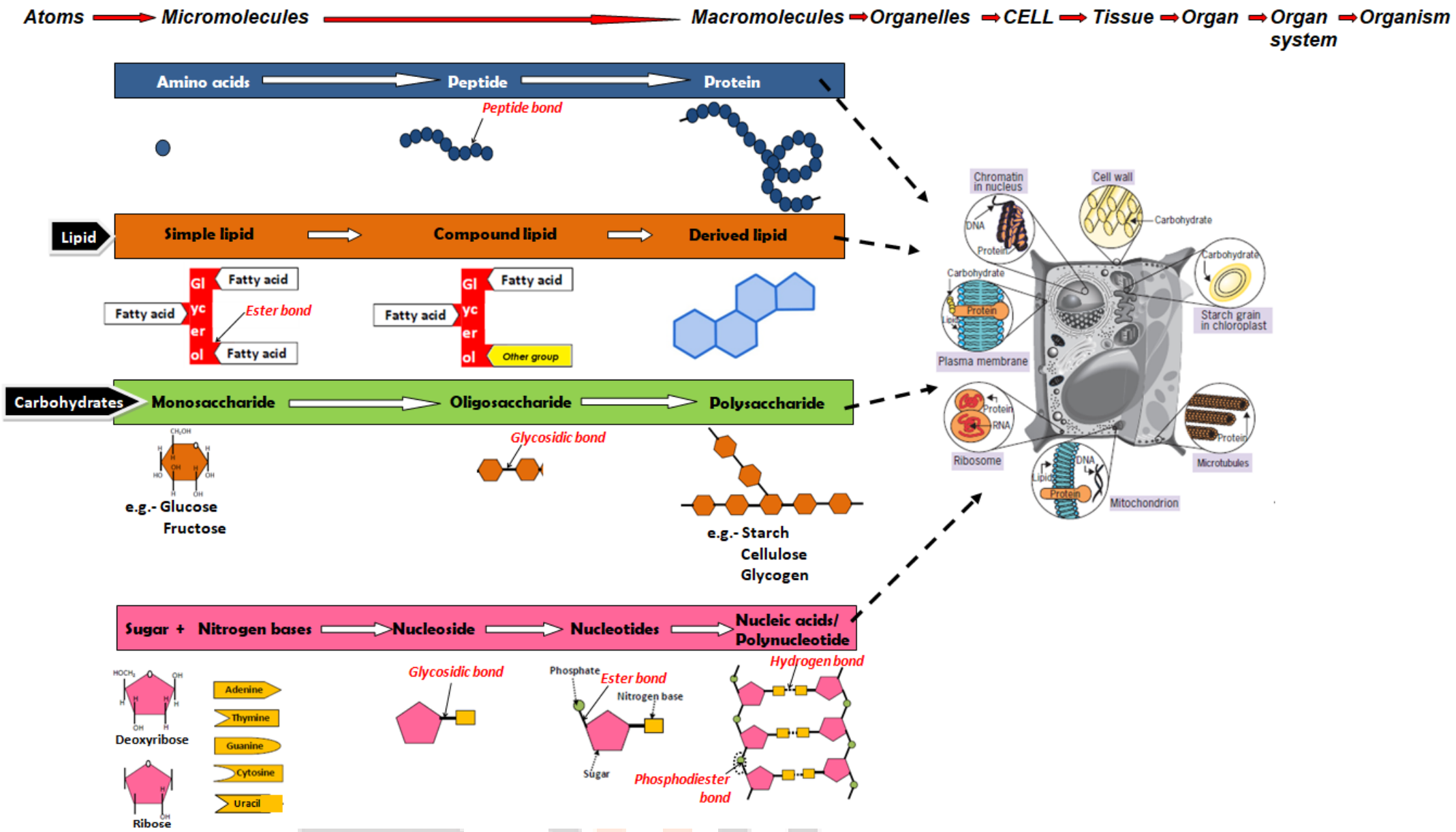
9. BIOMOLECULES

Biomolecules: Carbon compounds present in living systems.

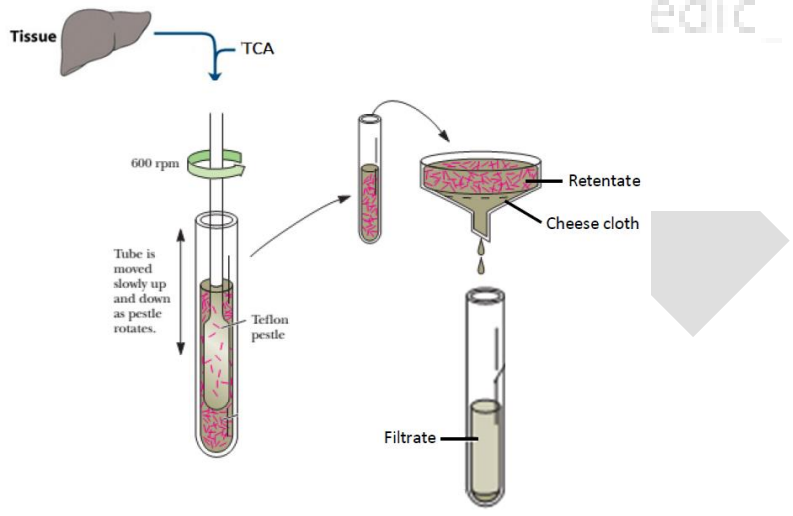


ANALYSIS OF ORGANIC COMPOUNDS

Acid Solubility test

(Step-1) Take a living tissue (a vegetable / piece of liver).

(Step-2) Grind it in **trichloroacetic acid (Cl_3CCOOH)** using a mortar and a pestle to get a thick slurry.



(Step-3) Filter the slurry through a cheesecloth or cotton; it will get separated to 2 fractions-

Filtrate / Acid-soluble pool / Micromolecules	**Retentate / Acid-insoluble fraction / Macromolecules**
Include: Amino acids, nucleotides, simple sugars, nitrogen bases etc.	Proteins, nucleic acids, polysaccharides and lipids*
Molecular weights: 18 -800 daltons	>10,000 Da (except lipids < **800 Da**)

Grinding tissue → Lipids form vesicles (water insoluble)
∴ lipids are not strictly macromolecules.

ANALYSIS OF INORGANIC COMPOUNDS

Ash test

Tissue —Dry / Burn→ **Ash** → **Elemental analysis**

(Step-1) Take a living tissue (leaf or liver)

(Step-2) Dry the tissue (to evaporate water).

(Step-3) Burn it to ash (to oxidize all the C-compounds to CO_2).

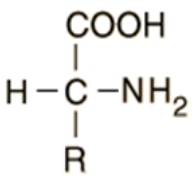
(Step-4) The ash is subjected to elemental analysis to know the **inorganic elements (Ca^{2+}, Mg^{2+}** etc**)** and **inorganic compounds (SO_4^{2-}, PO_4^{3-}** etc**)** contained in it**.**

BIOMICROMOLECULES

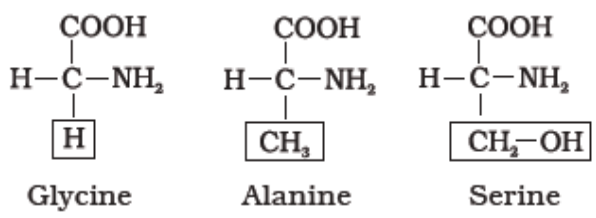
➔ Biomolecules having molecular weight **less than 1000 Da** are called **micromolecules.**

1. Amino acids

- **Amino acid** are organic compound containing an amino group ($-NH_2$), an acid group (-COOH), H & a variable group (R) attached to a C- atom (C_α).



- There are 20 amino acids used as building blocks for protein synthesis.
  E.g. -



Classification -

A. Based on **requirement** by animals, amino acids are 2 types:

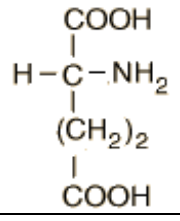
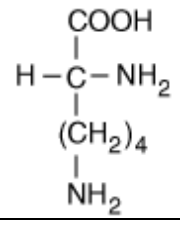
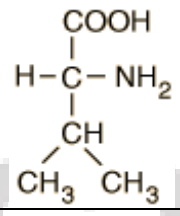
o **Essential amino acids** (should get through diet)

Lysine, leucine, isoleucine, methionine, phenylalanine, tryptophan, histidine, threonine, arginine and valine include in this type.

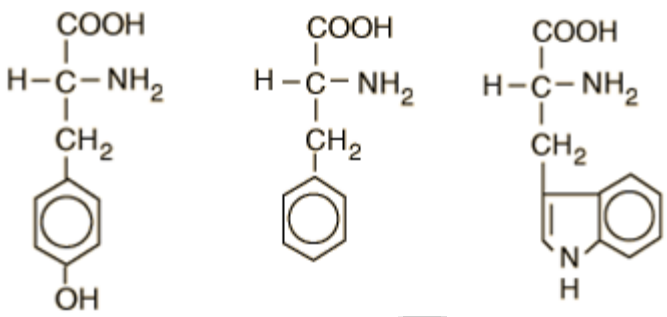
o **Non-essential amino acids** (body can synthesize)

Alanine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine, and tyrosine.

B. Based on the no. of amino & carboxyl group

Type	Definition	Example
Acidic	1 amino group and 2 carboxyl group OR have a acidic group (-COOH) in R	**Glutamic acid** 
Basic	2 amino group and 1 carboxyl group OR have a basic group ($-NH_2$) in R	**Lysine** 
Neutral	1 amino group and 1 carboxyl group OR have neither acidic nor basic group in R	**Valine** 

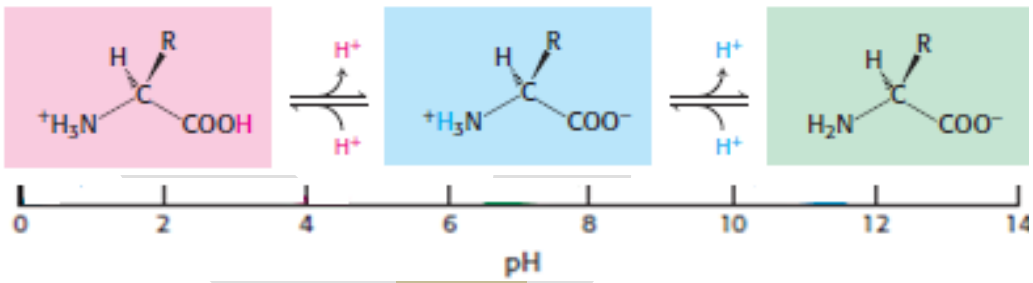
❖ **Aromatic** - Amino acids with cyclic structure in the variable group.



Ex: Tyrosine Phenyl alanine Tryptophan

➢ Amino acids have ionizable $-NH_2$ & –COOH groups. So its structure changes in solutions of diff. pH.

Amino acid, *at a particular pH*, possessing both NH_3^+ (cationic) & COO^- (anionic) is termed as ***zwitter ionic*** (***zwitter***^{German}= both).



2. Lipids

➢ **Lipids** are fatty acids esterified with various alcohols.

Classification -

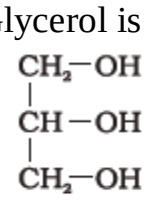
A. Based on melting point, lipids are 2 types: **fats** and **oils.**

Lipids (Triglycerides)
- → **Fats-** Solid @ room temp. (↑ mp)
- → **Oils-** Liquid @ room temp. (↓ mp)

B. Based on composition, lipids are ***3 types-***

I. The **simple lipids** are formed of ***alcohol*** like glycerol and ***fatty acids***

♥ Glycerol is *trihydroxy propane*.

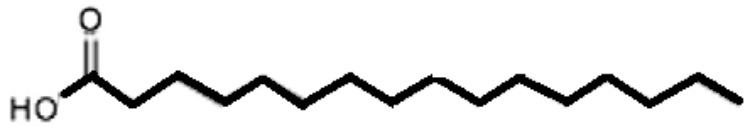


♥ Fatty acid are organic acids with hydrocarbon chain ending in a carboxyl group (–COOH).

Fatty acids are 2 types:

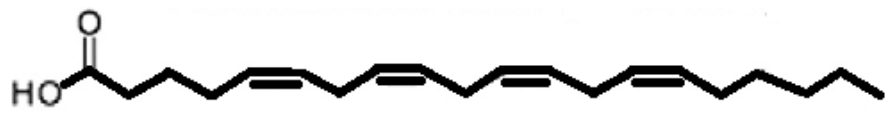
o **Saturated fatty acids:** No double bonds between C- atoms

Eg: Palmitic acid-16 C.



o **Unsaturated Fatty acids:** Have **o**ne or more C=C bonds

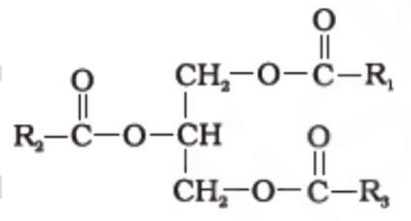
Eg: Arachidonic acid- 20 C, double bonds b/w C5-6, 8-9, 11-12 & 14-15.



It includes **monoglycerides**= glycerol + 1 fatty acid

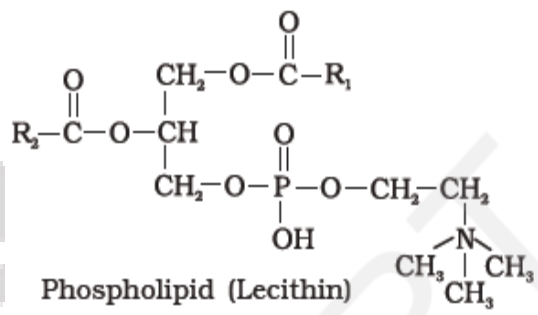
diglycerides = glycerol + 2 fatty acids

triglycerides = glycerol + 3 fatty acids

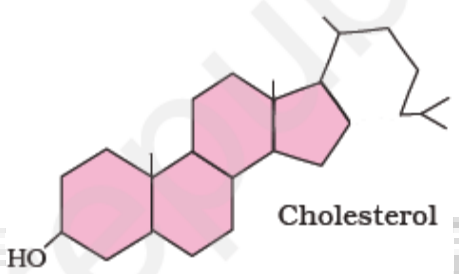


II. Phospholipids are lipids with phosphorus compounds

E.g. ***Lecithin*** (found in cell membrane)



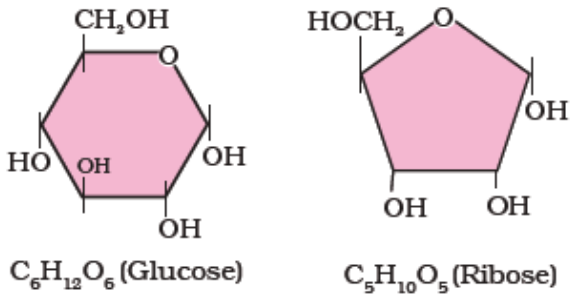
III. Sterols are compounds of fused hydrocarbon ring and a long hydrocarbon side chain. e.g.: Cholesterol ($C_{27}H_{46}O$)



3. Sugars/ Saccharide

➢ Sugars are sweet and water soluble carbohydrates.

E.g.:



$C_6H_{12}O_6$ (Glucose) $C_5H_{10}O_5$ (Ribose)

4. Nucleotides

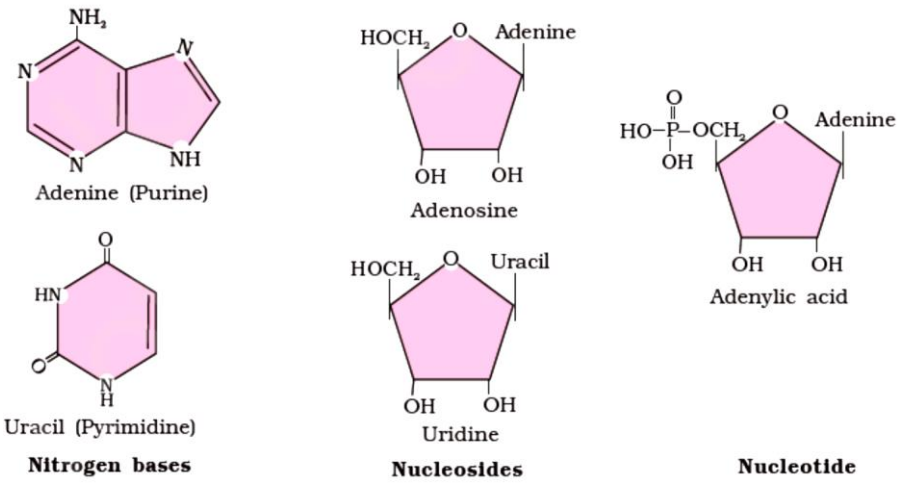
A nucleotide has 3 components:

1. **A nitrogenous base-** heterocyclic compound, 2 types -
   - **Purines:** It includes **Adenine** and **Guanine.**
   - **Pyrimidines:** It includes **Cytosine, Thymine** & **Uracil.**
2. **A pentose sugar (ribose** in **RNA & deoxyribose** in **DNA)**
3. **A phosphate group**

N-glycosidic linkage ester linkage

➢ Nitrogen base + Sugar = ***Nucleoside*** + phosphate = ***Nucleotide***

Adenine	+	’’	= ***Adenosine***	+	’’ = ***Adenylic acid***
Guanine	+	’’	= ***Guanosine***	+	’’ = ***Guanylic acid***
Cytosine	+	’’	= ***Cytidine***	+	’’ = ***Cytidylic acid***
Thymine	+	’’	= ***Thymidine***	+	’’ = ***Thymidylic acid***
Uracil	+	’’	= ***Uridine***	+	’’ = ***Uridylic acid***



BIOMACROMOLECULES

✓ Biomolecules having molecular weight **greater than 1000 Dalton (Da)** is called **macromolecules.**

1. Proteins /Polypeptides

- They are *heteropolymers of amino acids* linked by ***peptide bonds***.

Functions of protein:

- **Transport** nutrients across cell membranes (e.g. GLUT-4 enables glucose transport into cell).
- Acts as intercellular **ground substance** (e.g. collagen).
- Acts as **antibodies** to fight infectious organisms.
- Acts as **receptors** (e.g. receptors of smell, taste, hormones).
- Some are **hormones** (e.g. Insulin), **enzymes** (e.g. trypsin), etc.
  - Most abundant protein in animal world: **Collagen**
  - Most abundant protein in the biosphere: **RuBisCO**

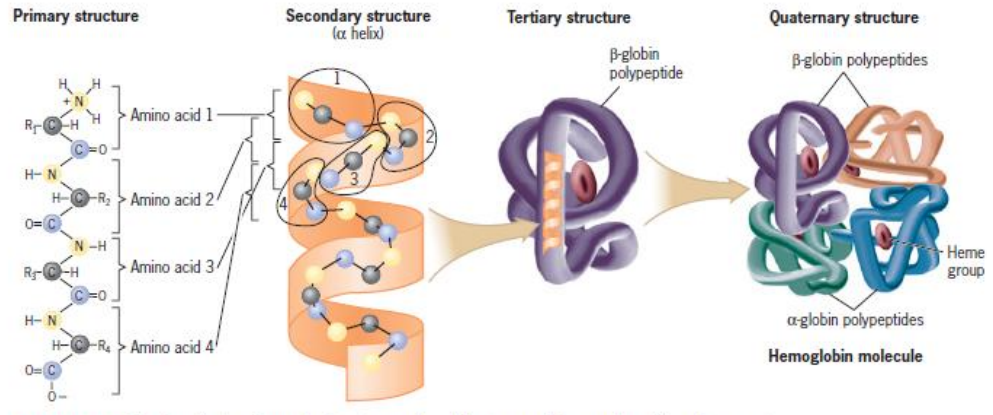
Structure of protein

4 levels of protein structure can be recognised:

1. **Primary structure:** Here, the amino acids are linked with each other to form a **thread-like** structure.
   The first amino acid (on left) is also called as N-terminal amino acid because its $-NH_2$ is free. The last amino acid (on right) is called the C-terminal amino acid because its –COOH is free.

2. **Secondary structure:** A protein thread is folded in the form of a **right-handed helix**.
3. **Tertiary structure:** Long protein chain is **folded** upon itself.
4. **Quaternary structure:** Protein that are an assembly of **more than one polypeptide** or **subunits**.

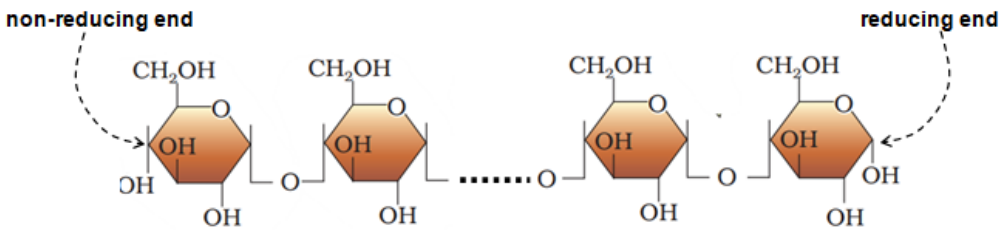
   E.g. Hb has 4 subunits (2 **α** and 2 **β**subunits)



2. Carbohydrates /Polysaccharides

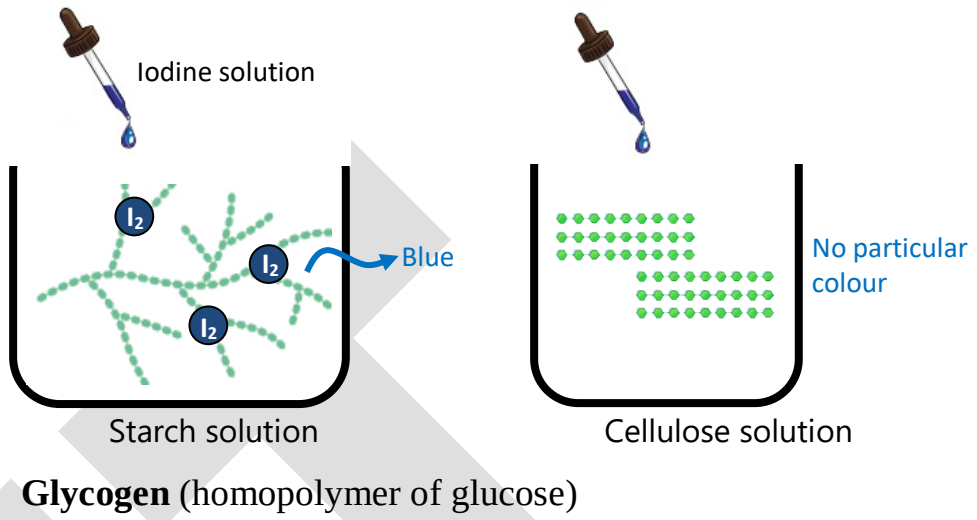
✓ These are polymers of sugars (monosaccharides) linked by ***glycosidic bond***.

In a polysaccharide chain, the right end is called the reducing end (∴ it contain an aldehyde group) and the left end is called the non-reducing end (∴ it does not contain an aldehyde group).



E.g:

- ❖ **Starch** (homopolymer of glucose)
  - Store energy in plant tissues
  - Forms helical structure and hence it can hold I_2 molecules in the helical portion giving blue colour.
- ❖ **Cellulose** (homopolymer of glucose)
  - Component of plant cell wall and cotton fibre
  - It is a straight chain and has no complex helices. So cannot hold I_2.



- ❖ **Glycogen** (homopolymer of glucose)
  - Reserve food in animals.
- ❖ Inulin (homopolymer of fructose)
- ❖ Chitin (homopolymer of N-acetyl glucosamine)
  - Found in cell wall of fungi & exoskeleton of arthropods

❖ ***N*-Acetylgalactosamine (GalNAc)**, is an amino sugar derivative of galactose.
N-Acetylgalactosamine is necessary for intercellular communication, and is concentrated in sensory nerve structures of both humans and animals.

3. Nucleic Acids /Polynucleotide

- Nucleic acids are **heteropolymer** of nucleotides which function as genetic material.
  2 types:
  - **DNA** (deoxyribonucleic acid): contains deoxyribose sugar
  - **RNA** (ribonucleic acid): contains ribose sugar.

METABOLITES

All the biochemical reactions taking place inside a living system together constitute **metabolism.**

➔ ***Metabolites*** *are the organic compounds taking part in metabolism*.
They are 2 types:-

METABOLITES

Primary metabolites: Compounds present in organisms with known role in normal physiology

E.g.

- amino acids
- sugars
- lipids
- nitrogen bases
- nucleoside
- nucleotides etc.

Secondary metabolites: Compounds other than primary metabolites, present in plants, fungi or microbes, which have no known physiological role

E.g.

- **Pigments:** Carotenoids, Anthocyanins etc.
- **Alkaloids:** Morphine, Codeine etc.
- **Terpenoides:** Monoterpenes, Diterpenes.
- **Essential oils:** Lemon grass oil etc.
- **Toxins:** Abrin, Ricin etc.
- **Lectins:** Concanavalin A.
- **Drugs:** Vinblastin, curcumin etc.
- **Polymeric substances:** rubber, gums, cellulose etc.

Pigments: Compounds which reflect only certain wavelengths of visible light.	
Carotenoids-	Plant pigments responsible for bright red, yellow and orange hues in many fruits and vegetables. **Use:** They help plants absorb light energy for use in photosynthesis.
Anthocyanins-	Responsible for the colours red, purple, and blue, are in fruits and vegetables. **Use:** They help plants reproduce by attracting pollinators and protect plants from UV light, drought and cold.
Alkaloids: Class of nitrogen-containing bases produced by mainly by plants for defence.	
Morphine-	Strong opiate that is found in a dark brown resin in opium poppy. **Use:** It is used as a pain medication, and is also used to induce an altered state of consciousness either for pleasure or for some pastime.
Codeine -	Prodrug of morphine found in the sap of poppy. **Use:** It is mainly used to treat pain, coughing, and diarrhea.
Terpenoides: Multicyclic structures that contain oxygen formed due to the enzymatic reactions of primary metabolites.	
Monoterpenes-	Found in many parts of different plants, such as barks, heartwood, leaves of coniferous trees, in vegetables, fruits and herbs. **Use:** Used in pharmaceutical, cosmetic, agricultural, and food industries.
Diterpenes-	They are of fungal or plant origin and are found in resins, gums etc.
Essential oils: A concentrated hydrophobic liquid containing volatile chemical compounds from plants. 'Essential' is in the sense that it contains the essence of the plant's fragrance.	
Lemon grass oil-	Pale yellow oil present in leaves and stalks of the lemongrass plant. **Use:** Used in traditional medicine for pain relief, stomach problems, fevers and in aromatherapy.
Toxins: Substances created by metabolic activities of plant and animal cells that are poisonous (toxic) to humans	
Abrin -	Found in the seeds of the *rosary pea*. **Effects:** Poisoning include diarrhea, vomiting, tachycardia and tremors. Death usually occurs after a few days due to kidney failure, heart failure, and/or respiratory paralysis.
Ricin -	Produced in the seeds of the *castor beans*. **Effects:** Within 2-5 days of exposure to ricin, its effects on the CNS, adrenal glands, kidneys, and liver appear.
Lectins: Carbohydrate-binding protein	
Concanavalin A-	From the *jack-bean*. **Effects:** It has the property to agglutinate RBCs and also triggers anticancer immune responses
Drugs: Class of chemical substance that causes a change in an organism's physiology or psychology when consumed.	
Vinblastin –	Found in *Madagascar periwinkle*. **Effects:** It slow or stop the growth of cancer cells.
Curcumin -	Bright yellow chemical produced in *turmeric*. **Use:** It is used as a herbal supplement, cosmetics ingredient, food flavoring and coloring.
Polymeric substances:	
Rubber-	Latex from the rubber tree. **Use:** It is used in medical devices, gloves, aircraft and car tires, toys, etc.
Gums-	A viscous secretion of some trees and shrubs that hardens on drying.
Cellulose -	Structural component of the cell wall of green plants, many forms of algae and the oomycetes.

ENZYMES

➔ **Enzymes** are proteins which catalyse biochemical reactions in the cells (***biological catalysts***).

Differences b/w enzyme and inorganic catalyst

Inorganic catalyst	*Enzymes*
At high temperature and high pressure- **works efficiently**	**Get damaged** (except enzymes from thermophilic organisms)
Substrate specificity- ***low***	***High***

- There are about 1300 enzymes are discovered from humans and 3000 in total.

Fastest enzyme

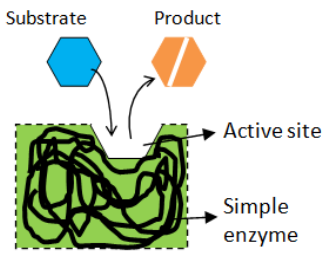
$$CO_2 + H_2O \xrightleftharpoons{\textit{Carbonic anhydrase}} H_2CO_3$$

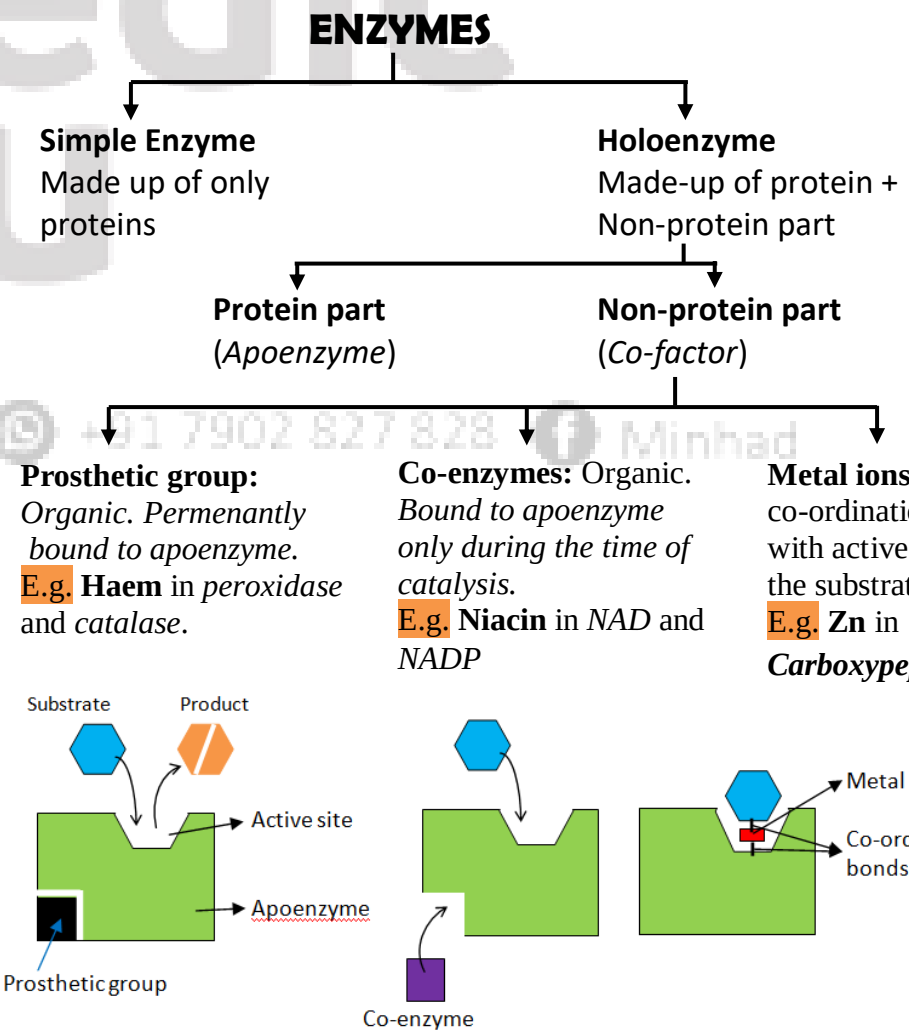
Carbonic acid

Turnover in the absence of enzyme: 200 molecules / hr
Turnover in the presence of enzyme: 6,00,000 molecules / sec

Structure of Enzyme

- **Enzymes** are made-up of 3^0 proteins.
  Exception: Sometimes RNA act as enzymes called ***Ribozymes***.
- The 3^0 structure of an enzyme has some pockets called **'active site'** into which the substrate fits.





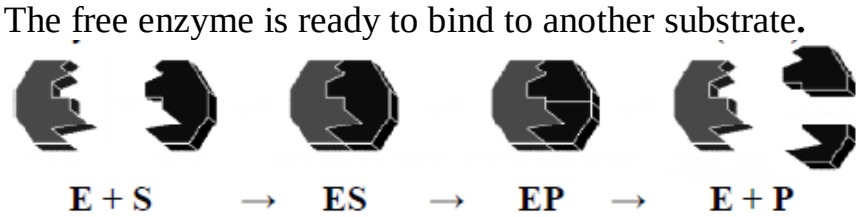
Enzyme action (Catalytic Cycle)

(Step-1) The substrate binds to the **active site** of enzyme **(E+S).**

(Step-2) Enzyme **alter its shape** and fits more tightly around the substrate **(ES).**

(Step-3) The active site **breaks / makes chemical bonds** of the substrate. As a result, new **enzyme-product complex** is formed **(EP).**

(Step-4) The **enzyme releases the products (E+P)**
The free enzyme is ready to bind to another substrate.

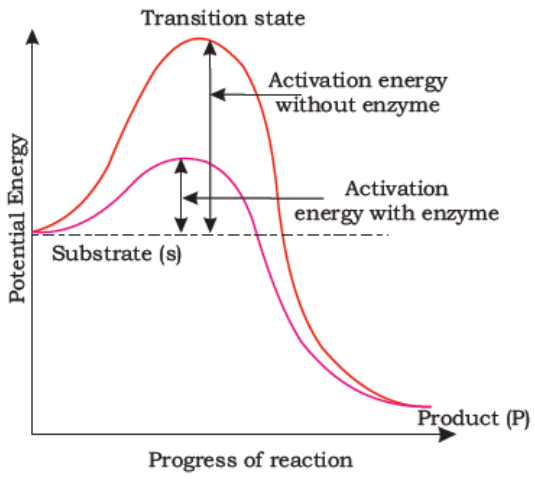


Mechanism of Acceleration (Concept of Activation Energy)

In a reaction, the substrate has to go through a much higher energy state. It is called **transition state energy.**

➔ **Activation energy** is the difference between average energy of substrate and transition state (ES) energy.

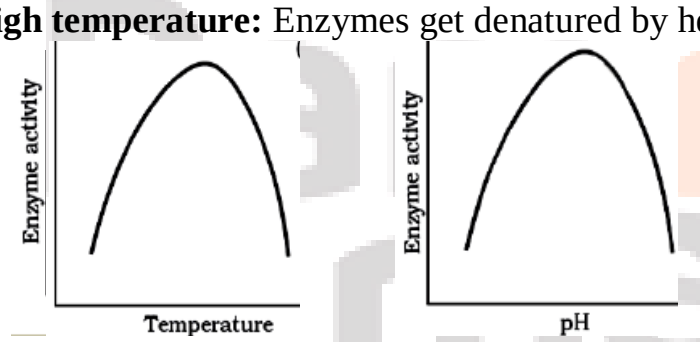
- ♥ **Role of enzyme**: In a biochemical reaction, ***enzymes lower the activation energy***. As a result, speed of the reaction increases.



Factors affecting enzyme activity

a) Temperature

- ♥ ***Optimum temperature***: Temperature at which particular enzymes show highest activity.
  Activity declines below and above optimum value.
  - **At low temperature:** Enzyme temporarily inactive.
  - **At high temperature:** Enzymes get denatured by heat.



b) pH

- ♥ ***Optimum pH***: pH at which particular enzymes show highest activity. Enzyme activity declines below and above optimum pH.

c) Concentration of substrate

Velocity of enzyme action rises with the increase in substrate concentration

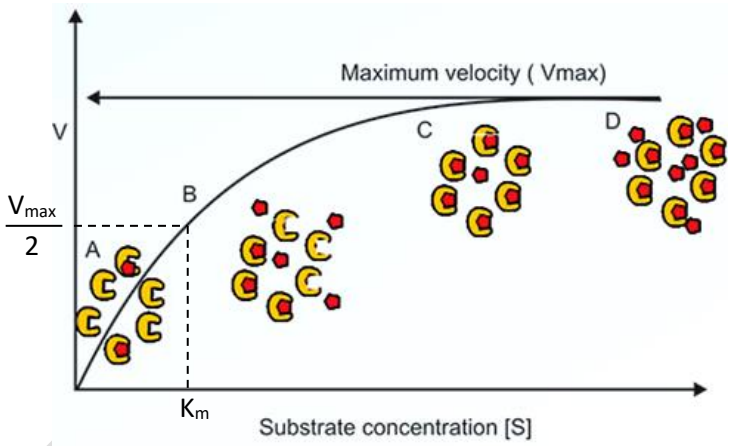
↓

Reaches a *maximum velocity (V_{max})*

↓

Velocity does not exceeded by further rise in concentration.

Reason: Enzyme molecules are fully saturated. i.e., *no active site is left free to bind with additional substrate molecules*.



K_m (Michaelis – Menten constant) is the substrate concentration at which the reaction rate is half of the V_{max}

d) Presence of inhibitors

- ▪ The binding of specific chemicals (**inhibitor**) shuts off enzyme activity. This is called **inhibition**.

➔ **Competitive inhibitor:** inhibitor which is similar to substrate.
It competes with the substrate for the binding site of the enzyme.

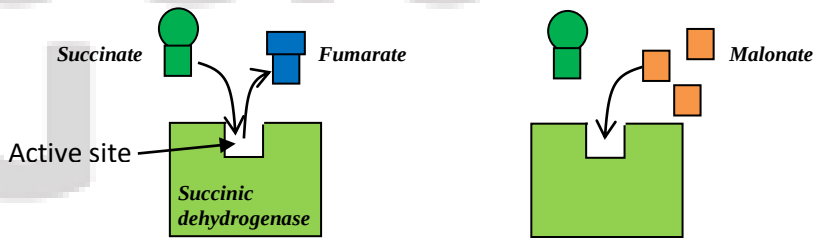
↓

Inhibitor binds with binding site (substrate cannot bind)

↓

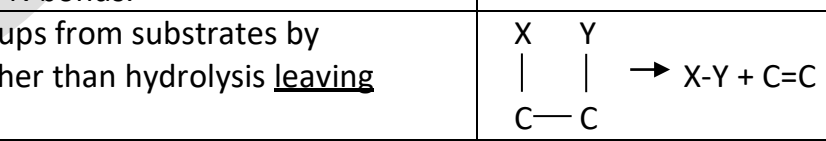
Enzyme action declines

e.g. Inhibition of ***succinic dehydrogenase*** by malonate.



- Competitive inhibitors are used in the control of bacterial pathogens.
  e.g. ***Penicillin*** is a competitive inhibitor that blocks the active site of an enzyme that many bacteria use to construct their cell wall.

Classification of Enzymes

Class	Type of reaction catalysed	Exemplified Reaction
1. Oxidoreductases/ dehydrogenases	Reaction involving the exchange of H_2 atom or ion between two substrates	$S_{red} + S'_{ox} \rightarrow S_{ox} + S'_{red}$
2. Transferases	Transfer of a group (other than H) between a pair of substrate S and S'	$S - G + S' \rightarrow S + S' - G$
3. Hydrolases	Hydrolysis of ester, ether, peptide, glycosidic, C-C, C-halide or P-N bonds.	$S + H_2O \rightarrow X + Y$
4. Lyases	Removal of groups from substrates by mechanisms other than hydrolysis leaving double bond.	
5. Isomerases	The rearrangement of molecular structure to form isomer.	$X \rightarrow Y$
6. Ligases	The linking together of 2 compounds of C-O, C-S, C-N, P-O etc. bonds.	$X + Y \rightarrow X - Y$

Chemical reactions

Chemical compounds undergo 2 types of changes:

i. Physical change: Change in shape without breaking bonds
ii. Chemical changes: Change involving breaking or making bonds
    a. Inorganic chemical reactions:
       e.g.: $Ba(OH)_2 + H_2SO_4 \rightarrow BaSO_4 + 2H_2O$
    b. Organic chemical reactions:
       e.g.: Hydrolysis of starch into glucose

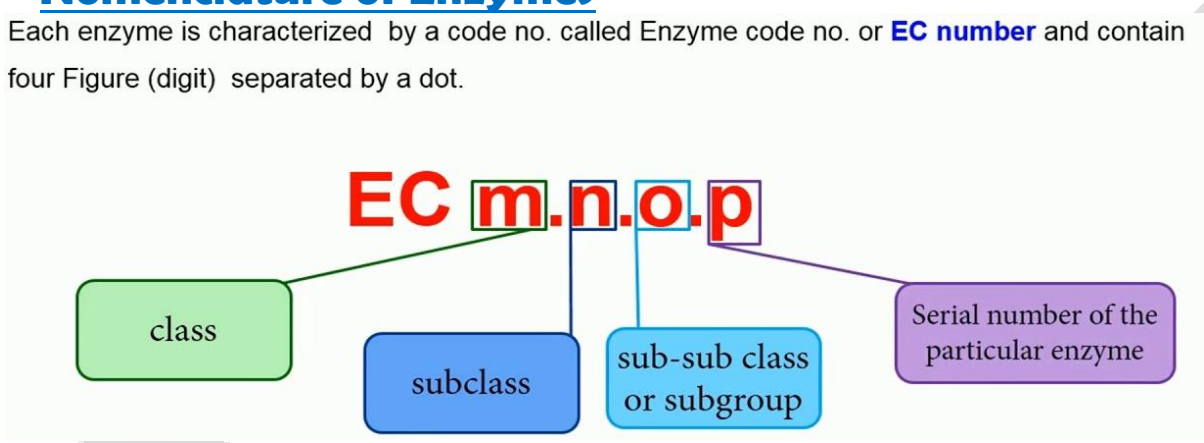
Rate of a reaction-

$$\text{rate} = \frac{\delta P}{\delta t}$$

Influence of temperature
- Rate doubles for 10^0C increase in temperature
- Rate halves for 10^0C decrease in temperature

Nomenclature of Enzymes

Each enzyme is characterized by a code no. called Enzyme code no. or **EC number** and contain four Figure (digit) separated by a dot.



1. Oxidoreductase
- 1.1 Acting on the CH-OH group of donors
  - 1.1.1 With NAD+ or NADP+ as acceptor
    - 1.1.1.1 alcohol dehydrogenase (NAD)
    - 1.1.1.2 alcohol dehydrogenase (NADP+)
    - ⋮
  - 1.1.2 With a cytochrome as acceptor
  - 1.1.3 With oxygen as acceptor
  - ⋮
- 1.2 Acting on the aldehyde or oxo group of donors
  - 1.2.1 With NAD+ or NADP+ as acceptor
  - 1.2.2 With a cytochrome as acceptor
  - 1.2.3 With oxygen as acceptor
  - ⋮

2. Transferase
- 2.1 Transferring one-carbon groups
  - 2.1.1 Methyltransferases
  - 2.1.2 Hydroxymethyl-, formyl- and related transferases
  - 2.1.3 Carboxy- and carbamoyltransferases
  - ⋮
- 2.2 Transferring aldehyde or ketonic groups
- 2.3 Acyltransferases
  - 2.3.1 Transferring groups other than aminoacyl groups
  - 2.3.2 Aminoacyltransferases
  - ⋮

3. Hydrolase
- 3.1 Acting on ester bonds
- 3.2 Glycosylases
- ⋮

4. Lyase
- 4.1 Carbon-carbon lyases
- 4.2 Carbon-oxygen lyases
- ⋮

5. Isomerase
- 5.1 Racemases and epimerases
- 5.2 cis-trans-Isomerases
- ⋮

6. Ligase
- 6.1 Forming carbon-oxygen bonds
- 6.2 Forming carbon-sulfur bonds
- ⋮