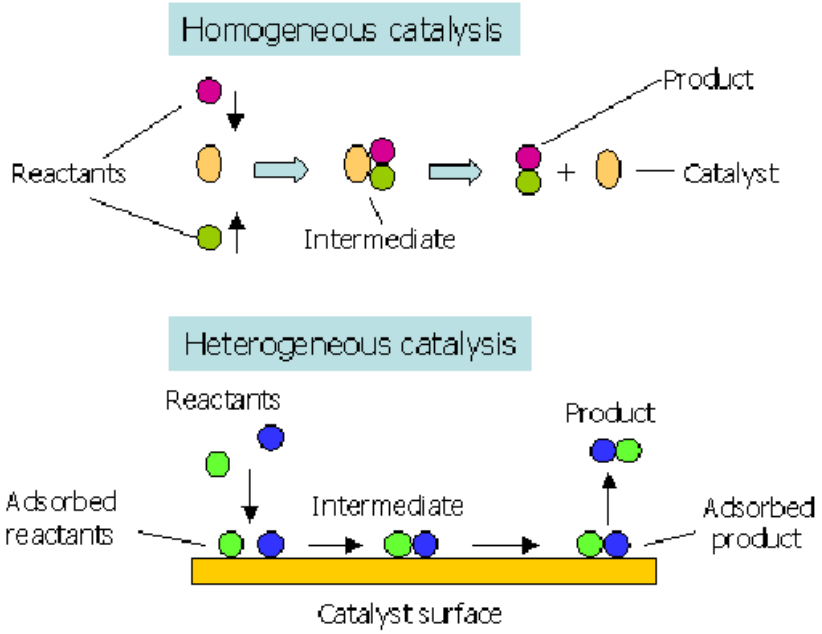
After separation, the catalyst is free again to form a bond with other reacting molecules.



Types of Catalysts

A **homogeneous** catalyst exists in the same phase as the reaction it catalyzes. It is often in the gas or liquid phase.

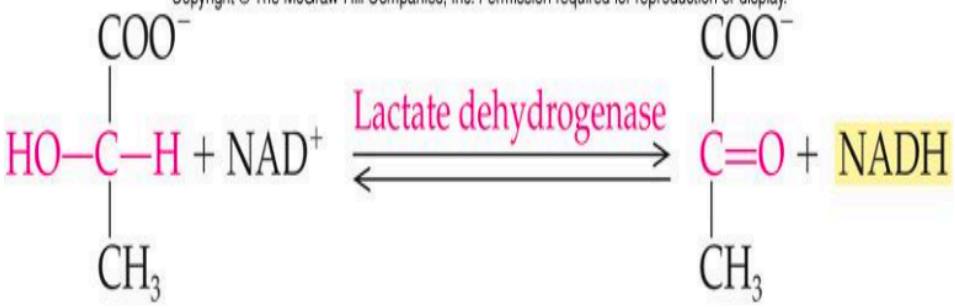
A **heterogeneous** catalyst exists in a different phase as the reaction it catalyzes. It often involves gaseous reactant molecules being adsorbed on a solid catalyst surface.



3.3 CLASSIFICATION AND NOMENCLATURE OF ENZYMES

Enzymes are primarily classified into 6 main classes based on the type of reaction catalyzed. Each class is further divided into subclasses and sub-subclasses based on functional groups, bonds, or specific mechanisms. Individual enzyme classes are systematically refined using the chemical name of the substrate and reaction.

1. Oxidoreductase: Catalyze oxidation-reduction (redox) reactions; subdivided into oxidases (O_2 as acceptor) and reductases.

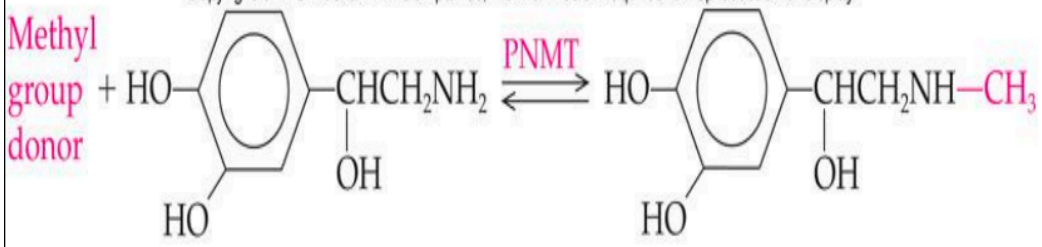


Lactate Pyruvate

2. Transferase: Transferases transfer a group from one molecule to another:

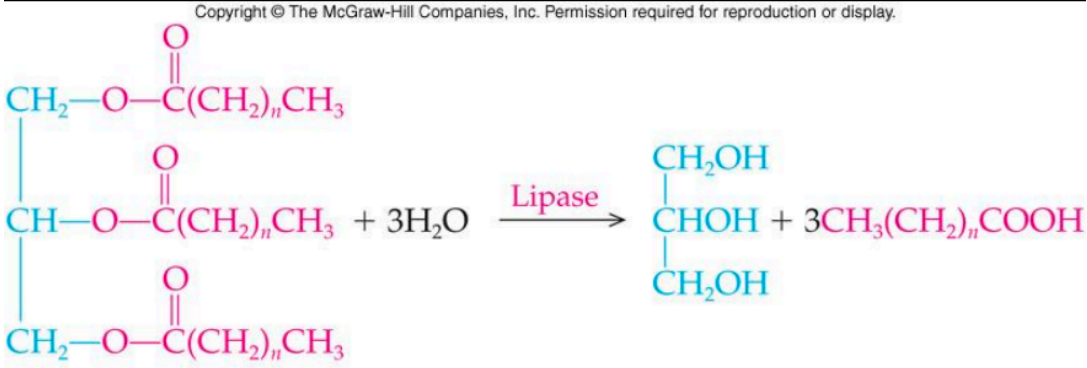
• Transaminases catalyze transfer of an amino group

• Kinases transfer a phosphate group.



Norepinephrine Epinephrine

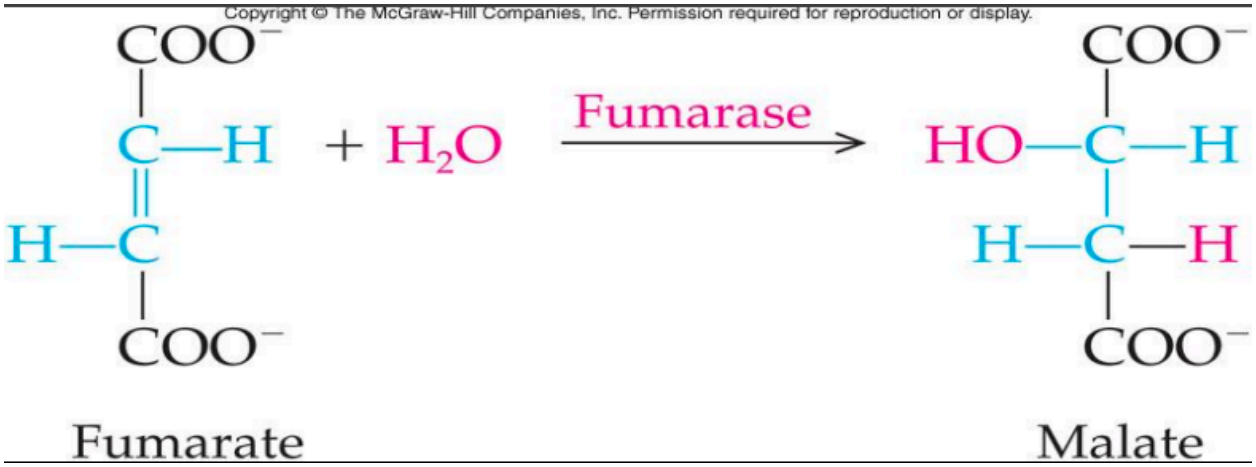
3. Hydrolase: Catalyze hydrolytic enzyme lipases, peptidases/proteases. These are of the general form: A-X + H2O ↔ X-OH + HA



4. Lyase: Lyases catalyze removal of groups to form double bonds or the reverse break double bonds:

• Decarboxylases

• Synthases

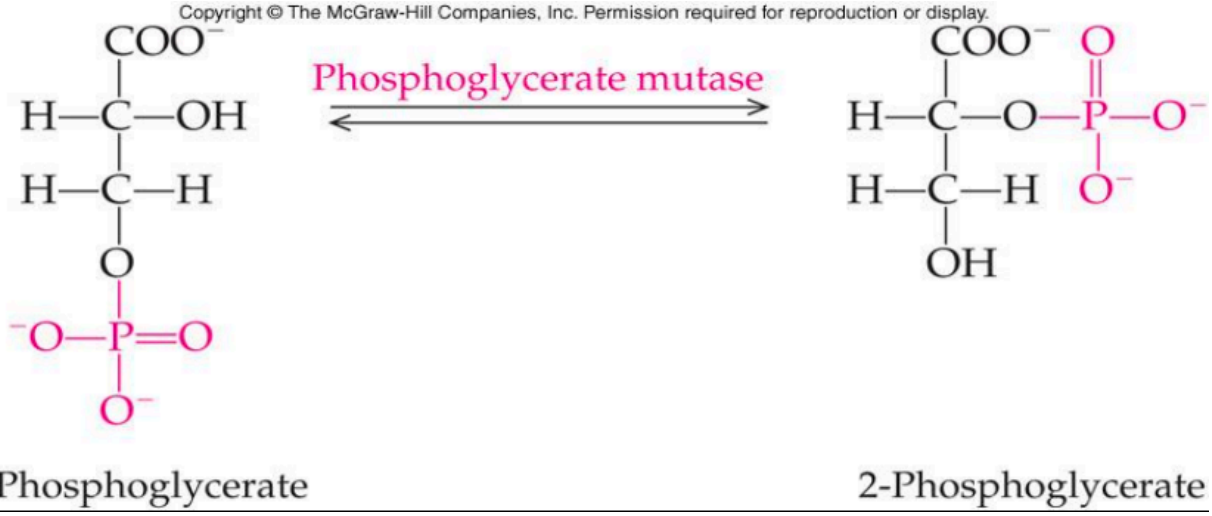


5. Isomerase: Rearrange atoms within a molecule (isomerization, including geometric/optical isomers):

Isomerases catalyze intramolecular rearrangements

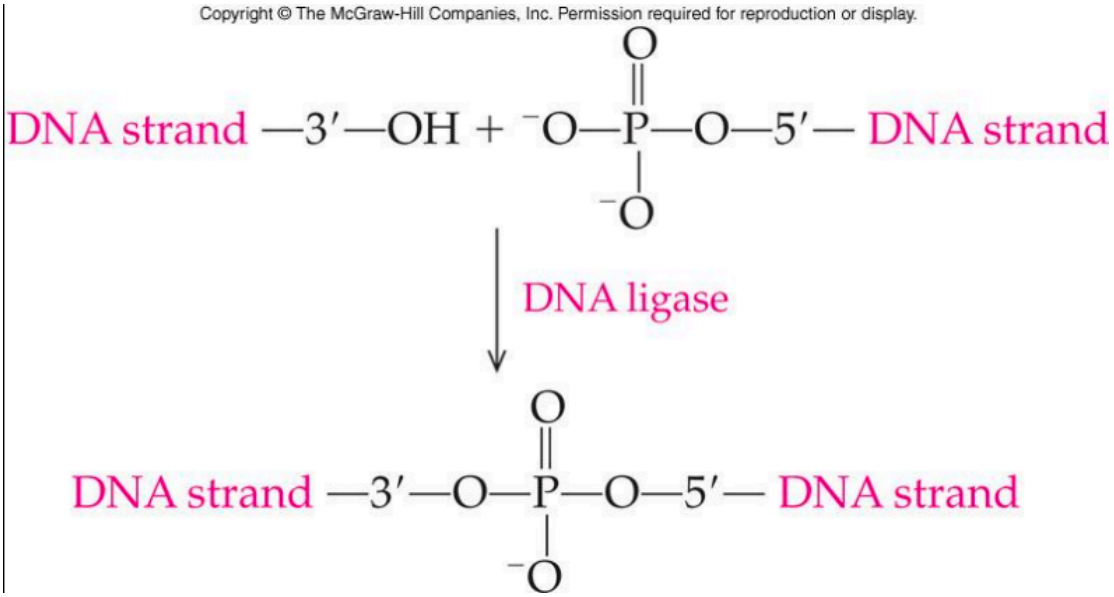
• Epimerases

• Mutases



6. Ligase: in two molecules (synthesis) using energy from ATP hydrolysis; e.g., forming C-C, C-O, C-N bonds:

Ligases catalyze a reaction in which a C-C, C-S, C-O, or C-N bond is made or broken



Nomenclature of Enzymes

In most cases, enzyme names end in "-ase" ; the common name for a hydrolase is derived from the substrate.

Urea: remove -a, replace with –ase :urease
Lactose: remove – ose , replace with -ase= lactase

Other enzymes are named for the substrate and the reaction catalyzed such as "Lactate dehydrogenase" and "Pyruvate decarboxylase".

Some names are historical - no direct relationship to substrate or reaction type, eg: Pepsin, Trypsin

3.4. FACTORS THAT AFFECT ENZYMES ACTIVITY

There are a number of factors that affect the rate of enzyme activity:

- Temperature
- pH
- Substrate concentration
- Enzyme concentration
- Presence of inhibitors
    - Competitive inhibition
    - Non-competitive inhibition
    - Feedback inhibition

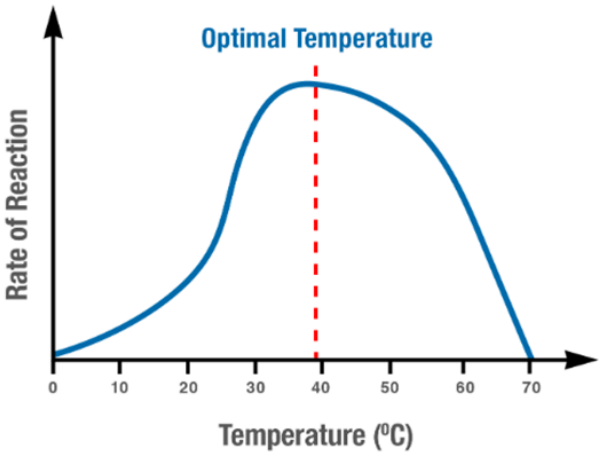
Temperature

- Temperature affects enzyme-catalysed reactions by influencing the rate at which they occur. For a reaction to occur between an enzyme and substrate the two molecules need to collide.

- At lower temperatures, the reaction rate is low as the enzymes and substrates do not collide frequently. As temperature increases, reaction rates generally rise due to more frequent collisions between enzyme and substrate molecules.

- Once the temperature exceeds an enzyme's optimal range, the enzyme can **denature**, losing its structure and function, which significantly decreases or stops the reaction.

The graph below shows this relationship between temperature and rate of reaction. Human enzymes have an optimal activity at about 37 degrees Celsius.

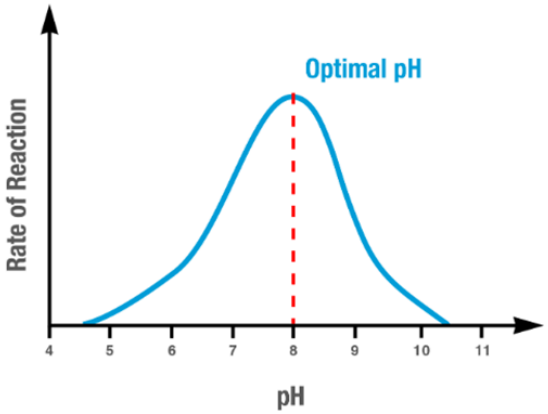


pH

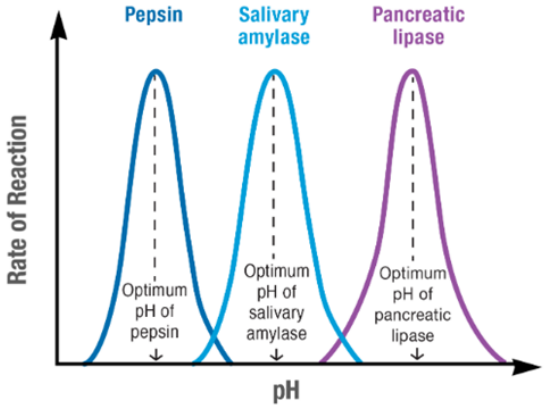
- Each enzyme has an optimal pH range in which it functions most effectively, and deviations from this range can lead to reduced activity or denaturation of the enzyme.

- Extremely high or low pH levels can cause enzymes to denature, meaning they lose their three-dimensional structure, changing the shape of the active site and resulting in the enzyme being ineffective. This loss of structure can prevent the substrate from binding properly.

This relationship between pH and rate of reaction is shown in the graph below. At a low pH the rate of reaction is low. It is high around the enzyme's optimal pH and then decreases as the pH above the optimal pH.



The diagram below shows the activity of three enzymes that are found in different parts of our body, showing their optimum pH.

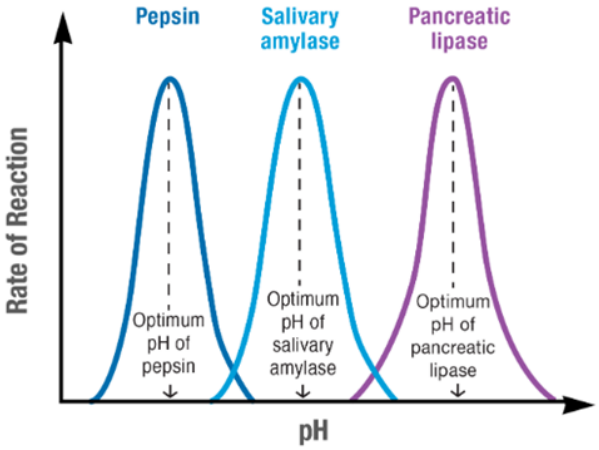


- ***Pepsin*** *is found in the stomach, which has a low pH due to the hydrochloric acid.*
- ***Salivary amylase*** *is found in the mouth, which is neutral.*
- ***Pancreatic lipase*** *acts in the small intestine, which has a high pH due to bicarbonate, which neutralises the stomach acid.*

Substrate concentration

- As substrate concentration increases, the rate of an enzyme-catalysed reaction initially rises as more substrate molecules are available to bind to enzyme active sites.

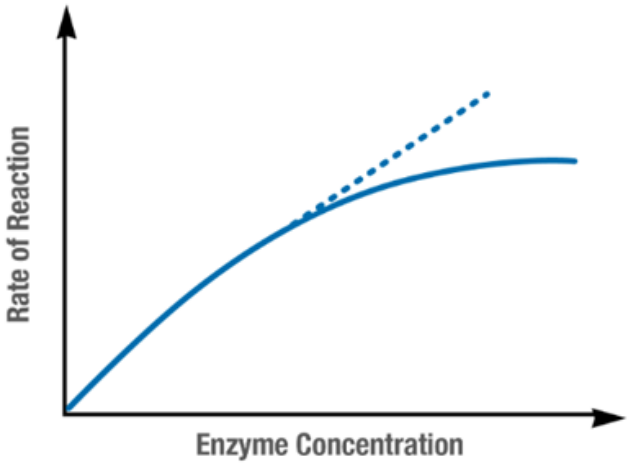
- However, if the amount of enzyme is limited, then the reaction will proceed until all enzymes become saturated with substrate. At this point the rate of reaction will plateau, as all active sites are occupied and unable to process additional substrate molecules any faster.



This plateau in reaction rate can continue until the substrate is exhausted and rate falls again, eventually to zero.

Enzyme concentration

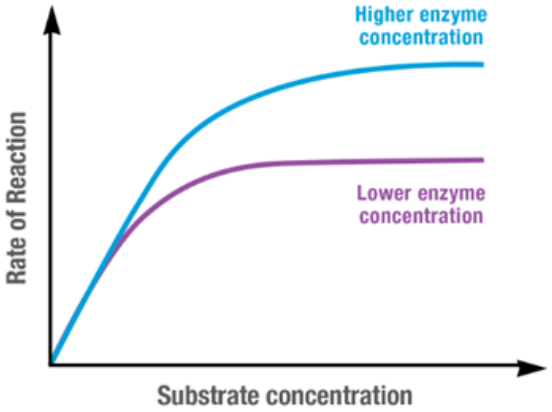
- Increasing enzyme concentration can increase the rate of reaction, as there are more enzyme molecules available to catalyse the reaction, leading to more frequent enzyme-substrate interactions. This is shown in the graph below by the dotted line



- Enzyme concentration directly affects the rate of reaction. More enzyme molecules mean more active sites for substrate binding, which leads to an increase in reaction rate, provided there is sufficient substrate.

- If the amount of substrate is limited, increasing enzyme concentration beyond a certain point will have little to no effect on enzyme activity, since there will be a surplus of unnecessary enzyme molecules.

The effect of enzyme concentration and substrate concentration on reaction rate



Note that in this case, the amount of enzyme is the limiting factor, so as the enzyme concentration increases, the reaction rate also increases until all the enzymes are saturated. At this point, the reaction rate plateaus, unless more enzymes are added to the reaction.

Presence of inhibitors

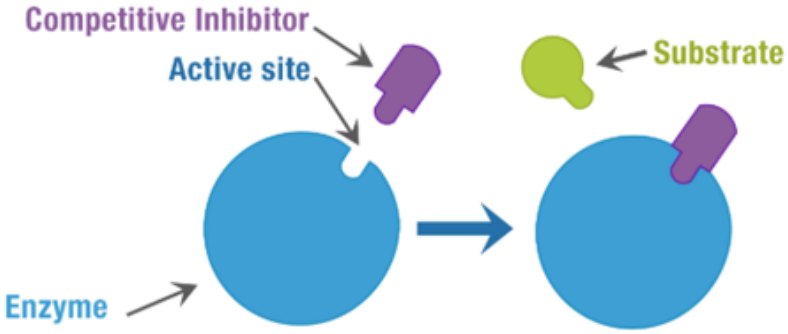
Enzyme function can be regulated by inhibitors in a number of ways:

- **Competitive inhibitors** that bind to the active site and block the substrate from binding
- **Non-competitive inhibitors** that bind to a different site on the enzyme and alter its shape, reducing its activity
- **Feedback inhibitors** is a regulatory mechanism where the end product of a metabolic pathway binds to an enzyme involved early in the pathway, reducing its activity to prevent overproduction.

Competitive Inhibition

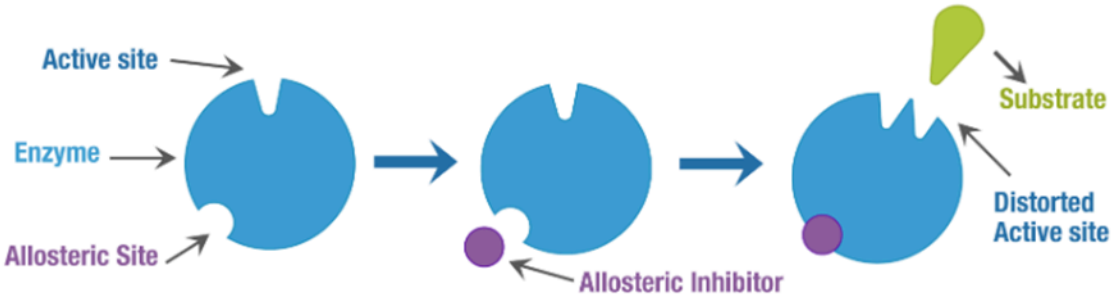
Competitive inhibitors are molecules that bind to the active site of an enzyme, directly competing with the substrate and preventing it from attaching.

The shape of the competitive inhibitor is complementary to the active site of the enzyme.



Non-Competitive Inhibition

Non-competitive inhibitors bind to a site other than the enzyme's active site, called the allosteric site, causing a change in the enzyme's shape and reducing its activity regardless of substrate concentration



Feedback Inhibition

Feedback inhibition is a regulatory mechanism where the end product of a biochemical pathway inhibits an enzyme involved early in the pathway, preventing overproduction and conserving resources.

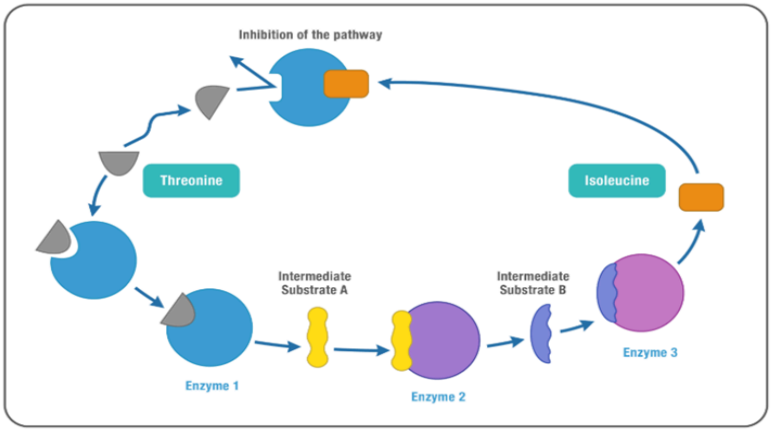
Example

- In bacteria, feedback inhibition occurs in the production of the amino acid isoleucine from the amino acid threonine in a series of enzyme catalysed reactions.

- When isoleucine levels become sufficiently high, it acts as an inhibitor by binding to the first enzyme in the pathway, threonine deaminase.

- By binding allosterically to threonine deaminase, isoleucine changes the enzyme's shape, reducing its activity and thereby slowing down or stopping the production of more isoleucine.

This ensures that the cell does not produce excess isoleucine, conserving energy and resources.



3.5 PROPERTIES OF ENZYME

What an enzyme is?

Enzymes are proteins that act as biological catalysts in their respective biological reactions. A substrate is a molecule upon which an enzyme can function, and a product is a molecule produced by the enzyme after the enzyme has converted the substrate into another molecule. In 1878, Wilhelm Kuhne coined the term “enzyme.” Enzymes are macromolecules that are extremely precise in their function. Enzymes are required for all metabolic activities in our bodies because they catalyse reactions at a quicker rate. In contrast to catalysts, enzymes are naturally created by living cells.

Enzymes as Biocatalysts

The following are some of the reasons why enzymes are referred to as “Biocatalysts”: Enzymes, like catalysts, increase the rate of a reaction by shortening the time it takes for the substrate to become the product of the reaction. Additionally, enzymes govern reaction specificity, which is the ability of an enzyme to produce a given product only when a specific substrate adheres to its active site. Like catalysts, enzymes just participate in the biological reaction and do not consume any resources or alter the equilibrium state of the system in which they function. The activation energy of a biological reaction is reduced by enzymes, and the transition energy from substrate to product is increased by enzymes.The following are some of the reasons why enzymes are referred to as “Biocatalysts”: Enzymes, like catalysts, increase the rate of a reaction by shortening the time it takes for the substrate to become the product of the reaction.

Physical Properties of enzymes

1. Physically enzymes behave as colloids or as substances of high molecular weight.
2. Enzymes are destroyed or inactivated at temperature below the boiling point of water.
3. At 60 degrees Celsius most enzymes in liquid medium are inactivated.
4. Dried enzymes extract can endure temperatures 100 degree Celsius to 120 degrees Celsius or even higher. Thus enzymes are thermos-labile.

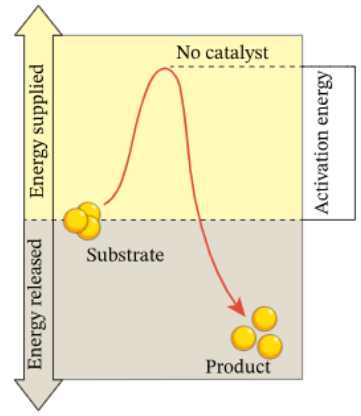
5. There is always a specific temperature of optimum activity of every enzyme, which usually ranges from 25 degrees Celsius to 45 degrees Celsius. Enzymatic action is highest at 37 degrees Celsius and enzymes become inactive when temperature rises above 60 degrees Celsius.

General properties of enzymes

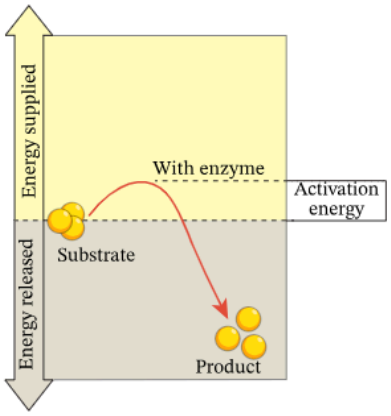
1. Catalytic property
2. Specificity
3. Reversibility
4. Sensitiveness to Heat and Temperature
5. Specific to Hydrogen Ion Concentration (pH)

1. **Catalytic Properties**

Enzymes are proteins (some may be catalytic RNA) that have a unique ability to accelerate chemical reactions within cells. They increase the rate of the reaction while remaining unchanged afterward. They work through a variety of ways, but they all increase reaction rate by lowering the activation energy of the reaction. Enzymes, like proteins, can coagulate with heat, alcohol, strong acids, and alkaline reagents. Over 2,000 enzymes have been found so far.



Progress of reaction without enzyme



Progress of reaction with enzyme

Figure 7. *Two diagrams depict the activation energy (the energy necessary for a reaction to occur) with and without an enzyme. The enzyme has a substantially lower activation energy, allowing the process to proceed more quickly.*

Using enzymes allows for more chemical reactions to occur over a given length of time than without the enzyme, increasing the rate of reaction. Figure 1 shows how much more energy is required for a reaction to proceed without an enzyme than with it. Consider this single reaction occurring thousands of times, and you'll understand why using an enzyme in biological reactions is so much more energy efficient. Many critical events in our cells are simply too sluggish to occur on their own. For example, if the enzymes involved in respiration did not work properly, we would be unable to release sufficient energy in our cells to survive.

2. Specificity

Enzymes are quite particular in their actions. Enzymes function on specific substrates only. Enzymes are also unique to certain types of reactions. In some rare circumstances, the specificity may be too low. Enzymes exhibit many sorts of specificity, as follows:

1. Bond Specificity: It is also called relative specificity. Here the enzymes are specific for a bond. eg; peptidase is specific or peptide bond, lipase is specific for ester bond in a lipid.
2. Group Specificity: It is also called structural specificity. Here the enzymes are specific for a group.
3. Substrate Specificity: It is also called absolute specificity. Here the enzyme acts only on a particular substrate. eg; arginase acts only on arginine; carbonic anhydrase acts only on carbonic acid.
4. Optical Specificity: It is also called stereo-specificity. This is the highest specificity shown by an enzyme. Here the enzymes are specific not only to the substrate but also to its optical configuration. e.g. L amino acid oxidase acts only on L-amino acids, not on D-amino acids. Similarly, the alpha-amylase acts only on

alpha-14 glycosidic linkage of starch and glycogen. It is not able to hydrolyse the beta-14 glycosidic linkage of cellulose.

5. Co-factor Specificity: This shows that enzymes are not only specific to the substrate but also specific to its co-factors.
6. Geometric Specificity: Here the specificity is very less. Some enzymes will work with a small range of similar substrates having similar structural geometry. e.g. alcohol dehydrogenase can oxidise methanol and n-propanol to aldehydes.

3. Reversibility

Enzymatic reactions are theoretically reversible, with enzymes capable of catalyzing both forward and backward reactions depending on substrate/product concentrations and cellular conditions. While many enzymes facilitate reversible processes in metabolic pathways, others function irreversibly due to high energy barriers.

4. Sensitiveness to Heat and Temperature

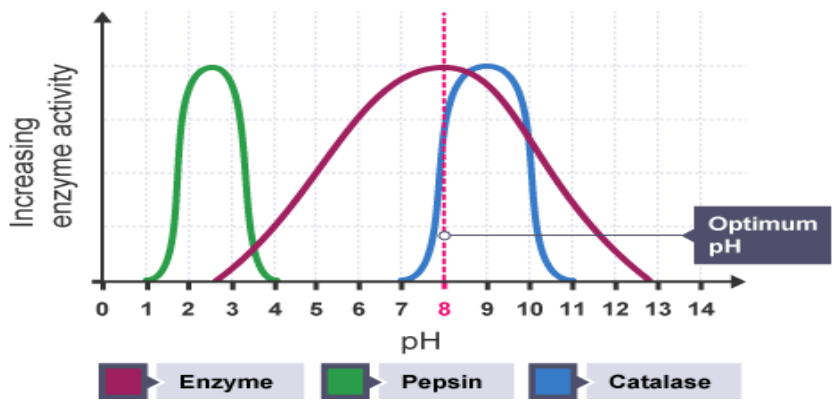
Enzymes are very sensitive to heat and temperature. They are thermolabile. The maximum activity of Associate in Nursing protein is at traditional temperature. The correct temperature for the utmost activity is termed optimum temperature. Enzymes will be inactive at very low temperatures; this is the reason for preserving food and vegetables in the refrigerator. The enzymatic activity increases with the increase in temperature up to a certain level. At higher temperature (60-70 degree Celsius), the enzyme is destroyed or denatured

5. Specific to Hydrogen Ion Concentration (pH)

Enzymes are sensitive to acidity and alkalinity. They don't work properly if an environment is too acidic or basic. For example, an enzyme in the stomach called pepsin breaks down proteins. If your stomach doesn't have enough acid, pepsin can't function optimally.

The pH scale is used to measure the acidity or alkalinity of a sample and describes how many hydrogen ions or hydroxides are present in the sample. The change of pH will lead to the ionization of amino acids, atoms and molecules, changing the shape and

structure of proteins, thus damaging the function of proteins. Enzymes are also proteins, which are also affected by changes in pH. Very high or very low pH will lead to the complete loss of the activity of most enzymes. The pH value at which the enzyme is most active is called the optimal pH value



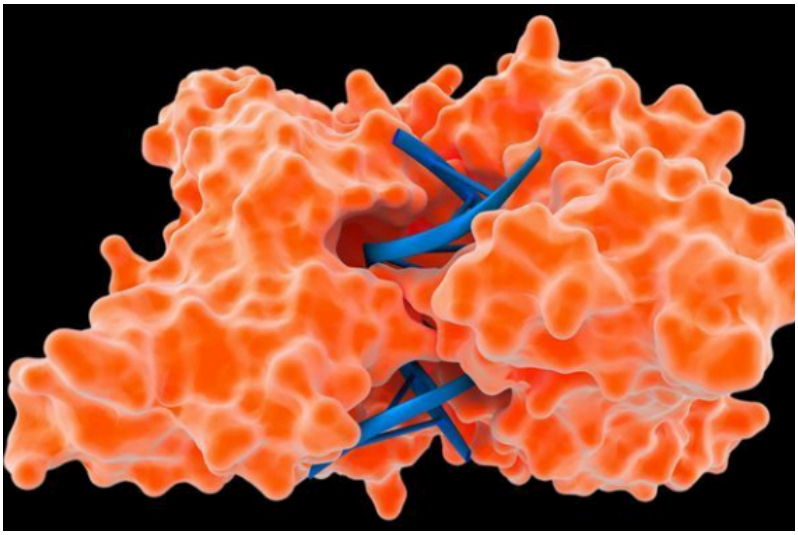
pH Effects Enzyme Activity

The enzyme's structure greatly influences its activity. In other words, changes in the structure of the enzyme influence the pace of chemical reactions. When the pH of the reaction media changes, the enzyme's shape and structure change. For example, pH can influence the ionization of acidic or basic amino acids. The side chains of acidic amino acids include carboxyl functional groups. Amine-containing functional groups can be found in the side chains of basic amino acids. If the ionized state of amino acids in a protein changes, so do the ionic interactions that keep the protein in its three-dimensional form. This may result in alterations in enzyme function.

pH Effects Substrates

pH affects not only the enzyme's activity, but also the charge and shape of the substrate, preventing it from binding to the active site or being catalyzed to produce a product. Enzyme and substrate structural and morphological changes may be reversible within a specific pH range. However, if the pH changes significantly, the enzyme and substrate may get denatured. In this situation, the enzyme and substrate do not recognize each other, hence there is no reaction.

3.6. DISEASES RELATED TO ENZYMES



Enzymes are biological catalysts that speed up chemical reactions in the body and are essential for life. They play a crucial role in processes such as digestion, metabolism, DNA replication, and energy production. Each enzyme has a specific function, and even a small change in its structure or activity can disrupt normal body processes. When enzymes are absent, deficient, or not functioning properly due to genetic mutations or other factors, various diseases can develop.

Diseases caused by enzyme dysfunction are often referred to as metabolic or enzyme-related disorders. These conditions occur when the body cannot properly break down or process certain substances, leading to the accumulation of harmful materials or the deficiency of important products. Many of these disorders are inherited and can affect different organs, including the brain, liver, and muscles. Understanding enzyme-related diseases is important because it helps in early diagnosis, proper treatment, and the development of targeted therapies such as enzyme replacement therapy.

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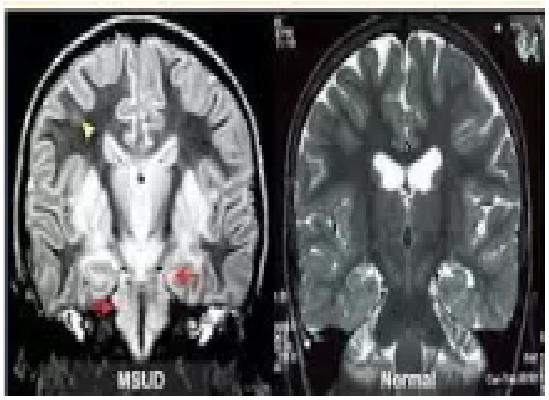
Enzymes are essential for regulating the chemical reactions in our bodies, and when they are absent or not functioning properly, various diseases can occur. Below is a list of some common diseases caused by enzyme dysfunction along with their characteristic symptoms.

Maple Syrup Urine Disease (MSUD) is a rare inherited metabolic disorder in which the body cannot properly break down certain branched-chain amino acids (leucine, isoleucine, and valine). These amino acids and their toxic byproducts build up in the blood and urine, causing serious health problems. The disease is named for the **sweet-smelling urine** characteristic of affected individuals, similar to maple syrup.

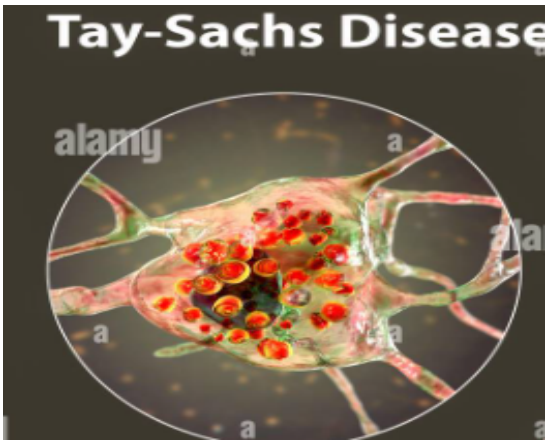


Cause: MSUD is caused by a deficiency of the branched-chain alpha-keto acid dehydrogenase complex, the enzyme complex responsible for breaking down leucine, isoleucine, and valine.

- It is inherited in an autosomal recessive pattern (both parents must pass the defective gene).

Symptoms: usually appear in the first few days of life and may include:

- Poor feeding and vomiting
- Lethargy or unusual sleepiness
- Irritability
- Sweet-smelling urine (like maple syrup)
- Developmental delays

• Seizures • If untreated, can lead to severe neurological damage or death	
Tay-Sachs disease is a rare, inherited genetic disorder that progressively destroys nerve cells (neurons) in the brain and spinal cord. It is a **lysosomal storage disease**, meaning the body cannot properly break down certain fatty substances, which then build up and cause damage to the nervous system. **Cause:** The disease is caused by a **deficiency of the enzyme hexosaminidase A**. This enzyme normally breaks down a fatty substance called **GM2 ganglioside**. When the enzyme is missing or not functioning properly, GM2 accumulates in nerve cells, leading to progressive neurological damage. • Inherited in an **autosomal recessive** pattern (both parents must pass the defective gene). **Symptoms:** usually appear in infancy and progressively worsen: • **Loss of motor skills** (difficulty crawling, sitting, or moving) • **Muscle weakness** • **Exaggerated startle** response • **Seizures** • **Vision** and **hearing loss**	

- **Cherry-red spot** on the retina (diagnostic sign)
- **Developmental delays**
- **Progressive neurodegeneration** leading to early childhood death (usually by age 4–5 in the severe infantile form)

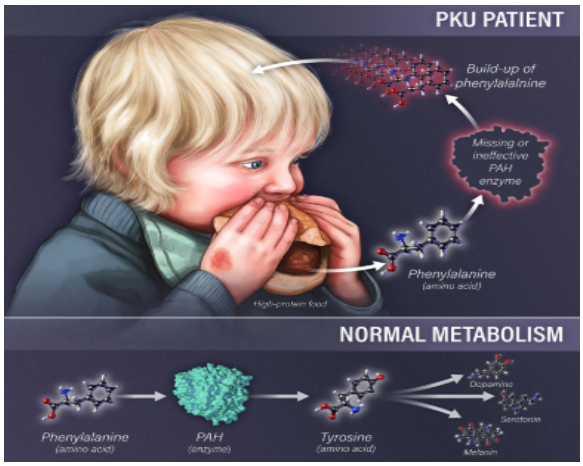
Phenylketonuria (PKU) is a rare inherited metabolic disorder in which the body cannot properly break down the amino acid **phenylalanine**. If untreated, phenylalanine builds up in the blood and brain, which can lead to intellectual disability and other serious health problems.

Cause: PKU is caused by a **deficiency of the enzyme phenylalanine hydroxylase**, which normally converts phenylalanine into another amino acid called tyrosine.

- It is inherited in an **autosomal recessive** pattern (both parents must pass the defective gene).

Symptoms: usually appear in infancy and may include:

- Intellectual disability (if untreated)
- Developmental delays
- Seizures
- Behavioral problems
- A musty or “mousy” odor in the body or urine



- Fair skin and hair compared to other family members (due to lack of melanin)

Alkaptonuria is a rare inherited metabolic disorder in which the body cannot properly break down certain amino acids, specifically **tyrosine and phenylalanine**. This leads to the accumulation of a substance called **homogentisic acid** in the body, which can deposit in tissues and cause damage over time.

Cause: The disease is caused by a **deficiency of the enzyme homogentisate oxidase**, which is needed to break down homogentisic acid.

- It is inherited in an **autosomal recessive** pattern (both parents must pass the defective gene).

Symptoms: appear in childhood or adulthood and may include:

- **Dark-colored urine** (especially when exposed to air)
- **Joint pain and stiffness** (due to pigment deposits in cartilage)
- **Discoloration of connective tissues** (such as ears, eyes, and skin, called ochronosis)
- Heart problems (in some cases, due to pigment deposits in heart valves)
- Kidney stones

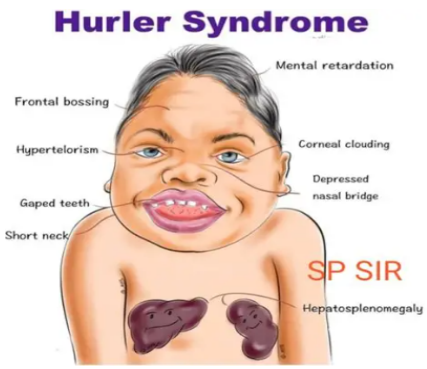
Hurler Syndrome is a rare **genetic disorder** and the most severe form of **mucopolysaccharidosis type I (MPS I)**. It is a **lysosomal storage disease**, meaning the body cannot properly break down certain complex sugars called **glycosaminoglycans (GAGs)**. The accumulation of these substances in cells leads to progressive damage to tissues and organs.

Cause: Hurler Syndrome is caused by a **deficiency of the enzyme alpha-L-iduronidase**, which is necessary to break down glycosaminoglycans.

- It is inherited in an **autosomal recessive** pattern (both parents must pass the defective gene).
- Without this enzyme, GAGs build up in organs, bones, and connective tissues, leading to widespread physical and developmental problems.

Symptoms: usually appear in early childhood and may include:

- **Developmental delay**
- **Skeletal abnormalities** (short stature, joint stiffness, and abnormal bone growth)
- **Enlarged organs** such as the liver and spleen
- **Coarse facial features**
- **Heart problems**
- **Vision and hearing impairment**
- Progressive physical and cognitive decline



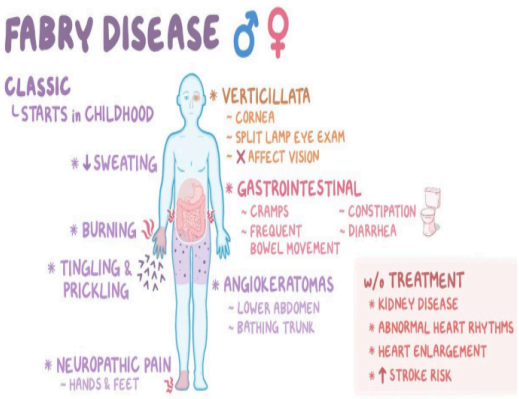
Fabry disease is a rare **genetic lysosomal storage disorder** in which the body cannot properly break down certain fatty substances called **globotriaosylceramide (GL-3 or Gb3)**. This leads to their buildup in blood vessel walls and various organs, causing progressive damage.

Cause: Fabry disease is caused by a **deficiency of the enzyme alpha-galactosidase A**, which is needed to break down Gb3.

- It is inherited in an **X-linked recessive** pattern, meaning the defective gene is on the X chromosome.
- Men are usually more severely affected, while women may have milder symptoms.

Symptoms: can vary but often include:

- **Pain, burning, or tingling in hands and feet** (neuropathic pain)
- **Small, dark red spots on the skin** called angiokeratomas
- **Decreased sweating** (hypohidrosis)
- **Corneal opacity** (clouding of the eyes, usually without affecting vision)
- **Gastrointestinal problems** (abdominal pain, diarrhea)
- **Kidney problems** (proteinuria, **kidney failure** in severe cases)
- **Heart problems** (heart enlargement, arrhythmias)



3.7 RECENT DEVELOPMENTS AND APPLICATIONS CONCERNING ENZYMES

The enzyme technology provided a new route for manufacturing bulk and high added-value products utilizing enzymes, in order to meet needs such as food (e.g., bread, cheese, beer, vinegar), fine chemicals (e.g., amino acids, vitamins), and pharmaceuticals. The enzyme based processes have gained momentum in various sectors such as in washing and bioremediation, or for analytical and diagnostic purposes.

According to the intended use food enzymes are categorized either as:

- Food additives having a technological function
- Processing aids present only in residual amounts in food and not having a function anymore there

Enzymes and Applications

Function	Enzyme	Application
Enzymes as Diagnostic Tool	Choline Esterase	Paralysis
Enzymes used to Diagnose Inheritable Genetic Disorder	HGPRT (Hypoxanthine guanine phosphorylase Adenosine Deaminase)	Immunodeficiency Disease
Enzymes as Analytical Agent	Urease Glutamate Dehydrogenase	Urea
Therapeutic Enzymes	L-asparaginase	Cancer Chemotherapy
Baking Enzymes	Baking Lipase	To improve dough strength, volume, and crumb whiteness.

Dietetics Enzymes	Amylase	Aid for digestion of dietary starch
Egg-processing Enzymes	Glucose Oxidase	Prevents browning
Flavoring Enzymes	Esterase, Lipase	Produces typical Cheddar-type flavor
Fruit and Vegetable Processing Enzymes	Alpha-amylase	Hydrolysis of starches during fruit processing

Table 3: *Application of Enzymes*

Recent developments in enzyme research focus on improving enzyme efficiency, stability, and specificity through techniques like genetic engineering, directed evolution, and artificial intelligence. These advances allow enzymes to work better under industrial conditions and perform new chemical reactions. Enzymes are widely used in medicine (drug and vaccine production), food processing (fermentation, flavor, and shelf life improvement), and environmental protection (waste treatment, biofuels, and plastic degradation). Overall, modern enzyme technology supports greener, safer, and more sustainable processes across many industries.

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