

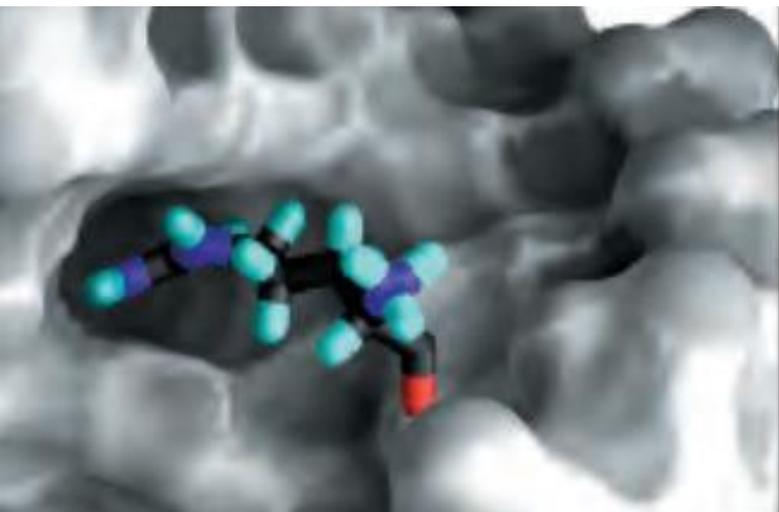
Lecture 1: Biochemistry I

Introduction to the macromolecules biochemistry

3rd stage

Anbar University-College of Pharmacy-Clinical Laboratory Sciences Department
2020-2021

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■ **References:**

- ✓ Harper's Illustrated Biochemistry
- ✓ Lippincott Biochemistry
- ✓ Lehninger Principles of Biochemistry
- ✓ Stryer Biochemistry



Objectives of this semester

- To integrate key concepts describing the traditional core topics of biochemistry:
structure and metabolism

At the end of the semester the students should be able to understand

- The chemical structure, and function of all biomolecules present in the living organisms



What is Biochemistry?

- **Biochemistry** is the science concerned with studying the various molecules that occur in living cells and organisms and with their chemical reactions. Because life depends on biochemical reactions, biochemistry has become the basic language of all biologic sciences.
- **Biochemistry and medicine** are intimately related. Health depends on a harmonious balance of biochemical reactions occurring in the body, and disease reflects abnormalities in biomolecules, biochemical reactions, or biochemical processes.
- **Biochemical processes** is the study of chemical processes within and relating to living organisms. By controlling information flow through biochemical signalling and the flow of chemical energy through metabolism.



- **Biochemical approaches** are often fundamental in illuminating the causes of diseases and in designing appropriate therapies. The judicious use of various biochemical laboratory tests is an integral component of diagnosis and monitoring of treatment.
- **Biochemistry spills** over into pharmacology, physiology, microbiology, toxicology, and clinical chemistry. In these areas, a biochemist may investigate the mechanism of a drug action; engage in viral research; conduct research pertaining to organ function; or use chemical concepts, procedures, and techniques to study the diagnosis and therapy of disease and the assessment of health.
- The study of biochemistry shows how the collections of inanimate molecules that constitute living organisms interact to maintain and perpetuate life animated solely by the physical and chemical laws that govern the nonliving universe



Introduction to the macromolecules biochemistry

- The cell is the fundamental unit of life
- Cells are composed of small molecules (water), **macromolecules** and organelles macromolecules fold into complex 3D structure
- **Macromolecules** can be classified into **4** different categories: **carbohydrates, proteins, lipids and nucleic acids**
- Each type possesses distinct chemical properties that suit it for the functions it serves in the cell

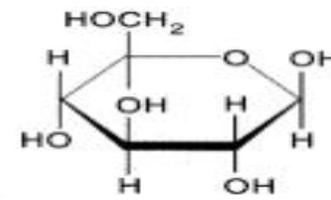
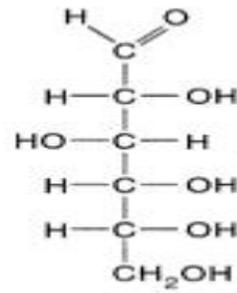


Carbohydrates

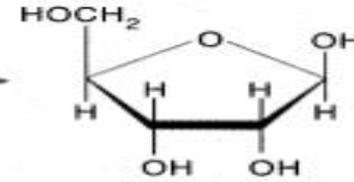
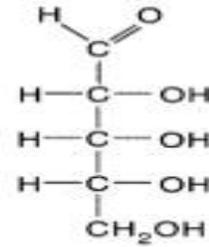
- **Carbohydrates**, contain a carbon backbone but they also contain many polar OH groups and are therefore soluble in water - large carbohydrate molecules called polysaccharides consist of many small ringlike sugar molecules, these sugar monomers are attached to one other by glycosidic bonds in a linear or branched array to form the sugar polymer
- **Carbohydrates** release chemical energy and may also provide carbon skeletons for the synthesis of other molecules.
- Important structural functions are also served by polysaccharides
- linear polysaccharides form a major component of plant cell walls and bacteria cell walls



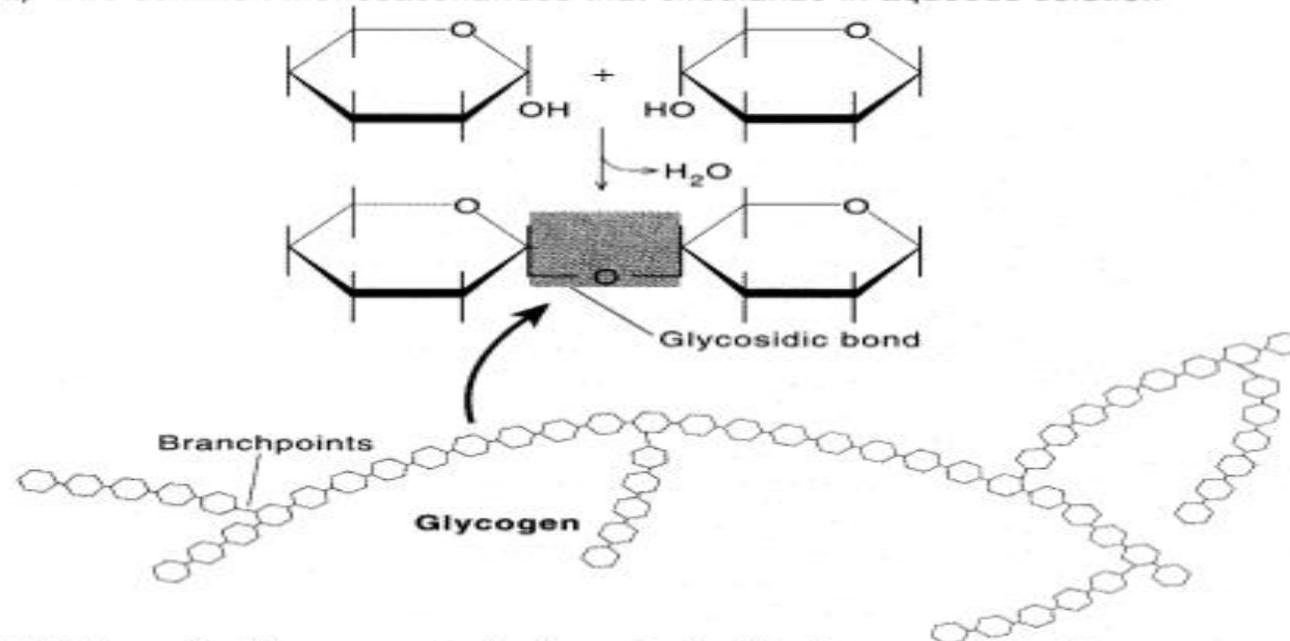
Glucose
(a common
hexose)



Ribose
(a common
pentose)



(a) Two common monosaccharides that circularize in aqueous solution



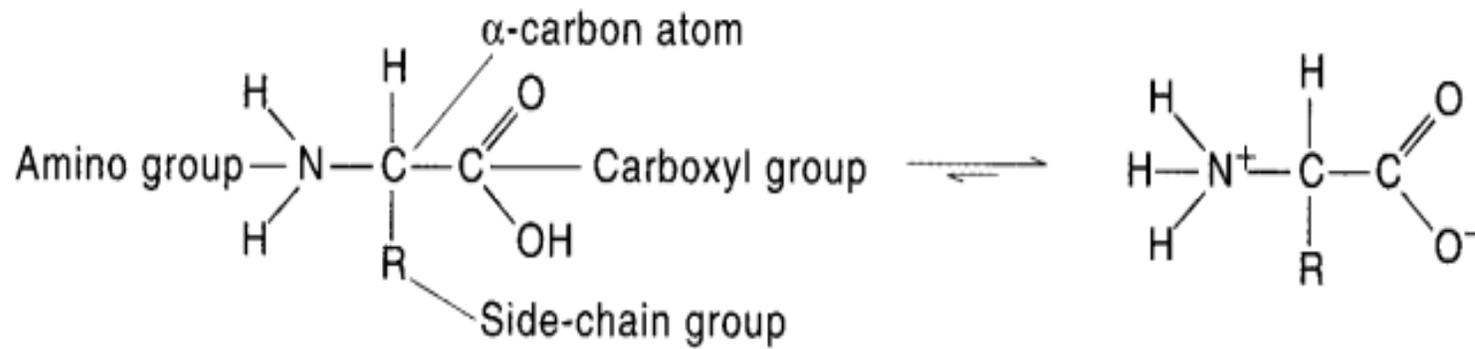
(b) Polysaccharides composed of covalently linked monosaccharides



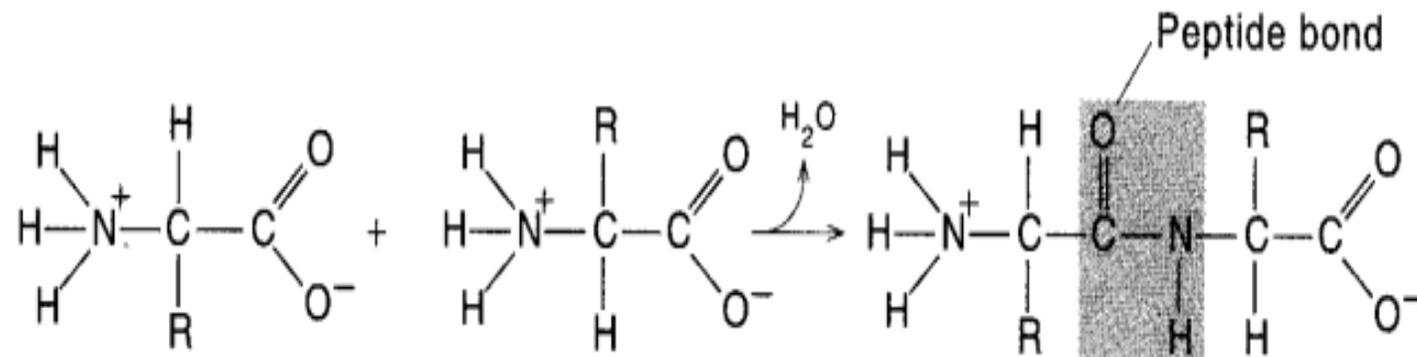
Proteins

- **Proteins** the most complex macromolecules found in the cell.
- They are composed of linear polymers called polypeptides, which contain amino acids connected by peptide bonds
- Each amino acid contains a central carbon atom attached to 4 groups:
carboxyl group, amino group, H atom and R group
- Some structural proteins interact with lipids in membrane structure. Others aggregate to form part of cytoskeleton that helps to give the cell its shape.
- Others are the chief components of muscle or connective tissue





(a) Generalized structure of amino acid



(c) Two amino acids reacting to form a peptide bond



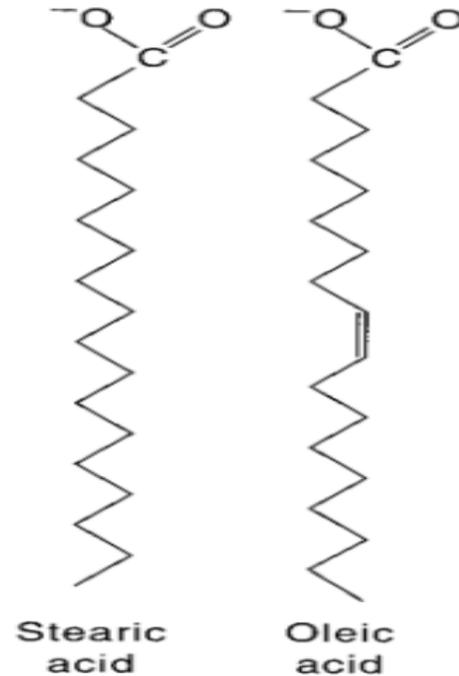
Enzymes

- Enzymes are major class of proteins which function as **catalysts** that accelerate chemical reactions by **lowering the activation energy**
- Enzymes are proteins consisting of one or more **polypeptide** chains.



Lipids

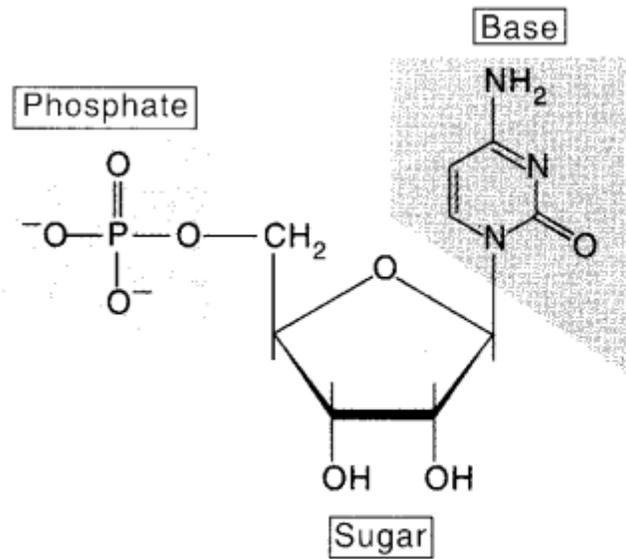
- Lipids are primarily hydrocarbon structure. They tend to be insoluble in water and are therefore particularly well suited to serve as a major component of the various membrane in structure found in cells.
- lipids also serve as storing chemical energy to drive the metabolism of the cells.
- Lipids include fats, oils, phospholipids, steroids, and cholesterol.
- Formed by dehydration synthesis of one glycerol for every 3 fatty acids.



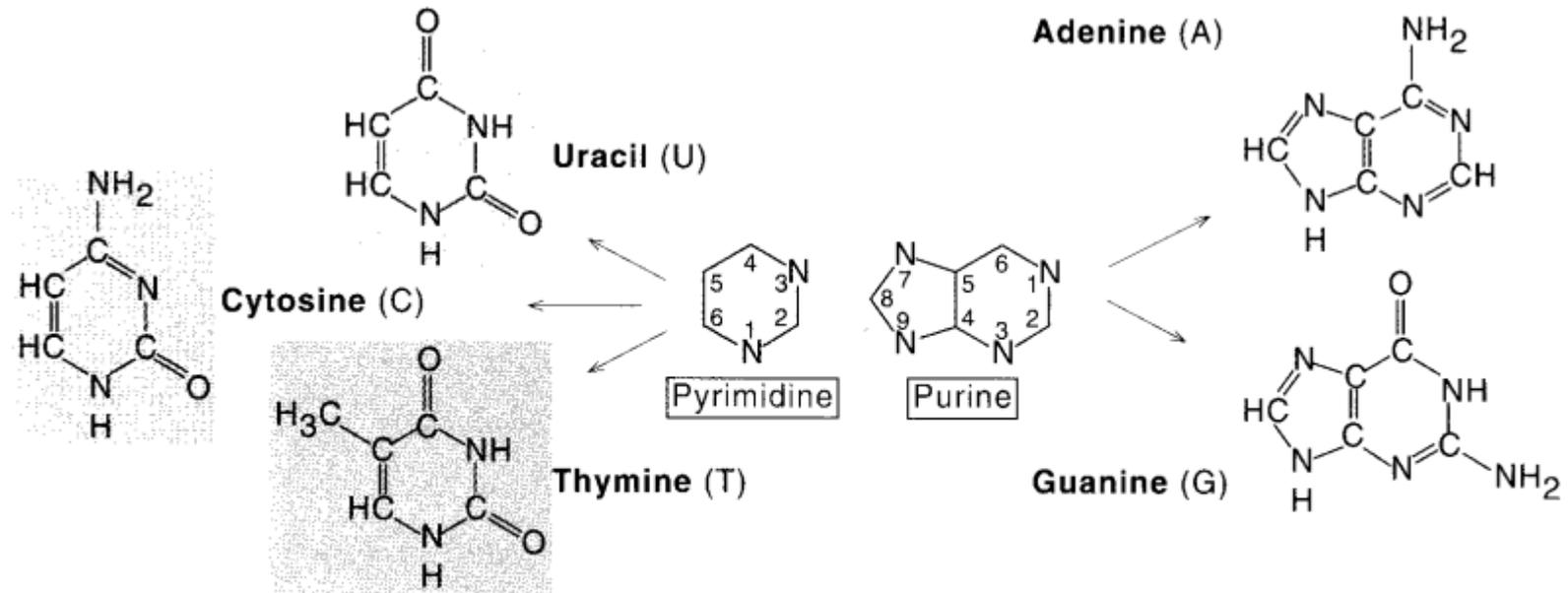
Nucleic acids

- Nucleic acids are the largest macromolecules in the cell.
- They are very long, linear polymers, called **polynucleotides**, composed of **nucleotides**
- A nucleotide contains
 - **5 carbon sugar** molecules
 - **One** or more **phosphate groups**
 - **Nitrogenous base**
- **Five** different type of nitrogenous bases are found in the **two** main type of nucleic acids, **deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)**
- **DNA** contains the **genetic information** that is inherited when cells divide and organisms reproduce
- This genetic information is used in the cells to make **ribonucleic acids and proteins**





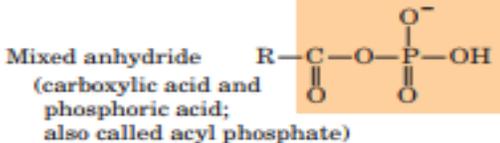
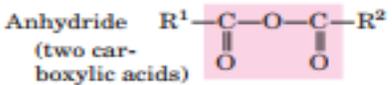
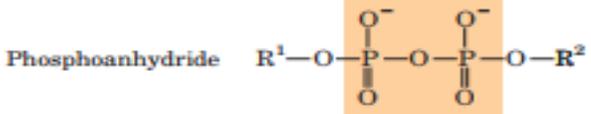
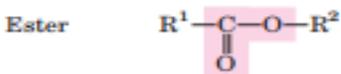
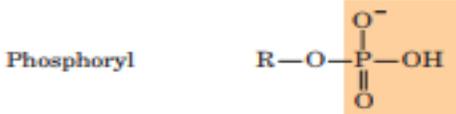
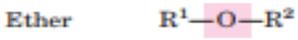
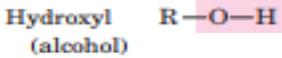
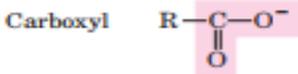
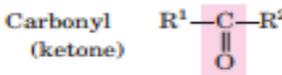
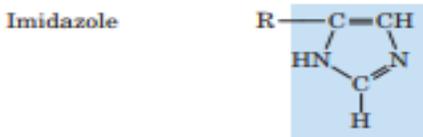
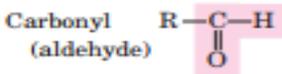
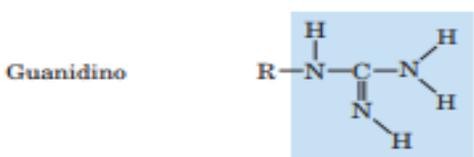
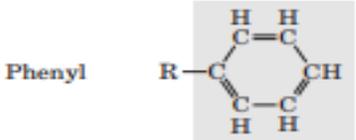
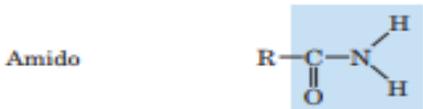
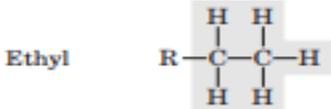
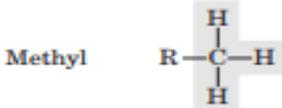
(a) Generalized structure of a nucleotide



(b) Different bases found in nucleotides



Some common functional groups in biomolecules



- **30** chemical elements are essential to organisms. The **four** most elements in living organisms are **hydrogen, oxygen, nitrogen, and carbon**, which together make up more than **99%** of the mass of most cells.

TABLE 1-1 Strengths of Bonds Common in Biomolecules

Type of bond	Bond dissociation energy* (kJ/mol)	Type of bond	Bond dissociation energy (kJ/mol)
Single bonds		Double bonds	
O—H	470	C=O	712
H—H	435	C=N	615
P—O	419	C=C	611
C—H	414	P=O	502
N—H	389		
C—O	352	Triple bonds	
C—C	348	C≡C	816
S—H	339	N≡N	930
C—N	293		
C—S	260		
N—O	222		
S—S	214		

*The greater the energy required for bond dissociation (breakage), the stronger the bond.



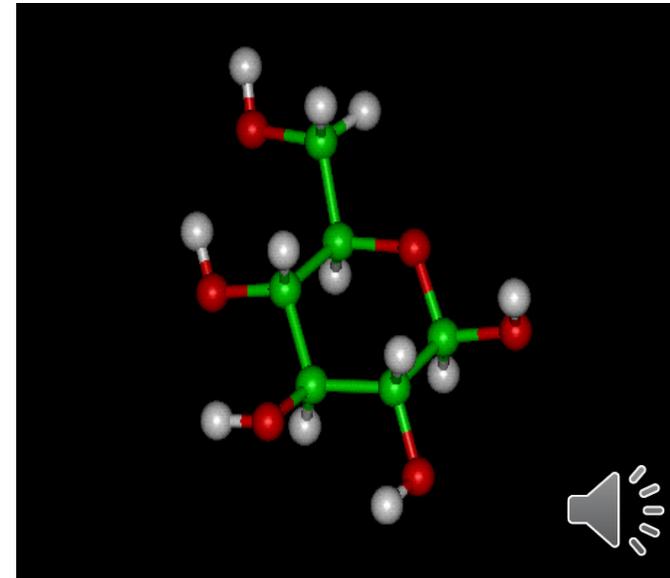
Lecture 2: Biochemistry I

Carbohydrates

3rd Class

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Learning outcomes

- To know the formation of carbohydrates
- To understand the nature of glycosidic bonds
- To understand the structural organisation of carbohydrates
- To appreciate the various functions of carbohydrates



Carbohydrates

- Carbohydrates, one of the four major classes of biomolecules along with **proteins, lipids, and nucleic acids.**
- Carbohydrates are built from monosaccharides (carbon atoms bound to hydroxyl groups, Empirical formula $C_n(H_2O)_n$)
- Monosaccharides are linked together by **glycosidic bonds** to form di, oligo- and a huge variety of polysaccharides
- Carbohydrates are aldehyde (CHO) or ketone ($C=O$) compounds with multiple hydroxyl groups (Carbon-oxygen double bonds make the sugars reactive)



Importance of carbohydrates

- Carbohydrates serve as **energy store** and **metabolic intermediates**.
- Ribose and deoxyribose sugars form part of the structural framework of **RNA and DNA**.
- Carbohydrates are important for **tissue formation**
- Carbohydrates form the basis of **human blood groups**
- Polysaccharides are **structural elements in the cell walls of bacteria and plants and in the connective tissues of animals..**
- Carbohydrates are linked to many proteins and lipids, where they play key roles in **mediating interactions among cells and interactions between cells and other elements in the cellular environment.**



Several classifications of carbohydrates

Basics	Types
Complexity	Simple Carbohydrates: monosaccharaides Complex Carbohydrates: disaccharides, oligosaccharides & polysaccharides.
Size	Tetrose: C ₄ sugars, Pentose: C ₅ sugars Hexose: C ₆ sugars Heptose: C ₇ sugars, Etc.
C=O Function	Aldose: sugars having an aldehyde function or an acetal equivalent. Ketose: sugars having a ketone function or an aketal equivalent.
Reactivity	Reducing: sugars oxidized by Tollens' reagent (or Benedict's or Fehling's reagents). Non-reducing: sugars not oxidized by Tollens' or other reagents.



Classification of Carbohydrates

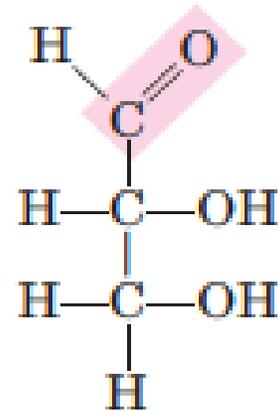
Carbohydrates can be classified into:

- 1- Monosaccharides** - simple sugars with multiple OH groups. Based on number of carbons.
- 2- Disaccharides:** two monosaccharides covalently linked by glycosidic bond.
- 3- Oligosaccharides: several monosaccharides (3-9) joined by glycosidic bonds.**
- 4- Polysaccharides:** polymers consisting of chains of monosaccharide or disaccharide units.

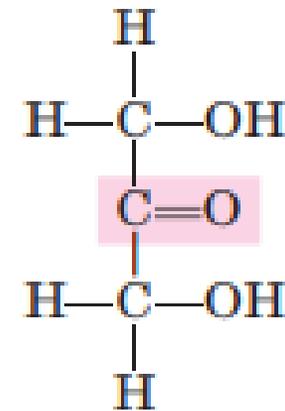


Monosaccharides

- The simplest of the carbohydrates
- Aldehydes or ketones
- Colorless, crystalline solids that are freely soluble in water but insoluble in nonpolar solvents.
- They are important fuel molecules as well as building blocks for nucleic acids.
- The smallest monosaccharides are :



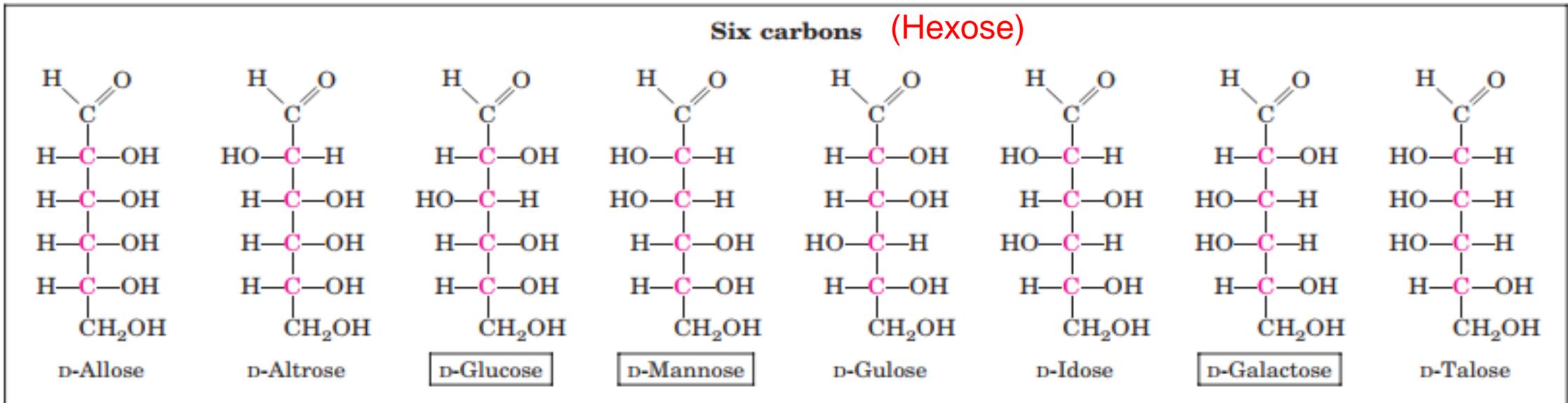
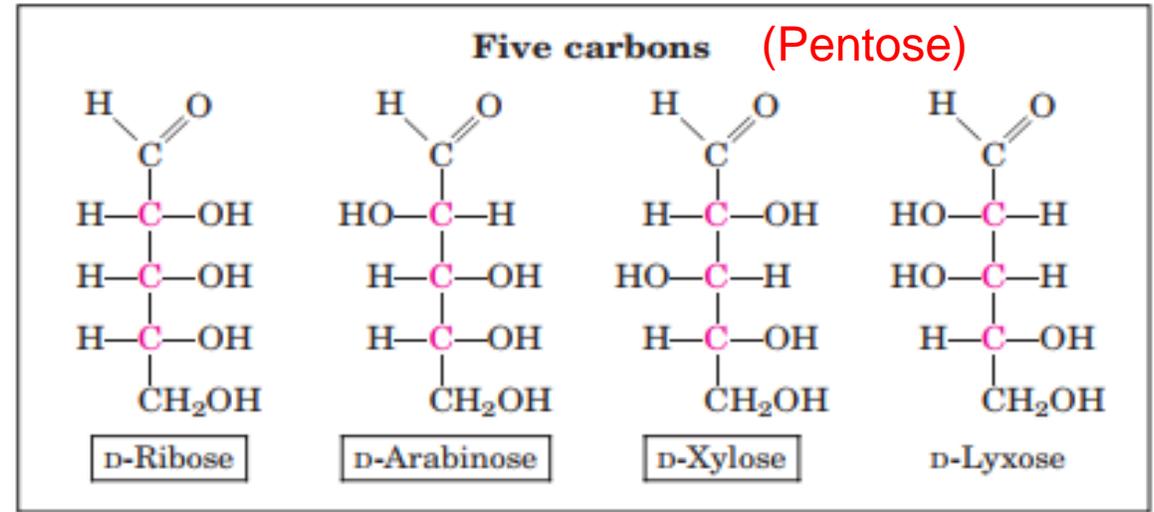
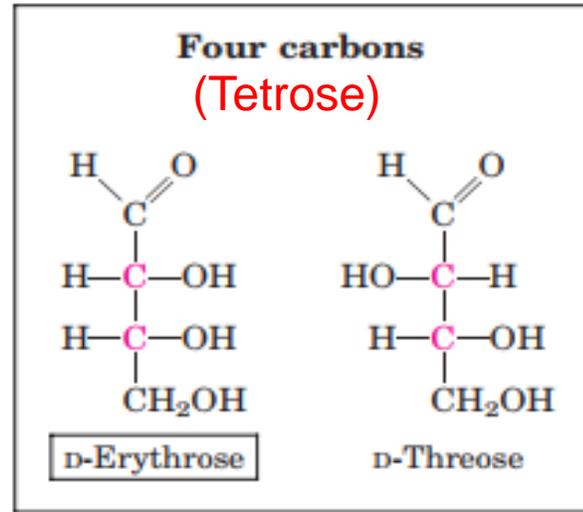
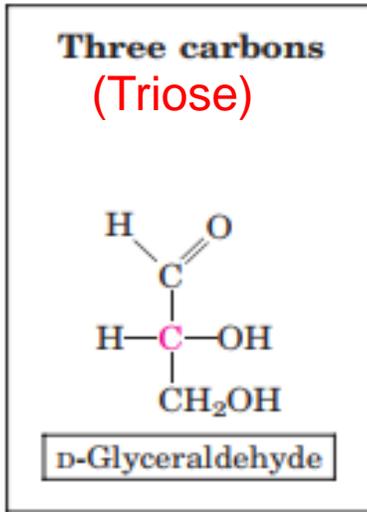
Glyceraldehyde,
an aldotriose



Dihydroxyacetone,
a ketotriose



The sugars named in boxes are the most common in nature

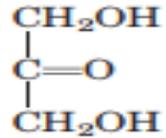


D-Aldoses



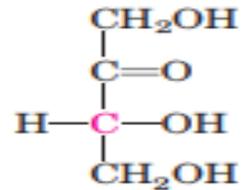
The sugars named in boxes are the most common in nature

Three carbons



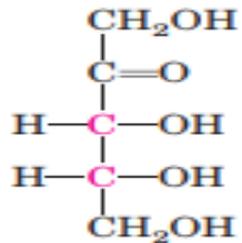
Dihydroxyacetone

Four carbons

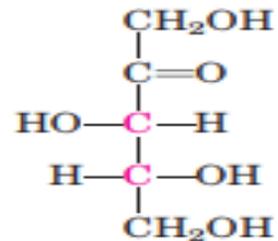


D-Erythrulose

Five carbons

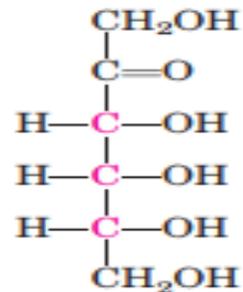


D-Ribulose

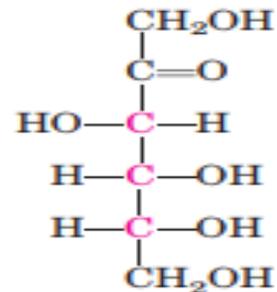


D-Xylulose

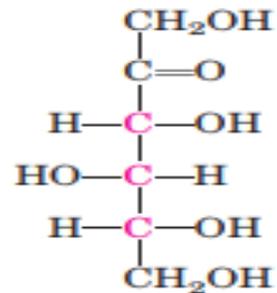
Six carbons



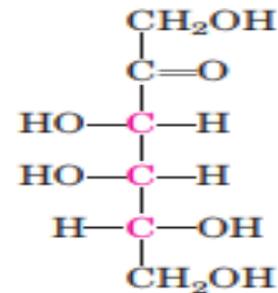
D- Psicose



D-Fructose



D-Sorbose



D-Tagatose

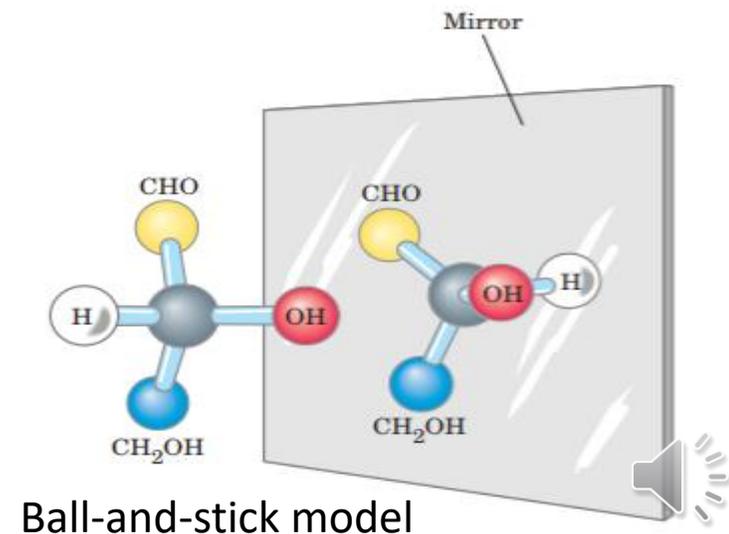
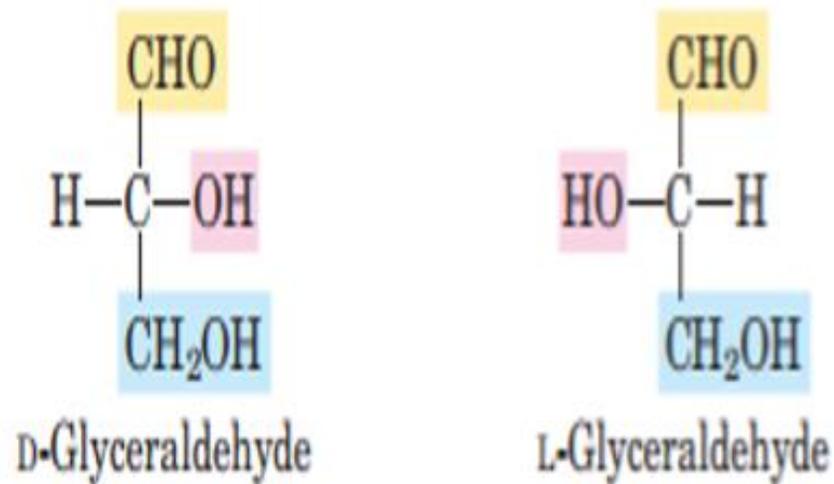
D-Ketoses

- The names of all sugars end in – **ose**
- **Hexoses** are the nutritionally important sugars.



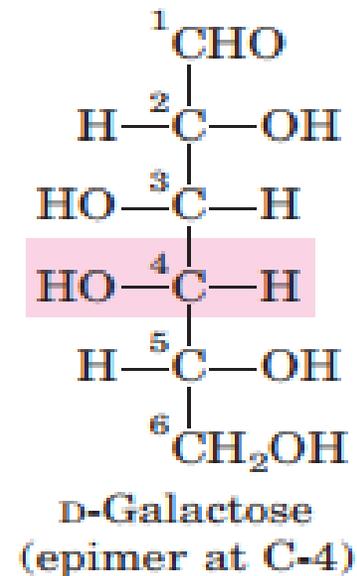
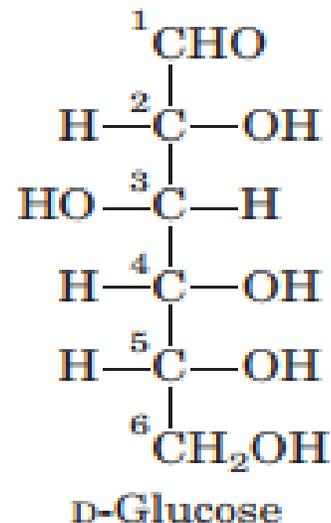
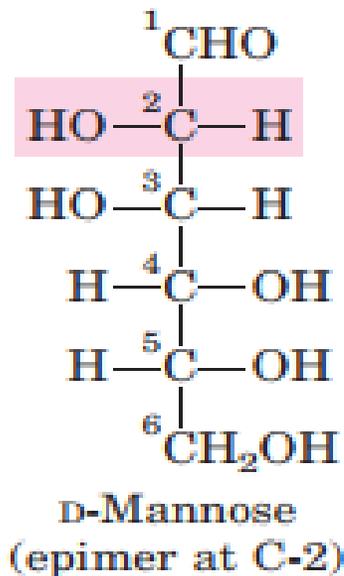
D and L isomerism

- **Isomers** are molecules with the **same kinds and numbers of atoms joined up in different ways**.
- A **carbon atom** that contains **4** different chemical groups forms an **asymmetric** (chiral) center.
- The prefixes **D** and **L** designate the absolute configuration of the asymmetric carbon **farthest** from the **aldehyde or keto group**.
- When the **OH group** on this carbon is on the **right**, the sugar is the **D-isomer**; when it is on the **left**, it is the **L-isomer**.
- Glyceraldehyde has **a single asymmetric carbon** and, thus, there are **2** stereoisomers of this sugar.
- D-Glyceraldehyde and L-glyceraldehyde are **mirror images** of each other (enantiomers).



Stereoisomers and Epimers

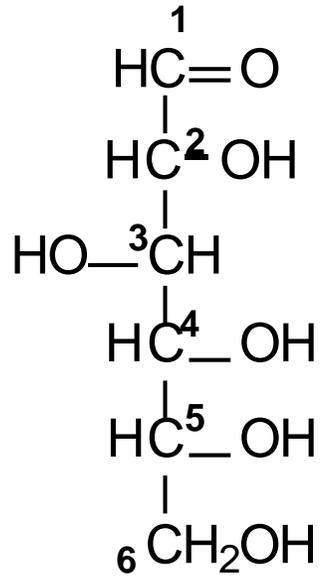
- **Stereoisomers** have the same chemical formula but differ in the **position** of the **OH group** on **1** or more of their asymmetric carbons.
- **Epimers are stereoisomers** that differ in the **position** of the **OH group** at only **1** of their asymmetric carbons.
- **D-glucose** and **D-galactose** are **epimers** of each other, differing only at **C4**, and can be interconverted in human cells by enzymes called **epimerase**.
- **D-mannose** and **D-glucose** are **also epimers** of each other, differing only at **C2**.



Fischer/Haworth projection

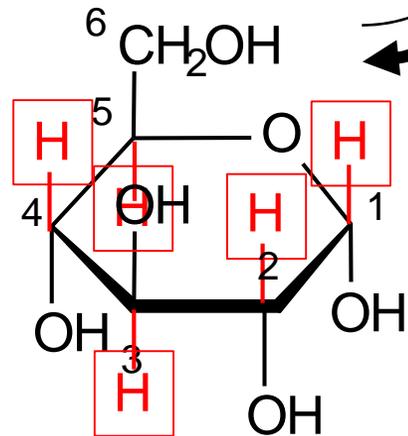
Glucose

(dextrose; grape sugar)



open-chain form
(Fischer projection)

- Glucose in solution exists mostly in the ring form at equilibrium, with less than 0.1% of the molecules in the open-chain form.
- Fischer projections are useful for depicting carbohydrate structures because they provide clear and simple views of the stereochemistry at each carbon center.



ring-form

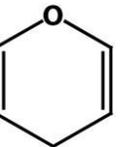
(Haworth projection)

glucose

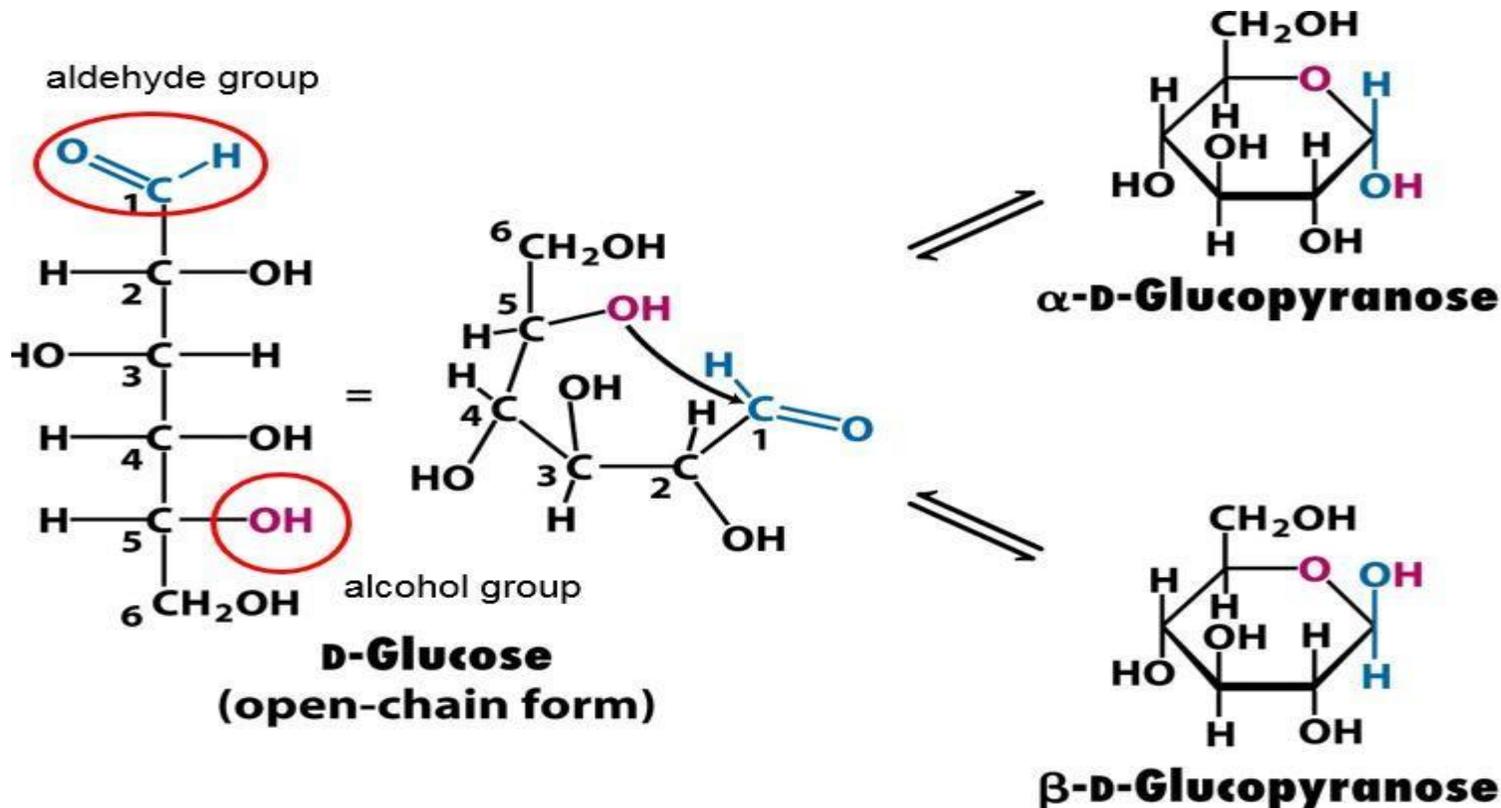


Cyclization of Monosaccharide

- The predominant forms of ribose, glucose, fructose, and many other sugars in solution are **not open chains**. Rather, the open-chain forms of these sugars cyclize into **rings**.
- For an aldohexose such as glucose, Formation of a **hemiacetal** by reaction of the **C-1** aldehyde group with the **C-5 hydroxyl group** to form an intramolecular hemiacetal. The resulting cyclic hemiacetal, a six-membered ring, is called **pyranose**. because of its similarity to pyran.

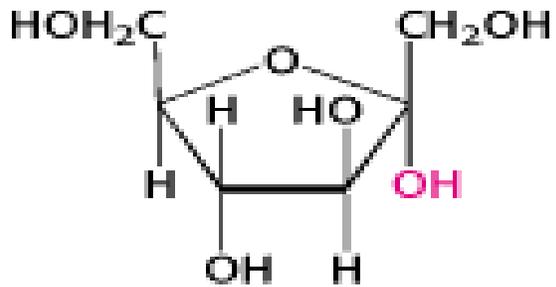


Pyran

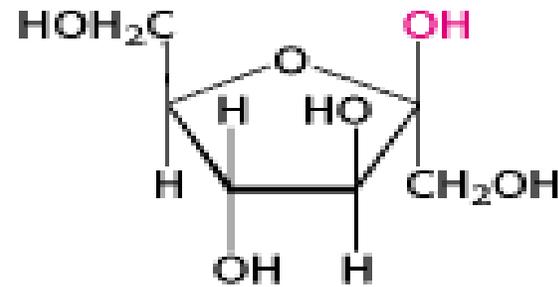


(Ring-form is energetically more stable)

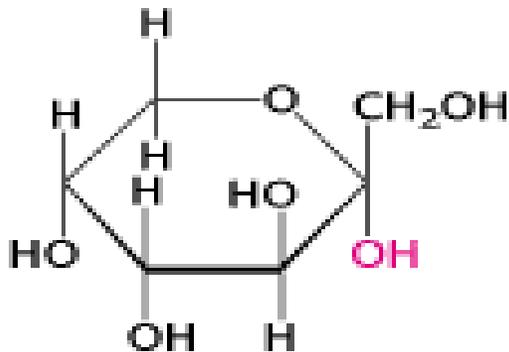
- Similarly, the **C-2** keto group in the open-chain form of a ketohexose, such as fructose, can form an intramolecular hemiketal by reacting with either the **C-6** hydroxyl group to form a six-membered cyclic hemiketal or the **C-5 hydroxyl group to form a five-membered cyclic hemiketal**. The five-membered ring is called a furanose because of its similarity to furan.



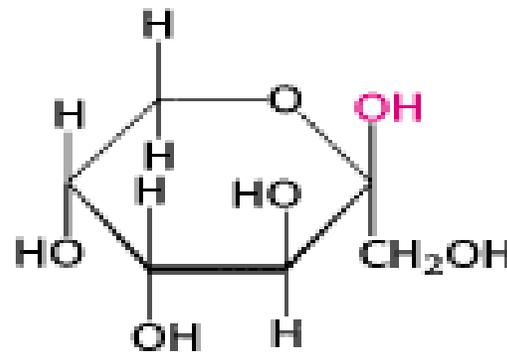
α -D-Fructofuranose



β -D-Fructofuranose



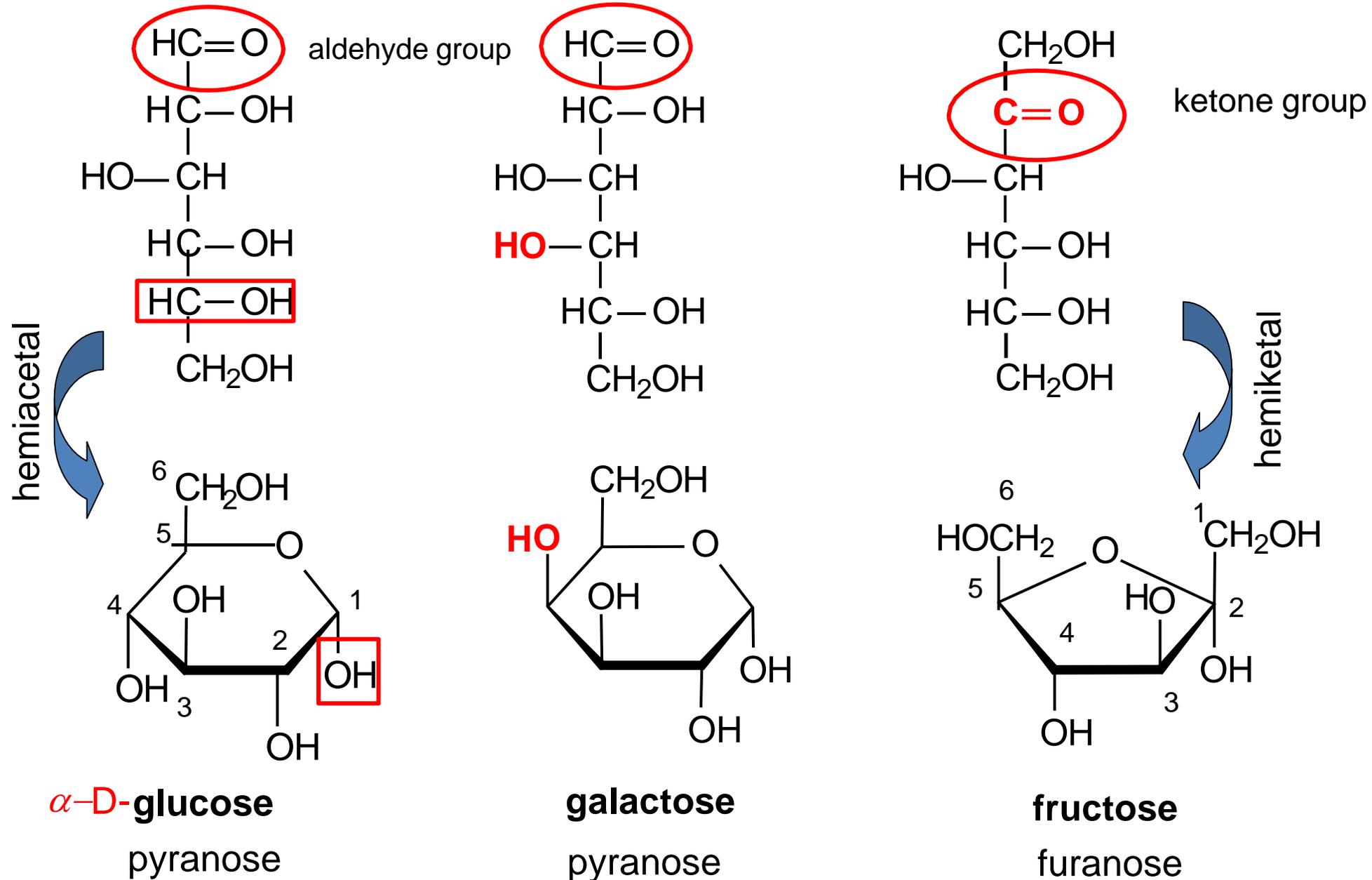
α -D-Fructopyranose



β -D-Fructopyranose

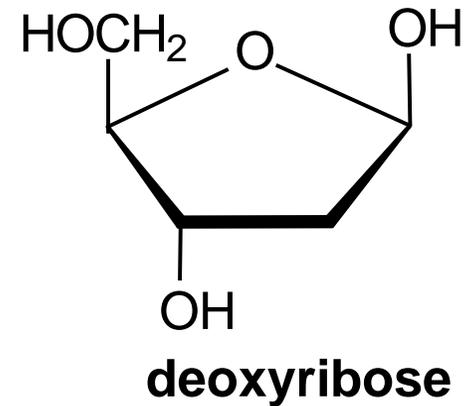
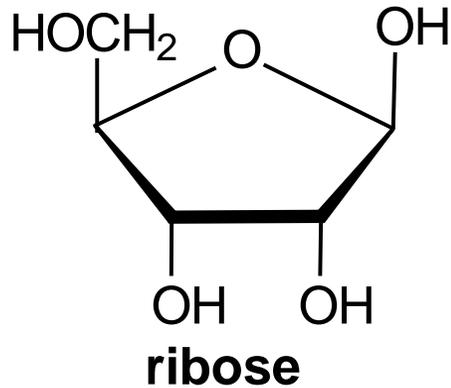
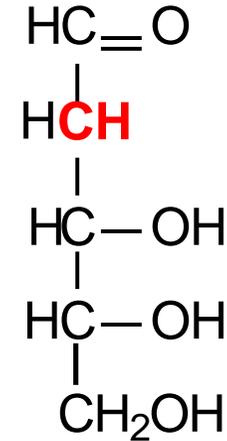
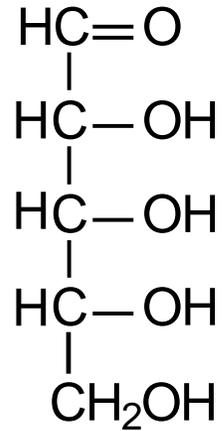


Pentoses and Hexoses Cyclize to Form Pyranose and furanose Rings



Pentose sugars

Pentoses such as D-ribose and 2-Deoxy-D-ribose form furanose rings, as we have seen in the structure of these units in **RNA** and **DNA**.



Components of RNA

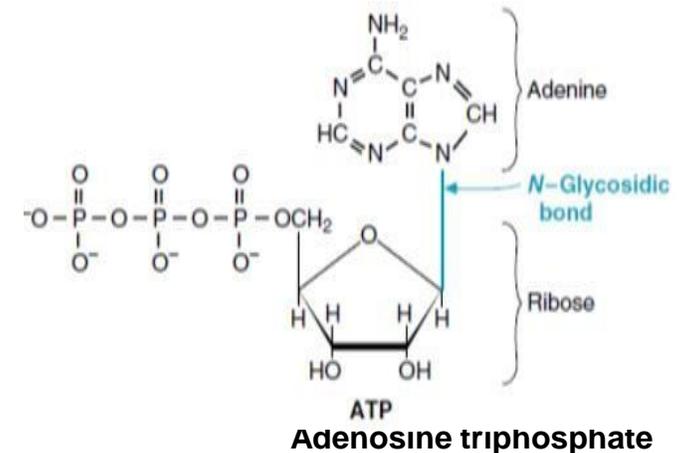
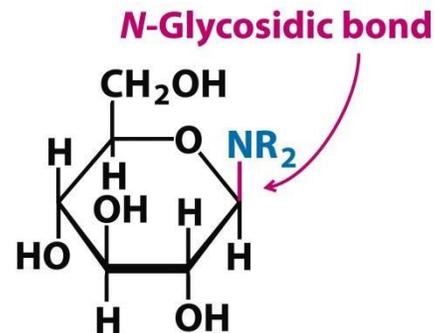
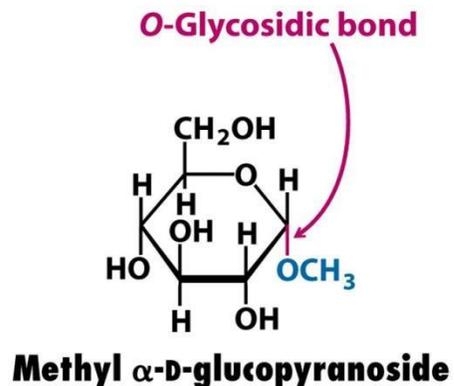
and

DNA



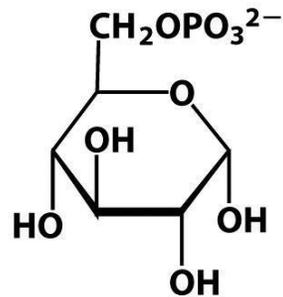
Monosaccharides joined to alcohols and amines Through Glycosidic Bonds

- Facilitate their metabolism
- Linked to alcohols, amines and phosphates
- The **OH group** on the **anomeric carbon of a cyclic sugar** can react with an $-OH$ or an $-NH$ group of another compound to form:
 - O-glycosidic bond between a monosaccharide and an alcohol or two monosaccharides or between a monosaccharide and a protein
 - N-glycosidic bond between a monosaccharide and a nitrogenous base or the amino acid lysine of a protein

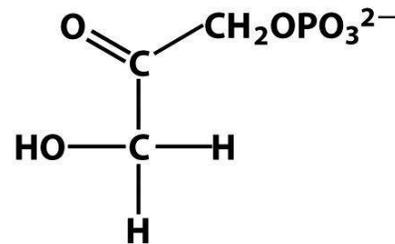


Phosphorylated sugars

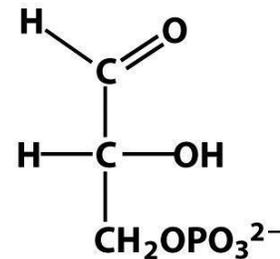
- Addition of a phosphoryl group to the monosaccharide
- Makes sugars anionic
- Trap sugar within the cell
- Creates a reactive intermediate of sugar metabolism



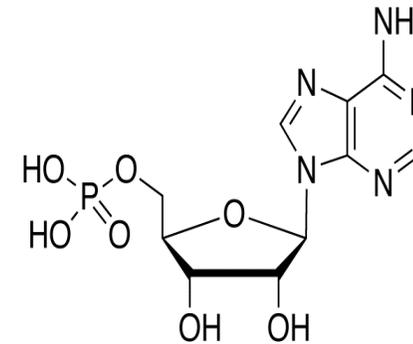
**Glucose 6-phosphate
(G-6P)**



**Dihydroxyacetone
phosphate
(DHAP)**



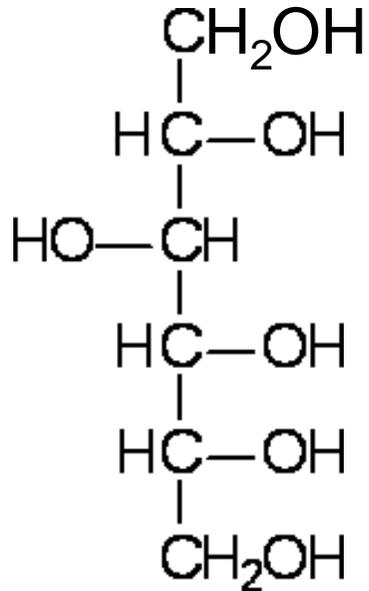
**Glyceraldehyde
3-phosphate
(GAP)**



Adenosine monophosphate



Sugar alcohols

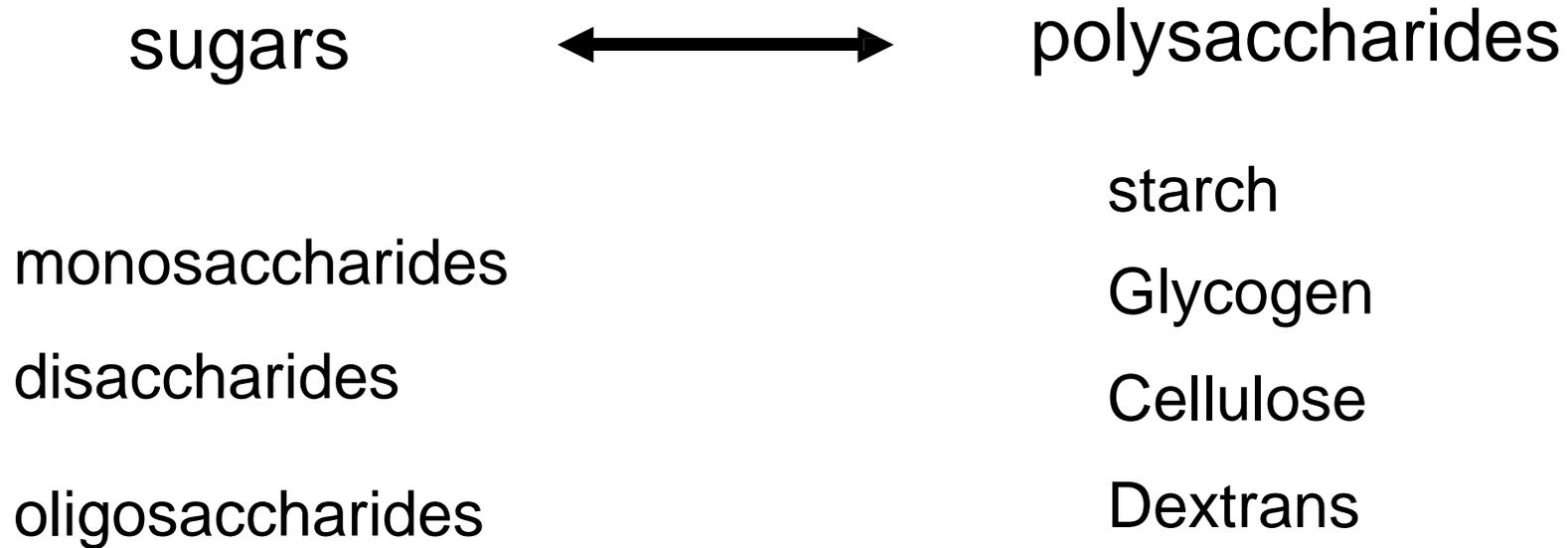


Sorbitol

- Formed by the **reduction of the aldehyde group of glucose to a hydroxyl group.**
- Used in foods suitable for diabetics as it tastes sweet. Also in cough syrup and sugar-free mints.
- Energy yield roughly half that of glucose.



Nutritional classification of carbohydrates



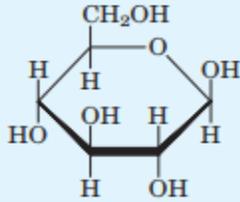
Disaccharide = condensation between two monosaccharides (**O-glycosidic bond**)

Oligosaccharides = 3 to 9 monosaccharides

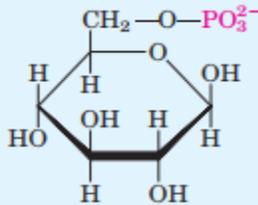


Some hexose derivatives important in biology

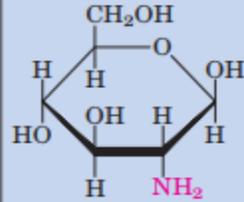
Glucose family



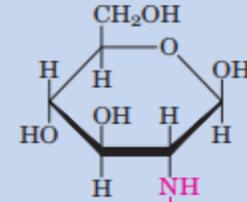
β -D-Glucose



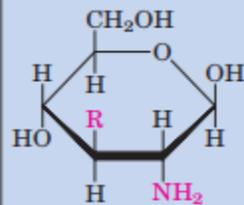
β -D-Glucose 6-phosphate



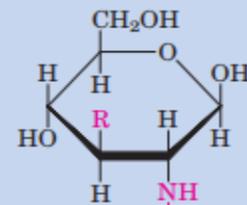
β -D-Glucosamine



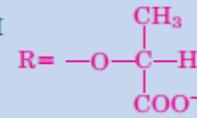
N-Acetyl- β -D-glucosamine



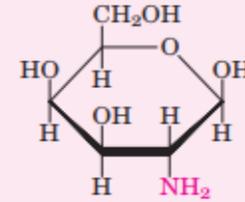
Muramic acid



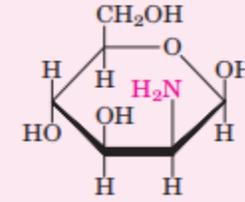
N-Acetylmuramic acid



Amino sugars

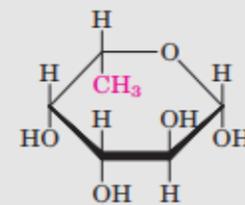


β -D-Galactosamine

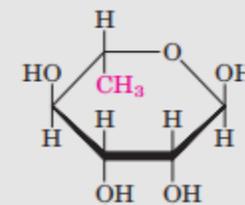


β -D-Mannosamine

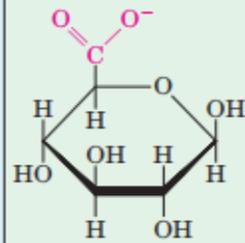
Deoxy sugars



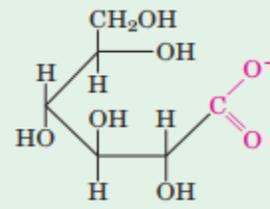
β -L-Fucose



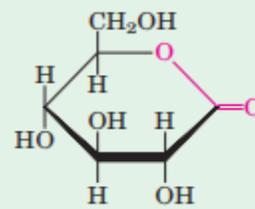
α -L-Rhamnose



β -D-Glucuronate

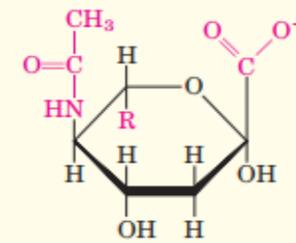


D-Gluconate

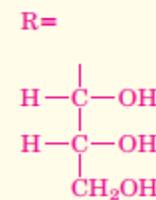


D-Glucono- δ -lactone

Acidic sugars



N-Acetylneuraminic acid
(a sialic acid)



Simple sugars

Glucose, mannose, galactose, fructose, sucrose, lactose and maltose.

The most common disaccharides are:

Sucrose (cane or beet sugar - made from one glucose and one fructose)

Maltose (made from two glucoses)

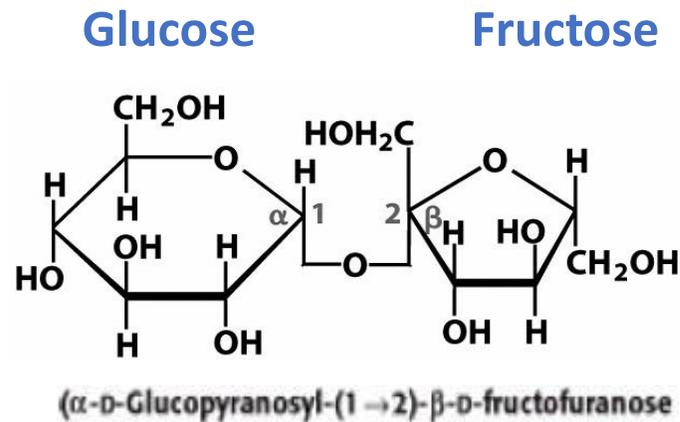
Lactose (milk sugar - made from one glucose and one galactose)

The formula of these disaccharides is $C_{12}H_{22}O_{11}$



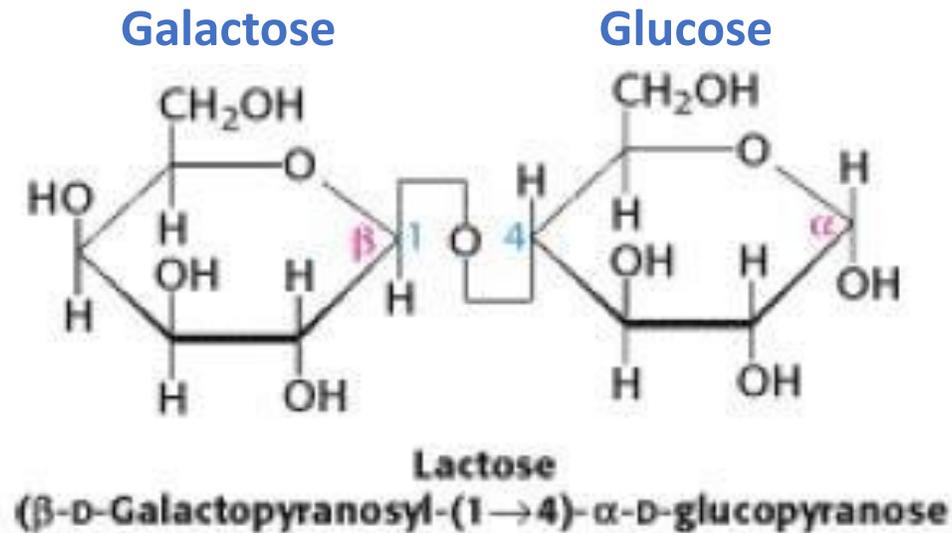
Sucrose

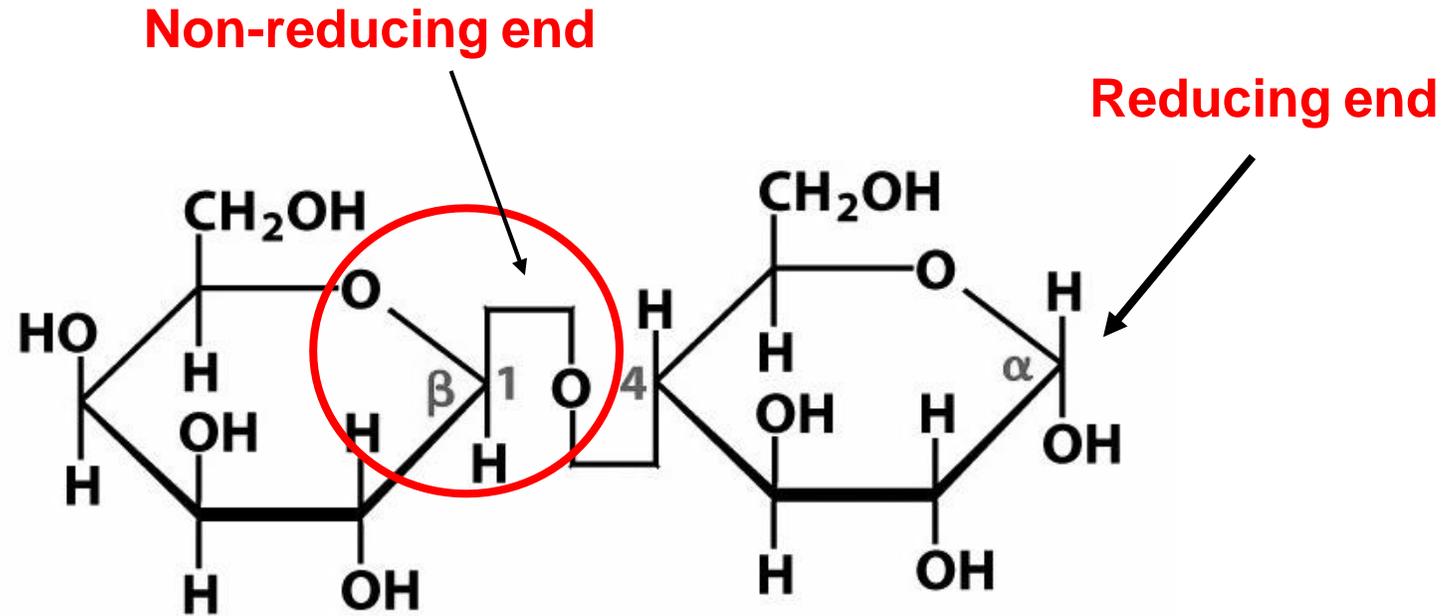
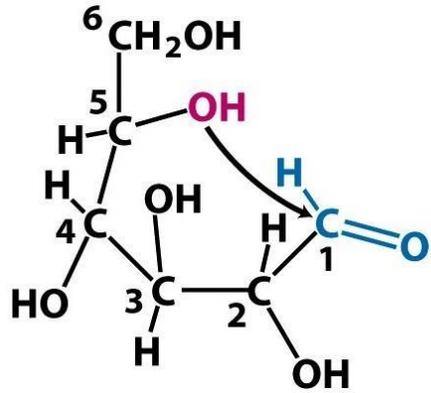
Sucrose (common table sugar) is obtained commercially from cane or beet. The anomeric carbon atoms of a glucose unit and a fructose unit are joined in this disaccharide; the configuration of this glycosidic linkage is α for glucose and β for fructose. Sucrose can be cleaved into its component monosaccharides by the enzyme **sucrase**.



Lactose: (Sugar of milk)

Lactose is the most important carbohydrate in the milk of mammals, Cow's milk contains 4.5% lactose, while human milk contains up to 7.5%, consists of galactose joined to glucose by a β -1,4-glycosidic linkage. Lactose is hydrolyzed to these monosaccharides by **lactase** in human beings and by **β -galactosidase** in bacteria.





Lactose

- ❖ An **acetal** is a molecule with **two single bonded oxygens attached to the same carbon atom**.
- ❖ This prevents opening of the chain to the aldehyde form and renders the modified residue non-reducing.

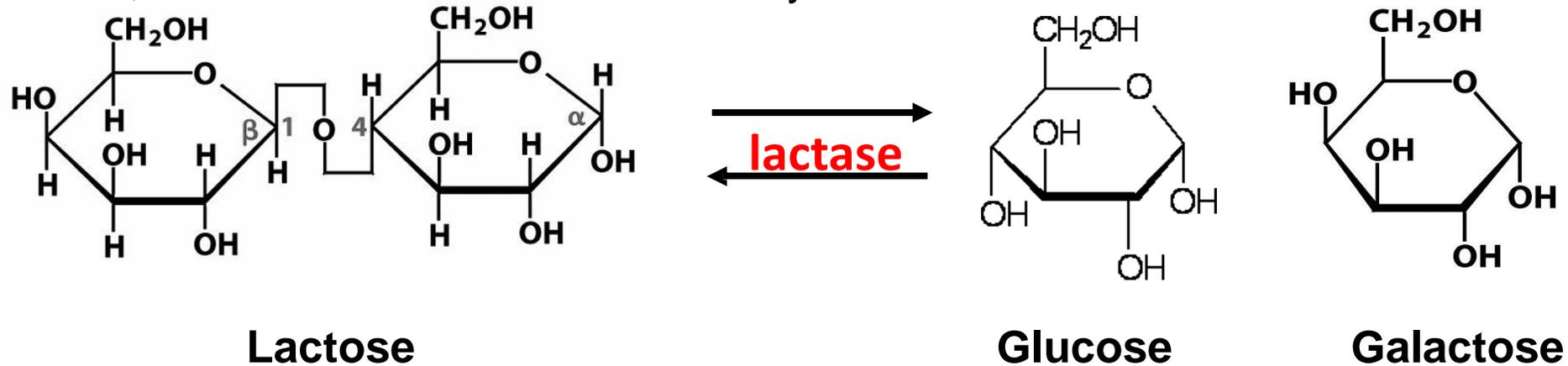


Milk intolerance

- Many adults are unable to metabolise the milk sugar **lactose** → gastrointestinal disturbances
- Decrease in lactase activity to 5 – 10% of the level at birth
- Lactose is used as **energy source** for **microorganisms in the colon**

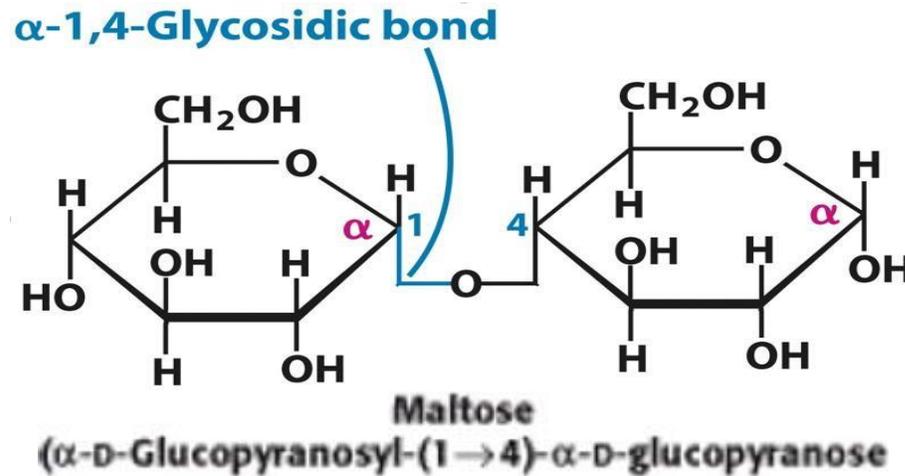
→ **Fermentation** to lactate with production of methane (CH_3OH) and hydrogen gas (H_2).

→ **Flatulence, diarrhea** as lactate is osmotically active and draws water into the intestine



Maltose – originally isolated from malt

Two D-glucose residues are joined by a glycosidic linkage between the α -anomeric form of C-1 on one sugar and the hydroxyl oxygen atom on C-4 of the adjacent sugar. Maltose comes from the hydrolysis of starch and is in turn hydrolyzed to glucose by **maltase**



Sucrase, lactase, and maltase are located on the outer surfaces of epithelial cells lining the **small intestine**.



Polysaccharides (also called glycans)

- Most carbohydrates found in nature occur as polysaccharides, polymers of medium to high molecular weight.
- Homopolysaccharides are polymers of a single monosaccharide, whereas heteropolysaccharides contain more than one type of monosaccharide ,Three important Polysaccharides are **starch, glycogen and cellulose**

Starch – large molecule with variable number of glucose units; storage carbohydrate of plant cells



Amylose – is a linear polymer of glucose linked with mainly (α -1,4) bonds

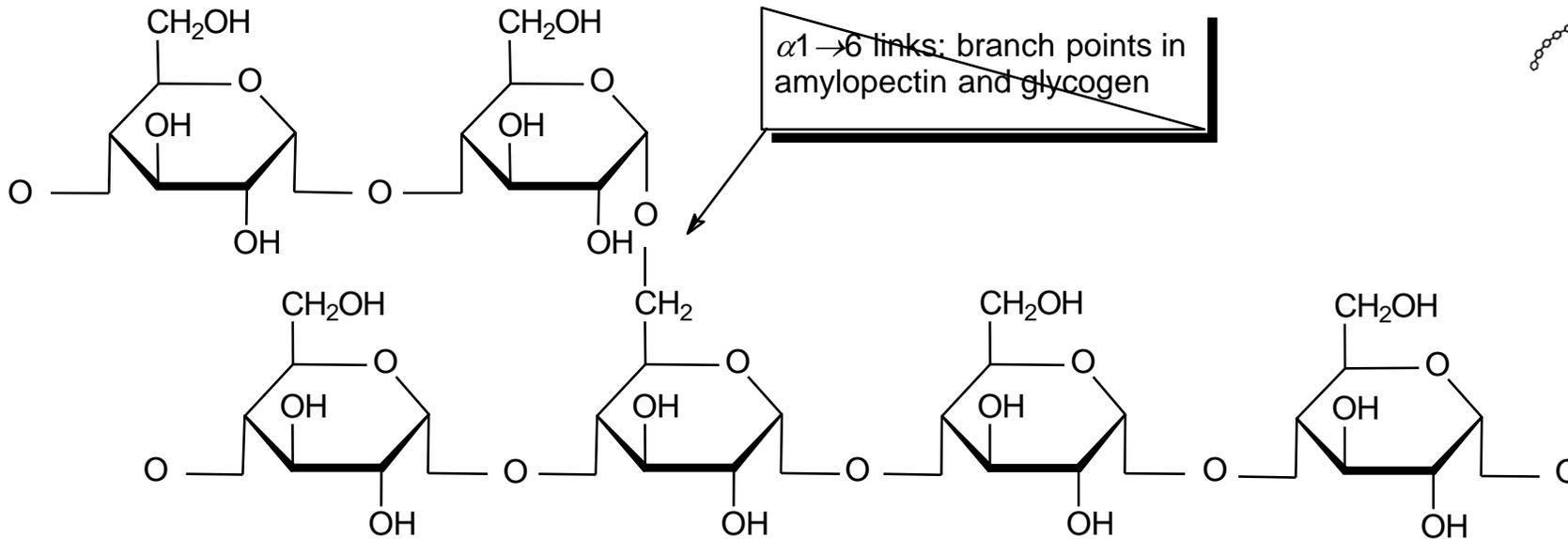
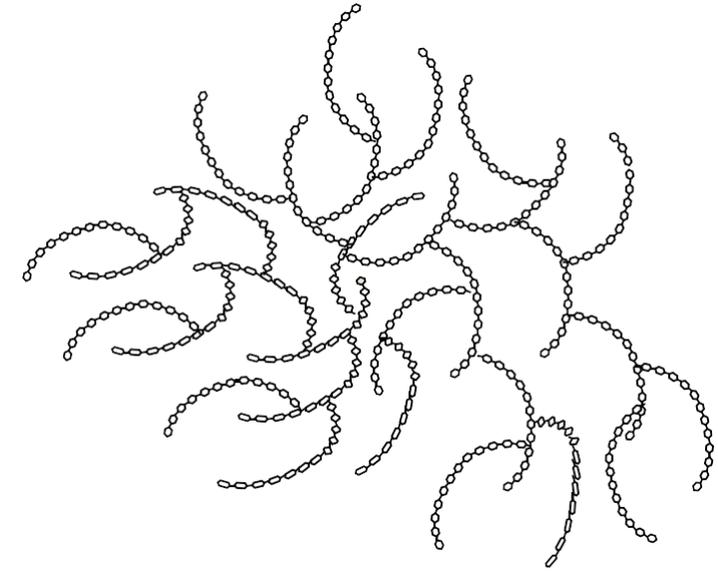
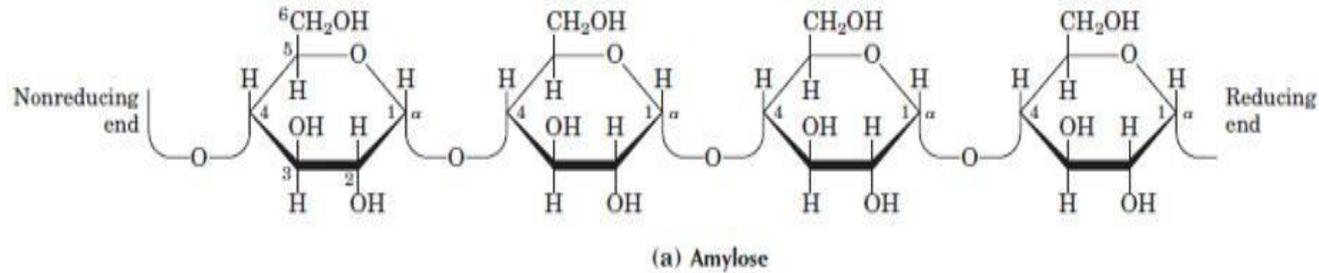
Amylopectin – chain of glucose molecules (α -1,4), every 30th glucose branch to other glucose residues (α -1,6)

Glycogen - storage carbohydrate in animal cells (muscle and liver)
- similar to amylopectine, but branch every 10th glucose

Non-starch polysaccharides – not digested by human enzymes
- e.g. Cellulose (glucose linked β -1,4), chitin, pectin



The end of the polysaccharide with an anomeric **C1** not involved in a glycosidic bond is called the reducing end.

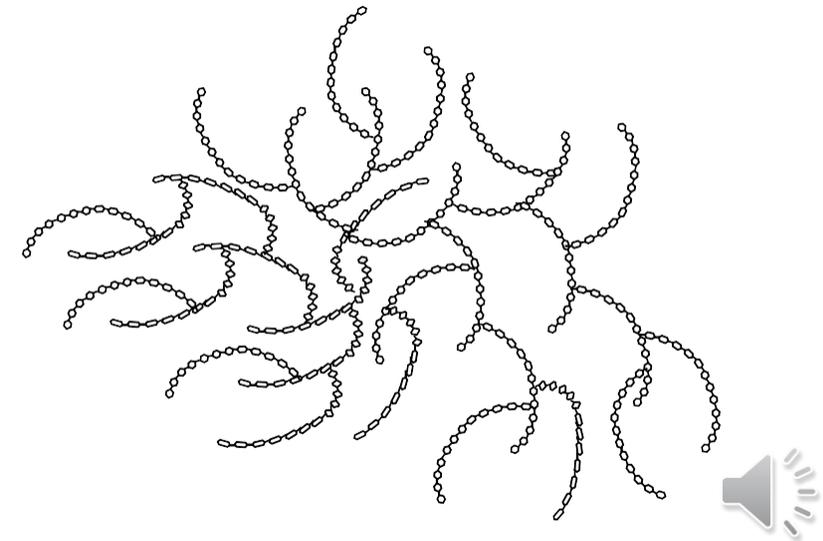
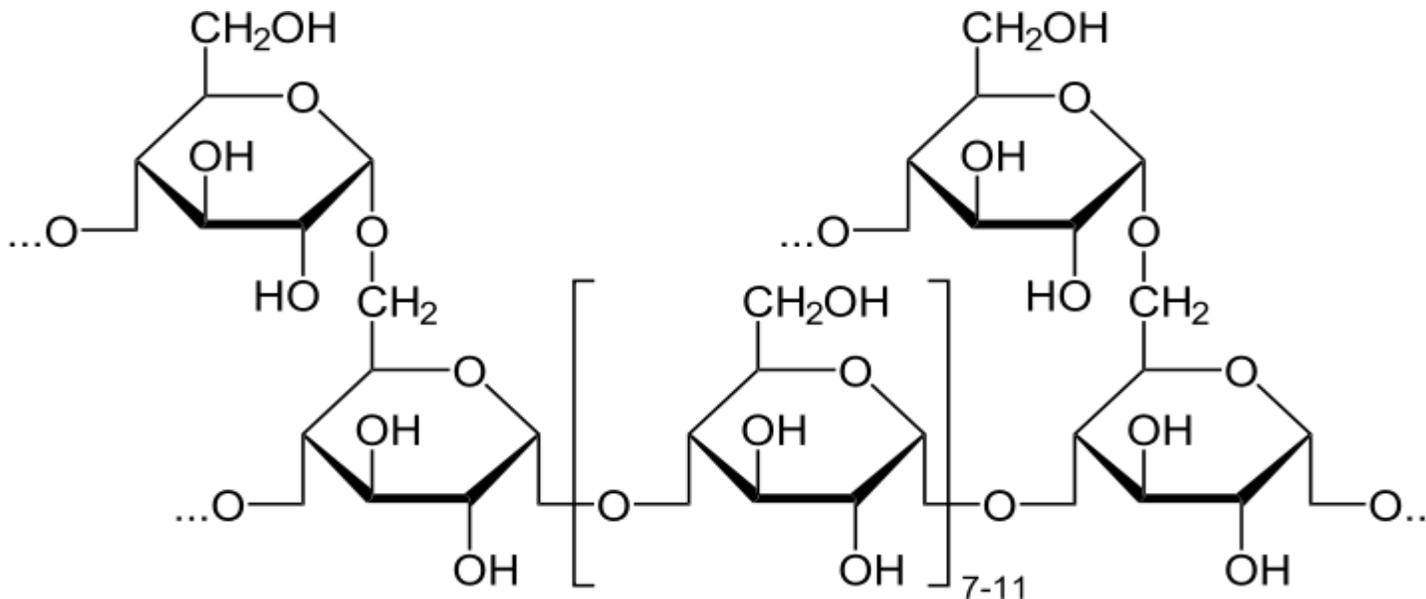


Hydrolysis of starch by amylase in saliva and pancreatic juice results in formation of Dextrans



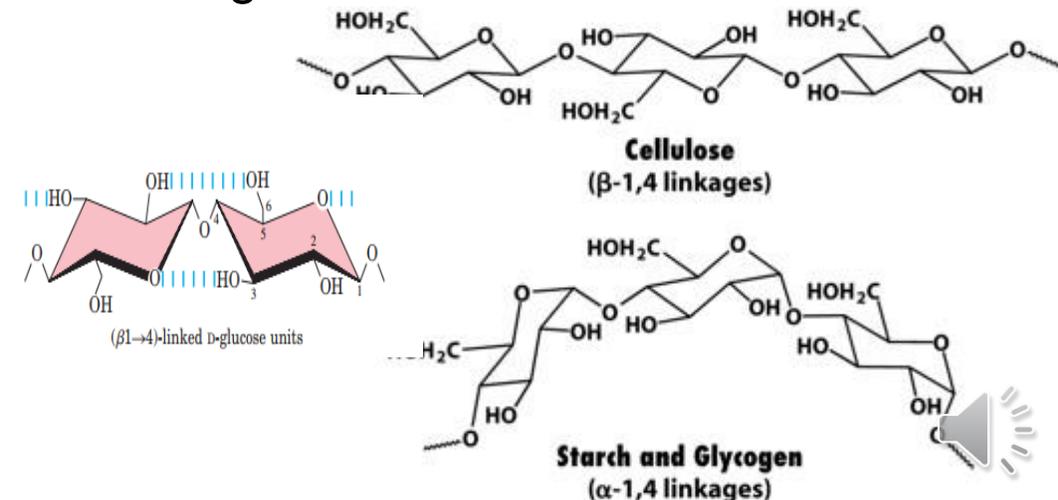
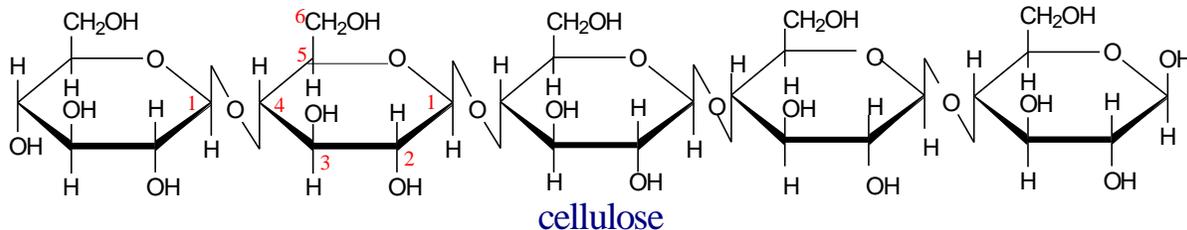
Glycogen

- Having a similar structure to amylopectin of starch, but more branches. and is commonly referred to as animal starch.
- Glycogen does not possess a **reducing end**.
- The „reducing end“ glucose residue is not free but is covalently bound to a protein termed **glycogenin**
- Main storage of glucose in **liver and skeletal muscle**.
- The glycogen granules contain both glycogen and the enzymes of glycogen synthesis (**Glycogenesis**) and degradation (**Glycogenolysis**).



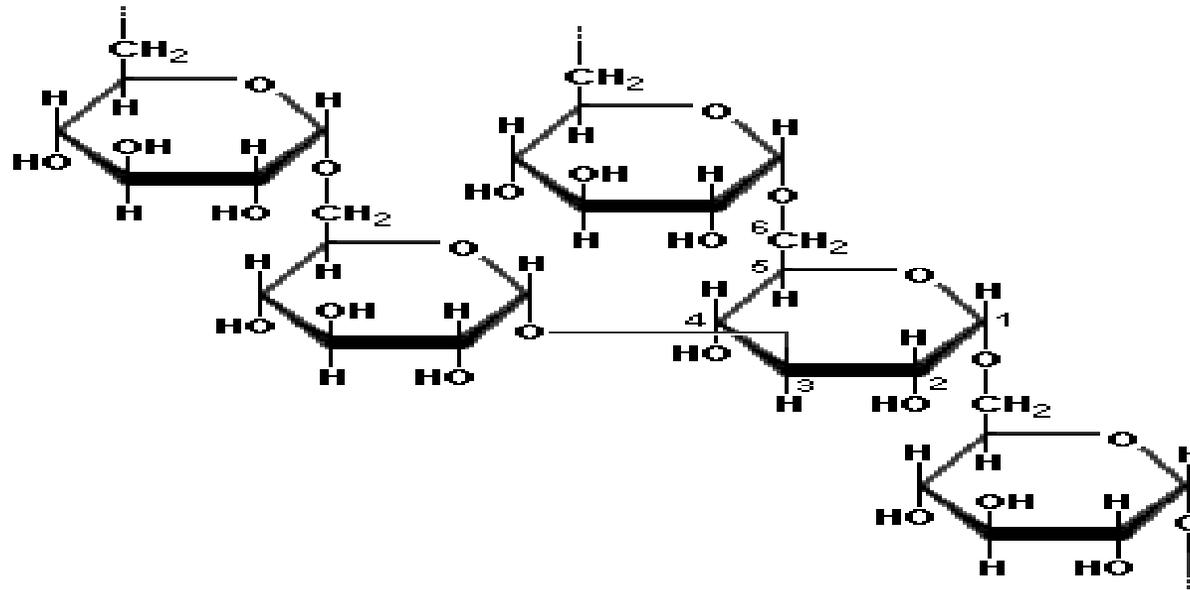
Cellulose

- **Cellulose** is a polysaccharide of glucose found in **plants**, consists of linear chains of glucose units. It is an **unbranched polymer** of glucose residues joined by **β -1,4 linkages**.
- The β configuration allows cellulose to form very long, straight chains. Fibrils are formed by parallel chains that interact with one another through hydrogen bonds.
- The α -1,4 linkages in glycogen and starch produce a very different molecular architecture from that of cellulose. A hollow helix is formed instead of a straight chain.
- These differing consequences of the α and β linkages are biologically important. The straight chain formed by β linkages is optimal for the construction of fibers having a high tensile strength.
- **Mammals lack cellulase** and therefore cannot digest wood and vegetable fibers.



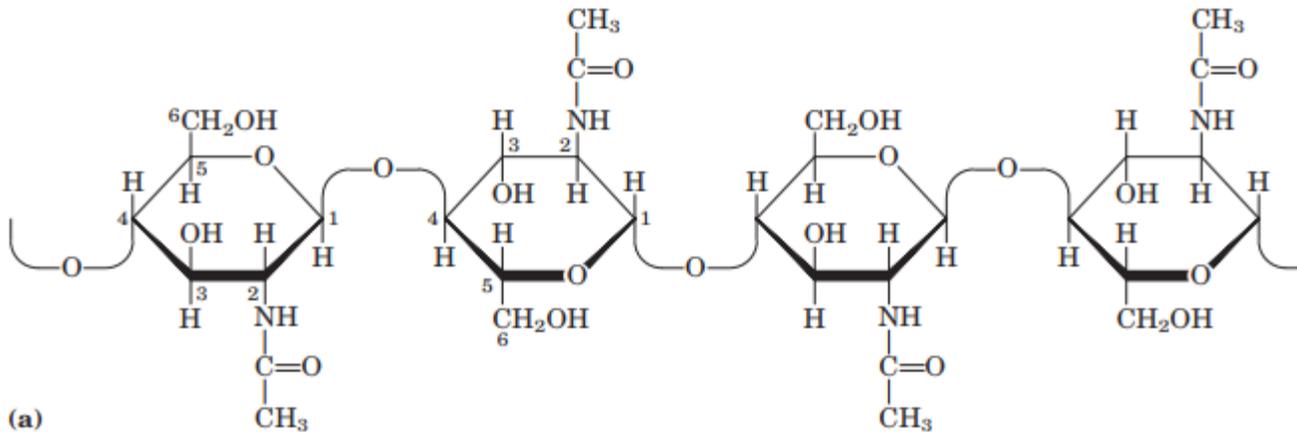
Dextrans

- **Dextrans** are bacterial and yeast polysaccharides made up of **($\alpha 1 \rightarrow 6$)-linked poly-D-glucose**; all have **($\alpha 1 \rightarrow 3$)** branches, and some also have **($\alpha 1 \rightarrow 2$)** or **($\alpha 1 \rightarrow 4$)** branches.
- Dental plaque, formed by bacteria growing on the surface of teeth, is rich in dextrans. Synthetic dextrans are used in several commercial products (for example, Sephadex) that serve in the fractionation of proteins by size-exclusion chromatography.



Chitin

- (a) A homopolymer of N-acetyl-D-glucosamine units in β -1,4 linkage, strengthens the exoskeletons of arthropods
- (b) A spotted beetle (*Pellidnota punetata*), showing its surface armor (exoskeleton) of chitin.



(a)



(b)



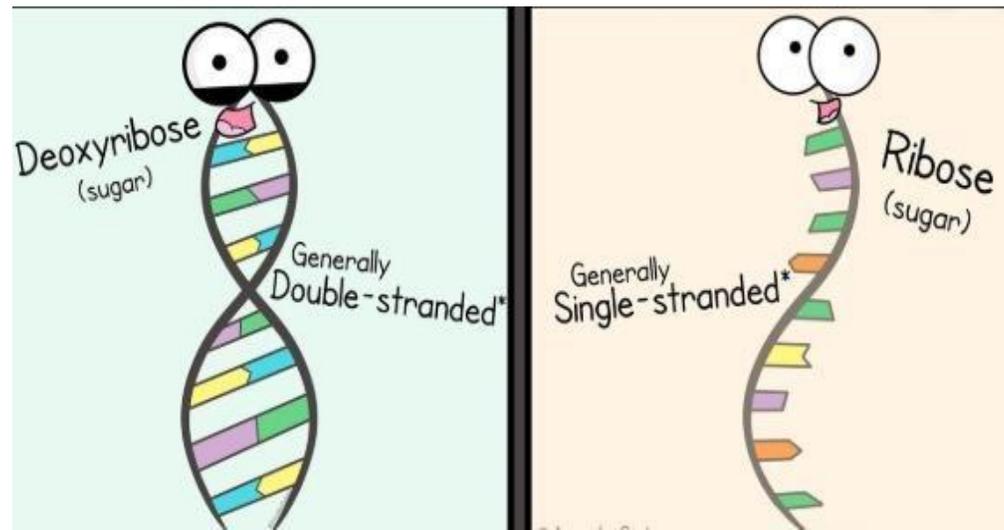
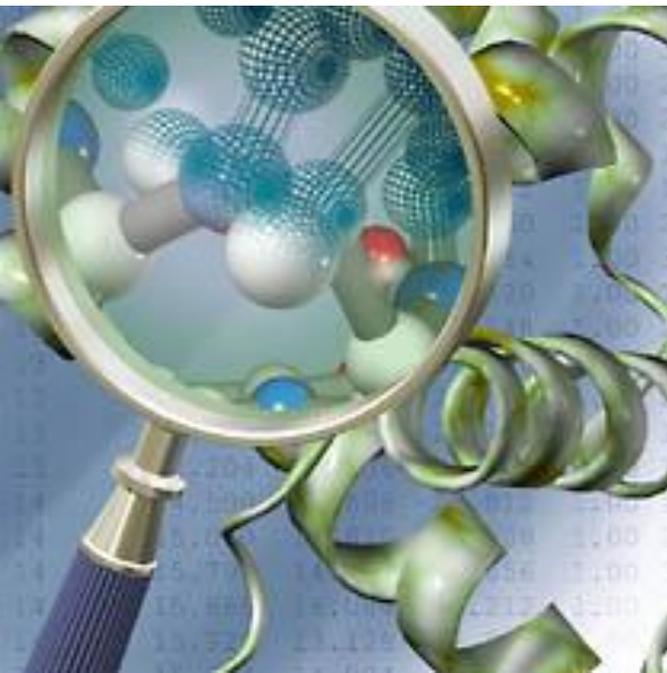
Lecture 3: Biochemistry I

Biochemistry of Nucleic acids

3rd stage

Anbar University-College of Pharmacy-Clinical Laboratory Sciences Department
2020-2021

Dr. Yousif H. Khalaf



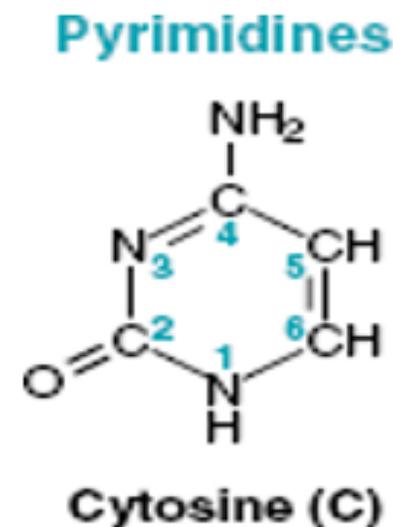
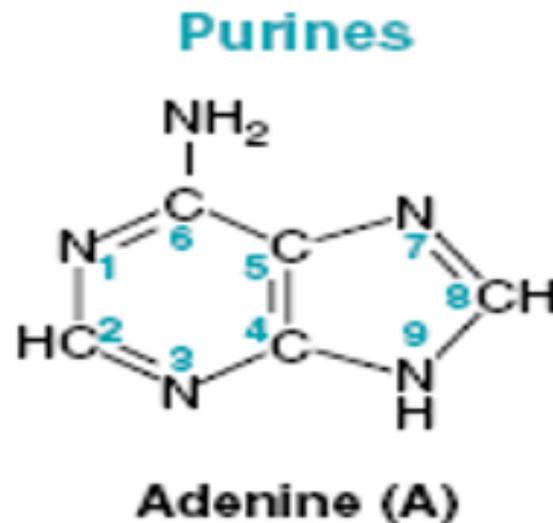
Nucleic acids

- **Two types** of Nucleic acids: Deoxyribonucleic acid (**DNA**) and ribonucleic acid (**RNA**).
- **Storage** and expression of **genetic information**.
- **DNA**: DNA is organic chemical of complex molecular structure that is found in all prokaryotic and eukaryotic cells and in many viruses. It is also found in mitochondria and the chloroplasts of the plants.
- The genetic information found in DNA is copied and transmitted from one generation to the next through **DNA replication**.
- **Nucleotides** are the monomeric units of the **DNA and RNA**
 - **Heterocyclic nitrogenous base**
 - **Sugar**
 - **Mono, di, or triphosphate**.

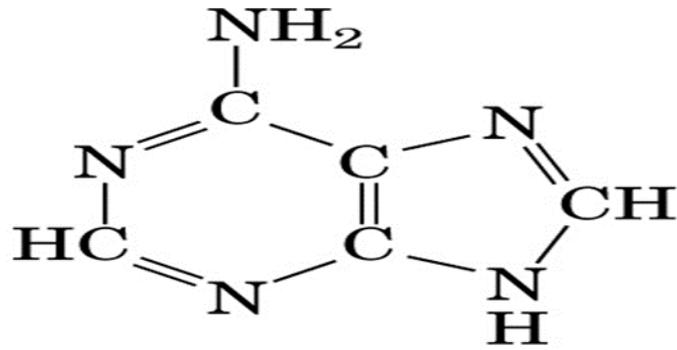


Bases present in DNA and RNA

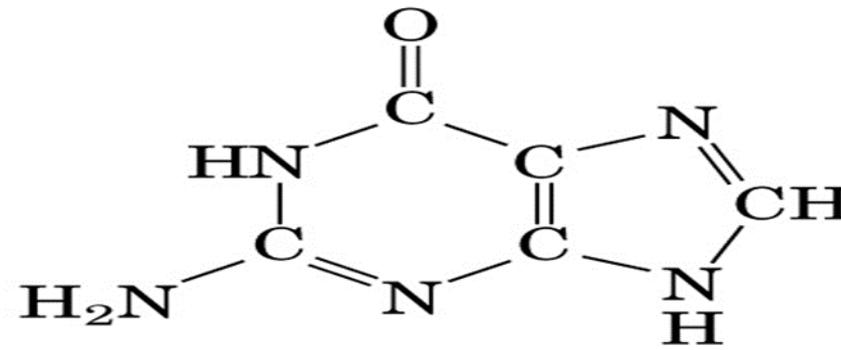
- **DNA** and **RNA** contain the same **purine bases**: Adenine (**A**), Guanine (**G**), and **pyrimidine base** - Cytosine (**C**)
- But they **differ** in their **second pyrimidine base**: **DNA** contains thymine (**T**), while **RNA** contains uracil (**U**).
- The atoms in the rings of the bases are numbered **1 to 6 in pyrimidines** and **1 to 9 in purines**.



Bases present in DNA and RNA

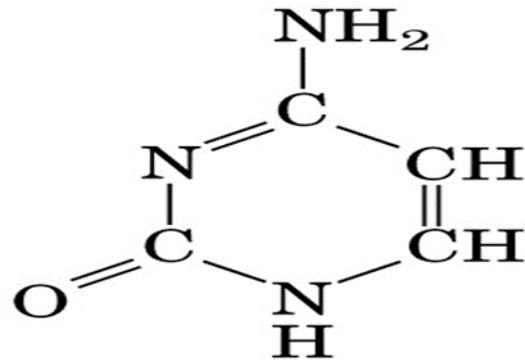


Adenine

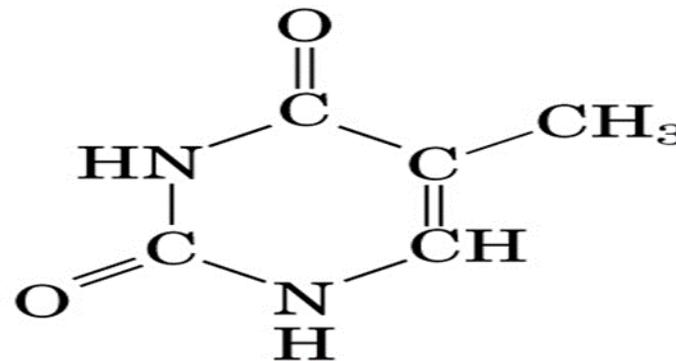


Guanine

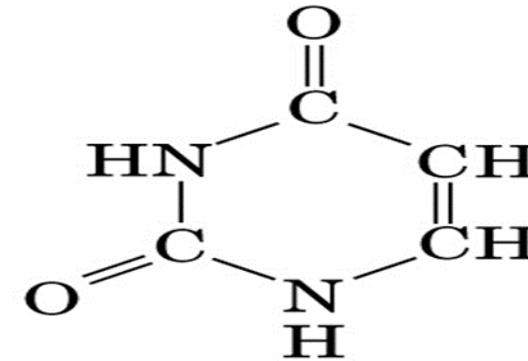
Purines



Cytosine



Thymine
(DNA)



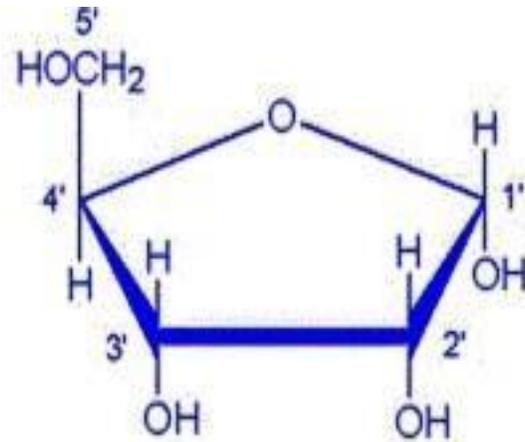
Uracil
(RNA)

Pyrimidines

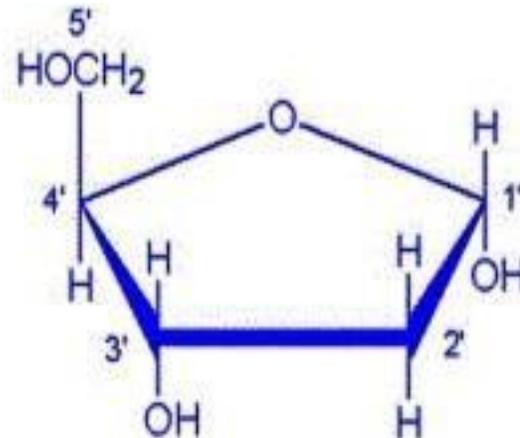


Sugars present in DNA and RNA

- Pentoses (5-C sugars)
- Numbering of sugars is primed



ribose
found in RNA



2'-deoxyribose
found in DNA

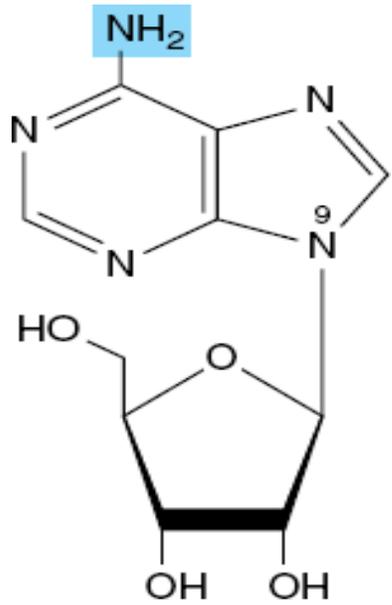


Nucleosides

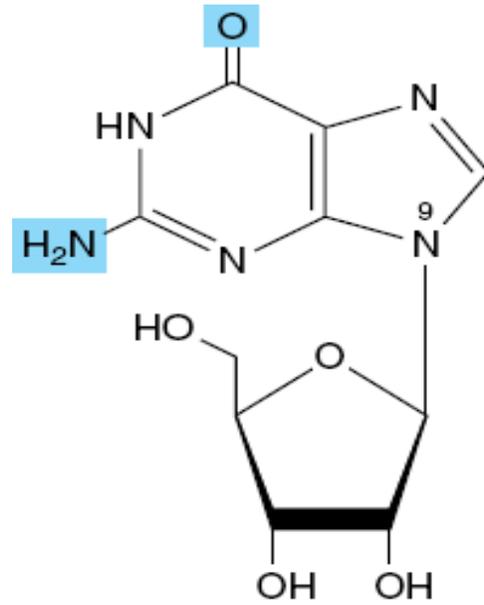
- linking one of the **sugars** with a **purine** or **pyrimidine** base through an **N-glycosidic bond**
- **Purines** are bonded to **C1** of the sugar at their **N9** atom.
- **Pyrimidines** are bonded to **C1** of the sugar at their **N1** atom.
- If the sugar is **D-ribose**, a **ribonucleoside** is produced , if the sugar is **2-deoxyribose** a **Deoxyribonucleoside** is produced.
- **DNA**: Deoxyadenosine, Deoxyguanosine, Deoxycytidine, and Deoxthymidine
- **RNA**: Adenosine, Guanosine, Cytidine, and Uridine



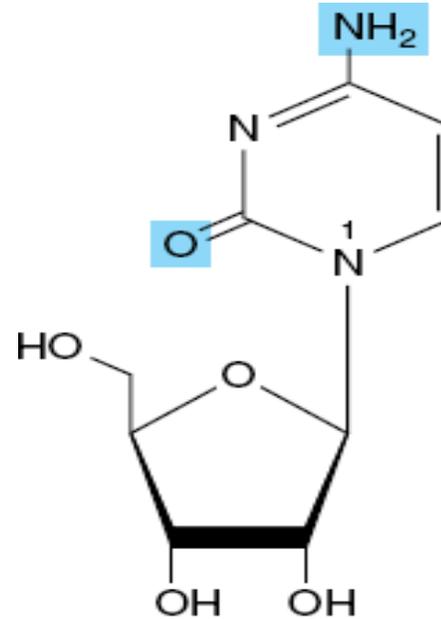
Ribonucleosides



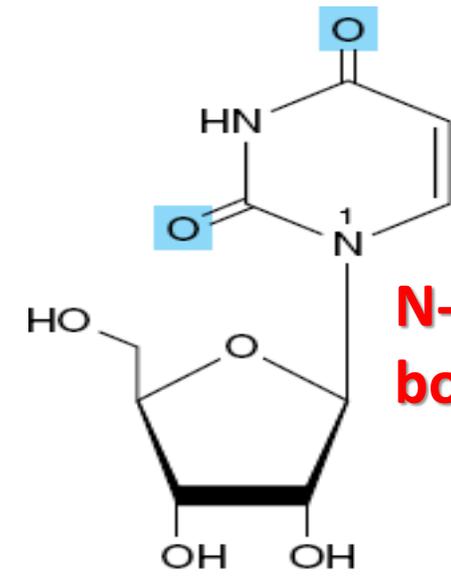
Adenosine



Guanosine



Cytidine



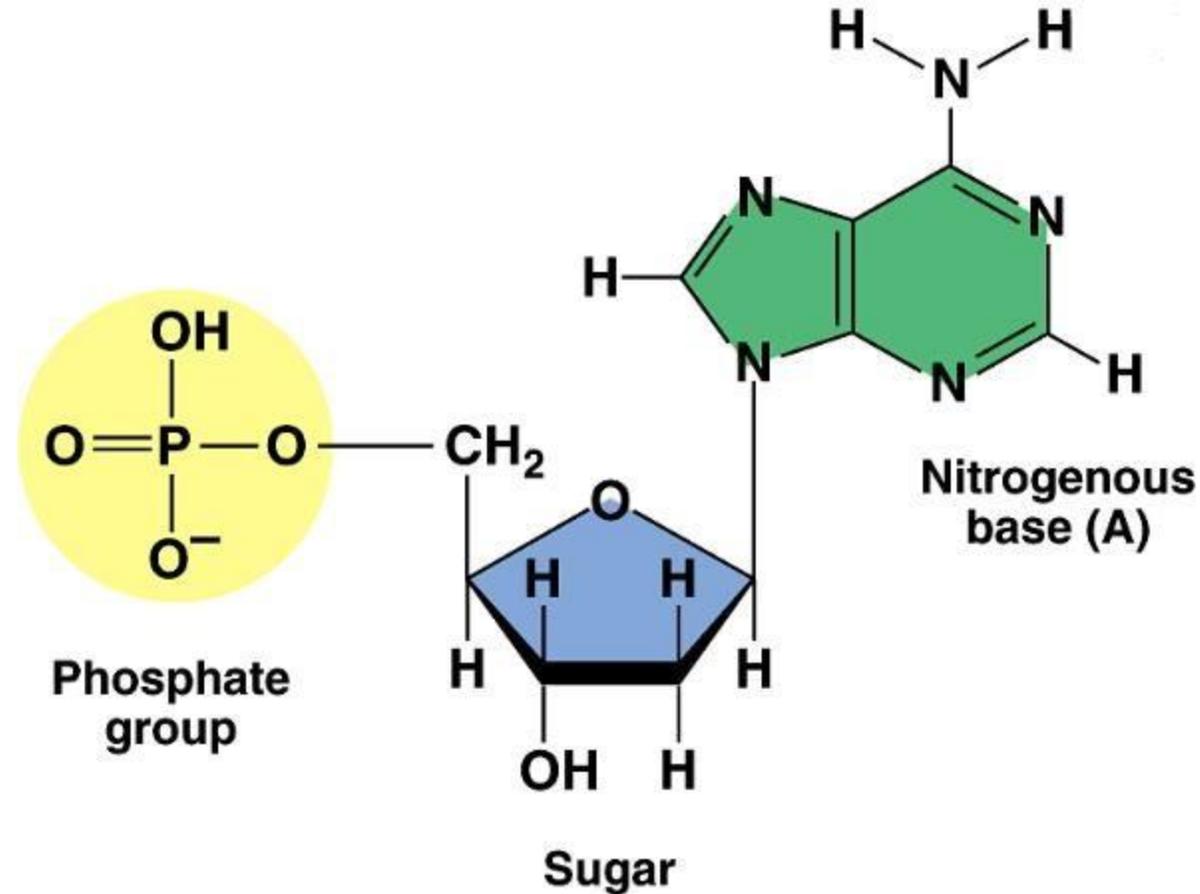
Uridine

N-glycosidic bond



Phosphate Group

- Mono, di- or triphosphates
- **Phosphates** can be bonded to either **C3** or **C5** atoms of the **sugar**

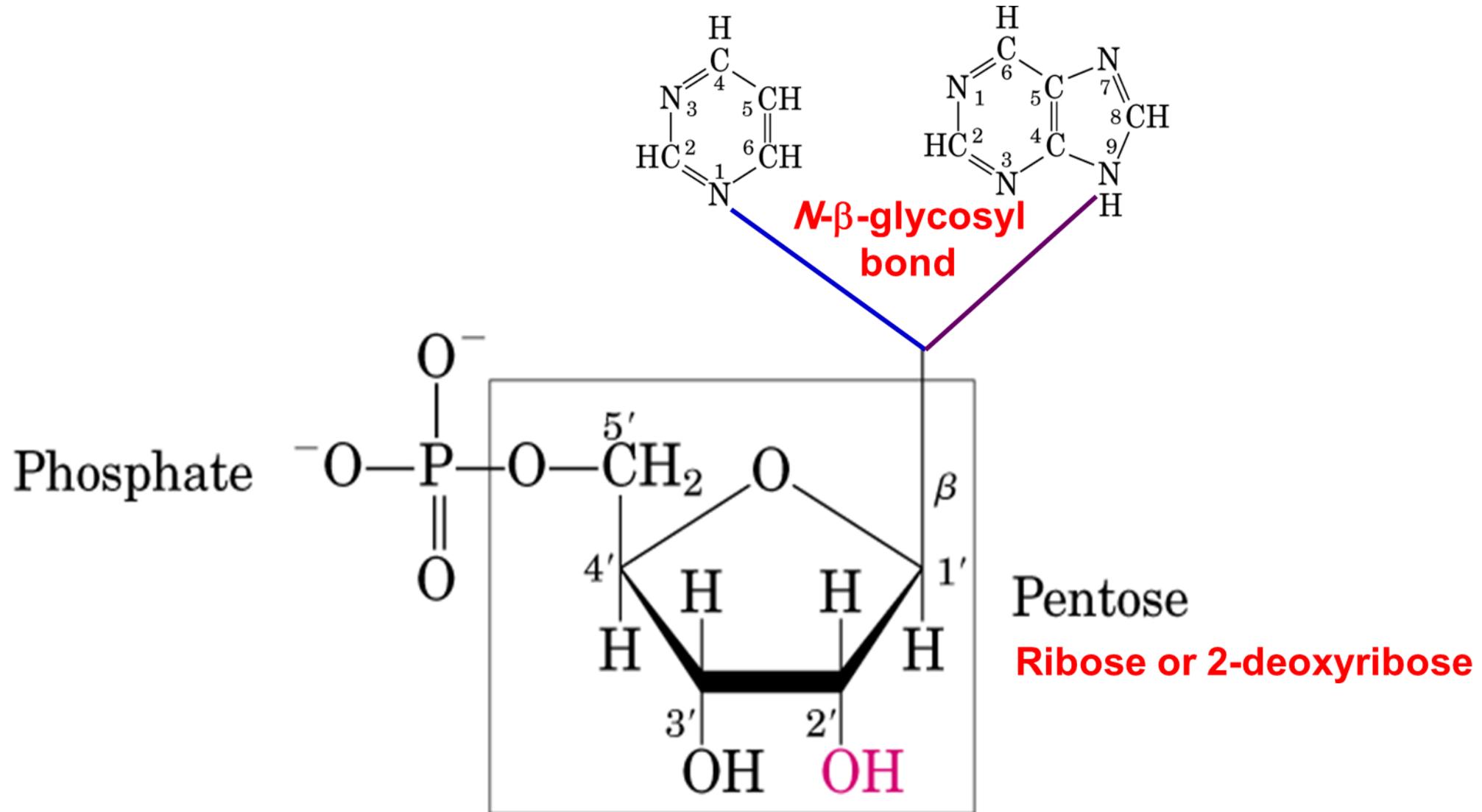


Nucleotides

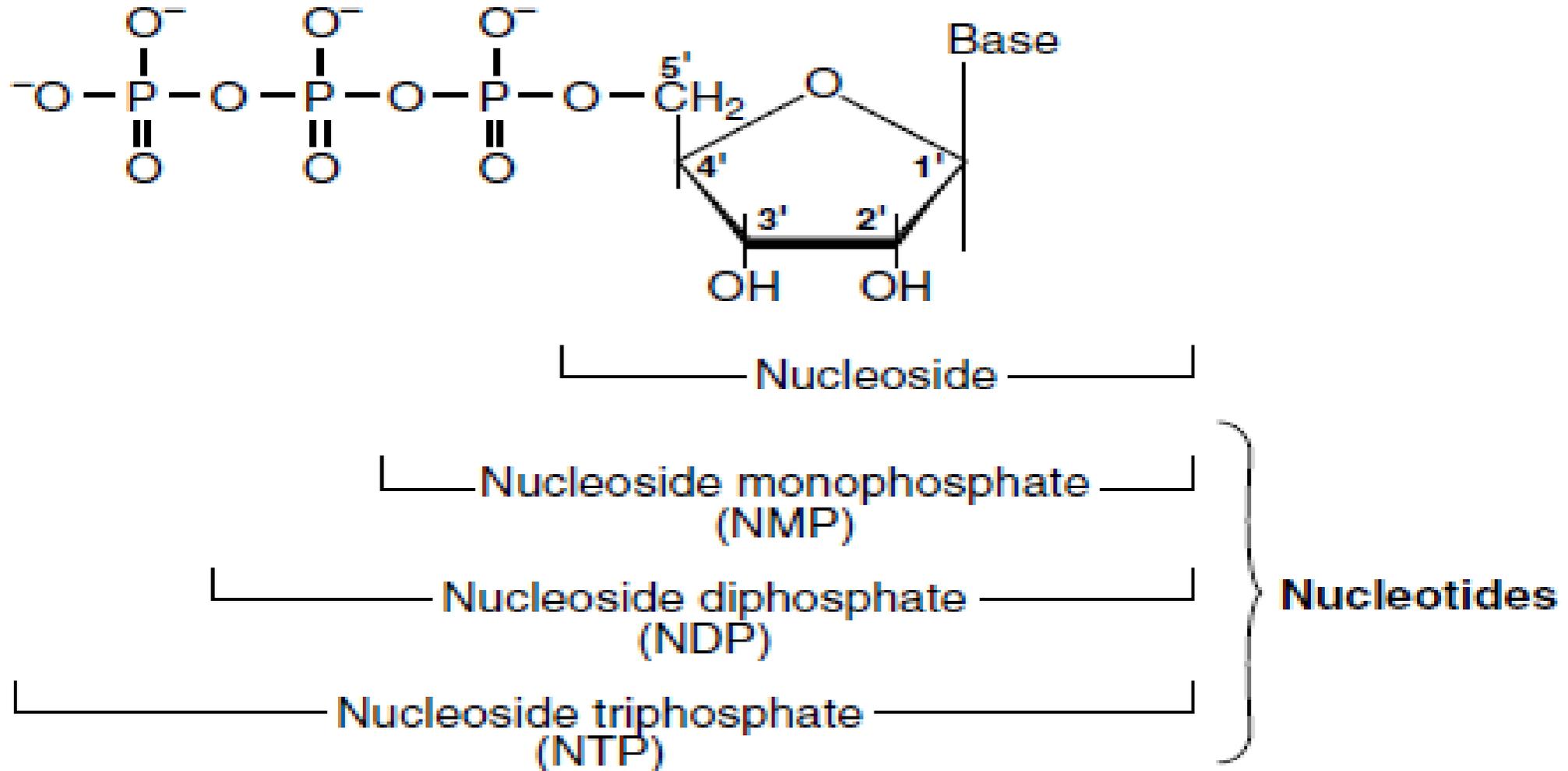
- A nucleotide is a **nucleoside** with an **inorganic phosphate** attached to a 5-hydroxyl group of the **sugar** in **ester linkage**.
- The **nitrogenous base** is linked by an **N-glycosidic bond** to the **anomeric carbon** of the sugar, either **ribose** or **deoxyribose**
- The names and abbreviations of nucleotides specify the base, the sugar, and the number of phosphates attached.



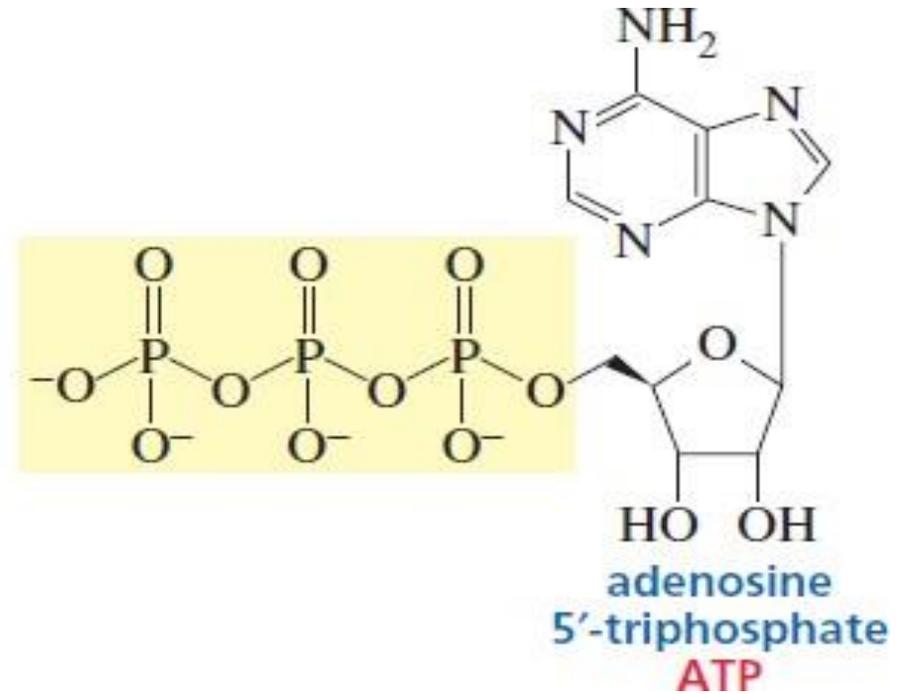
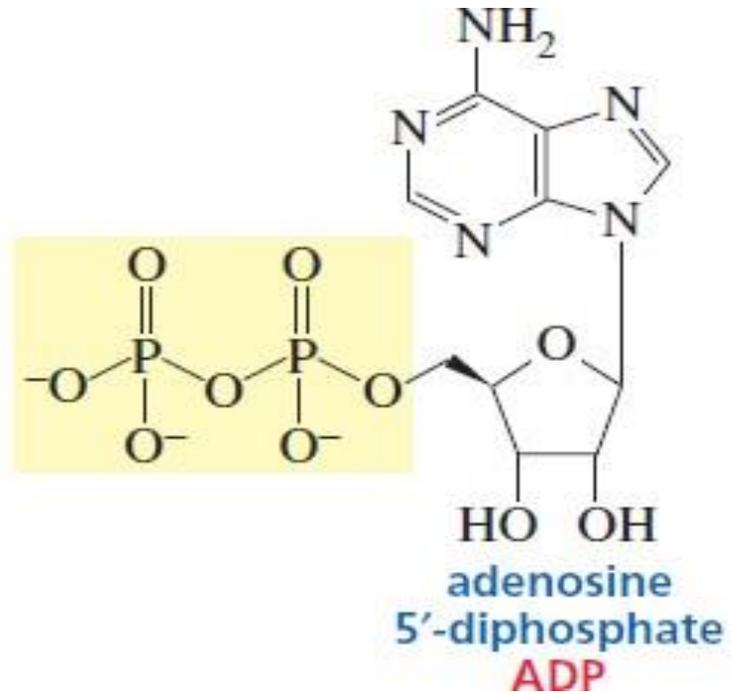
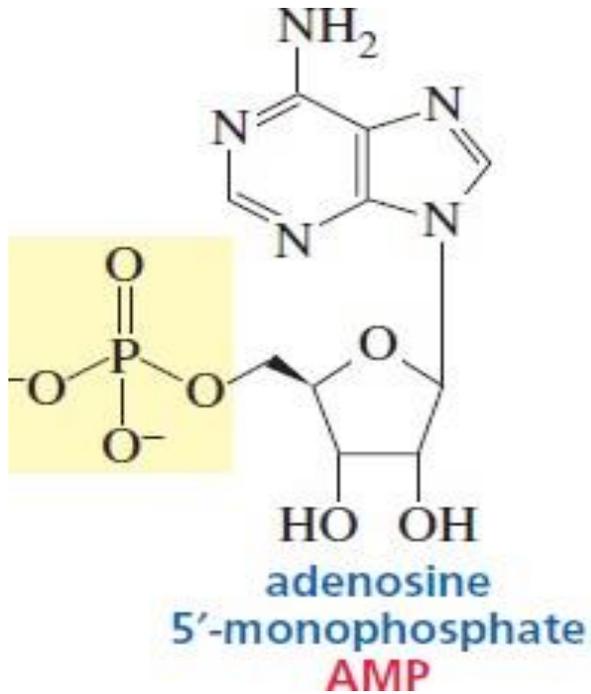
Structure of nucleotides



Structure of nucleotides

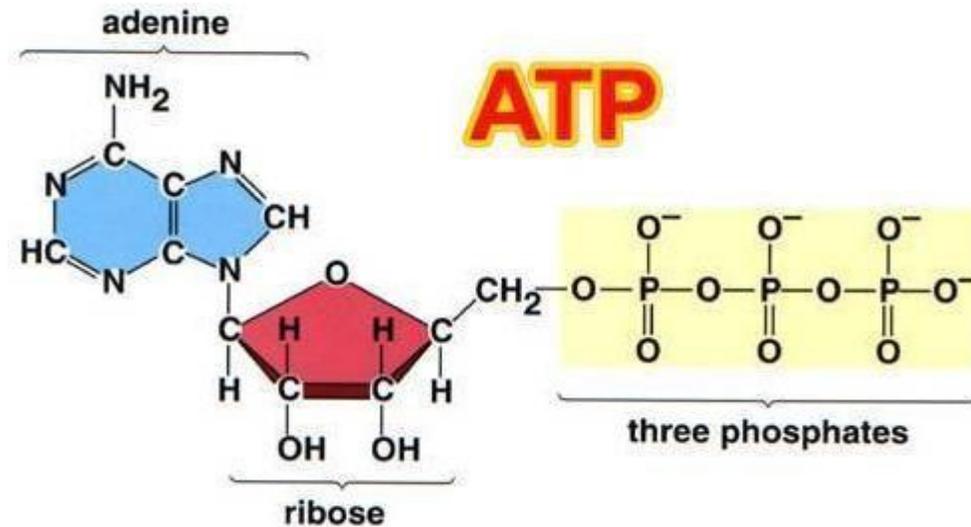


Structure of nucleotides



Nucleotides

- In **deoxynucleotides**, the prefix “d” precedes the abbreviation. For example, **ADP** is **Adenosine diphosphate** (the base **Adenine** attached to a **ribose** that has **two phosphate groups**) and **dATP** is **deoxyadenosine triphosphate** (the base **adenine** attached to a **deoxyribose** with **three phosphate groups**).



DNA is a polymer of deoxyribonucleotide

- Deoxyadenosine triphosphate (dATP)
- Deoxyguanosine triphosphate (dGTP)
- Deoxycytidine triphosphate (dCTP)
- Deoxythymidine triphosphate (dTTP)

RNA is a polymer of ribonucleotide

- Adenosine triphosphate (ATP)
- Guanosine triphosphate (GTP)
- Cytidine triphosphate (CTP)
- Uridine triphosphate (UTP)

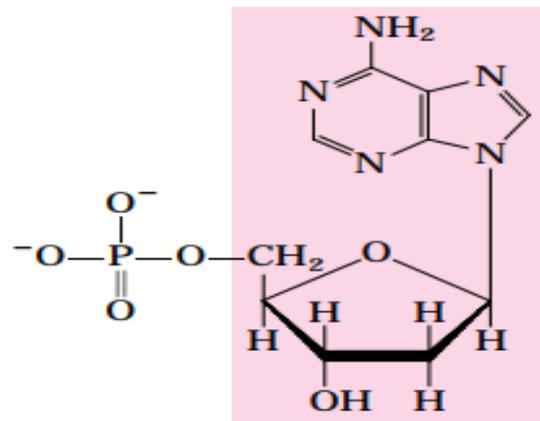


Nucleotides

Abbreviations of ribonucleoside 5'-phosphates			
Base	Mono-	Di-	Tri-
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

Abbreviations of deoxyribonucleoside 5'-phosphates			
Base	Mono-	Di-	Tri-
Adenine	dAMP	dADP	dATP
Guanine	dGMP	dGDP	dGTP
Cytosine	dCMP	dCDP	dCTP
Thymine	dTMP	dTDP	dTTP

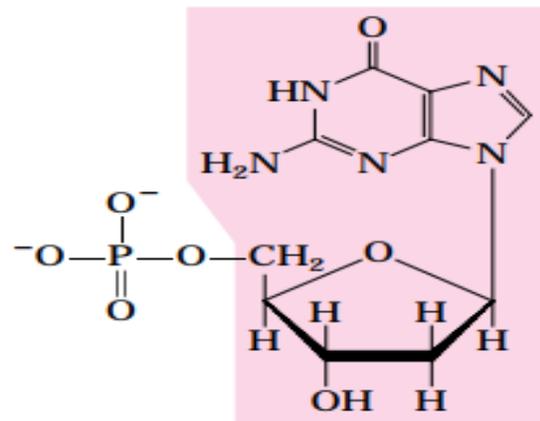




Nucleotide: Deoxyadenylate
(deoxyadenosine
5'-monophosphate)

Symbols: A, dA, dAMP

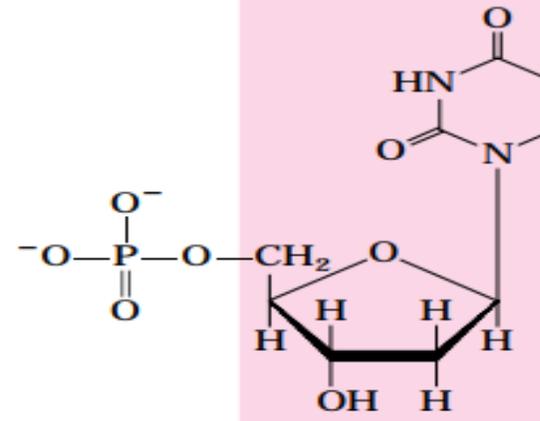
Nucleoside: Deoxyadenosine



Nucleotide: Deoxyguanylate
(deoxyguanosine
5'-monophosphate)

Symbols: G, dG, dGMP

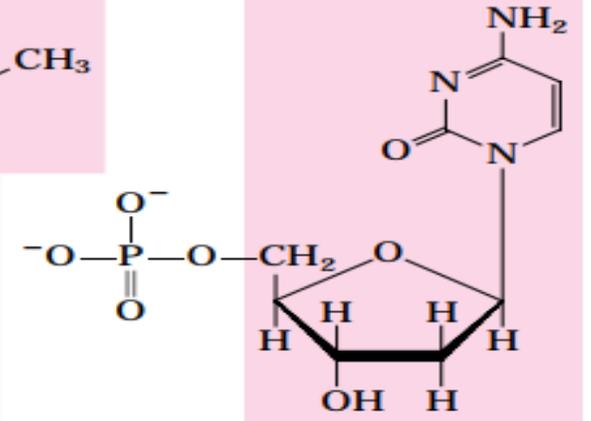
Nucleoside: Deoxyguanosine



Nucleotide: Deoxythymidylate
(deoxythymidine
5'-monophosphate)

Symbols: T, dT, dTMP

Nucleoside: Deoxythymidine

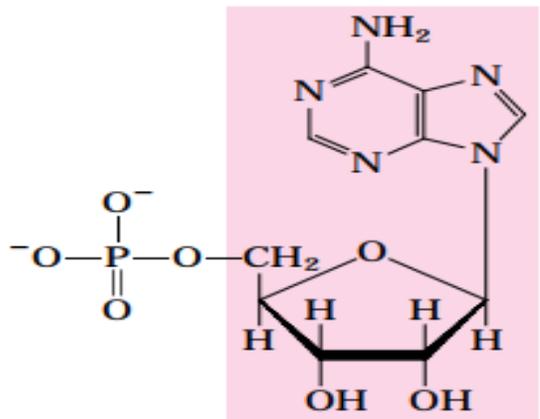


Nucleotide: Deoxycytidylate
(deoxycytidine
5'-monophosphate)

Symbols: C, dC, dCMP

Nucleoside: Deoxycytidine

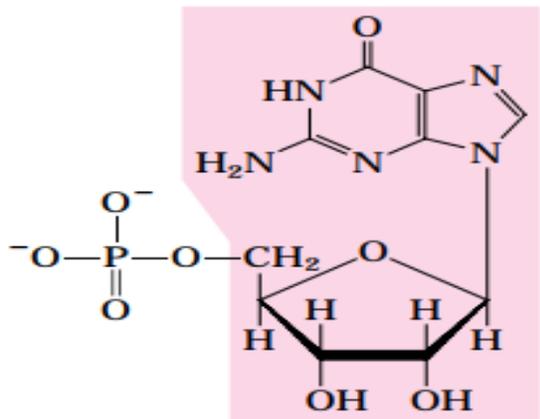
(a) Deoxyribonucleotides



Nucleotide: Adenylate (adenosine
5'-monophosphate)

Symbols: A, AMP

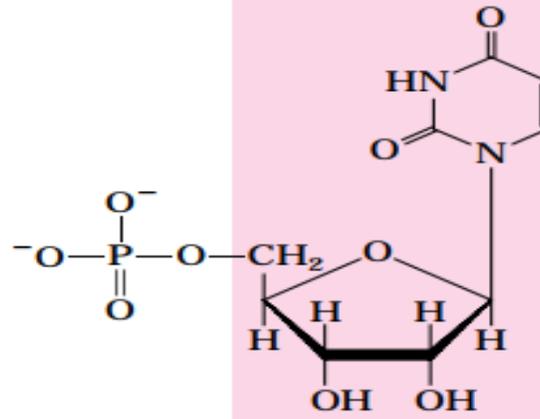
Nucleoside: Adenosine



Nucleotide: Guanylate (guanosine
5'-monophosphate)

Symbols: G, GMP

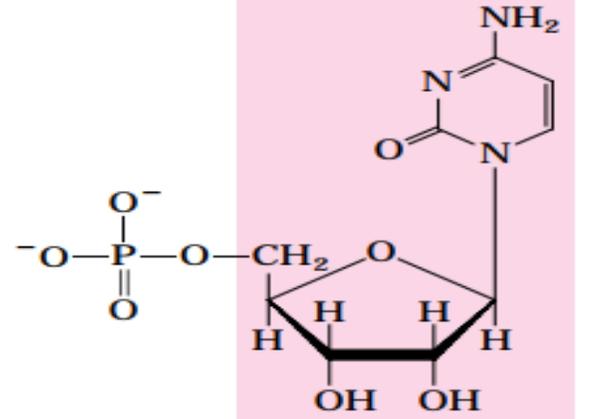
Nucleoside: Guanosine



Nucleotide: Uridylate (uridine
5'-monophosphate)

Symbols: U, UMP

Nucleoside: Uridine



Nucleotide: Cytidylate (cytidine
5'-monophosphate)

Symbols: C, CMP

Nucleoside: Cytidine

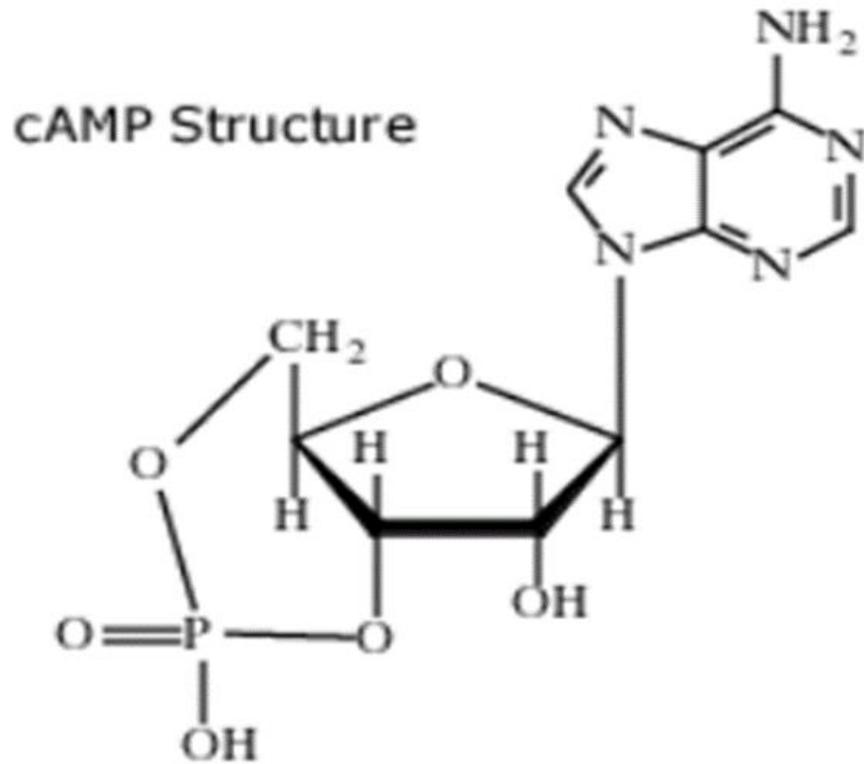
(b) Ribonucleotides



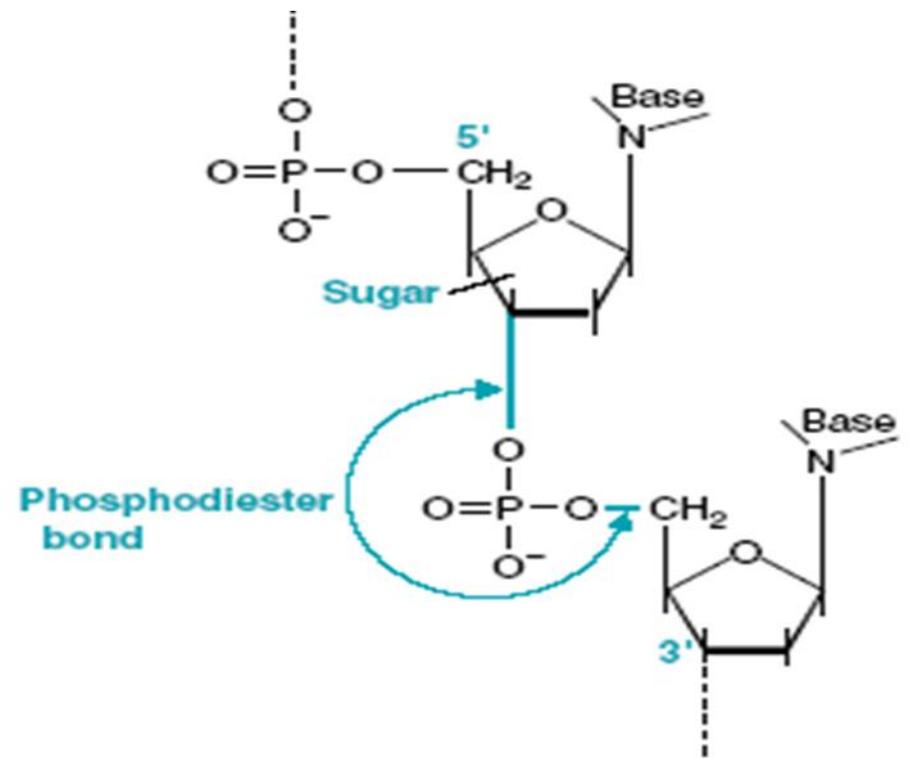
Significances of nucleotides

- Building blocks of **DNA** and **RNA**.
- Essential carriers of **chemical energy**, especially **ATP**
- Components of the **cofactors NAD, FAD**, and **coenzyme A**
- Formation of activated intermediates such as **UDP-glucose** and **CDP-diacylglycerol**.
- **cAMP** and **cGMP**, are also **cellular second messengers**.





- Cyclic AMP (**cAMP**), formed from **ATP** in a reaction catalyzed by adenylyl cyclase, is a common **second messenger produced in response to hormones** and other chemical signals, used for **intracellular signal transduction**, such as transferring into cells the effects of hormones like **glucagon** and **adrenaline**, which **cannot pass** through the plasma membrane



- Nucleic acids: **Phosphates** can be bonded to **C3** and **C5** atoms of the sugar by **phosphodiester bond**



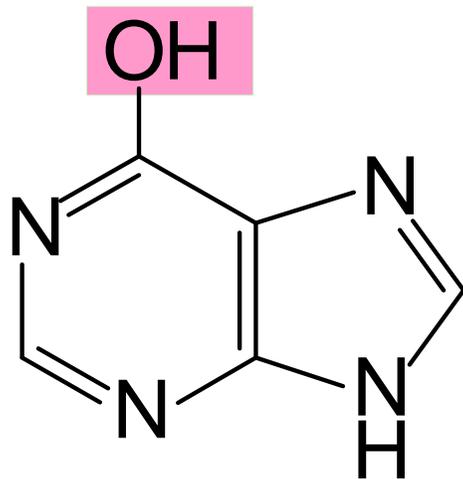
Anti-metabolites of nucleotides

- Anti-metabolites of **purine/pyrimidine** nucleotides are structural **analogs** of **purines** and **pyrimidines**.
- They can **interfere or inhibit** synthesis pathway of purine or pyrimidine nucleotides and further **block** synthesis of **DNA, RNA, and proteins**.
- Widely used to **control cancer**.

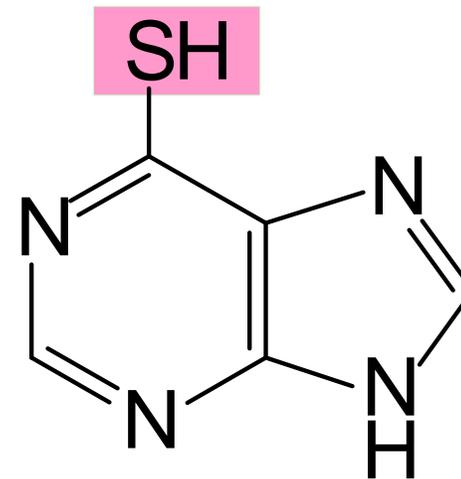


Purine analogs

- **6-Mercaptopurine (6-MP)** is analog of **hypoxanthine**.



hypoxanthine

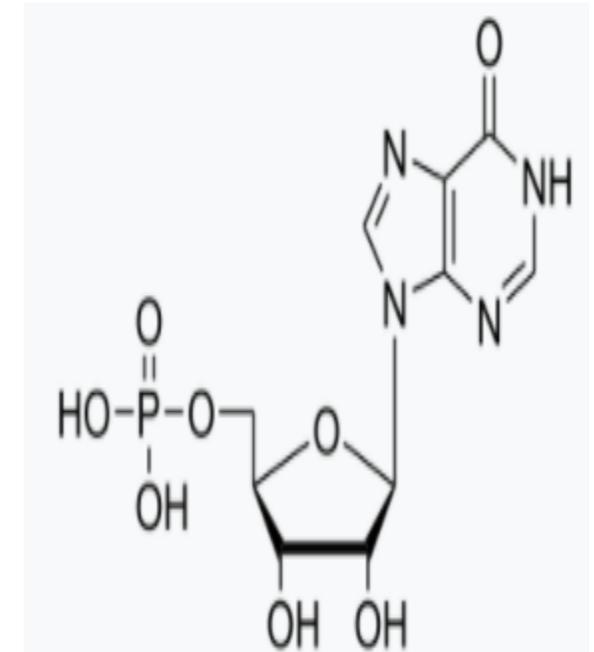


6-MP



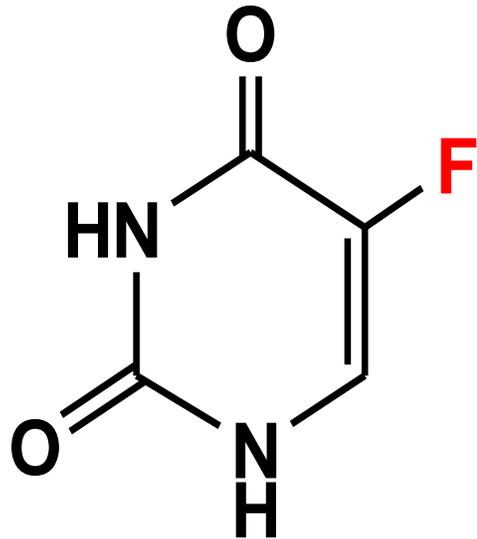
6-MP nucleotide is analog of IMP

- Inosine monophosphate (**IMP**) or Inosinic acid is important in metabolism. It is the ribonucleotide of **hypoxanthine** and the **first nucleotide** formed during the **synthesis of purine nucleotides**.
- It can also be **formed** by the **deamination** of adenosine monophosphate by **AMP deaminase**

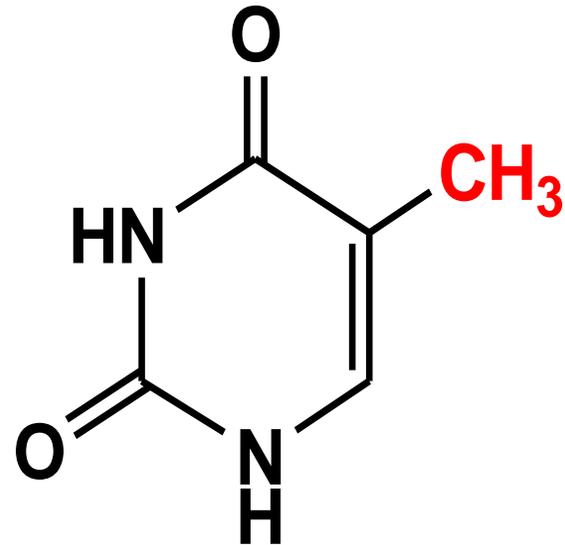


Pyrimidine analogs

- **5-fluorouracil (5-FU)** is analog of thymine.
- Destroy structure of **RNA**



5-FU



thymine

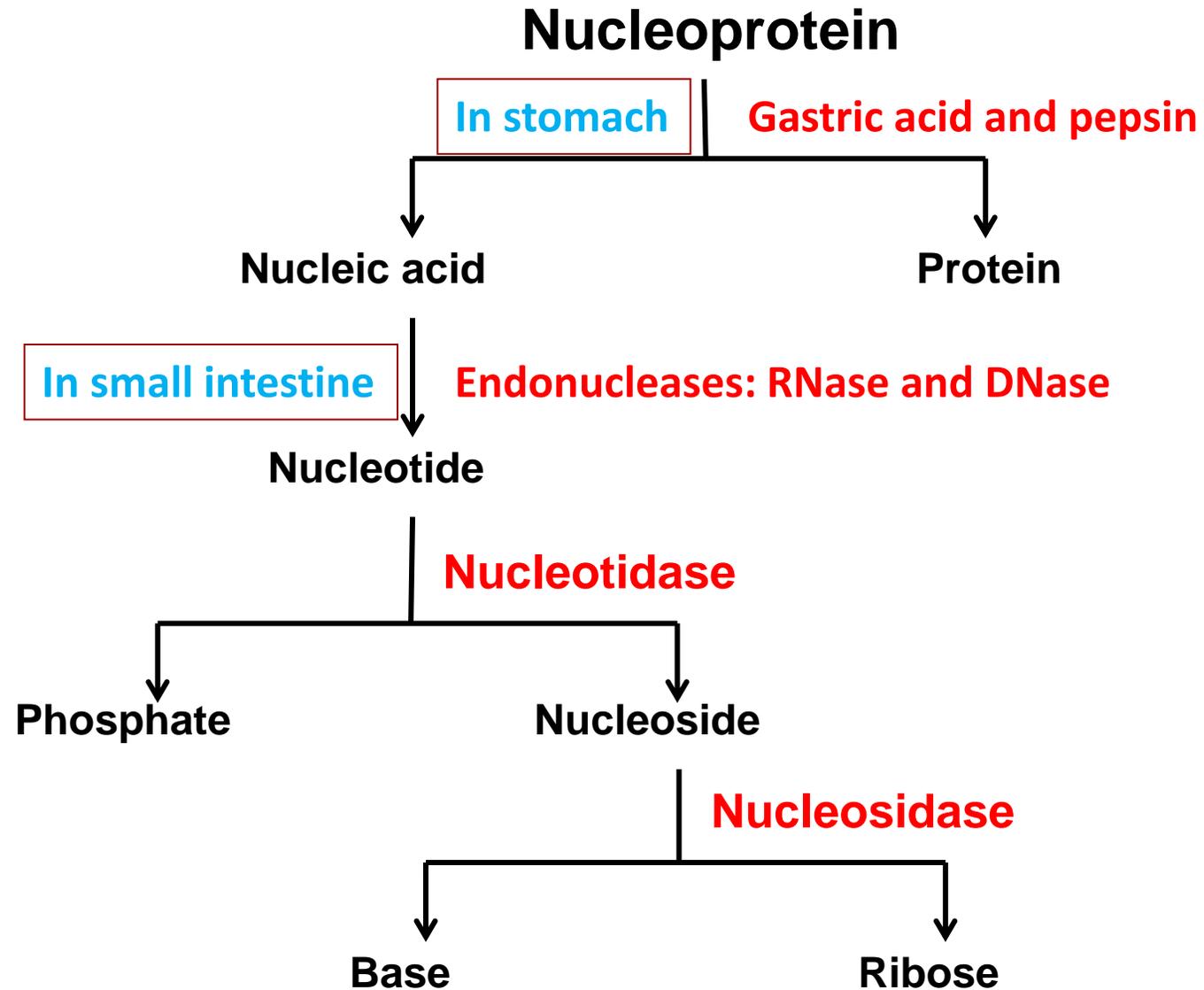


There are two pathways leading to nucleotides

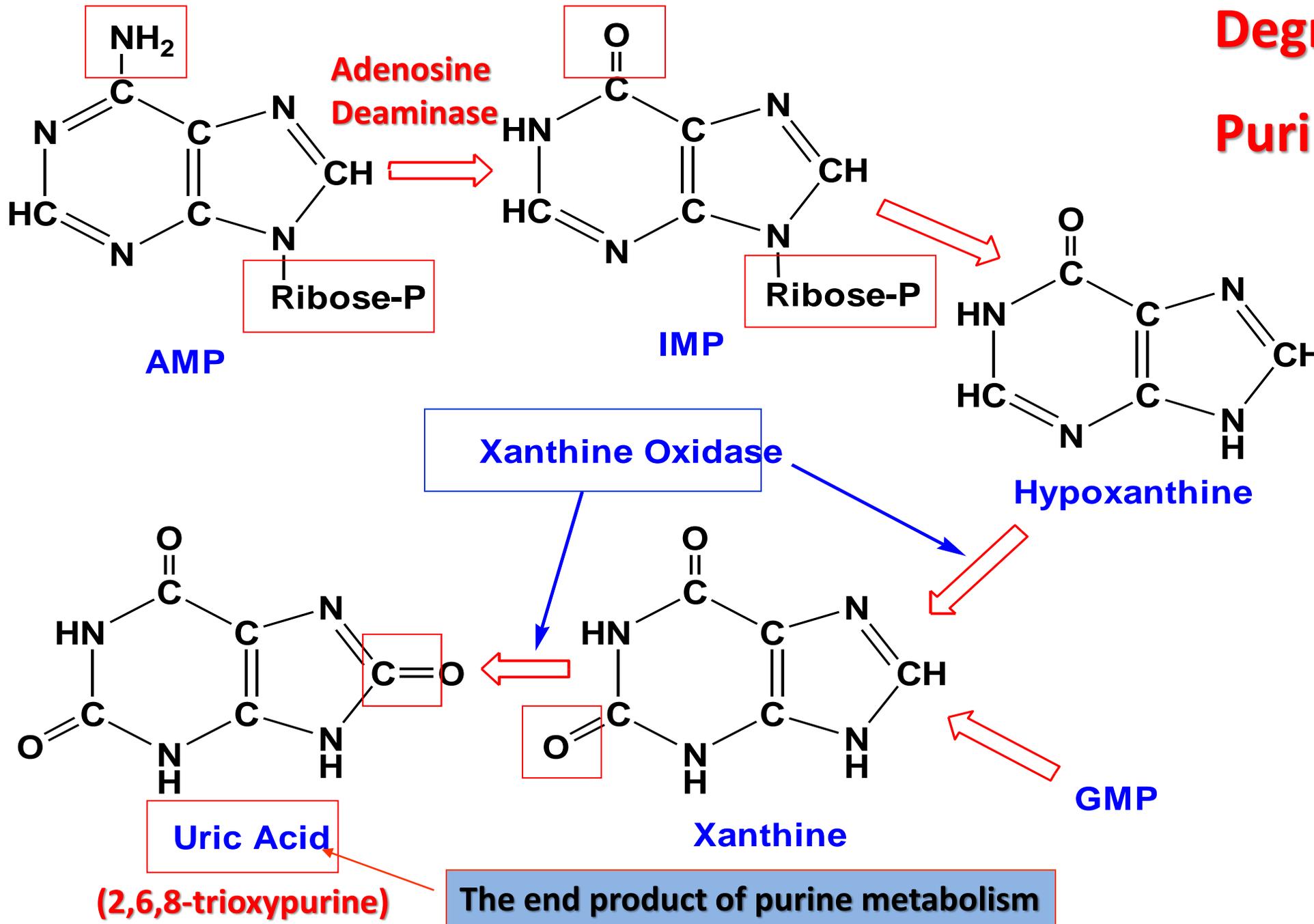
- **De novo pathway:** The synthesis of nucleotides begins with their metabolic precursors: **amino acids, ribose-5-phosphate, CO₂, and one-carbon units.**
- **Salvage pathway:** The synthesis of nucleotide by **recycle the free bases or nucleosides** released from nucleic acid breakdown.
- Some tissues and organs such as **brain** and **bone marrow** are **only able** of synthesizing nucleotides by **salvage pathway.**



Degradation of nucleic acid

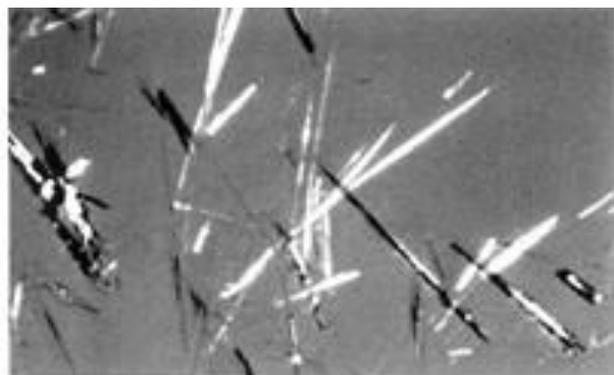


Degradation of Purine Nucleotides



Gout

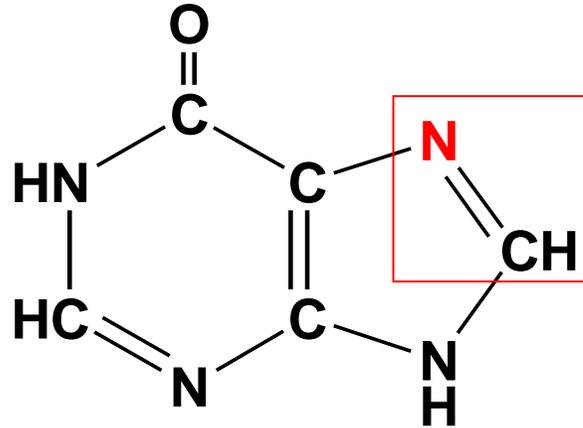
- Gout is a **disorder** of purine metabolism, occurs when **uric acid crystallizes** in the form of **monosodium urate**
- A disease of the **joints**, usually in **males**, caused by an **elevated** concentration of **uric acid** in the **blood** and **tissues**.
- The **joints** become **inflamed** and **painful** due to the abnormal deposition of crystals of **monosodium urate**.
- The **kidneys** are also affected, because **excess uric acid** is deposited in the **kidney tubules**.



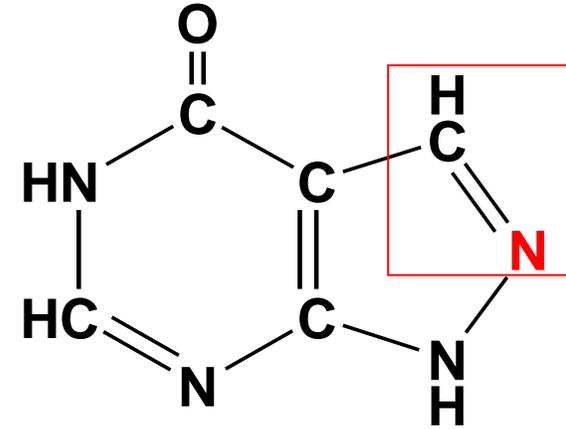
Sodium Urate Crystals



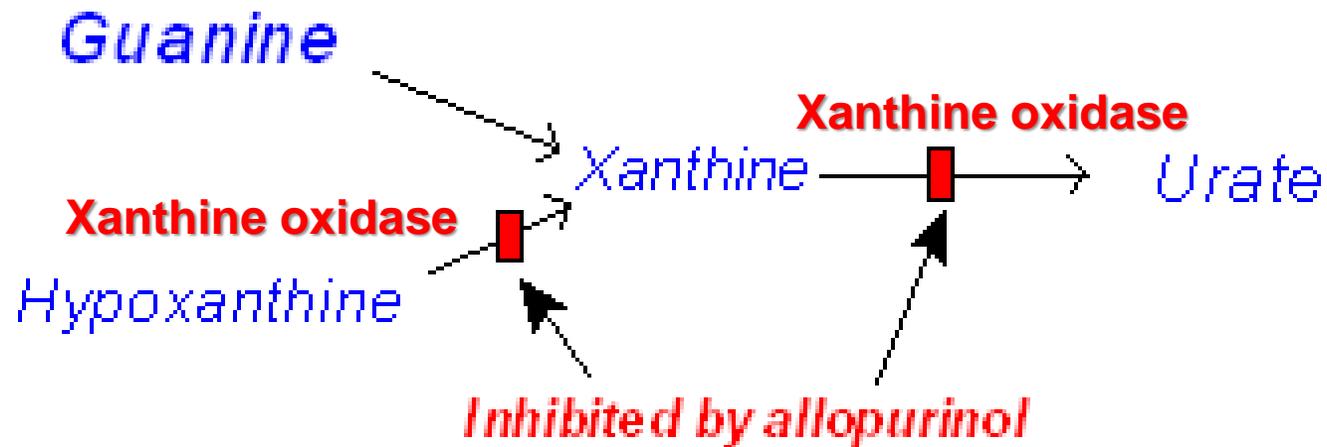
Allopurinol: inhibitor used to treat Gout



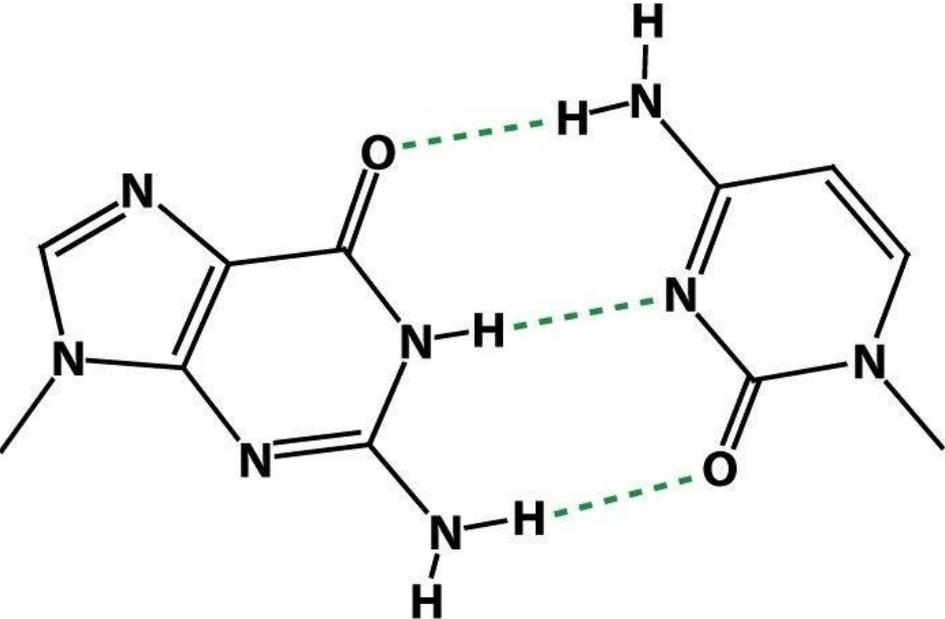
Hypoxanthine



Allopurinol



Bases in DNA make hydrogen bonds

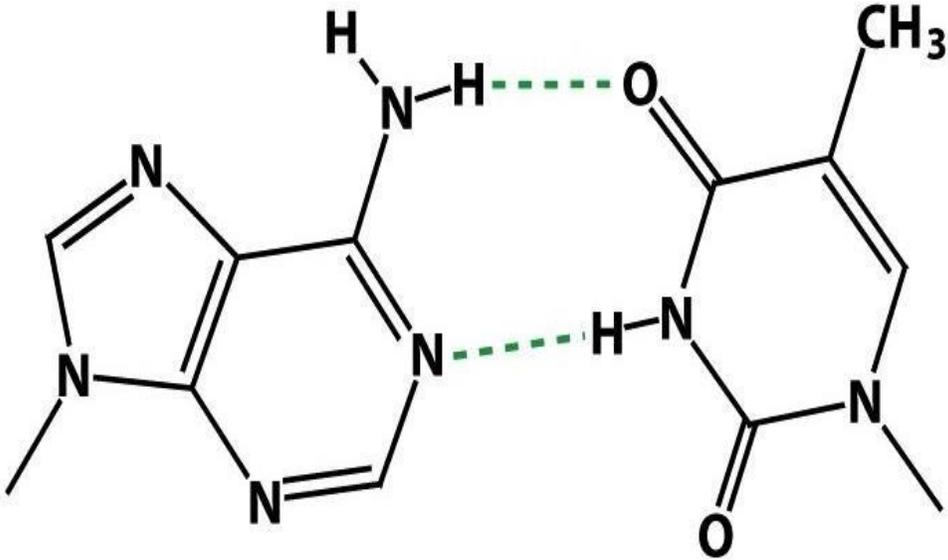


Guanine

Cytosine

PURINE

PYRIMIDINE



Adenine

Thymine

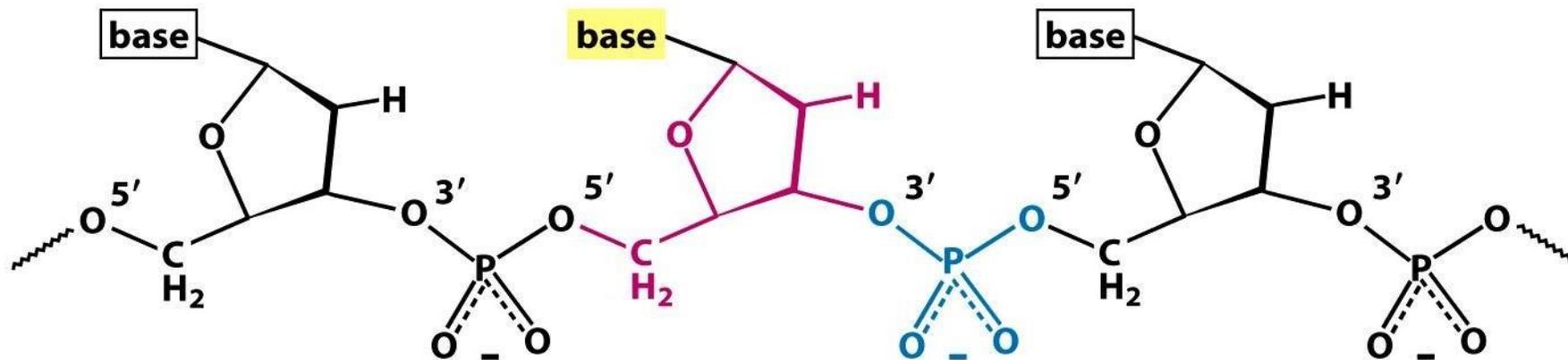
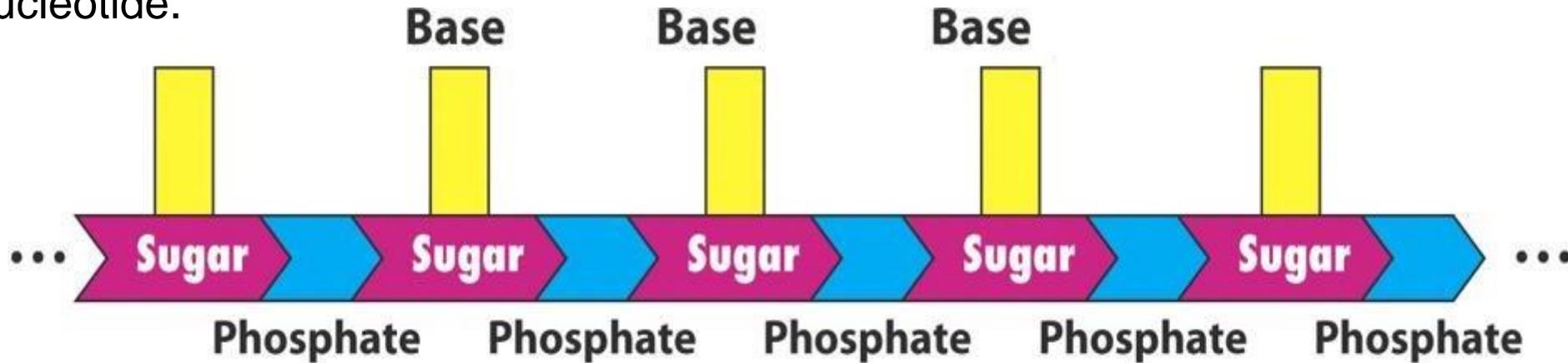
PURINE

PYRIMIDINE

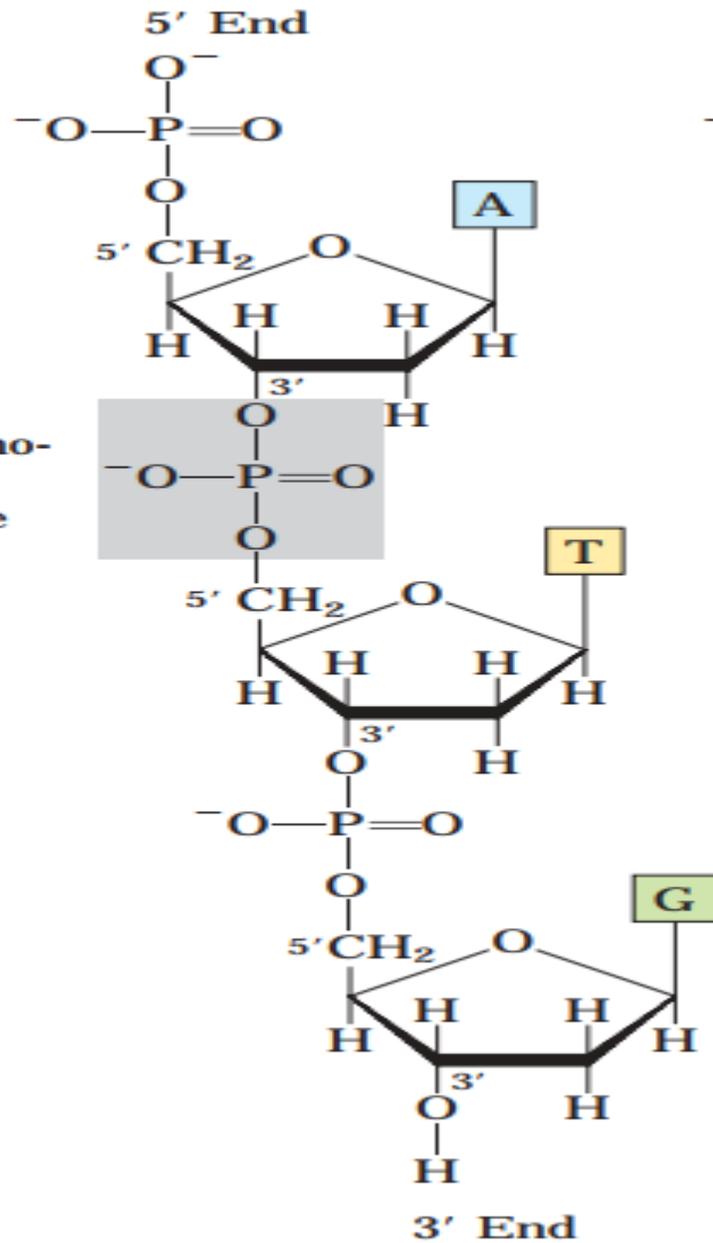


DNA Structure

- DNA is a **poly deoxyribonucleotide** that contain many **monodeoxyribonucleotide** covalently linked by **phosphodiester bonds**.
- Phosphodiester bonds join the **3'-OH** group of **one** nucleotide to the **5'-OH** group of the **next** nucleotide.



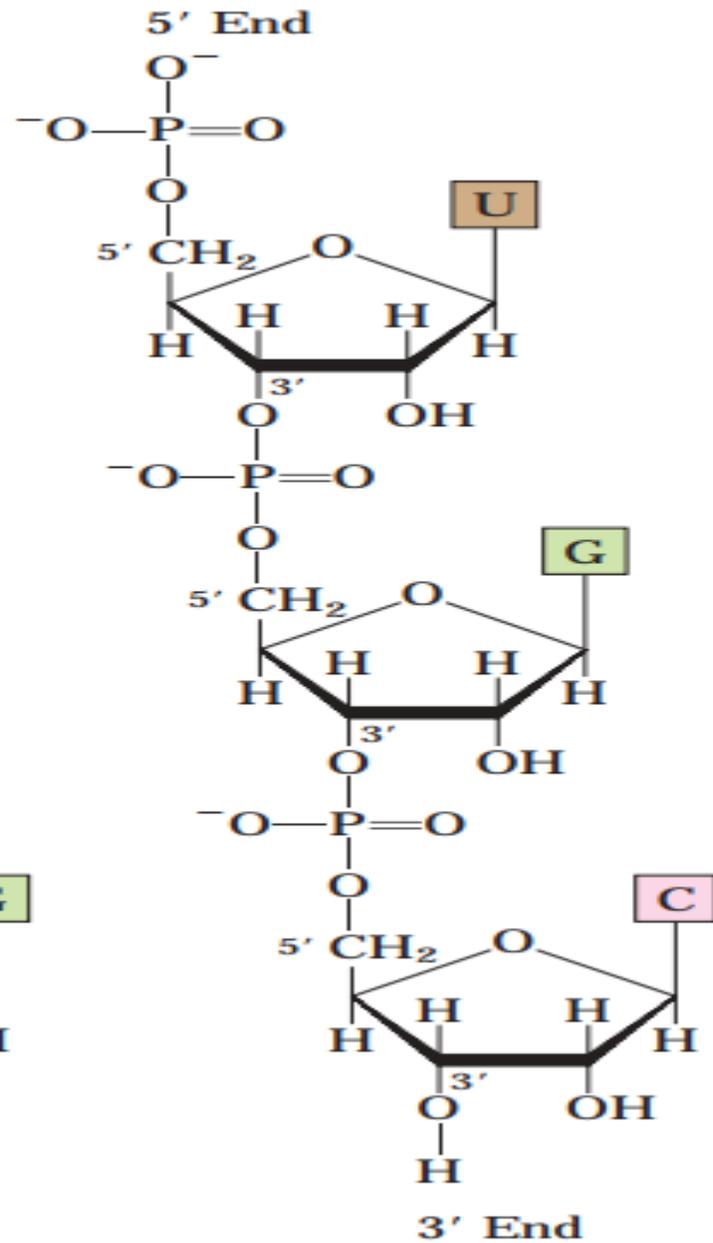
DNA



Phosphodiester linkage

5'
↓
3'

RNA

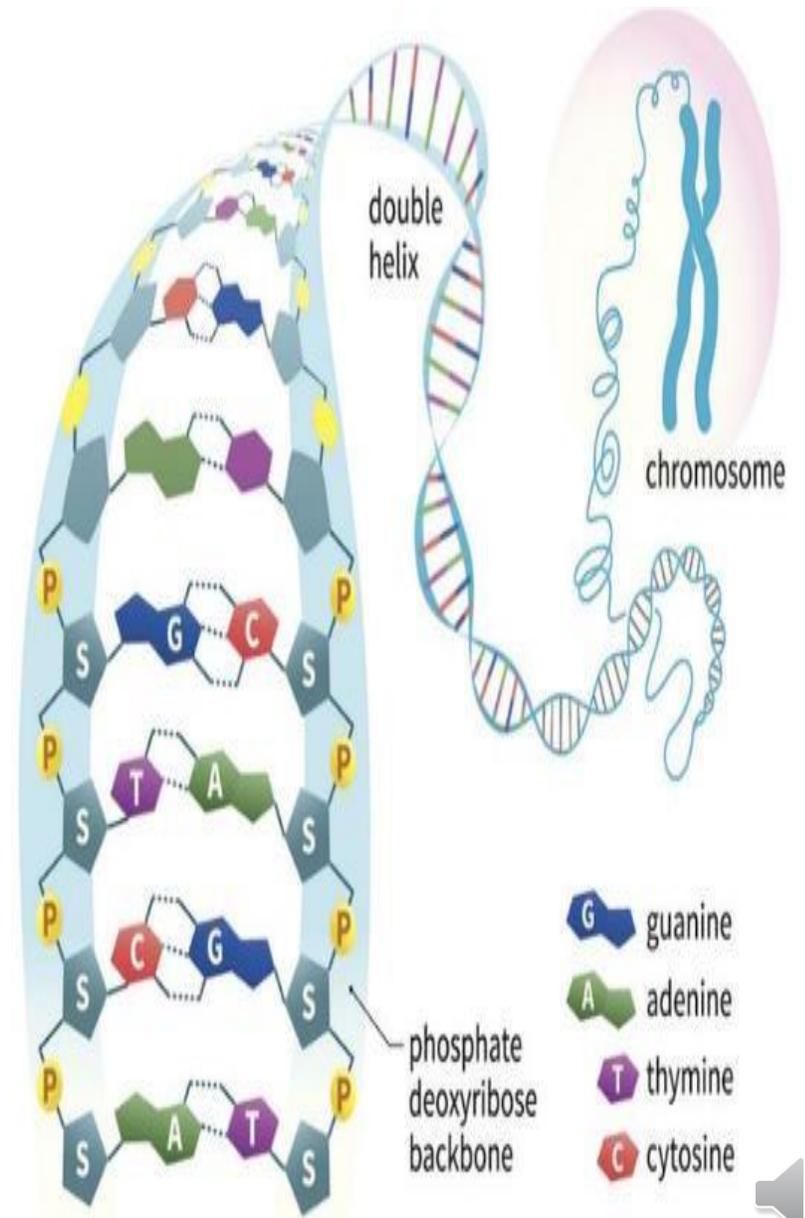
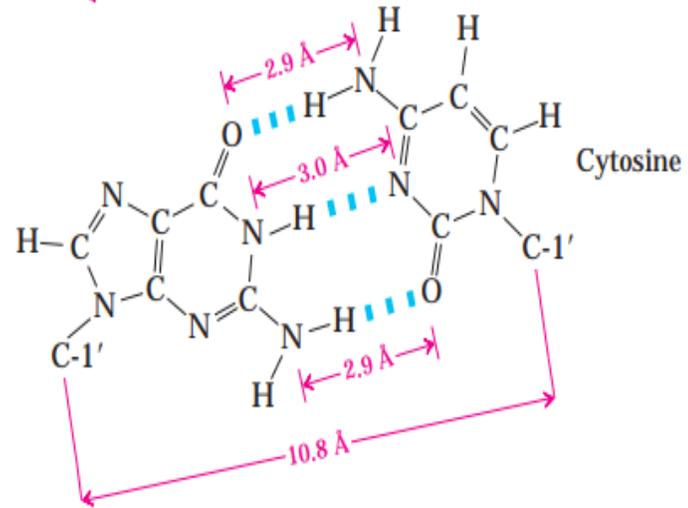
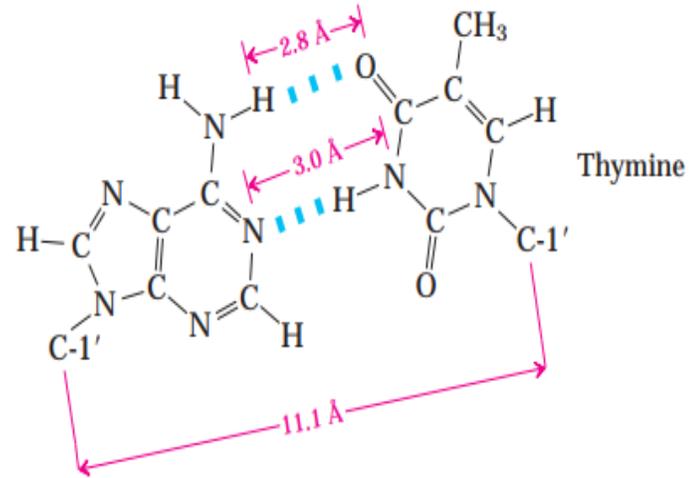
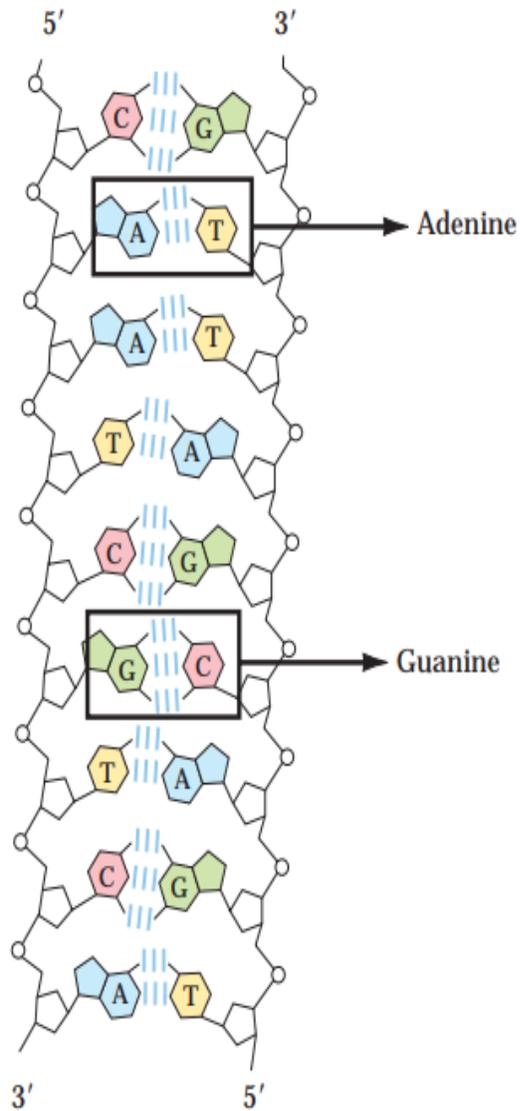


Watson – Crick model of DNA structure

- 1953, Watson and Crick proposed a 3D for the DNA:
 - Two helical polynucleotide chains, coiled about a common axis.
 - The chains (strands) are anti-parallel, the 5-end of one strand is paired with the 3- end of the other strand.
 - The hydrophilic deoxyribose-phosphate of each chain is on the outside of the molecule, while the hydrophobic bases are stacked on the inside.
 - A purine on one strand forms H bonds with a pyrimidine on the other strand
 - The bases of one strand of DNA are paired with the bases of the second strand, an Adenine is always paired with a Thymine, while a Cytosine is always paired with a Guanine. Therefore one polynucleotide chain of the DNA double helix is always the complement of the other. Given the sequence of bases on one chain, the sequence of bases on the complementary chain can be determined.



Watson – Crick model of DNA structure



Chargaff's rules

- In any samples of double-strand DNA:
 - The amount of **Adenine** equals the amount of **Thymine**
 - The amount of **Guanine** equals the amount of **Cytosine**
 - The **total amount of purines (A + G) equals the total amount of pyrimidines (T + C)**.
- The base pairs are held together by **hydrogen bonds**: **two** between **A** and **T** and **three** between **G** and **C**.

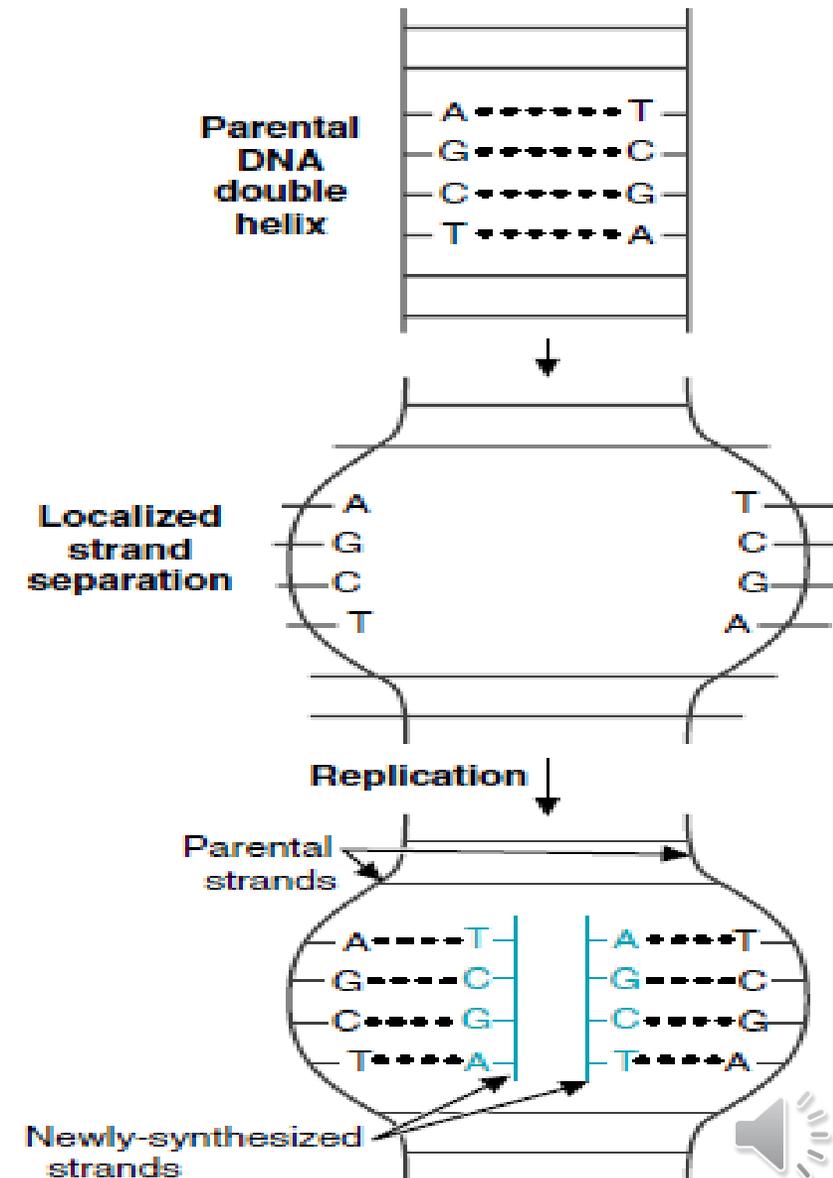
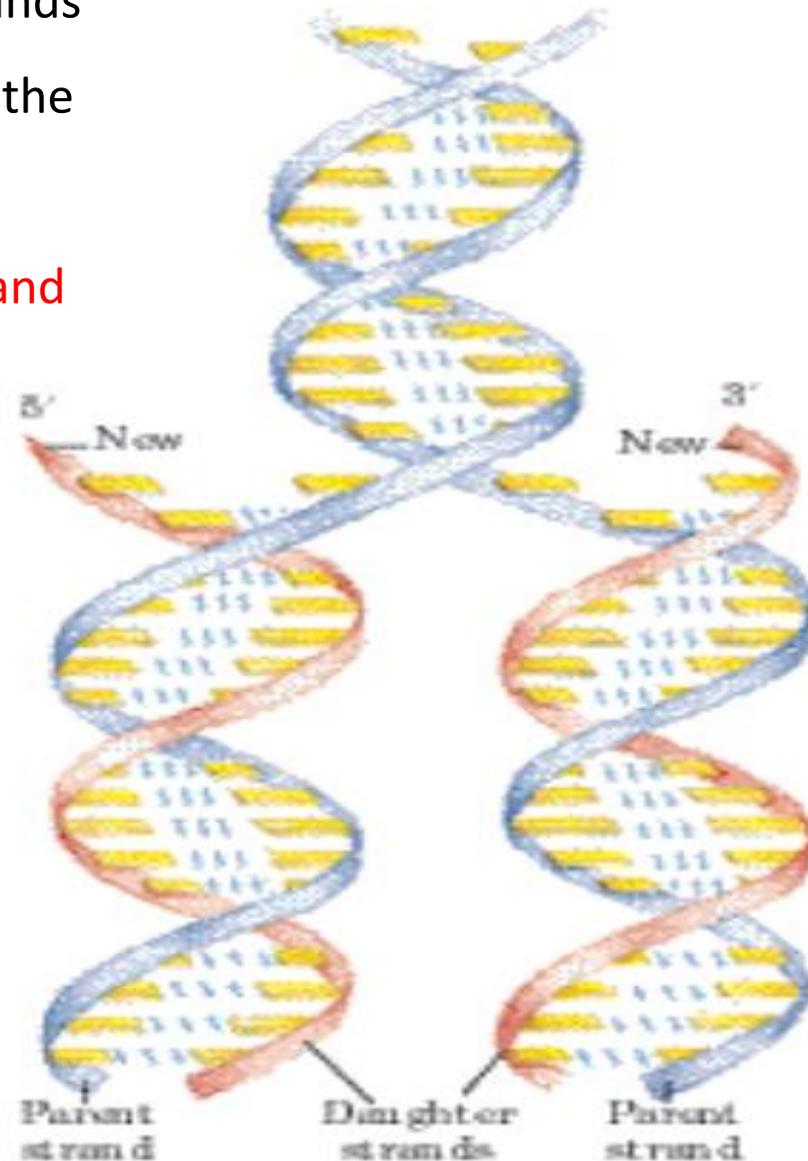


- **Base-pairing** proved to be essential for determining the mechanism of **DNA replication** (in which the copies of DNA are produced that are distributed to daughter cells) and the mechanisms of **transcription** and **translation** (in which mRNA is produced from genes and used to direct the process of **protein synthesis**).
- As Watson and Crick suggested, **base-pairing** allows **one strand** of DNA to serve as a **template** for the **synthesis** of the **other strand**.
- **Base pairing** also allows a strand of **DNA** to serve as a **template** for the synthesis of a complementary **strand of RNA**.



Replication of DNA as suggested by Watson and Crick

- The **preexisting** or “parent” strands become **separated**, and each is the **template** for biosynthesis of a **complementary** “daughter” strand



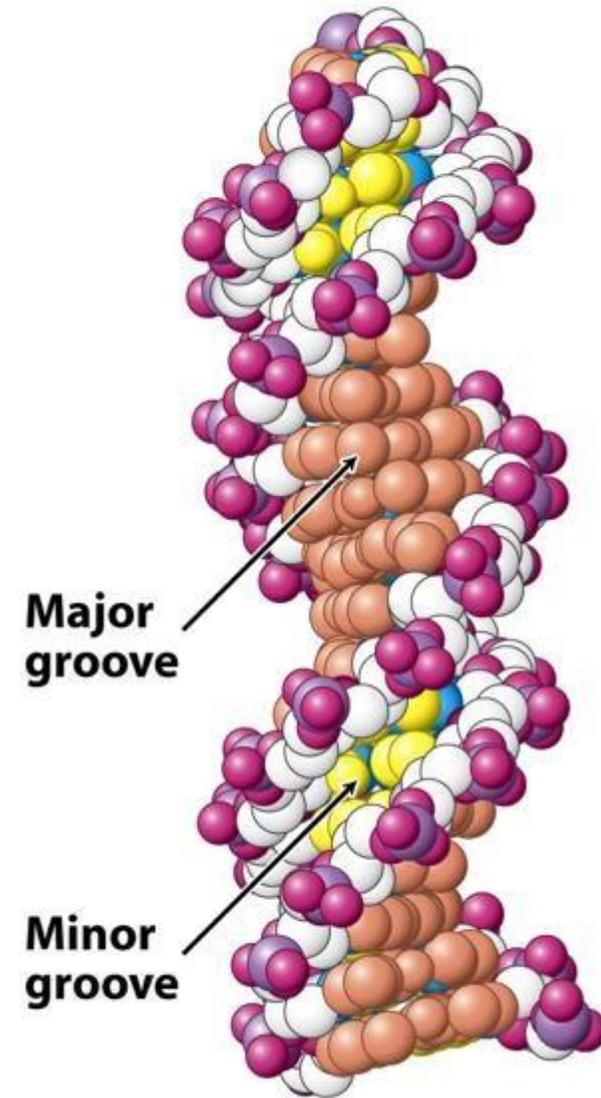
DNA can occur in different three-Dimensional (3D) Forms

DNA is a **flexible molecule**. Considerable **rotation** is possible around a **number of bonds** in the sugar–phosphate (**phospho-deoxyribose**) backbone. Many significant forms are described by Watson-Crick of DNA structure that are found in cellular DNA. These structural variations generally **do not affect** the key properties of DNA defined by Watson and Crick: **strand complementary, anti-parallel strands**, and the requirement for A=T and **C≡G** base pairs.



The B form (DNA double helix)

- **Two helical** polynucleotide chains, coiled about a common axis.
- Helix direction = **Right-handed**
- Strands are **anti-parallel**
- Diameter is **20 Å** between base pairs
- **Sugar-phosphates** on the **outside**; **purine and pyrimidine** bases of both strands on the **inside** of double helix .
- The offset pairing of the two strands creates a **major groove** and **minor groove** on the surface of the double-helical
- **H bonds** between nitrogen bases and the **van der Waals** forces between the stacked bases **stabilize** the structure of the double helix.
- Approx. **10** bases per helix turn and **34 Å** between base pairs.
- Rise/turn of helix = **3.4 Å**



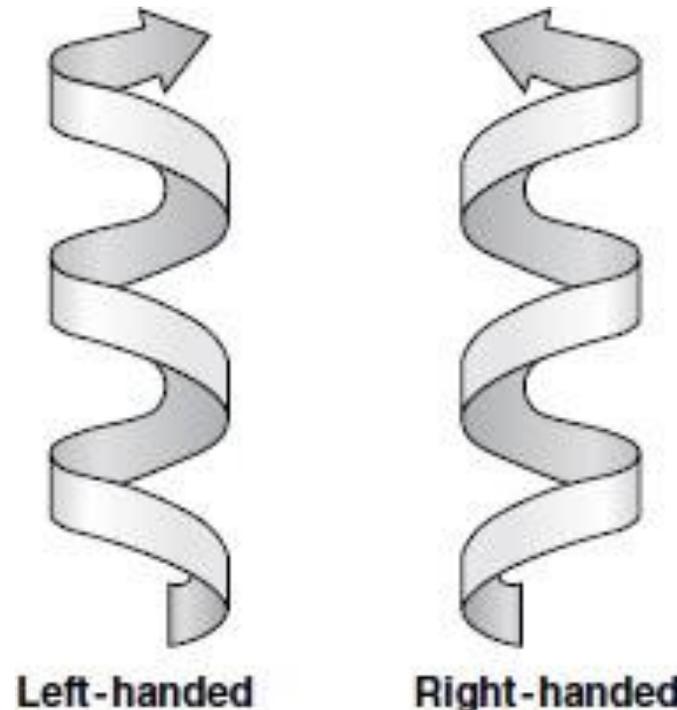
DNA can exist in several structural forms. Two variations of the Watson-Crick form (B-DNA), are A- and Z-DNA. These structural changes **deepen** the **major groove** while making the **minor groove shallower**.

- **The A form** (devoid of water).

- Right-handed double helix, but the helix is wider (Diameter is **26 Å** between base pairs)
- **11** base pairs per helical turn.
- Rise/turn of helix = **2.6 Å**

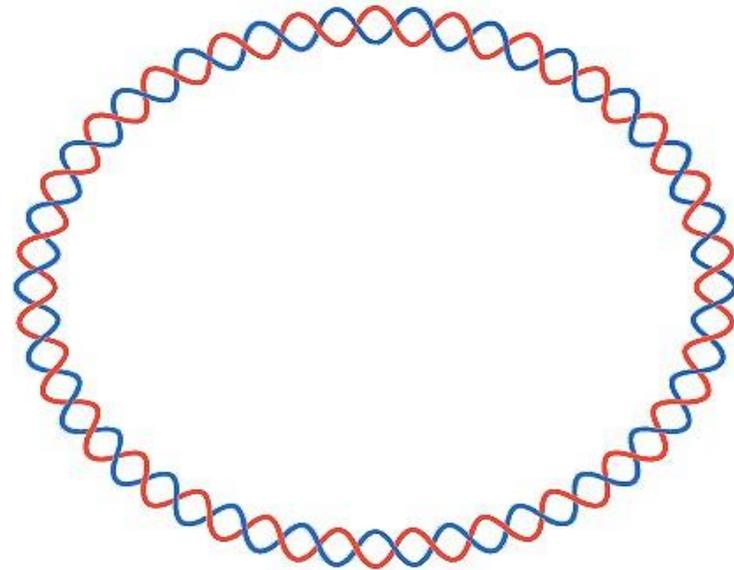
- **The Z form**

- Diameter is **18 Å** between base pairs
- left-handed helix.
- **12** base pairs per helical turn
- Rise/turn of helix = **3.7 Å**



DNA Supercoiling

Supercoiling means the **coiling of a coil**. A **telephone cord**, for example, is typically a **coiled wire**. The **path** taken by the **wire** between the **base of the phone** and the **receiver** often includes **one or more supercoils**. **DNA is coiled** in the form of a **double helix**, with both strands of the DNA coiling **around an axis**. The **further coiling** of that axis upon itself produces **DNA supercoiling**. When there is no net bending of the DNA axis upon itself, the DNA is in a **relaxed state**.

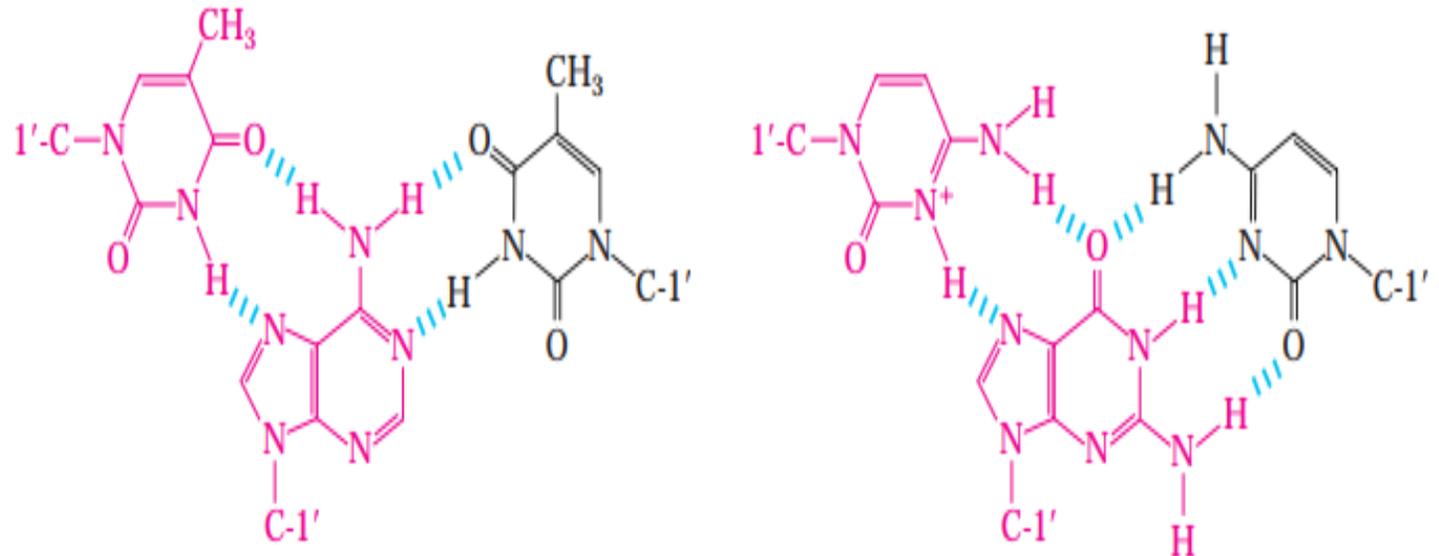


"relaxed" double-helical segment of DNA



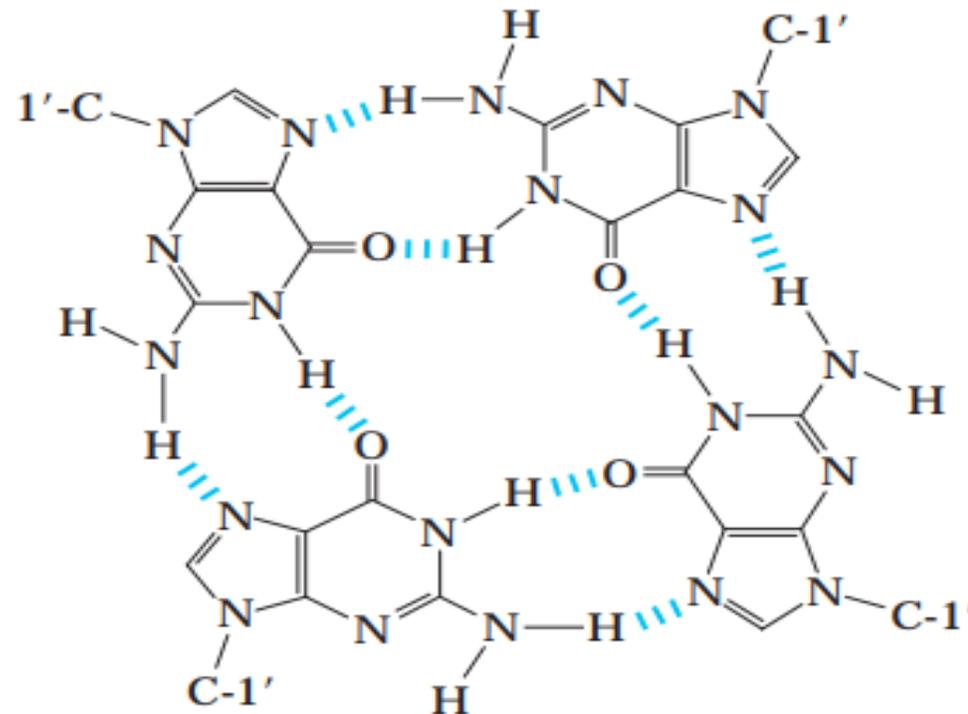
Alternative structures triplex

- Several **unusual DNA structures** involve **three** or even **four DNA strands** can form a number of **additional hydrogen bonds**
- **Triple helical DNA** containing **2 pyrimidine** strands (T) and **1 purine** strand (A)
- For example, **Cytidine** can pair with **Guanosine** $C \equiv G$ and **Thymidine** can pair with **Adenosine** (A=T).
- The **N-7**, **O-6**, and **N-6** of **purines**, the atoms that participate in the **hydrogen bonding of triplex DNA**, are often referred to as **Hoogsteen positions**, so called Hoogsteen pairing (Karst Hoogsteen 1963). Hoogsteen pairing allows the formation of triplex DNAs.



Alternative structures tetraplex (Quadruplex)

- Base-pairing pattern in the **guanosine tetraplex** structure.
- **4 DNA strands** can also pair to form a tetraplex (quadruplex), but this occurs readily only for **DNA sequences** with a **very high proportion** of **guanosine residues**.
- The guanosine tetraplex, is **stable** over a wide range of conditions.



Guanosine tetraplex



Several types of RNA and RNA polymerases

Messenger RNA (mRNA)

template for protein synthesis
RNA polymerase II (Pol II)

Transfer RNA (tRNA)

carries activated amino acids to ribosomes
RNA polymerase III (Pol III)

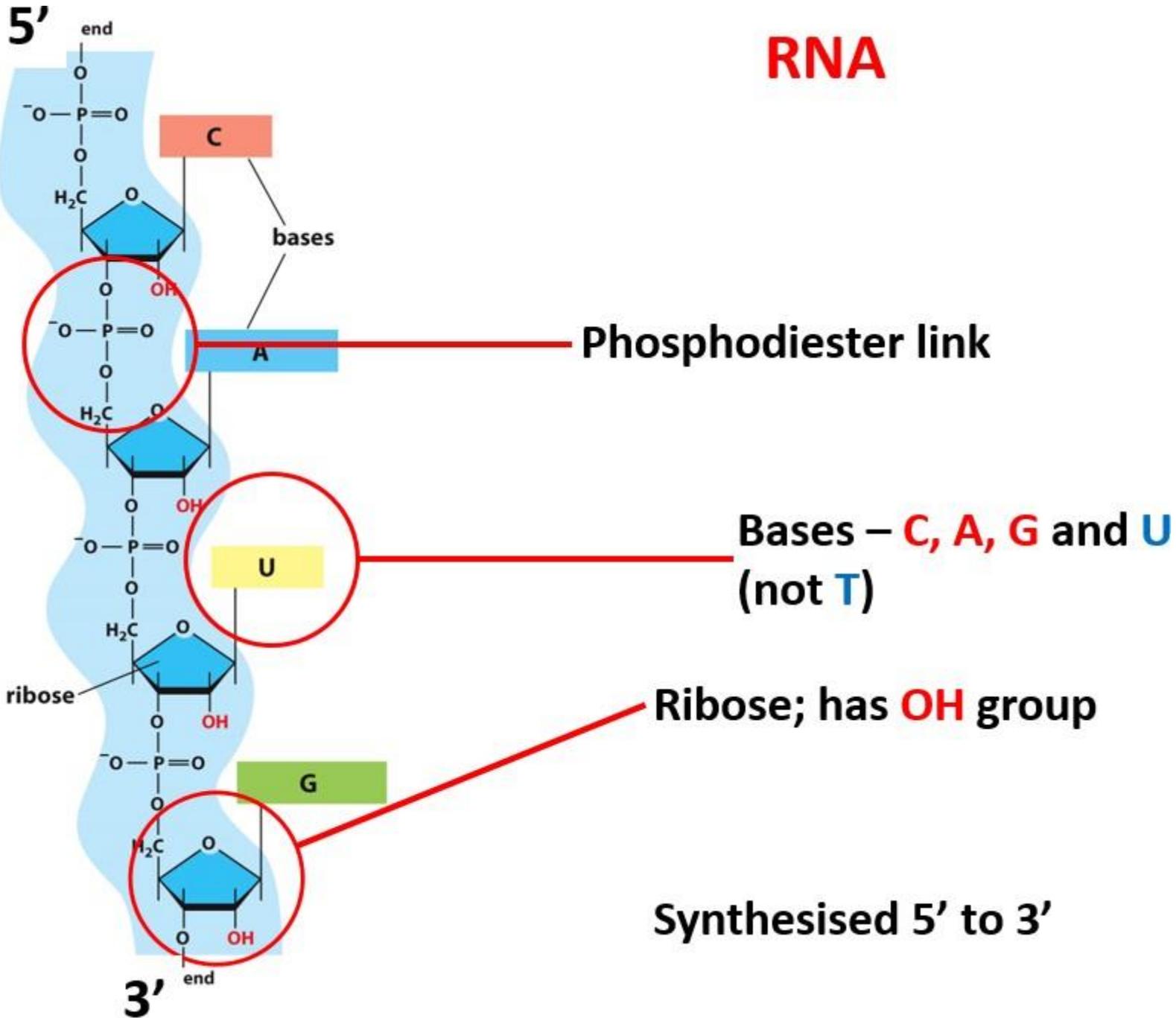
Ribosomal RNA (rRNA)

major component of ribosomes
RNA polymerase I (Pol I)

All require template DNA, ribonucleotides (ATP, GTP, UTP, CTP) and a divalent metal ion (Mg^{2+})

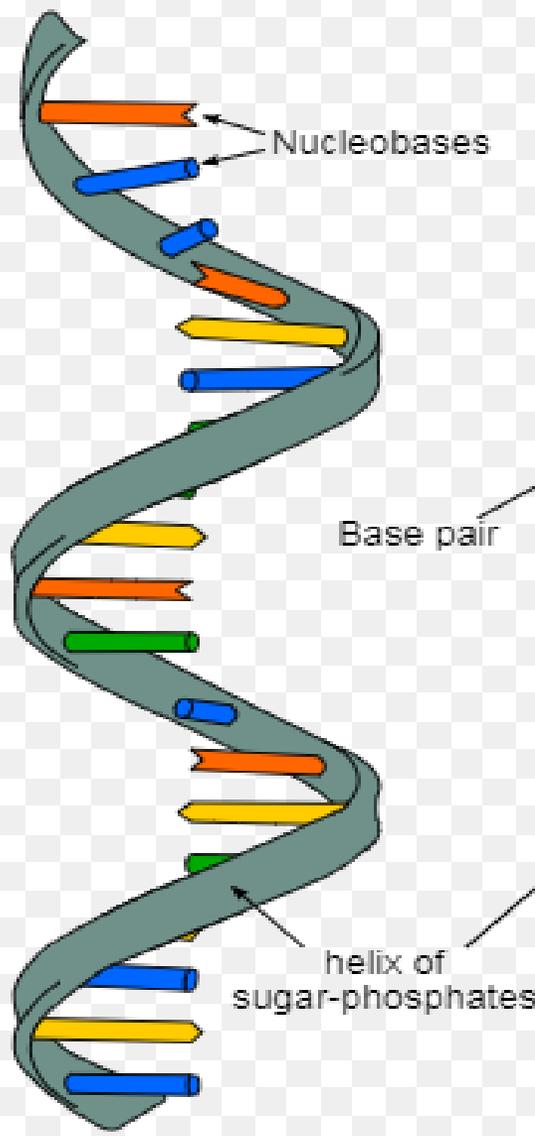
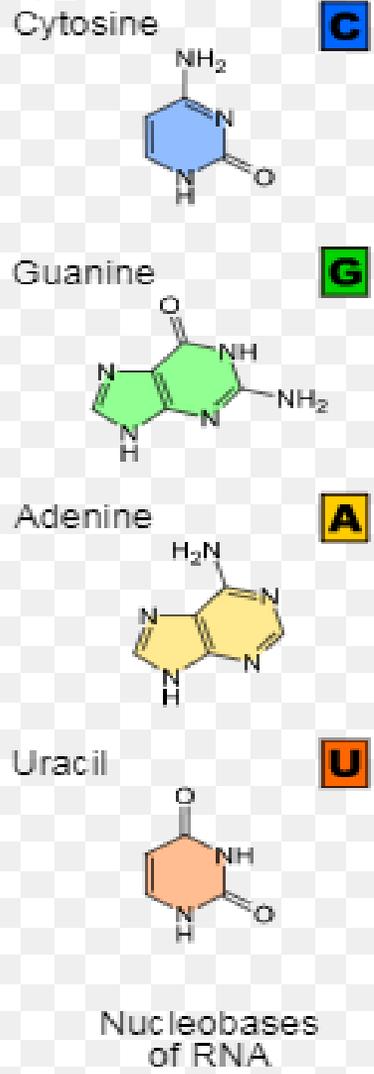


RNA

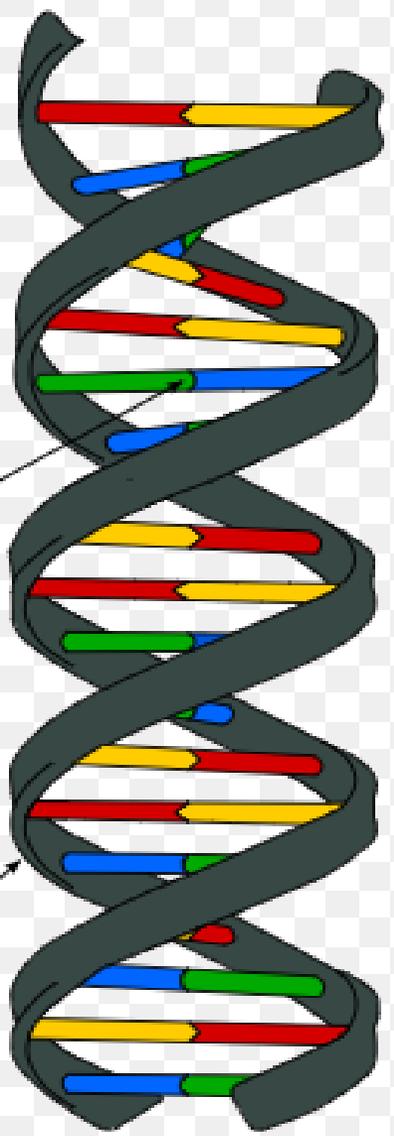


RNA

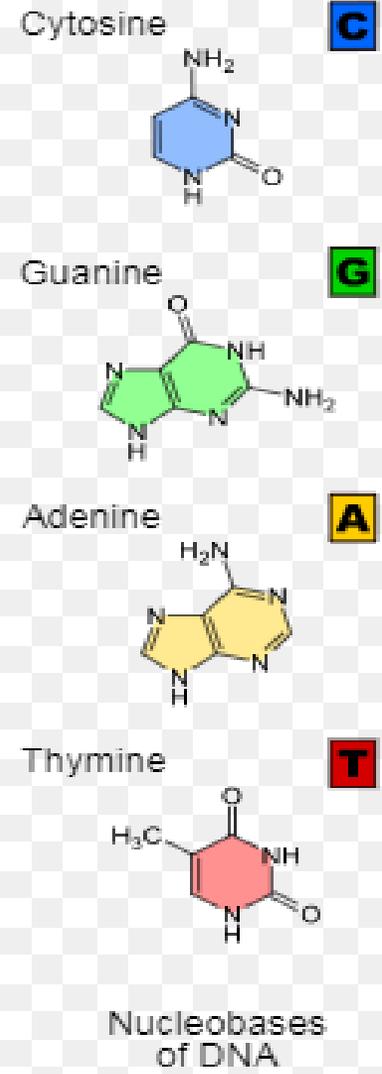
DNA



RNA
Ribonucleic acid



DNA
Deoxyribonucleic acid



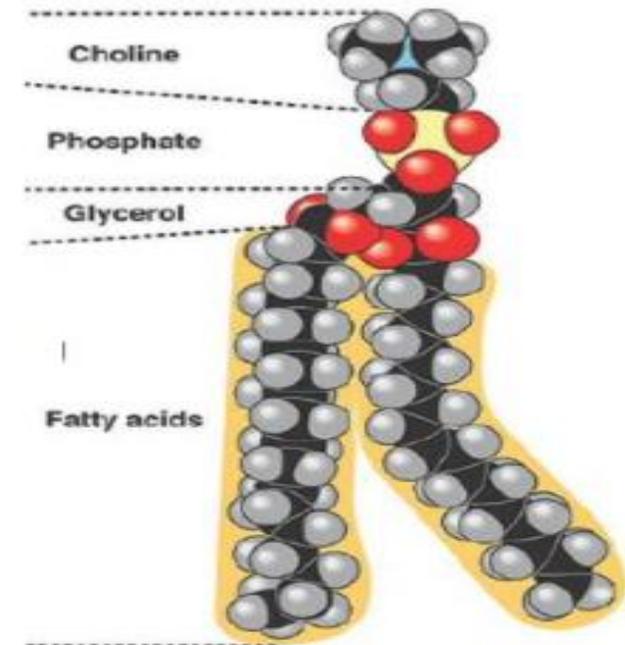
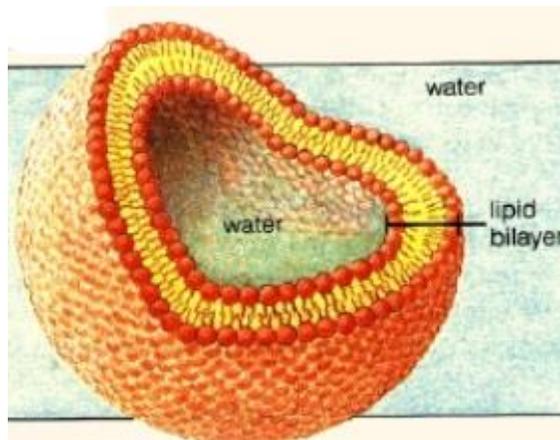
Lecture 4: Biochemistry I

Biochemistry of Lipids

3rd stage

Anbar University-College of Pharmacy-Clinical Laboratory Sciences Department
2020-2021

Dr. Yousif H. Khalaf



Lipids

- Organic compounds found every type of plant and animal cells. They contain **Carbon, Hydrogen, and Oxygen**.
- Unlike the polysaccharides, proteins, and nucleic acids, lipids are **not polymers**. They are mostly **small molecules**.

General properties of lipids:

- **Insoluble** in **water**.
- **Soluble** in **nonpolar solvents** such as chloroform and methanol.
- Water-hating nature is due to the predominance of **hydrocarbon chains**
- **(-CH₂-)_n** in their structure.

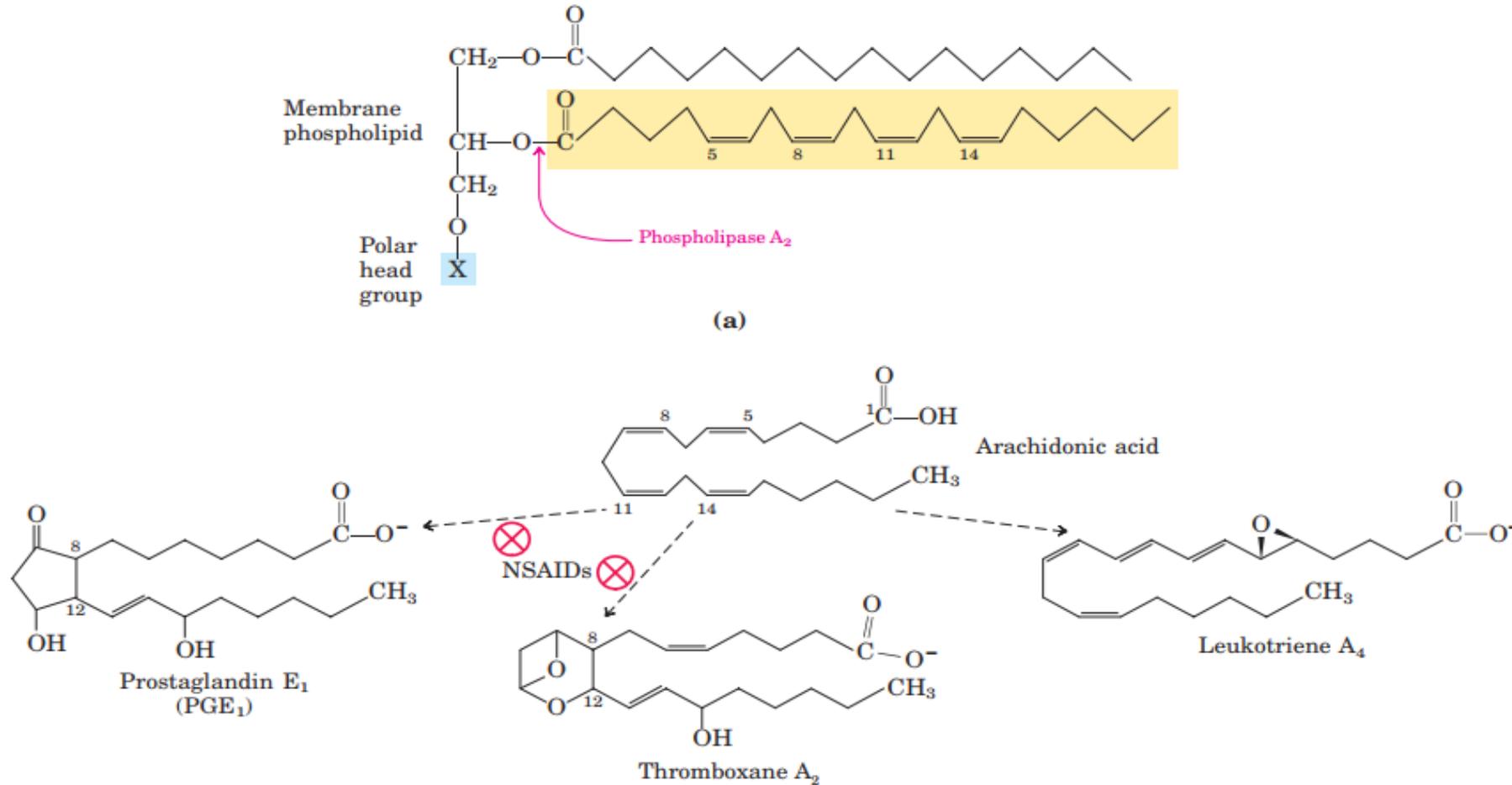


Function of lipids

- Energy storage molecules. Lipids are a source of high energy value:
 - **Fat**: 1 gram = **9** calories
 - **Protein**: 1 gram = **4** calories
 - **Carbohydrate**: 1 gram = **4** calories
- Major structural elements of **cell membrane** and regulate the membrane permeability
- Serve as a source of **fat soluble vitamins** (A, D, K and E)
- Important as **cellular metabolic regulators** (steroid hormones and prostaglandins).
- **Protect the internal organs**, serve as **insulating materials** and give **shape** and **smooth** appearance to the body .
- **Inner mitochondrial membranes**, phospholipids participate in **electron transport chain**.



Arachidonic acid and some eicosanoid derivatives



- In response to hormonal signals, phospholipase A₂ cleaves arachidonic acid-containing membrane phospholipids to release arachidonic acid, the precursor to various Eicosanoids. These compounds include prostaglandins such as PGE₁, thromboxane A₂, and leukotriene A₄. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen block the formation of prostaglandins and thromboxanes from arachidonate by inhibiting the enzyme cyclooxygenase (prostaglandin synthase)

Fatty acids

- **Fatty acids** are **carboxylic acids** with hydrocarbon chains ranging from 4 to 36 carbons (**C4 to C36**). The **numbering** of carbons in fatty acids begins with the **C of the carboxylate group (COOH)**.
- The most commonly fatty acids have **even numbers** of carbon atoms in an **unbranched** chain of **12 to 24** carbons; they are either **saturated** or **unsaturated (one or more double bonds)**.
- The configuration of the **double bonds** in most unsaturated fatty acids is **cis**. Trans fatty acids correlate with increased blood levels of **LDL** (bad cholesterol) and decreased **HDL** (good cholesterol)
- **Short chain** and **unsaturation** enhance the **fluidity** of fatty acids and their derivatives by **lowering** the **melting temperature**.
- Fatty acids that contain **multiple** sites of unsaturation are termed **polyunsaturated fatty acids (PUFAs)**. The double bonds are separated by at least **one methylene group**.



Nomenclature of fatty acids

- The general rule is that **total number of carbon** atoms are written **first**, followed by the **number of double bonds**, separated by a **colon**; for example, the 16-carbon **saturated palmitic acid** is abbreviated **16:0**, and the 18-carbon **oleic acid**, with **one double bond**, is **18:1**
- The **positions of double bonds**, starting from the **carboxyl end**, are specified by **superscript numbers following the symbol Δ (delta)**; 18-carbon fatty acid with **one double bond** between **C-9** and **C-10** and **another** between **C-12** and **C-13** is designated **18:2 ^{Δ 9,12}**



Some Naturally Occurring Fatty Acids

Number of carbons	Number of double bonds	Common name	Systematic name	Formula
12	0	Laurate	<i>n</i> -Dodecanoate	$\text{CH}_3(\text{CH}_2)_{10}\text{COO}^-$
14	0	Myristate	<i>n</i> -Tetradecanoate	$\text{CH}_3(\text{CH}_2)_{12}\text{COO}^-$
16	0	Palmitate	<i>n</i> -Hexadecanoate	$\text{CH}_3(\text{CH}_2)_{14}\text{COO}^-$
18	0	Stearate	<i>n</i> -Octadecanoate	$\text{CH}_3(\text{CH}_2)_{16}\text{COO}^-$
20	0	Arachidate	<i>n</i> -Eicosanoate	$\text{CH}_3(\text{CH}_2)_{18}\text{COO}^-$
22	0	Behenate	<i>n</i> -Docosanoate	$\text{CH}_3(\text{CH}_2)_{20}\text{COO}^-$
24	0	Lignocerate	<i>n</i> -Tetracosanoate	$\text{CH}_3(\text{CH}_2)_{22}\text{COO}^-$
16	1	Palmitoleate	<i>cis</i> - Δ^9 -Hexadecenoate	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}^-$
18	1	Oleate	<i>cis</i> - Δ^9 -Octadecenoate	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}^-$
18	2	Linoleate	<i>cis, cis</i> - Δ^9, Δ^{12} - Octadecadienoate	$\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_2(\text{CH}_2)_6\text{COO}^-$
18	3	Linolenate	<i>all-cis</i> - $\Delta^9, \Delta^{12}, \Delta^{15}$ - Octadecatrienoate	$\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_6\text{COO}^-$
20	4	Arachidonate	<i>all-cis</i> - $\Delta^5, \Delta^8, \Delta^{11}, \Delta^{14}$ - Eicosatetraenoate	$\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_2\text{COO}^-$



Saturated and unsaturated lipids

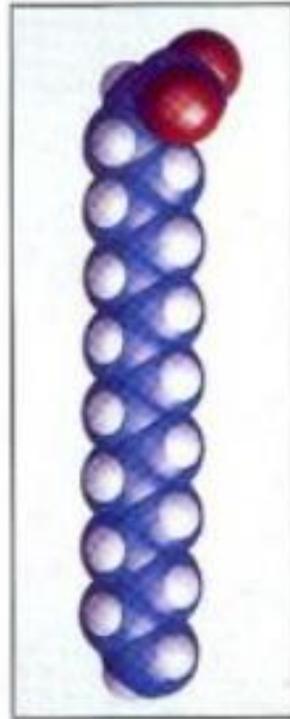
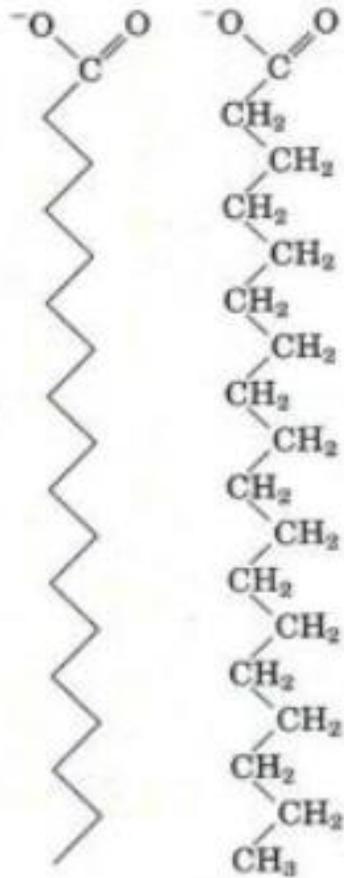
- **Saturated** fatty acids have **no double bond**, general formula $\text{CH}_3\text{-(CH}_2\text{)}_n\text{-COOH}$
- **Unsaturated** fatty acids have **one or more double bonds**
- In the **fully saturated** compounds, **free rotation** around each carbon–carbon bond gives the hydrocarbon chain great **flexibility**; the most **stable conformation** is the **fully extended form**, in which the steric hindrance of neighboring atoms is minimized.
- **Double bond** in oleic acid does **not** permit **rotation** and introduces a **rigid bend** in the hydrocarbon tail
- In vertebrates, **free fatty acids circulate** in the **blood bound** noncovalently to a protein carrier, **serum albumin**



Saturated and unsaturated fatty acid

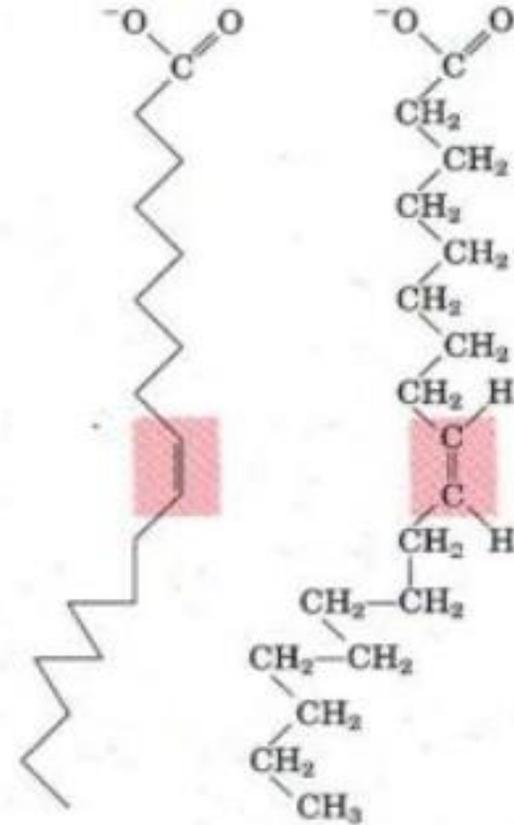
Carboxyl group

Hydrocarbon chain



(a)

a. Stearic acid

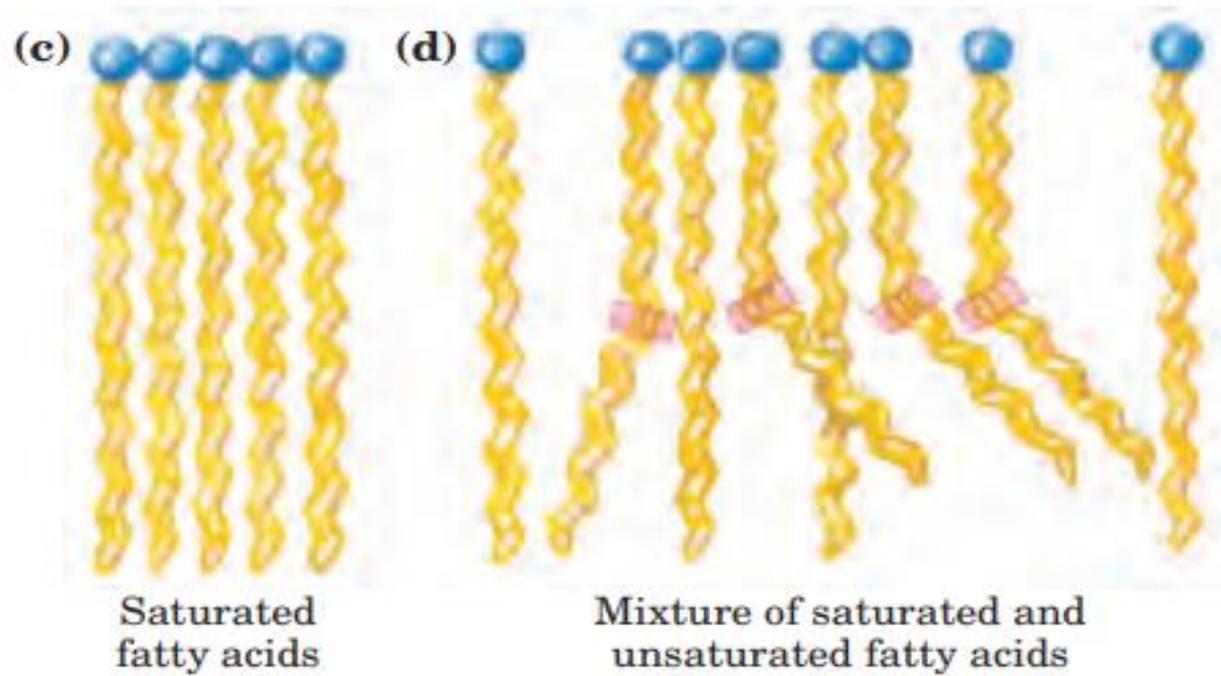


(b)

b. Cis double bond (shaded) in oleic acid



Saturated and unsaturated fatty acid



- c. Stabilized by many hydrophobic interactions**
- d. less stable aggregates**



Essential fatty acids (EFA)

- The fatty acids **can not** be synthesized by the body and, therefore, should be supplied in the **diet**
- Only two fatty acids are known to be **essential for humans**: **alpha-linolenic acid** (Omega-3 fatty acid) and **linoleic acid** (Omega-6 fatty acid)

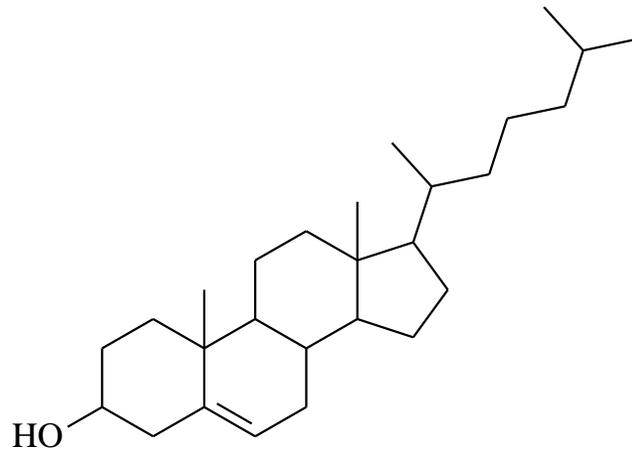
Functions of EFA

- **Membrane** structure and functions.
- **Transport** and **oxidation** of **cholesterol**. EFA tend to **lower** plasma cholesterol.
- Prevention of **fatty liver**.
- They are also needed for synthesis of eicosanoids (prostaglandins, prostacyclins).



Types of fat in our diet and body

- **Triacylglycerols (triglycerides)**
(3 fatty acids attached to glycerol)
- **Phospholipids**
(2 fatty acids and a head group attached to glycerol)
- **Cholesterol**
(4 ring hydrocarbon)



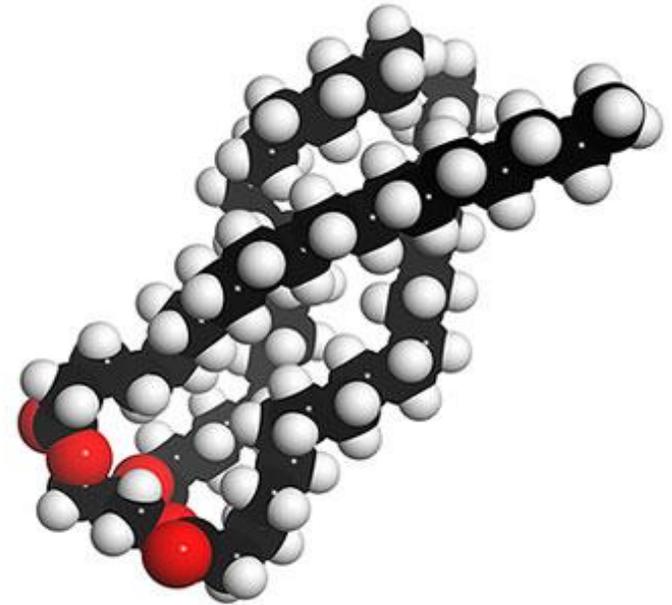
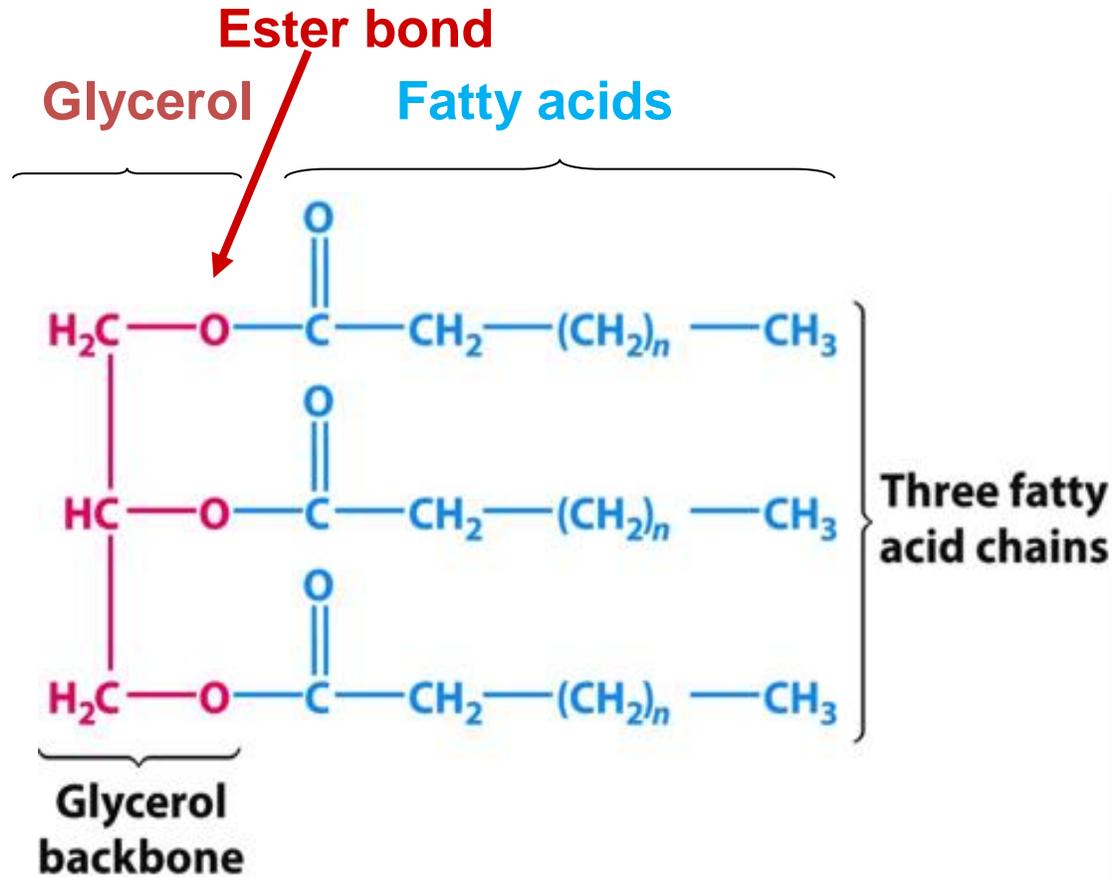
Triacylglycerols

- Also referred to as **triglycerides**, **fats**, or **neutral fats**.
- Triacylglycerols are fatty acid esters of glycerol. They are composed of **3 fatty acids** each in **ester linkage** with a **single glycerol**.
- Triacylglycerols containing only **saturated fatty acids**
- **Simple** triacylglycerols contain only **one type** of fatty acid; **mixed** triacylglycerols, **two or three types**.
- Triacylglycerols are **primarily storage fats**; they are present in many foods
- In some animals, triacylglycerols **stored under the skin** serve not only as **energy stores** but as **insulation against low temperatures**.

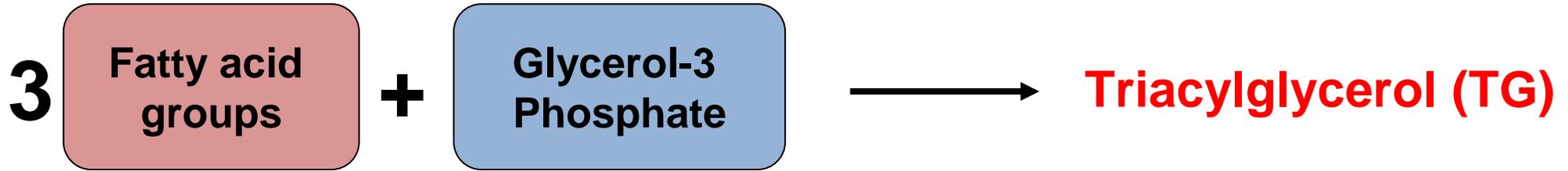


Triacylglycerols (TGs)

- A compact, **High-energy** storage biomolecule



TGs are synthesised in a number of tissues: **Liver, adipose tissue, intestinal tract**



2 types:

- **TG synthesis** from fatty acids made using **glucose** (glycolysis) or **amino acid** side chains (**in Liver**)
- TG synthesis during **transport of dietary fat** (**in intestines and adipose tissue**)
- TG **cannot** cross cell membranes: has to be broken down with the help of **lipases** to get in and out of cells.
- TG are **carried** around the body by **lipoproteins** in the **plasma**:
 - Very low density lipoprotein (**VLDL**): from liver to peripheral tissues.
 - **Chylomicrons**: from intestine to peripheral tissues.



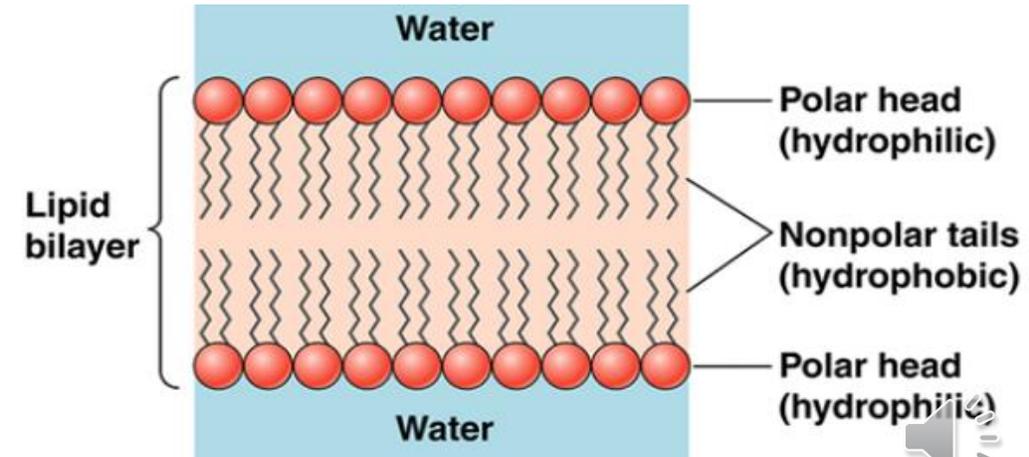
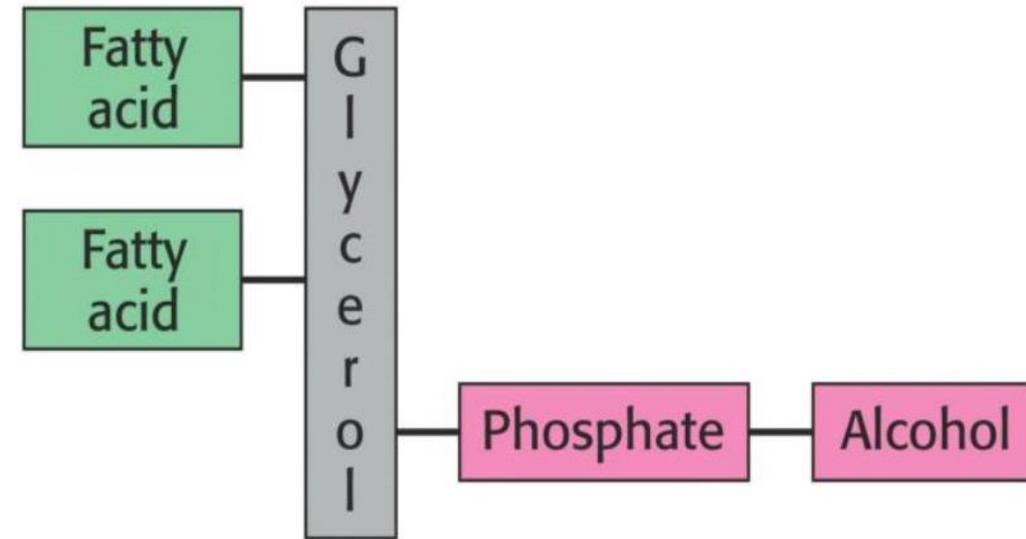
Triacylglycerols as stored fuels

- Two significant advantages to using triacylglycerols as stored fuels, rather than polysaccharides such as glycogen and starch.
- **Carbon atoms** of fatty acids are **more reduced** than those of sugars, oxidation of triacylglycerols yields **more than twice as much energy**, gram for gram, as the oxidation of carbohydrates.
- Triacylglycerols are **hydrophobic** and therefore unhydrated, the organism that carries fat as fuel **does not have to carry the extra weight of water** of hydration that is associated with stored polysaccharides (2 g per gram of polysaccharide).



Structure of phospholipid

- Phospholipid as triglyceride except **one** molecule of fatty acid is **replaced** by a **phosphate group**
- The phosphate group is **polar** and so is attracted to water- therefore the **phospholipid** has **two distinct ends**:
 - A **hydrophilic end** (head) that dissolves in **water**
 - A **hydrophobic end** (tail) that is (repels) **repelled by water**
- This property causes phospholipids to spontaneously form **bilayer**

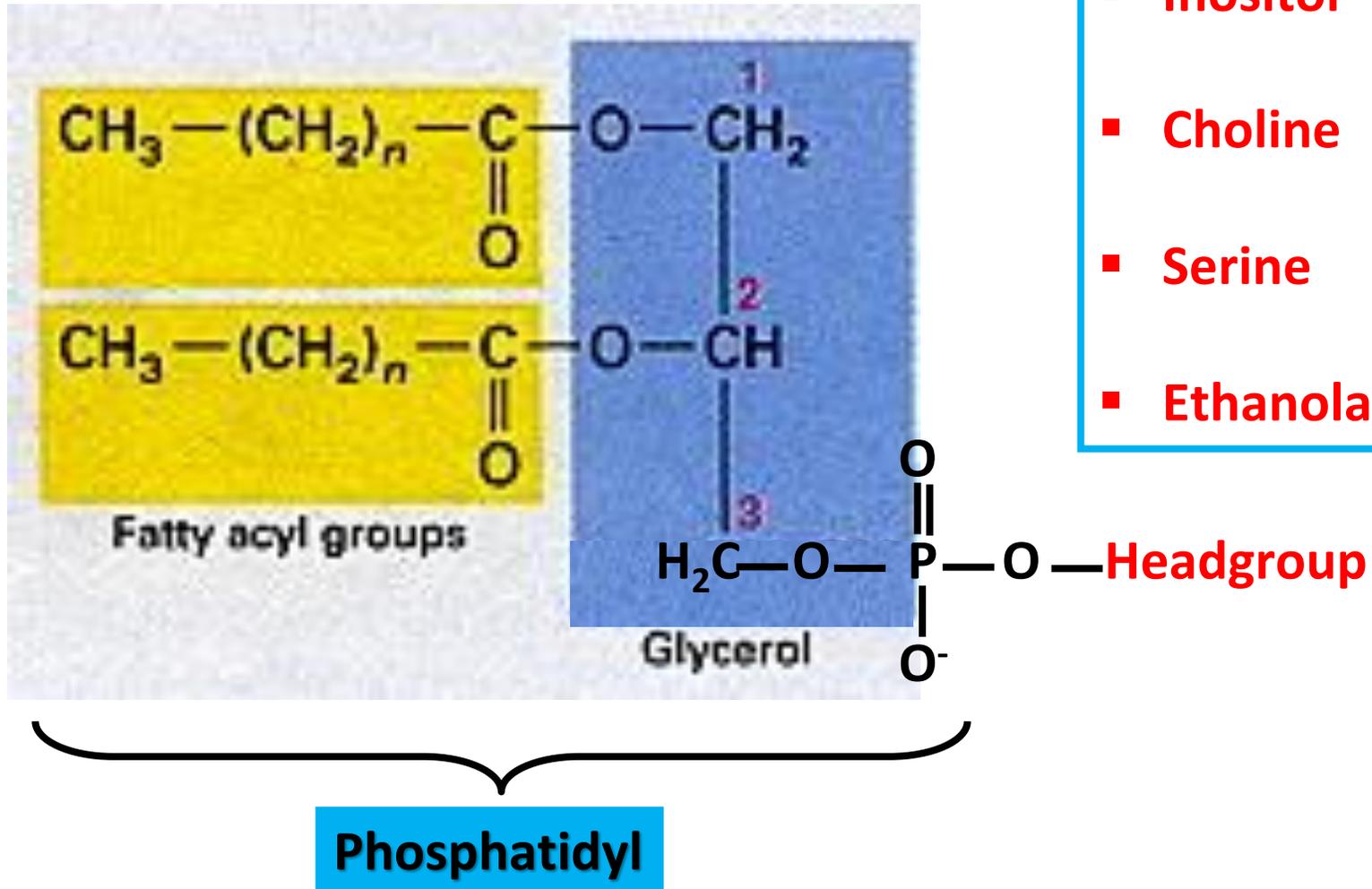


Glycerophospholipids – general structure

- Most common phospholipid in animals tissues is phosphatidylcholine

Common headgroups

- Inositol
- Choline
- Serine
- Ethanolamine



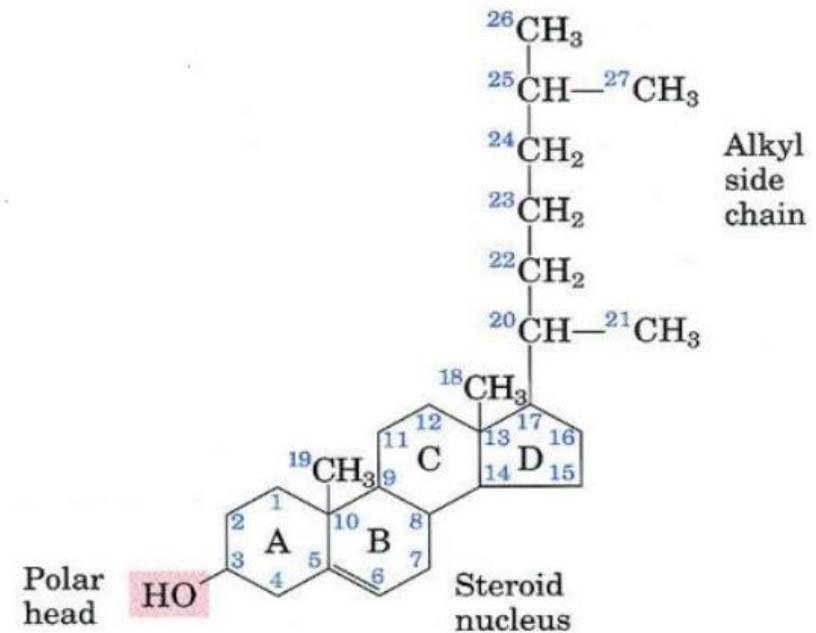
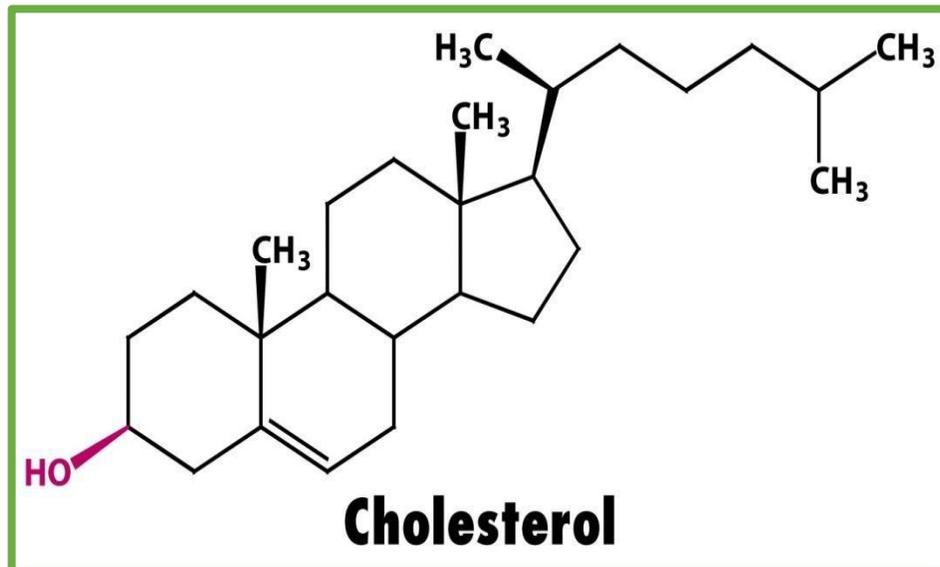
Cellular Lipids – relative amounts and some major functions

NAME		% GPL	Comment
Phosphatidylinositol	PI	10	Precursor of signal molecules
Phosphatidylethanolamine	PE	30	Donor of functional group to membrane proteins; required for certain ATPases
Phosphatidylcholine	PC	50	Structural component of membrane; precursor of some signal molecules
Phosphatidylserine	PS	20	Important signal in apoptosis
GPL = glycerophospholipids			
Sphingomyelin	SM		'parent' sphingolipid; precursor of some signal molecules; stabilises 'lipid rafts'
Cholesterol			50-60% of all membrane lipids; important precursor of other molecules



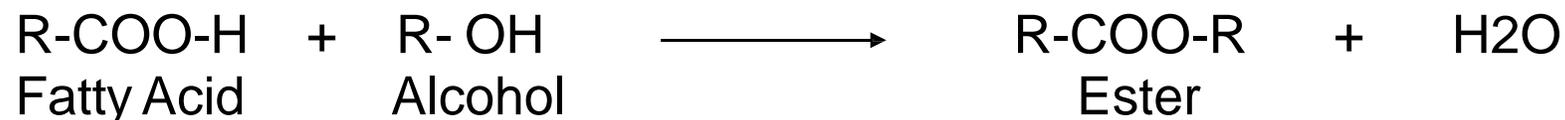
Cholesterol

- A **27 carbon** lipid, it makes up **50-60%** of all membrane lipid
- Cholesterol is exclusively found in **animals tissues**
- **Amphipathic**, with a **polar head** group (hydroxyl group) and a **nonpolar hydrocarbon body** (the steroid nucleus and the hydrocarbon side chain at C-17)
- Major site of cholesterol synthesis is the **liver** with significant synthesis in **intestines**
- Precursor of **steroid hormones**, **bile salts** and **Vitamin D**

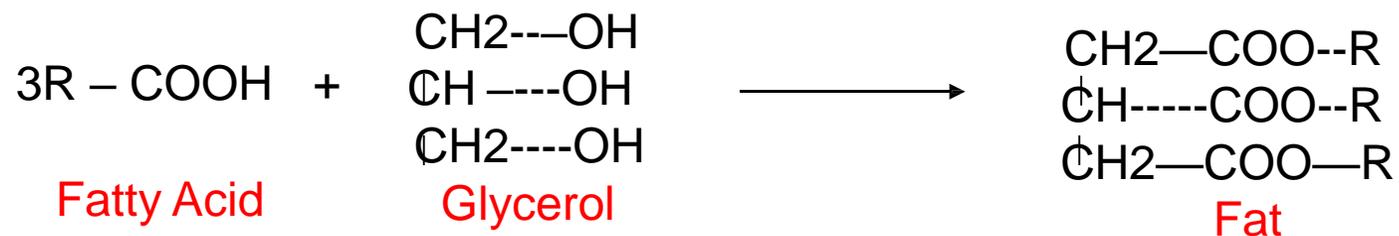


Classification of Lipids

- Simple lipids
- Compound (complex) lipids
- Derived lipids
- Simple lipids are esters of fatty acids with various alcohols.



- a. **Fats and Oils** are esters of fatty acids with **glycerol**. Oil is a liquid unsaturated, tend to be available in plants. while fat is a solid at room temperature .



- b. **Waxes**: esters of fatty acids (usually long chain) with **alcohol** other than glycerol
- Biological waxes are using in the **pharmaceutical** and **cosmetic**. Lanolin, beeswax , carnauba wax are widely used in the manufacture of lotions and ointments

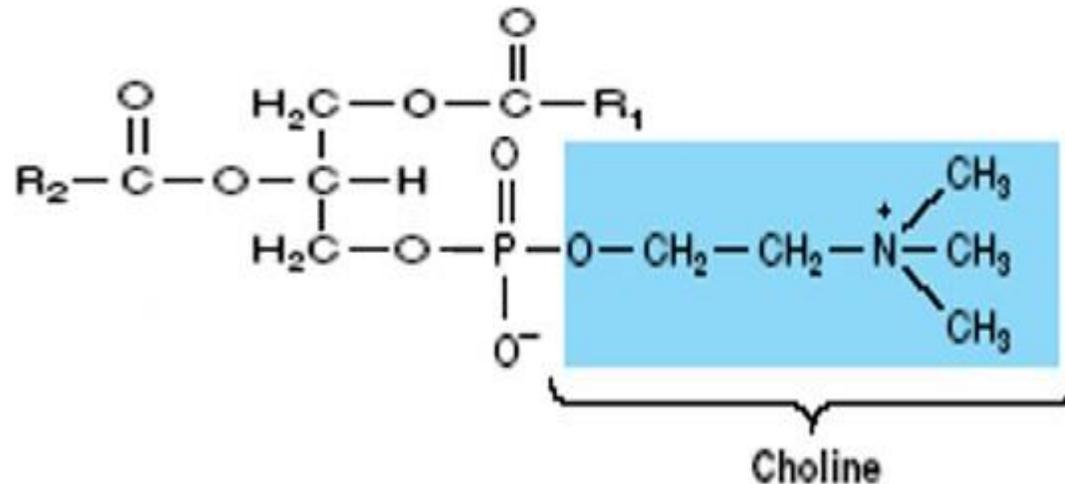


- **Compound Lipids:** esters of fatty acids with **alcohols** containing additional groups such as **phosphate, nitrogenous base, carbohydrate, protein** etc.

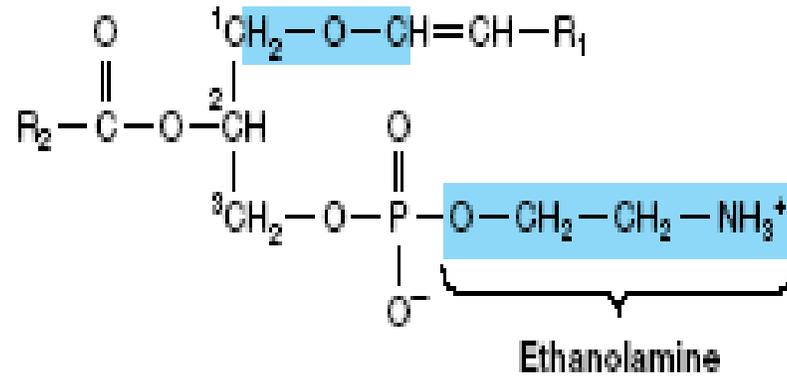
They are divided into :

A- Phospholipids

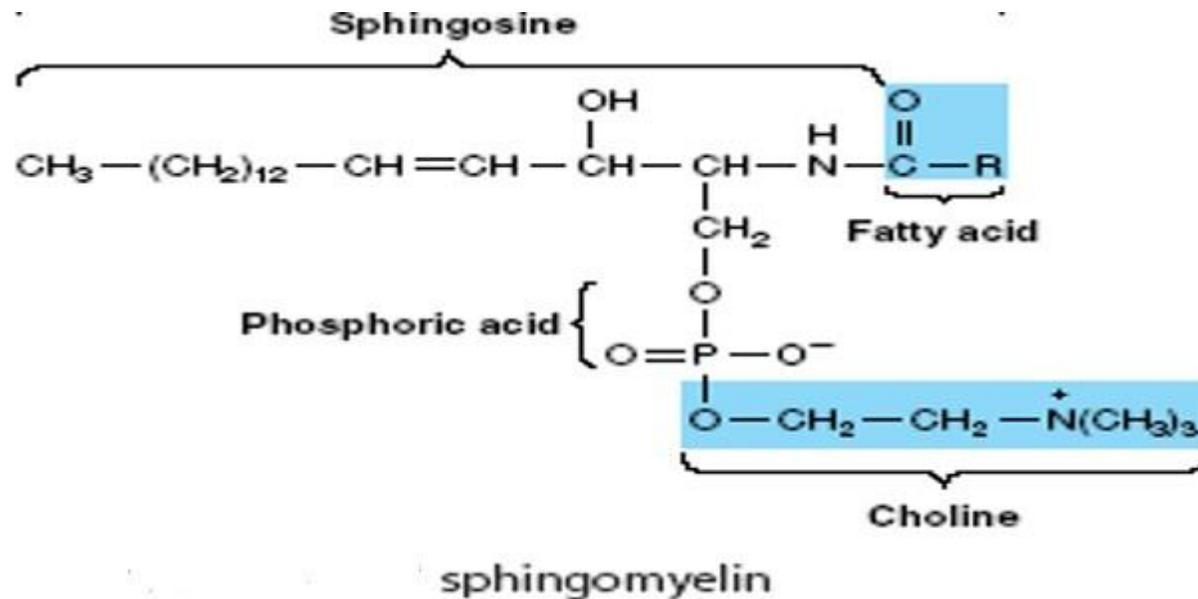
- There are two classes of phospholipids:
- **1- Glycerophospholipids:** These phospholipids contain **glycerol** as the alcohol e.g.,
- **Lecithin** (glycerol + saturated fatty acid + phosphate+ **choline**).



➤ **Cephalin:** (glycerol + unsaturated fatty acid + phosphate+ **ethanolamine**)



2- Sphingophospholipids: **Sphingosine** is the alcohol in this group of **phospho-lipids** e.g., Sphingomyelin (Sphingosine + fatty acid + phosphate + choline).

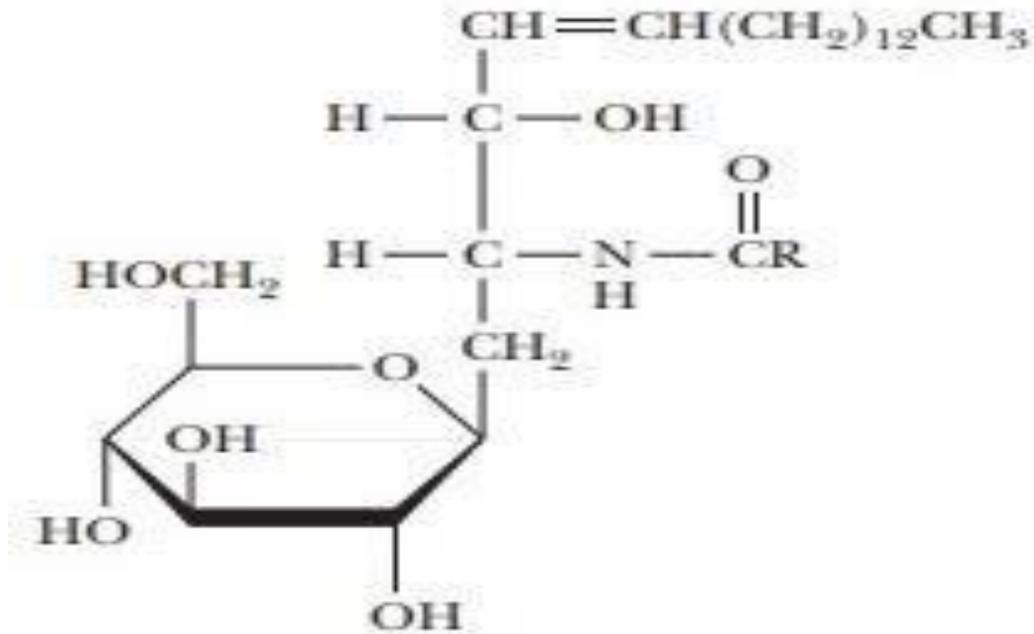


- Sphingomyelin is an **insulating material** for **nerve fibres**
- It has a double behaviour where it dissolves in fat and polar solvents.
- It is found in the brain, kidney, liver and blood



Compound Lipids:

- b) **Glycolipids:** These lipids contain a **fatty acid and carbohydrate**. The alcohol is Sphingosine (also called as glycosphingolipids) e.g., Cerebrosides (**Sphingosine + Cerebronic acid + Glucose or Galactose**).
- Cerebroside is a key component of brain, spinal cord, and nervous cells



β -Gluco-cerebroside



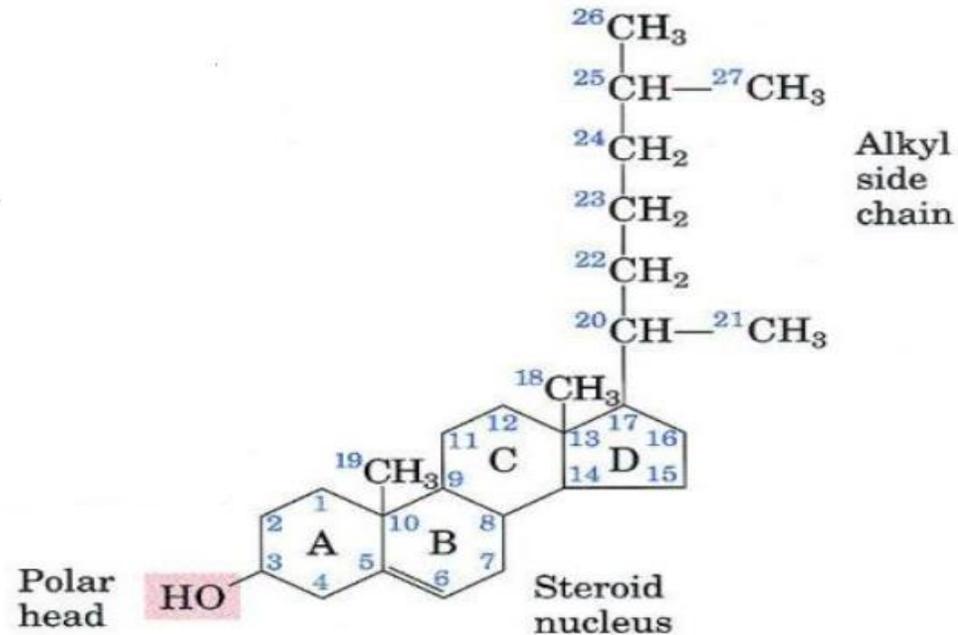
C) Lipoproteins (Lipid transport to tissues)

- Lipids are hydrophobic and insoluble in aqueous environments. Therefore, special methods are needed to transport them round the body.
- **Lipoproteins**: Macromolecular complexes of **lipids** and **proteins**. They are transport fat molecules, such as **triglycerides**, **phospholipids**, and **cholesterol** within the water-based solution of the bloodstream to all the cells and tissues of the body. **Five types of lipoproteins**
 - **Chylomicrons**, transport dietary lipids (exogenous) from **intestine** to peripheral tissues.
 - **Very low density lipoproteins (VLDL)**, transport the lipids (endogenously synthesized) mainly **TG** from liver to peripheral tissues.
 - **Low density lipoproteins (LDL)** ("bad" cholesterol), transport **cholesterol** from liver to peripheral tissues.
 - **High density lipoproteins (HDL)** ("good" cholesterol), carry **cholesterol** from peripheral tissues to **liver**.
 - **Intermediate density lipoproteins (IDL)**.



3- Derived Lipids: are the derivatives obtained by the hydrolysis of simple and compound lipids. These include fatty acids, alcohols, mono-and diacylglycerol, lipid soluble vitamins and steroids. The most common derived lipids are steroids.

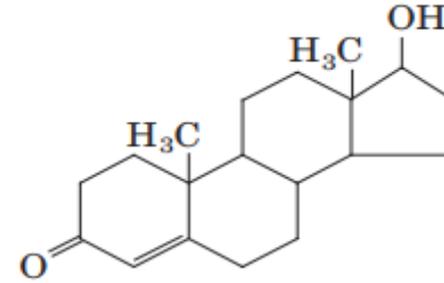
- **Sterols** are structural lipids present in the membranes of most eukaryotic cells. The characteristic structure is the steroid nucleus, consisting of **four** fused rings, **three with six carbons** and **one with five**
- There are several steroids in the biological system. These include **cholesterol, bile acids, vitamin D, sex hormones, adrenocortical hormones**



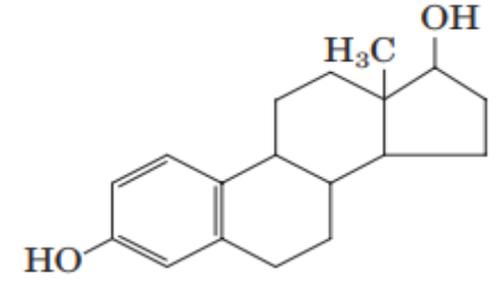
4- Neutral lipids : The lipids which are uncharged are referred to as neutral lipids e.g. **triacylglycerols**

Derivatives of cholesterol – steroid hormones

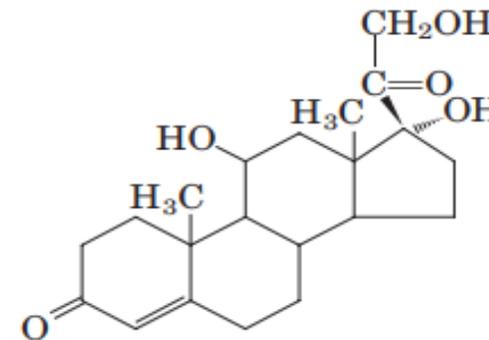
- **Testosterone**, the **male sex hormone**, is produced in the **testes**.
- **Estradiol**, one of the **female sex hormones**, is produced in the **ovaries** and **placenta**.
- **Cortisol** and **aldosterone** are hormones synthesized in the cortex of the **adrenal gland**; they regulate **glucose metabolism** and **salt excretion**, respectively.
- **Prednisolone** and **prednisone** are synthetic steroids used as **anti inflammatory agents**.



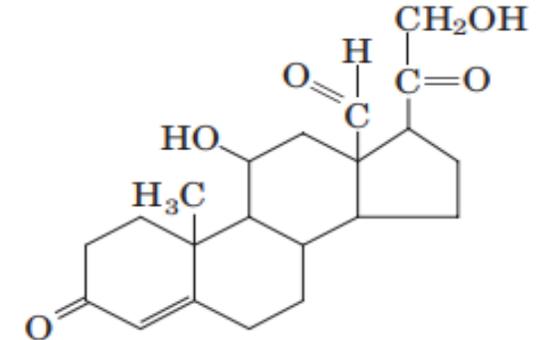
Testosterone



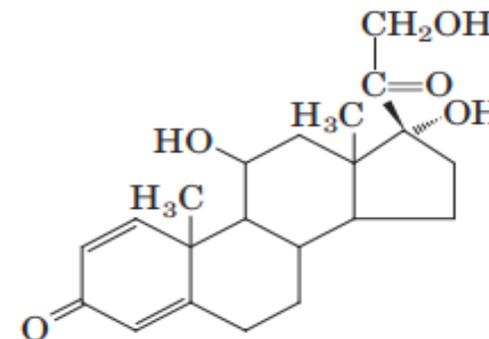
Estradiol



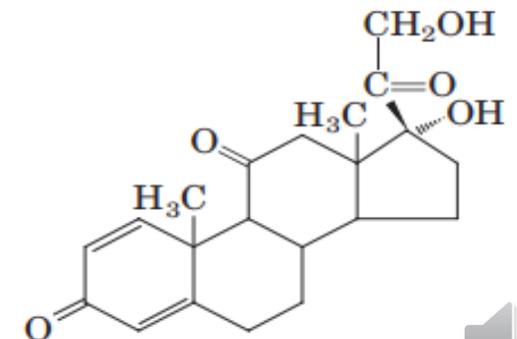
Cortisol



Aldosterone



Prednisolone



Prednisone

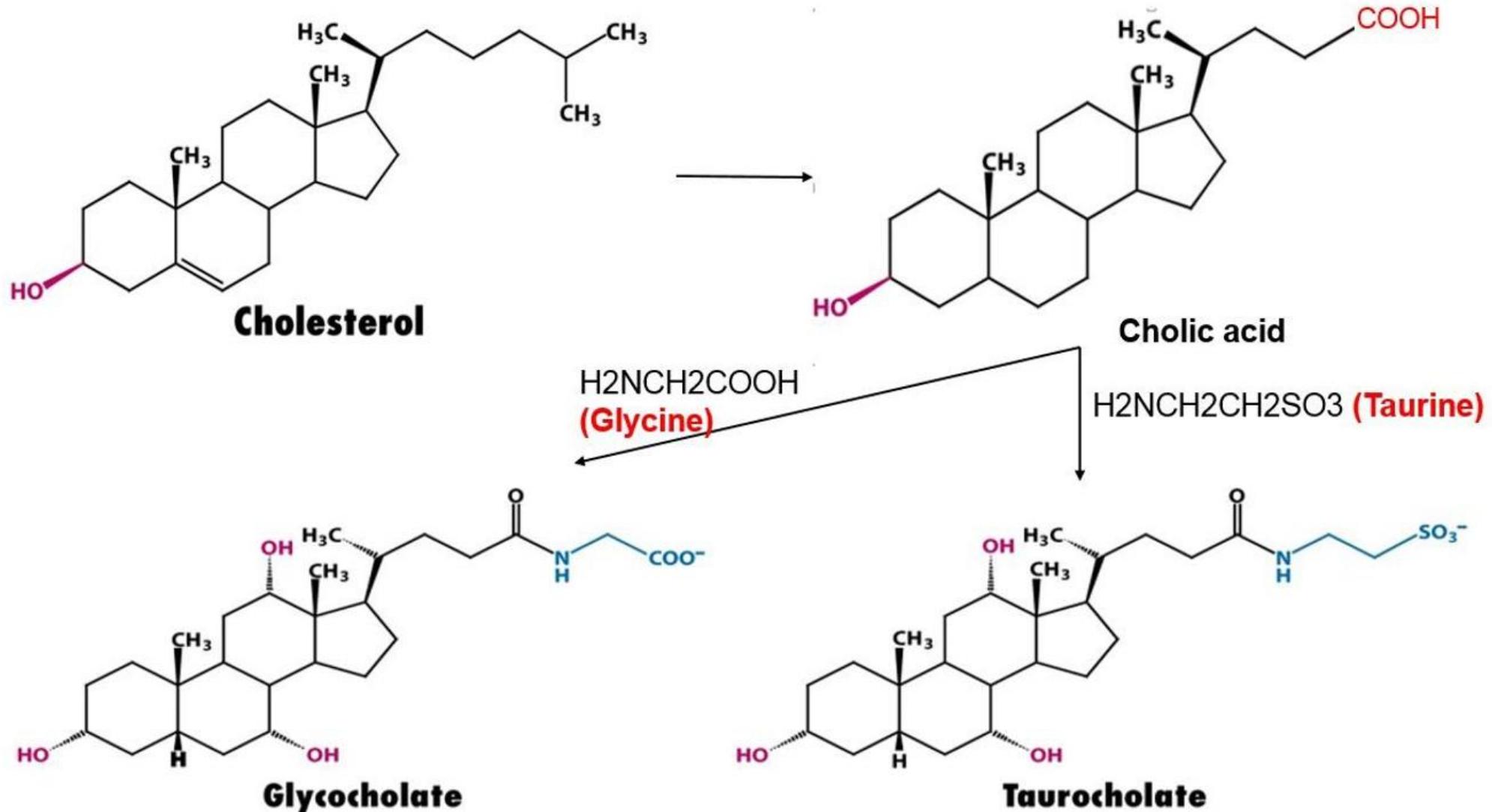


Bile acids

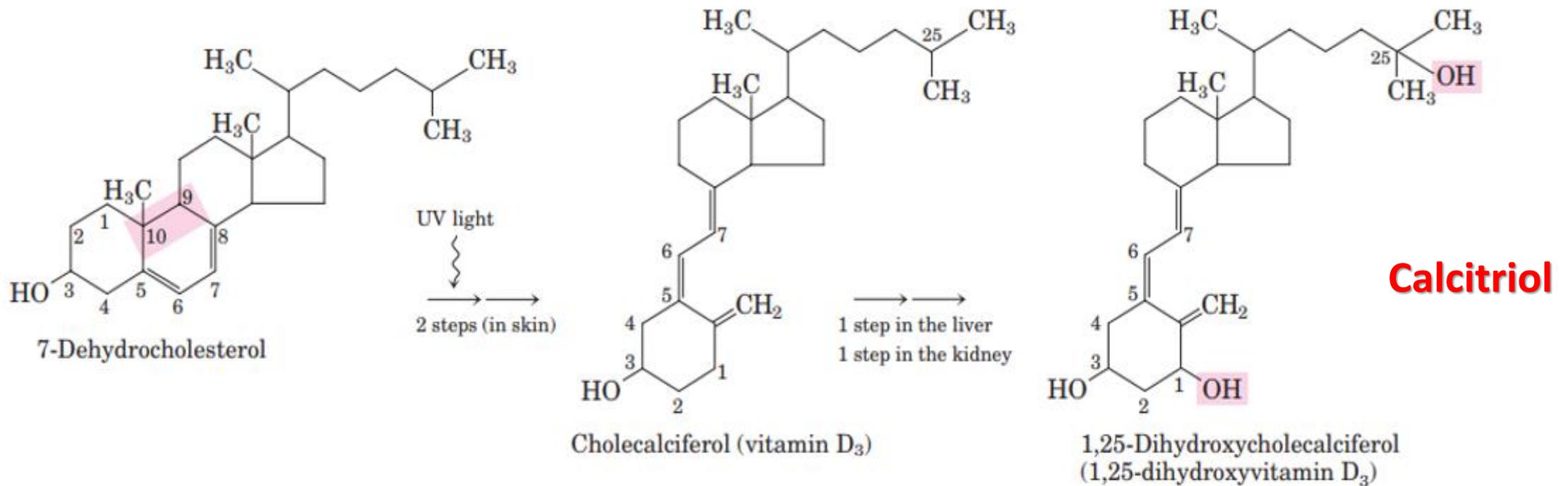
- Bile acids are polar derivatives of cholesterol that stored in the gallbladder after secretion by the liver, and then release into small intestine to aid in the processes of digestion and absorption of fats (emulsifying dietary fats to make them more readily accessible to digestive lipases).



Derivatives of cholesterol – bile salts



Vitamin D (Solar vitamin)



- **Skin**, UV irradiation **breaks** the bond between **C9-C10** of 7 dehydrocholesterol
- **liver**, a hydroxyl group is added at **C-25**
- **kidney**, a **second hydroxylation** at **C-1** produces the active hormone



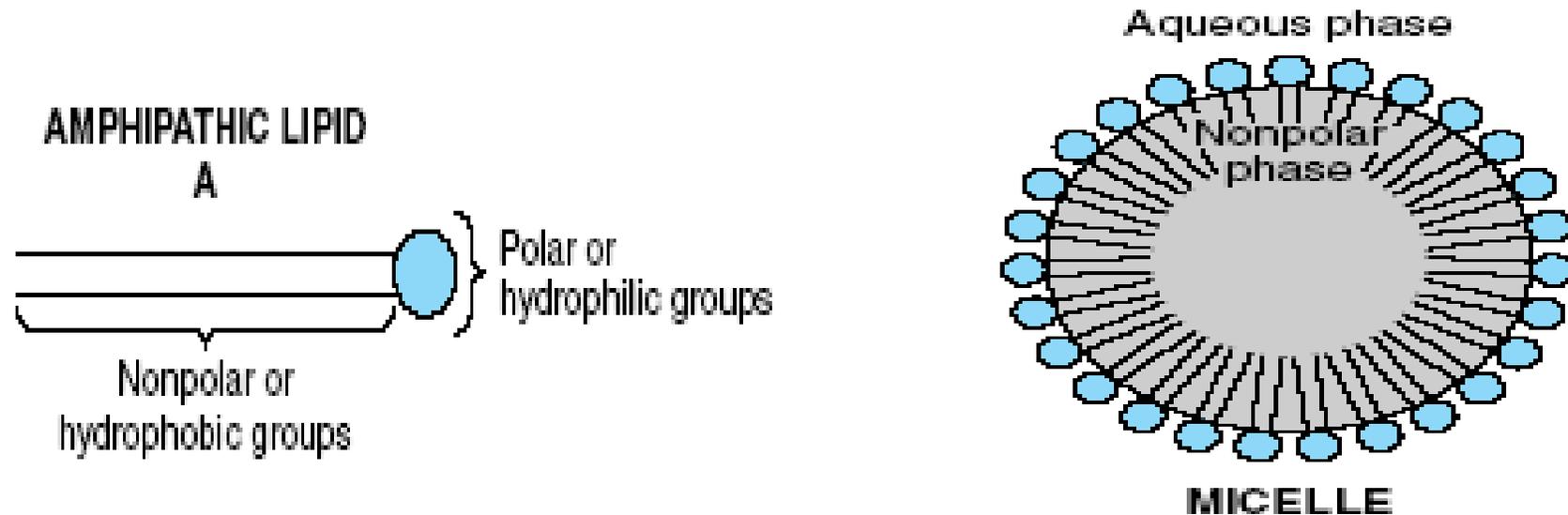
Orientation amphipathic lipids

- Depending on the precise **conditions** and the **nature** of the **lipids**, three types of lipid aggregates can form when amphipathic **lipids are mixed with water**
- **Micelles**
- **Lipid bilayer**
- **liposome**



Micelles

- When the **amphipathic lipids** are mixed in water (aqueous phase), the **polar groups (heads)** orient themselves towards the **aqueous phase** while the **non-polar (tails)** orient themselves towards the **opposite directions**. This leads to the formation of **micelles**. Micelles formation facilitated by **bile salts** is very important for **lipid digestion and absorption**.

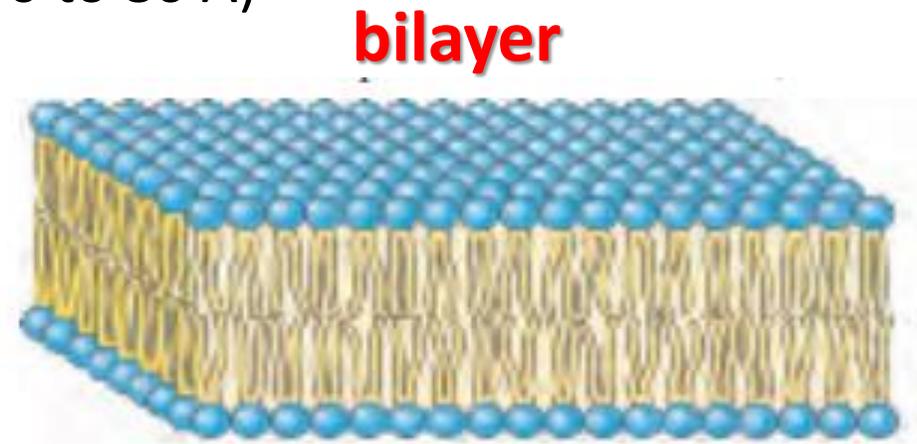


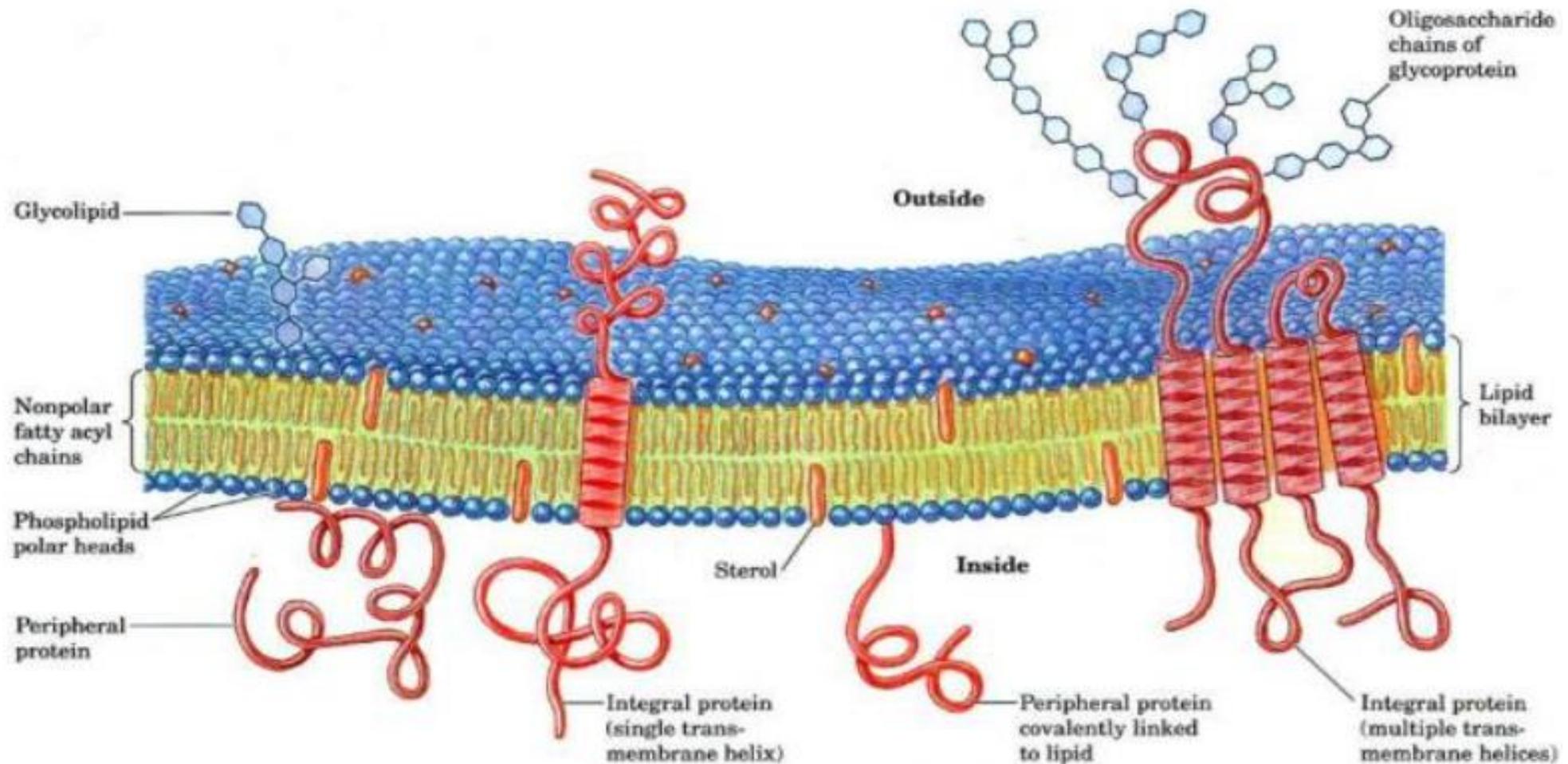
- Micelles** are water soluble molecules formed from aggregation of **bile salts, fatty acids and mono-glycerides** in the intestine.



Lipid bilayer

- The **hydrophobic** portions in each monolayer, **excluded from water**, **interact with each other**.
- The **hydrophilic head** groups **interact with water at each surface** of the bilayer.
- Because the **hydrophobic regions** at its **edges** are transiently in **contact with water**, the bilayer sheet is relatively **unstable** and spontaneously forms a third type of lipid aggregate:
 - bilayer **folds back on itself** to form a hollow sphere, a **vesicle** or **liposome**
- Lipid bilayer are **highly impermeable** to **ions** and most **polar molecules**, but **permeable to nonpolar compounds**; they are **5 to 8 nm** (50 to 80 Å)





- Fluid model for **membrane structure**. The **fatty acyl chains in the interior** of the membrane form a fluid, hydrophobic region. The **carbohydrate** moieties attached to some **proteins** and **lipids** of the plasma membrane are exposed on the **extracellular surface** of the membrane.



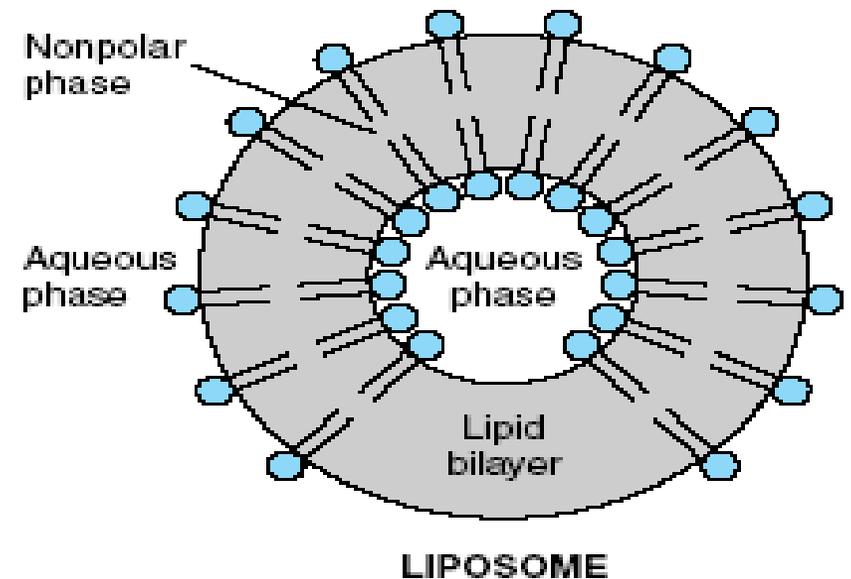
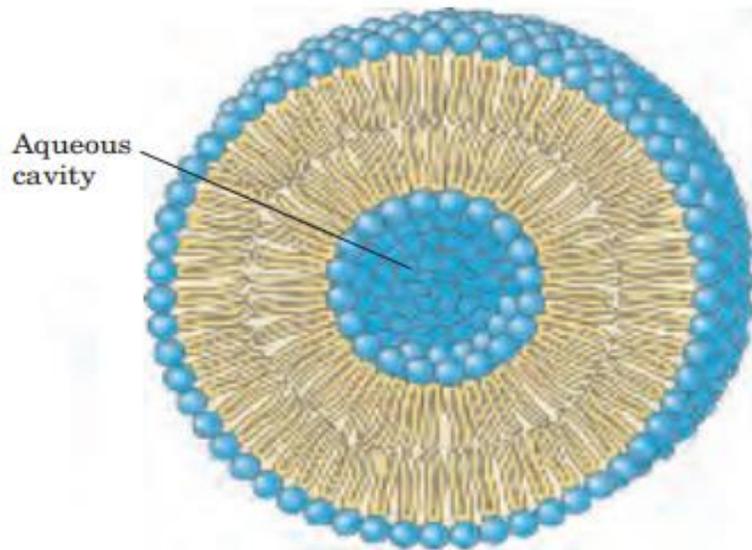
lipid bilayer

- The central feature of biological membranes is a **double layer** of lipids, which are composed of **lipids** and **proteins**
- Many **membrane proteins** contain covalently attached **oligosaccharides**. Plasma membrane **glycoproteins** are always oriented with the **carbohydrate-bearing domain** on the **extracellular surface**
- Some of the **roles of oligosaccharides** in the cell surface:
 - **Viruses** that infect animal cells, such as the **influenza virus**, **bind to cell surface glycoproteins** as the first step in **infection**.
 - **Bacterial toxins**, such as the **cholera** and **pertussis** toxins, **bind to a surface glycolipid** before **entering**



Liposomes

- By forming a sphere-shaped vesicles (liposomes), bilayers lose their hydrophobic edge regions, achieving maximal stability in their aqueous environment. These bilayer liposomes (phospholipid) enclose water, creating a separate aqueous compartment.
- The ability of liposomes to encapsulate hydrophilic or lipophilic drugs have allowed to become useful drug delivery systems, e.g., Liposomes are used as carriers of drugs to target certain tissue in cancer therapy.



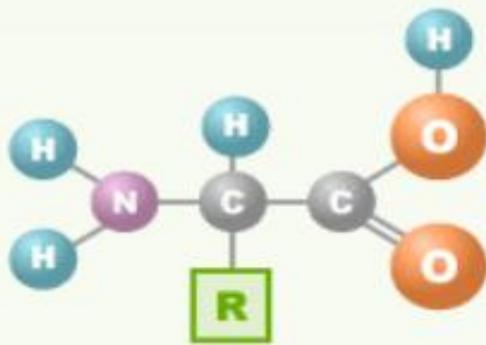
Lecture 5: Biochemistry I

Amino Acids

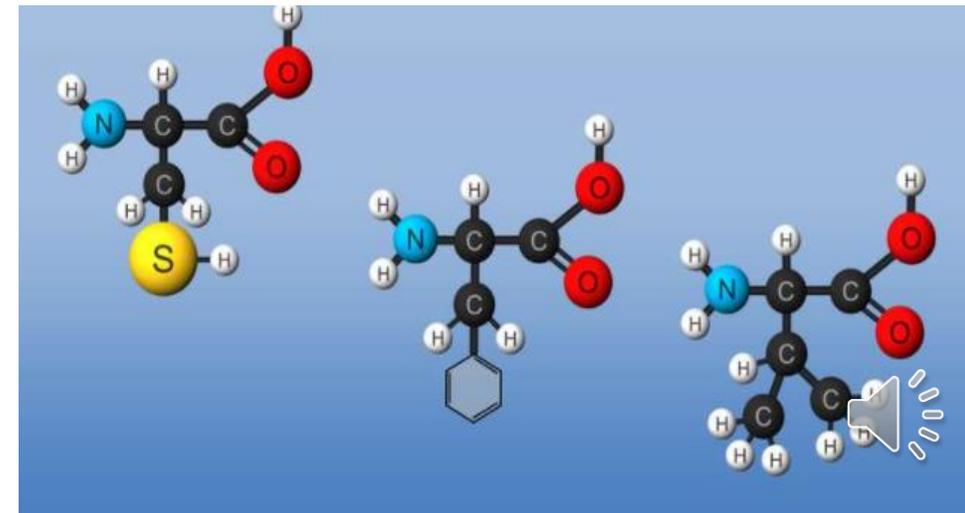
3rd Class

Anbar University-College of Pharmacy-Clinical Laboratory Sciences Department
2020-2021

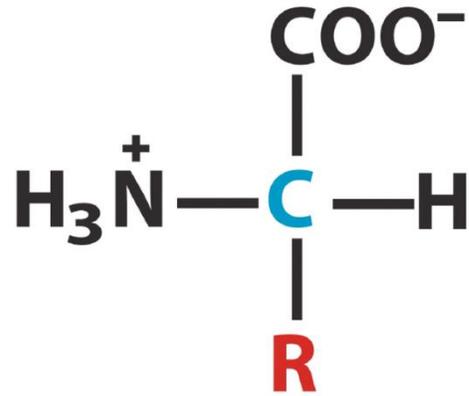
Dr. Yousif H. Khalaf
Ph.yhks1980@uoanbar.edu.iq



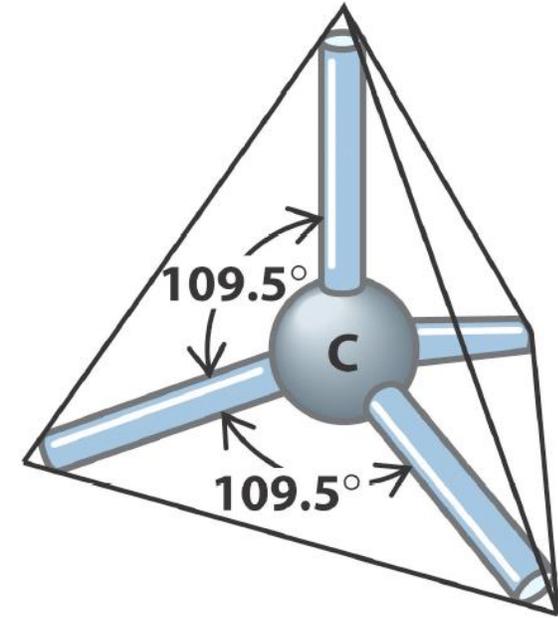
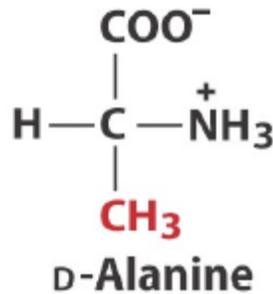
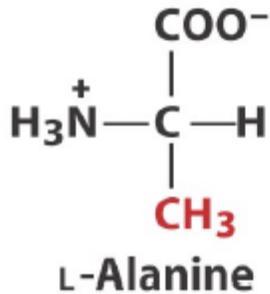
Amino acid



Structure of an amino acid



Example:



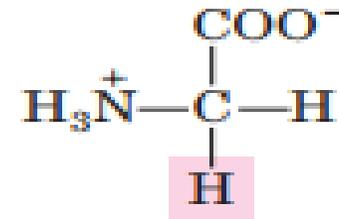
asymmetric carbon atom (α -carbon), C is connected to 4 different atoms.

- They have a **carboxyl group** and an **amino group** bonded to the same carbon atom.
- They differ from each other in their side chains, or **R groups**. which vary in structure, size, and electric charge, and which influence the solubility of the amino acids in water



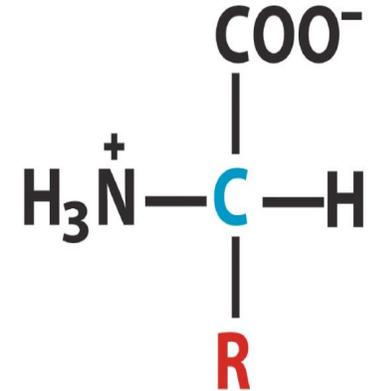
Structure of an amino acid

- For all the common amino acids **except glycine**, the **α -carbon** is bonded to **4 different groups**:
 - Carboxyl group
 - Amino group
 - R group
 - Hydrogen atom
- In glycine, the **R group is another hydrogen**
- **20** different amino acids are commonly found in **proteins**
- The common amino acids of proteins have been assigned **three-letter abbreviations**
- **Single**-letter abbreviations are usually used to denote the amino acids in a **polypeptide sequence**

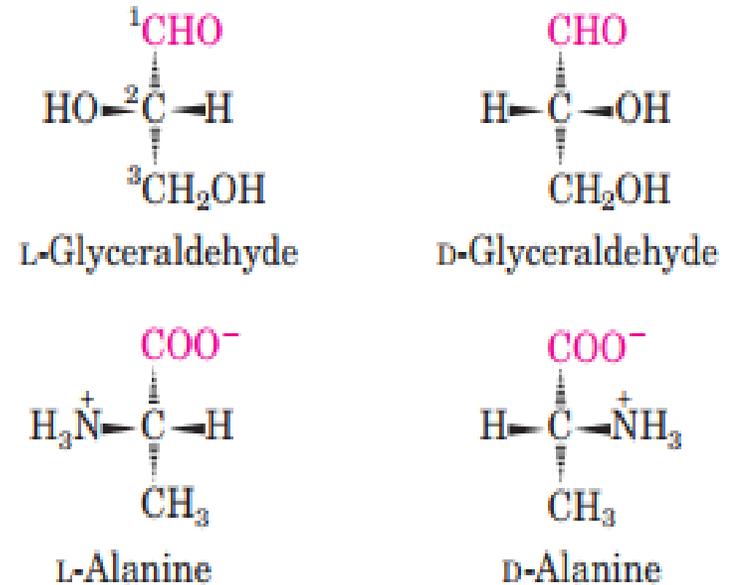
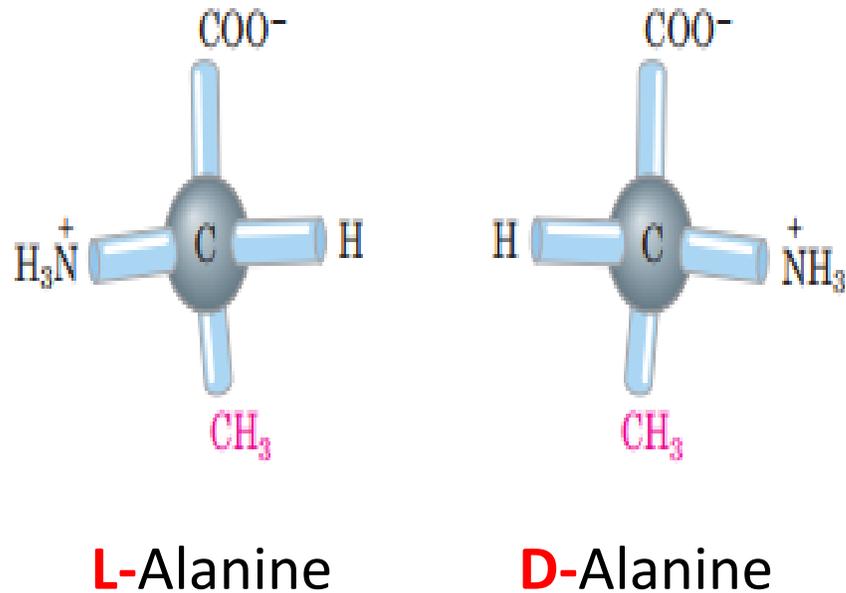


Glycine

Gly G



- When a carbon atom has 4 different groups, they can be arranged in 2 stereoisomers that represent mirror images of each other (enantiomers). This asymmetric carbon atom is called a chiral center.



- L-Amino acids are those with the amino group on the left, and D-amino acids have the amino group on the right
- All amino acid residues in proteins are L- stereoisomers



Classification of Amino Acids

- Polar and Non-polar Amino Acids
- Chemical Classification of Amino Acids
- Nutritional classification of amino acids
- Metabolic classification of amino acids



Polar and Non-polar Amino Acids

- Amino acids can be grouped into **two** main classes based on **polarity of the “R” groups** , or their tendency to interact with water at biological pH (near pH 7.0)
 - **polar amino acids**: polar and hydrophilic (water-soluble)
 - **Nonpolar amino acids**: nonpolar and hydrophobic (water-insoluble)



polar amino acids

- **Uncharged R Groups:** They contain functional groups that form **hydrogen bonds with water**
 - Serine (hydroxyl group)
 - Threonine (hydroxyl group)
 - Cysteine (sulfhydryl group)
 - Asparagine (amide group)
 - Glutamine (amide group)
- **Positively Charged (Basic) R Groups:** The most hydrophilic R groups are those that are either **positively or negatively charged**.
 - Lysine (second primary amino group)
 - Arginine (guanidino group)
 - Histidine (imidazole group)
- **Negatively Charged (Acidic) R Groups:** Each of which has a **second carboxyl group**
 - Aspartate
 - Glutamate



Nonpolar amino acids

- **Aliphatic amino acids** are **nonpolar and hydrophobic**. They tend to cluster together within proteins, stabilizing protein structure by means of hydrophobic interactions
- **Methionine**, amino acids containing **sulfur**, has a nonpolar thioether group in its side chain
- **Proline** has an aliphatic side chain with a distinctive cyclic structure
- **Aromatic R Groups: phenylalanine, tyrosine, and tryptophan**, with their aromatic side chains, are relatively nonpolar (hydrophobic). All can participate in hydrophobic interactions. However, **the hydroxyl group of tyrosine can form hydrogen bonds (polar)**

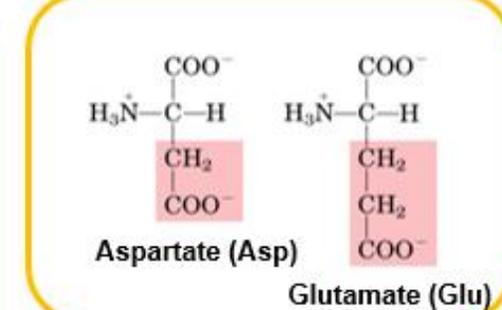
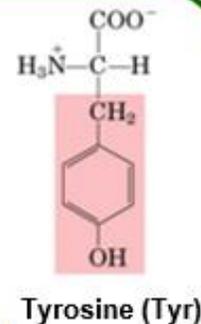
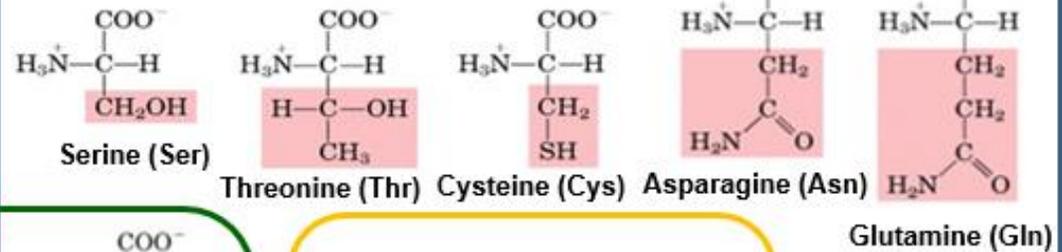
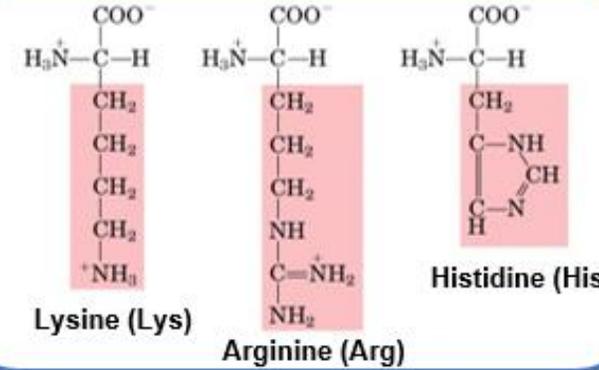
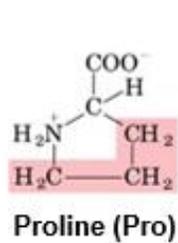
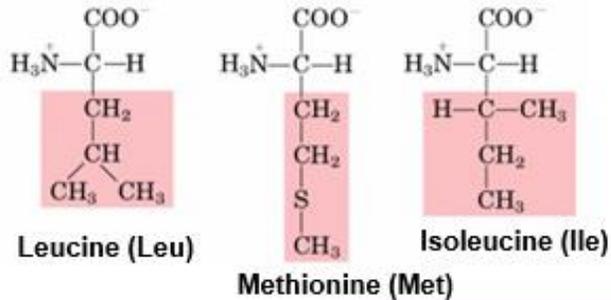
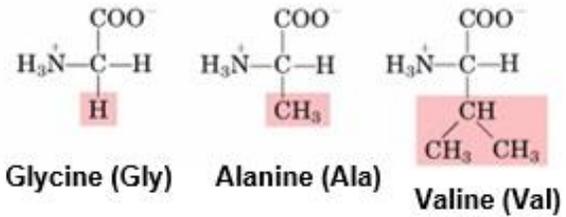


Polar and Non-polar Amino Acids

Nonpolar R groups

20

Polar R groups



Chemical Classification of Amino Acids

- **20 amino acids found in proteins are also divided into 7 distinct groups:**

1- Amino acids with **Aliphatic** side chains:

- Glycine (Gly)
- Alanine (Ala)
- Valine (Val)
- Leucine (Leu)
- Isoleucine (Ile)

2- Amino acids with side chains containing **Hydroxylic (OH)** groups:

- Serine (Ser)
- Threonine (Thr)
- Tyrosine (Tyr)

3- Amino acids with side chains containing **Sulfur atoms**:

- Cysteine (Cys)
- Methionine (Met)



4- Amino acids with side chains containing **Acidic groups**:

- Aspartic acid (Asp)
- Asparagine (Asn)
- Glutamic acid (Glu)
- Glutamine (Gln)

5- Amino acids with side chains containing **Basic groups**:

- Arginine (Arg)
- Lysine (Lys)
- Histidine (His)

6- Amino acids with side chains containing **Aromatic rings**:

- Phenylalanine (Phe)
- Tyrosine (Tyr)
- Tryptophan (Trp)

7- **Imino Acid**:

- Proline (Pro)



Nutritional classification of amino acids

➤ Non-essential amino acids (NEAAs):

- can be synthesized inside the body

➤ Essential amino acids (EAAs):

- can not be synthesized by the body
- Their deficiencies in the diet result in diseases

○ phenylalanine

○ valine

○ threonine

○ tryptophan

○ methionine

○ leucine

○ isoleucine

○ lysine

○ histidine



Metabolic classification of amino acids

- Amino acids are classified according to their metabolic fate into:
 - **Ketogenic amino acids:**
 - Leucine and lysine
 - **Both Ketogenic and glucogenic “mixed amino acids “**
 - Phenylalanine
 - Tryptophan
 - Tyrosine
 - Isoleucine
 - **Glucogenic amino acids:**
 - All the remaining 14 amino acids are glucogenic .



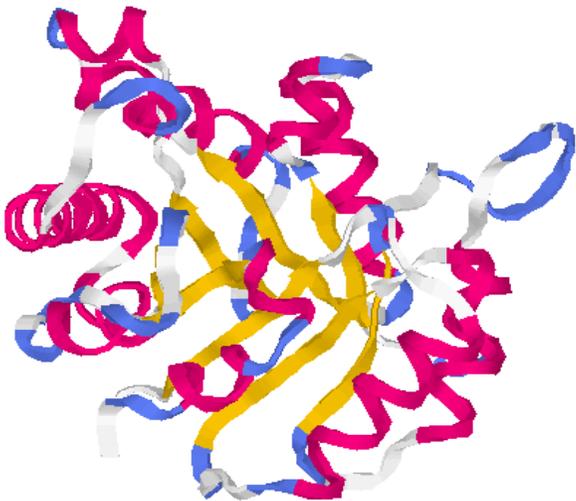
Lecture 6: Biochemistry I

Amino Acids, Peptides and Proteins

3rd Class

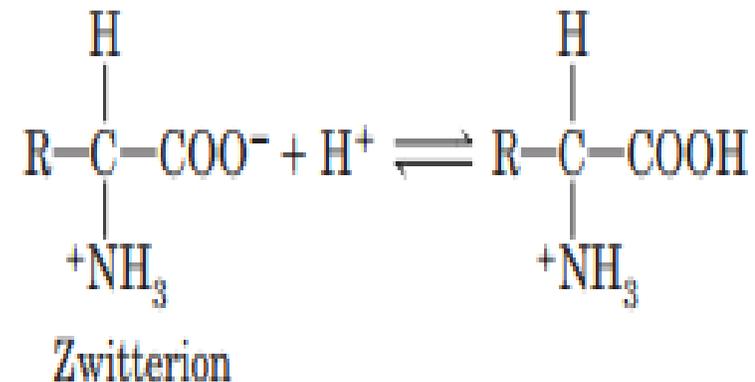
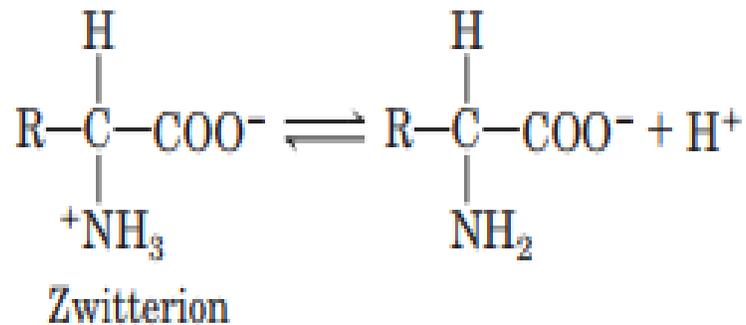
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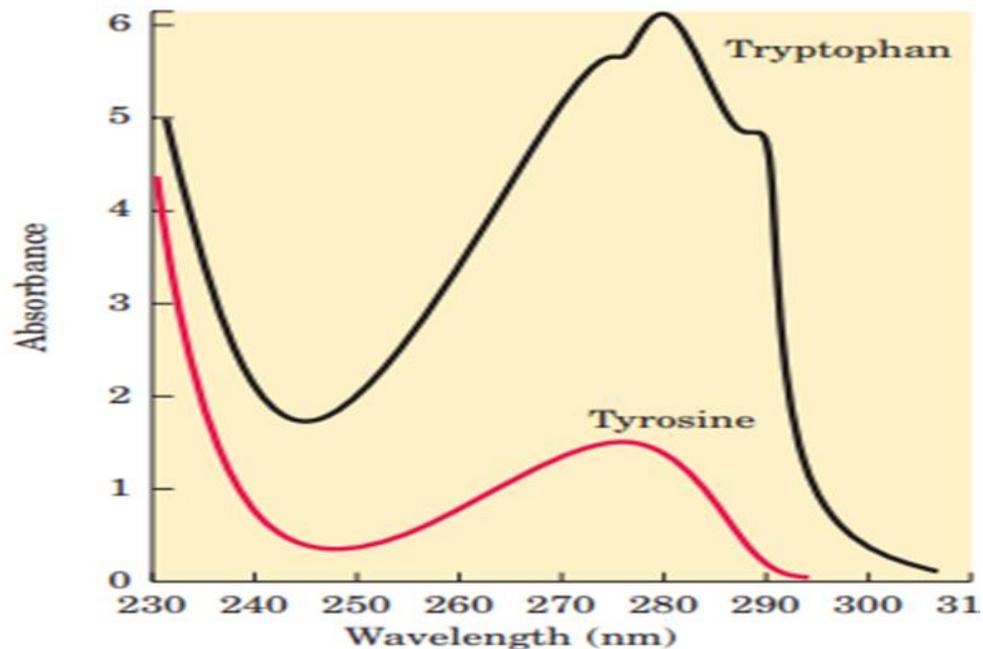


Amino Acids Can Act as Acids and Bases

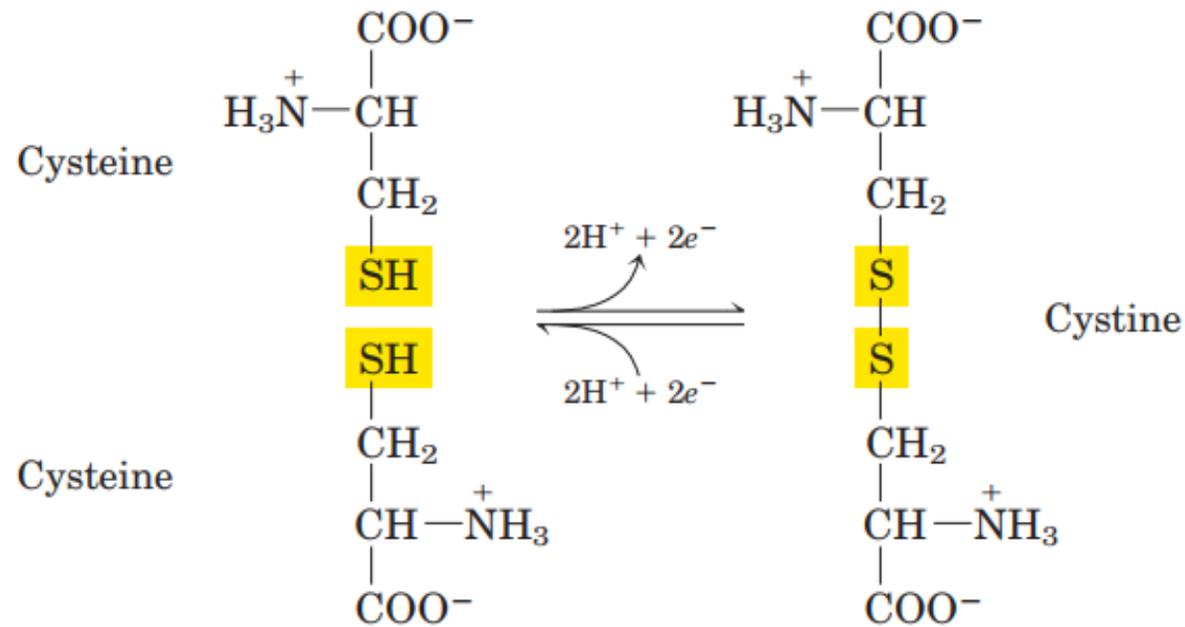
- In solution, the free amino acids exist as the **dipolar ion**, or **zwitterion**, which can act as either an acid (proton donor) or a base (proton acceptor). Ions in which the **amino group** is **positively charged** and the **carboxylate group** is **negatively charged**.
- Substances having this dual nature are called **Amphoteric**



- Amino acids differ from each other in their **side-chains (R)** .Thus, the chemical properties of the side chains determine how the **protein folds**, how it **binds specific ligands**, and how it interacts with **its environment** (such as the aqueous medium of the cytoplasm).
- **Tryptophan** and **tyrosine**, and to a much lesser extent **phenylalanine**, **absorb Ultraviolet light** . This accounts for the characteristic strong absorbance of light by most proteins at a wavelength of 280 nm, a property exploited by researchers in the characterization of proteins.



- Cysteine is readily oxidized to form a covalently linked dimeric amino acid called cystine, in which two cysteine molecules or residues are joined by a disulfide bond.



- Reversible formation of a disulfide bond by the oxidation of two molecules of cysteine.

Disulfide bonds between Cys residues stabilize the structures of many proteins



Titration Curves of Weak Acids

- Acid-base titration involves the gradual addition or removal of protons
- Titration is used to determine the amount of an acid in a given solution. A measured volume of the acid is titrated with a solution of a strong base, (NaOH), of known concentration. The NaOH is added in small increments until the acid is consumed (neutralized), as determined with an indicator dye or a pH meter. The concentration of the acid in the original solution can be calculated from the volume and concentration of NaOH added
- A plot of pH against the amount of NaOH added (a titration curve) reveals the pKa of the weak acid.
- At the midpoint of the titration, at which exactly 0.5 equivalent of NaOH has been added, one-half of the original acid has undergone dissociation, so that the concentration of the proton donor, , now equals that of the proton acceptor. At this midpoint, pH is exactly equal to the pKa
- The end point of the titration occurs at about pH 7.0: all the acid has lost its protons to OH



Titration Curves of Weak Acids

- **Weak acids** partially ionize to release a **hydrogen ion**, thus **lowering the pH** of the aqueous solution. **Weak bases accept a hydrogen ion**, **increasing the pH**, and is expressed as a **dissociation constant, K_a**
- The **pKa** expresses, on a logarithmic scale, the relative strength of a weak acid or base
- The stronger the acid, the lower its pKa; the stronger the base, the higher its pKa.
- The **pKa** can be determined experimentally; it is the **pH** at the **midpoint of the titration curve** for the acid or base.

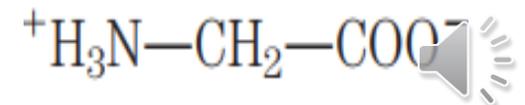


Amino Acids Have Characteristic Titration Curves

- At a physiologic pH of 7.4, the amino group on the amino acids carries a positive charge, and the carboxylic acid group is negatively charged.
- the pKa, 50% of the molecules are dissociated into carboxylate anions and protons and at a physiologic pH of 7.4, , more than 99% of the molecules are dissociated
- pKa values: pKa of the -COOH group in the range of 1.8 to 2.4, and pKa of the $-\text{NH}_3^+$ group in the range of 8.8 to 11.0
- The characteristic pH at which the net electric charge is zero is called the isoelectric point or isoelectric pH, designated pI
- Amino acids with an ionizable R group have more complex titration curves, with three possible ionization steps (three stages); thus they have three pKa, values the pKa of the R group is designated here as pK_R

Titration curve of the diprotic form of glycine

- Glycine has **two distinct stages**.
- **At low pH**, the predominant ionic species of glycine is the fully protonated form, $^+\text{H}_3\text{N}-\text{CH}_2-\text{COOH}$.
- The **first stage** of the titration, as the **pH is increased by the addition of OH**, the **-COOH** group of glycine **loses its proton**, the proton dissociates from the carboxylic acid group, and its charge changes from **zero to negative** $^+\text{H}_3\text{N}-\text{CH}_2-\text{COO}^-$
- At the **midpoint of the titration**, a **point of inflection** is reached, where the **pH is equal to the pKa**
- The **pH at the midpoint** is **2.34**, thus its **-COOH** group has a **pKa** (labeled **pK1**) of **2.34**.
- As the titration proceeds, another important point is reached at **pH 5.97**. Here there is **another point of inflection**, at which **removal of the first proton** is essentially complete (**COOH**) and **removal of the second** ($-\text{NH}_3^+$) has just begun. At this pH glycine is present largely as the **dipolar**

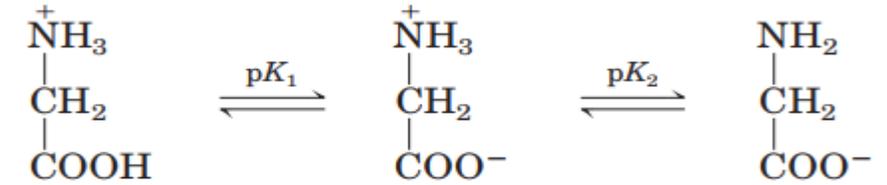


Titration curve of the diprotic form of glycine

- The **second stage** is removal of a proton from the $-\overset{+}{\text{N}}\text{H}_3$ group of glycine.
- The **pH** at the **midpoint** of this stage is **9.60**, equal to the **pKa** (labeled **pK2**) for the $-\overset{+}{\text{N}}\text{H}_3$ group.
- The titration is essentially complete at a **pH** of about **12**, at which point the predominant form of glycine is $\text{H}_2\text{N}-\text{CH}_2-\text{COO}^-$.
- The characteristic **pH** at which the **net electric charge is zero** is called the **isoelectric point** or **isoelectric pH**, designated **pI**.



- Glycine has **no ionizable group** in its side chain, the **isoelectric point** is simply the arithmetic mean of the **two pKa values**:



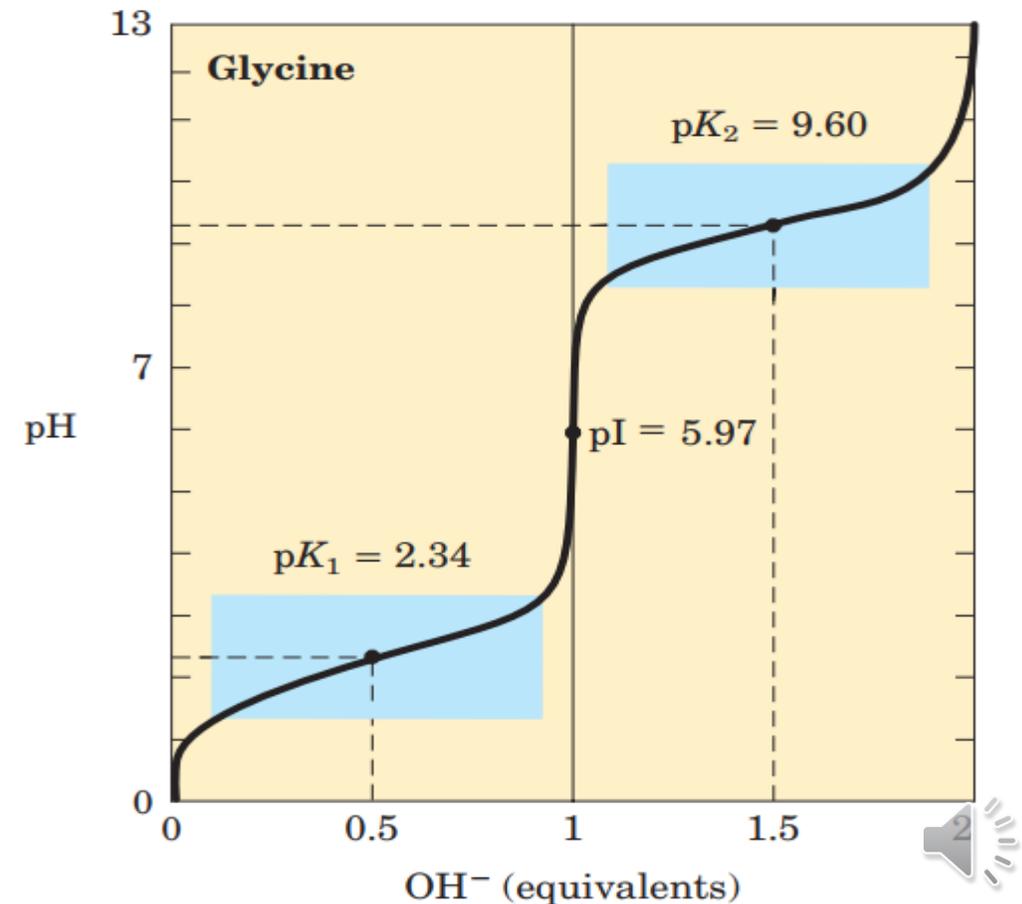
pK₁ carboxylic acid = **2.34**

pK₂ amino group = **9.60**

$$\text{pI} = (\text{p}K_1 + \text{p}K_2) / 2$$

$$\text{pI} = (2.34 + 9.60) / 2$$

pI = 5.97



- Glutamine has a pI of 3.22, lower than that of glycine. This is due to the presence of two carboxyl groups (Negatively charged)

pK_1 carboxylic acid = 2.19

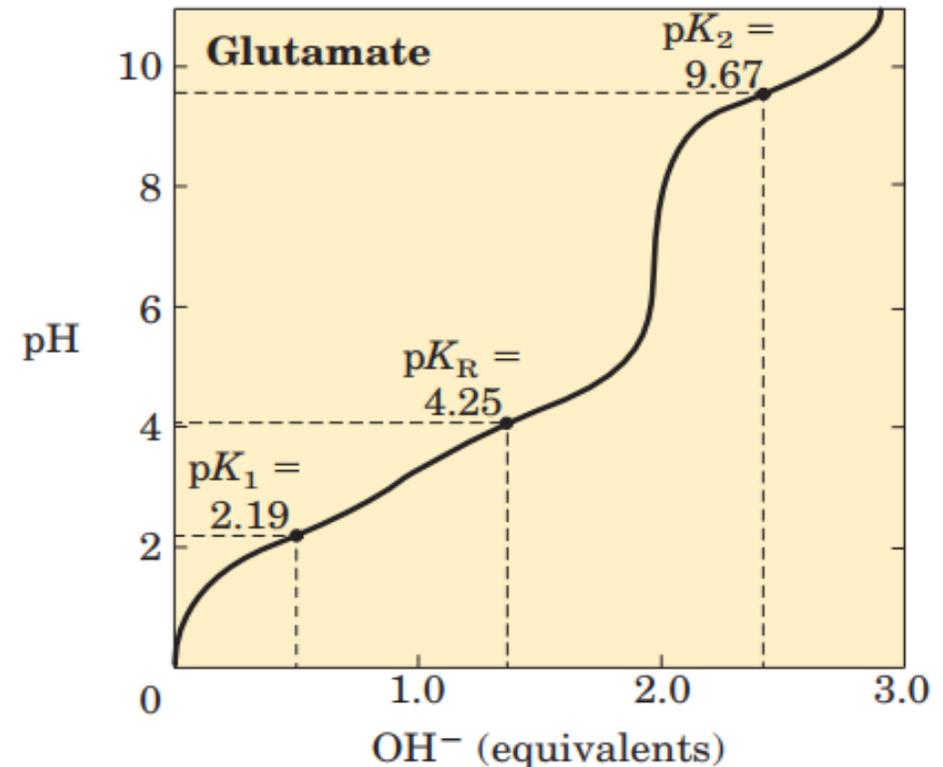
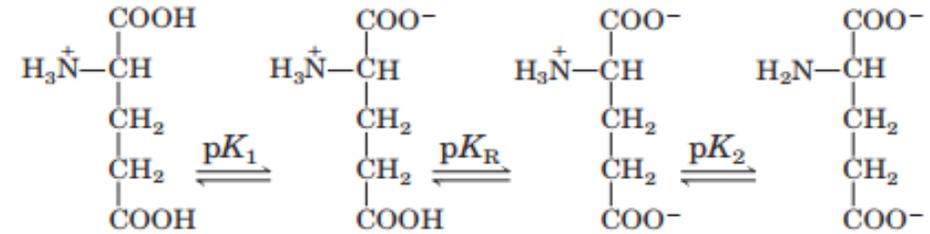
pK_R R group = 4.25

pK_2 amino group = 9.67

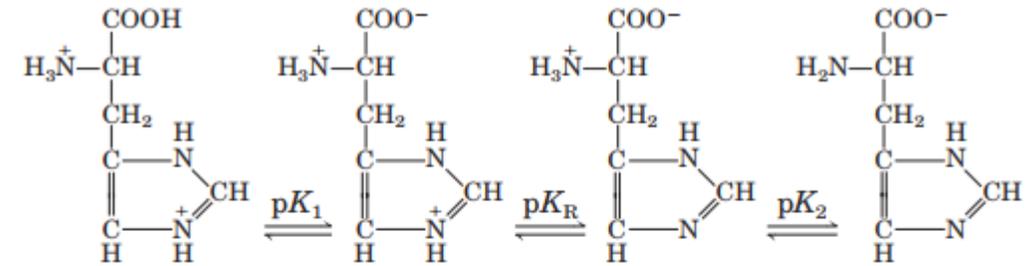
$pI = (pK_1 + pK_R)/2$

$pI = (2.19 + 4.25)/2$

$pI = 3.22$



- **Histidine** has a **pI** of **7.59** (the average of the **pKa values** of the **amino** and **imidazole groups**) ,much higher than that of glycine. This is due to the presence of **two amino group** (positively charged)



pK_1 carboxylic acid = 1.82

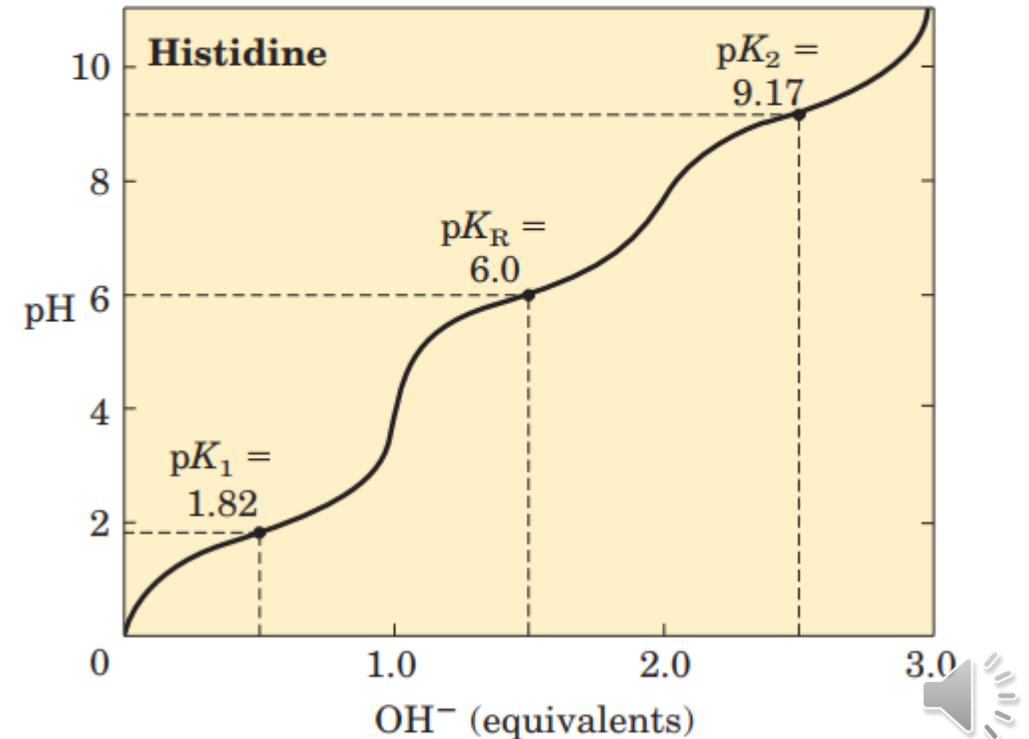
pK_R R group = 6.0

pK_2 amino group = 9.17

$pI = (pK_R + pK_2)/2$

$pI = (6.0 + 9.17)/2$

$pI = 7.59$



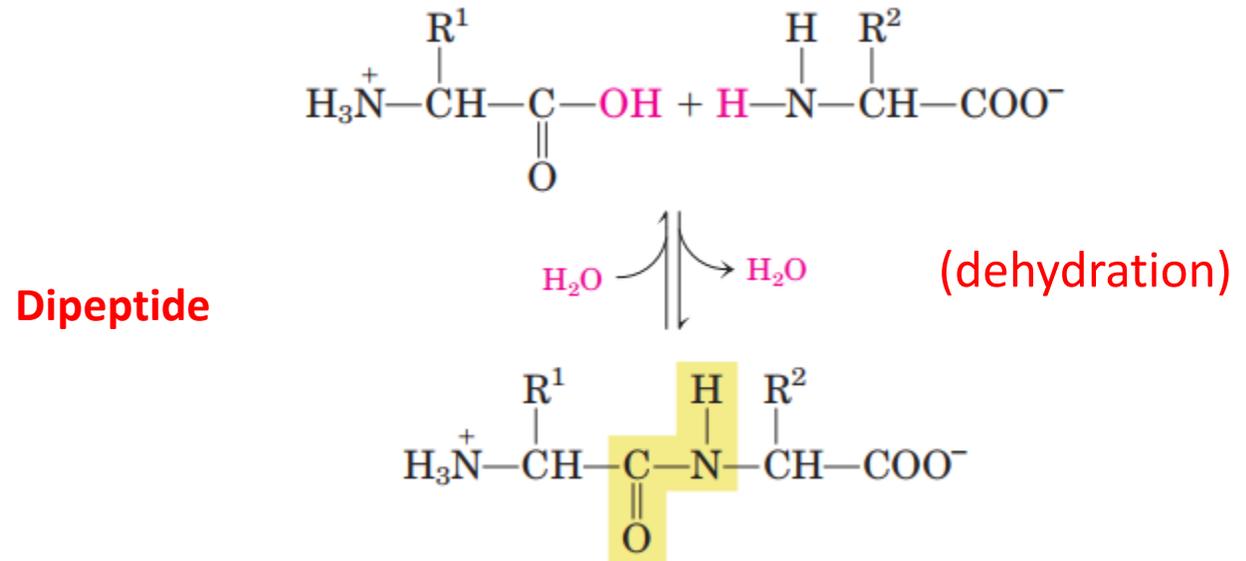
pKa for the common amino acids found in protein

Amino acid	Abbreviation/ symbol	M_r	pK_a values			pI	Hydropathy index*	Occurrence in proteins (%) [†]
			pK_1 (—COOH)	pK_2 (—NH ₃ ⁺)	pK_R (R group)			
Nonpolar, aliphatic								
R groups								
Glycine	Gly G	75	2.34	9.60		5.97	-0.4	7.2
Alanine	Ala A	89	2.34	9.69		6.01	1.8	7.8
Proline	Pro P	115	1.99	10.96		6.48	1.6	5.2
Valine	Val V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups								
Phenylalanine	Phe F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr Y	181	2.20	9.11	10.07	5.66	-1.3	3.2
Tryptophan	Trp W	204	2.38	9.39		5.89	-0.9	1.4
Polar, uncharged								
R groups								
Serine	Ser S	105	2.21	9.15		5.68	-0.8	6.8
Threonine	Thr T	119	2.11	9.62		5.87	-0.7	5.9
Cysteine	Cys C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn N	132	2.02	8.80		5.41	-3.5	4.3
Glutamine	Gln Q	146	2.17	9.13		5.65	-3.5	4.2
Positively charged								
R groups								
Lysine	Lys K	146	2.18	8.95	10.53	9.74	-3.9	5.9
Histidine	His H	155	1.82	9.17	6.00	7.59	-3.2	2.3
Arginine	Arg R	174	2.17	9.04	12.48	10.76	-4.5	5.1
Negatively charged								
R groups								
Aspartate	Asp D	133	1.88	9.60	3.65	2.77	-3.5	5.3
Glutamate	Glu E	147	2.19	9.67	4.25	3.22	-3.5	6.3



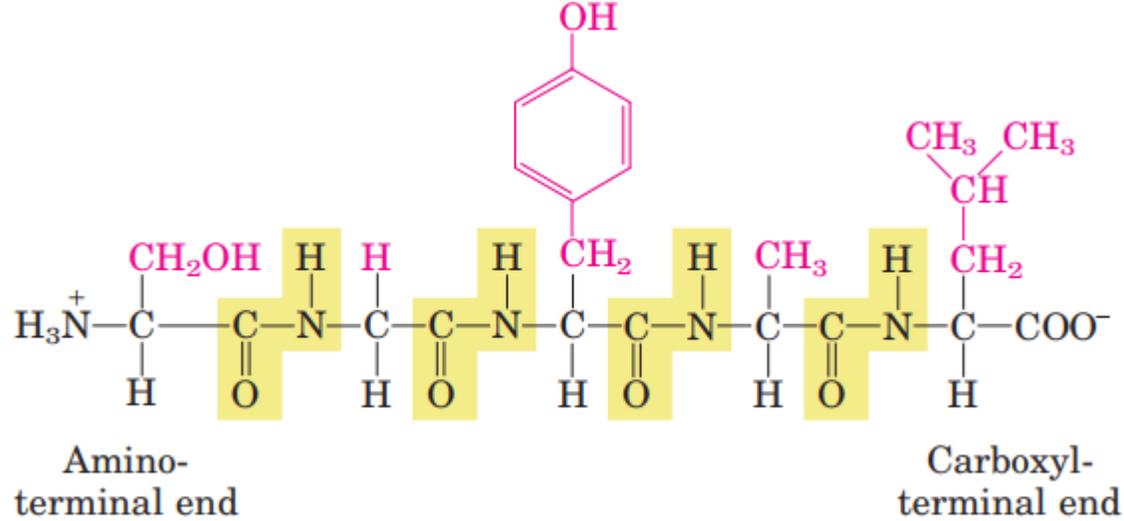
Peptides

- Peptides are chains of **amino acids**
- Two amino acid** molecules can be covalently joined by **peptide bond** to yield a dipeptide.
- Three amino acids** can be joined by **two peptide bonds** to form a **tripeptide** and so forth
- An amino acid unit in a peptide is often called **a residue** (the part left over after **losing a hydrogen atom** from its **amino group** and the **hydroxyl** (OH) moiety from its carboxyl group)



Formation of a peptide bond by condensation





The pentapeptide Ser-Gly-Tyr-Ala-Leu

- In a **peptide** amino acid residue at the end with a **free amino group** is the amino terminal or (**N-terminal**), the residue at the other end, which has a **free carboxyl group** is the carboxyl terminal (**C-terminal**).
- Peptides are named beginning with the amino terminal and the **sequence of amino acids in a polypeptide** is written starting with the **amino terminal** as **number one**.
- **Polymerization** of the 20 amino acids into polypeptide chain in cells is catalyzed by **enzymes** and is associated with the ribosomes (protein translation).



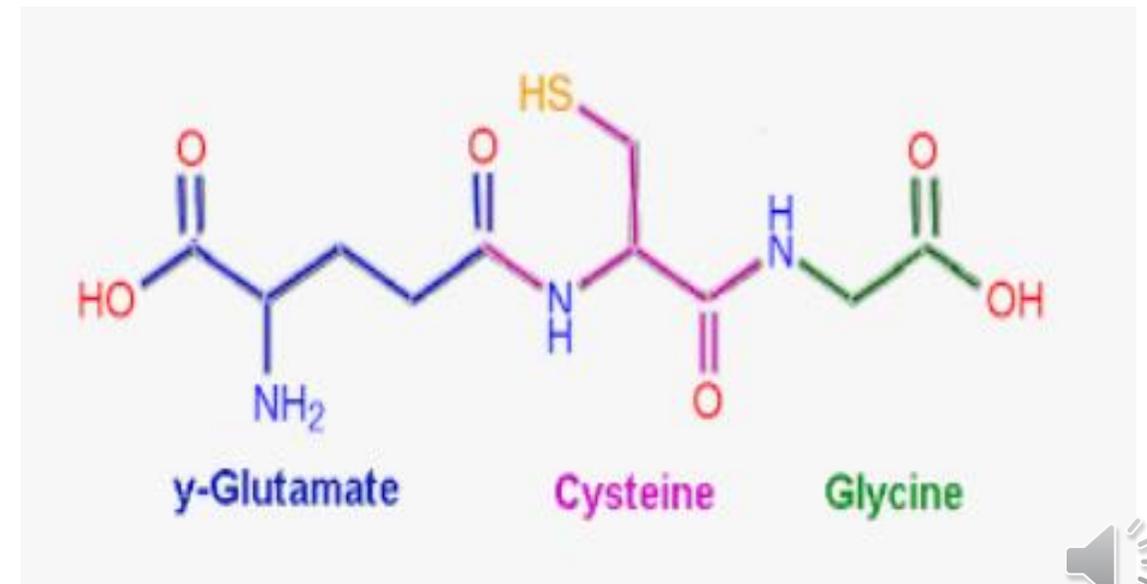
Classification of peptides

- **Oligopeptides:** Short peptides containing 2 to 10 amino acid units
- **Polypeptides:** More than 10 amino acid units

Biological Activity of small Peptides

- **Glutathione (GSH)** is an **antioxidant** in plants, animals, fungi, and some bacteria.
- Glutathione is capable of **preventing damage to important cellular components** caused by Reactive Oxygen Species (ROS) such as **free radicals, peroxides, lipid peroxides, and heavy metals**

Glutathione: tripeptide (Glutamic acid-Cysteine-Glycine)



Biological Activity of small Peptides

Oxytocin and Vasopressin

- Oxytocin and Vasopressin are a **nonapeptide hormones** contain **9 amino acids** which released by the **posterior pituitary**.
- **Oxytocin** is released into the bloodstream as a hormone in labor. Thus, It plays a role in **birth** (muscular contraction), **bonding with the baby, and milk production**.
- **Vasopressin** helps **water reabsorption by renal tubules**. It called “antidiuretic hormone **ADH**”



Proteins

- Proteins are **polymers** of amino acids
- Proteins range in size from **small peptides** to **very large polypeptides** chains of 100 to several thousand amino acid residues
- Proteins are the most abundant biological macromolecules occurring in all cells.

Classification of Proteins

Proteins can be classified according to

- **Protein Function**
- **Shape**
- **Chemical Composition**



Functions of Proteins

- **Enzymes** (Catalytic function)
 - Hexokinase, mitogen-activated protein (MAP) kinases, and cysteine proteases
- **Structural Proteins**
 - Collagen and tubulin
- **Transport oxygen in blood and muscles**
 - Hemoglobin and myoglobin
- **Receptors**
 - Toll-like receptors, insulin receptor
- **Membrane Transport Proteins**
 - Na⁺/K⁺-ATPase, porins
- **Hormones and Cytokines** (Regulatory function)
 - insulin and IL-1
- **Carrier proteins**
 - Albumin and ferritin
- **Antibodies** (Protective function)
 - IgG, IgM (immunoglobulin)



Shape of Protein

- **Fibrous proteins**

- Water insoluble
- Have a role as structural elements, e.g. Collagen, elastin, α -keratin and silk fibroin

- **Globular proteins**

- water soluble
- biologically active, e.g. Insulin, albumin, globulins and many enzymes



Chemical Composition of Proteins

Proteins are classified according to their chemical composition into

- Simple Proteins.
 - Conjugated Proteins.
 - Derived Proteins
-
- Simple Proteins
 - Contain only amino acid residues and no other chemical constituents for example the enzymes ribonuclease A and chymotrypsinogen



■ Conjugated Proteins

- They are combinations of proteins with a **non- protein part**
- The **non-amino acid part** of a conjugated protein is usually called its **prosthetic group**
- **Conjugated proteins are classified** on the basis of the chemical **nature of their prosthetic groups** (table below)
- Usually the **prosthetic group** plays an important role in the **protein's biological function**

<i>Class</i>	<i>Prosthetic group</i>	<i>Example</i>
Lipoproteins	Lipids	β_1 -Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin



- **Derived proteins:**

- They are degradation products of native proteins as denatured proteins or hydrolytic products as peptones and peptide.



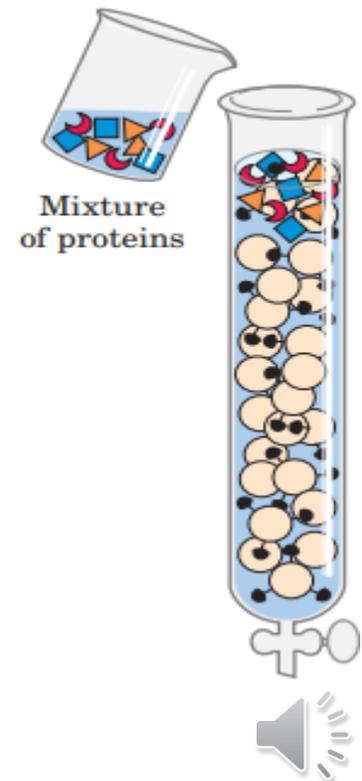
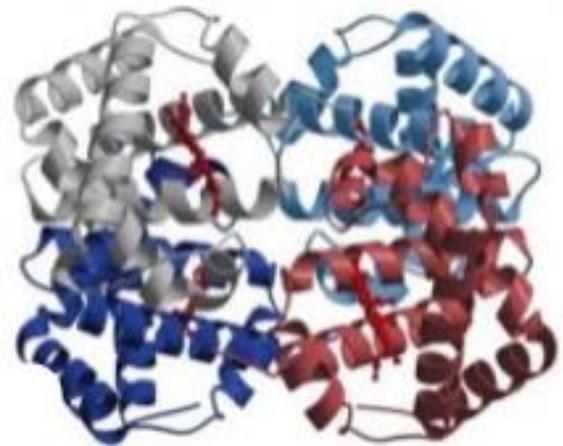
Lecture 7: Biochemistry I

Biochemistry of Proteins

3rd Class

Anbar University-College of Pharmacy-Clinical Laboratory Sciences Department
2020-2021

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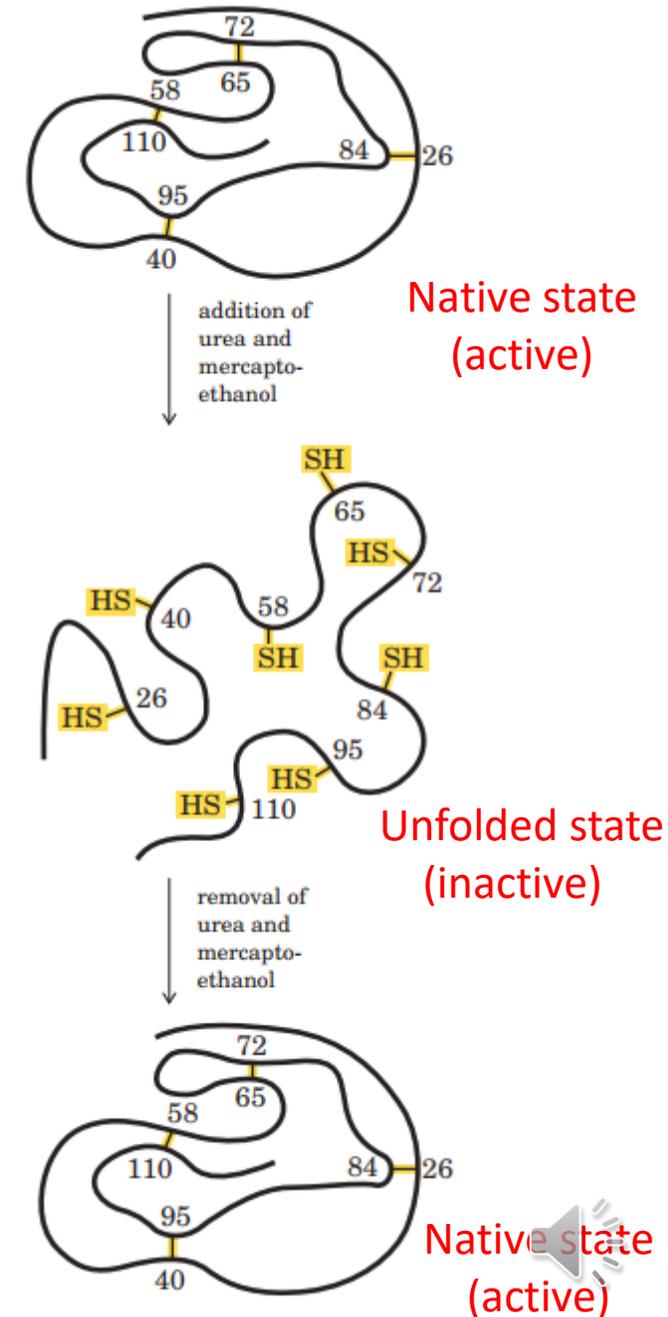
Lecture outline

- **Protein Denaturation**
- **Protein Purification**
- **Amino Acid Sequences**
- **Protein Structure**



Protein Denaturation

- A loss of 3-dimensional structure sufficient to cause loss of function is called **denaturation**.
- Most proteins can be denatured by:
 - **Heat**, which affects the weak interactions in a protein (**primarily hydrogen bonds**)
 - **Extremes of pH**
 - **Organic solvents** such as alcohol or acetone
 - **Solutes** such as urea and guanidine hydrochloride
 - **Detergents**
- ❖ **Organic solvents, urea, and detergents** act primarily by **disrupting the hydrophobic interactions** that make up the **stable core** of globular proteins
- ❖ **Extremes of pH** alter the **net charge on the protein**, causing electrostatic repulsion and the disruption of some **hydrogen bonding**.
- ❖ Loss of **protein structure** results in **loss of function**
- Denaturation of some proteins is **reversible**. This process is called **renaturation**.



Protein purification

- A **pure protein** is essential before its **properties and activities** can be determined.
- **How can one protein be purified from a complex mixture of proteins?**
- Proteins can be purified according to **solubility, size, charge, and binding properties**
- Usually, protein mixtures are subjected to a series of separations, each based on a different property to yield a pure protein.
- The **source of a protein** is generally **tissue or microbial cells**. The first step in any protein purification procedure is to **break open** these cells, releasing their proteins into a solution called a **crude extract** (Centrifugation)
- Commonly, the extract is subjected to treatments that separate the proteins into different fractions based on a property such as **size or charge**, a process referred to as **fractionation**



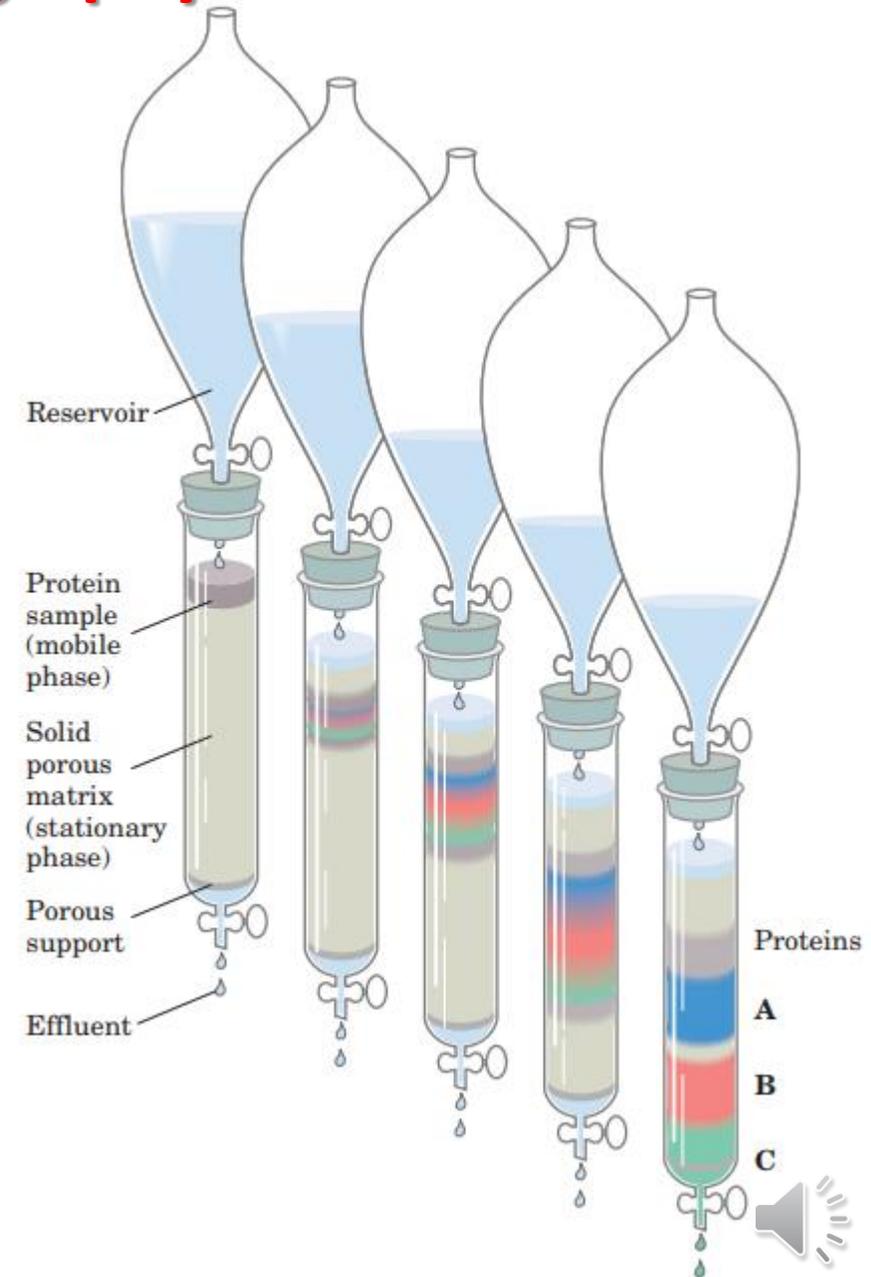
Protein Purification

- Early **fractionation steps** in a purification utilize differences in **protein solubility**, which is a complex function of **pH, temperature, salt concentration** ($(\text{NH}_4)_2\text{SO}_4$), and other factors
- The **solubility of proteins** is generally **lowered** at **high salt concentrations** “**salting out.**” For example, **0.8 M** ammonium sulfate precipitates **fibrinogen**, a blood-clotting protein, whereas a concentration of **2.4 M** is needed to precipitate **serum albumin**.
- Proteins can be separated from **small molecules** by **dialysis** through a semipermeable membrane (bag or tube), such as a cellulose membrane with pores. **Molecules** having dimensions significantly **greater** than the pore diameter are **retained inside the dialysis bag**, whereas **smaller molecules and ions** traverse the pores of such a membrane and emerge in the dialysate outside the bag. This technique is useful for **removing a salt** or other small molecule from the protein preparation



Fractionating Proteins by Column Chromatography

- The standard elements of a chromatographic column include a solid porous material supported inside a column, generally made of plastic or glass.
- The solid material (matrix) makes up the stationary phase through which flows a solution (the mobile phase)
- The solution that passes out of the column at the bottom (the effluent) is constantly replaced by solution supplied from a reservoir at the top
- The protein solution to be separated is layered on top of the column and allowed to percolate into the solid matrix
- Additional solution is added on top. The protein solution forms a band within the mobile phase
- As proteins migrate through the column, they are retarded to different degrees by their different interactions with the matrix material. The overall protein band thus widens as it moves through the column
- Individual types of proteins (such as A, B, and C) gradually separate from each other, forming bands within the broader protein band
- Separation improves (resolution increases) as the length of the column increases. However, each individual protein band also broadens with time due to diffusional spreading, a process that decreases resolution.
- In this example, protein A is well separated from B and C, but diffusional spreading prevents complete separation of B and C under these conditions.



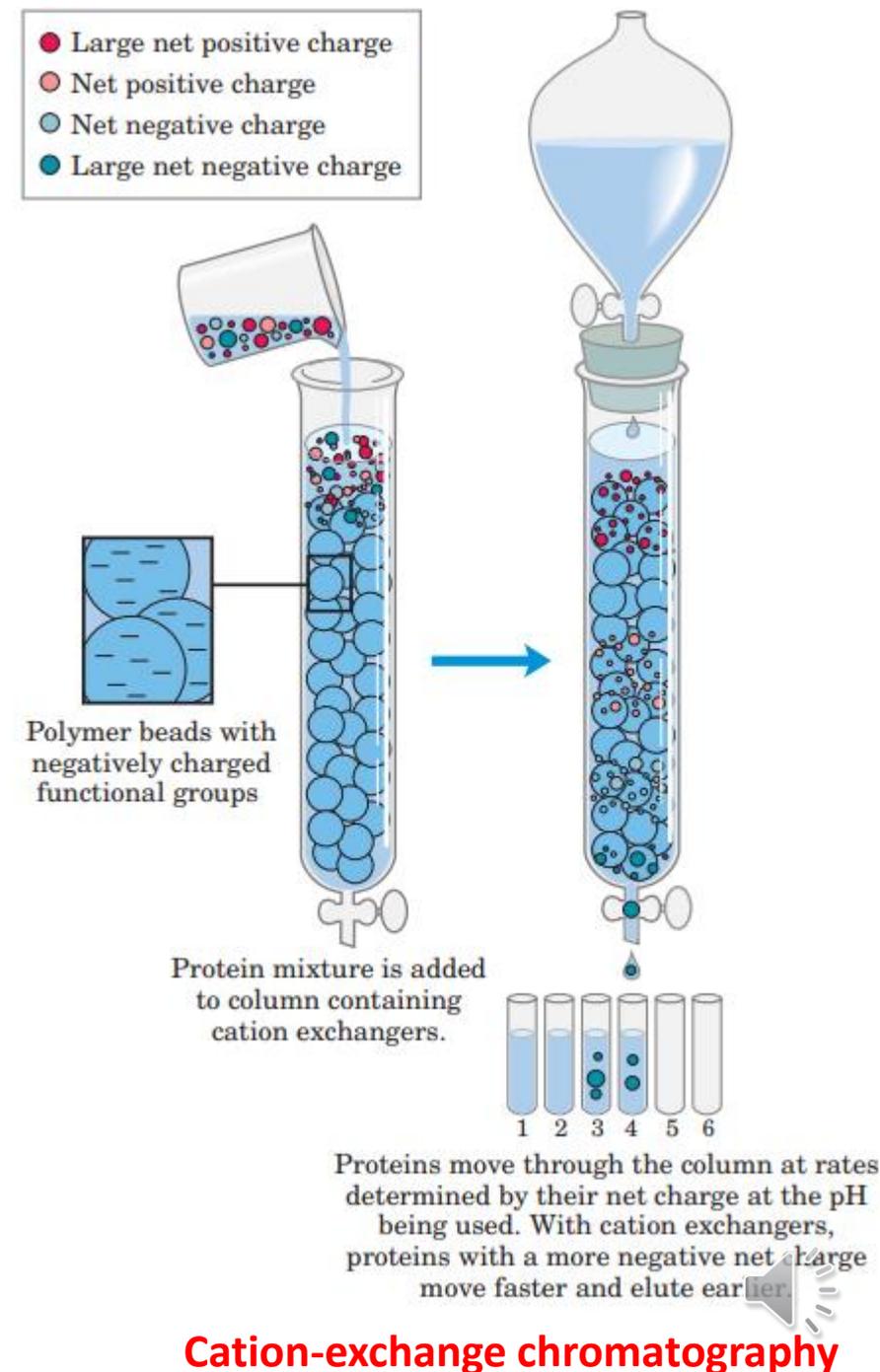
Column Chromatography

- Ion-exchange chromatography
 - Size exclusion chromatography (also called gel filtration)
 - Affinity chromatography
 - High-performance liquid chromatography (HPLC)
- In most cases, several different methods must be used sequentially to purify a protein completely, and many protocols may be tried before the most effective one is found



Ion-exchange chromatography

- Proteins can be separated on the basis of their **net charge** at a **given pH**
- The **column matrix (stationary phase)** is a synthetic polymer containing **bound charged groups**; those with **bound anionic groups** are called **cation exchangers**, and those with **bound cationic groups** are called **anion exchangers**
- In the **mobile phase** (protein solution), **proteins with a net positive charge migrate** through the matrix more **slowly** (due to its **interaction with the stationary phase**) than those with a net negative charge
- The rate at which the **protein solution can flow** through the **column** usually decreases with **column length (More time)**
- The resolution can decline as a result of diffusional spreading within each protein band



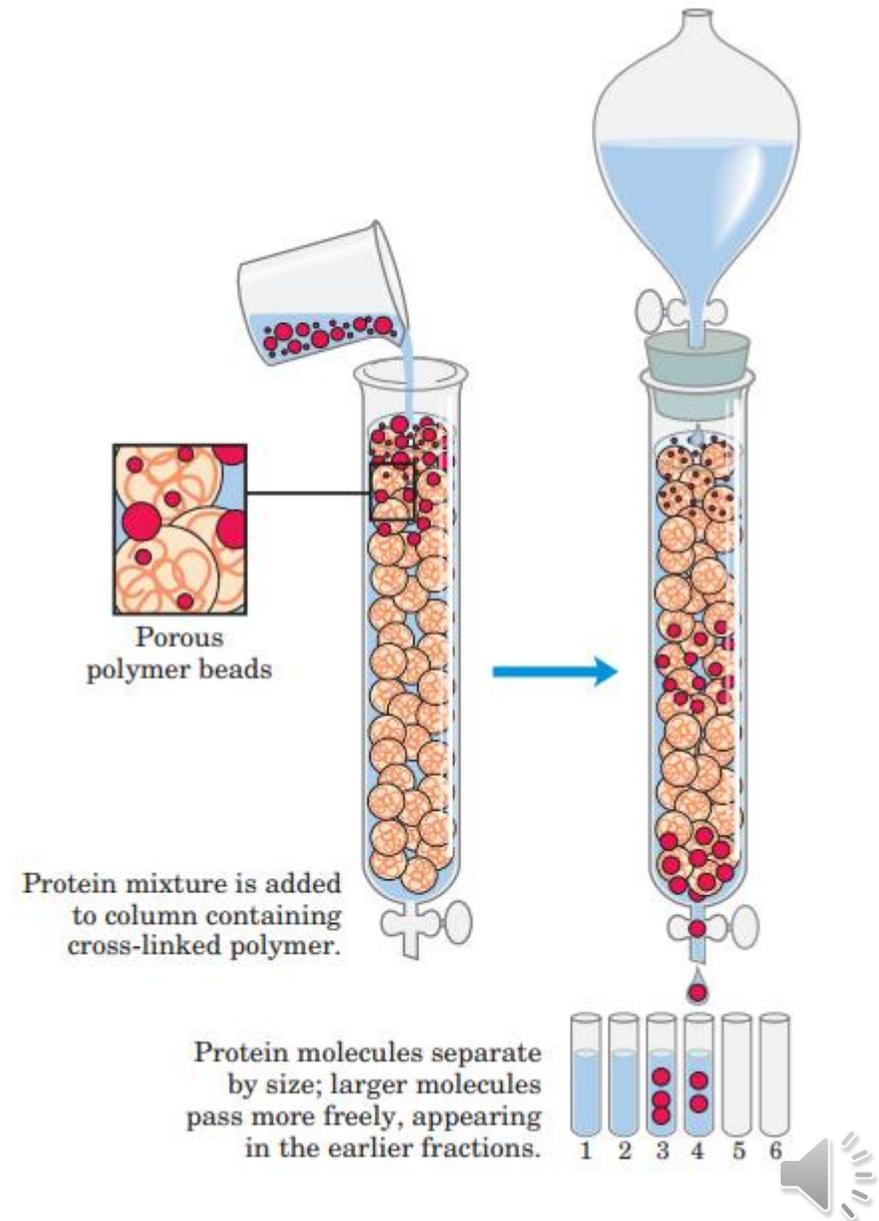
Ion-exchange chromatography

- The **affinity** of each protein is affected by the **pH** (which determines the ionization state of the molecule) and the concentration of competing **free salt ions** in the surrounding solution.
- Separation can be optimized by gradually **changing the pH and/or salt concentration** of the mobile phase
- **Proteins** that have a **low density** of **net positive charge** will tend to **emerge first**, followed by those having a **higher charge density**
- **Positively charged proteins** (cationic proteins) can be separated by chromatography on negatively charged carboxy methyl-cellulose (**CM-cellulose**) Columns
- **Negatively charged proteins** (anionic proteins) can be separated by chromatography on positively charged Diethylaminoethyl cellulose (DEAE-C) columns



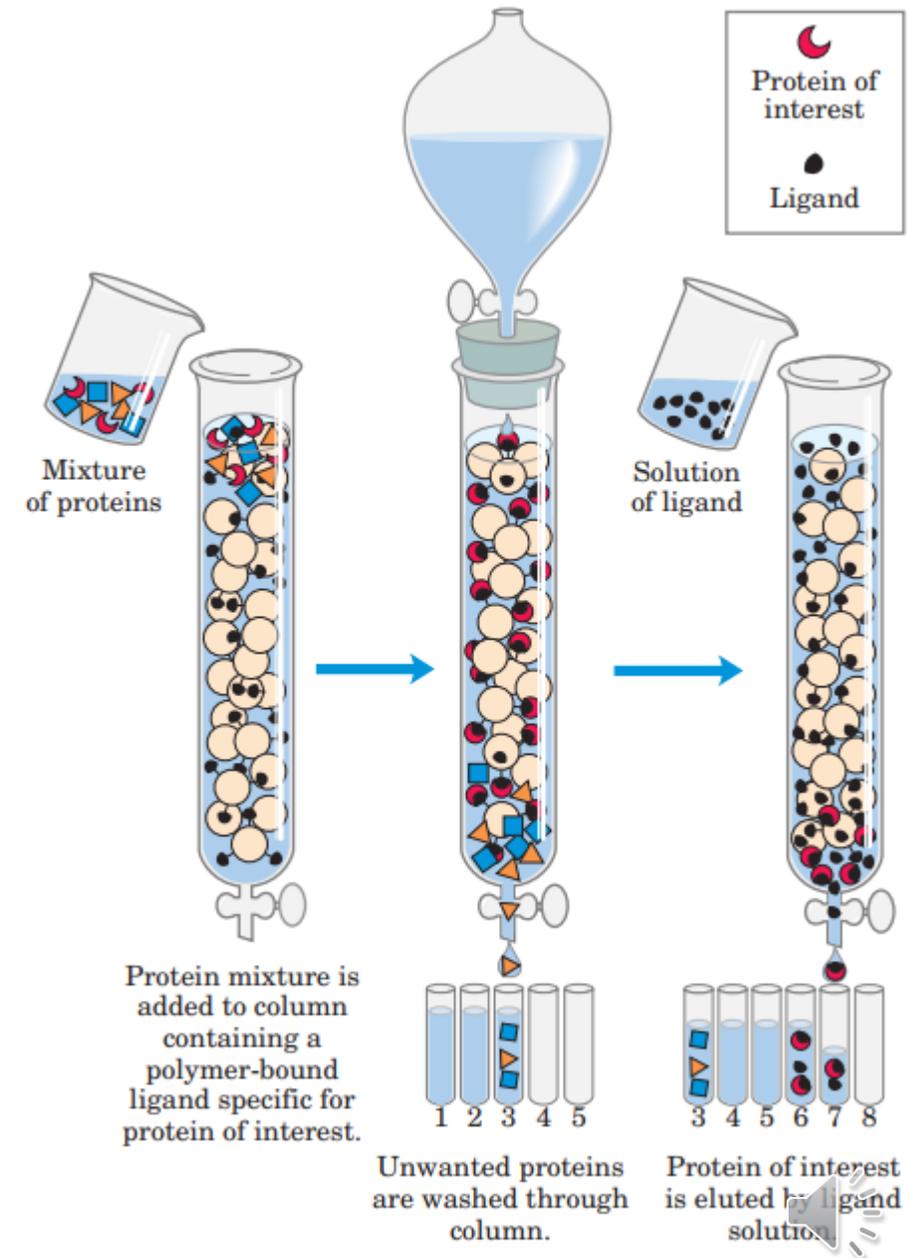
Size exclusion chromatography (also called gel filtration)

- Separates proteins according to **size**
- The sample is applied to the **top of a column** matrix (solid phase) consisting of **porous beads made of an insoluble** but highly hydrated polymer such as **dextran or agarose** (which are carbohydrates) or **polyacrylamide**
- **Larger proteins** migrate **faster** than **smaller** ones, because they are too large to enter the pores in the beads and hence take a more **direct route** through the column, around the beads
- **Small proteins** enter the cavities, and migrate through the column more **slowly** as a result



Affinity chromatography

- It is based on the binding **affinity** of a protein.
- The **beads** in the column have a **covalently attached** chemical group.
- A **protein with affinity** for this particular chemical group will **bind to the beads** in the column (bind to a ligand cross-linked to the beads), and its migration will be **retarded** as a result
- After proteins that do not bind to the **ligand** are washed through the column, the **bound protein** of particular interest is eluted (washed out of the column) by a solution containing free ligand



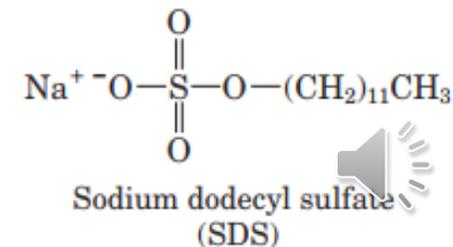
high-performance liquid chromatography (HPLC)

- HPLC is a modern chromatographic methods
- The column materials themselves are much more finely divided and, as a consequence, there are more interaction sites and thus greater resolving power.
- HPLC makes use of high-pressure pumps that speed the movement of the protein molecules down the column
- It has Higher-quality chromatographic materials that can withstand the crushing force of the pressurized flow. By reducing the transit time on the column
- HPLC can limit diffusional spreading of protein bands and thus greatly improve resolution.



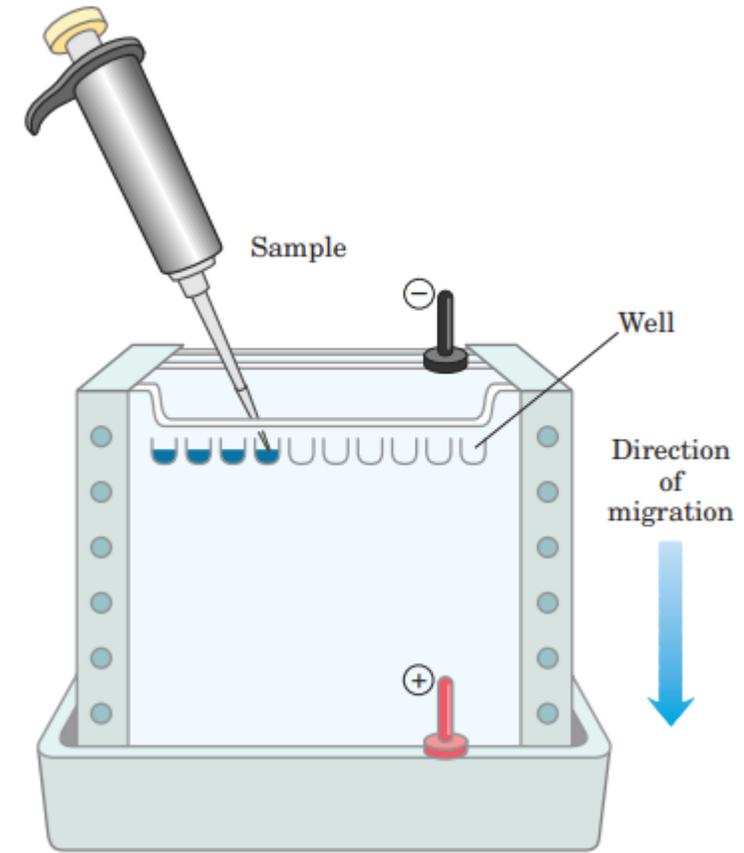
Proteins can be separated by Electrophoresis

- It is **based** on the migration of charged proteins in an **electric field**.
- **Electrophoresis of proteins** is generally carried out in **gels** made up of the cross-linked polymer **polyacrylamide**
- The **polyacrylamide gel** acts as a **molecular sieve**, **slowing the migration** of proteins approximately in proportion to their **charge-to-mass ratio**
- **Sodium dodecyl sulfate** (SDS) is employed for estimation of purity and molecular weight
- **SDS binds to most proteins** in amounts roughly proportional to the **molecular weight** of the protein, about **one** molecule of SDS for every **two** amino acid residues
- **The bound SDS** contributes a **large net negative charge**, rendering the intrinsic charge of the protein insignificant. In addition, the **native conformation** of a protein is **altered** when SDS is bound, and most proteins assume a **similar shape**
- **Electrophoresis** in the presence of **SDS** therefore **separates proteins** almost exclusively on the basis of **mass (molecular weight)**. Smaller polypeptides migrating more rapidly



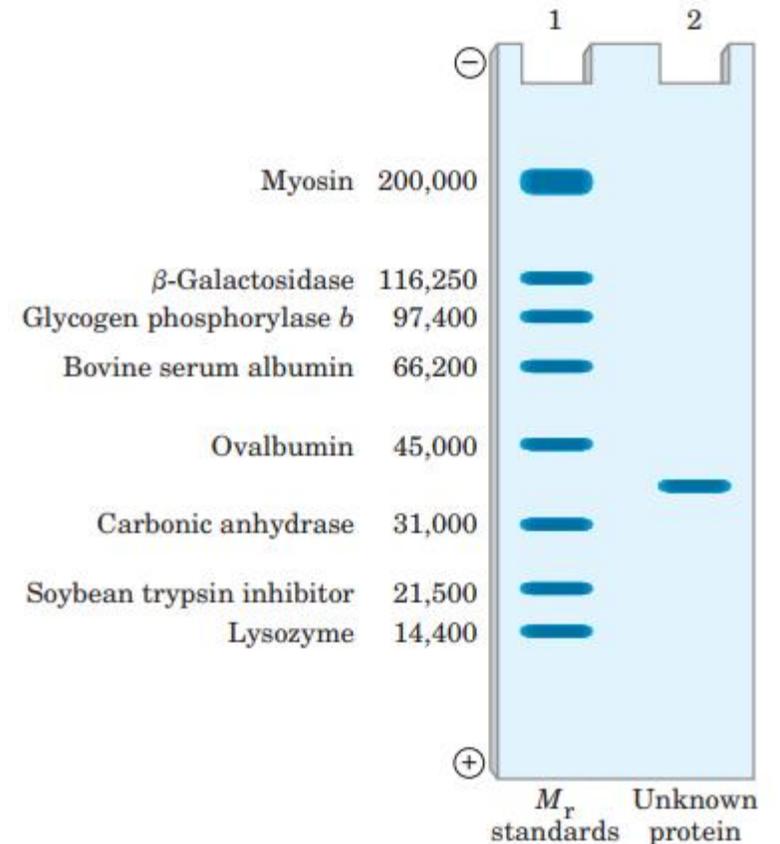
Electrophoresis

- **Electrophoresis** separates proteins on the basis of **molecular weight**
- Different samples are loaded in the wells at the **top** of the **polyacrylamide gel**.
- The **proteins move** into the **gel** when an **electric field is applied**.
- The **gel** minimizes convection currents caused by **small temperature gradients**, as well as protein movements other than those induced by the electric field.
- Proteins can be **visualized** after electrophoresis by treating the gel with a **stain** such as **Coomassie blue**, which binds to the proteins but not to the gel itself
- Each band on the gel represents a **different protein** (or protein subunit); **smaller proteins** move through the gel **more rapidly** than **larger proteins** and therefore are found nearer the **bottom of the gel**.



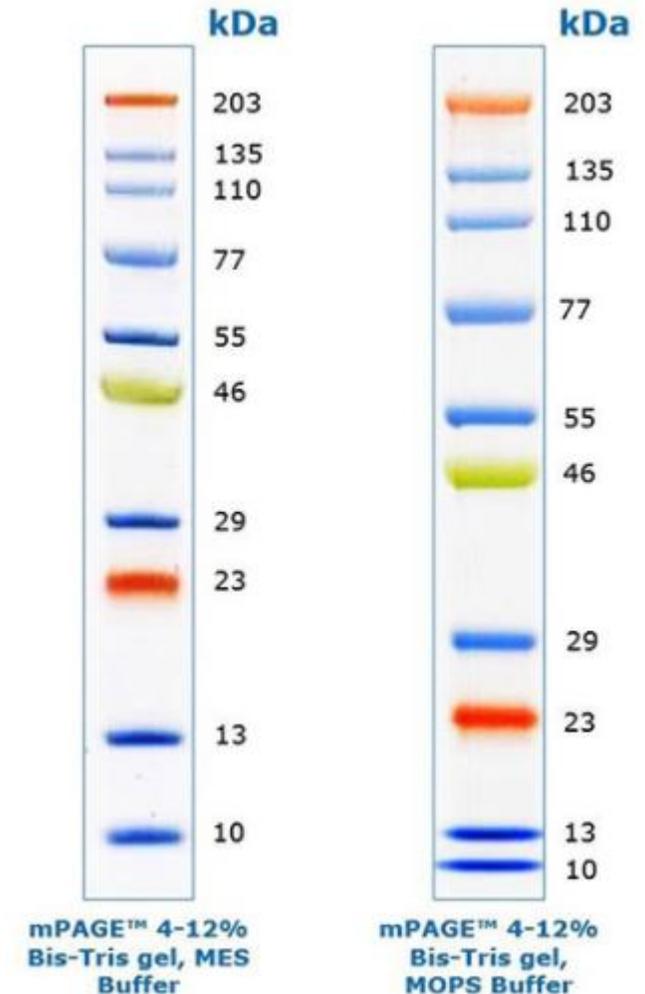
Estimating the molecular weight of a protein

- The **electrophoretic mobility** of a protein on an SDS polyacrylamide gel is related to its **molecular weight**
- **Standard proteins** of known molecular weight are subjected to electrophoresis (**lane 1**). These marker proteins can be used to estimate the molecular weight of an **unknown protein (lane 2)**.
- **Protein marker** (also called a protein molecular weight marker, a protein MW marker, or a protein ladder) is used to **estimate the size of proteins** resolved by gel electrophoresis and to **monitor transfer efficiency from gel to blotting membrane**.



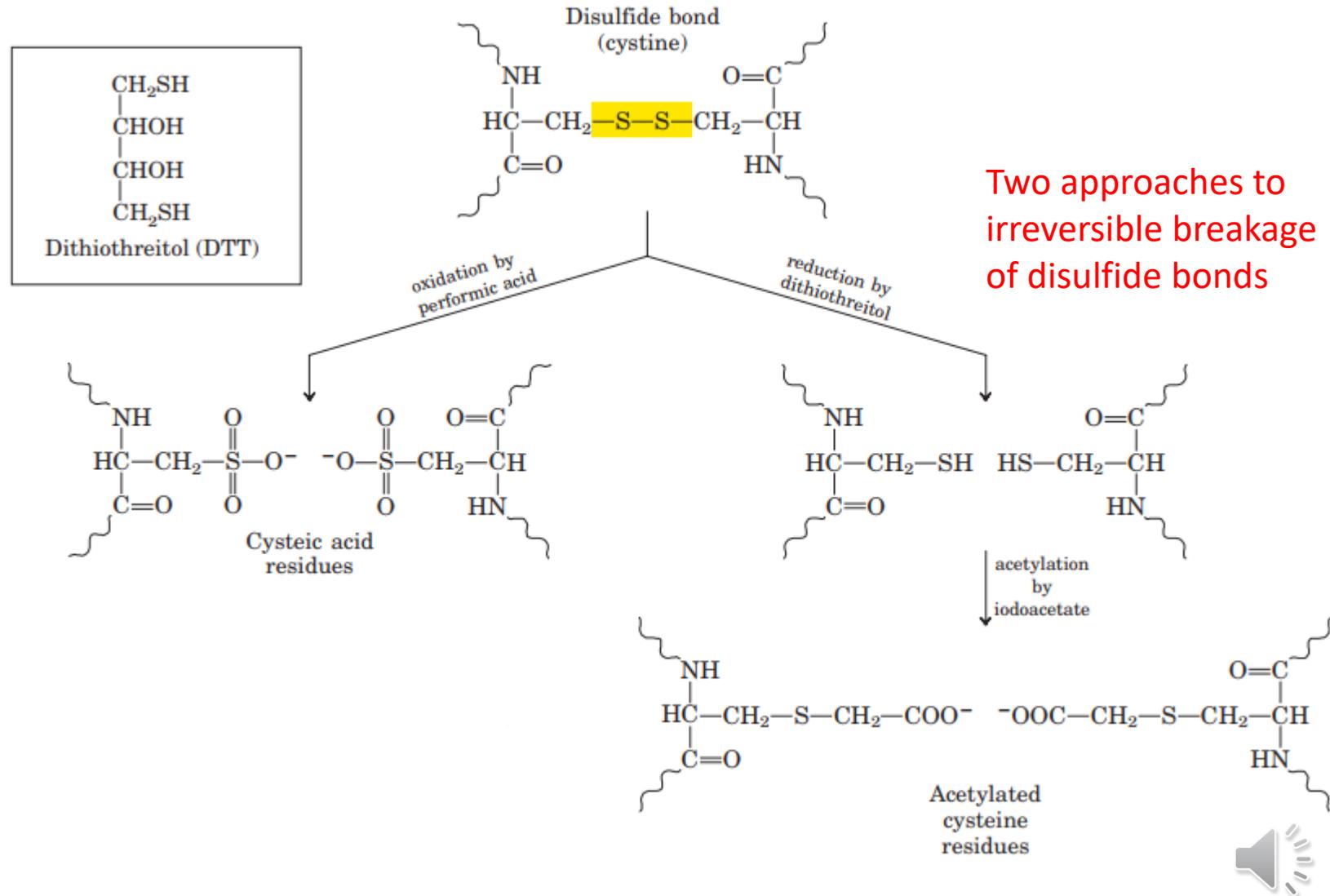
Colour Protein Standard

- mPAGE™ Color Protein Standard is a mixture of ten recombinant prestained proteins from 10 kDa to 203 kDa, covalently coupled with different chromophores. It is designed for observing protein separation during SDS-PAGE, verifying membrane transfer efficiency, and approximating the molecular weight of proteins.



Determination of the amino acid composition of a polypeptide

- **Sanger method:** Sanger was the first to determine the **sequence of a polypeptide**.
- Identification of the **amino-terminal residue** can be the first step in sequencing a polypeptide.
- **Insulin** consists of the **21 amino acids (A chain)** and the **30 amino acids (B chain)** joined by **disulfide bonds**.
- Sanger reduced the disulfide bonds by either **oxidation** or **reduction** followed by acetylation



- Separated the A and B chains, and cleaved each chain into smaller peptides using trypsin, chymotrypsin, and pepsin
- The resulting peptides were then isolated and treated with acid to hydrolyze peptide bonds and generate peptides with as few as two or three amino acids
- Each peptide was reacted with 1-fluoro-2,4-dinitrobenzene (FDNB) (Sanger's reagent), which derivatizes the exposed α -amino group of amino terminal residues. The amino acid content of each peptide was then determined.



Amino acid sequences can be determined by Edman Degradation

- The **Edman degradation** procedure **labels and removes** only the **amino-terminal residue** from a peptide, leaving all other **peptide bonds intact**
- The peptide is reacted with **phenylisothiocyanate** (Edman's reagent) under mildly **alkaline** conditions, which converts the **amino terminal** amino acid to a **phenylthiocarbamoyl (PTC)**
- The **peptide bond** next to the PTC adduct is then cleaved in a step carried out in **anhydrous trifluoroacetic acid**, with removal of the **amino-terminal amino** acid as an **anilinothiazolinone** derivative
- The derivatized amino acid is extracted with **organic solvents**, converted to the more stable **phenylthiohydantoin** derivative by treatment with aqueous acid, and then **identified**.
- After removal and identification of the amino terminal residue, the **new amino-terminal** residue so exposed can be **labeled, removed, and identified through the same series of reactions**
- **This procedure is repeated** until the entire **sequence is determined**
- **Edman degradation** is carried out on a machine, called a **sequenator**,
- **Edman degradation** procedure reveals the **entire sequence of a peptide (shorter peptides, 50-70 amino acids)**, larger polypeptides are often fragmented into smaller peptides for sequencing



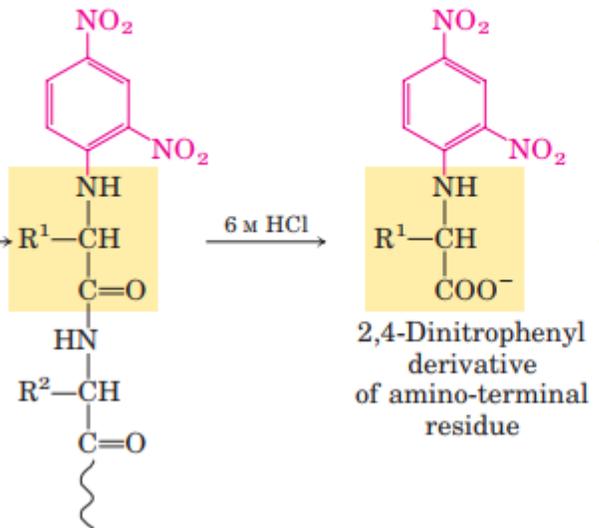


Polypeptide

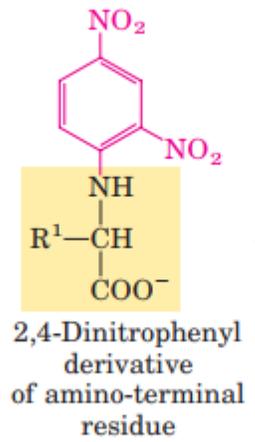
Sanger's method



2,4-Dinitrophenyl derivative of polypeptide



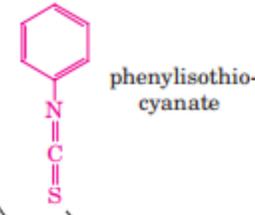
6 M HCl



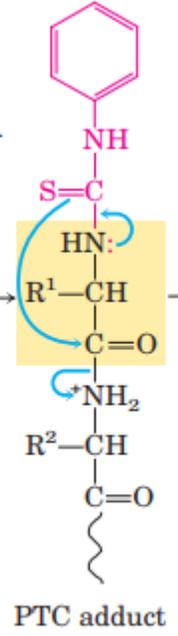
+ Free amino acids

Identify amino-terminal residue of polypeptide.

Edman Degradation

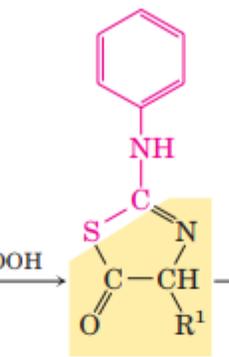


⁻OH



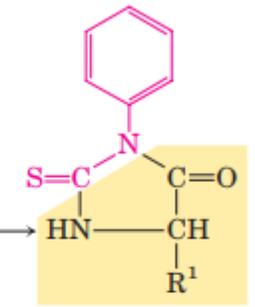
PTC adduct

CF₃COOH



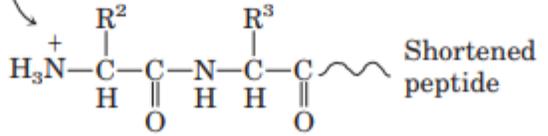
Anilinothiazolinone derivative of amino acid residue

H⁺



Phenylthiohydantoin derivative of amino acid residue

Identify amino-terminal residue; purify and recycle remaining peptide fragment through Edman process.



Shortened peptide



Structural levels of proteins

- Proteins regardless of their function or biologic activity are built up from the same basic set of the 20 standard amino acids
- ❖ **What gives a protein enzymatic activity, another protein hormonal action , and antibody activity ?**
- Quite simply proteins differ from each other because each **has a distinctive amino acid sequence (primary structure)** which direct folding of the protein once synthesized in the cell.



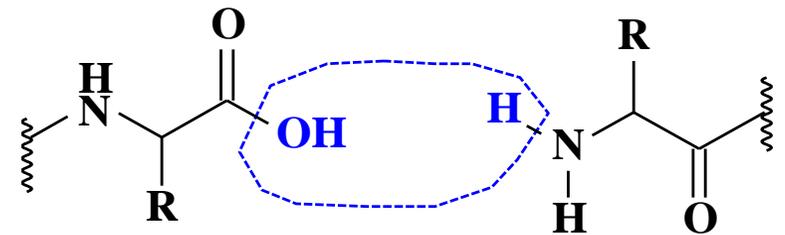
Structural levels of proteins

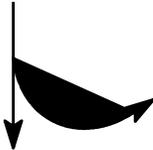
- **Four levels** of protein structure are commonly defined
 - **Primary structure** involves the linking of L-amino acids by **peptide bonds** between **-COOH** and **-NH₂** groups.
 - **Secondary structure:** Proteins are highly organized structures with some degree of rigidity.
 - **Tertiary structure:** Proteins are not loose, floppy chains of amino acids.
 - **Quaternary structure:** The higher levels of structure are critical for determining a protein's function.

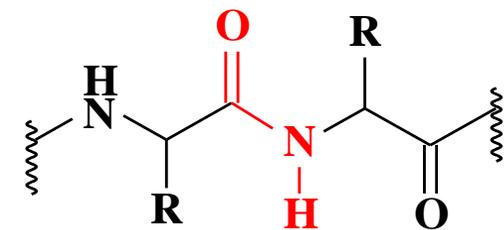


Primary Structure and Peptide Bonds

- The primary structure of a protein is **the sequence of the amino acids present in polypeptide**.
- It is written from the **N-terminus** to the **C-terminus**.
- The amino acids are joined by **peptide bonds** between $-\text{COOH}$ and $-\text{NH}_2$ groups
- The primary structure of a protein is the linear sequence of amino acids in the polypeptide chain.



Condensation
reaction  H_2O



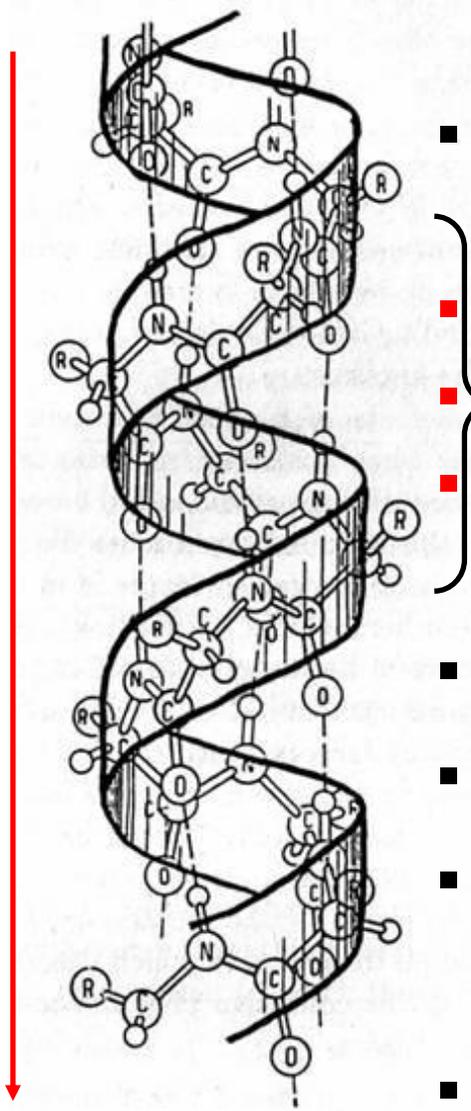
Secondary Structure

- Secondary structure refers to the **special stable arrangements** of amino acid by twisting of the polypeptide chain.
- There are **3 main types** of secondary structure:
 - **α -Helix**
 - **β -Sheet**
 - **Loops and Turns**
- These structures are formed by **bending or coiling** of the amino acid chain, which involves **rotation** around the **N-C α** and the **C α -C** bonds.
- The amount of rotation allowed depends on steric hindrance from the R (rest) groups of the amino acids. Hence the **primary structure** also determines the **secondary structure**



The α -Helix

N-term

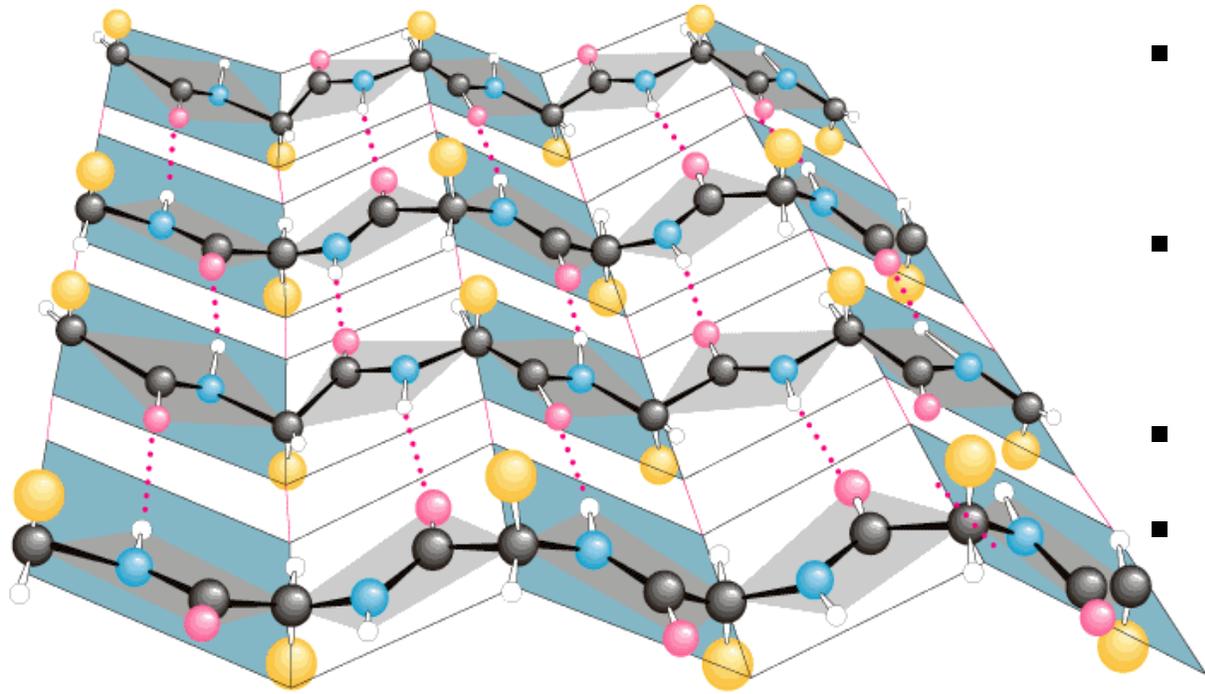


C-term

- The peptide bonds form the backbone (**primary structure**)
- **right-handed helix**
- **3.6** residues / turn
- **5.4 Å** pitch (distance)
- R groups stick outside the spiral structure
- The helical structure is **stabilized by intrachain hydrogen bonds**
- H-bonding is between the **C=O** of one amino acid and the **N-H** of another **four** residues forward in the chain
- Typically about 12-15 residues long, i.e. 3-4 turns and ~ 18 Å (1.8 nm).
- Segments, are found in many globular protein like myoglobin

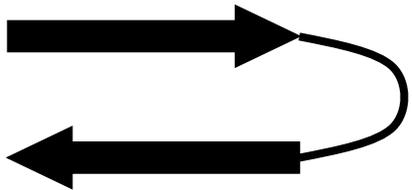


β -Pleated Sheet Structure (β -sheet)



- The polypeptide chains in the β -pleated sheets is fully extended into **zigzag structure**.
- The zigzag polypeptides are arranged side by side to form a series of **pleats**.
- Typically: **6-10** residues per strand and **2-10 strands / sheet**
- Can occur between **separate peptide chains** (e.g. silk fibroin) or between **segments** of the same peptide chain where it fold back upon itself " β -turn".
- **Two types** of β -pleated sheets exist : **parallel and antiparallel**
- The H-bonded structure is relatively strong and rigid so it is an important part of many fibrous proteins.
- They are stabilized by **H bond between N-H and carbonyl** groups of adjacent chains

- Parallel β -sheet: H-bonded chains extend in the same direction
- Antiparallel β -sheet: opposite direction



antiparallel β -sheet



parallel β -sheet

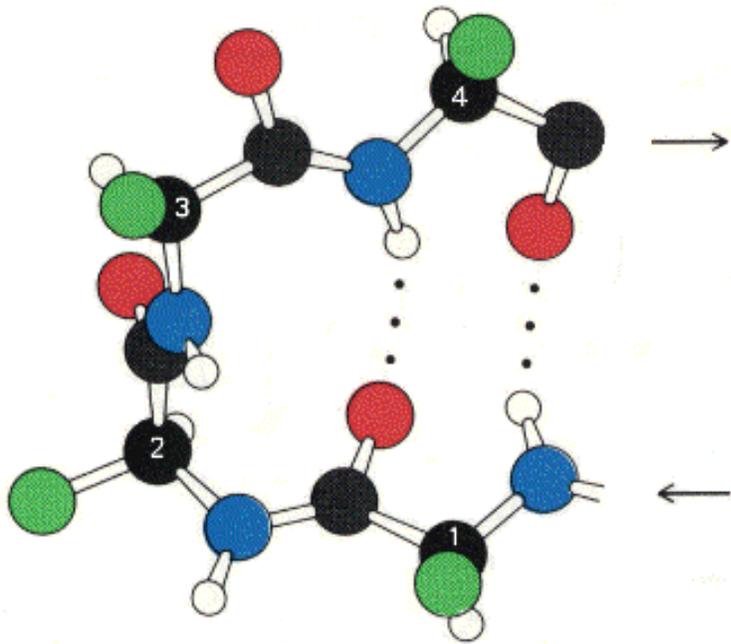


Loops and Turns

- α -helices and β -sheets are **stiff structures** that don't bend easily.
- More flexible parts to connect them are needed for a globular structure – **loops and turns**.
- Non-repetitive structures, but still quite ordered.
- These often contain **Gly** and **Pro**, which are “helix-breakers” (permit an acute angle at turn)
- **Turns** are short and ordered, containing 3-4 residues
- **Loops/ Coils** are longer (>6 residues) and more disordered

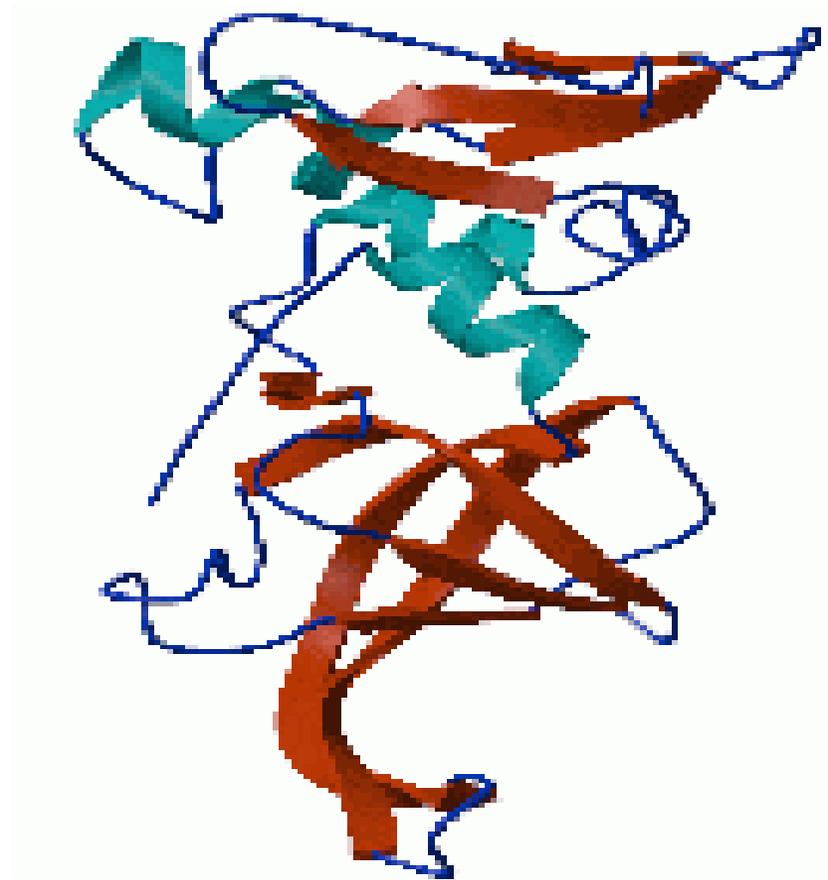


The β -Turn



The **C=O** group of residue **1** of the **tetrapeptide** forms a hydrogen bond with **N-H** of residue **4** and causes a **hairpin turn**

Loops & Coils



Tertiary Structure of Protein

- Defined as: **3-D** folding of the secondary structural elements to give the final, **native conformation**
- The tertiary structure is maintained by
 - Ionic bonds
 - Hydrogen bonds
 - Hydrophobic interactions
 - Van der Waals forces
 - Disulfide bridges.
- Most proteins contain both **α -helices** and **β -sheets** (on average 30% of each)



General Rules About 3 Structure

- Helices and sheets run across the protein.
- Turns and loops are usually at the edges.
- The core is densely packed and water is excluded
- The core has some flexibility but the edges are much looser.
- Cytosolic proteins are polar on the surface and hydrophobic inside.
- Membrane proteins are hydrophobic on the surfaces that are in contact with the membrane.



3 Structure is determined by sequence

- Some simple principles contribute to determining the tertiary structure :
 - **Hydrophobic side chains** do not like contact with water, and prefer to be buried **inside proteins**.
 - **Polar side chains** are hydrophilic and prefer to be **on the surface in contact with water**.
- Unless it is a membrane protein, in which case the external environment is hydrophobic, so the opposite is true.

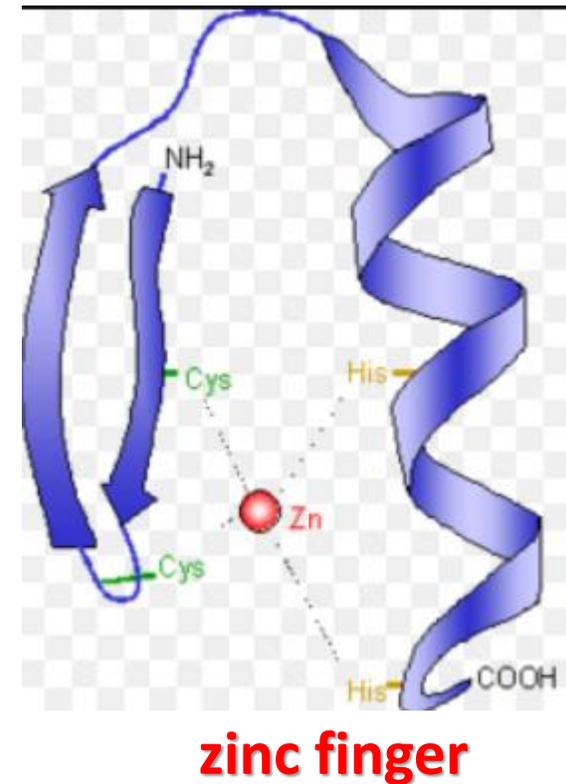


- There are **two** general classes of proteins based on **tertiary structure**: **fibrous and globular**.
- **Fibrous proteins**, which serve mainly structural roles, have **simple repeating elements of secondary structure**.
- **Globular proteins** have **more complicated** tertiary structures, often containing several types of **secondary structure in the same polypeptide chain**.

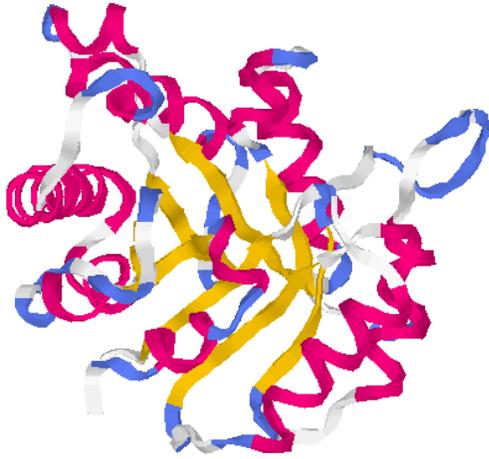


Within 3 Structure there are also Motifs and Domains

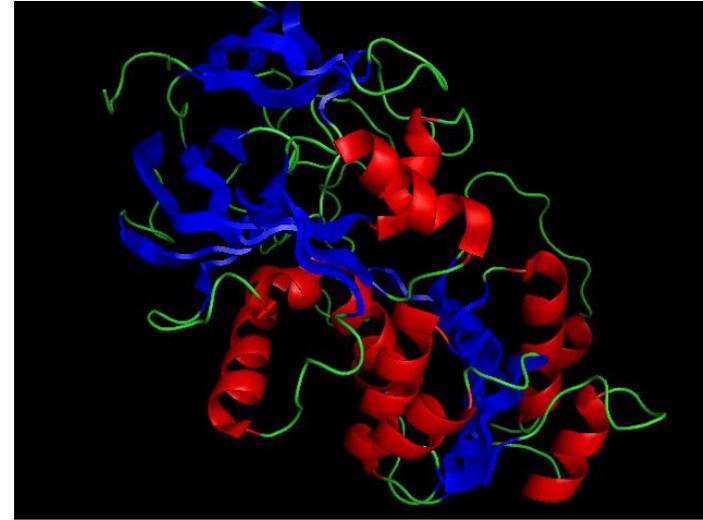
- **Motifs** are also called **Super-secondary elements**. They are combinations of a few elements of secondary structure. Examples: **helix-loop-helix** and **zinc finger**
- **Domains** are larger structures can fold stably and independently, often with discrete functions or activities. Examples: dehydrogenase domain
- **Motifs** and **domains** are important in providing help in **folding and providing stability to the protein**. They have also been important in the **evolution of proteins**.



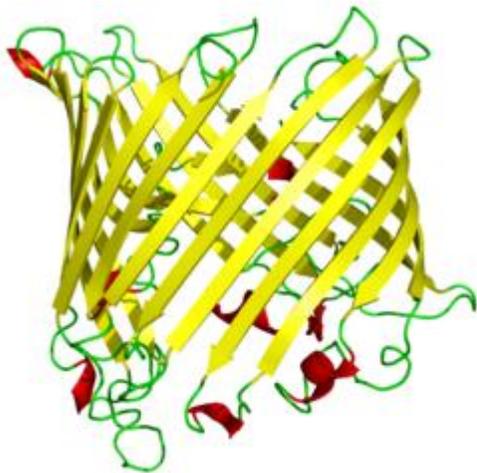
Some examples of globular 3 Structure



Triose Phosphate Isomerase



Liver Alcohol Dehydrogenase



Sucrose Porin



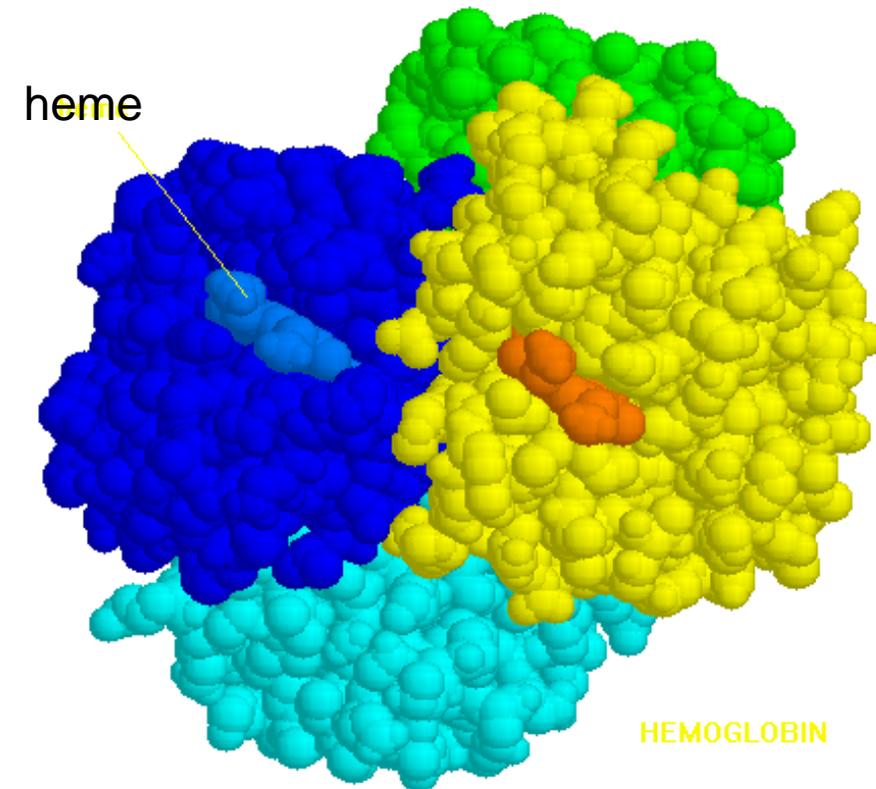
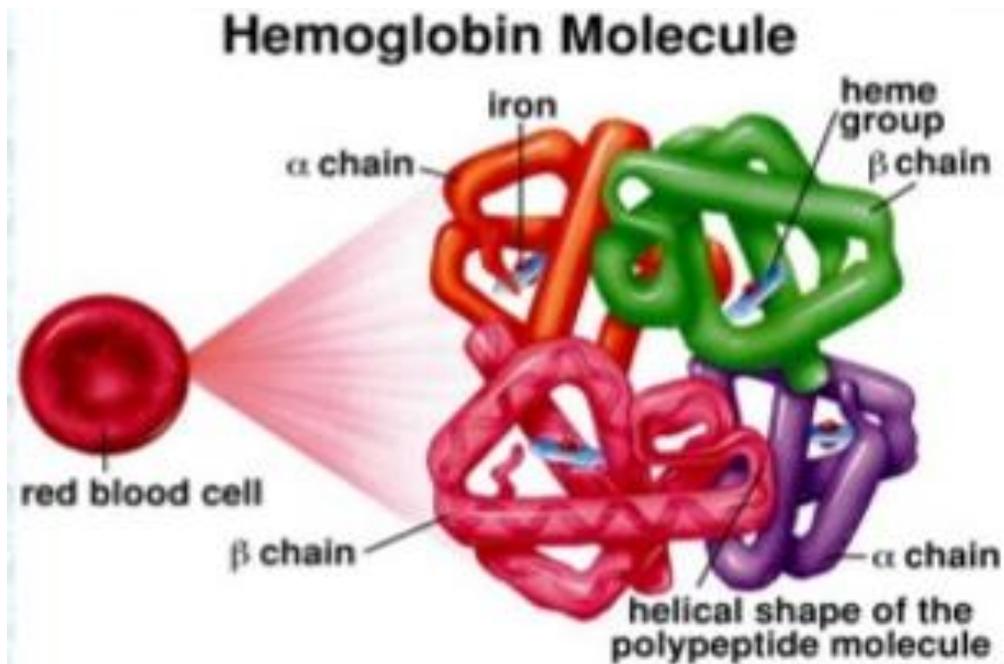
Quaternary structure of protein

- **Combination of two or more protein** to form a large, biologically active protein
- **Two kind** of quaternary structure, both are multi-subunit proteins
 - **Homodimer:** association between identical polypeptide chains
 - **Heterodimer:** interactions between subunits of very different structures

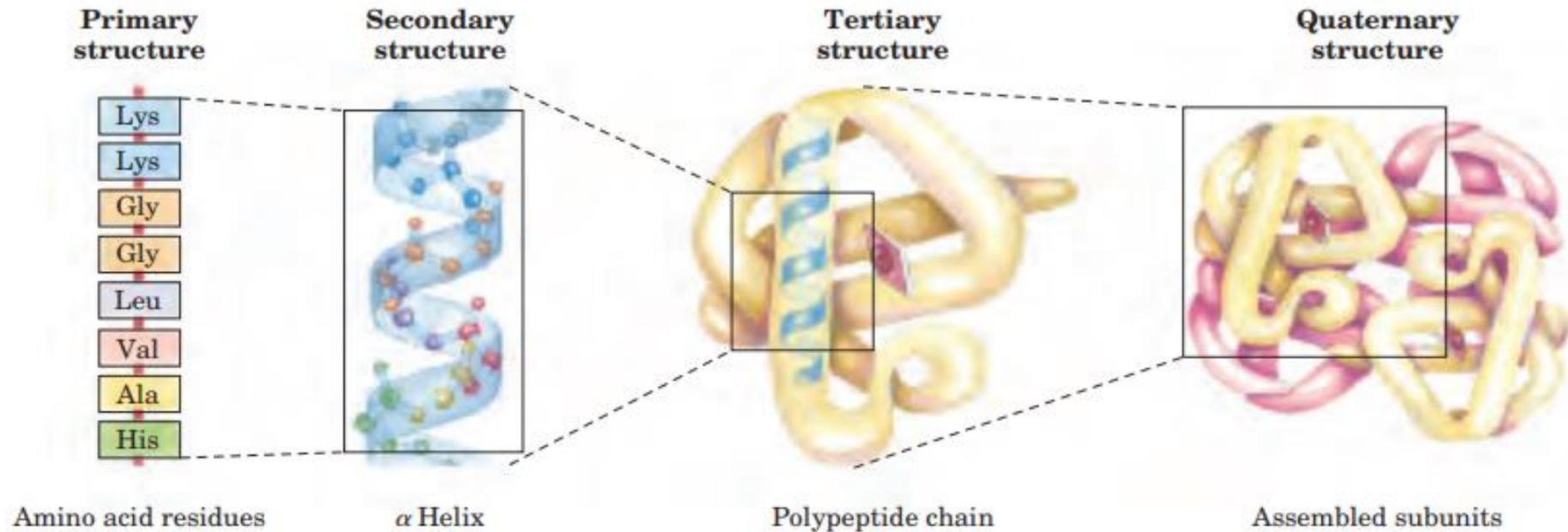


4 structure (multiple subunits)

- Hemoglobin is a globular protein with 4 polypeptide chains bonded together.
- The 4 polypeptide chains consist of 2 alpha and 2 beta chains
- 4 haem groups each contain iron
- Each haem group can carry one molecule of oxygen



Levels of structure in proteins



The primary structure consists of a sequence of amino acids linked together by peptide bonds and includes any disulfide bonds. The resulting polypeptide can be coiled into units of secondary structure, such as an α -helix.

The helix is a part of the tertiary structure of the folded polypeptide, which is itself one of the subunits that make up the quaternary structure of the multi-subunit protein



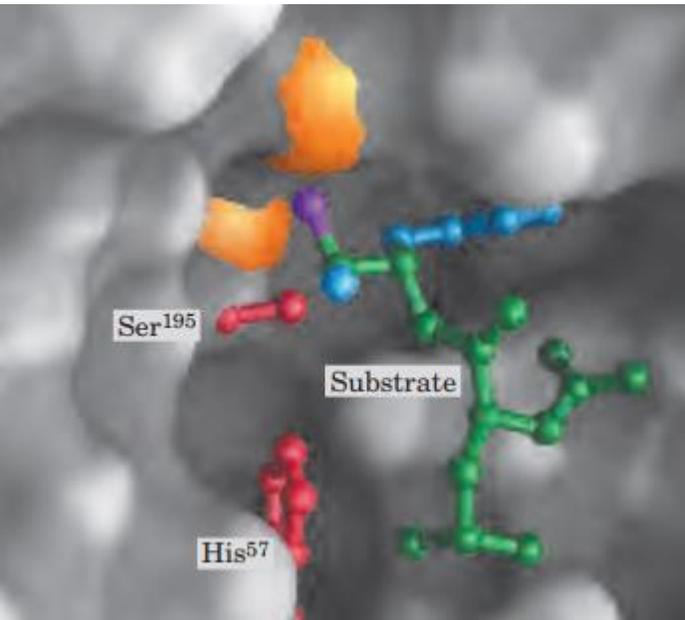
Lecture 8: Biochemistry I

Enzymes

3rd Class

Anbar University-College of Pharmacy-Clinical Laboratory Sciences Department
2020-2021

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Learning outcomes

- To understand the **function** of the **active site** of enzymes
- To understand the role of **cofactors** in enzymes
- To know **enzyme activity** and **specificity**
- To understand the **factors affecting enzyme activity**
- To understand **enzyme inhibition**



Enzymes

- With the exception of a few catalytic RNAs, all known enzymes are **proteins** that act as catalysts to **increase the rate of biochemical reactions**
- Enzyme-catalyzed reactions have three basic steps: **Enzyme** catalysts bind reactants (**substrates**), **convert** them to **products** and **release the products**:



- Enzymes, provide speed, specificity, and regulatory control to reactions in the body.
- Enzymes return to their original form at the end of reaction
- Have M.Weight ranging from about **12,000** to more than **1 million**
- Substrate is the substance upon which the enzyme act. The substrates are bound to **specific substrate binding sites on the enzyme** through interactions with the amino acid residues of the enzyme.
- Each **enzyme selective** for its **substrates** and ensures that only **specific products** are formed
- An enzyme-catalyzed reaction is take place within the confines of a pocket on the enzyme called the **active site**



Enzymes

- Enzymes provide a means for regulating the rate of metabolic pathways in the body
- In some diseases, especially **genetic disorders**, there may be **deficiency** or a total absence of **one or more enzymes**
- Measurements of the **activities of enzymes** in blood plasma, erythrocytes, or tissue samples are important in **diagnosing certain illnesses**
- **Many drugs** exert their biological effects through **interactions with enzymes**
- If an enzyme is **broken down** into its component amino acids, **its catalytic activity is always destroyed**. Thus the primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity.



- Many enzymes require an additional chemical component called a **cofactor**—either one or more **inorganic ions** or a **complex organic, or metalloorganic** molecule called a **coenzyme**, some enzymes require **both** a coenzyme and one or more metal ions for activity

Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

- Coenzyme act as transient carriers of specific functional groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biotin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5'-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:\text{H}^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)



- A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a prosthetic group (a nonprotein group forming part of or combined with a protein.)
- Active enzyme together with its bound coenzyme and/or metal ions is called a holoenzyme.
The protein part of such an enzyme is called the apoenzyme or apoprotein



International Classification of Enzymes

- Enzymes usually have both a **common name** and a **systematic** classification that includes a **formal name** and an **Enzyme Commission (EC) number**.
- **The common names** for most enzymes derive from their ability to catalyze a specific chemical reaction. In general, an enzyme's name consists of a term that identifies the **type of reaction catalyzed** followed by the **suffix-ase**. e.g **dehydrogenases** remove hydrogen atoms, **proteases** hydrolyze proteins, and **isomerases** catalyze rearrangements in configuration.
- **Systematic name:** each enzyme has a unique **name and code number** that reflect the **type of reaction** catalyzed and the **substrates** involved. e.g “hexokinase” is designated “ATP:D-hexose-6-phosphotransferase E.C. **2.7.1.1**.” This identifies **hexokinase** as a member **of class 2** (transferases), **subclass 7** (transfer of a **phosphoryl group**), **sub-subclass 1** (**alcohol** is the **phosphoryl acceptor**). Finally, the term “**hexose-6**” indicates that the **alcohol phosphorylated** is that of carbon **six** of a hexose.



International Classification of Enzymes

Biochemists have adopted a system for naming and classifying enzymes. This system divides enzymes into six classes

No.	Class	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage



1- **Oxidoreductases**: oxidation and reduction reactions

- dehydrogenases \longrightarrow addition or removal of H
- peroxidases \longrightarrow use as H_2O_2 as oxygen donor, forming H_2O

2- **Transferases**: transfer a chemical group from one substrate to another

- kinases \longrightarrow transfer phosphate from ATP onto substrate

3- **Hydrolases**: hydrolysis (water splits the bond) of C-O, C-N, O-P and C-S bonds (e.g. esterases, proteases, phosphatases, deamidases)

4- **Lyases**: catalyze cleavage of C-C, C-O, C-N and other bonds by elimination, leaving double bonds, and also add groups to double bonds (e.g. dehydratases, hydratases, decarboxylases)

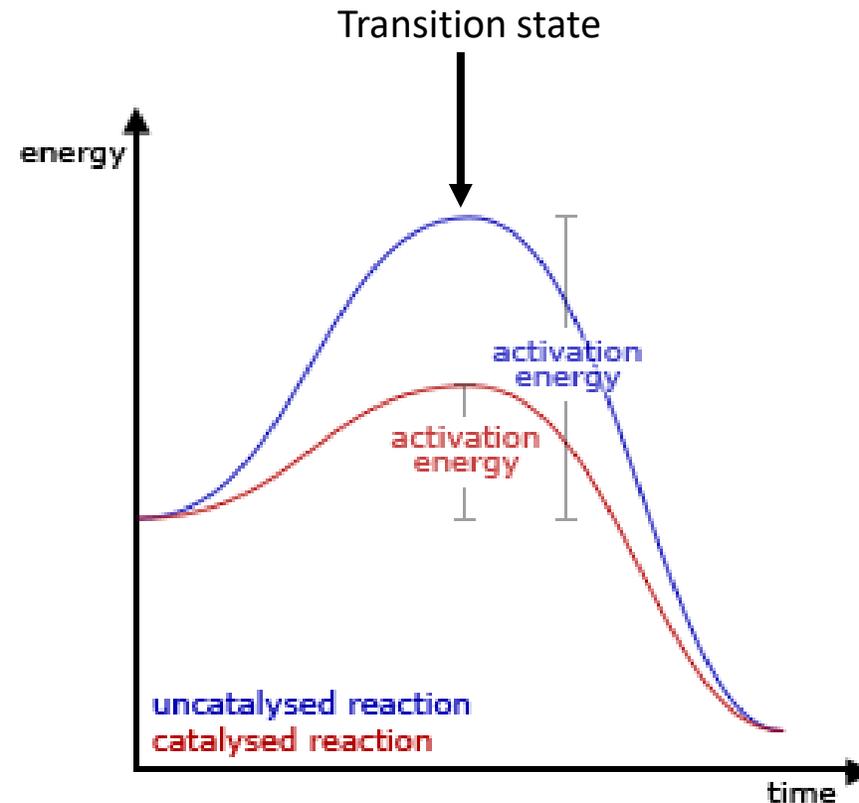
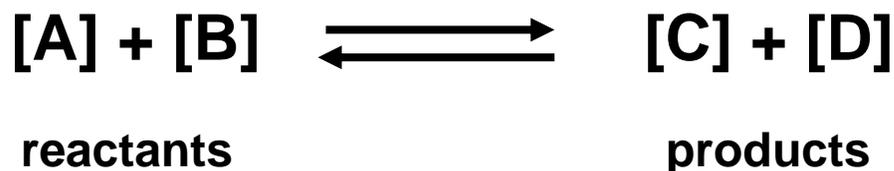
5- **Isomerases**: intramolecular rearrangements (catalyze geometric or structural changes within a single molecule)

6- **Ligase (Synthetases)** : formation of bonds between two substrates (frequently linked to utilization of ATP)



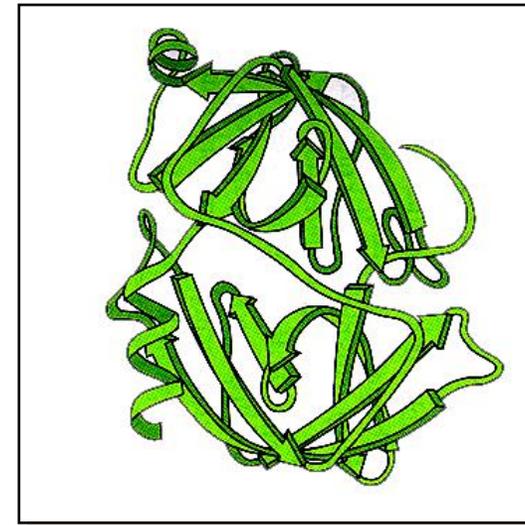
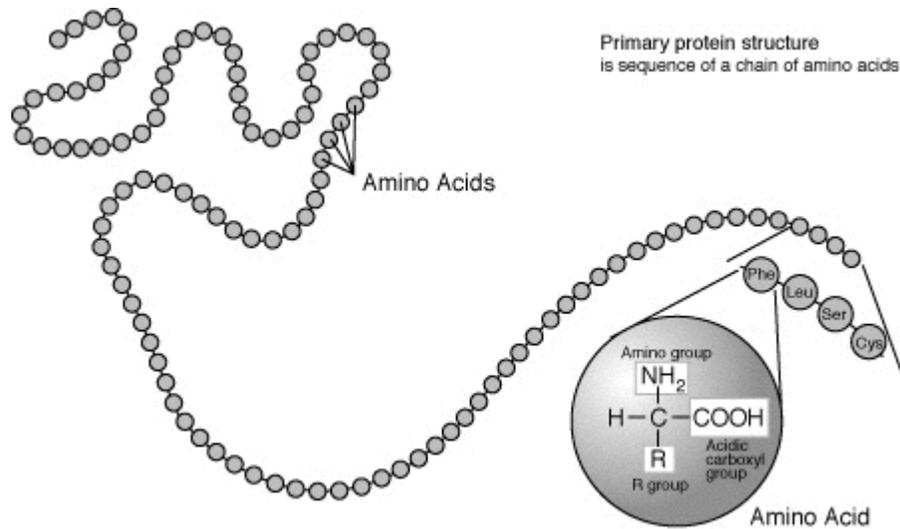
What is a catalyst ?

- The functional groups in the catalytic site of the enzyme activate the **substrates** and **decrease the energy** needed to form the high-energy intermediate stage of the **transition state complex**
- The essence of catalysis is **specific stabilisation of the transition state**.
 - **lowers** the activation energy
 - **increased** rate of reaction
 - is **not** consumed in the reaction
 - does **not** affect the reaction equilibrium



Enzymes as catalysts

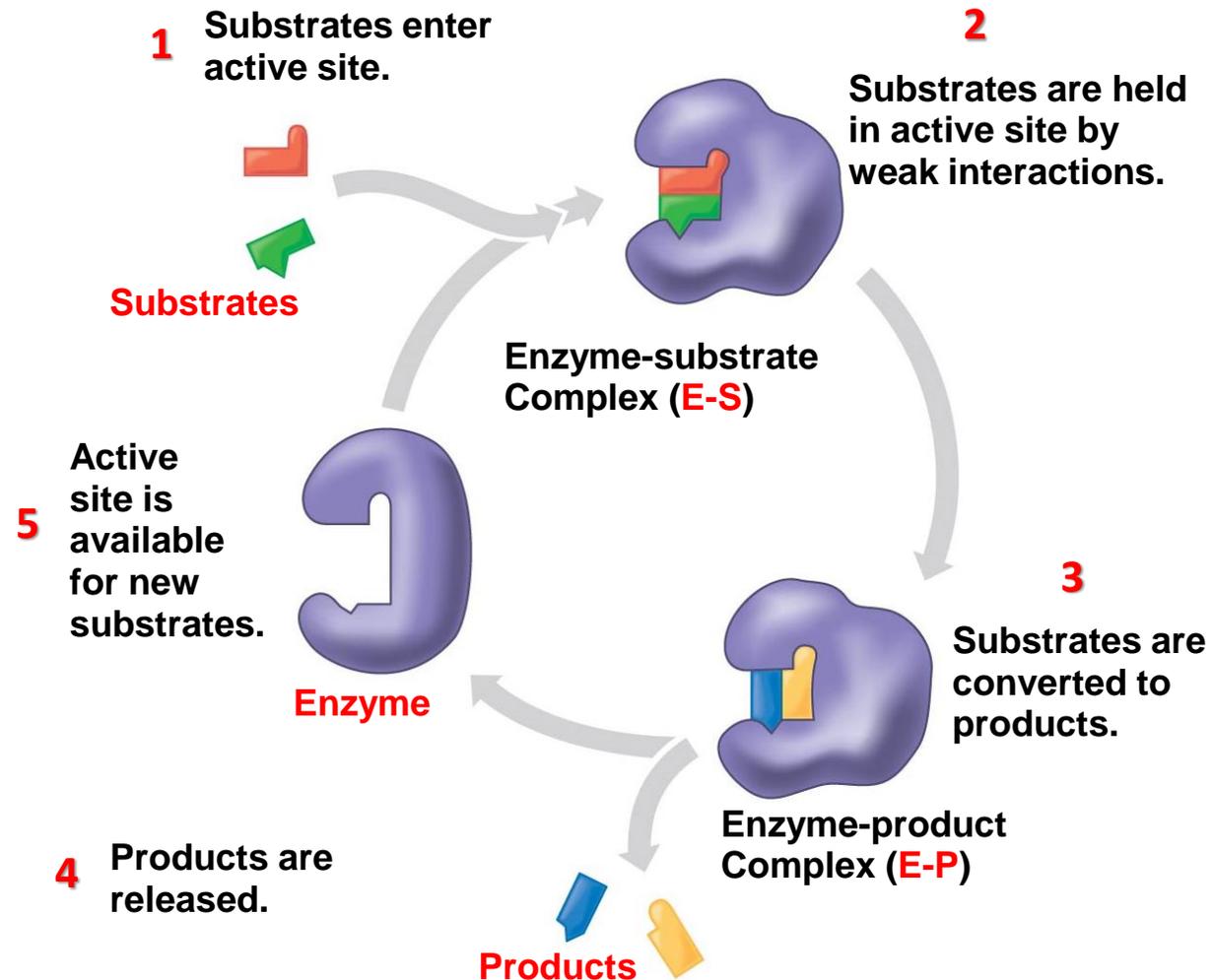
- The enzymatic catalysis of reactions is essential to living systems
- **Protein** – large organic compound made of amino acids arranged in a **linear chain** and **folded** into a **3-D structure**
- **Folding** of the protein brings **side-chains** of various amino acids that may be **far apart** in the primary sequence into **close juxtaposition**, forming an **active site**.



Tertiary structure



Stages in an enzyme-catalysed reaction



- **E-S & E-P:** Transient complexes

Overall it is a three-step reaction



How Enzymes Work

Properties of the active site

- **Positioning of substrate** molecules in the most favourable relative orientation for the reaction to occur
- The active site is perfectly **complementary** to the **transition state**
- Amino acid side chains of the active site **stabilise** the electron distribution of the transition state
- The **substrate is strained** on binding to the active site
 - **lowers the activation energy**
 - **increases the reaction rate**
- The **transition state** is rapidly converted to the **product(s)**
- The products bind **less tightly** to the enzyme and are released



The active site of an enzyme – substrate binding site

- **Non-covalent interaction between the substrate and the amino side –chain of the enzyme:**
 - Basic groups (Lys, His, Arg) → **ionic bonds**
 - Acidic groups (Asp, Glu) → **ionic bonds**
 - **Hydrophobic interactions** (Ala, Leu, Ile, Val, Met)
 - **Hydrophilic interactions with –OH** or alcoholic groups (Ser, Thr, Tyr)
 - **Hydrophilic interactions with –SH** or thiol groups (Cys)
 - **Hydrophilic interactions with amide** groups (Asn, Gln)
 - **Aromatic interactions** (Phe, Tyr, Trp)



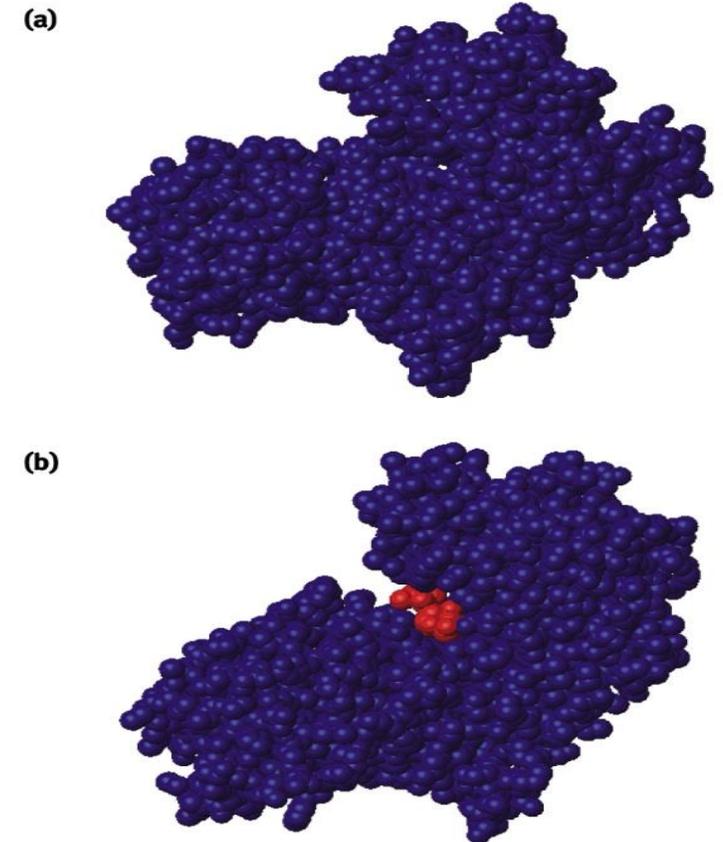
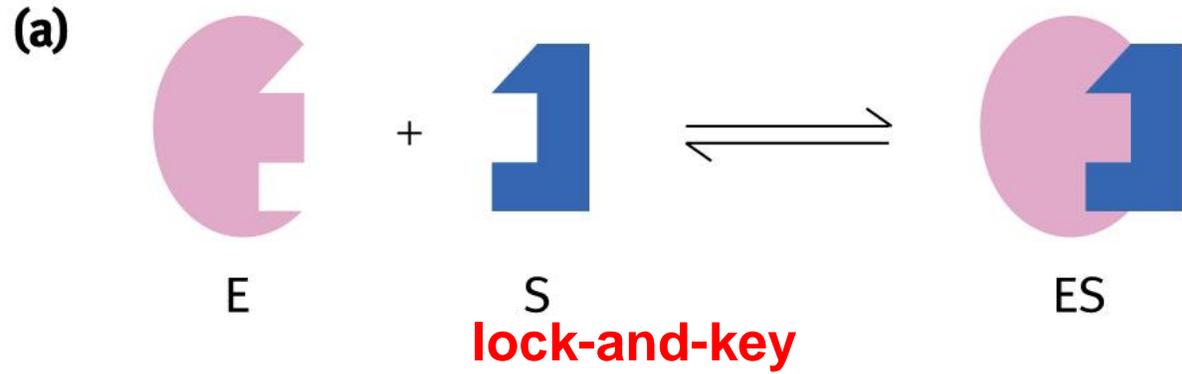
The active site of an enzyme – catalytic site

Reactive groups at the enzyme surface catalyse the reaction by:

- Donating or withdrawing **electrons**
- Stabilizing or generating **free radical intermediates**
- Forming **temporary covalent bonds** (a transition state intermediate)



Enzyme-substrate interaction



Yeast hexokinase phosphorylation of glucose



lock-and-key model

- The substrate binding site contains amino acid residues arranged in a complementary three-dimensional surface that “recognizes” the substrate and binds it through multiple **hydrophobic interactions, electrostatic interactions, or hydrogen bonds**. The amino acid residues that bind the substrate can come from very different parts of the linear amino acid sequence of the enzyme. In the lock-and-key model, the complementarity between the substrate and its binding site is compared to that of a key fitting into a rigid lock.

Induced fit model

- As the substrate binds, enzymes undergo a **conformational change** (“induced fit”) that repositions the side chains of the amino acids in the active site and increases the number of binding interactions . The induced fit model for substrate binding recognizes that the substrate binding site is a dynamic surface created by the flexible overall three-dimensional structure of the enzyme.



- In addition to **reactive groups from amino acids** enzymes may contain **non-protein** molecules also called **cofactors and coenzyme**:
 - **Metal group** (e.g. hexokinase **Mg²⁺**)
 - **Coenzyme** – tightly but not covalently bound organic molecule (**NAD**)
 - **Prosthetic group** – covalently bound organic molecule (heme)
- Enzyme **with** prosthetic group = **holoenzyme** – **catalytically active**
- Enzyme protein **without** prosthetic group
= **apoenzyme** – **catalytically inactive**



Units of enzyme activity – catalytic activity

- How much **substrate** can be converted (**product formed**) in a given **time**
- Number of micromoles (**μmol**) of **substrate** converted to product per **minute**
under standard optimized conditions at **30°C**
- **1 enzyme unit (EU) = 1 μmol min⁻¹**



Specificity

What does specificity mean ?

- The ability of an enzyme to select just **one substrate** and distinguish this substrate from a group of very similar compounds is referred to as **specificity**, e.g. Glucokinase catalyzes the transfer of a phosphate from **ATP** to **carbon 6 of glucose**
- Enzymes catalyse only **one** specific reaction. The enzyme converts this substrate to just **one product**
- **Shape, charge** and **conformation** of the **substrate** are critically important for binding to an **enzyme**
- **Specificity and speed** of enzyme catalyzed reactions result from the unique **sequence** of specific amino acids that form the three-dimensional (3D) structure of the enzyme.

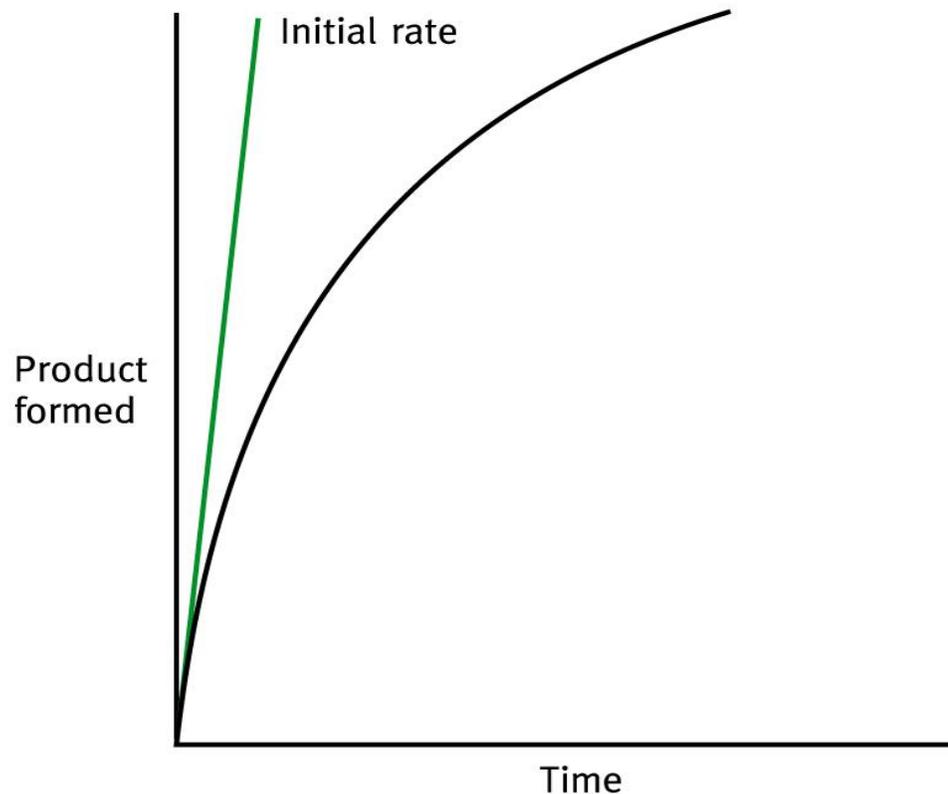
Specific activity

- **Activity of an enzyme per milligram (mg) of total protein** (expressed in $\mu\text{mol min}^{-1}\text{mg}^{-1}$)
- Specific activity gives a **measurement of the purity of the enzyme**.



The reaction rate

- Generation of the **reaction product** with **time**
- Measured at a **fixed enzyme concentration**
- Defined **temperature** and **pH**



Why is it hyperbolic ?

- Accumulation of product
- Depletion of substrate
- Denaturation of enzyme



Factors affecting enzyme activity

- pH
- Temperature
- Concentration of enzyme
- Concentration of substrate
- Covalent modification of enzyme
- Inhibitors and activators



pH

- pH is a measure of the acidity or alkalinity of a solution
- Every enzyme has an optimum pH (or pH range) at which it has maximal activity

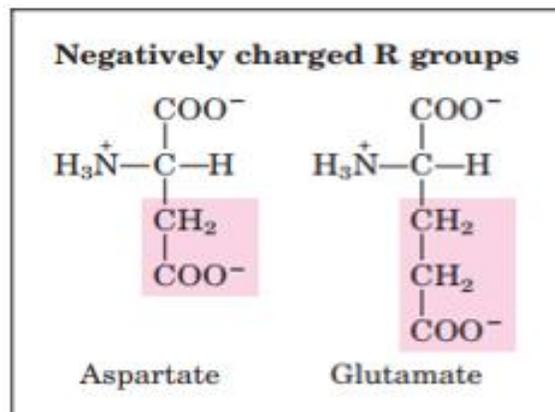
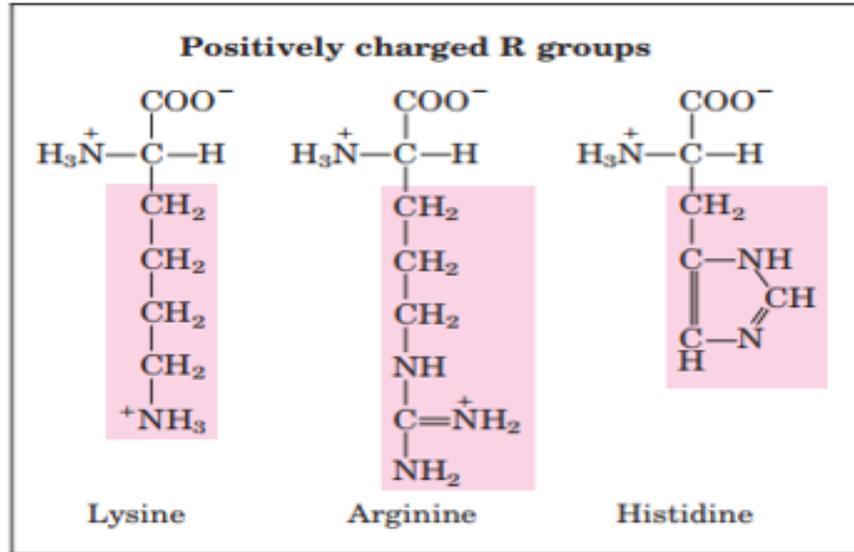
- **Acidic** < 7.00 < **Basic**

- **Neutral = pH 7.00**

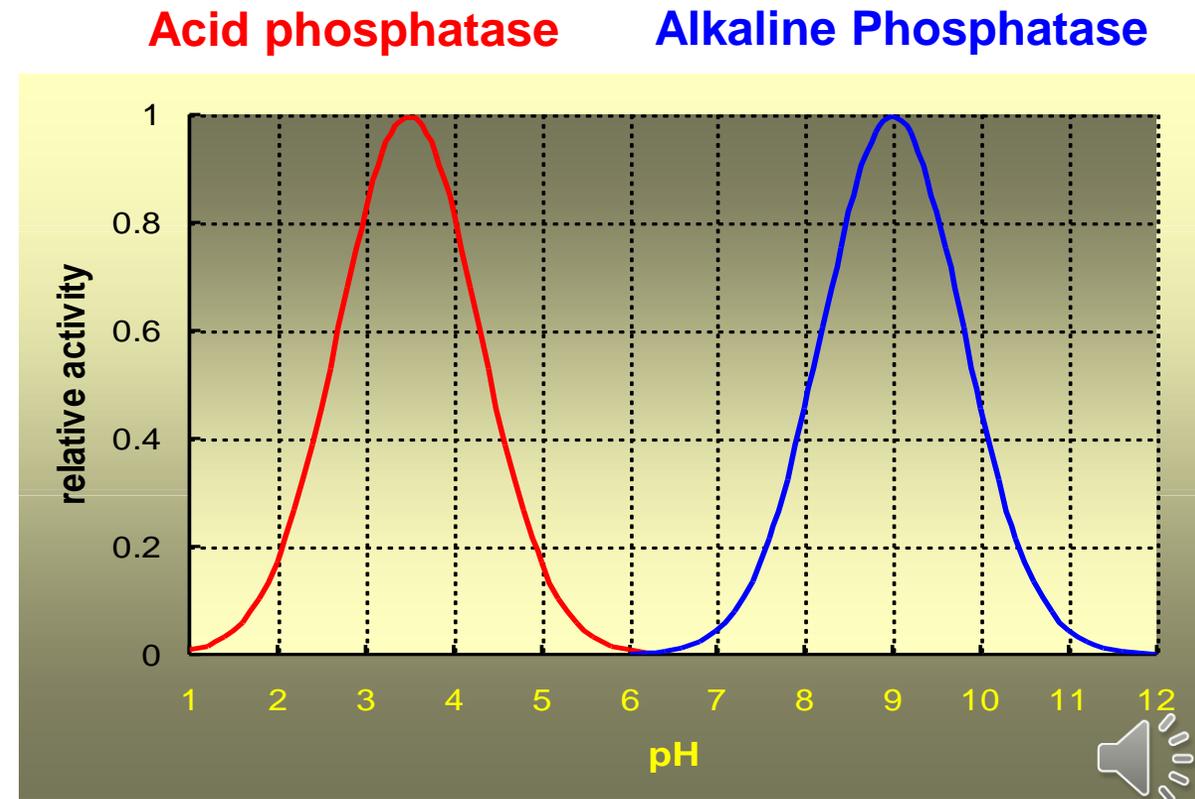


pH dependence of an enzyme-catalysed reaction

- **Ionization** state of amino acid **side chains** depends on the **pH** of the solution



- Binding of the **substrate** and **catalysis** depend on **pH**
- e.g. **pH** optima for **phosphatases** in the **blood plasma**



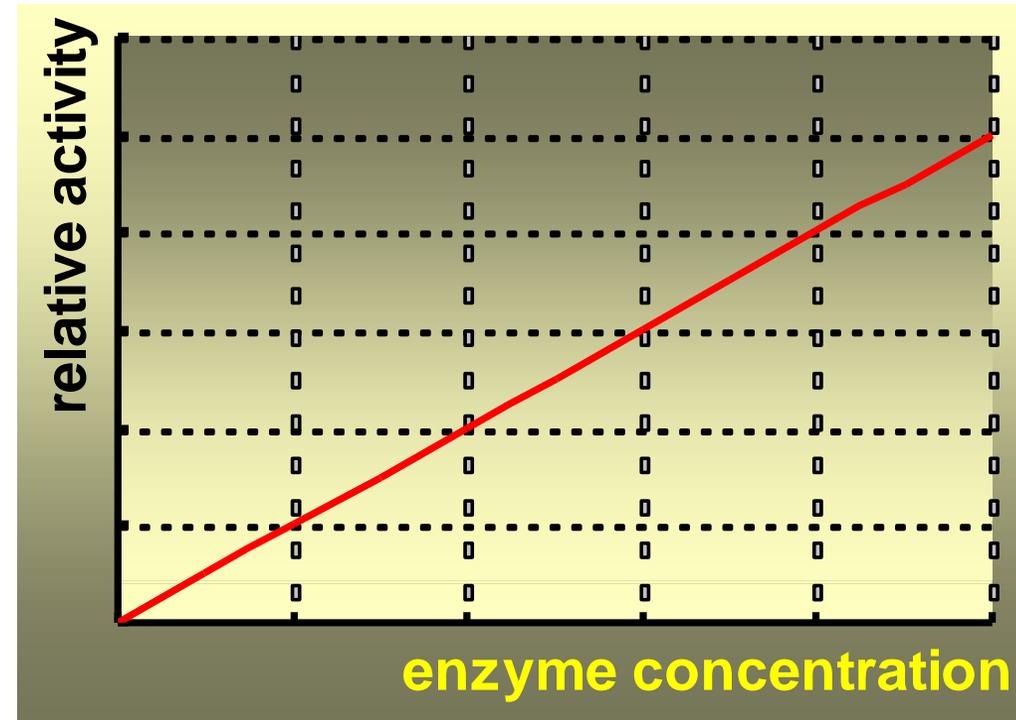
Temperature dependence of an enzyme-catalysed reaction

- Most **human enzymes** function optimally at a temperature of approximately **37°C**
- Chemical reactions **proceed faster at higher temperatures**:
 - molecules **move faster**, greater chance to **collide**
 - **electrons gain activation** energy easier
- **Denaturation of the enzyme** → loss of hydrogen bonding → unfolding
→ precipitation → loss of activity
- The temperature optimum depends on the time of incubation

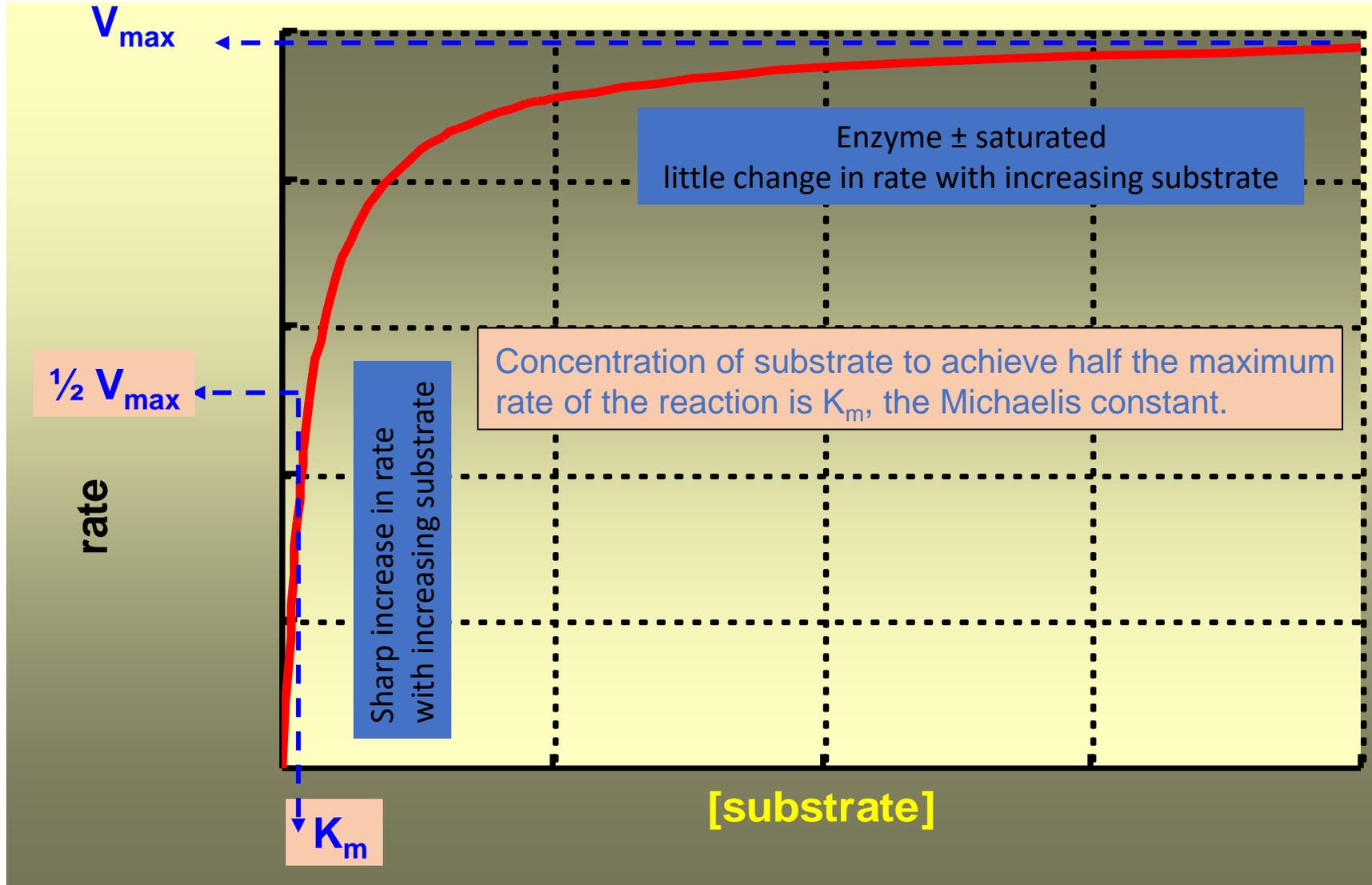


The effect of varying the amount of enzyme

- Predictable **linear increase** in product formation with **increasing amount of enzyme**



Substrate concentration dependence of an enzyme-catalysed reaction



- At a constant concentration of enzyme, the reaction rate increases with increasing substrate concentration until a maximal velocity is reached
- K_m is an important characteristic of enzyme-substrate interactions and is independent of enzyme and substrate concentrations



Kinetic Properties of Enzymes

- The equations of enzyme kinetics provide a quantitative way of describing the dependence of **enzyme rate on substrate concentration**.

Michaelis-Menten equation

- The **Michaelis-Menten model** of enzyme kinetics applies to a **simple reaction** in which the enzyme and substrate form an **enzyme–substrate complex** (ES) that can dissociate back to the free enzyme and substrate.
- Relates the velocity (v) to the concentration of substrate $[S]$ and the two parameters K_m and V_{max}



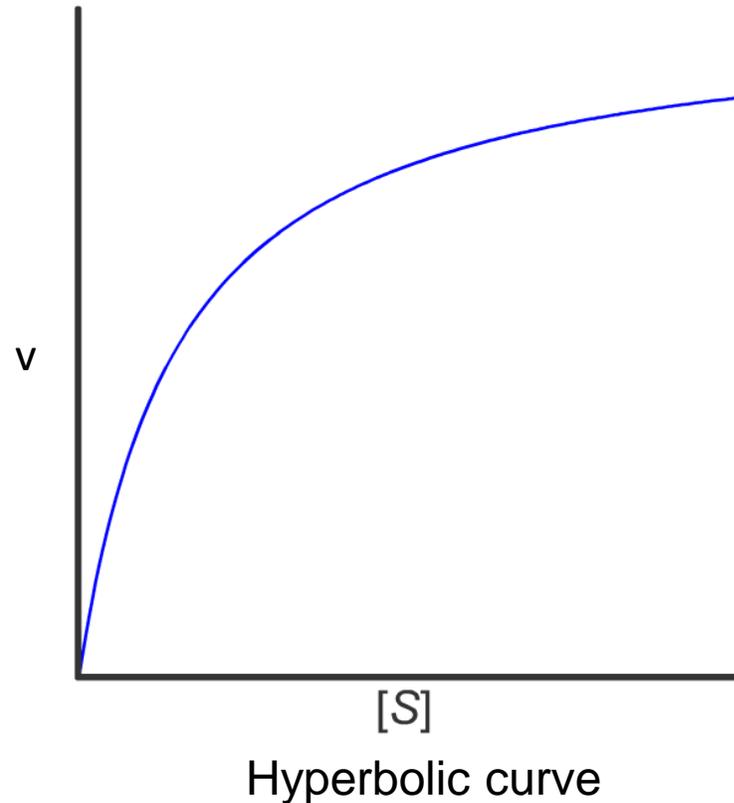
Michaelis-Menten equation (single-substrate reaction)

- Describes the dependence of **rate of reaction** on **concentration of substrate** at **steady state** (ES formation balanced by its removal) and **vast molar excess of substrate over enzyme** $[S] \gg [E]$.

Michaelis-Menten equation:

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

v rate of reaction
 V_{\max} maximal rate of reaction
 $[S]$ concentration of substrate
 K_m Michaelis constant

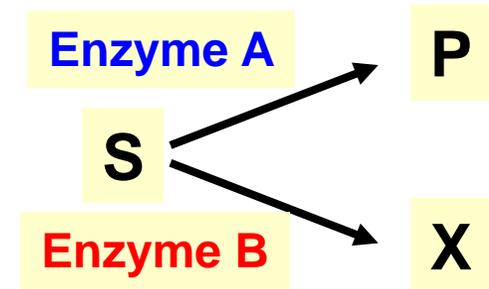
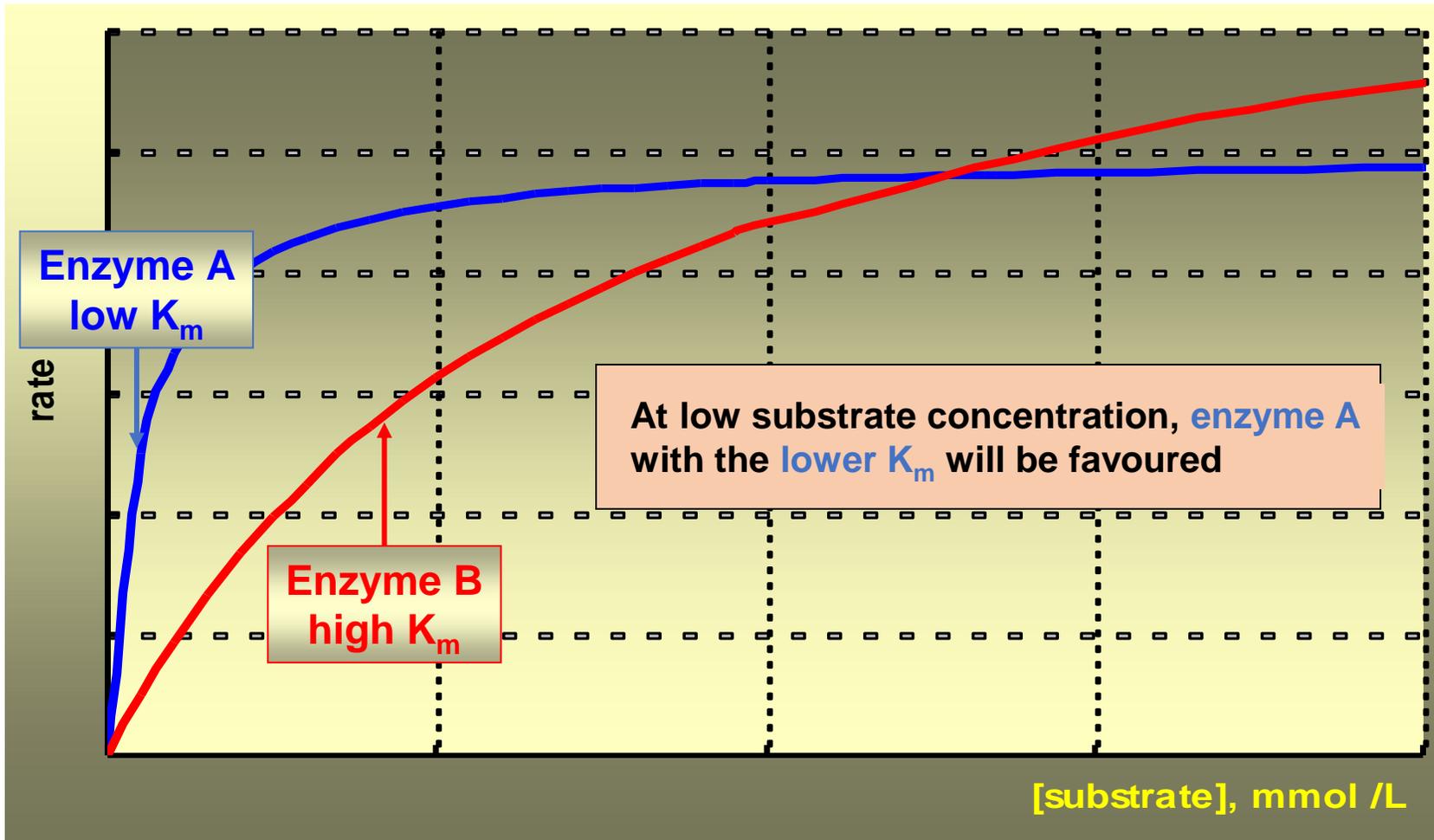


K_m , the Michaelis constant

- Low K_m corresponds to **high affinity** for the substrate
- High K_m corresponds to **low affinity** for the substrate
- Enzymes with a **low K_m** compared with the concentration of substrate **[S]** in the cell act at their **maximum rate**
→ modest **changes** in the concentration of substrate **[S]** have **no effect** on the **rate of reaction**
- Enzymes with a **high K_m** + **small change** in the concentration of substrate **[S]**
→ **large change** in the **rate of reaction**
- Typical K_m values:
 - Pyruvate carboxylase 60 μM for ATP
 - Chymotrypsin 5 mM for peptide substrate
 - Protein kinase 12 μM for ATP



The relevance of K_m : two enzymes “competing” for substrate [S]



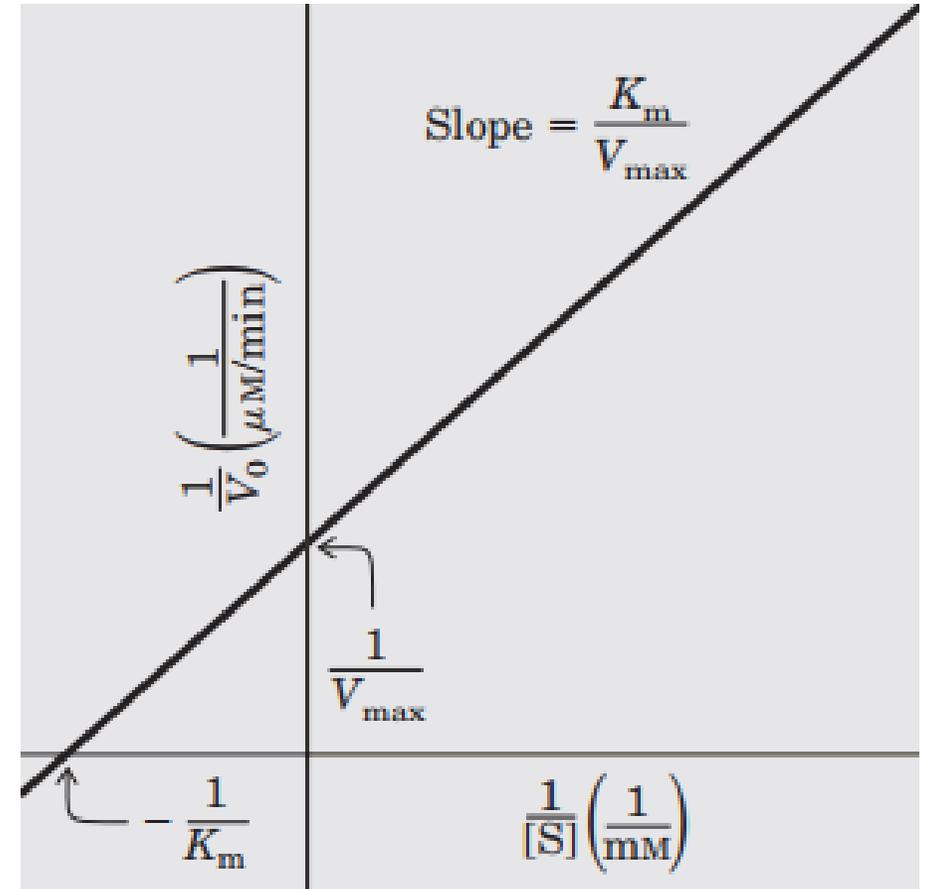
Experimental determination of K_m and V_{max}

The Lineweaver-Burk double reciprocal plot

- Is transformations of the Michaelis-Menten Equation
- The K_m and V_{max} for an enzyme can be visually determined from a plot of $1/v_0$ versus $1/S$, called a Lineweaver-Burk or a double reciprocal plot
- More accurate determination of V_{max}
- Distinguishing between certain types of enzymatic reaction mechanisms and in analyzing enzyme inhibition

$$\frac{1}{V_0} = \frac{K_m}{V_{max} [S]} + \frac{1}{V_{max}}$$

(v_0 = Initial velocity)

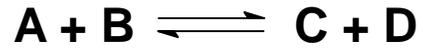


Most Biochemical Reactions Include Multiple Substrates

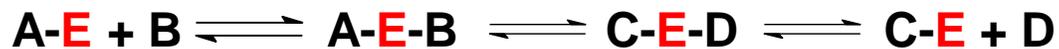
- Most reactions in biological systems usually include **two substrates** and **two products**
- **Multiple substrate reactions** can be divided into two classes: **sequential displacement** and **double displacement**



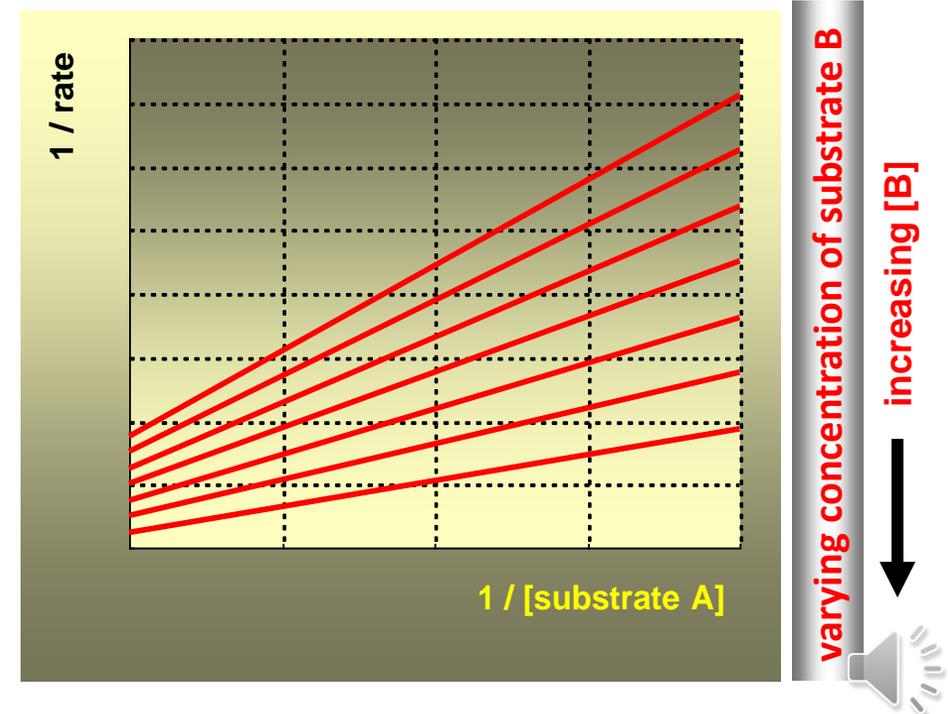
Enzymes with two substrates (sequential displacement)



- All substrates must bind to the enzyme before any product is released. Consequently, a ternary complex of the enzyme and both substrates forms
- **Sequential reaction** – each substrate binds in turn
(ternary complex = complex containing three different molecules A-E-B)



converging lines



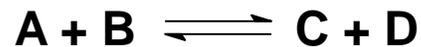
Enzymes with two substrates (double displacement)

- In double-displacement, or **Ping-Pong**, reactions, **one or more products are released** before all substrates bind the enzyme
- The defining feature of double-displacement reactions is the existence of a **substituted enzyme intermediate**, where the altered enzyme forms a second complex with another substrate molecule, and the **second product leaves**
- **Substrate 1** may **transfer a functional group to the enzyme** (to form the covalently modified E), which is **subsequently transferred to substrate 2**. This is called a **Ping-Pong** or double-displacement mechanism.
- Reactions that **shuttle amino groups** between **amino acids and α -ketoacids** are classic examples of double-displacement mechanisms.

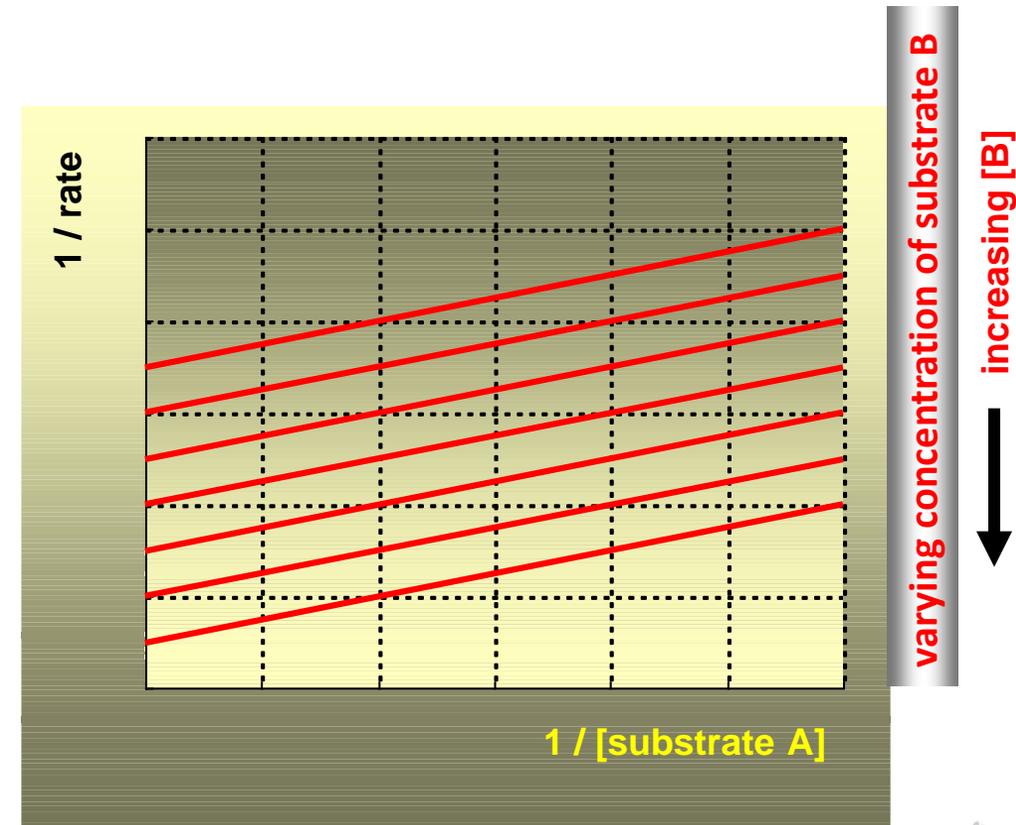


Enzymes with two substrates (double displacement)

- The Michaelis Menten equation is also applicable to bisubstrate reactions, which occur by ternary-complex or Ping-Pong (double-displacement) pathways
- Ping-pong reaction – one substrate reacts, and modifies enzyme, then second substrate reacts with modified enzyme



parallel lines



Allosteric enzymes

- Allosteric enzymes- enzymes with **cooperative substrate binding**
- contain binding sites **“other” (“allo”)** than substrate **binding sites**.
- often in **multi-subunit complex**, more than one active site in the complex.
- binding of substrate to the active site of the first subunit leads to **change in conformation** facilitating binding of substrate to the other active sites



Enzyme inhibitors

- **Decrease** the enzyme's ability to bind substrate or/and lower the enzyme's catalytic activity
- Many drugs and toxic agents act by inhibiting enzymes

Type of enzyme inhibitors

- Reversible inhibitors
- Irreversible inhibitors (inactivators)



Enzyme inhibitors

Reversible inhibitors

- **Non-covalent** binding to enzyme
- Many are relatively **unspecific**
- Mechanism: **blocking substrate binding** or **hindering catalytic steps**

Irreversible inhibitors

- Tightly Bind to enzyme **covalently**
- Many are substrate **analogues**
- Undergo part of reaction
- **Transition state** covalent intermediate does not **break down**



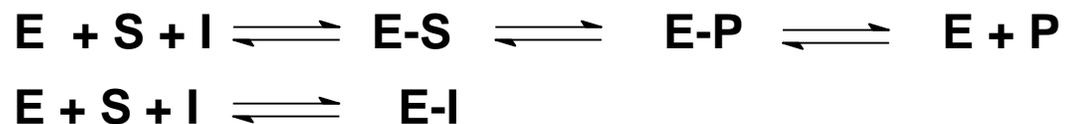
Irreversible inhibitors

- Dissociates **very slowly** from its target enzyme because it has become tightly bound to the enzyme
- Some irreversible inhibitors are important drugs. **Penicillin acts by covalently modifying** the enzyme **transpeptidase**, thereby **preventing** the synthesis of **bacterial cell walls** and thus killing the bacteria. **Aspirin** acts by covalently modifying the enzyme **cyclooxygenase**, **reducing** the synthesis of **inflammatory signals**.



Competitive inhibitor

- Competes with the substrate for binding at the active site
- Inhibition is a function of the relative affinities of the substrate and the inhibitor for binding the enzyme
- Inhibition is a function of the relative concentrations of substrate and inhibitor

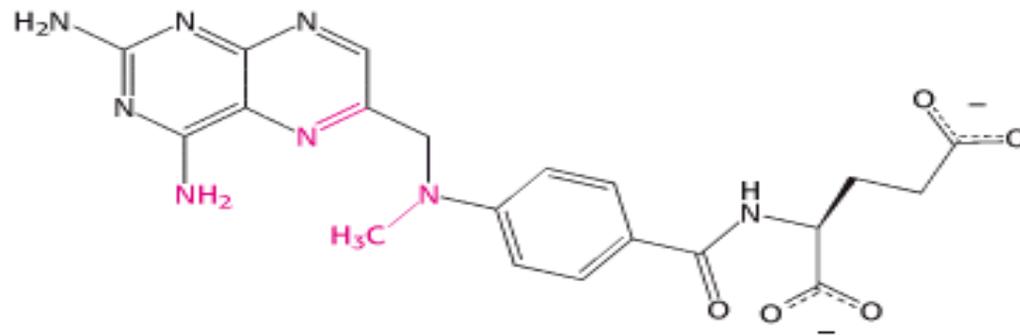


- V_{\max} is unchanged, K_m is increased
- If enough substrate is added, it overcomes the inhibitor

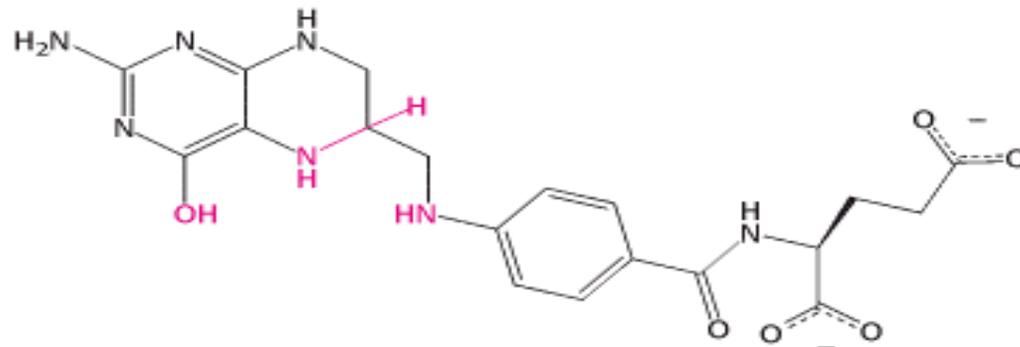


Competitive inhibitor

- **Methotrexate** is a structural analog of **tetrahydrofolate**, a coenzyme for the enzyme **dihydrofolate reductase**, which plays a role in the **biosynthesis of purines and pyrimidines**. It binds to dihydrofolate reductase **1000-fold** more tightly than the **natural substrate** and inhibits nucleotide base synthesis. It is used to **treat cancer**.



Methotrexate



Tetrahydrofolate



Non-competitive inhibitor

- Binds to the enzyme at a position **separate** from the **active site**
- **No competition** for binding with the **substrate**
- The apparent **affinity** for the substrate is **unchanged**, but the rate of reaction is **slowed**



- **K_m** is unchanged, **V_{max}** is decreased
- Adding more **substrate** has **no effect** on the rate of reaction



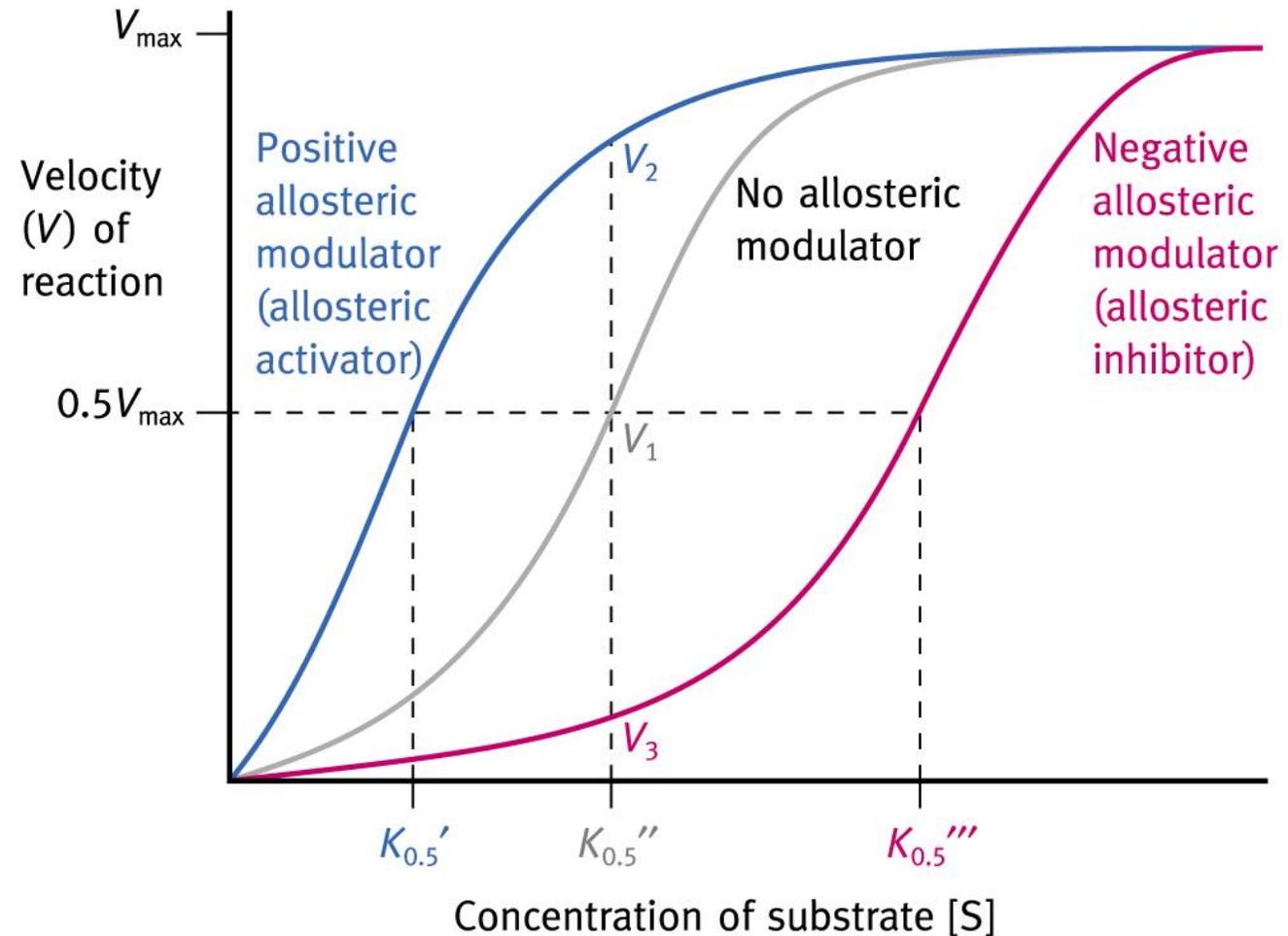
Mixed inhibitors

- Mixed inhibitors **do not bind** in the **active site**
- Inhibitor can **bind prior to substrate** or to the enzyme-substrate complex
- Mixed inhibitors **distort the substrate binding site** which affects:
 - apparent substrate affinity
 - catalytic turn-over (slowing catalysis)
- Mixed inhibitors can either:
 - **increase or decrease K_m**
 - **decrease V_{max}**



Allosteric inhibitors

- Increase the K_m and hence lower the apparent affinity of the enzyme for its substrate
- A decrease in the substrate affinity leads to a decrease of enzyme activity (at subsaturating levels of substrate present in the cell)



Lecture 9: Biochemistry I

Hormones

3rd Class

Anbar University-College of Pharmacy-Clinical Laboratory Sciences Department
2020-2021

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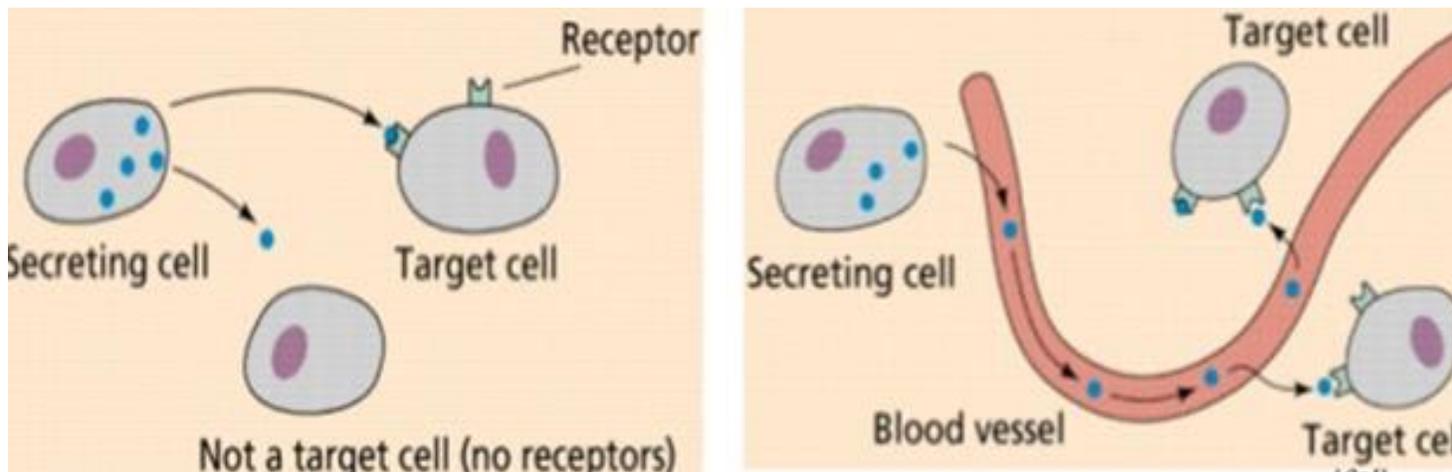


Hormones

- **Hormones** are **chemical messengers** formed in specific tissues. They are transferring information and instructions from one set of cells to another, and serve to **coordinate metabolic activities, regulate growth, maintain homeostasis of essential nutrients, sexual function, and prepare the organisms for reproduction**
- **Hormone** is **released** in small amount from **glands**, and is transported in the bloodstream to target organs or other cells to **modify their structures and functions**.
- **Hyposecretion** or **hypersecretion** of any hormone can be **harmful to the body**. **Controlling the production** of hormones can **treat many hormonal disorders** in the body

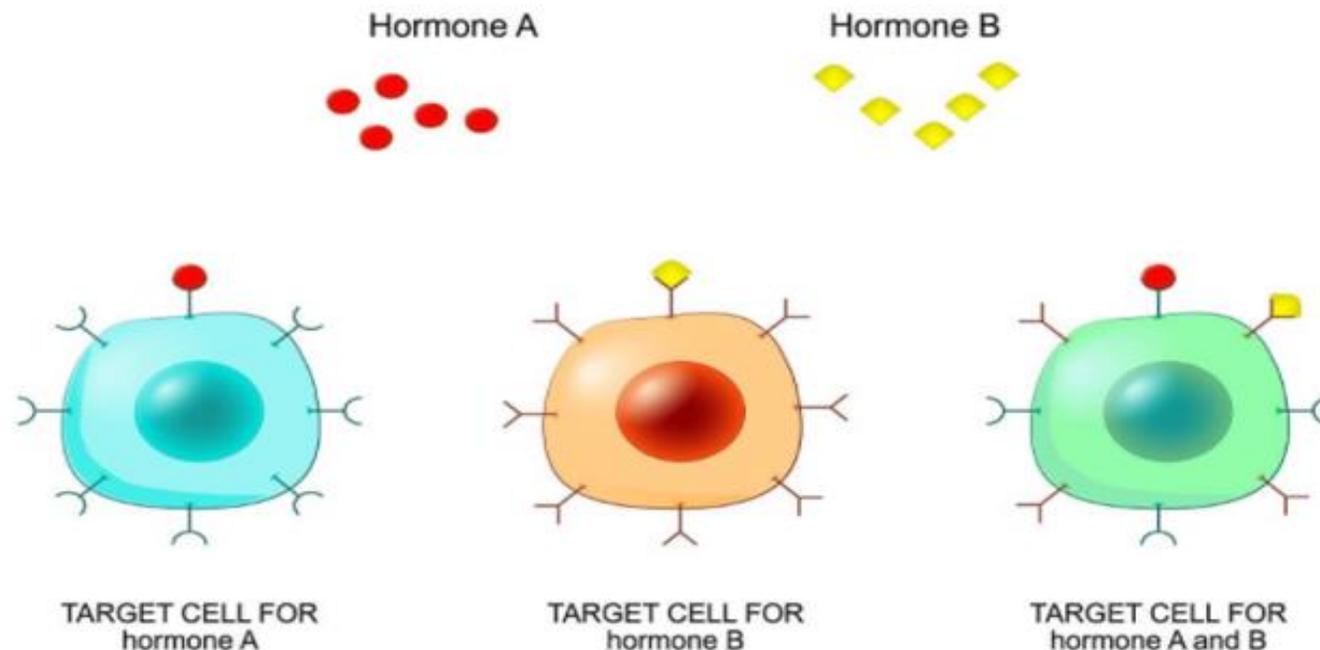


- Most **glands** of the body delivery their secretions by means of ducts. These are called **exocrine glands**.
- There are few other glands that produce chemical substance that they directly secrete into the blood stream for transmission to various target tissues, which have cells possessing the appropriate receptor. These are ductless or **endocrine glands**. The secretions of endocrine glands are called as **hormones**.



Hormones and target cells

- Most hormones **circulate in blood**, coming into contact with essentially **all cells**. However, a given hormone usually affects only a limited number of cells, which are called **target cells**. A target cell responds to a hormone when it expresses a **specific receptor** for that hormone.
- The hormone **binds to the receptor protein**, resulting in the **activation of a signal transduction mechanism** that ultimately leads to cell **type-specific responses**



- A target cell is defined by its ability to selectively bind a given hormone to its receptor.

Several biochemical features of this interaction:

- binding should be specific
- binding should be saturable
- binding should occur within the concentration range of the expected biologic response



The target cell concept and hormone receptors

Several factors determine the response of a target cell to a hormone.

- Factors that affect the concentration of the hormone at the target cell
- Factors that affect the actual response of the target cell to the hormone



The target cell concept and hormone receptors

■ Factors that affect the concentration of the hormone at the target cell

- The rate of **synthesis** and **secretion** of the hormones.
- The **proximity** of the target cell to the **hormone source** (dilution effect).
- The **dissociation constants** of the hormone with specific plasma transport proteins .
- The conversion of **inactive** or **sub-optimally active** forms of the hormone into the **fully active form**.
- The **rate of clearance** from plasma by other tissues or by digestion, metabolism, or excretion.



The target cell concept and hormone receptors

■ Factors that affect the actual response of the target cell to the hormone

- The **number, relative activity**, and state of occupancy of the specific receptors on the plasma membrane or in the cytoplasm or nucleus.
- The **metabolism** (activation or inactivation) of the hormone in the target cell
- The presence of other **factors** within the cell that are necessary for the hormone response.
- **Up- or down-regulation** of the receptor consequent to the interaction with the ligand.



- Although their varying actions and differing specificities depending on the target organ, the **hormones** have several characteristics in common with **enzymes**:
 - Act as body **catalysts**.
 - **Not consumed** in the reaction
 - Required only in **small quantities**
- Hormones **differ** from **enzymes** in the following ways:
 - Hormones are **produced in an organ other** than that which they ultimately perform their action
 - Hormones are secreted in blood **prior to use**. Because of the small amounts of the hormones required , blood levels of the hormones are extremely low
 - Structurally, Hormones are **not always proteins**.



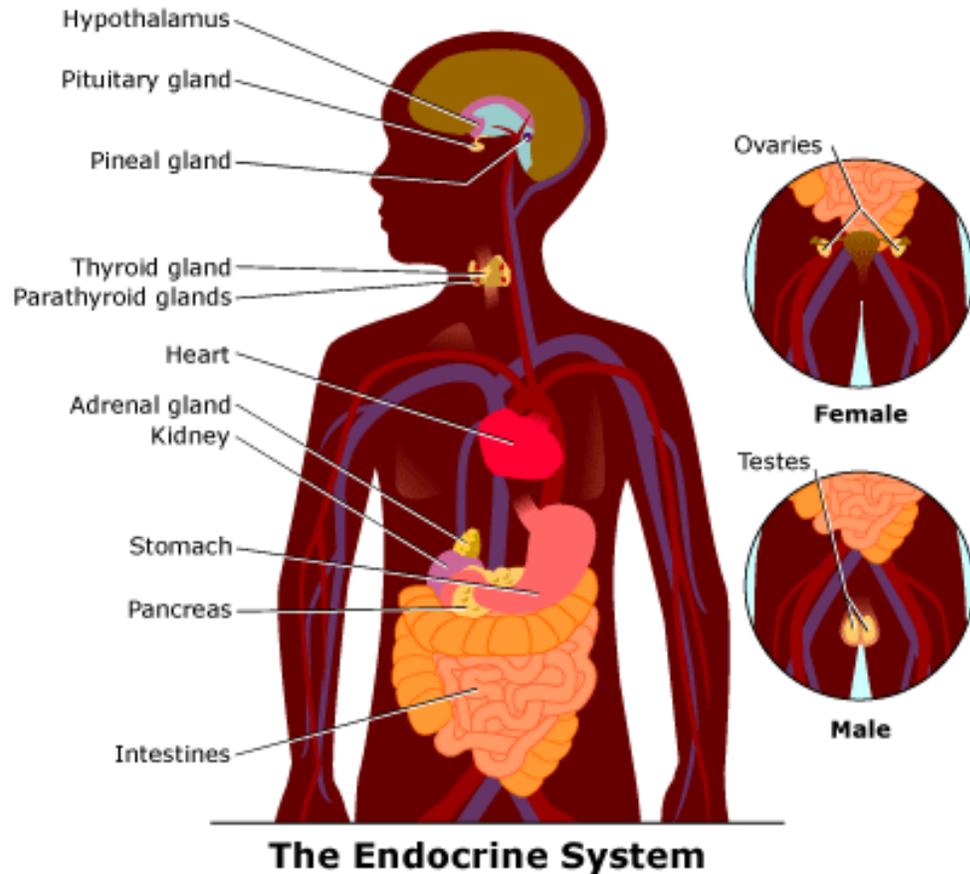
What is the endocrine system?

- The endocrine system is made up of **glands** and the **hormones** that they secrete.
- Although the endocrine glands are the primary hormone producers, they can also produce and release by:
 - brain
 - Heart
 - Lungs
 - Liver
 - Skin
 - Thymus
 - gastrointestinal mucosa
 - placenta
- The endocrine system **regulates all biological processes in the body** from conception through adulthood and into old age, including the development of the brain and nervous system, the growth and function of the reproductive system, as well as the metabolism and blood sugar



What is the endocrine system?

- The primary endocrine glands are:
 - The pituitary (the master gland)
 - Pineal, thyroid, parathyroid
 - Islets of Langerhans
 - Adrenals
 - Ovaries in the female
 - Testes in the male



General mechanisms of hormone action

- Hormone action begins with the **binding** of the **hormone to a receptor on (or in) a target cell**, binding of hormone molecule induces a conformational change in its receptor. Hence, the hormone-receptor interaction can be transduced from one molecule to another, cell may then
 - **Synthesis** new molecules
 - **Change** permeability of membrane
 - **Alter** reaction
- Each target cell **responds to hormone differently**
- Liver cells: **insulin** stimulates **glycogen synthesis**
- Adipose cells: **insulin** stimulates **triglyceride synthesis**



Regulation of hormones action

- Regulated by signals from **nervous system**, chemical changes in the blood or by other hormones
- **Negative feedback control** (most common)
 - Increase/decrease in blood level is reversed (maintains hormone levels within narrow ranges)
- **Positive feedback control**
 - The change production by hormone causes more hormone to be released
- Disorders involve either **hyposecretion** or **hypersecretion** of hormone



Diseases associated with the Endocrine system

- **Overproduction** of a hormone
- **Underproduction** of a hormone
- **Non-functional receptor** that cause target cells to become insensitive to hormones

- Knowledge of hormone biosynthesis, secretion, and interaction with target cells is essential to an understanding of biochemical basis of these disorders



Thyroid hormones

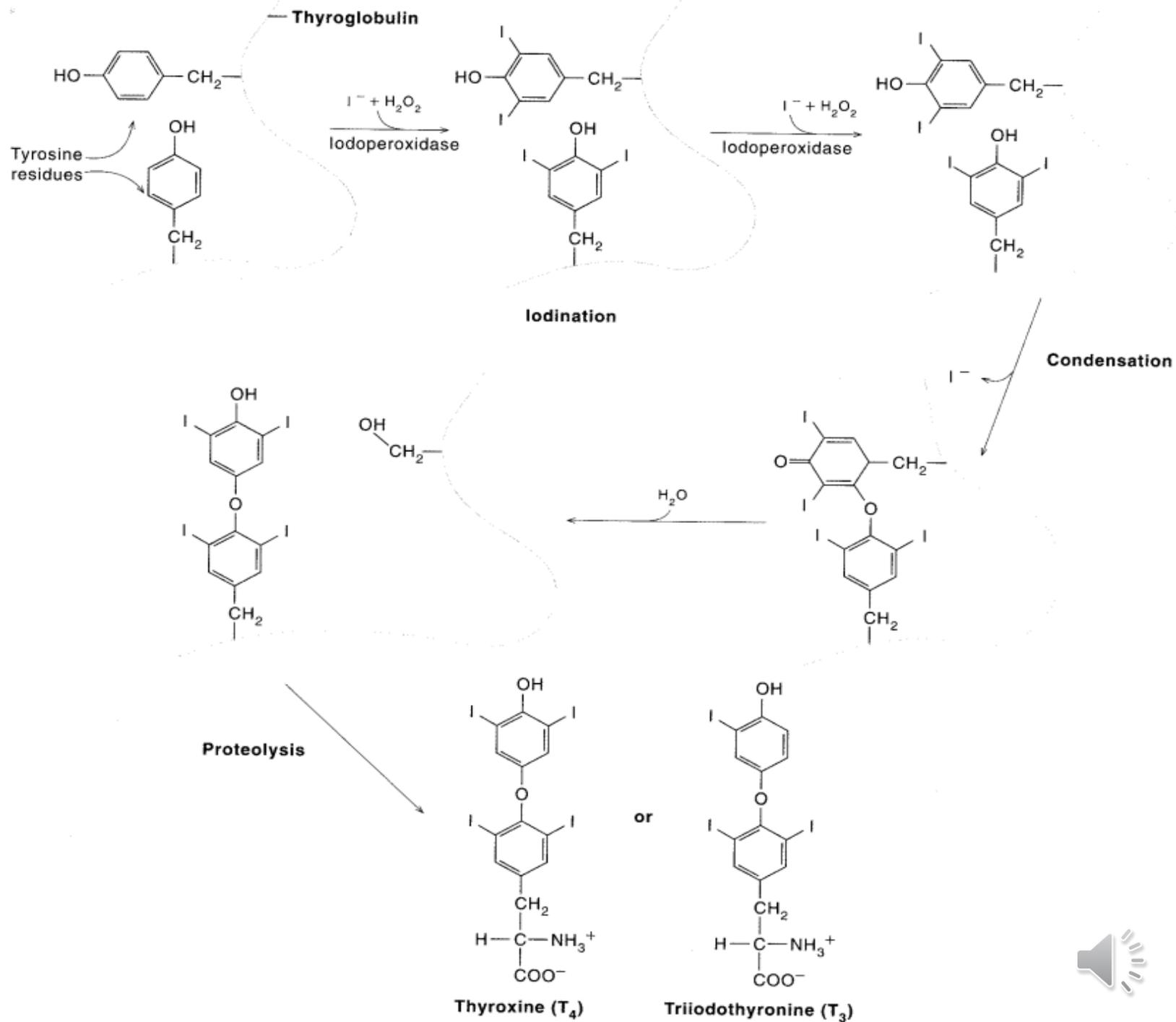
- **T3 and T4:** thyroid hormones responsible for our **metabolic rate, protein synthesis, breakdown of fats, use of glucose for ATP production**
- **Calcitonin (CT)** a hormone secreted by the thyroid that has the effect of **lowering blood calcium**, responsible for **building of bone**



- Most hormones fall into three classes:
 - **Polypeptide** (synthesised from large precursors)
 - **Steroids hormones** are derivatives of cholesterol
 - **Amino acids derivatives** (thyroids and epinephrine are amino acids derivatives)

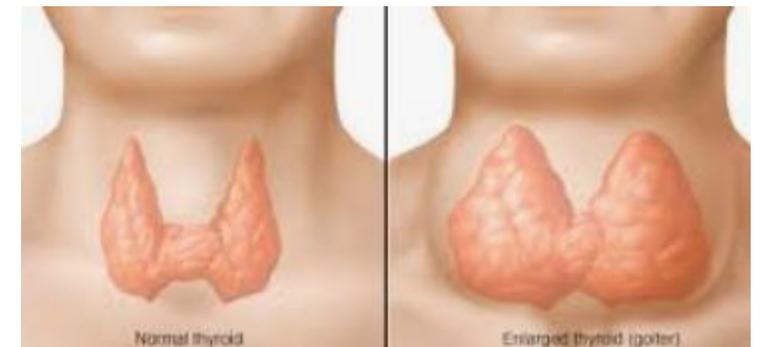
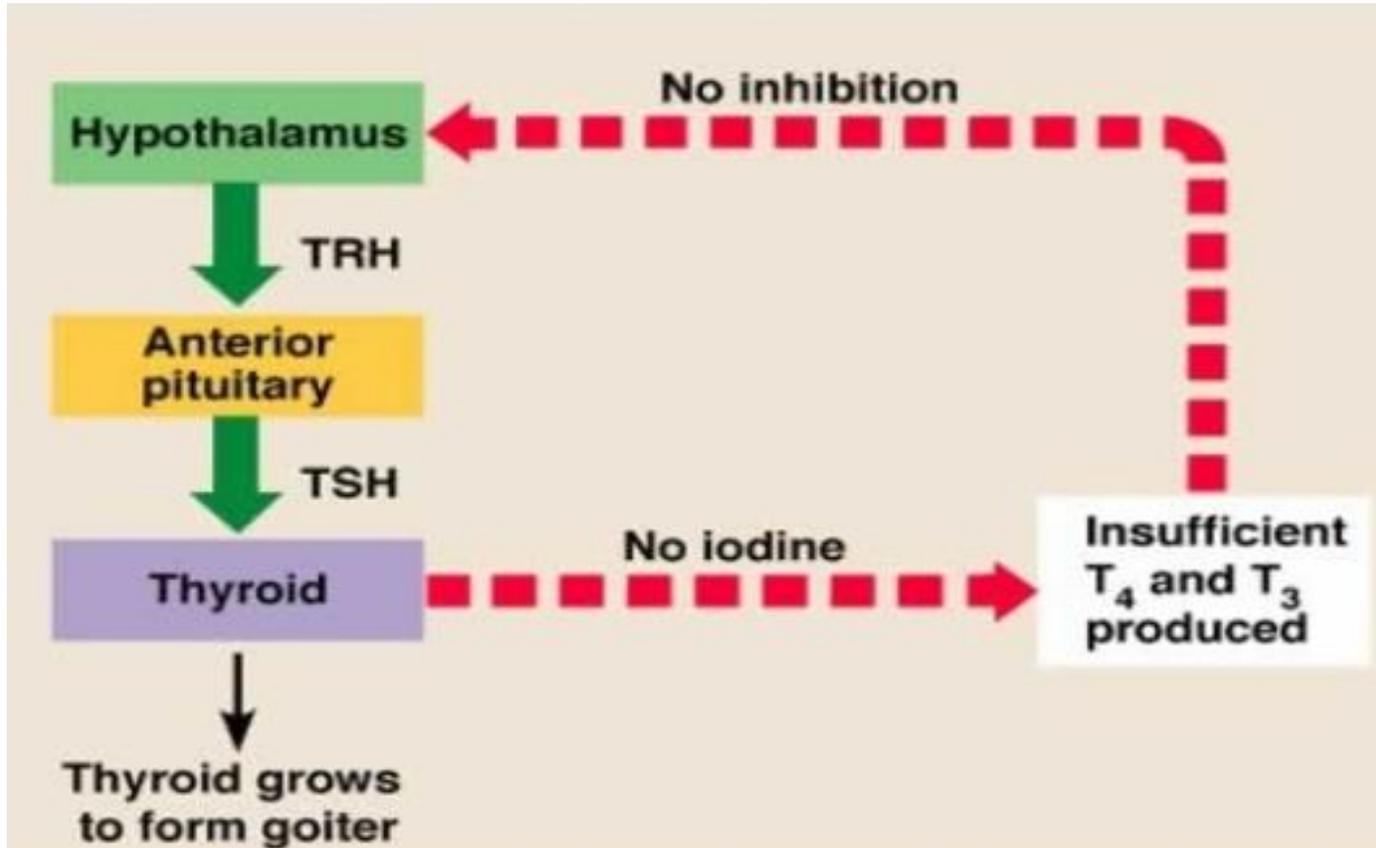


Pathway of thyroxine (**T4**) and triiodothyronine (**T3**) synthesis. Thyroid cells actively transport iodine (**I⁻**), which is incorporated into a few tyrosine residues of thyroglobulin by the enzyme **iodoperoxidase**. After condensation of iodinated tyrosine residues, the **thyroglobulin is proteolytically degraded** liberating T4 and T3



Goiter

- **Iodine deficiency** causes thyroid to enlarge as it tries to produce thyroxine
- Mechanism:



- **Hyperthyroidism** means **too much** thyroid hormone
- **Hypothyroidism** means **too little** thyroid hormone and is a common problem
- **Thyroid cancer** is a fairly common malignancy, however, the vast majority have excellent long term survival
- **Thyroiditis** is an **inflammatory** process ongoing within the thyroid gland
- **Solitary thyroid nodules**: defined as **swelling benign** within an normal gland



Diseases associated with the adrenal cortex

Two diseases associated with the adrenal cortex

- Cushing's disease
- Addison's disease

- Cushing's disease: **adenoma** in the **pituitary gland** produces large amount of Adrenocorticotrophic hormone (**ACTH**) causing the adrenal gland to produce elevated level of **cortisol**

- **Symptoms:**
 - Weigh gain
 - Hair loss
 - Hyperpigmentation
 - Hypercalcemia



Addison's disease

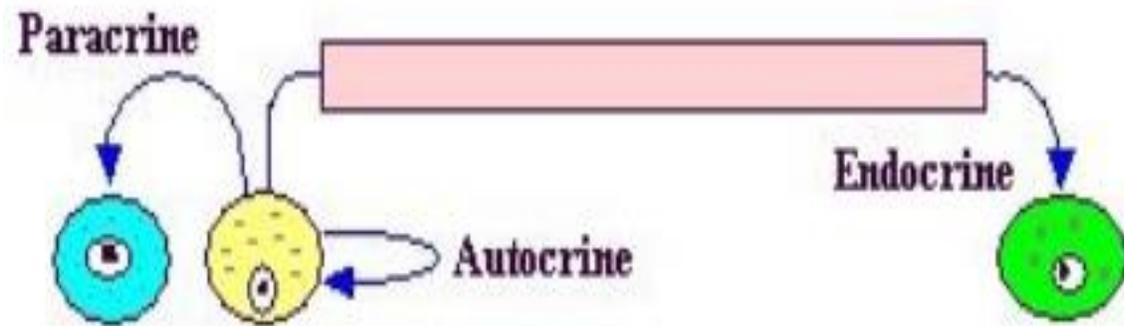
- Addison's disease (Chronic adrenal insufficiency, hypocortisolism, and hypoadrenalism)
- is a rare, chronic endocrine disorder in which the adrenal gland **do not produce sufficient steroid hormones** (glucocorticoids and often mineralocorticoids)
- It characterized by a number of relatively nonspecific symptoms such as **abdominal pain and weakness**

Symptoms:

- Nausea
- Fever
- Vomiting
- Fatigue



- **Three actions** were defined to describe how the signal is distributed for a particular hormonal pathway
- **Endocrine action:** the hormone is distributed in blood and binds to distant target cells.
- **Paracrine action:** the hormone acts **locally by diffusing** from its source to target cells in the neighborhood.
- **Autocrine action:** the hormone acts on the same cell that produced it.



Two important terms are used to refer to **molecules that bind to the hormone-binding sites of receptors:**

Agonists are molecules that bind the receptor and **induce** all the post-receptor events that lead to a **biologic effect**.

Antagonists are molecules that bind the receptor and **block** binding of the agonist, but **fail** to trigger intracellular signaling events. Hormone antagonists are widely used as drugs.



- The hormones are categorized based on the location of their specific cellular receptors and the type of signals generated.
- **Group I hormones** interact with an **intracellular receptor** and **group II hormones** with receptor recognition sites located on the extracellular surface of the plasma membrane of target cells.
- The hormones **generate signals** at or within **target cells**, and these signals regulate a variety of **biologic processes** which provide for a coordinated response to the stimulus.



Hormone Action and Signal Transduction

- A hormone-receptor interaction results in generation of an **intracellular signal** that can either **regulate the activity of genes**, thereby altering the amount of certain proteins in the target cell or affect the **activity of specific proteins**, including enzymes and transporter proteins.
- The **signal** can influence the **location of proteins** in the cell and can affect general processes such as **protein synthesis**, **cell growth**, and **replication**, often through effects on **gene expression**
- Many pharmacotherapeutic agents are aimed to target **signal transduction pathways**



Lecture 10: Biochemistry I

Vitamins

3rd Class

Anbar University-College of Pharmacy-Clinical Laboratory Sciences Department
2020-2021

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Objectives

- **Vitamins**
 - Introduction
 - General characteristics
 - General functions
 - Classification
 - Structure
 - Individual characteristics
 - Individual function
 - Deficiency



Definition of Vitamins

- Vitamins are **organic compounds in food that are** required in small amounts for **growth** and **maintaining good health**. They are **released** , **absorbed** and **transported** with the fat of the diet.
- The word **vitamin** comes from the Latin word **vita**, means **life**
- Organic nutrients with **on-caloric**
- Help body processes; digestion, absorption, metabolism, growth etc.
- Some appear in food as **precursors** or **provitamins**



Classification of vitamins

On the basis of their solubility, there are **2 classes** of vitamins

- **Fat** soluble vitamins
- **Water** soluble vitamins



Fat soluble vitamins

- are **A, D, E and K**
- Found in the **fats** and **oils** of food
- Absorbed into the **lymph** and carried in **blood with protein transporters = chylomicrons.**
- Vitamins that **dissolve in fat**, because fat is easily stored on our body, these vitamins can be stored within our fat
- They can **accumulate** and be **saved** for later use
- Stored in **liver** and **body fat** and can become **toxic** if large amounts are consumed.

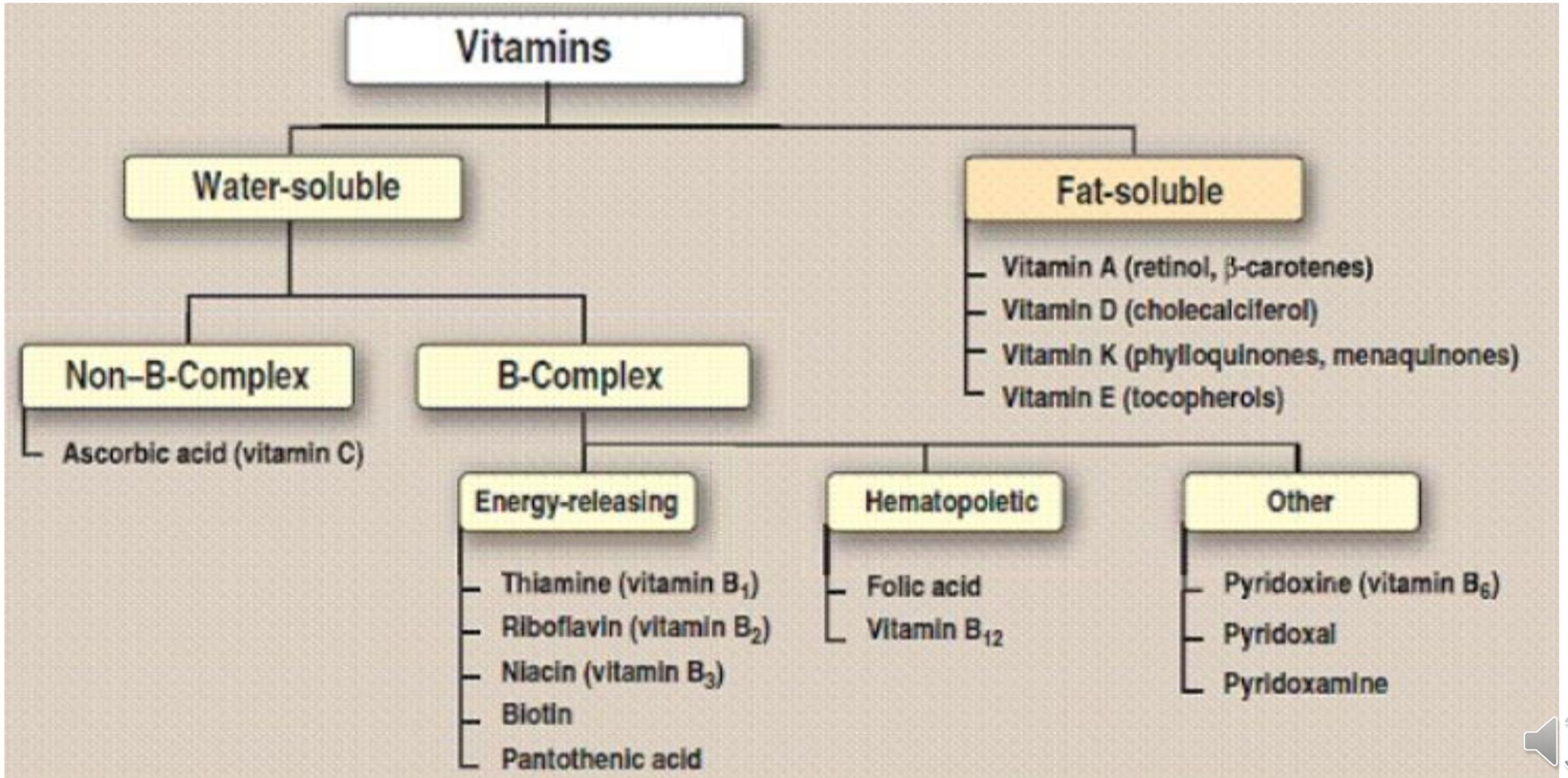


Water soluble vitamins

- Include **B complex** (B1, B2, B3, B5, B6, B7, B9 and B12) and **vitamin C**
- **Found** in vegetables, fruit and grains, meat
- Absorbed **directly** into the **blood stream**
- Vitamins that **dissolve in water**, because our body is a **watery environment**, these vitamins can **move** through our body **pretty easily**, and they can also be **flushed out the kidneys**
- **Not stored** in the body and **toxicity is rare**, smoking cause decreased absorption.



Classification of vitamins



Fat versus Water Soluble Vitamins

	Water Soluble	Fat Soluble
Absorption	Directly to blood	Lymph via CM
Transport	free	Require carrier
Storage	Circulate freely	In cells with fat
Excretion	In urine	Stored with fat
Toxicity	Less likely	More Likely
Requirements	Every 2-3 days	Every week

CM= Chylomicrons



General characteristics of vitamins

- Some of vitamin **cannot** to be **synthesized** by the body
- Significant amounts of **fat soluble vitamins** can be **stored** in human **adipose tissues and the liver**
- Water soluble vitamins **cannot be stored** in human **tissues**, their excess is excreted with **urine**
- **Synthetic** vitamins are identical to **natural vitamins**
- Following growth and development, **vitamins remain essential nutrients** for the healthy maintenance of the cells, tissues and organs



General Functions of vitamins

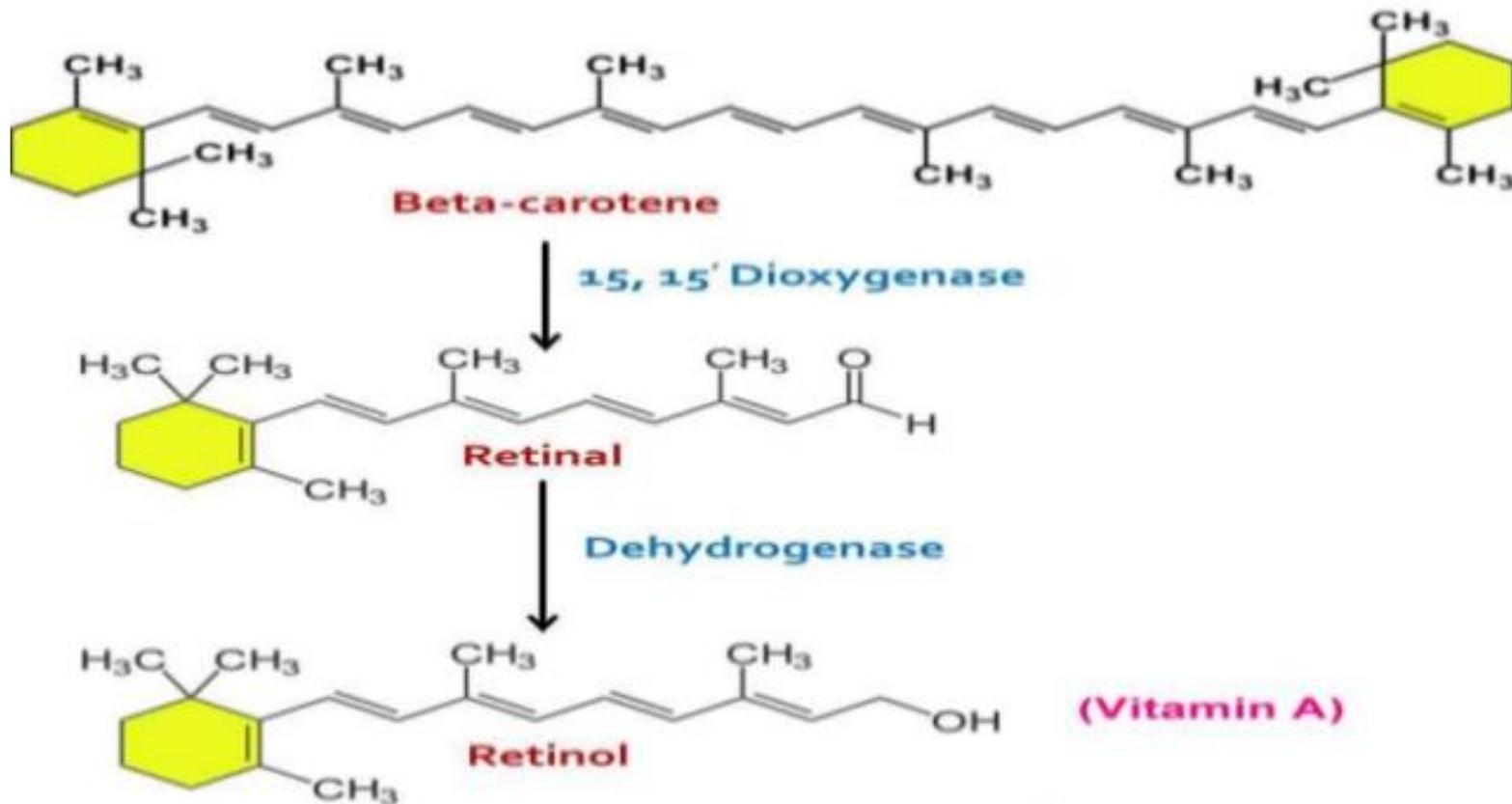
Vitamins are helpful for the health and life of the body in the following respects

- Help **health protection** by build up the **resistance** of the body **against diseases**
- **Prevent** and cure various **diseases** caused by **deficiency of vitamins**
- Help the **digestion** and utilization of **mineral salts** and **carbohydrates** in the body
- Help **maintenance** of **health** and **normal growth**
- **Stimulate** and give **strength** to **digestive** and **nervous system**



Vitamin A

- Vitamin **A** is a group of **unsaturated** nutritional organic compounds that includes, **retinol, retinal, retinoic acid**, and several provitamin A carotenoids (**beta-carotene**)
- **Retinol** is the active form of vitamin A, which is found only in **animal sources**
- Found in animal and plant sources



○ **Vitamin A Functions**

- Vision (11-cis-retinol bound to rhodopsin detects light in our eyes)
- Gene transcription
- Bone growth
- Embryonic development and Reproduction
- Cell division and differentiation
- Healthy Skin
- Regulate Immune System
- Antioxidant activity

○ **Vitamin A deficiency**

- Night blindness
- Xerophthalmia (Development of a peculiar condition around the eyes)
- Decreased resistance to infections
- Dry skin, hair or nails

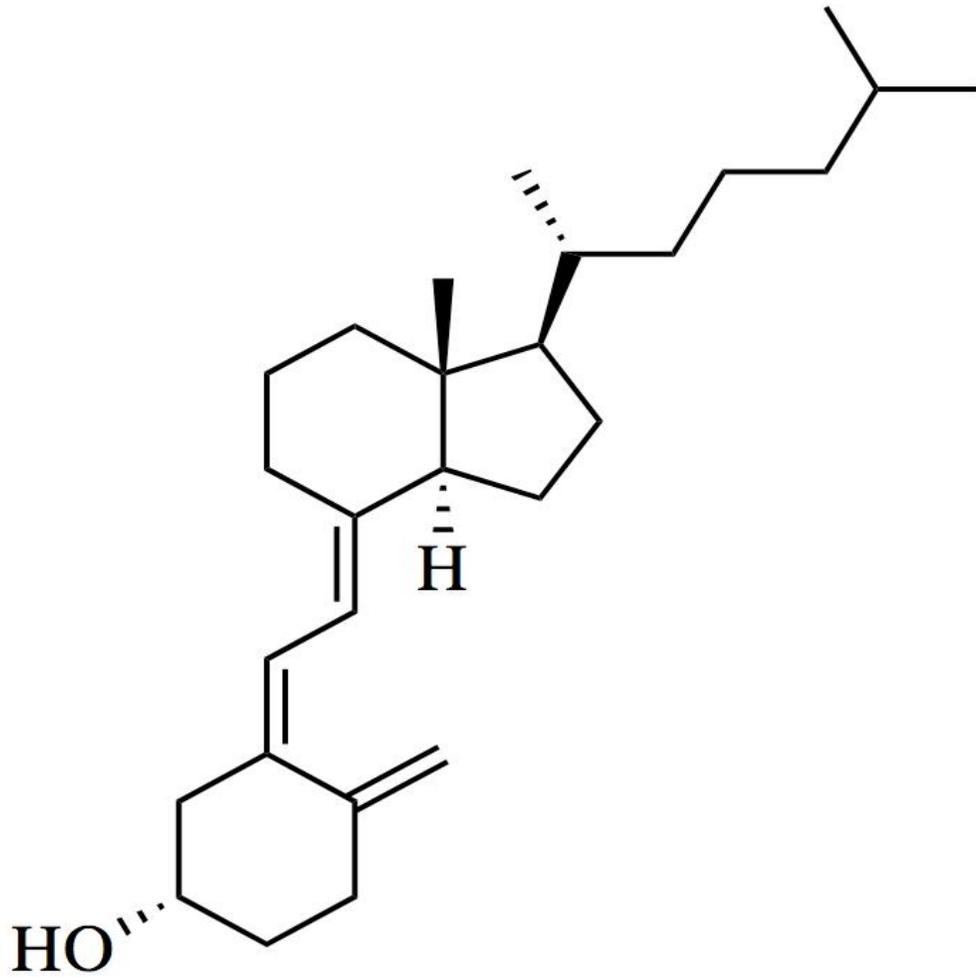


Vitamin D

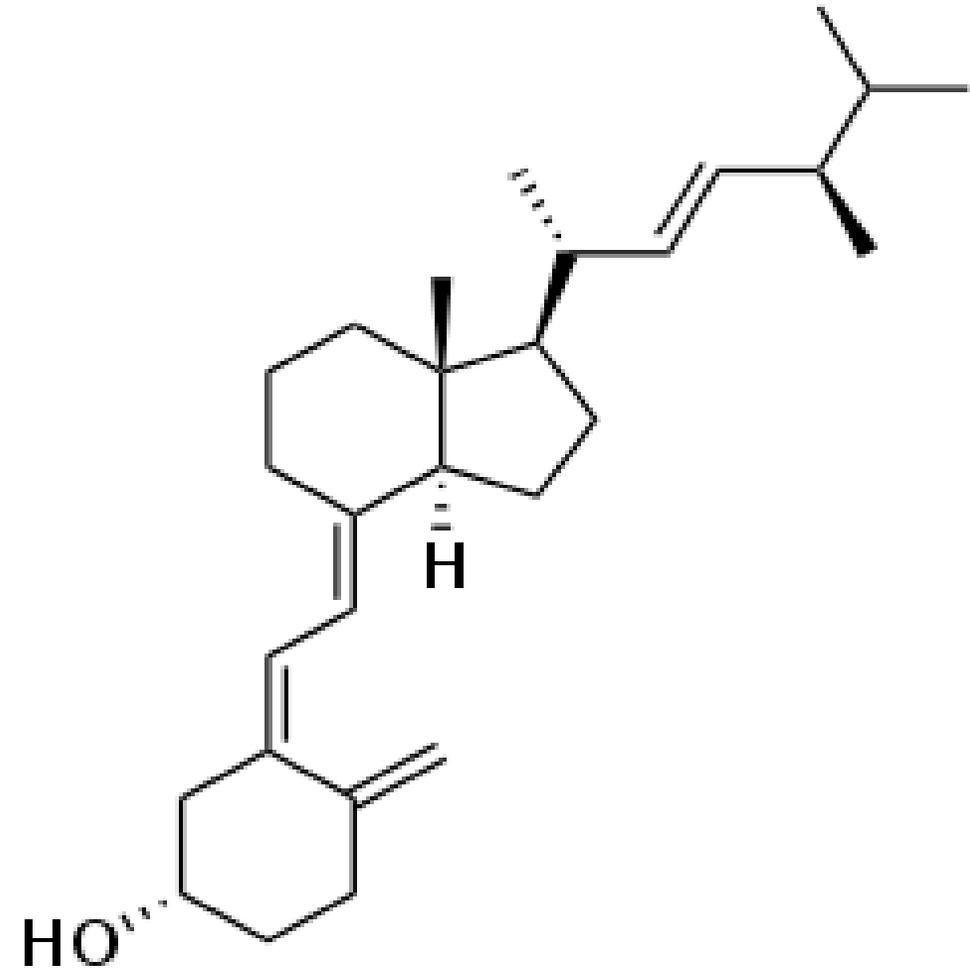
- **Vitamin D** refers to a group of **fat soluble secosteroids** (similar lipid-soluble molecules) that have a **hormone-like function**
- The active molecule binds to **intracellular receptor proteins**
- The most prominent actions-of the active molecule are to **regulate the plasma levels of calcium and phosphorus.**
- **Vitamin D** – precursor is **cholesterol**, converted by **UV** from **sunlight** exposure, therefore is a “non-essential” vitamin. It also called **sunshine vitamin**
- Vitamin D is available in **two forms**:
 - **Cholecalciferol (Vitamin D3)** is made from 7- dehydrocholesterol in the skin of animals and humans
 - **Calciferol (Vitamin D2)** is obtained artificially by irradiation of ergo-sterol and is called ergocalciferol



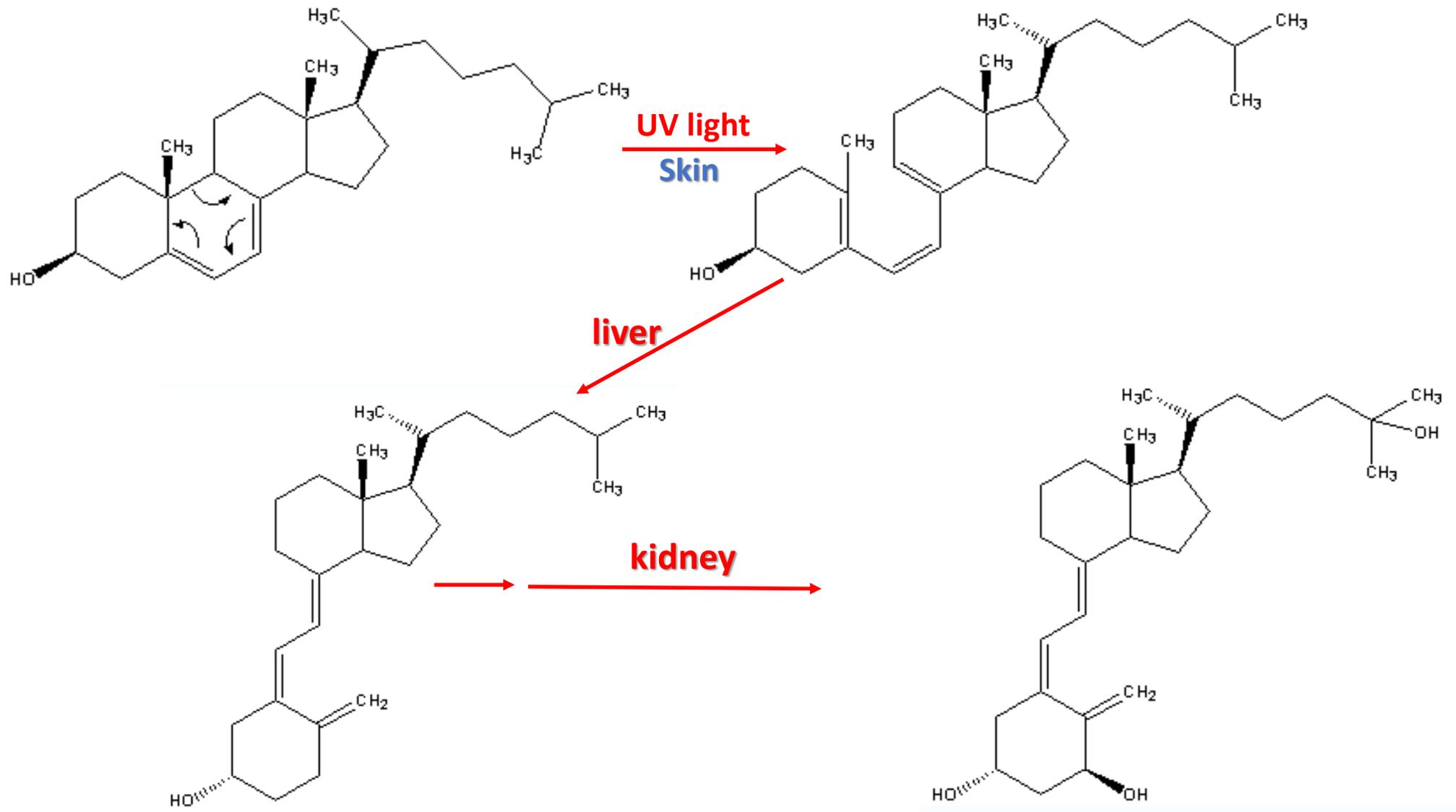
Vitamin D3 (cholecalciferol)



Vitamin D2 (ergocalciferol)



Vitamin D3 can be obtained in diet, or derived from **cholesterol** in a reaction that requires **UV light**.



Vitamin D3

calcitriol (1,25-dihydroxycholecalciferol)



Role of vitamin D

- Maintaining adequate plasma levels of calcium (**Calcium balance**):
 - **Stimulates** calcium **removal** from bone
 - **Increasing** uptake of calcium by the **intestine**
 - Minimizing **loss** of calcium by the **kidney**
- is essential for the proper utilization of calcium and phosphorus to produce normal, **healthy bones and teeth**
- Promotes **bone growth** and maintenance
- **Cell differentiation**
- Stimulates **maturation** of cells – heart and brain
- Important for **immune system function**
- **Blood pressure** regulation



Vitamin D Deficiencies

- Rickets (children)
- Osteomalacia (adults)

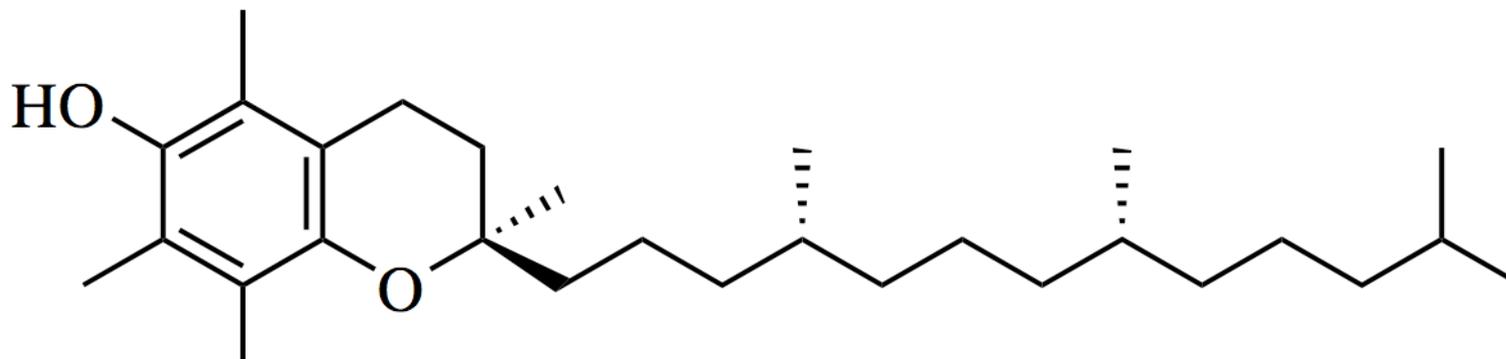
Toxicity of Vitamin D

- Vitamin D can be stored in the body and is **slowly metabolized**.
- **High doses** can cause loss of appetite, nausea, thirst, stupor.
- Enhanced calcium absorption and bone resorption results in **hypercalcemia** which can lead to **deposition of calcium** in many organs particularly the **arteries and kidney**.



Vitamin E

- Vitamin E refers to a group of compounds that include both **tocopherols** and **tocotrienols**.
- The word tocopherol is derived from the word **toco** meaning **child birth** and **peros** meaning **to bear**
- Essential for **normal reproduction** in many animals, hence known as **anti-sterility vitamin**
- It is also called **anti-aging factor**



α-tocopherol



Function of vitamin E

- **Anti-oxidant** (minimize the damage of free radicals)
- protects **cell membrane**
- Enhances **immune system**
- Protects **lipids** and prevents the oxidation of **polyunsaturated fatty acids** in various tissues
- Protects **RBC** from hemolysis by oxidizing agents



Vitamin K

- It naturally produced by **intestinal bacteria**
- It is essential for product of a type of protein called **prothrombin** and other factor involve in **blood clotting** mechanism
- It includes **two natural vitamins**
 - **K1 phylloquinone**
 - **K2 menaquinones**



Function of vitamin K

- Vitamin K is fat soluble vitamin with a specific **coenzyme function**. It is required in the **hepatic synthesis of prothrombin and blood clotting factors**.
- Vitamin K helps **blood clotting**, essential to **stop** bleeding from wounds.
- Necessary for the maintenance of **normal blood coagulation**.
- It prevents hemorrhage only in cases when there is **defective production of prothrombin**.
- **Oxidative phosphorylation**
- It acts as a **cofactor** in oxidative phosphorylation associated with **lipid**



Vitamin K deficiency

- Deficiencies are **rare** but
 - Seen in **infants**, Newborns are given a **dose of vitamin K at birth**.
 - After **prolonged antibiotic therapy**, and in patients with **decreased bile production**.
 - People with vitamin K deficiency may experience **easy bruising nosebleeds**
-
- ❖ Toxicities **>1000 mg/day**:
 - Rupture of **RBCs**
 - **Jaundice**



Water soluble vitamins

- Essential coenzymes required in energy releasing mechanisms
- Act as coenzymes for metabolism of carbohydrates, proteins, and fats
- ✓ **B1-thiamine**
- ✓ **B2-riboflavin**
- ✓ **B3-Niacin**
- ✓ **B5-pantothenic acid**
- ✓ **B6- pyridoxine**
- ✓ **B7-Biotin**
- ✓ **B9-folic acid**
- ✓ **B12-cobalamin**
- ✓ **Ascorbic acid**

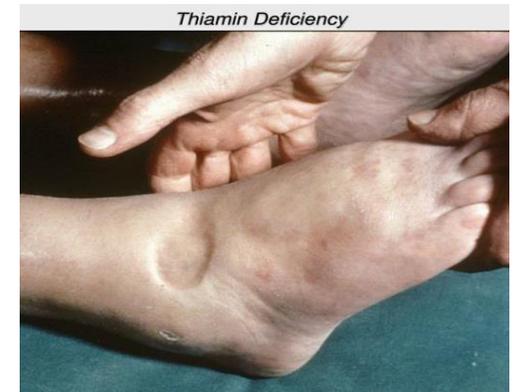
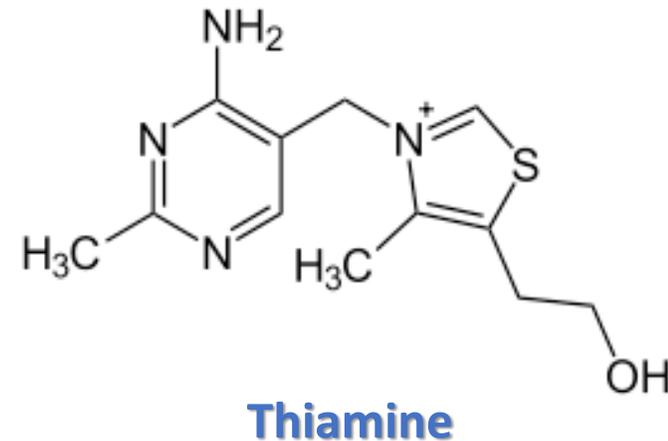


Thiamine (vitamin B1)

- It is colourless basic organic compound composed of a **sulphated pyramiding ring**
- It is synthesized only in **bacteria, fungi and plants**
- Thiamine pyrophosphate (**TPP**) is the biologically **active form** of the vitamin. It serves as a coenzyme in the **oxidative decarboxylation of α -keto acids**.

○ Important in:

- Producing **energy** from **carbohydrates**
- **Nerve function**



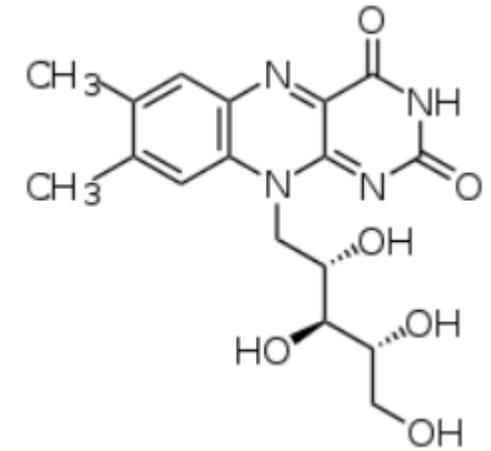
- **Deficiency** of vitamin 1 causes **beriberi** (Muscle atrophy, neurological problems)
- **Wernicke-korsakoff syndrome** (Characterized by apathy, loos of memory)



Riboflavin (vitamin B2)

- It is **yellowish green** fluorescent compound
- It is widely involved in **oxidation reduction** reaction
- Riboflavin is a precursor for **FAD** and **FMN**.
- The word Riboflavin derived from **2 sources**
 - **Ribose**-mean many **ribose sugar** found in several vitamins
 - **Flavin**-yellow
- **Light** can destroy riboflavin, so purchase milk in opaque containers.

- ❖ **NAD**= Nicotinamide adenine dinucleotide
- ❖ **FMN**= Flavin mononucleotide



Riboflavin (vitamin B2)

Important in:

- Energy production
- Carbohydrate, fat, and protein metabolism
- Formation of antibodies and red blood cells (RBCs)
- Cell respiration
- Maintenance of good vision, skin, nails, and hair

Deficiency of vitamin B2 can causes:

- Itching and burning eyes (eye abnormalities)
- Cracks and sores in mouth and lips (fissuring at the corners of the mouth)
- Glossitis (the tongue appearing smooth and purplish).



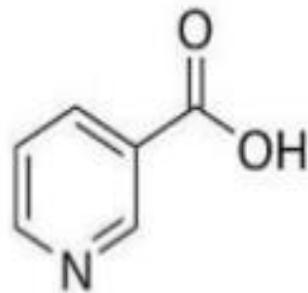
Niacin (vitamin B3)

- Vitamin B3 **niacin** or **nicotinic acid**
- It is a **pyridine** derivative and is precursor of the **NAD** (Nicotinamide adenine dinucleotide)
- Essential for **metabolism** of carbohydrate, fat and protein

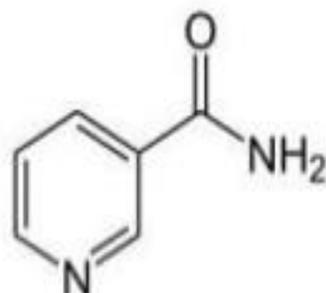
NAD⁺ A cofactor to remember.

Deficiency of vitamin B3 can causes:

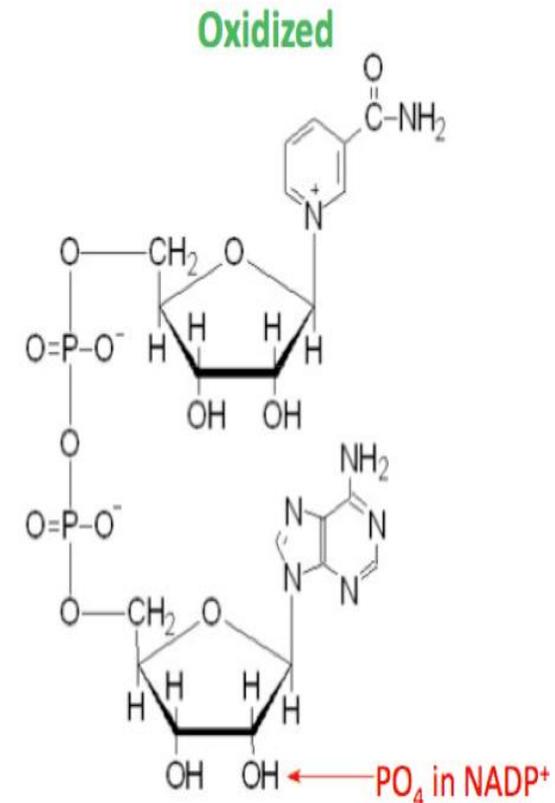
- **Pellagra**
- **Dermatitis**



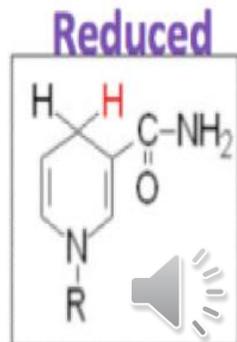
Niacin



Nicotinamide

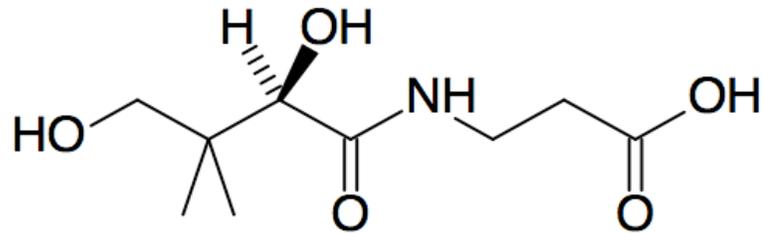


- Made up of nicotinamide attached to an AMP
- Can accept two electrons when reduced
- Gets reduced to NADH



Pantothenic acid (vitamin B5)

- This word derived from Greek word **pantos** meaning **everywhere**
- Part of **coenzyme A**
- Essential for **metabolism** of carbohydrate, fat and protein

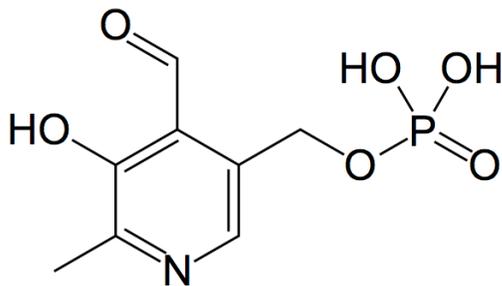


Pantothenic acid

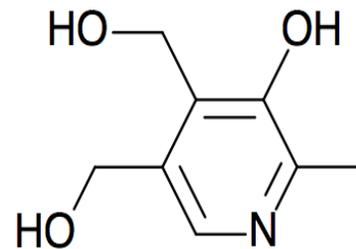


Pyridoxine (vitamin B6)

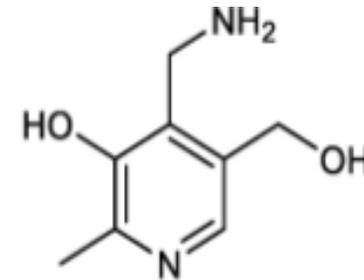
- Vitamin B6 refers to a group of chemical very similar compounds, **pyridoxine**, **pyridoxal** and **pyridoxamine**, all derives of pyridine, which can be interconverted in biological systems
- Its active form of B complex, **pyridoxal 5-phosphate (PLP)** serves as a cofactor in many enzyme reactions in **amino acid**, **glucose**, and **lipid metabolism**
- **PLP** is a covalently linked cofactor to **transaminases**, and some **decarboxylases**, and **glycogen phosphorylase**; these are called “PLP-dependent enzymes



pyridoxal 5-phosphate



pyridoxine



pyridoxamine



Pyridoxine (vitamin B6)

Important in:

- Production of red blood cells (RBCs)
- Enhances immune system
- Nervous system functions
- Reducing muscle spasms
- Keep blood sugar (glucose) in normal ranges
- Maintaining proper balance of sodium and phosphorous in the body
- Coenzyme for a large number of enzymes particularly those that catalyze reactions involving amino acids (transamination, deamination, decarboxylation).



Deficiency symptoms of B6

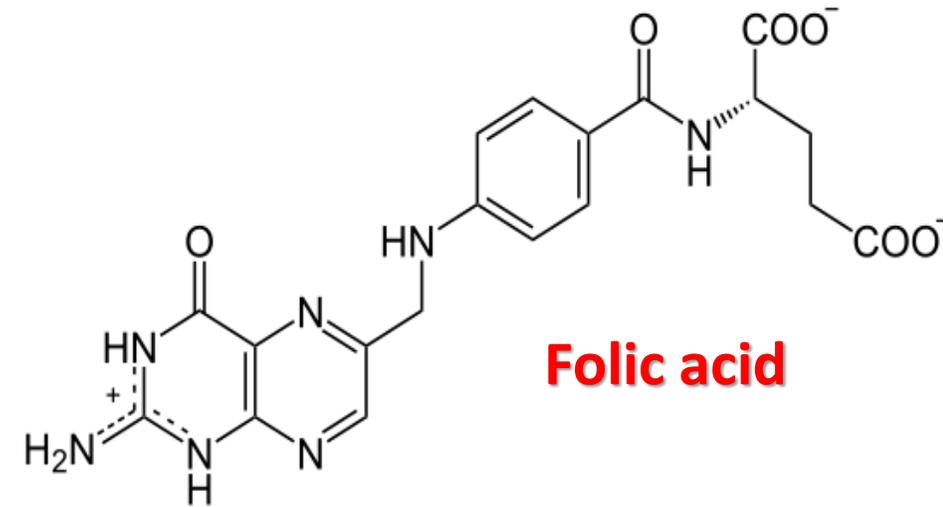
- Associated with **neurological** symptoms such as **depression, irritability, nervousness and mental confusion** due to decrease synthesis of serotonin, Gamma Aminobutyric Acid (GABA), adrenaline norepinephrine.
- **Anemia**



Folic acid (vitamin B9)

Important in:

- Formation of red blood cells (RBCs)
- Nerve function
- DNA reproduction
- Necessary for growth and division of all body cells
- Prevention of anemia
- Essential for the health of skin and hair
- Fat and proteins metabolism to energy production
- **Pregnancy**- Folic acid is an important nutrient for **pregnant women** and her developing **fetus**, also it improves the **lactation**
- Very important in **early pregnancy** (important for **rapidly dividing cells**)



Deficiency of vitamin B9

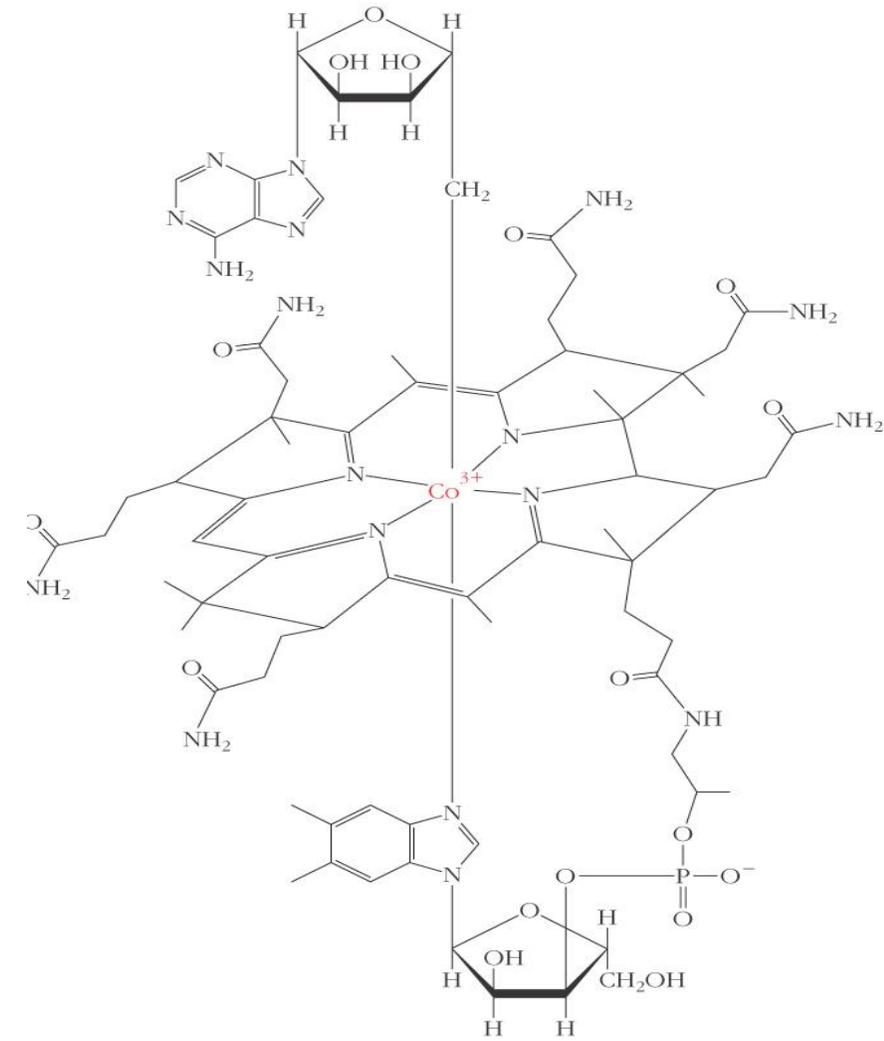
Deficiency of folic acid can causes:

- Anemia
- Nerve damage
- Hypersensitive skin



Cobalamin (vitamin B12)

- Also known as **anti-pernicious anemia** vitamin.
- synthesized only by **microorganisms**
- **Production of red blood cells**
- **Nervous: It improves concentration, memory and balance**
- Promotes **growth** and increase **appetite**
- Important for metabolism of carbohydrate, protein, fat and folic acid



Cobalamin



Deficiency of vitamin B12

Deficiency of vitamin B12 can causes:

- **Pernicious anemia** due to defect to absorb the vitamin from the **intestine**, not by an absence of the vitamin in the diet.
- Low levels of **hemoglobin**
- Irreversible **nerve cell death**

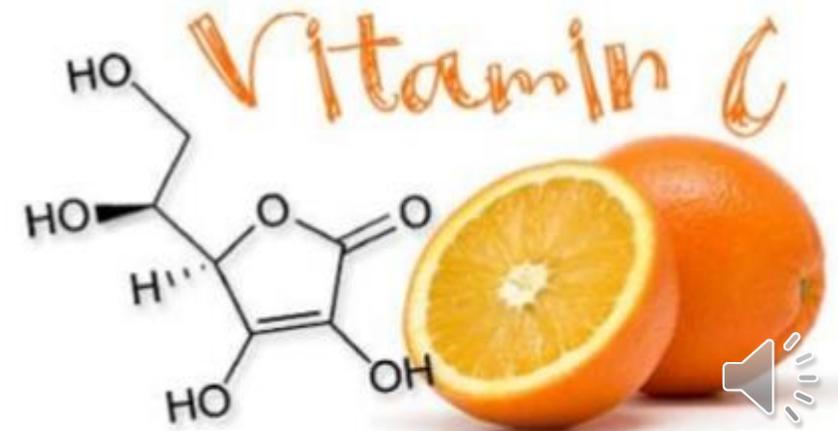


Ascorbic acid (vit C)

- Ascorbic acid (Toxic to **viruses, bacteria**, and some **malignant tumor cells**)
- In almost all organisms, ascorbic acid is synthesized from **glucose**

Functions of vitamin C in the body

- Protects the body from free radicals (**antioxidant**)
- Maintenance of bones and proper adrenal thyroid gland
- Helps form connective tissue (**Collagen**), and as a **cofactor for several enzymes**.
- Helps **healing of wounds**
- Helps in **absorbing iron**
- keep the **gums healthy**
- **Stimulates immune functions**
- prevention of **heart disease and cancer**



Deficiency of vitamin C can causes

- Weight loss
- **Scurvy** (swollen and painful joints and bleeding gums, poor wound healing, and bleeding from the skin may occur, and finally death from infection or bleeding)
- Reduced resistance to colds and infections
- poor wound healing and fractured bones
- Spongy gums loose of teeth
- **Harmful effects** in larger doses: (over 1000mg/ dose)
- **Diarrhea**
- ❖ Chewable tablets (may cause damage to teeth)

