Madenet Al elm University College
Department of Biology
2nd Year Biochemistry I 2021 – 2022

BIOCHEMISTRY

Dr. Hamza Yaseen Isa
Lecture 1

Biochemistry

- Definition: The chemistry of life
  - The science concerned with the chemical basis of life.
  - The science concerned with the various molecules that occur in living cells and organisms and with their chemical reaction.
  - Anything more than a superficial comprehension of life – in all its diverse manifestation - demands a knowledge of biochemistry.

Biochemistry

Why studying biochemistry

to describe and explain, *in molecular terms*, all chemical processes of living cells, including:

- Structure-function
- Metabolism and Regulation
- Genetics

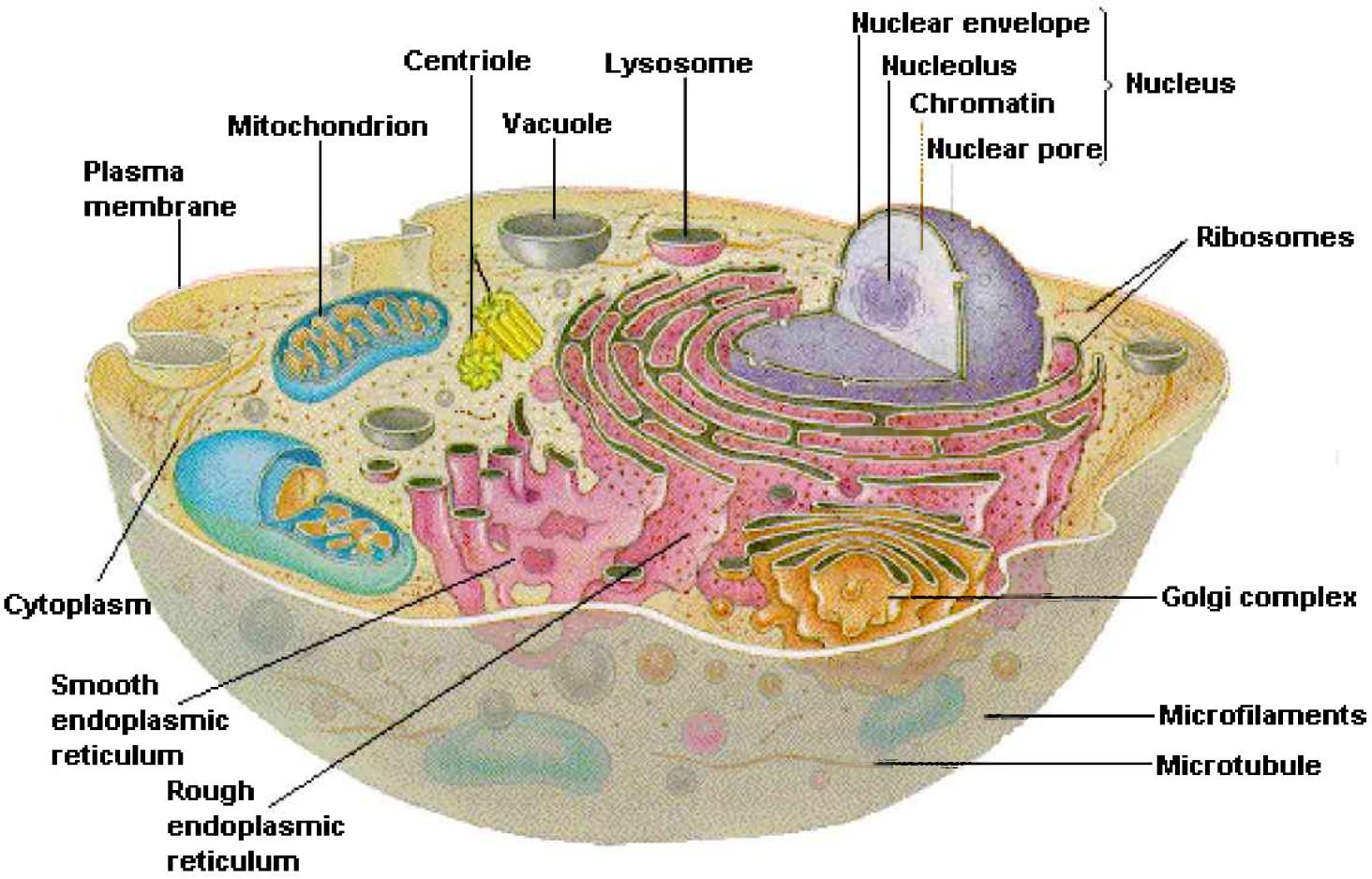
Biochemistry

- Significance: be essential to all life sciences as the common knowledge
  - Genetics; Cell biology; Molecular biology
  - Physiology and Immunology
  - Pharmacology and Pharmacy
  - Toxicology; Pathology; Microbiology
  - Zoology and Botany

What dose the Biochemistry discuss?

1. structure and function of cellular components
   - proteins, carbohydrates, lipids, nucleic acids and other biomolecules
2. Metabolism and Regulation
3. Gene expression and modulation

Cell Structure

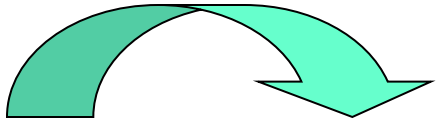


Bio – organic compounds

1. Carbohydrates.
2. Lipids.
3. Nucleic acids.
4. Amino acids.
5. Proteins.
6. Enzymes.
7. Hormones.
8. Vitamines.
9. Inorganic minerals.

Polymers and Monomers

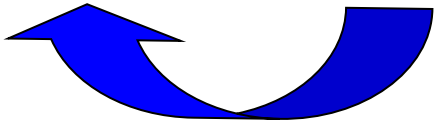
- Each of these types of molecules are polymers that are assembled from single units called monomers.
- Each type of macromolecule is an assemblage of different types of monomer.



Anabolic

- Building block
  - Simple sugar
  - Amino acid
  - Nucleotide
  - Fatty acid
- Macromolecule
  - Polysaccharide
  - Protein (peptide)
  - RNA or DNA
  - Lipid

Catabolic

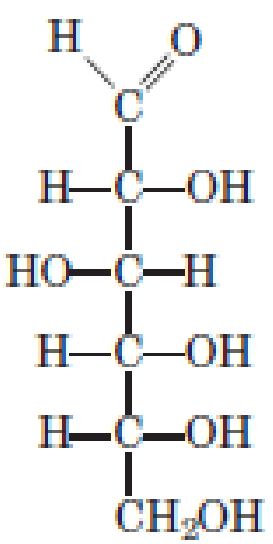


CARBOHYDRATES

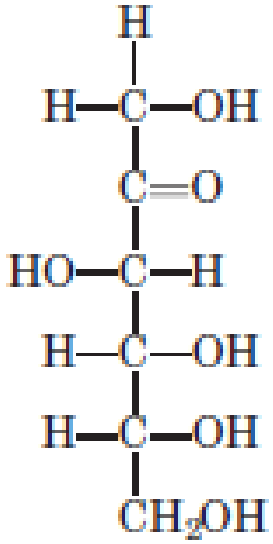
- Living things use carbohydrates as a key source of ENERGY!
- Plants use carbohydrates for structure (CELLULOSE)
  - include sugars and complex carbohydrates (starches)
  - contain the elements carbon, hydrogen, and oxygen (the hydrogen is in a 2:1 ratio to oxygen)

Monosaccharides (simple sugars)

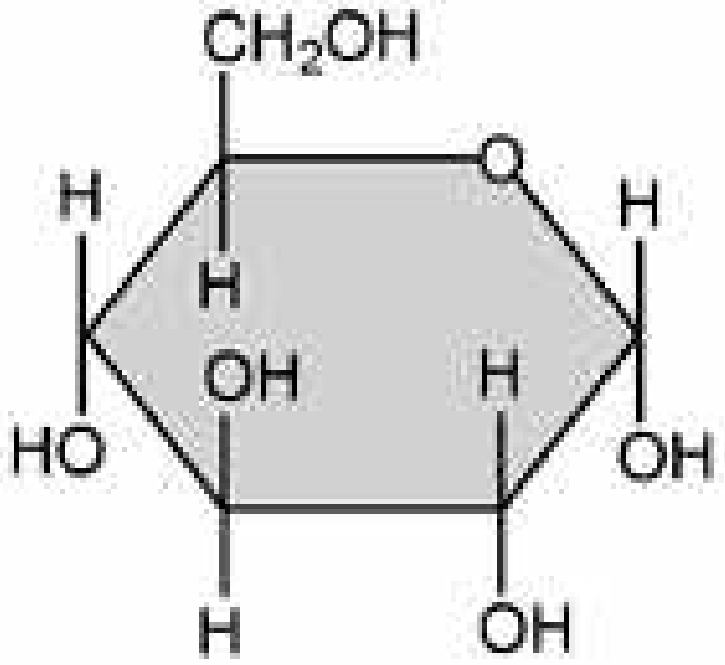
- all have the formula C6 H12 O6
- all have a single ring structure
  - (glucose is an example)



D-Glucose,
an aldohexose

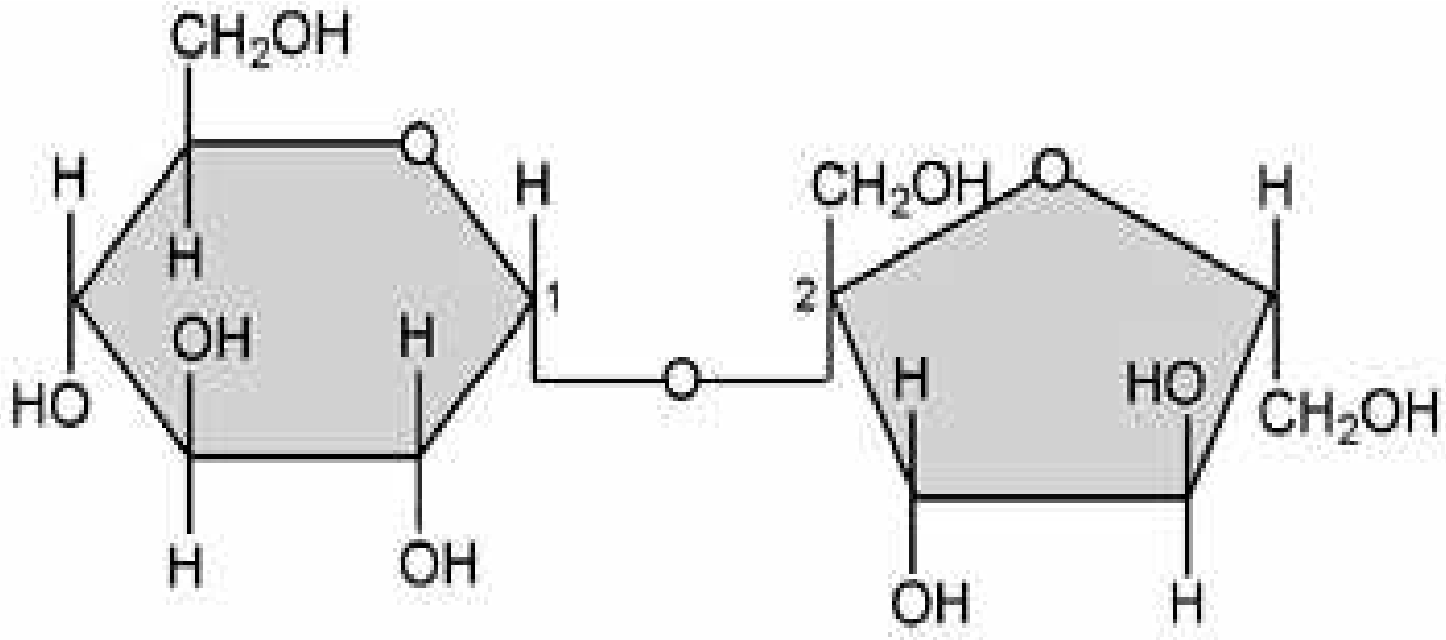


D-Fructose,
a ketohexose



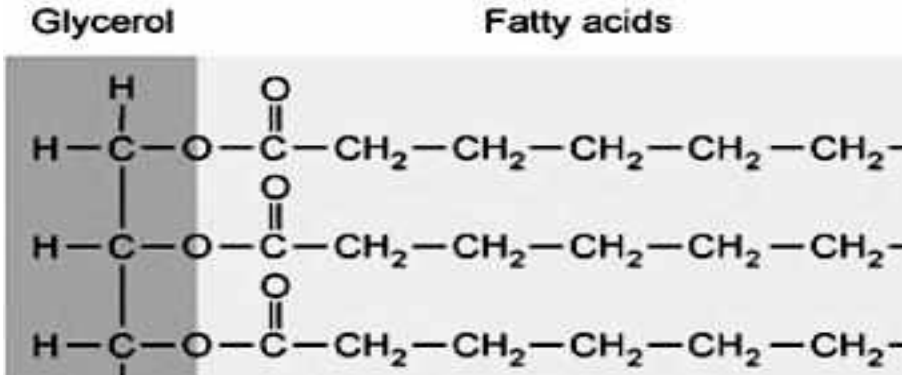
Disaccharides (double sugars)

- all have the formula C12 H22 O11
- sucrose (table sugar) is an example

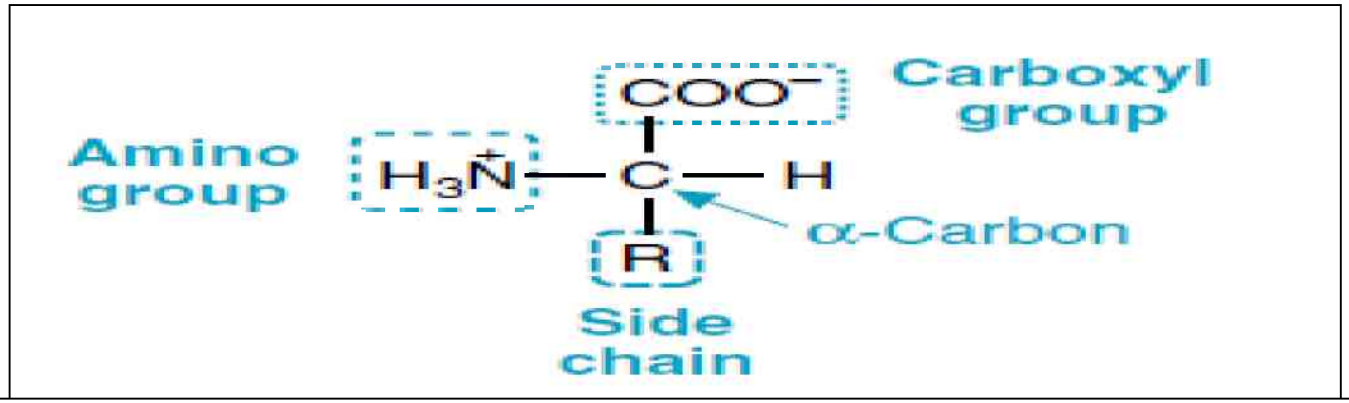


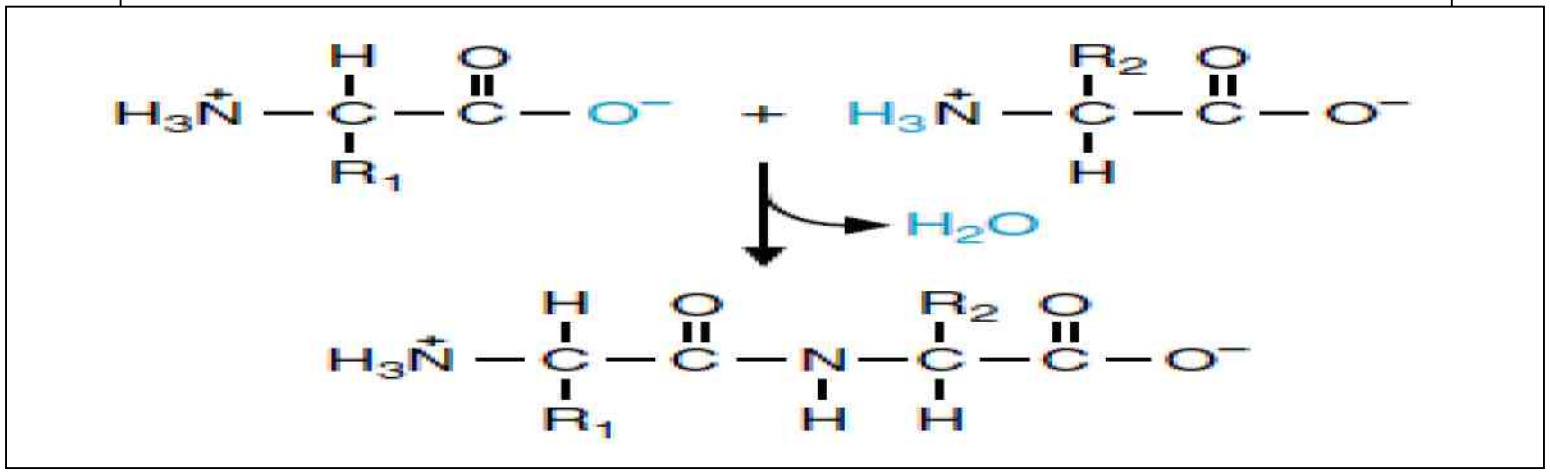
Lipids (Fats)

- Fats, oils, waxes, steroids
- Chiefly function in energy storage, protection, and insulation
- Contain carbon, hydrogen, and oxygen but the H:O is not in a 2:1 ratio
- Tend to be large molecules -- an example of a neutral lipid is below



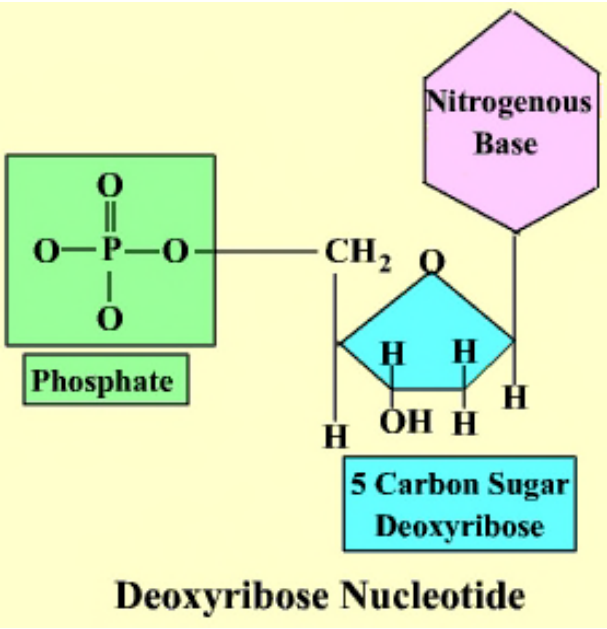
Amino acids





DNA (deoxyribonucleic acid)

- contains the genetic code of instructions that direct a cell's behavior through the synthesis of proteins
- found in the chromosomes of the nucleus (and a few other organelles)



Cells

- Basic building blocks of life
- Smallest living unit of an organism
- Cell volume is 10 microns and its weight is one Nano gram.
- One cell contains 10000 types of chemical compounds.
- A cell may be an entire organism (unicellular) or it may be one of billions of cells that make up the organism (multicellular).
- Grow, reproduce, use energy, adapt, respond to their environment
- Many cannot be seen with the naked eye
  - a typical cell size is 10μm; a typical cell mass is 1 nano gram.)

Chemical composition of a normal man (weight 65 kg)

Constituent	Percent (%)	Weight (kg)
Water	61.6	40
Protein	17.0	11
Lipid	13.8	9
Carbohydrate	1.5	1
Minerals	6.1	4

Similarities among all types of cells

- All cells use nucleic acids (DNA) to store information
  - Except RNA viruses, but not true cells (incapable of autonomous replication)
- All cells use nucleic acids (RNA) to access stored information
- All cells use proteins as catalysts (enzymes) for chemical reactions
- All cells use lipids for membrane components
  - Different types of lipids in different types of cells
- All cells use carbohydrates for cell walls (if present), recognition, and energy generation

What is molecular biology?

- **The attempt to understand biological phenomena in molecular terms**
- **The study of gene structure and function at the molecular level**
- **As a result, It is the study of molecular basic of the process of replication, transcription and translation of the genetic material.**
- **Molecular biology mainly concerns itself with:**

Understanding of interactions between the various systems of a cell, including the interactions between DNA, RNA and protein biosynthesis

- **learning how these interactions are regulated.**

Definitions

Nucleic acids are polymers of nucleotides

Nucleotides are carbon ring structures containing nitrogen linked to a 5-carbon sugar (a ribose)

5-carbon sugar is either a ribose or a deoxy-ribose making the nucleotide either a ribonucleotide or a deoxy ribonucleotide

In eukaryotic cells nucleic acids are either:

- Deoxyribose nucleic acids (DNA)
- Ribose nucleic acids (RNA)
  - Messenger RNA (mRNA)
  - Transfer RNA (tRNA)
  - Ribosomal RNA (rRNA)

Nucleic Acid Function

DNA

Genetic material - sequence of nucleotides encodes different amino acids

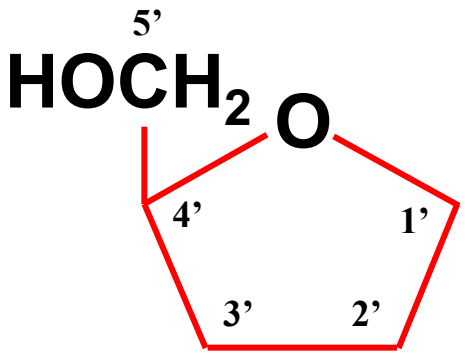
RNA

Involved in the transcription/translation of genetic material (DNA)

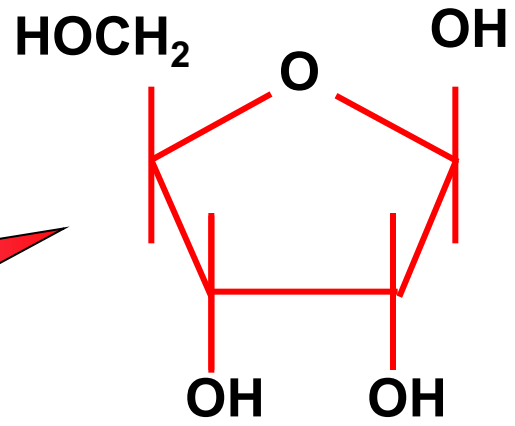
Genetic material of some viruses

Nucleotide Structure: 1- Sugars

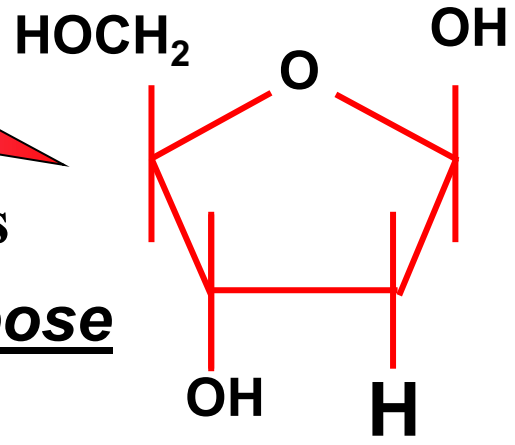
Generic Ribose Structure



Ribose

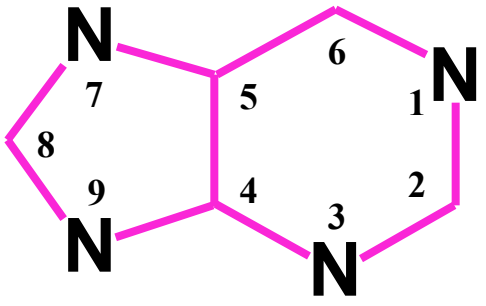


Deoxyribose

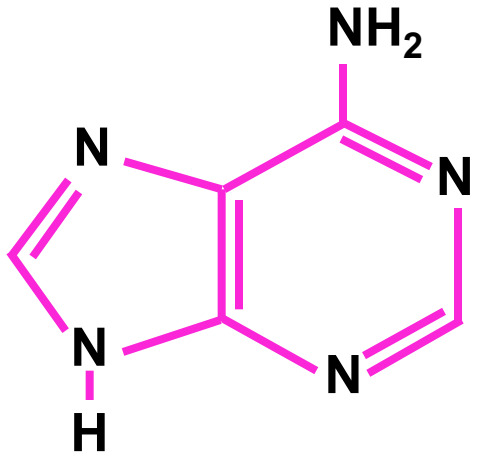


N.B. Carbons are given numberings as a prime.

Nucleotide Structure : 2- Bases - Purines

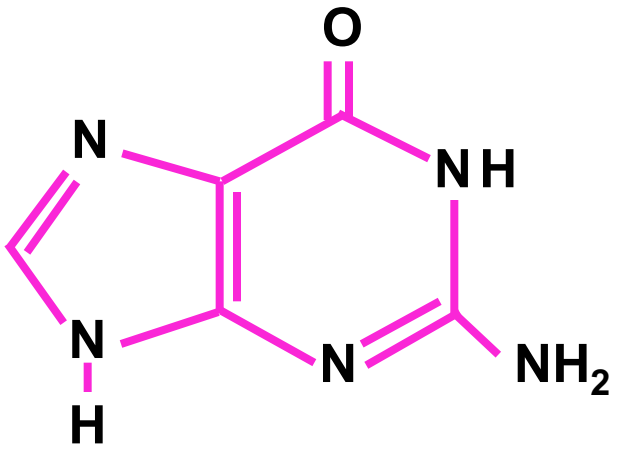


Adenine



A

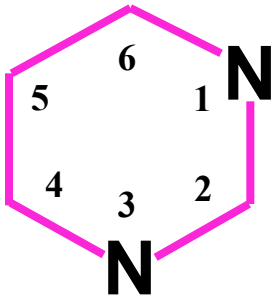
Guanine



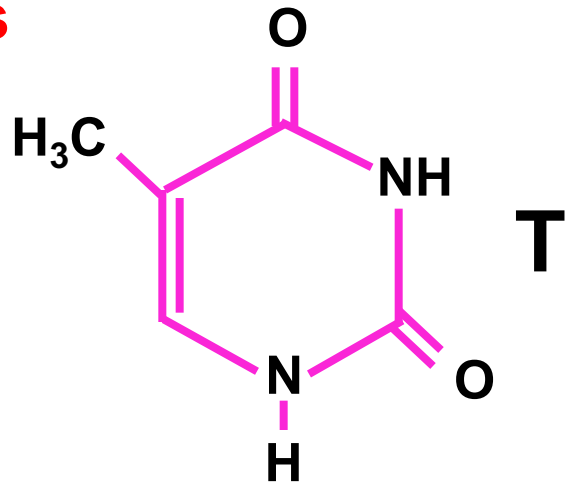
G

Nucleotide Structure :

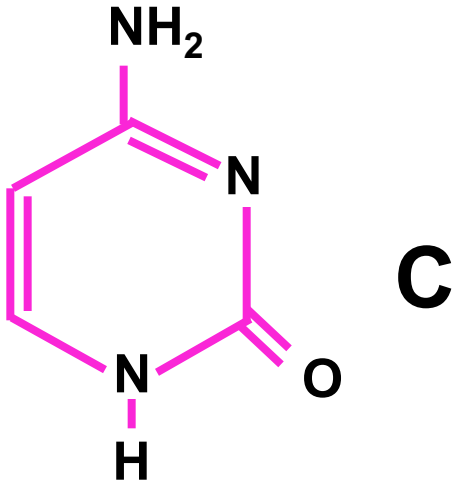
3- Bases - Pyrimidines



Thymine



Cytosine

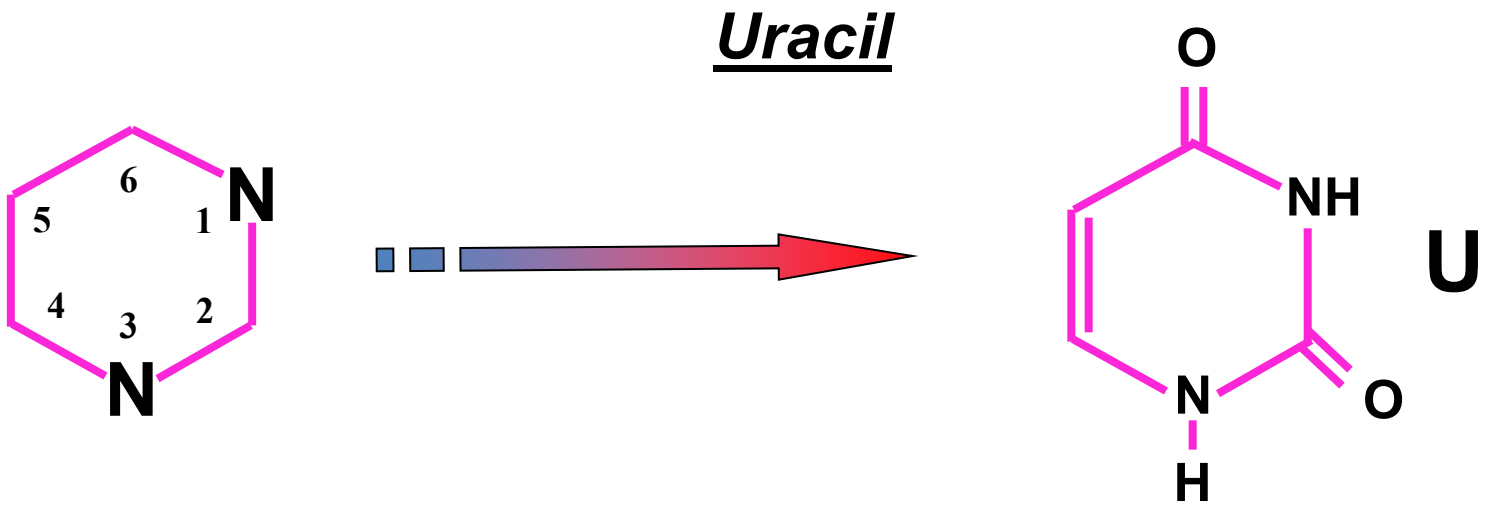


Nucleotide Structure :
3 - Bases - Pyrimidines

Thymine is found ONLY in DNA.

In RNA, thymine is replaced by uracil

Uracil and Thymine are structurally similar

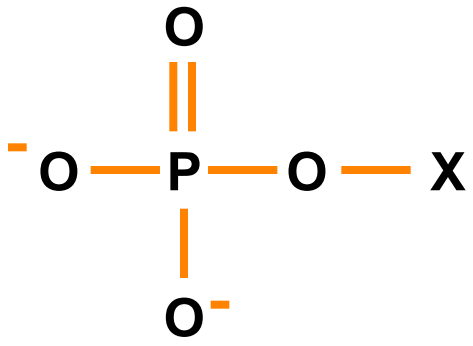


Nucleotide Structure:
4- Phosphate Group

Phosphate groups are what makes a nucleoside a nucleotide

Phosphate groups are **essential** for nucleotide polymerization

Basic structure:



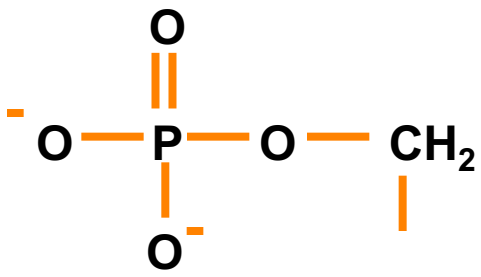
Nucleotide Structure:

4- Phosphate Groups

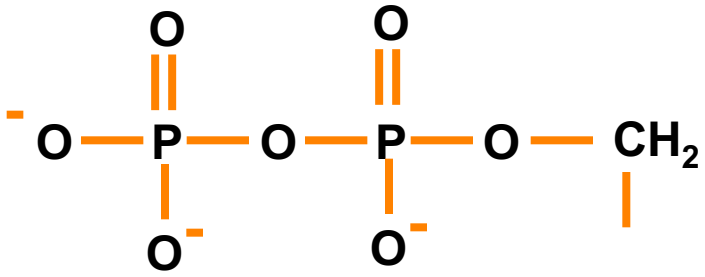
Number of phosphate groups determines nomenclature

Monophosphate
e.g. AMP

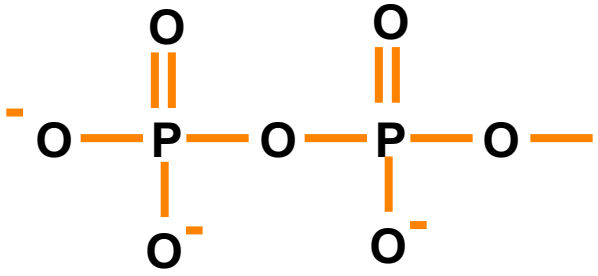
Free = inorganic phosphate (Pi)



Diphosphate
e.g. ADP



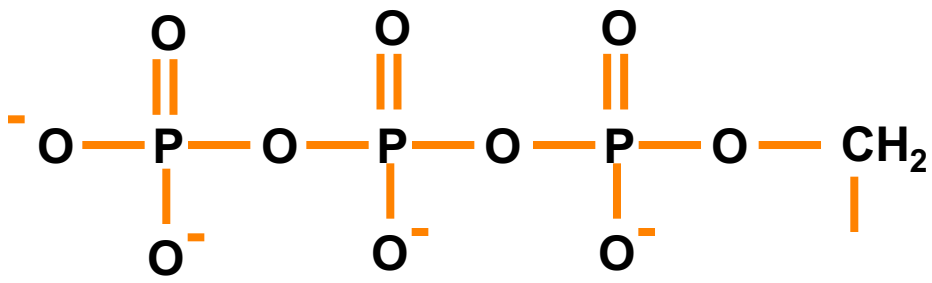
Free = Pyro-phosphate (PPi)



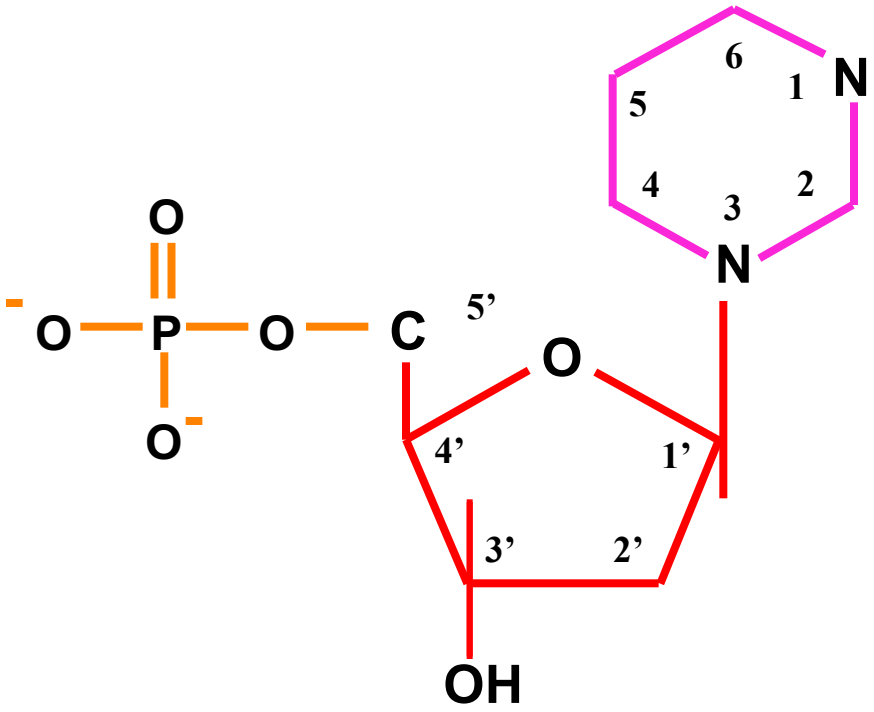
Nucleotide Structure:
4- Phosphate Groups

Triphosphate
e.g. ATP

No Free form exists

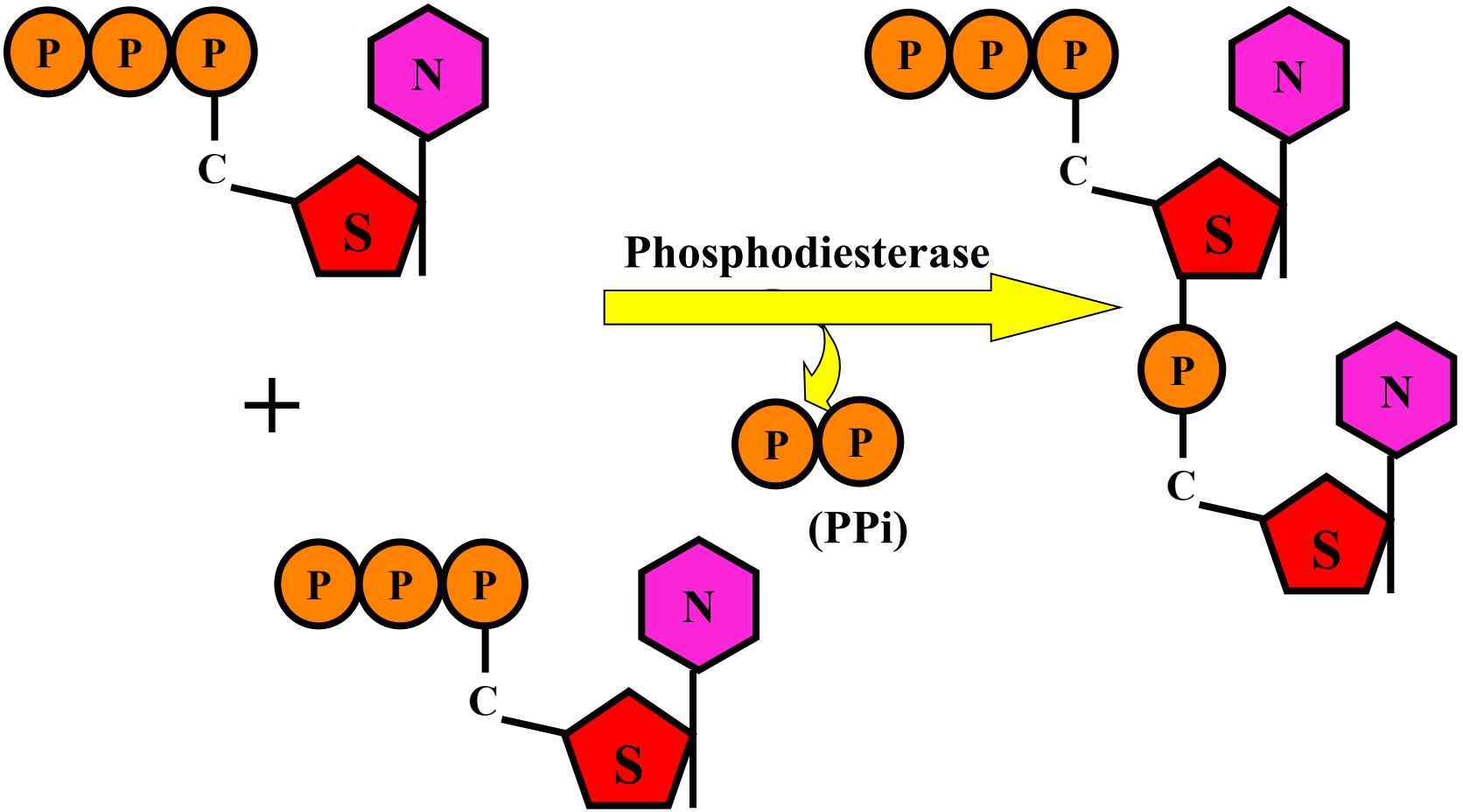


Nucleotide Structure:
5- Base-Sugar-PO_4^{2-}



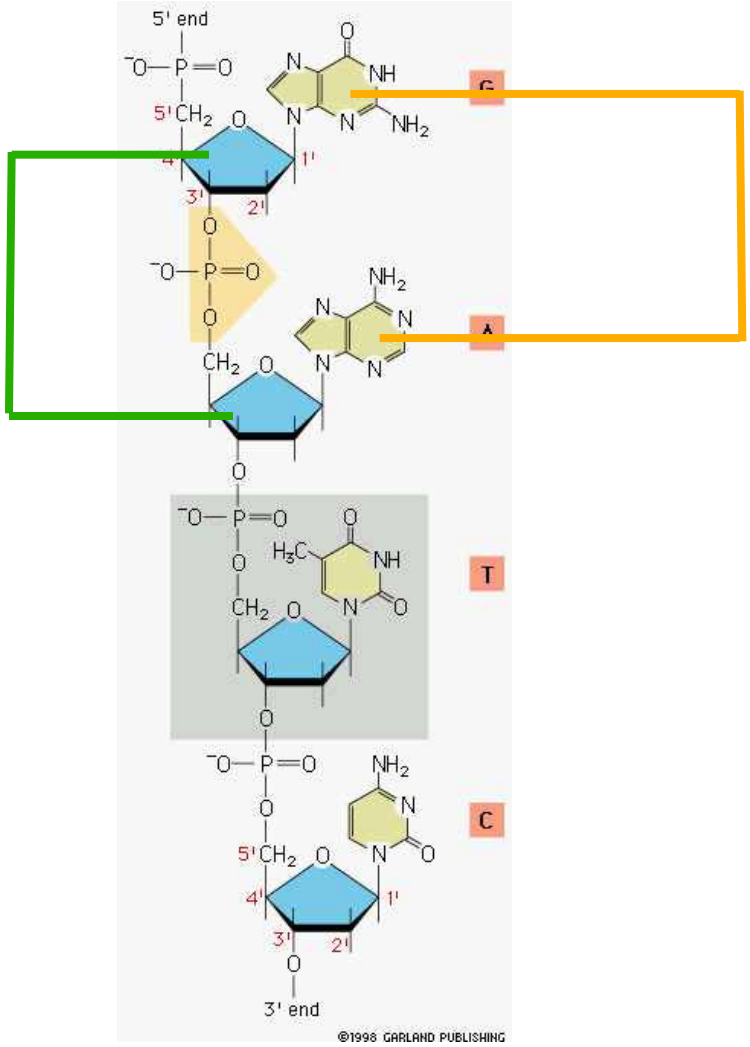
Monophosphate

Nucleic Acid Structure
Polymerization



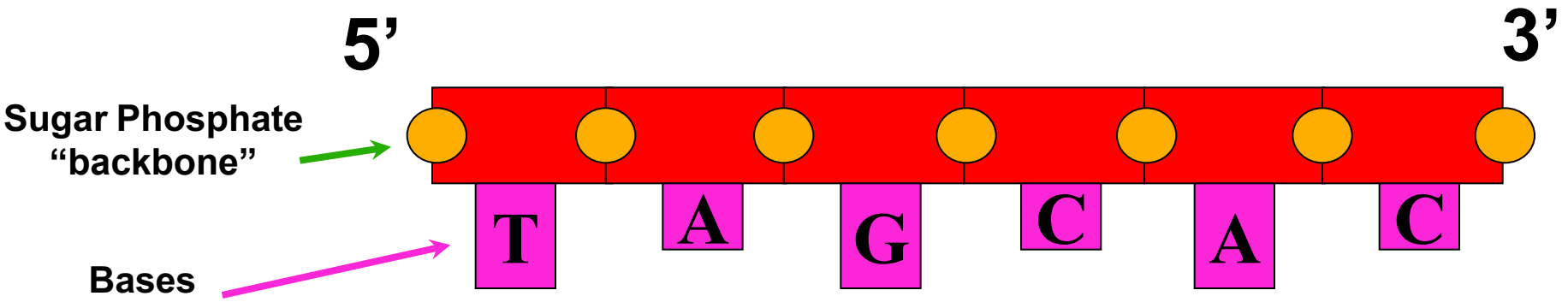
Nucleic Acid Structure
Polymerization

Sugar Phosphate
"backbone"



Nucleotide

Nucleic Acid Structure
Polymerization



5’ TAGCAC 3’

Nucleic Acid Structure

"Base Pairing"

RNA [normally] exists as a single stranded polymer

DNA exists as a double stranded polymer

DNA double strand is created by hydrogen bonds between nucleotides

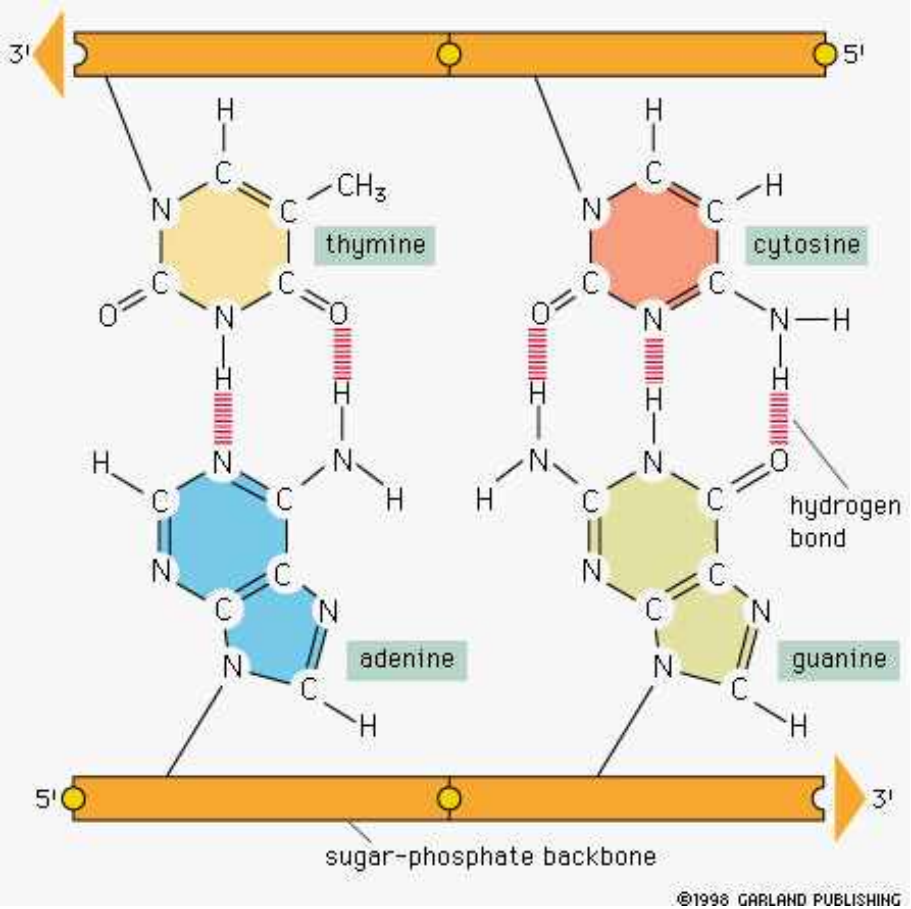
Nucleotides always bind to complementary nucleotides

A ⟷ **T** **(2 H-bonds)**

G ⟷ **C** **(3 H-bonds)**

Nucleic Acid Structure

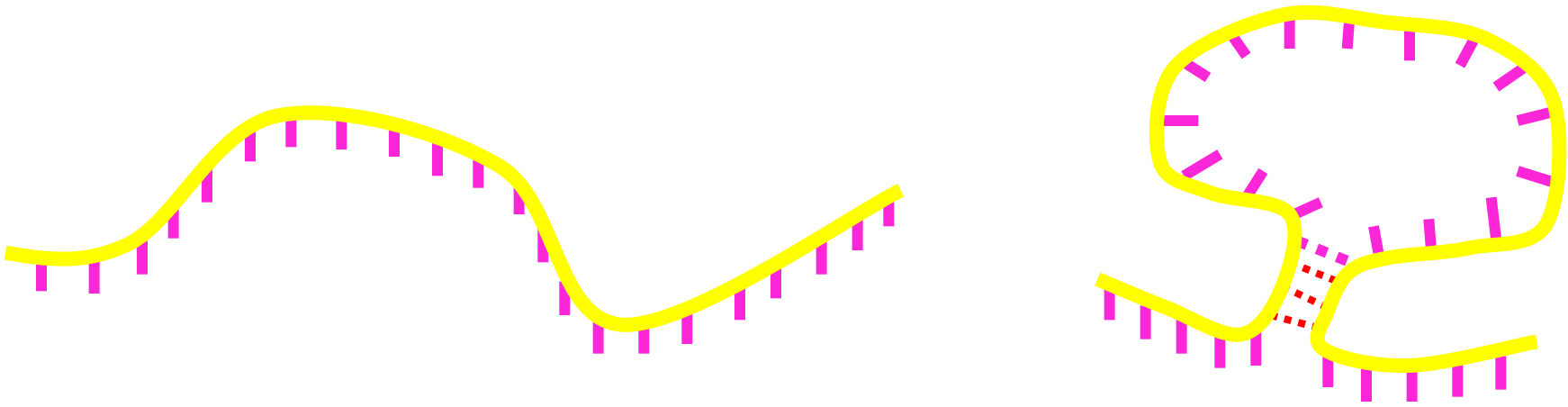
"Base Pairing"



Nucleic Acid Structure
"Base Pairing"

RNA is [usually] single stranded

Base pairing can occur in RNA but is usually within the same strand



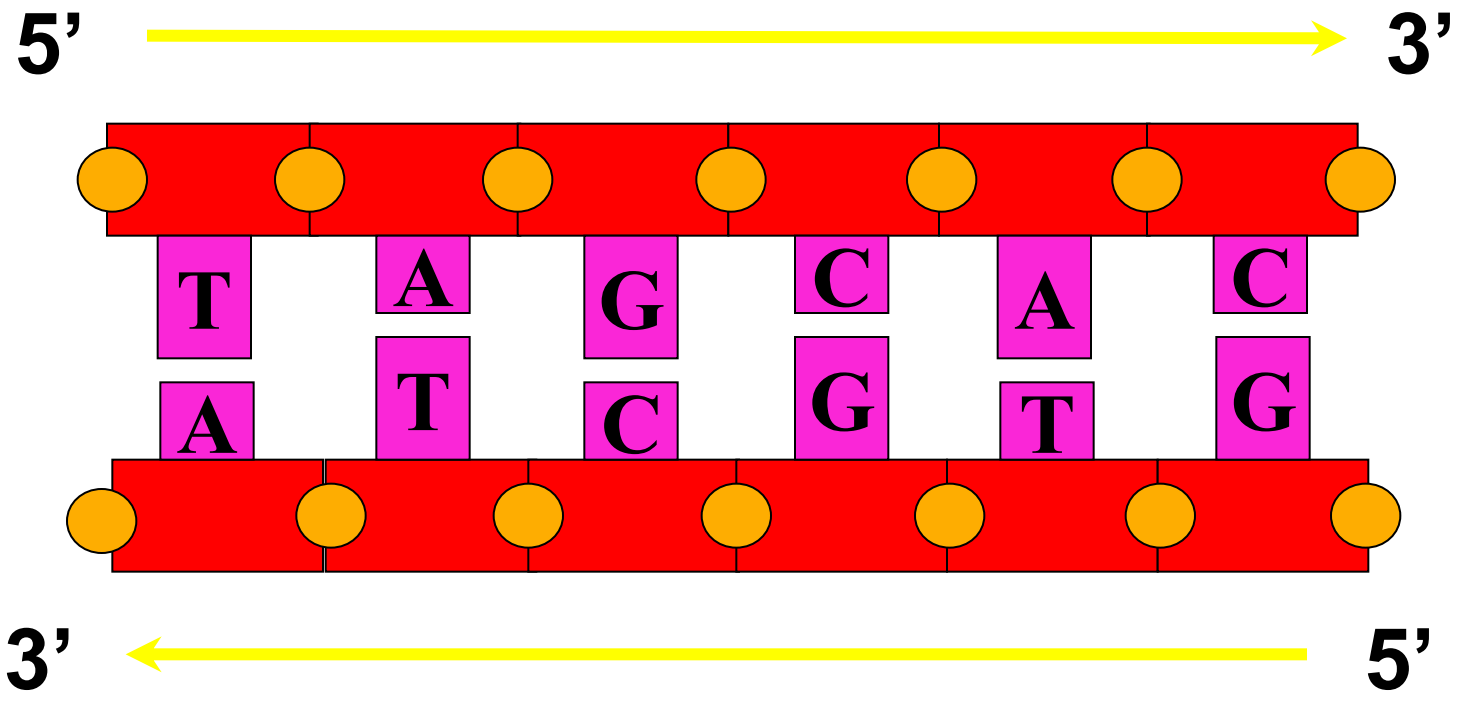
Difference between RNA & DNA

RNA	DNA
RNA nucleotides contain ribose sugar	DNA contains deoxyribose
RNA has the base uracil	DNA has the base thymine
presence of a hydroxyl group at the 2' position of the ribose sugar.	Lacks of a hydroxyl group at the 2' position of the ribose sugar.
RNA is usually single-stranded	DNA is usually double-stranded

Nucleic Acid Structure
"Base Pairing"

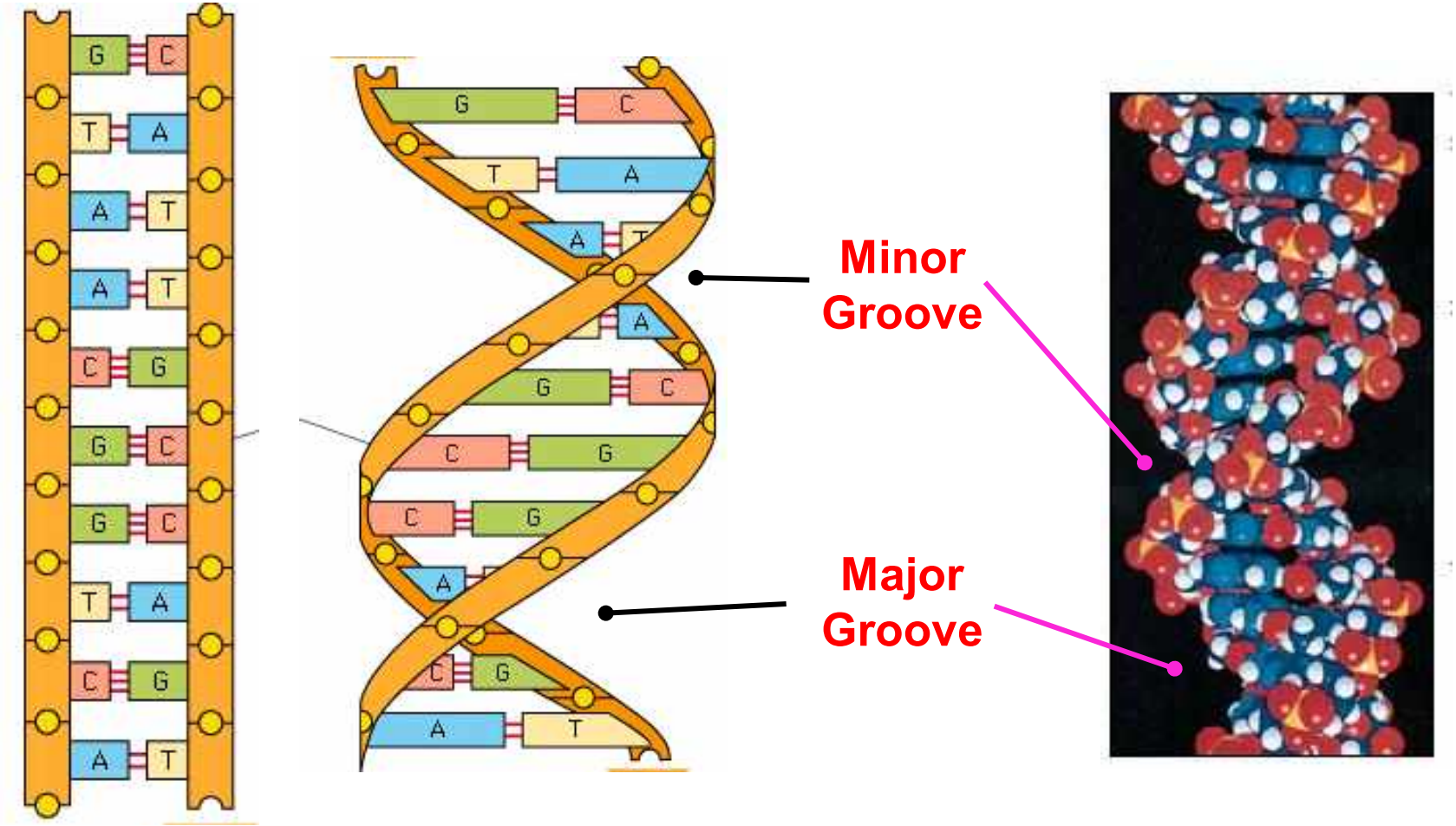
DNA base-pairing is **antiparallel**

i.e. 5' - 3' (l-r) on top : 5' - 3' (r-l) on bottom



Nucleic Acid Structure

The double helix



Madenet Alelm University College
Department of Biology
2nd Year Biochemistry I 2021 – 2022

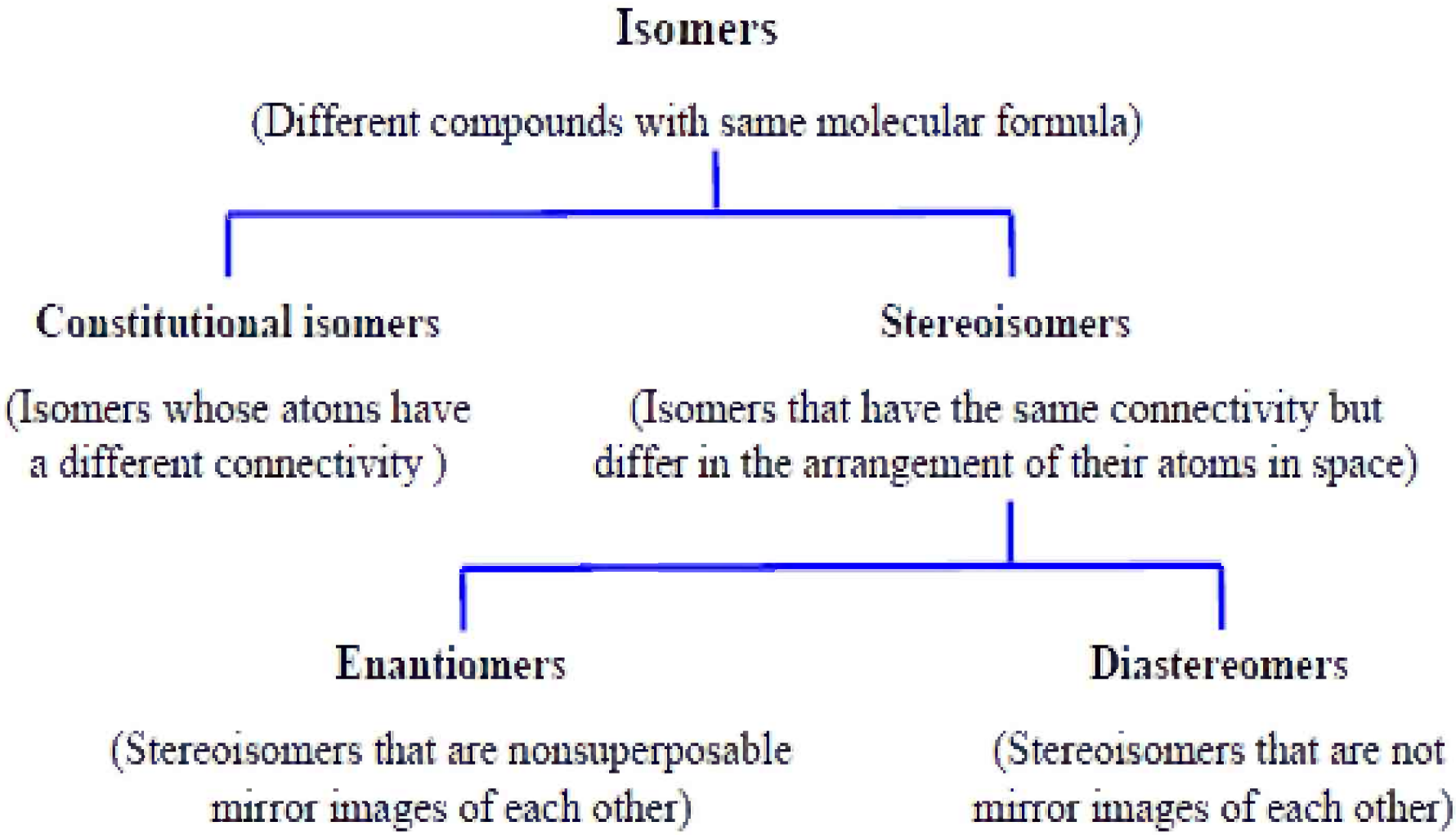
CHEMISTRY OF BIOMOLECULES

Dr. Hamza Yaseen Isa
Lecture 2 I 2

Stereochemistry

- Some objects are not the same as their mirror images (technically, they have no plane of symmetry)
  - A right-hand glove is different than a left-hand glove
  - The property is commonly called “handedness”
- Organic molecules (including many drugs) **have handedness that results from substitution patterns on sp^3 hybridized carbon**

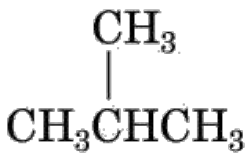
SUBDIVISION OF ISOMERS



Constitutional Isomers

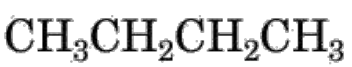
- Different order of connections gives different carbon backbone and/or different functional groups

Different carbon skeletons



Isobutane

and



Butane

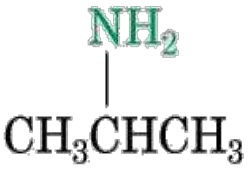
Different functional groups

Ethyl alcohol

and

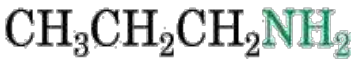
Dimethyl ether

Different position of functional groups



Isopropylamine

and



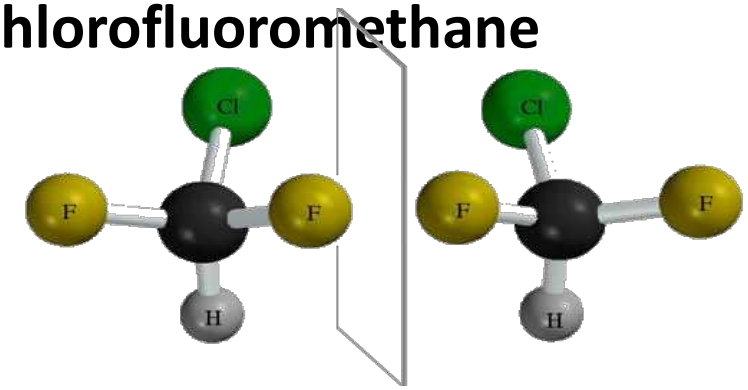
Propylamine

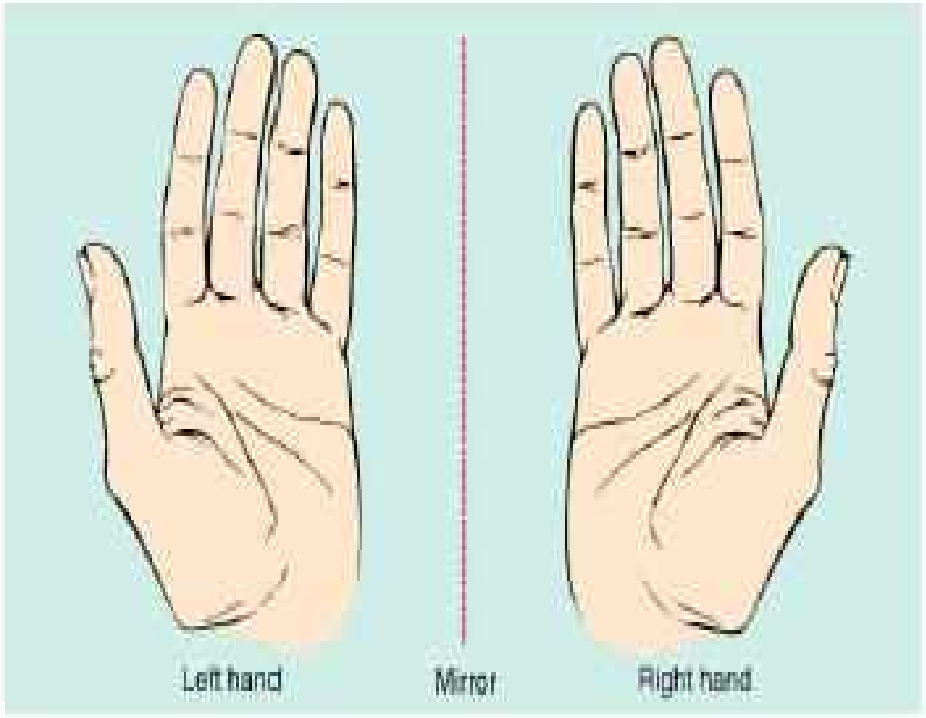
1. A chiral molecule is one that is *not identical with its mirror image.*

2. Objects (and molecules) that *are* superposable on their mirror images are *achiral.*

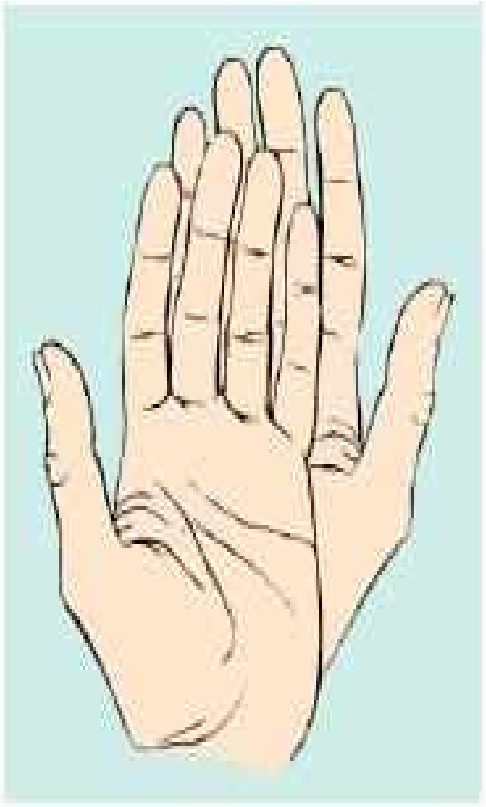
Enantiomers and the Tetrahedral Carbon

- **Enantiomers** are molecules that are not the same as their mirror image
- They are the "same" if the positions of the atoms can coincide on a one-to-one basis (we test if they are *superimposable*)
- This is illustrated by enantiomers of **bromochlorofluoromethane**





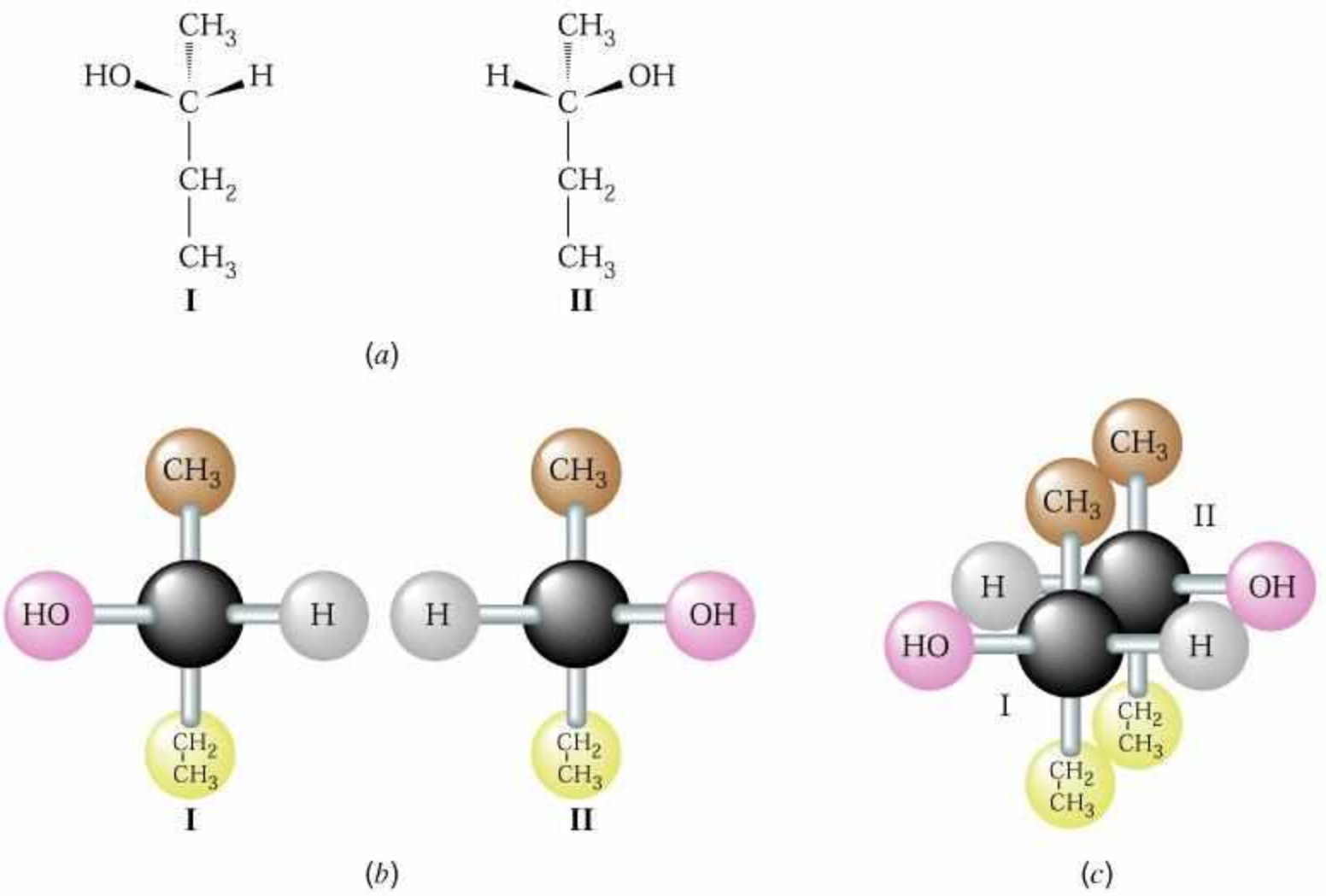
The mirror image of a left hand is aright hand.



Left and right hands are not superposable

Chirality

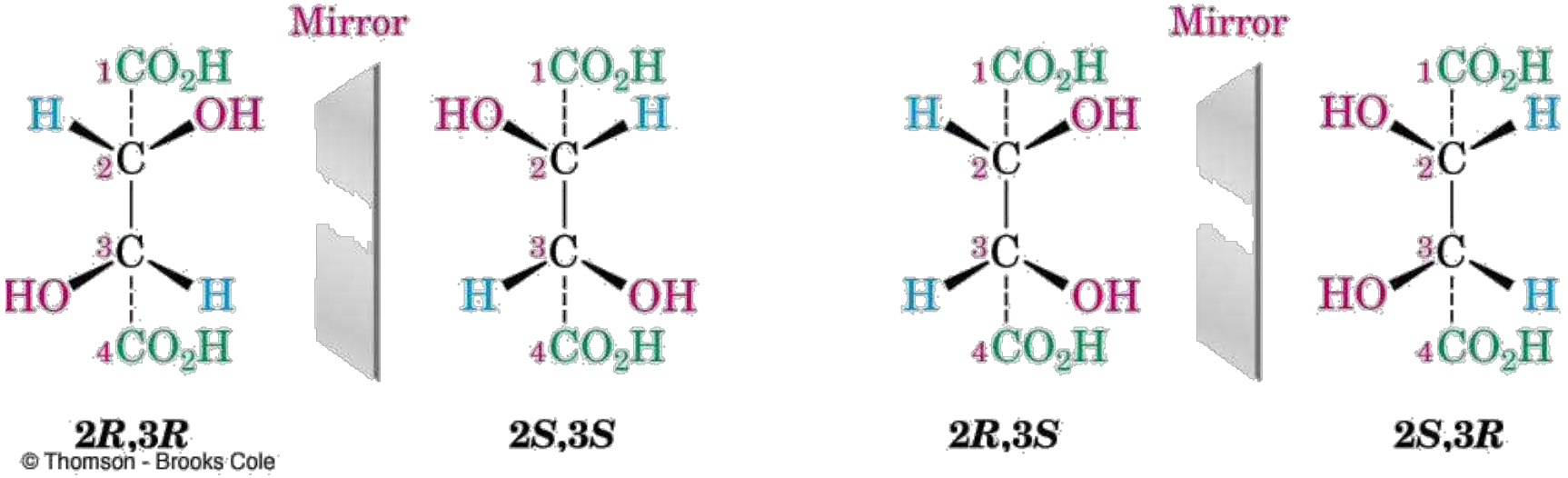
- If an object has a plane of symmetry it is necessarily the same as its mirror image
- The lack of a plane of symmetry is called “handedness”, **chirality**
- Hands, gloves are prime examples of chiral object
  - They have a “left” and a “right” version



(a) Three-dimensional drawings of the 2-butanol enantiomers I and II. (b) Models of the 2-butanol enantiomers. (c) An unsuccessful attempt to superpose models of I and II.

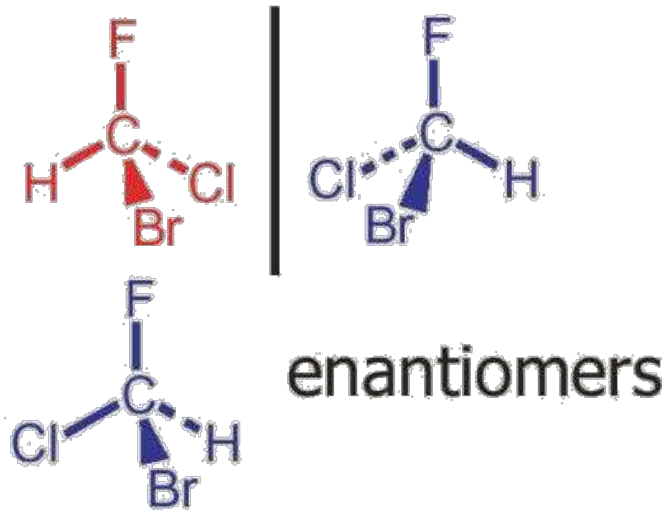
Meso Compounds

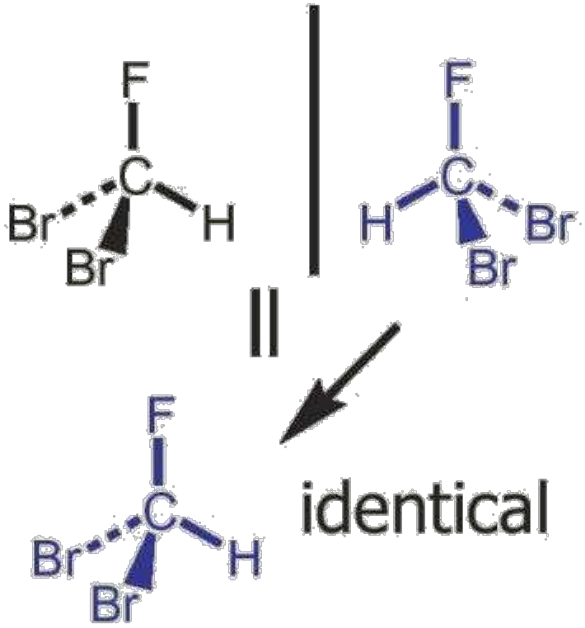
- Tartaric acid has two chiral carbons and two diastereomeric forms
- One form is chiral and the other is achiral, but both have two chiral carbons
- An achiral compound with chiral carbons is called a *meso* compound – it has a plane of symmetry
- The two structures on the right in the figure are identical so the compound (*2R, 3S)* is achiral



Examples of Enantiomers

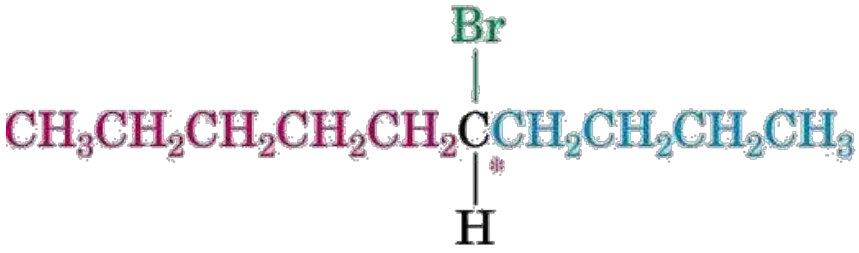
- Molecules that have one carbon with 4 (count-em 4) different substituents have a nonsuperimposable mirror image – enantiomer





Chiral Carbons

- A point in a molecule where four different groups (or atoms) are attached to carbon is called the **chiral carbon**
- There are two nonsuperimposable ways that 4 different different groups (or atoms) can be attached to one carbon atom
  - If two groups are the same, then there is only one way
- A chiral molecule usually has at least one chiral carbon



5-Bromodecane (chiral)

Substituents on carbon 5

—H

—Br

—$CH_2CH_2CH_2CH_3$ (butyl)

—$CH_2CH_2CH_2CH_2CH_3$ (pentyl)

Optical Activity

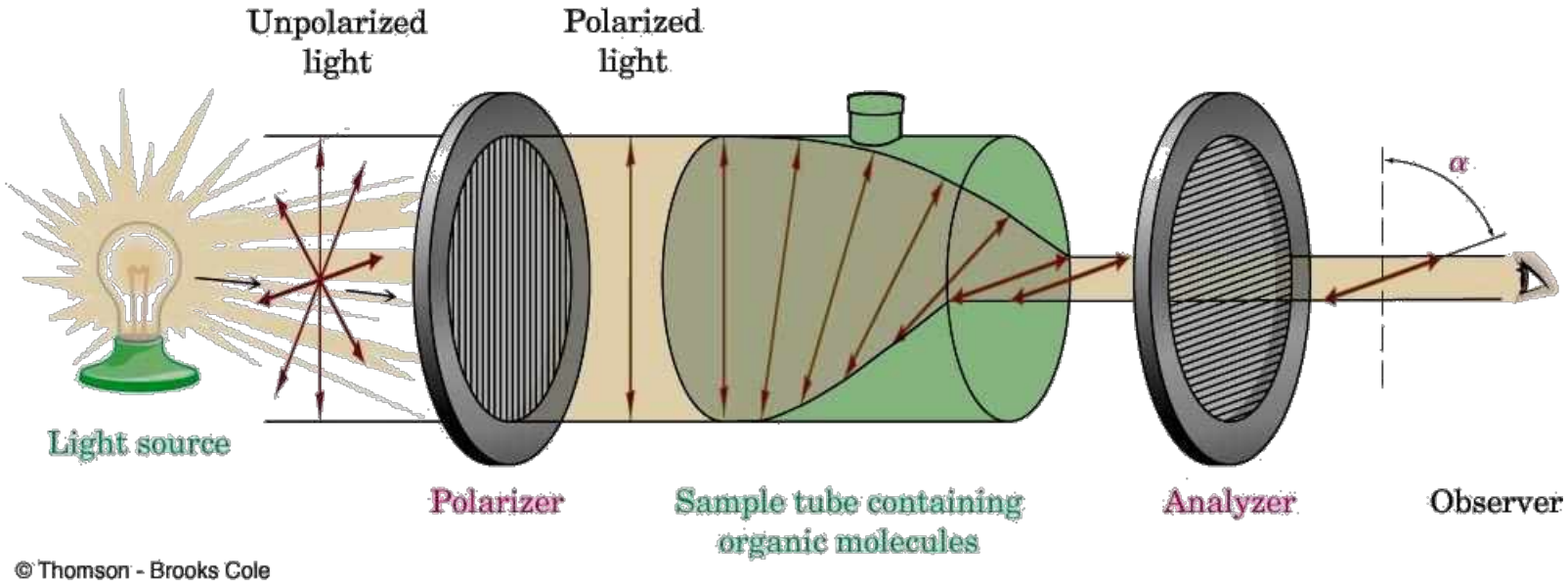
- Light restricted to pass through a plane is *plane-polarized*
- Plane-polarized light that passes through solutions of achiral compounds remains in that plane
- Solutions of chiral compounds rotate plane-polarized light and the molecules are said to be *optically active*
- Phenomenon discovered by Biot in the early 19^{th} century

Optical Activity

- Light passes through a plane polarizer
- Plane polarized light is rotated in solutions of optically active compounds
- Measured with polarimeter
- Rotation, in degrees, is $[\alpha]$
- Clockwise rotation is called **dextrorotatory**
- Anti-clockwise is **levorotatory**

Measurement of Optical Rotation

- A *polarimeter* measures the rotation of plane-polarized that has passed through a solution
- The source passes through a *polarizer* and then is detected at a second polarizer
- The angle between the entrance and exit planes is the optical rotation.



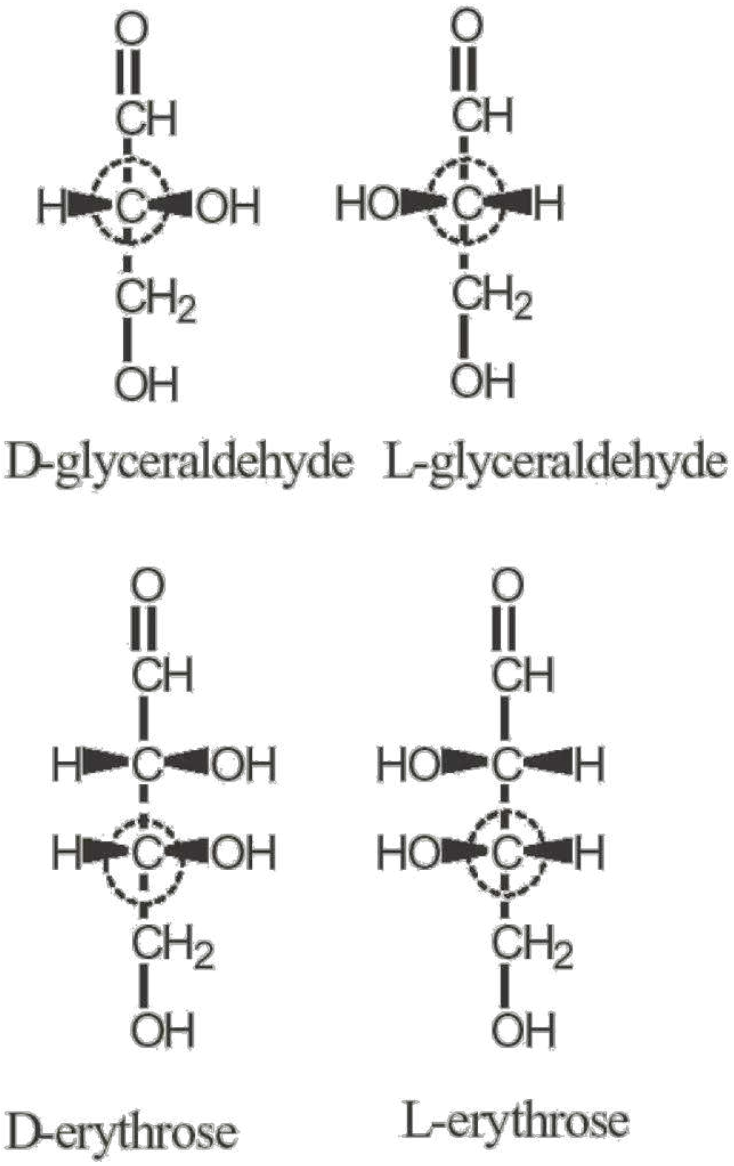
Specific Rotation

- To have a basis for comparison, define **specific rotation, $[\alpha]_D$** for an optically active compound

- $[\alpha]_D$ = observed rotation/(pathlength x concentration)
  = α/(*l x C) = degrees/(dm x g/mL)*

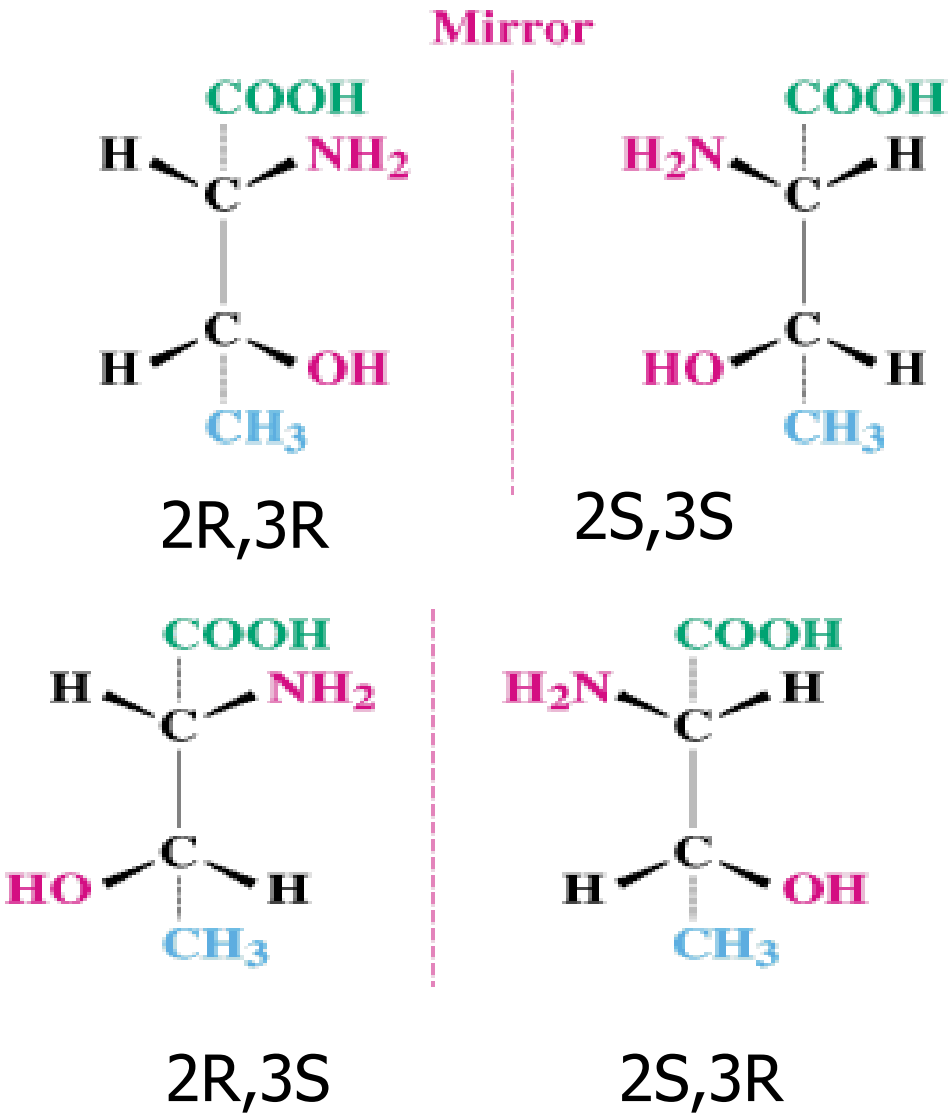
Relative 3-Dimensional Structure

- is the mirror image of L-erythrose
- This does not apply in general The original method was a correlation system, classifying related molecules into “families” focused on carbohydrates
  - Correlate to D- and L-glyceraldehyde
  - D-erythrose



Diastereomers

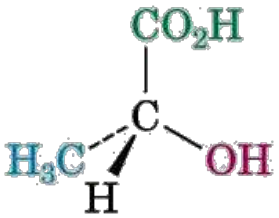
- Molecules with more than one chiral carbon have mirror image stereoisomers that are enantiomers
- In addition they can have stereoisomeric forms that are not mirror images, called **diastereomers**



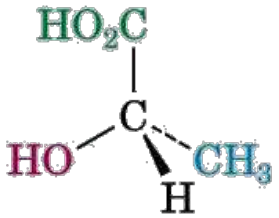
Stereoisomers

- **Same connections, different spatial arrangement of atoms**
  - **Enantiomers (nonsuperimposable mirror images)**
  - **Diastereomers (all other stereoisomers)**
    - **Includes cis, trans and configurational**

Enantiomers
(nonsuperimposable mirror-image stereoisomers)



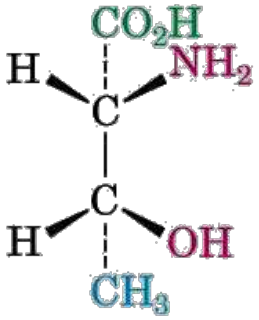
(*R*)-Lactic acid



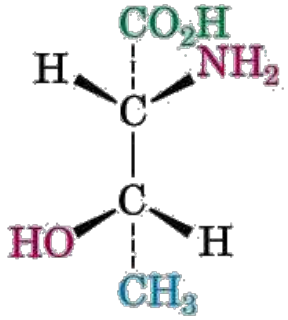
(*S*)-Lactic acid

Diastereomers
(nonsuperimposable, non–mirror-image stereoisomers)

Configurational diastereomers

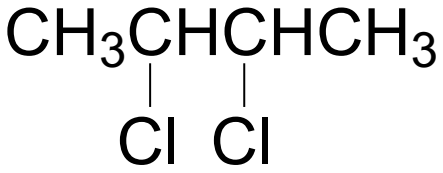


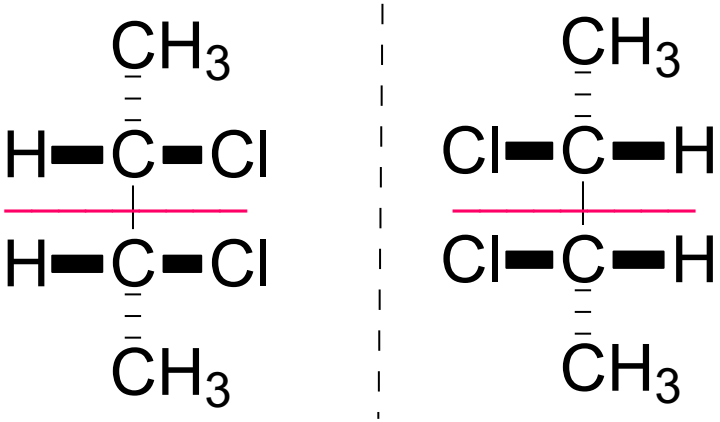
2*R*,3*R*-2-Amino-3-hydroxybutanoic acid



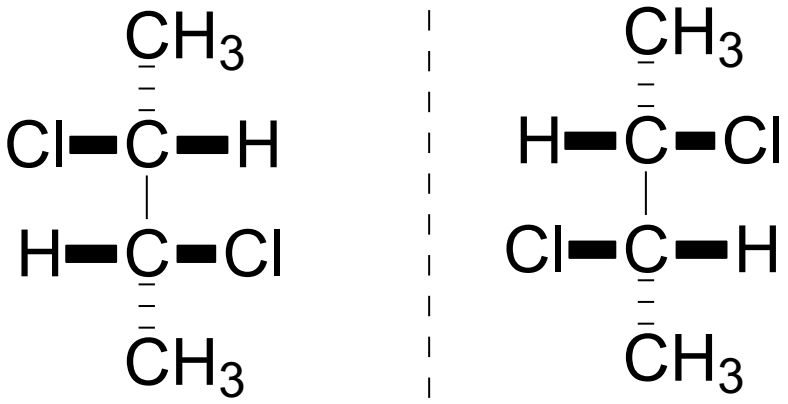
2*R*,3*S*-2-Amino-3-hydroxybutanoic acid

Stereoisomers of 2,3-Dichlorobutane



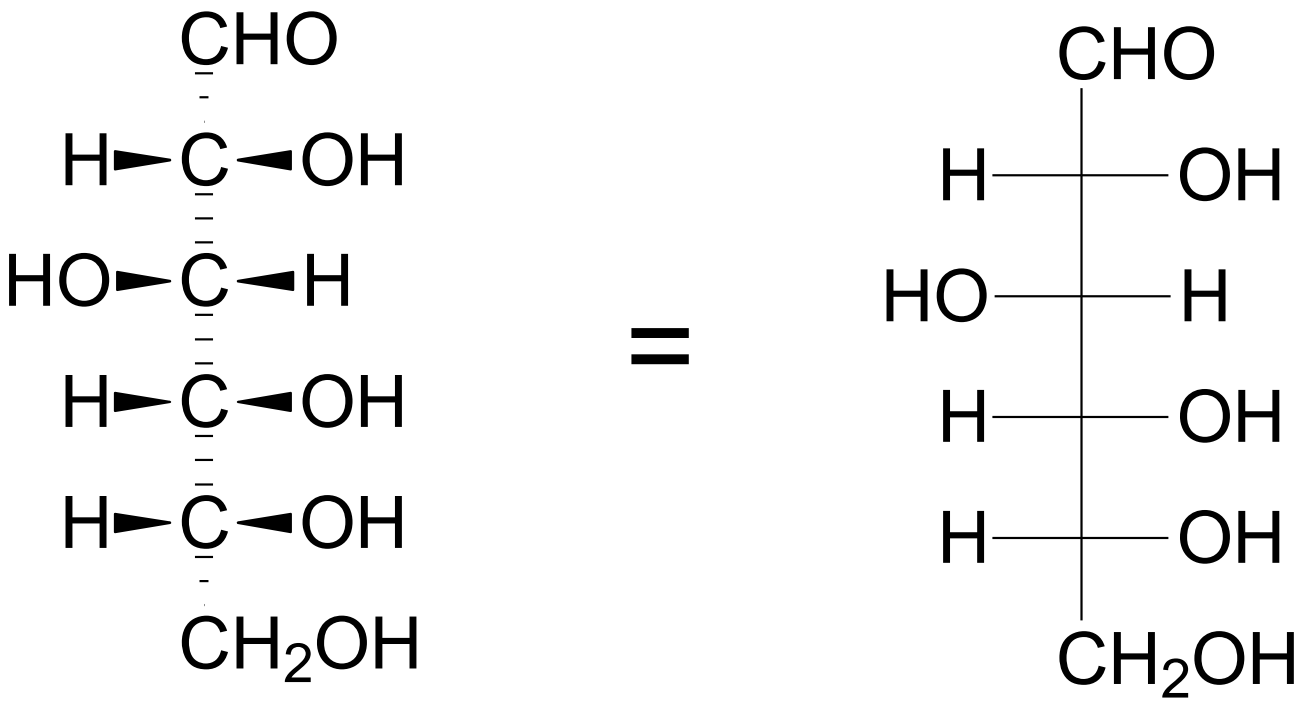


Meso Compound



Enantiomers

Fisher Projections

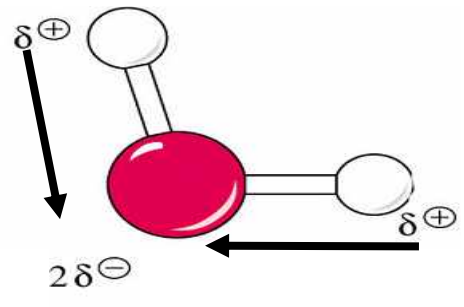


Vertical – Bonds are going away from you

Horizontal – Bond are coming toward you

Water in Biochemistry

Properties of water



- Very polar
- Oxygen is highly electronegative(electronegativity 3.5)
- H-bond donor and acceptor(electronegativity 2.1)
- High b.p., m.p., heat of vaporization, surface tension
- Water constitutes 65 to 70 % of human body while it constitutes 60 to 95 % of human cells.

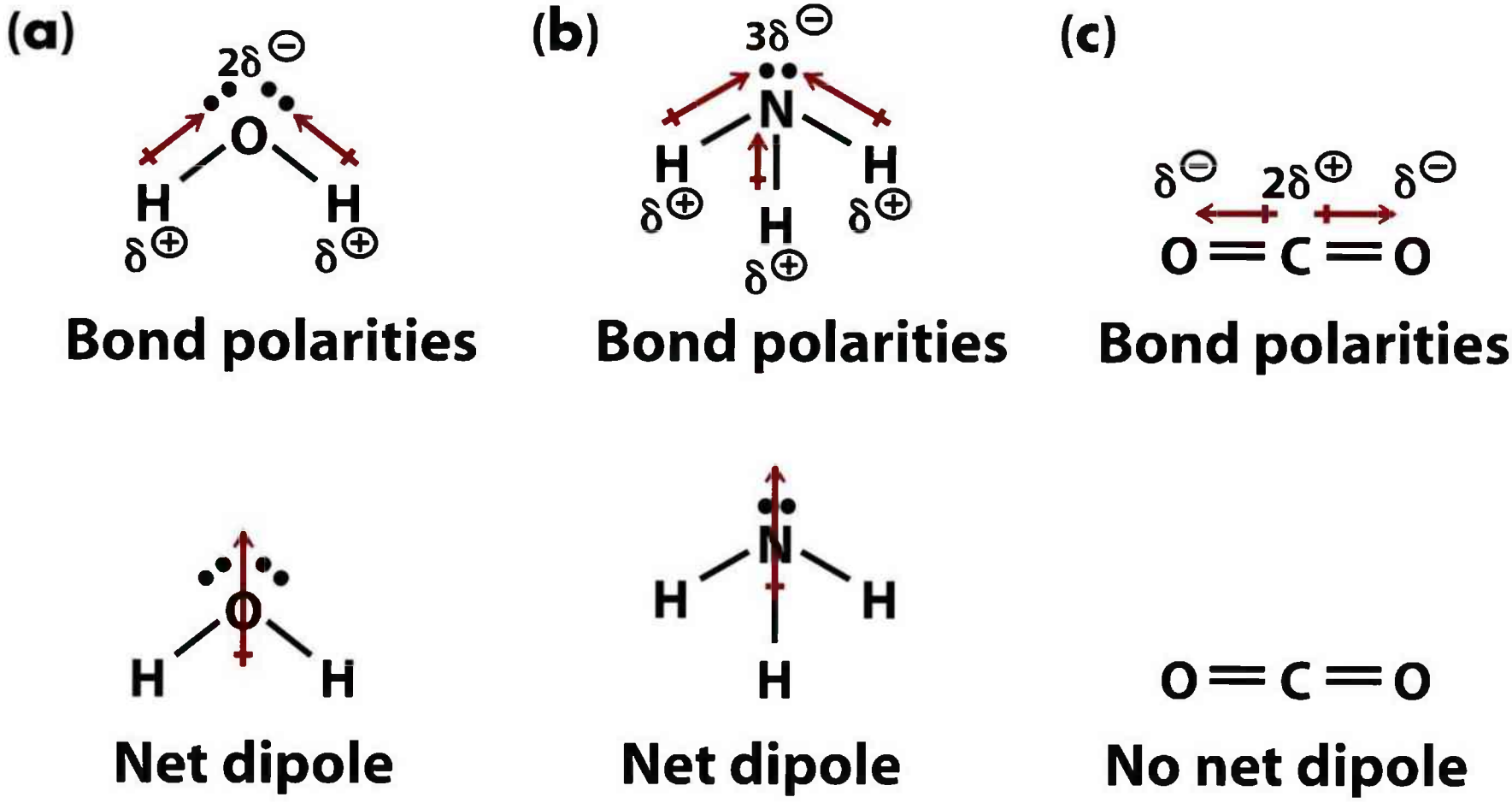
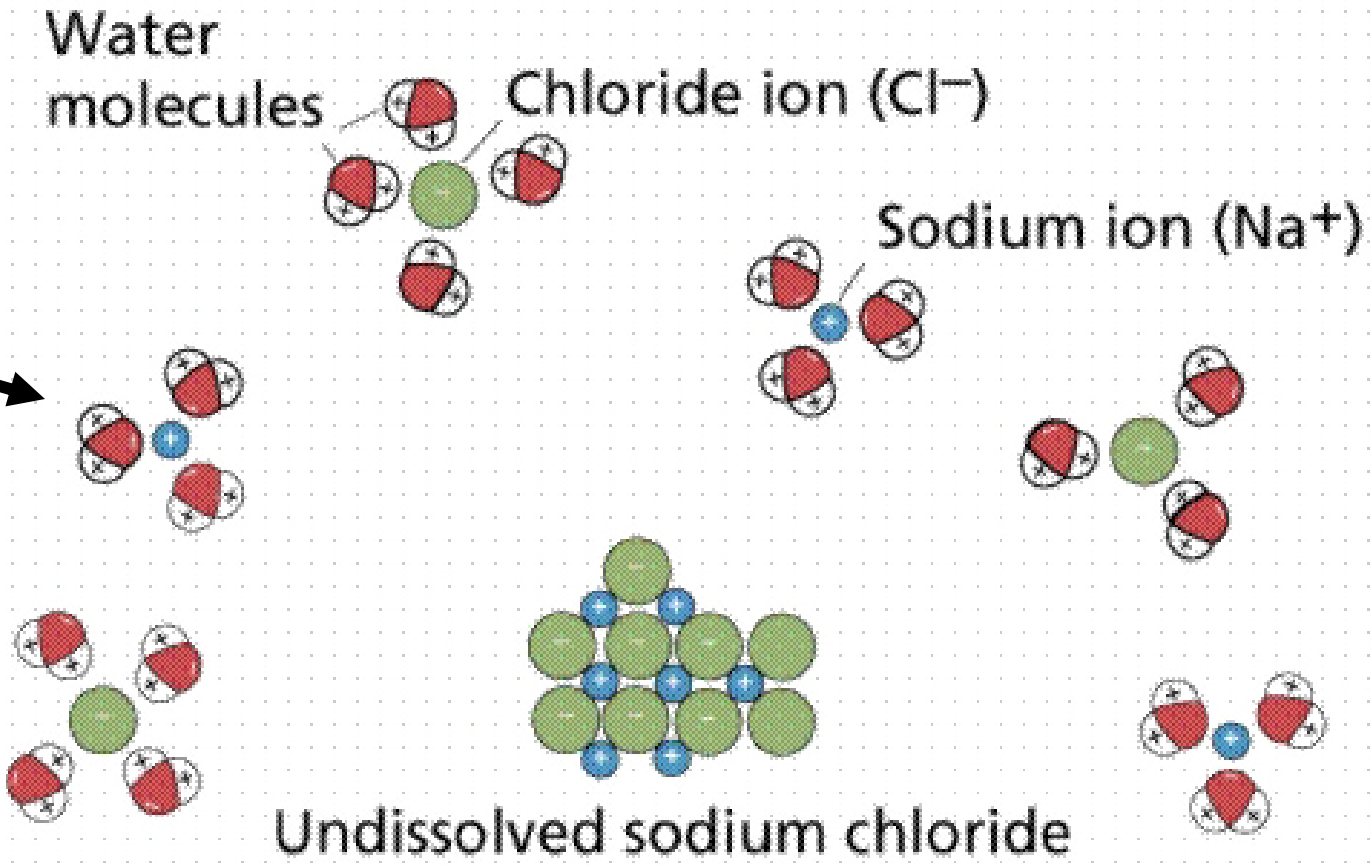


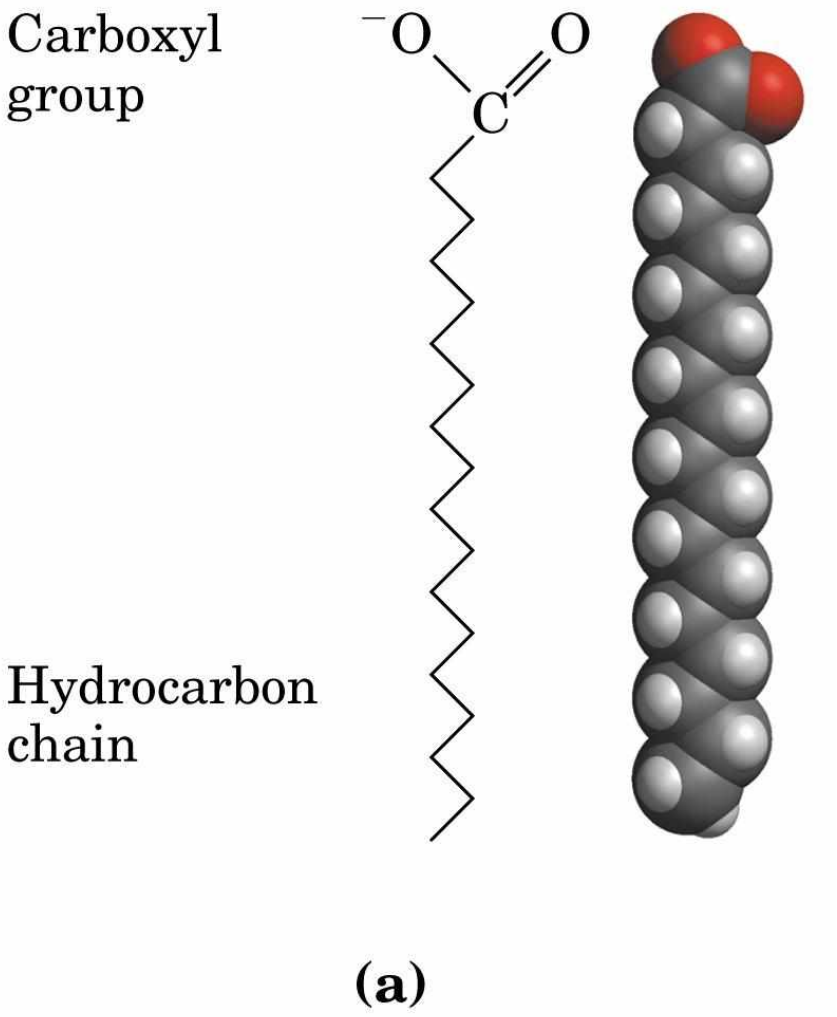
Figure 2-2 Principles of Biochemistry, 4/e

Water dissolves polar compounds

solvation shell
or
hydration shell



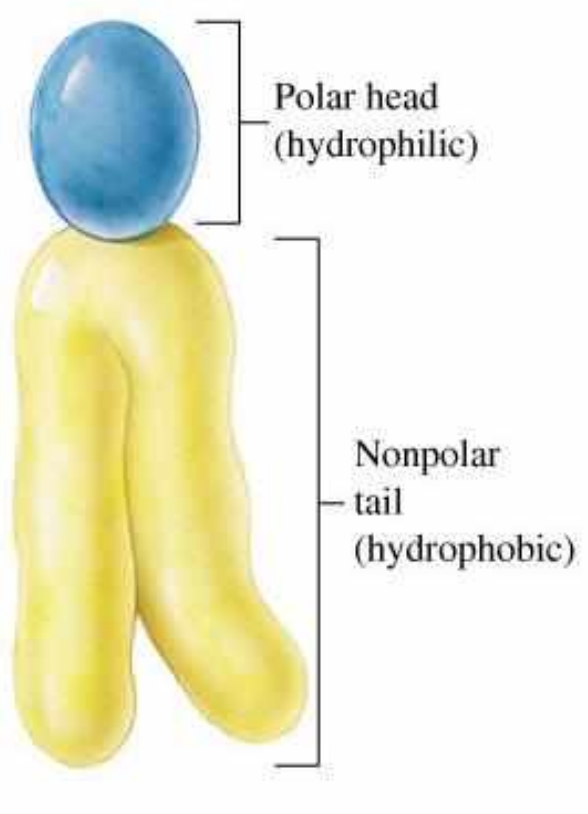
Stearic acid

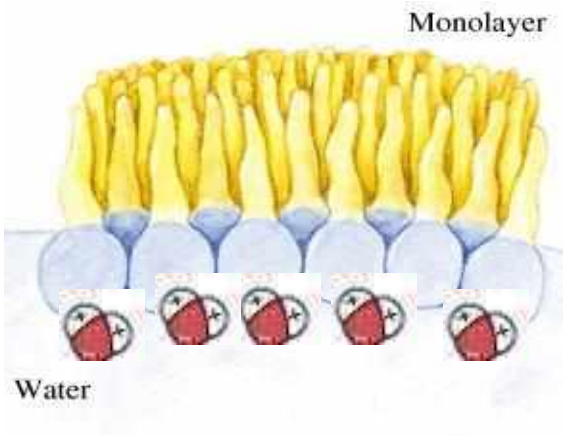


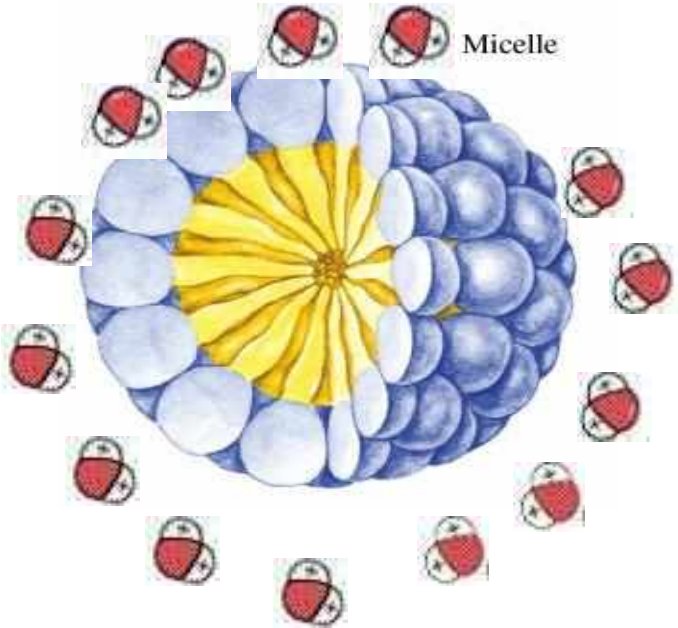
The packing of fatty acids depends on their degree of saturation. Stearic acid is shown here in its usual extended conformation. Saturated fatty acids are tightly packed and stabilized by many hydrophobic interactions

Non-polar substances are insoluble in water

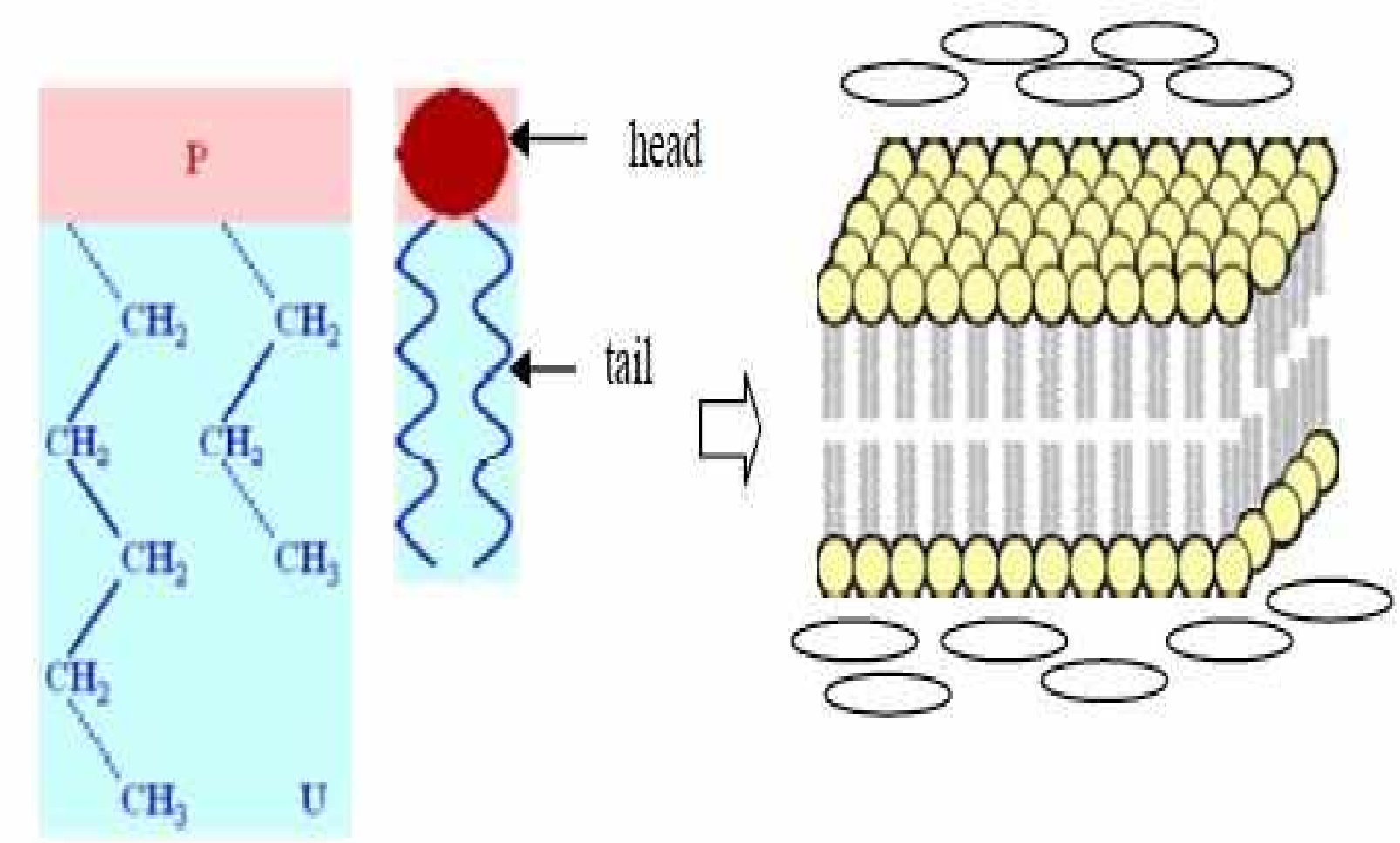
Many lipids are **amphipathic**







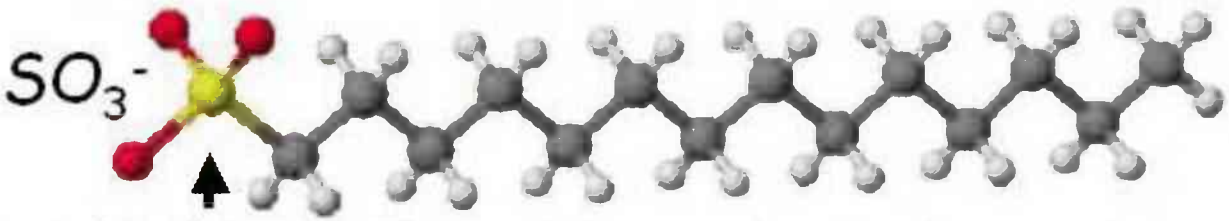
The bilayer system of lipids in aqueous solution



How detergents work?

Micelle Action

Hydrophilic Hydrophobic

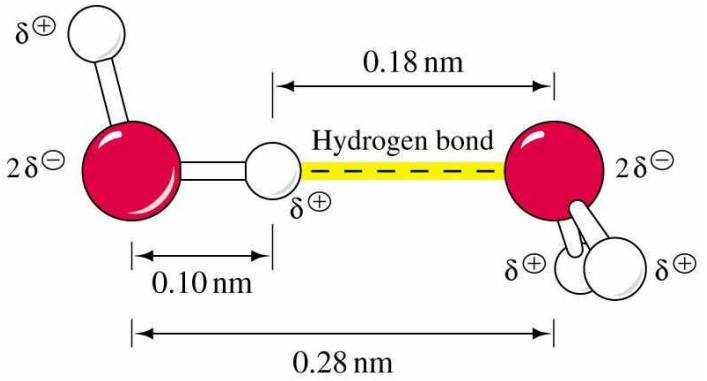


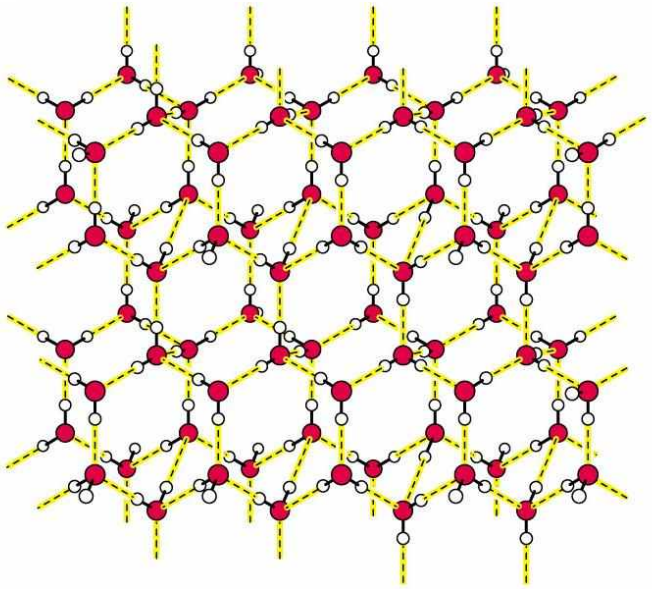
Polar

Essentially Non Polar

Non Polar Grease

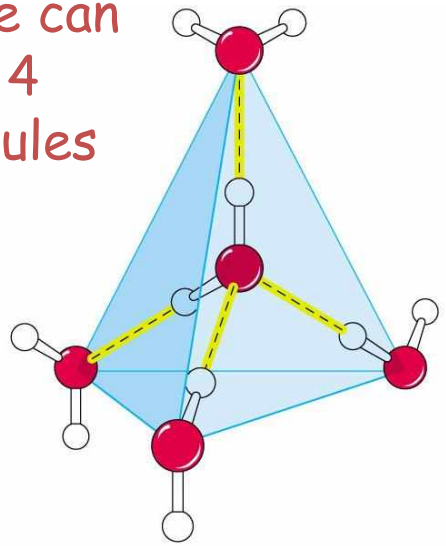
Hydrogen Bonding of Water





Crystal lattice of ice

One H_2O molecule can associate with 4 other H_2O molecules



- **Ice: 4 H-bonds per water molecule**
- **Water: 2.3 H-bonds per water molecule**

Biological Hydrogen Bonds

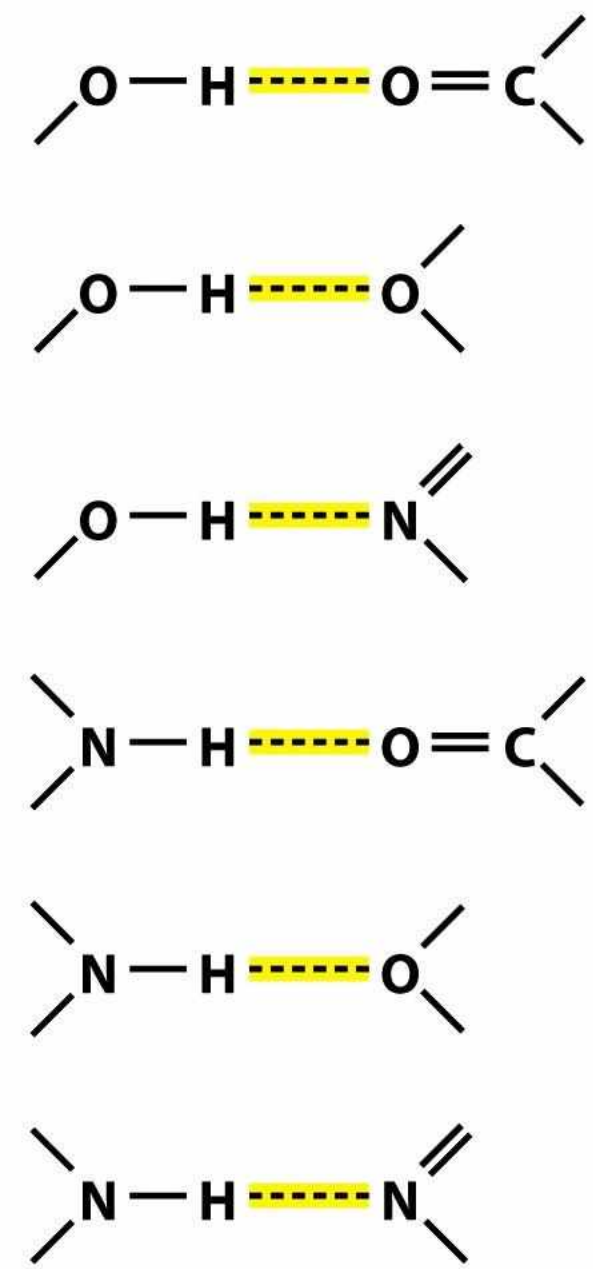


Figure 2-10b Principles of Biochemistry, 4/e

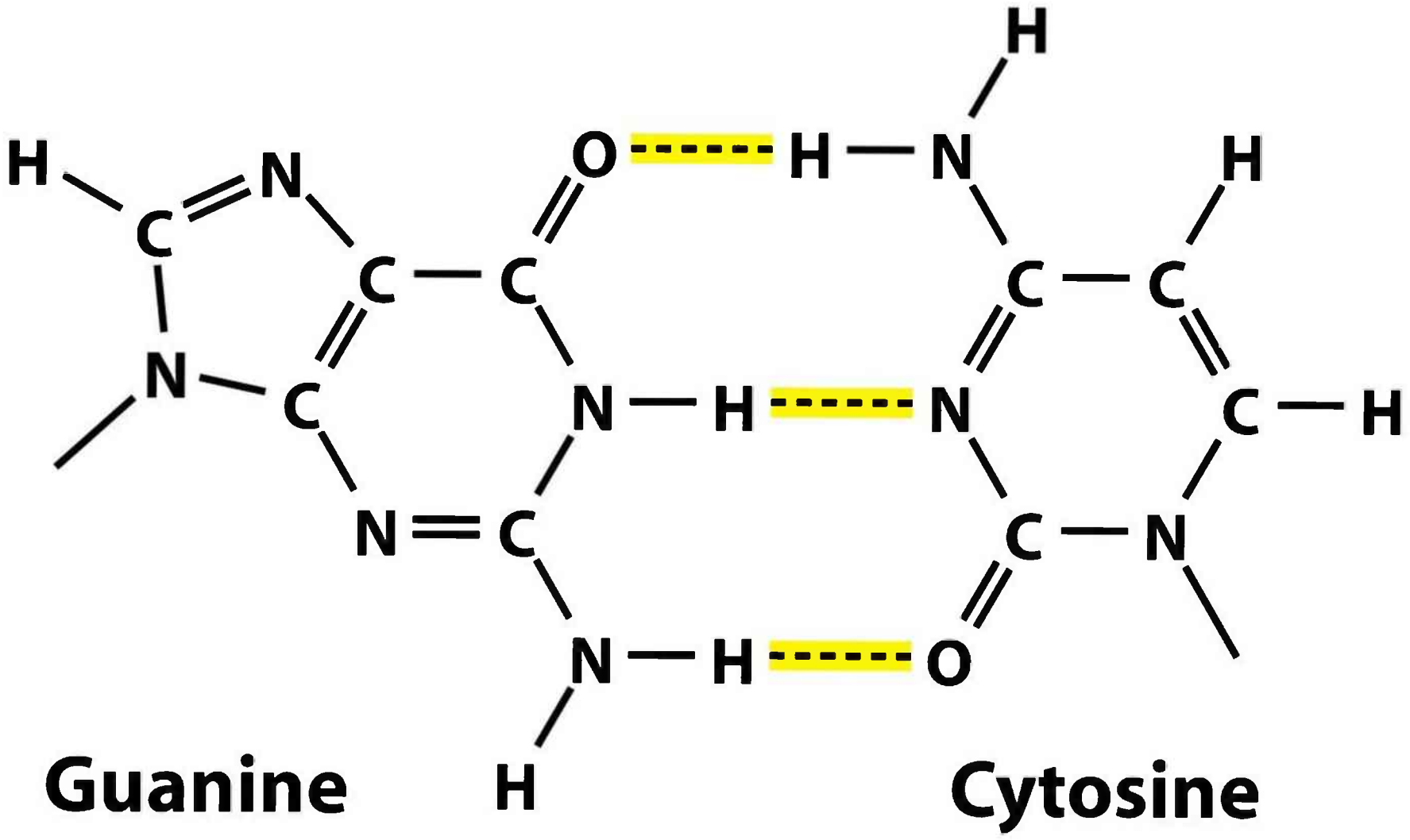
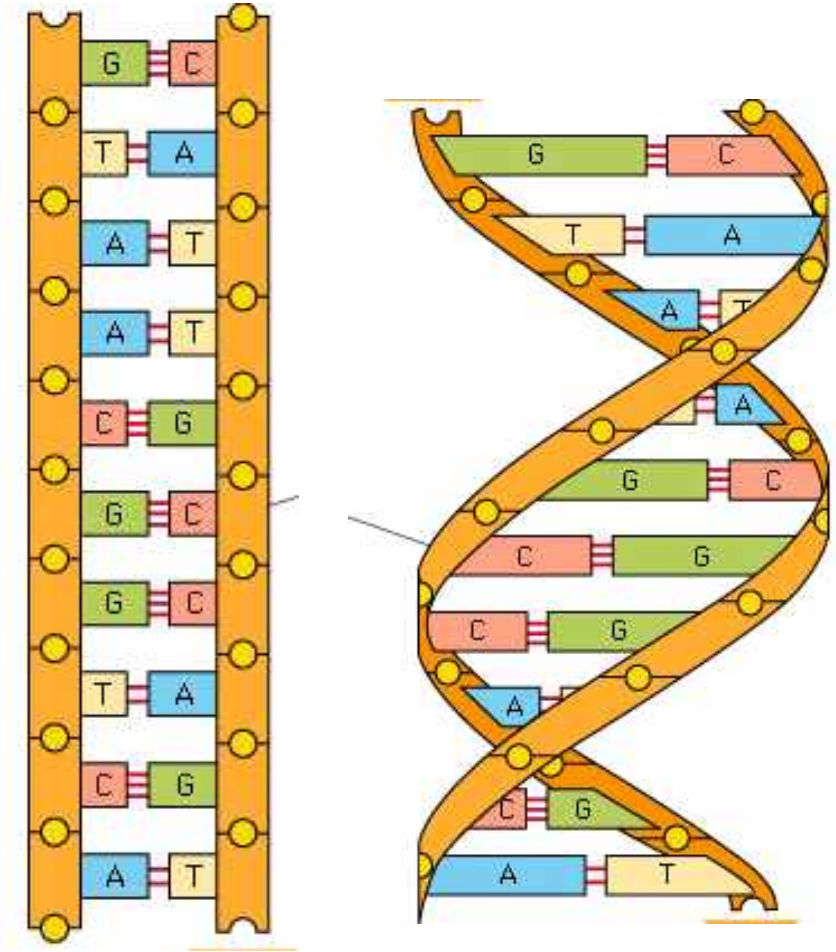
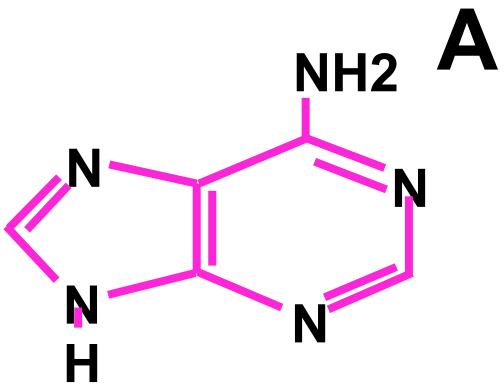


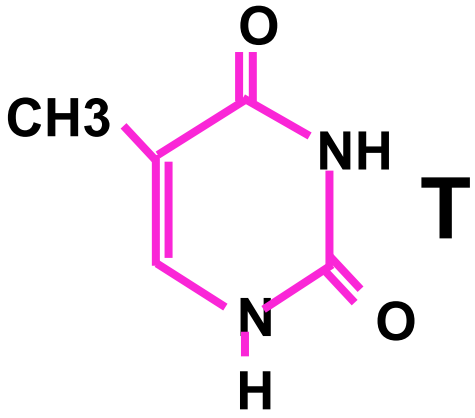
Figure 2-11 Principles of Biochemistry, 4/e

Nucleic Acid Structure

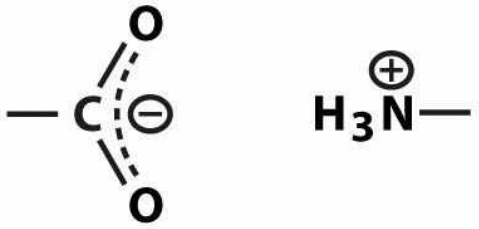
The double helix





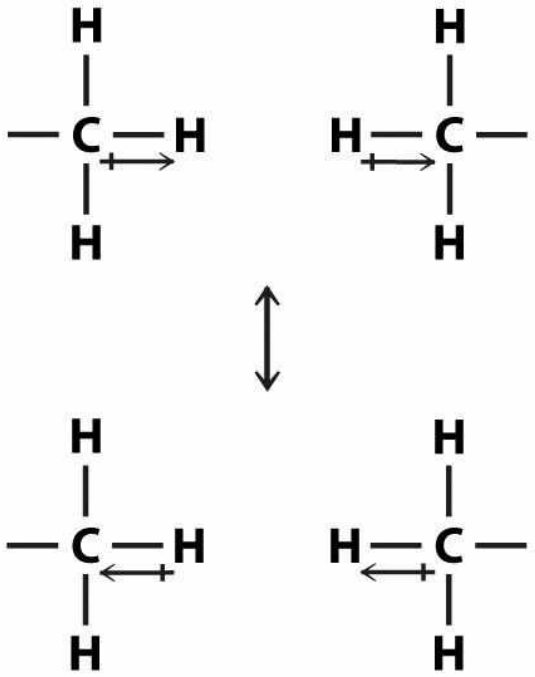


non-covalent interactions



Charge-charge interaction
~40 to 200 kJ mol^{-1}

Hydrogen bond
~2 to 20 kJ mol^{-1}



van der Waals interaction
~0.4 to 4 kJ mol^{-1}

Hydrophobic interaction
~3 to 10 kJ mol^{-1}

Figure 2-13 Principles of Biochemistry, 4/e

Ionization of Water

$$H_20 + H_20 \rightleftharpoons H_3O^+ + OH^-$$

$$H_20 \rightleftharpoons H^+ + OH^-$$

$$K_{eq} = \frac{[H^+]\,[OH^-]}{[H_2O]}$$

$K_{eq} = 1.8 \times 10^{-16} M$

$[H_2O] = 55.5\ M$

$[H_2O]\ K_{eq} = [H^+]\,[OH^-]$

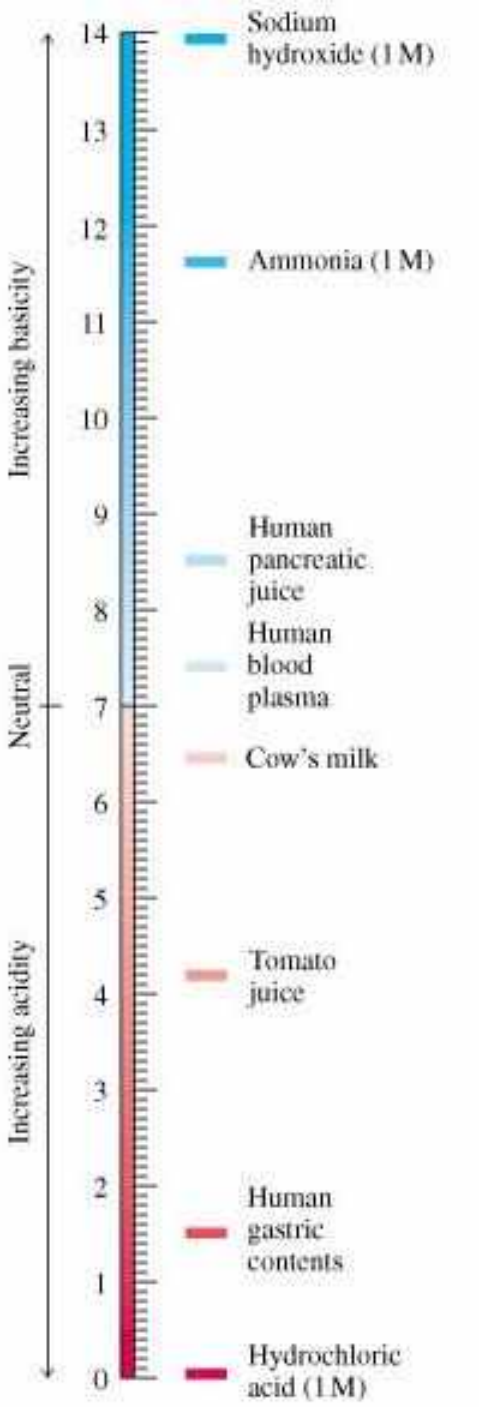
$(1.8 \times 10^{-16} M)(55.5\ M) = [H^+]\,[OH^-]$

$1.0 \times 10^{-14}\ M^2 = [H^+]\,[OH^-] = K_w$

If $[H^+]=[OH^-]$ then $[H^+] = 1.0 \times 10^{-7}$

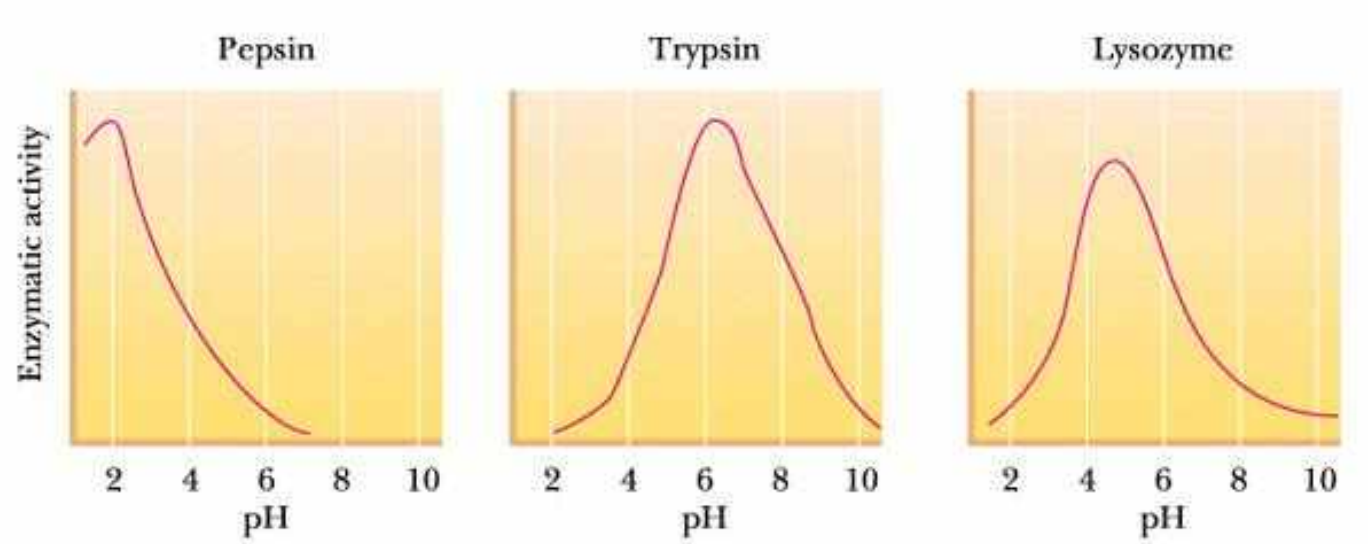
pH Scale

- Devised by Sorenson (1902)
- [H+] can range from 1M and 1×10^{-14}M
- using a log scale simplifies notation
- $pH = -\log [H^+]$
- Neutral pH = 7.0



Weak Acids and Bases Equilibria

- Strong acids / bases – disassociate completely
- Weak acids / bases – disassociate only partially
- Enzyme activity sensitive to pH
- weak acid/bases play important role in protein structure/function



Acid/conjugate base pairs

$$HA + H_2O \rightleftharpoons A^- + H_3O^+$$

$$HA \rightleftharpoons A^- + H^+$$

HA = acid (donates H^+)(Bronstad Acid)

A^- = Conjugate base (accepts H^+)(Bronstad Base)

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

K_a & pK_a value describe tendency to loose H^+

large K_a = stronger acid
small K_a = weaker acid

$$pK_a = - \log K_a$$

pKa values determined by titration

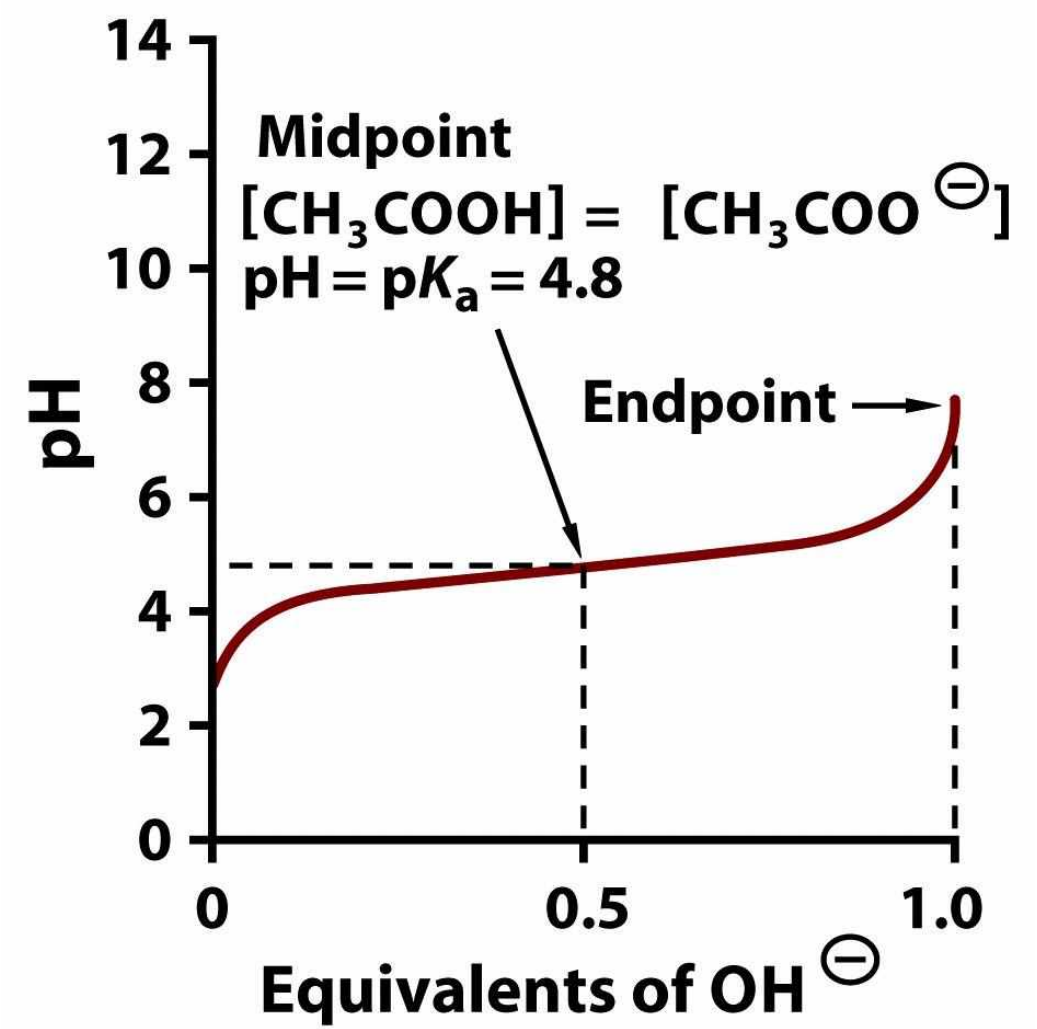
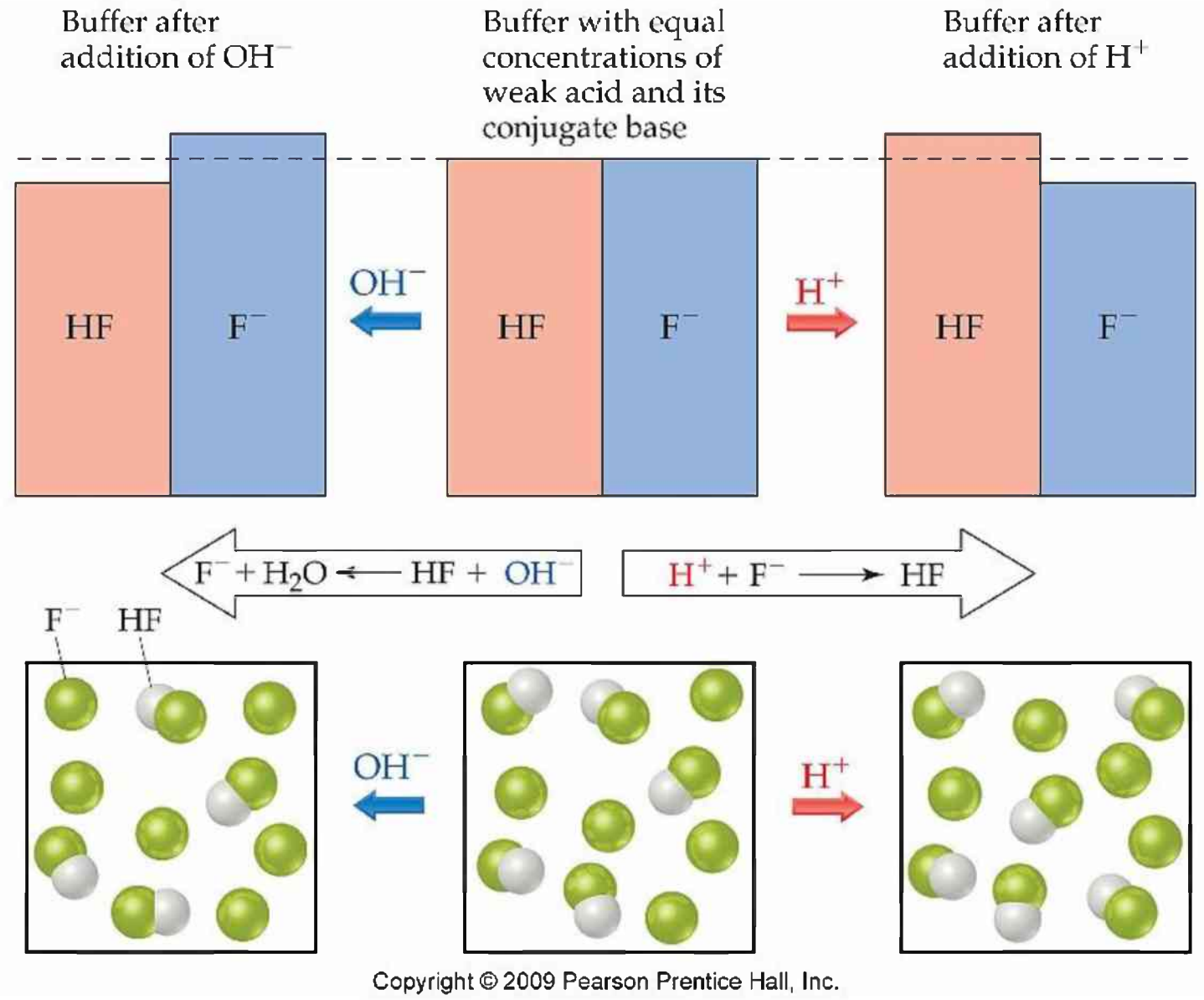


Figure 2-16 Principles of Biochemistry, 4/e

Buffers

- **Buffers are aqueous systems that resist changes in pH when small amounts of a strong acid or base are added.**
- **A buffered system consist of a weak acid and its conjugate base.**
- **The most effective buffering occurs at the region of minimum slope on a titration curve**

  (i.e. around the pKa).
- **Buffers are effective at pHs that are within +/-1 pH unit of the pKa**



How buffers work

- Strong acid + strong base → salt + water (neutral)

$HCl(aq) + NaOH(aq) \rightarrow NaCl(aq) + HOH(l)$

Net ionic: $H^+(aq) + OH^-(aq) \rightarrow HOH(l)$

The solution is neutral because both the Na ions and the Cl ions are neutral.

- Strong acid + weak base → weak acid + salt
- $HCl(aq) + NaC_2H_3O_2(aq) \rightarrow NaCl(aq) + HC_2H_3O_2(aq)$

Net ionic: $H^+(aq) + C_2H_3O_2^-(aq) \rightarrow HC_2H_3O_2(aq)$

Weak acid

Buffers, continued

- Strong base + weak acid → weak base

- $NaOH(aq) + HC_2H_3O_2(aq) \rightarrow NaC_2H_3O_2(aq) + HOH(l)$

  Net ionic:

- $OH^-(aq) + HC_2H_3O_2(aq) \rightarrow C_2H_3O_2^-(aq) + HOH(l)$

- A weak acid does not react appreciably with a conjugate weak base.

$[H_3O^+]$ and pH of Weak Acids

- In weak acid solutions, $[H_3O^+] < [HA]_0$;
- $[H_3O^+]$ and pH can be calculated from the initial concentration of the acid and its K_a value.

- For example, in 0.100 *M* acetic acid, CH_3COOH, with $K_a = 1.8 \times 10^{-5}$, $[H_3O^+]$ and pH can be calculated using the "**ICE**" table.

- $Ka = \frac{[C_2H_3O_2^-][H^+]}{[HC_2H_3O_2]} = \frac{(x)(x)}{(0.10 - x)}$

- If x is small compared to 0.10, then $0.10 - x \approx 0.10$

- So, $Ka = \frac{x2}{0.10}$ $\quad 1.8 \times 10^{-5} = \frac{x2}{0.10}$

- $x = [H_3O^+] = 3.6 \times 10^{-3}$
- $pH = -\log[H_3O^+] = -\log(3.6 \times 10^{-3}) = 2.75$

Henderson-Hasselbach Equation

1) $K_a = \frac{[H^+][A^-]}{[HA]}$

2) $[H^+] = K_a \frac{[HA]}{[A^-]}$

3) $-\log[H^+] = -\log K_a - \log \frac{[HA]}{[A^-]}$

4) $-\log[H^+] = -\log K_a + \log \frac{[A^-]}{[HA]}$

5) $pH = pK_a + \log \frac{[A^-]}{[HA]}$

HA = weak acid

A^- = Conjugate base

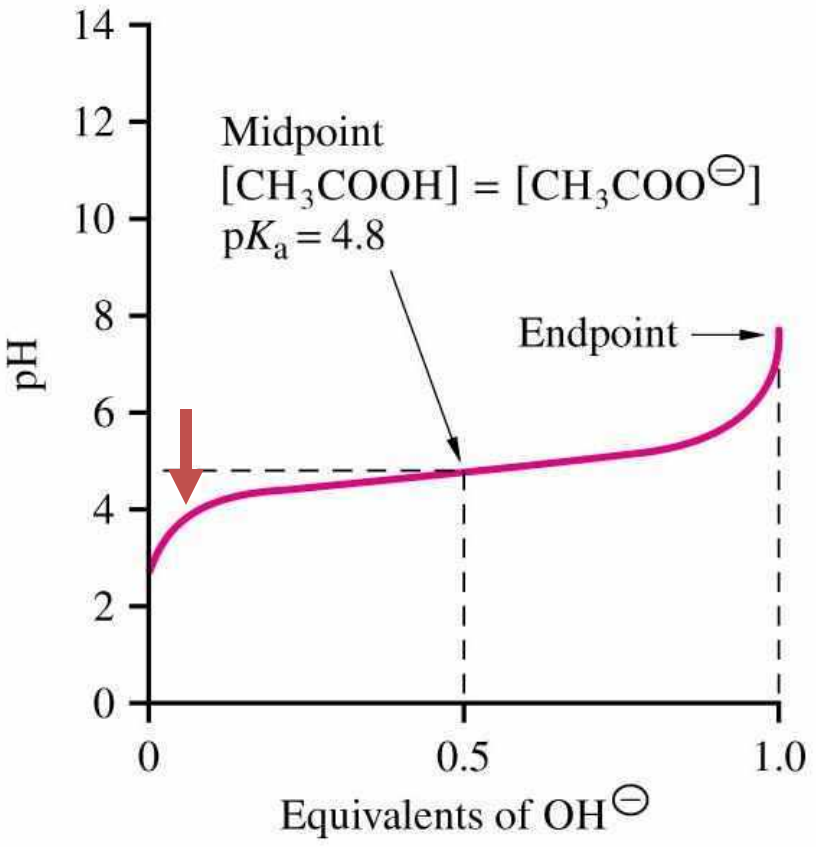
* H-H equation describes the relationship between pH, pKa and buffer concentration

Calculating the pH of Buffers

- Because the ICE tables for all buffer solutions are the same, the equation used in all buffer calculations can be generalized:
- $K_a = \frac{x[\text{base}]}{[\text{acid}]}$ pH = pKa + log([base]/[acid])
- K_a is the ionization constant of the weak acid,
- x is the molar concentration of $[H^+]$, [base] is the initial concentration of the weak base, and [acid] is the initial concentration of the weak acid.

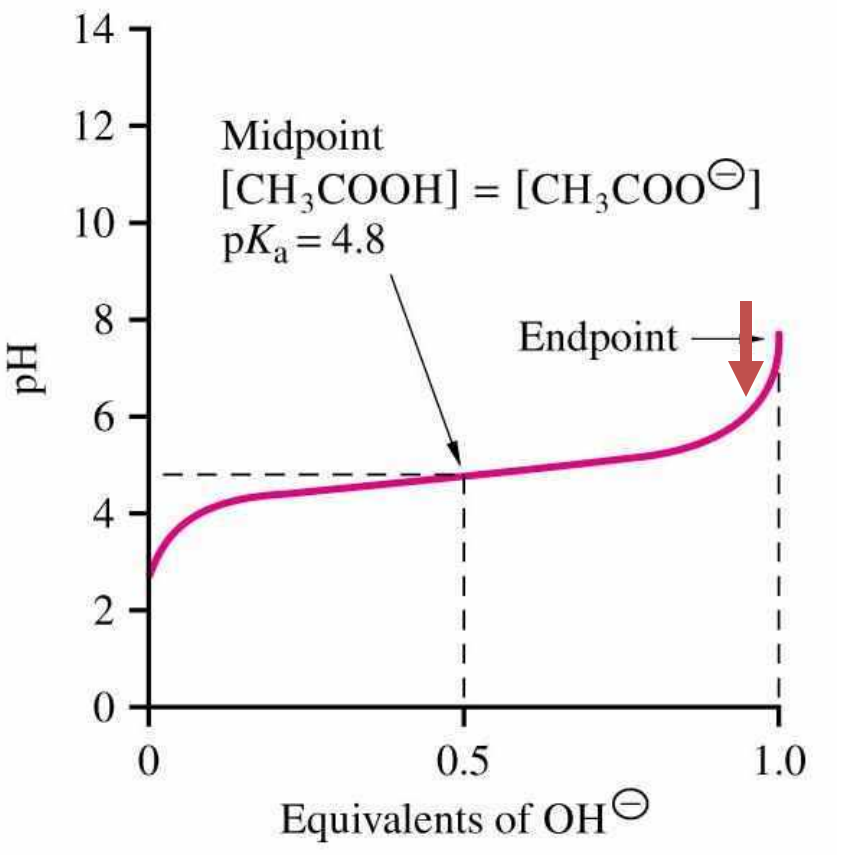
Case where 10% acetate ion 90% acetic acid

- $pH = pK_a + \log_{10} \frac{[0.1]}{[0.9]}$
- $pH = 4.76 + (-0.95)$
- $pH = 3.81$



Case where 90% acetate ion 10% acetic acid

- $pH = pK_a + \log_{10} \frac{[0.9]}{[0.1]}$
- $pH = 4.76 + 0.95$
- $pH = 5.71$



Aqueous phase
of blood cells
passing through
capillaries in lung

Air space
in lung

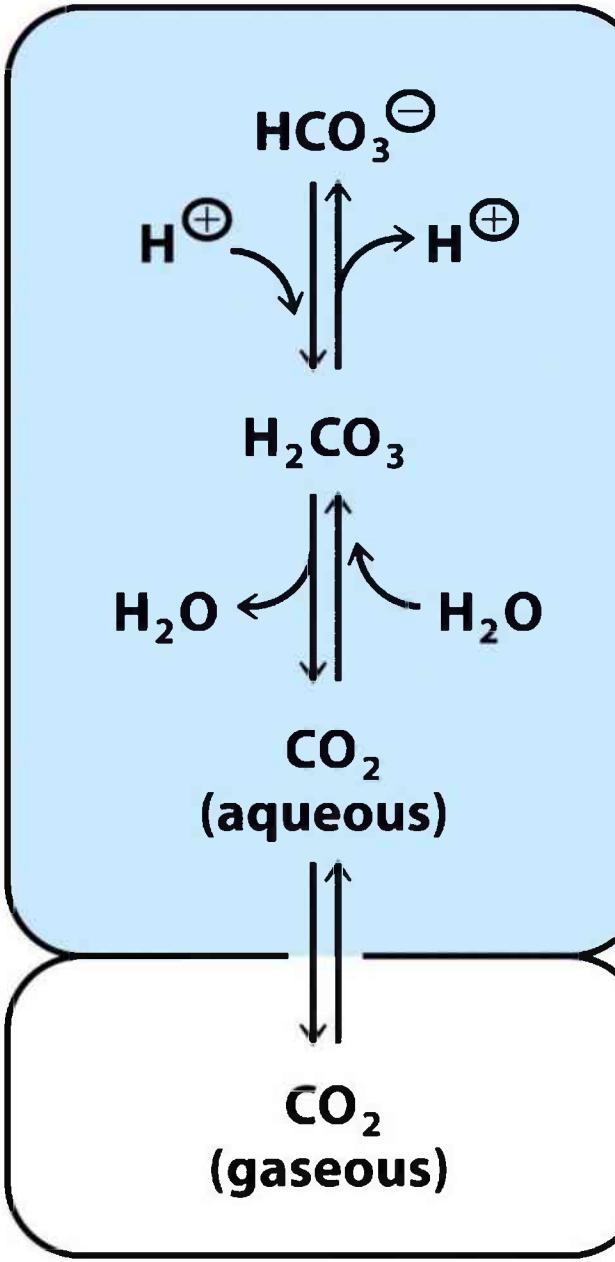


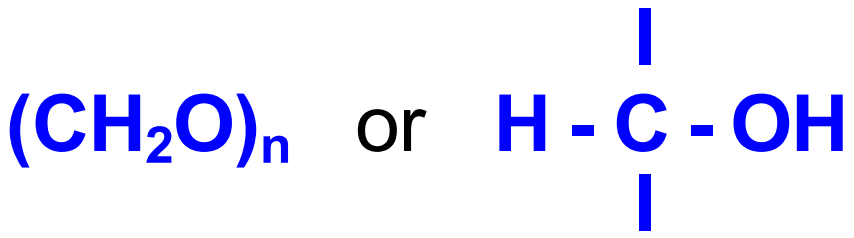
Figure 2-20 Principles of Biochemistry, 4/e

Madenet Alelm University College
Department of Biology
2nd Year Biochemistry I 2021 – 2022

Carbohydrates

Dr. Hamza Yaseen Isa
Lecture 3 I 2

Carbohydrates (glycans) have the following basic composition:

$(CH_2O)_n$ or 

1. Simple Sugars:

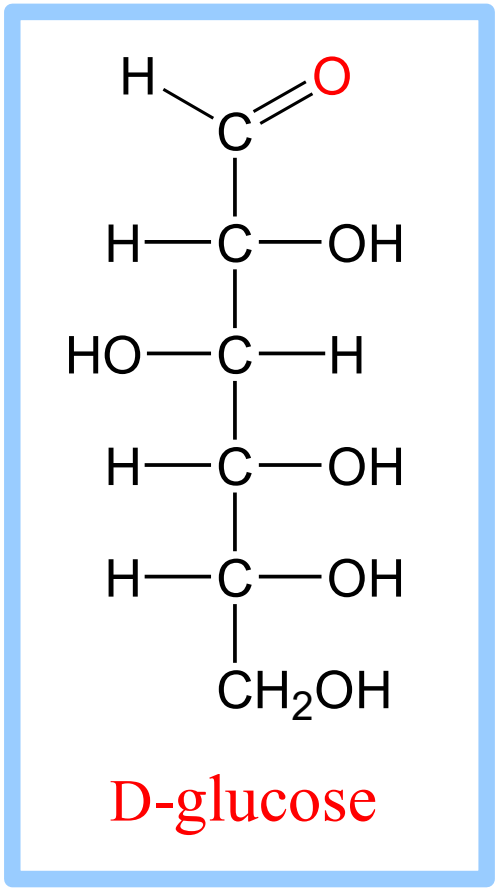
- Monosaccharides - simple sugars with multiple OH groups. Based on number of carbons (3, 4, 5, 6), a monosaccharide is a triose, tetrose, pentose or hexose.

2. Complex Sugars(contain more than one unit):

- Disaccharides - 2 monosaccharides covalently linked.
- Oligosaccharides - a few monosaccharides covalently linked(3-9units).
- Polysaccharides - polymers consisting of chains of monosaccharide or disaccharide units.

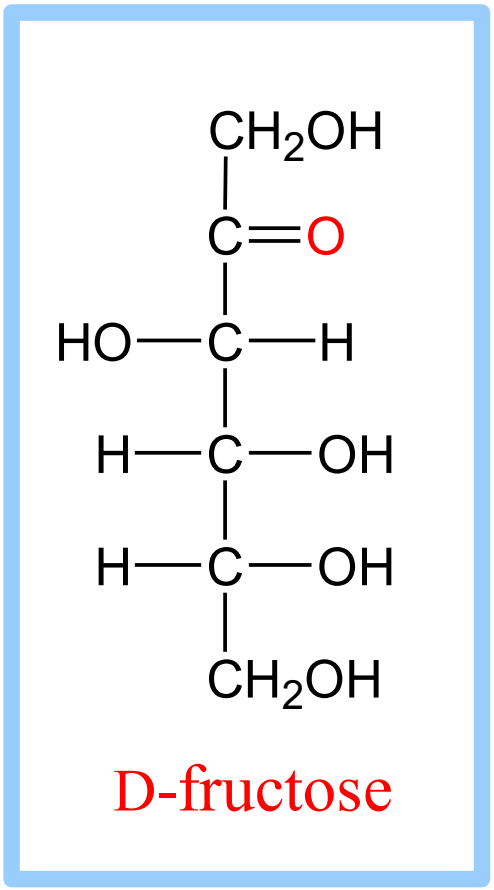
Monosaccharides

Aldoses (e.g., glucose) have an aldehyde group at one end.



D-glucose

Ketoses (e.g., fructose) have a keto group, usually at C2.



D-fructose

Epimers

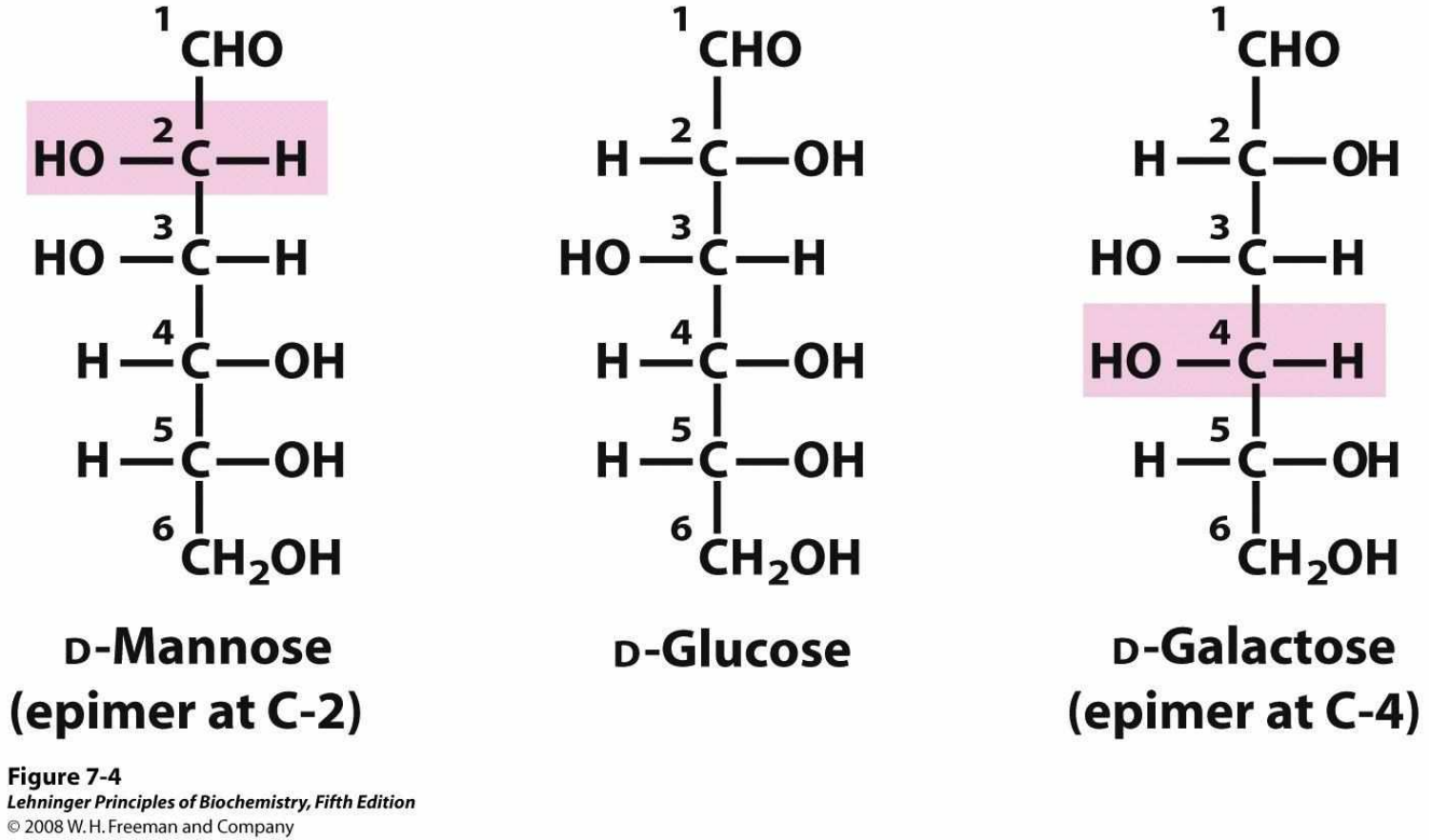


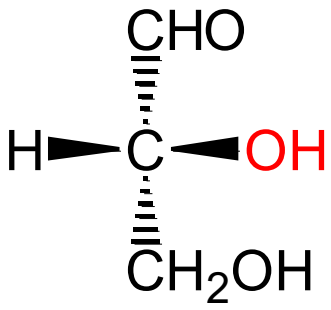
Figure 7-4
Lehninger Principles of Biochemistry, Fifth Edition

D-Glucose and two of its epimers are shown as projection formulas. Each epimer differs from D-glucose in the configuration at one chiral center (shaded pink).

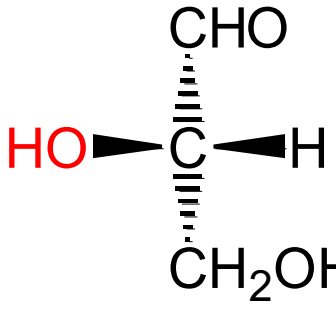
D vs L Designation

D & L designations are based on the configuration about the single asymmetric C in glyceraldehyde.

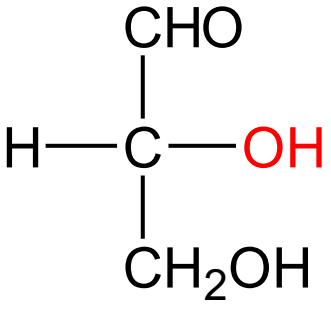
The lower representations are Fischer Projections.



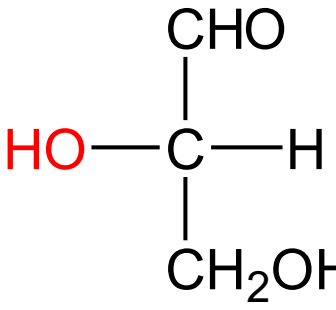
D-glyceraldehyde



L-glyceraldehyde



D-glyceraldehyde

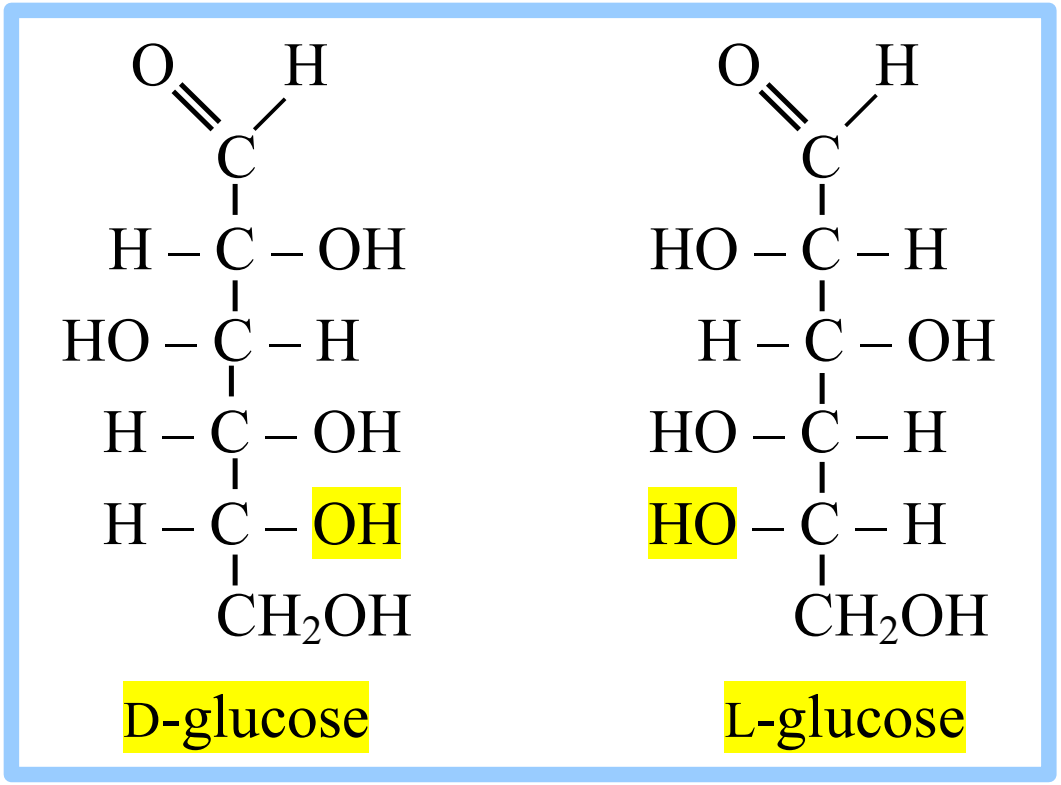


L-glyceraldehyde

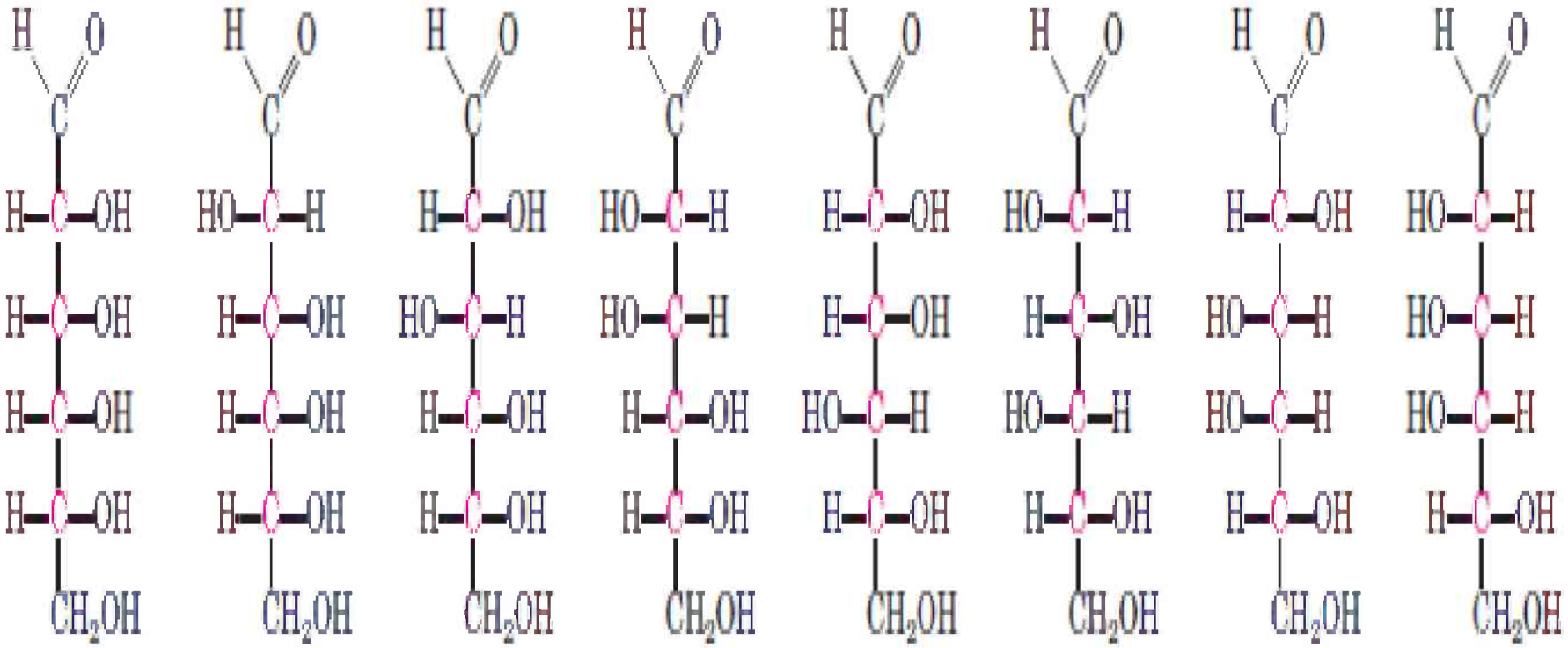
Sugar Nomenclature

For sugars with more than one chiral center, **D** or **L** refers to the asymmetric **C** farthest from the aldehyde or keto group.

Most naturally occurring sugars are D isomers.



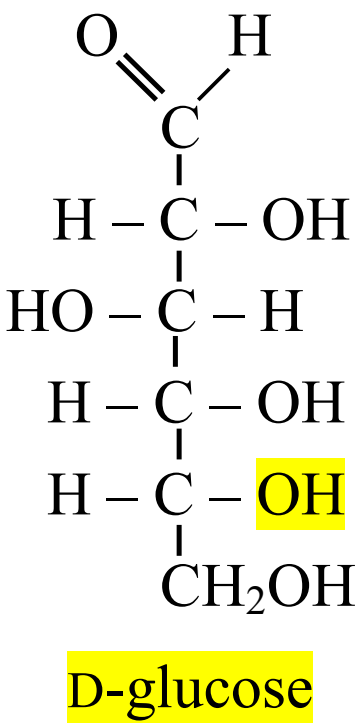
Six carbons


D-Allose
D-Altrose
D-Glucose
D-Mannose
D-Gulose
D-Idose
D-Galactose
D-Talose

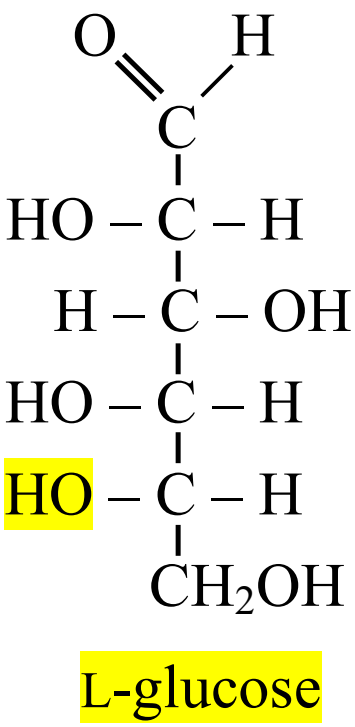
D & L sugars are mirror images of one another.

They have the same name, e.g., D-glucose & L-glucose.

Other stereoisomers have unique names, e.g., glucose, mannose, galactose, etc.



D-glucose



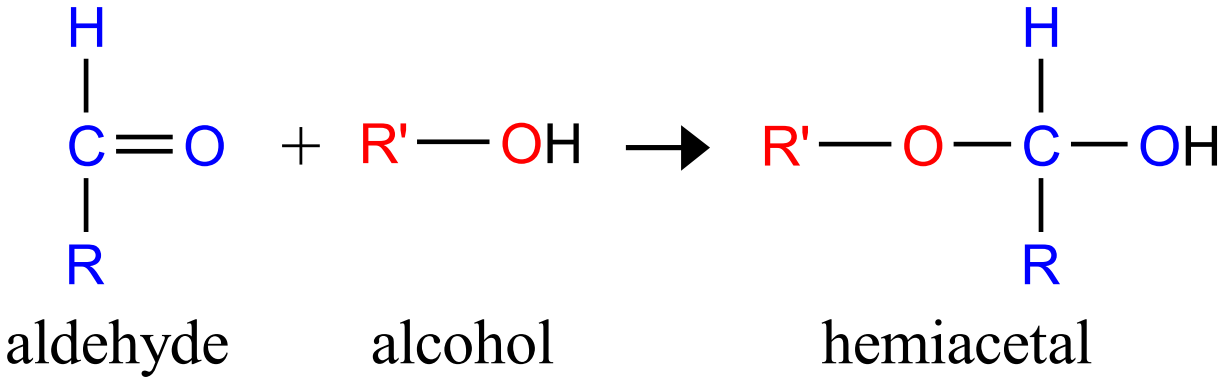
L-glucose

The number of stereoisomers is 2^n, where n is the number of asymmetric centers.

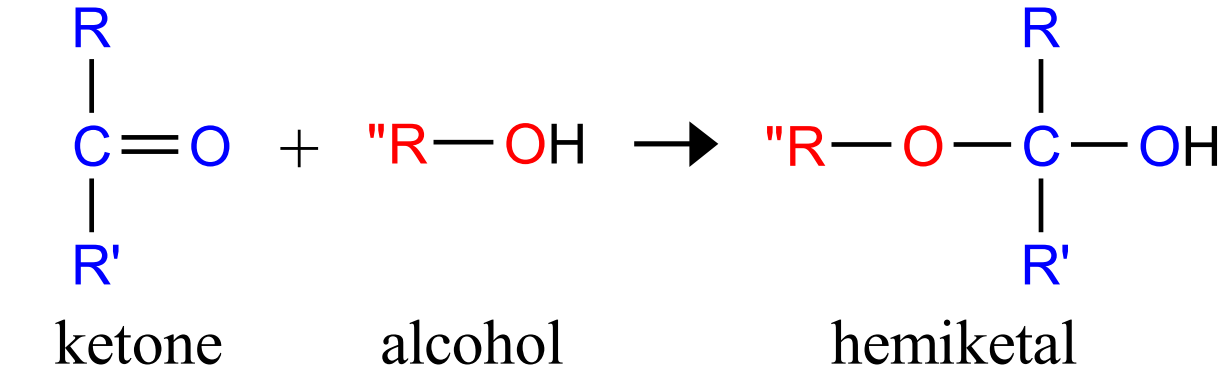
The 6-C aldoses have 4 asymmetric centers. Thus there are 16 stereoisomers (8 D-sugars and 8 L-sugars).

Hemiacetal & hemiketal formation

An aldehyde can react with an alcohol to form a hemiacetal.



A ketone can react with an alcohol to form a hemiketal.



Formation of hemiacetals and hemiketal

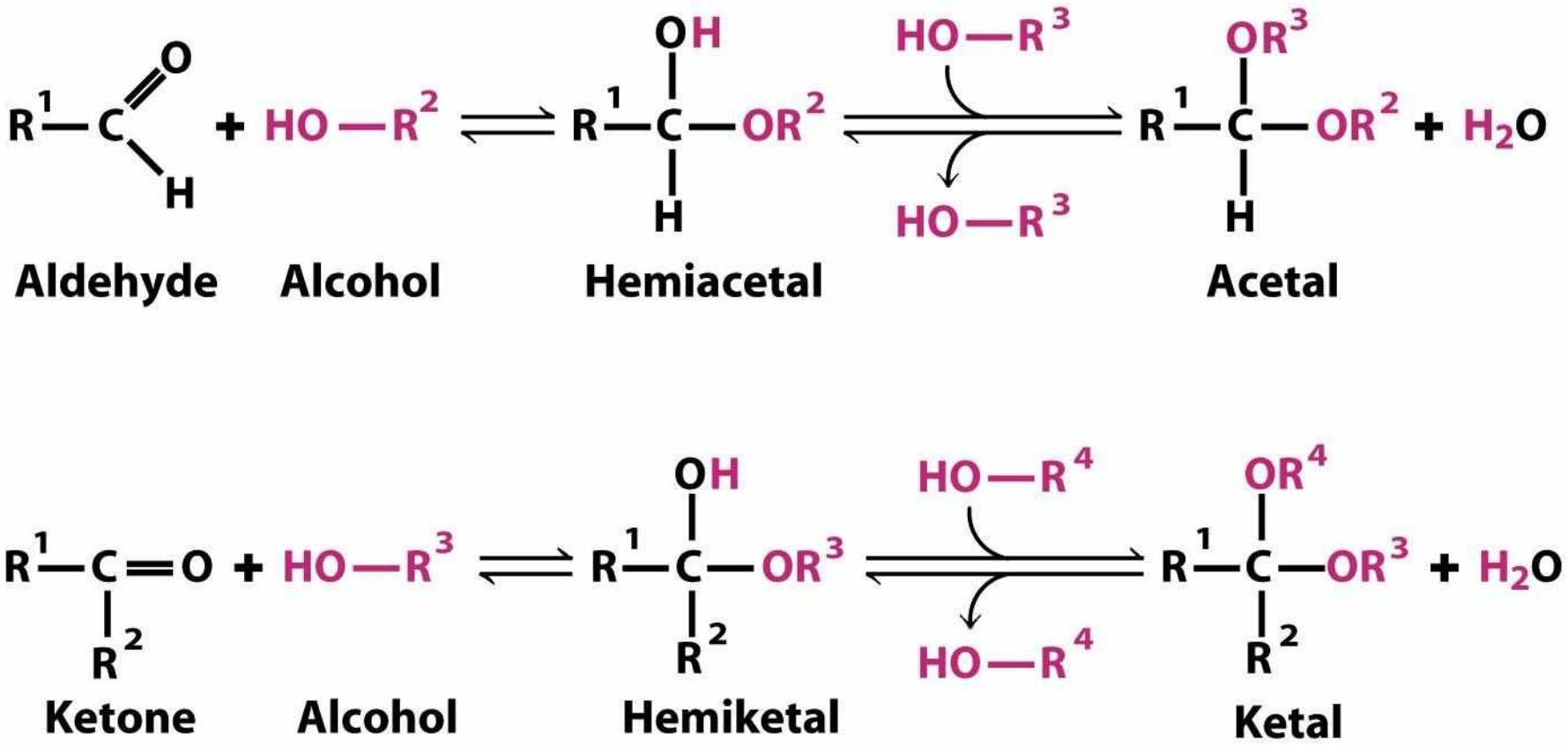
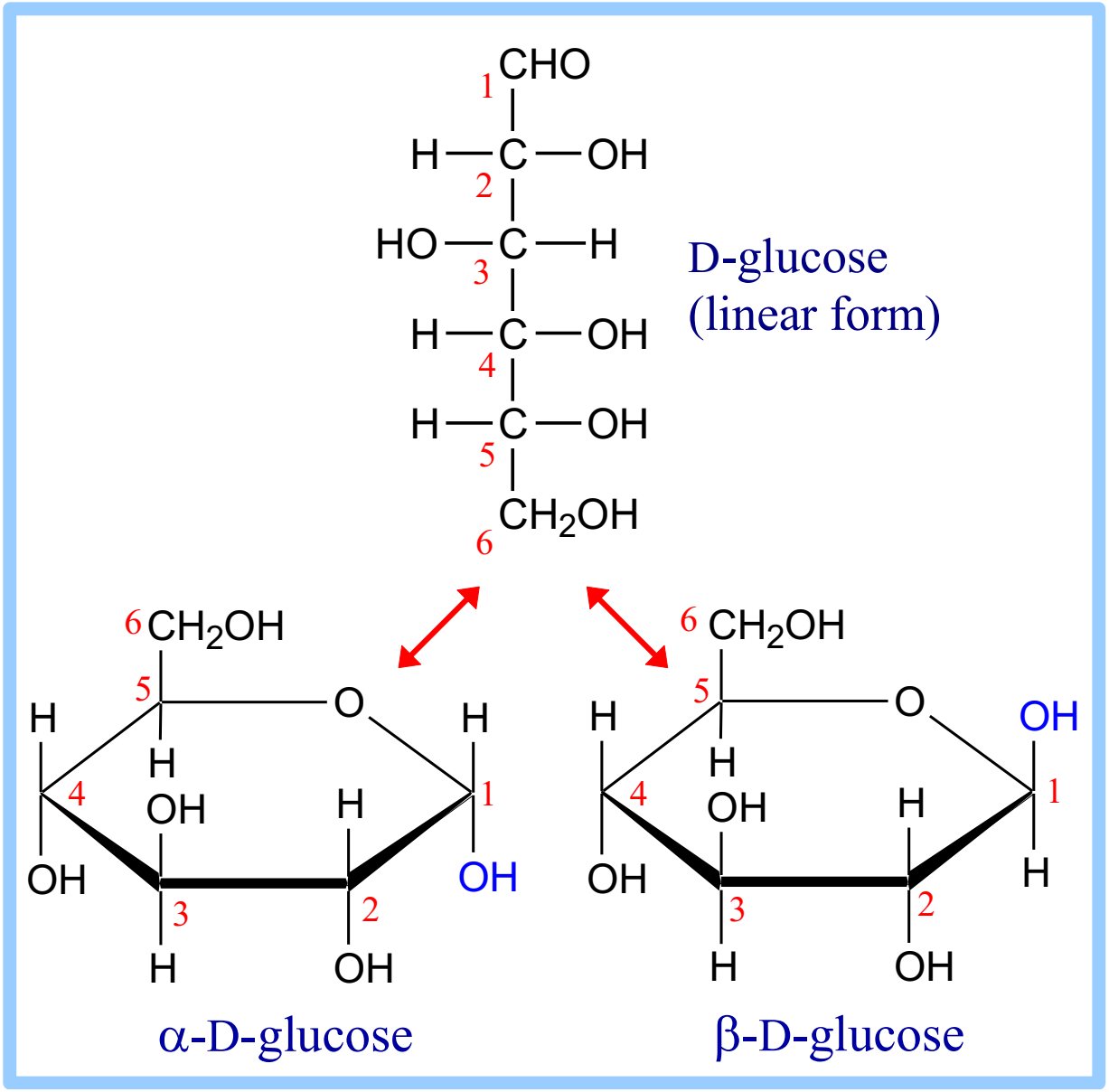


Figure 7-5
Lehninger Principles of Biochemistry, Fifth Edition

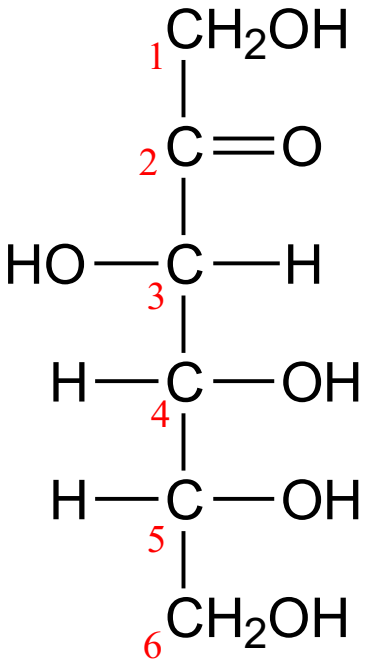
An aldehyde or ketone can react with an alcohol in a 1:1 ratio to yield a hemiacetal or hemiketal, respectively, creating a new chiral center at the carbonyl carbon. Substitution of a second alcohol molecule produces an acetal or ketal. When the second alcohol is part of another sugar molecule, the bond produced is a glycosidic bond.

Pentoses and hexoses can cyclize as the ketone or aldehyde reacts with a distal OH.

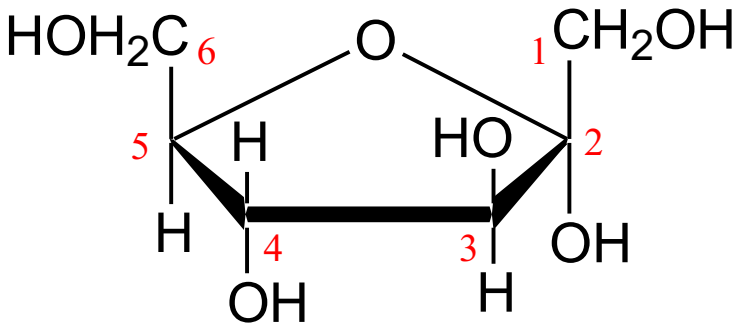
Glucose forms an intra-molecular hemiacetal, as the C1 aldehyde & C5 OH react, to form a 6-member pyranose ring, named after pyran.



These representations of the cyclic sugars are called Haworth projections.



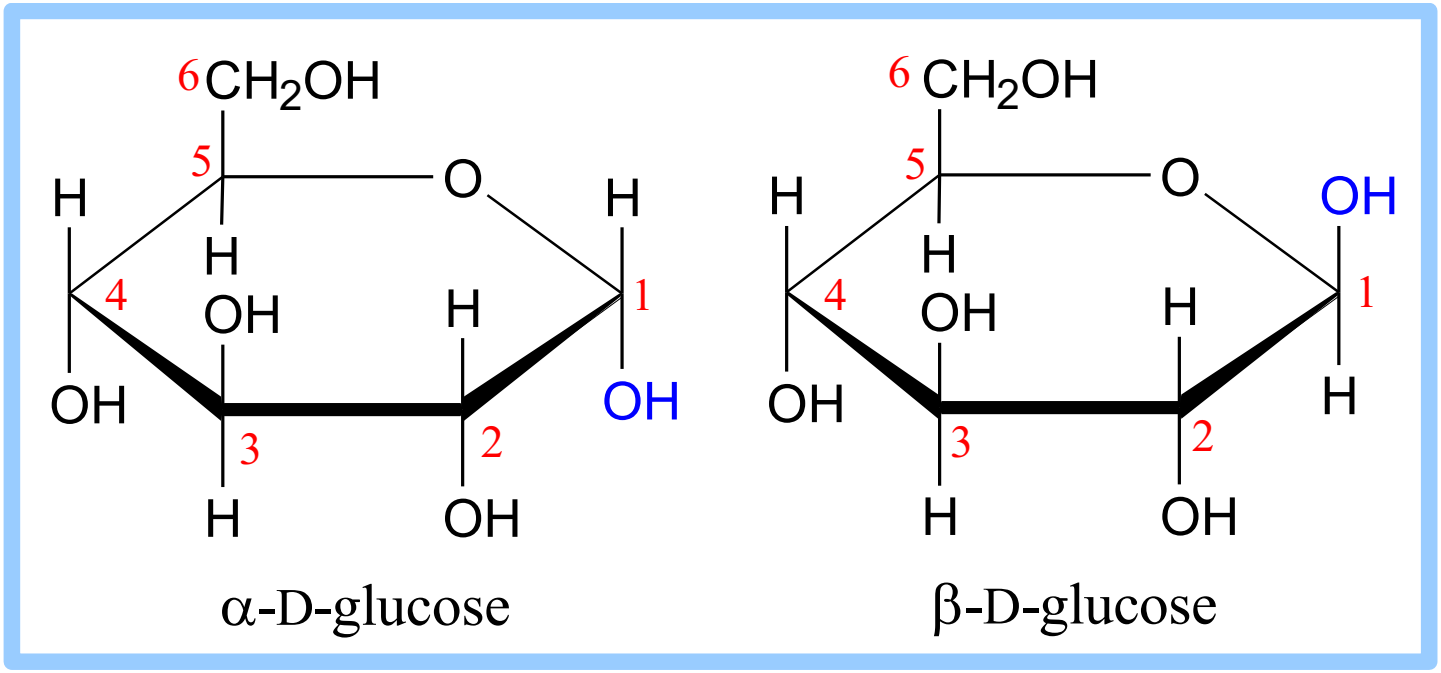
D-fructose (linear)



α-D-fructofuranose

Fructose forms either

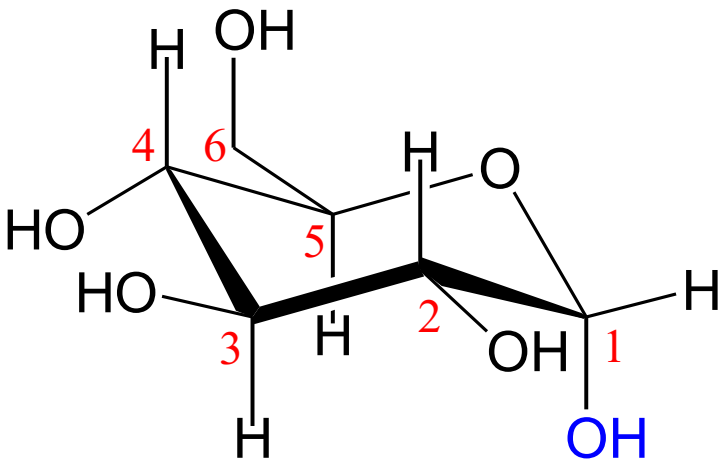
- a 6-member pyranose ring, by reaction of the C2 keto group with the OH on C6, or
- a 5-member furanose ring, by reaction of the C2 keto group with the OH on C5.



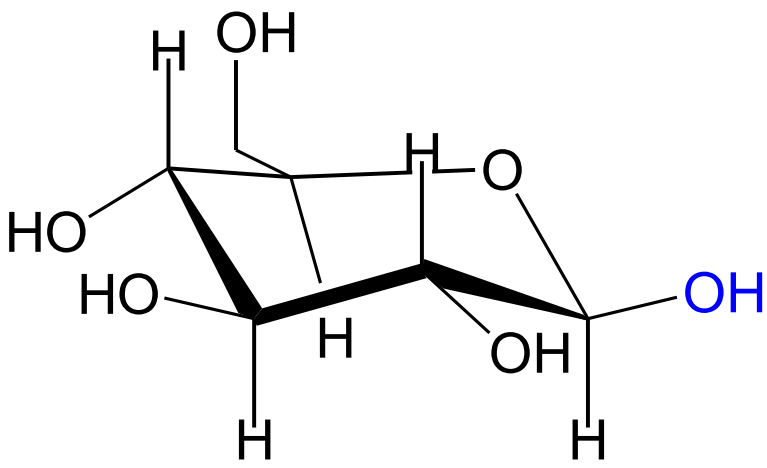
Cyclization of glucose produces a new asymmetric center at C1. The 2 stereoisomers are called anomers, α & β.

Haworth projections represent the cyclic sugars as having essentially planar rings, with the OH at the anomeric C1:

- α (OH below the ring)
- β (OH above the ring).



α-D-glucopyranose

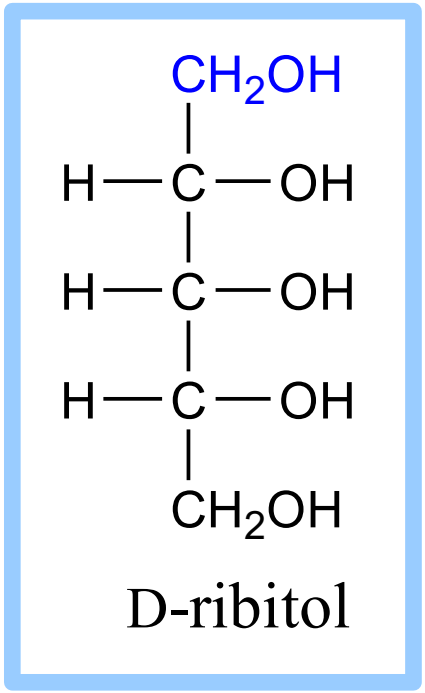


β-D-glucopyranose

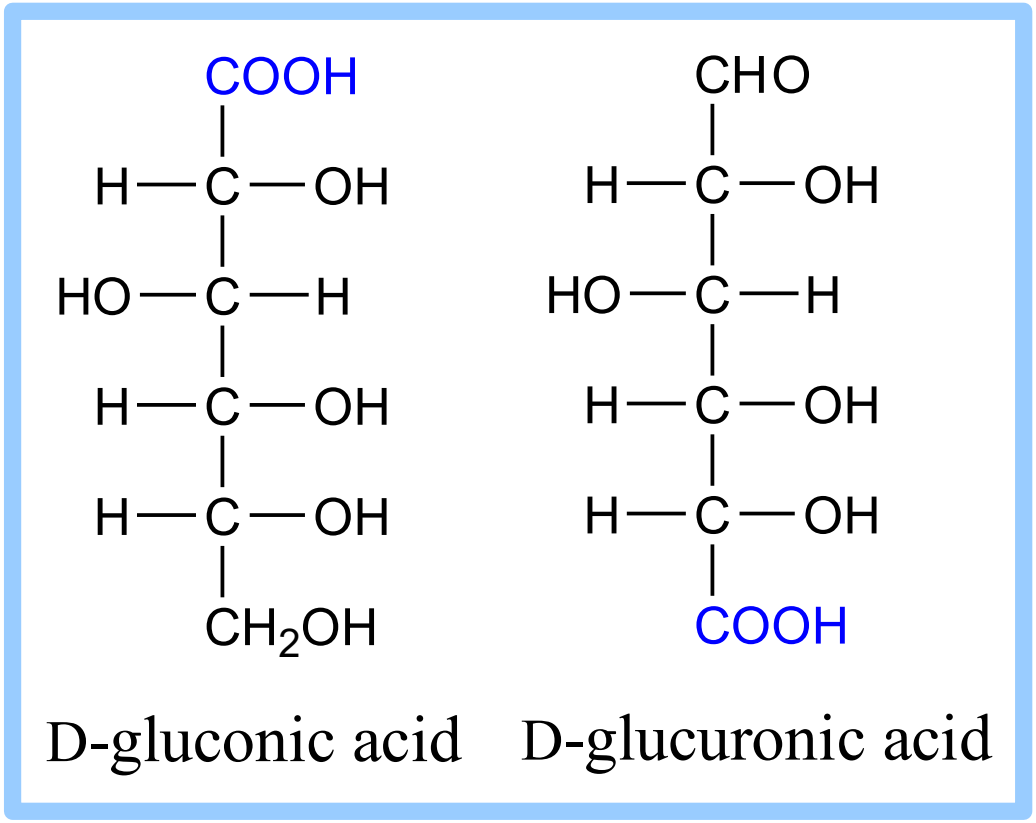
Because of the tetrahedral nature of carbon bonds, pyranose sugars actually assume a "chair" or "boat" configuration, depending on the sugar.

The representation above reflects the chair configuration of the glucopyranose ring more accurately than the Haworth projection.

Sugar derivatives



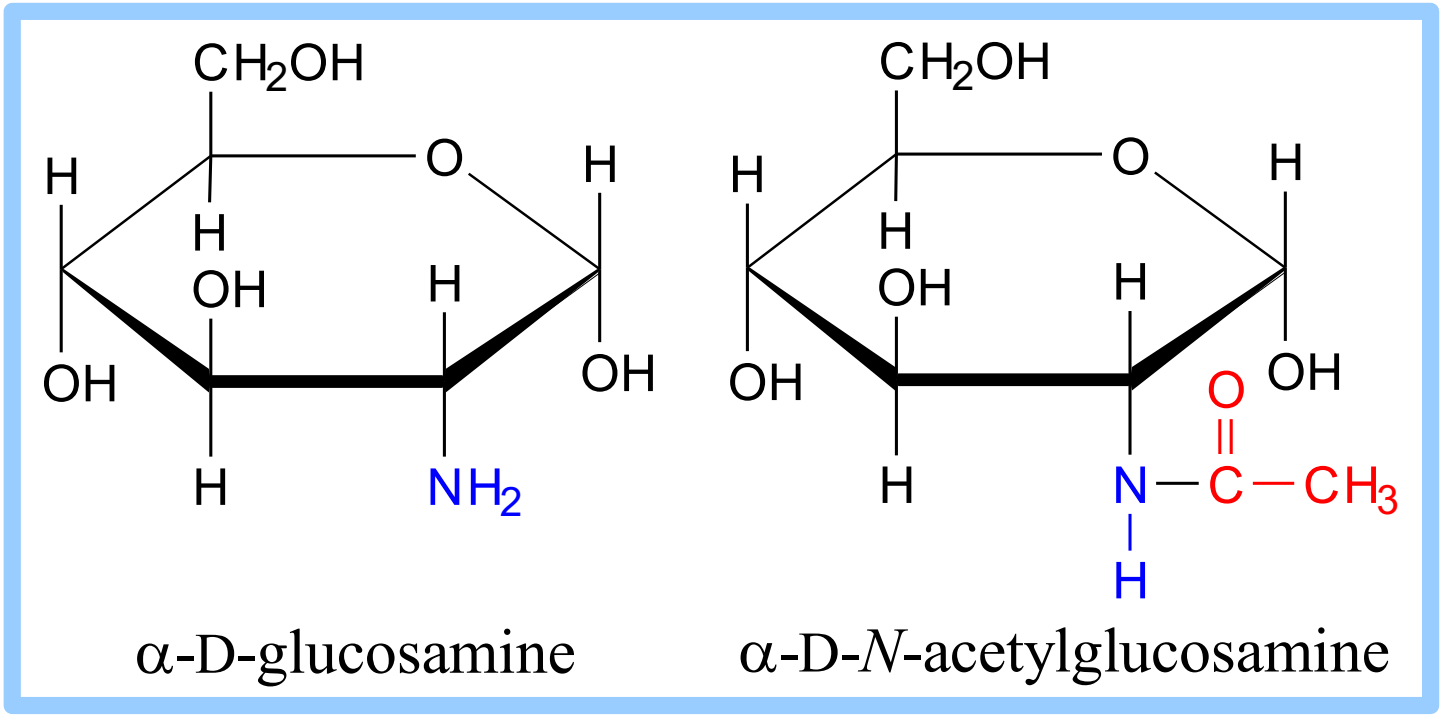
D-ribitol



D-gluconic acid D-glucuronic acid

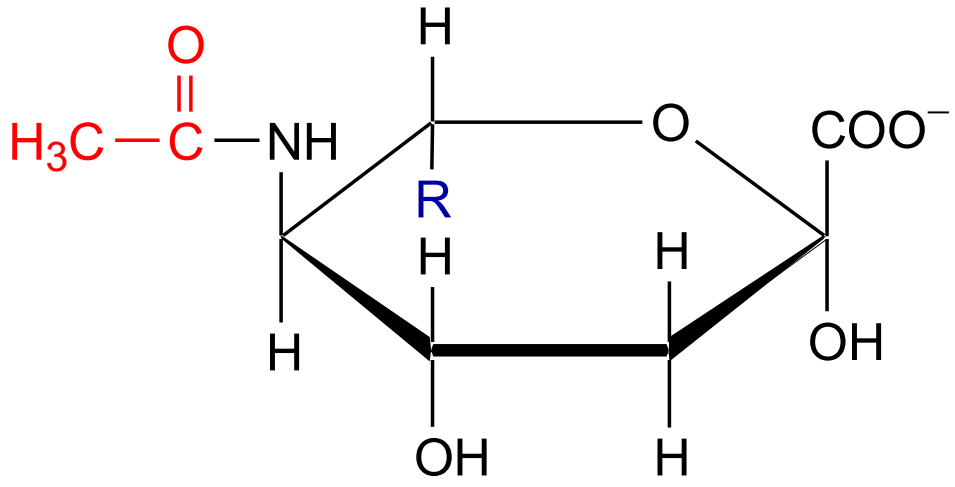
- sugar alcohol - lacks an aldehyde or ketone; e.g., ribitol.
- sugar acid - the aldehyde at C1, or OH at C6, is oxidized to a carboxylic acid; e.g., gluconic acid, glucuronic acid.

Sugar derivatives



amino sugar - an amino group substitutes for a hydroxyl. An example is glucosamine.

The amino group may be acetylated, as in *N*-acetylglucosamine.



N-acetylneuraminate (sialic acid)

N-acetylneuraminate (N-acetylneuraminic acid, also called sialic acid) is often found as a terminal residue of oligosaccharide chains of glycoproteins.

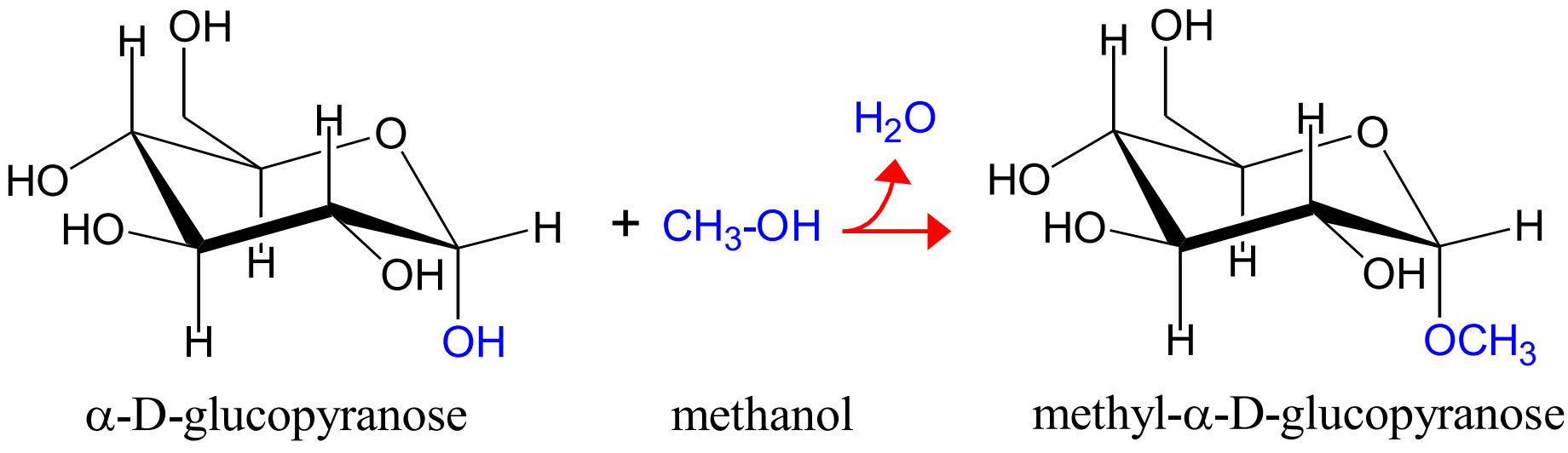
Sialic acid imparts negative charge to glycoproteins, because its carboxyl group tends to dissociate a proton at physiological pH, as shown here.

Glycosidic Bonds

The anomeric hydroxyl and a hydroxyl of another sugar or some other compound can join together, splitting out water to form a glycosidic bond:

R-OH + HO-R' → R-O-R' + H_2O

E.g., methanol reacts with the anomeric OH on glucose to form methyl glucoside (methyl-glucopyranose).

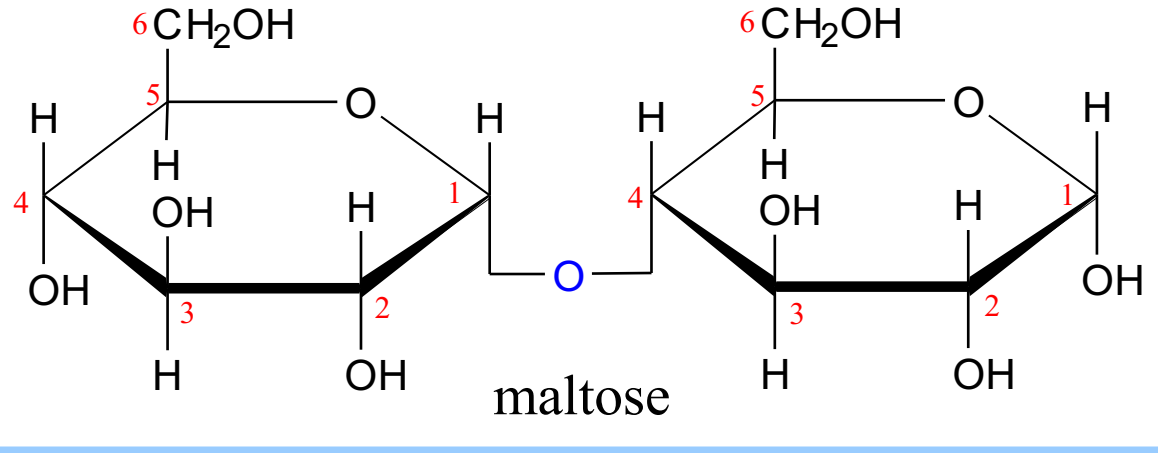


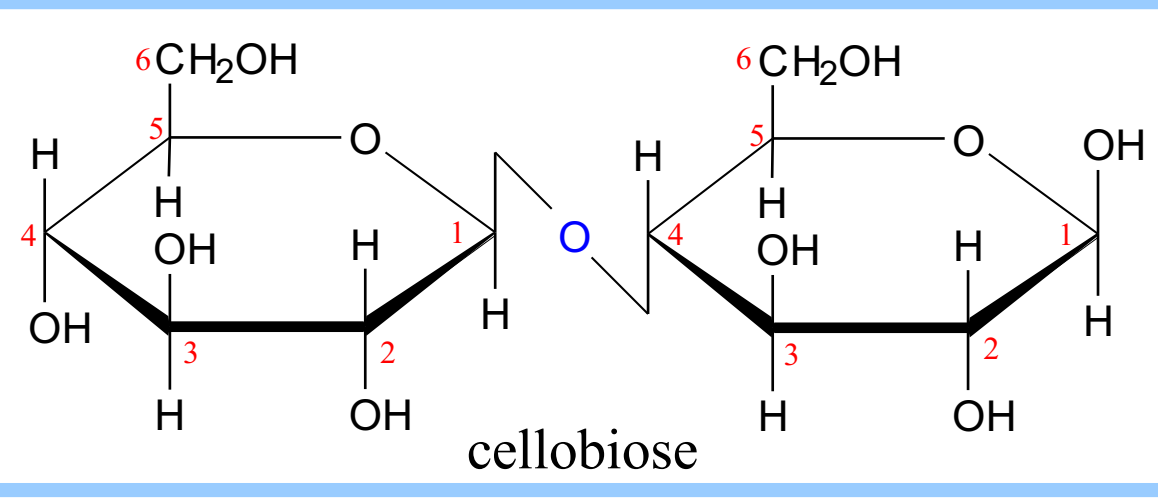
α-D-glucopyranose methanol methyl-α-D-glucopyranose

Disaccharides:

Maltose, a cleavage product of starch (e.g., amylose), is a disaccharide with an $\alpha(1\rightarrow 4)$ glycosidic link between C1 - C4 OH of 2 glucoses.

It is the **α** anomer (C1 O points down).





Cellobiose, a product of cellulose breakdown, is the otherwise equivalent **β** anomer (O on C1 points up).

The $\beta(1\rightarrow 4)$ glycosidic linkage is represented as a zig-zag, but one glucose is actually **flipped over** relative to the other.

Other **disaccharides** include:

- **Sucrose**, common table sugar, has a glycosidic bond linking the anomeric hydroxyls of glucose & fructose.

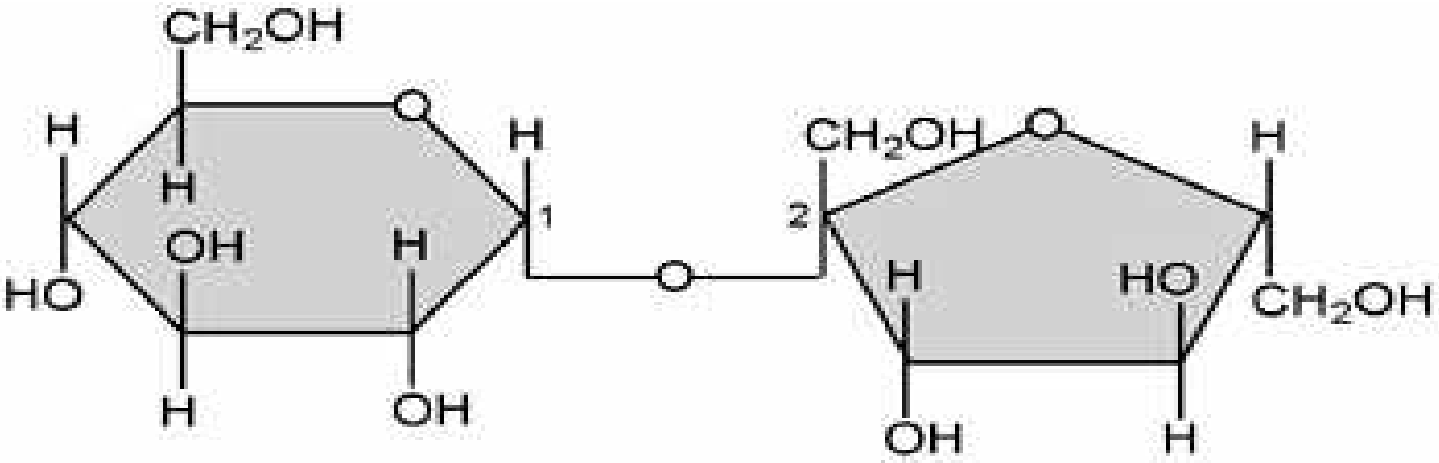
  Because the configuration at the anomeric C of glucose is α (O points down from ring), the linkage is α(1→2).

  The full name of sucrose is α-D-glucopyranosyl-(1→2)-β-D-fructopyranose.)

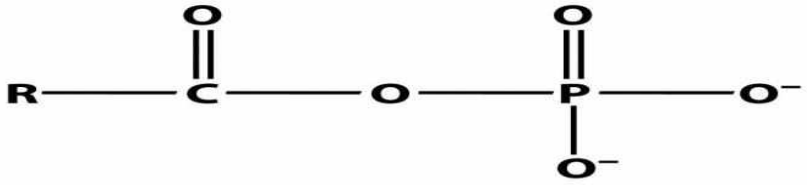
- **Lactose**, milk sugar, is composed of galactose & glucose, with β(1→4) linkage from the anomeric OH of galactose. Its full name is β-D-galactopyranosyl-(1→ 4)-α-D-glucopyranose

Disaccharides (double sugars)

- all have the formula C12 H22 O11
- sucrose (table sugar) is an example

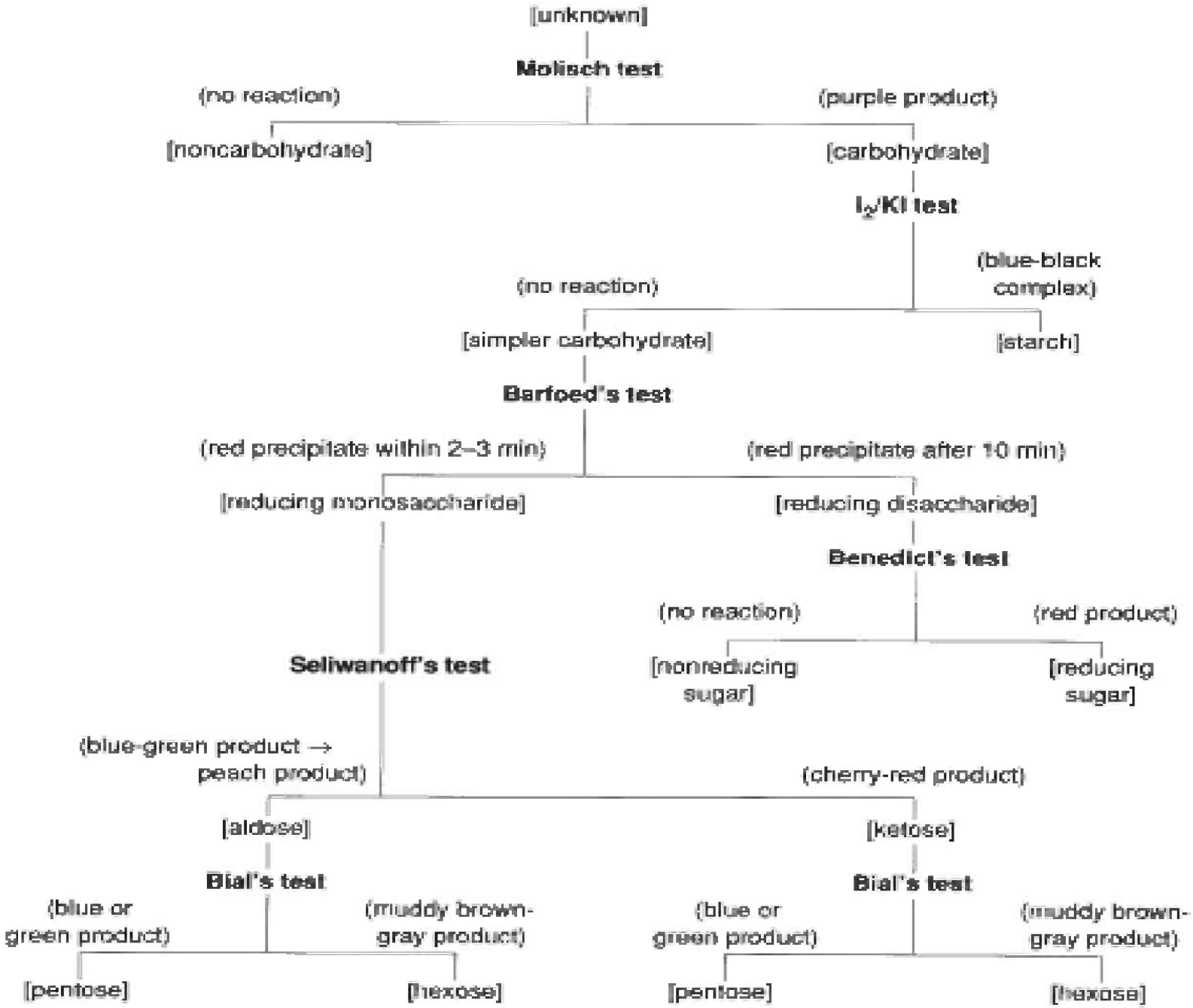


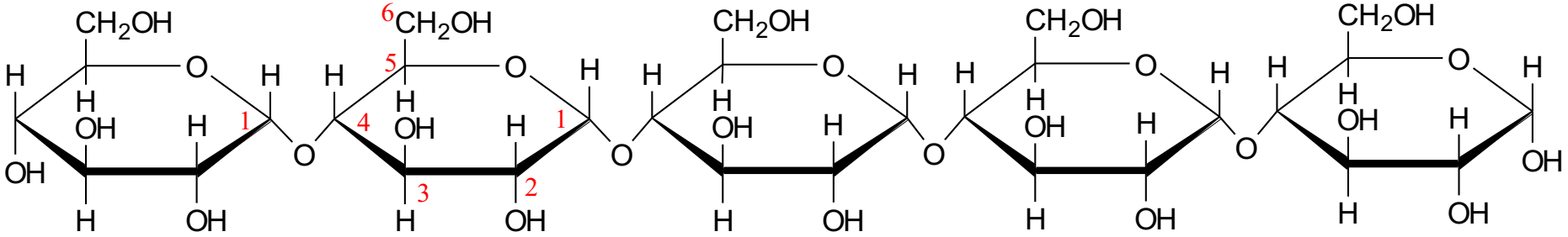
An ester



A phosphoester

Phosphoester


[unknown]
Molisch test
(no reaction)
(purple product)
[noncarbohydrate]
[carbohydrate]
I_2/KI test
(blue-black complex)
(no reaction)
[simpler carbohydrate]
[starch]
Barfoed's test
(red precipitate within 2–3 min)
(red precipitate after 10 min)
[reducing monosaccharide]
[reducing disaccharide]
Benedict's test
(no reaction)
(red product)
[nonreducing sugar]
[reducing sugar]
Seliwanoff's test
(blue-green product → peach product)
(cherry-red product)
[aldose]
[ketose]
Bial's test
Bial's test
(blue or green product)
(muddy brown-gray product)
(blue or green product)
(muddy brown-gray product)
[pentose]
[hexose]
[pentose]
[hexose]



Polysaccharides:

Plants store glucose as **amylose** or **amylopectin**, glucose polymers collectively called starch.

Glucose storage in **polymeric** form **minimizes osmotic effects**.

Amylose is a glucose polymer with **α(1→4)** linkages.

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

Polysaccharides

Polysaccharides can have one, two or many different monosaccharides

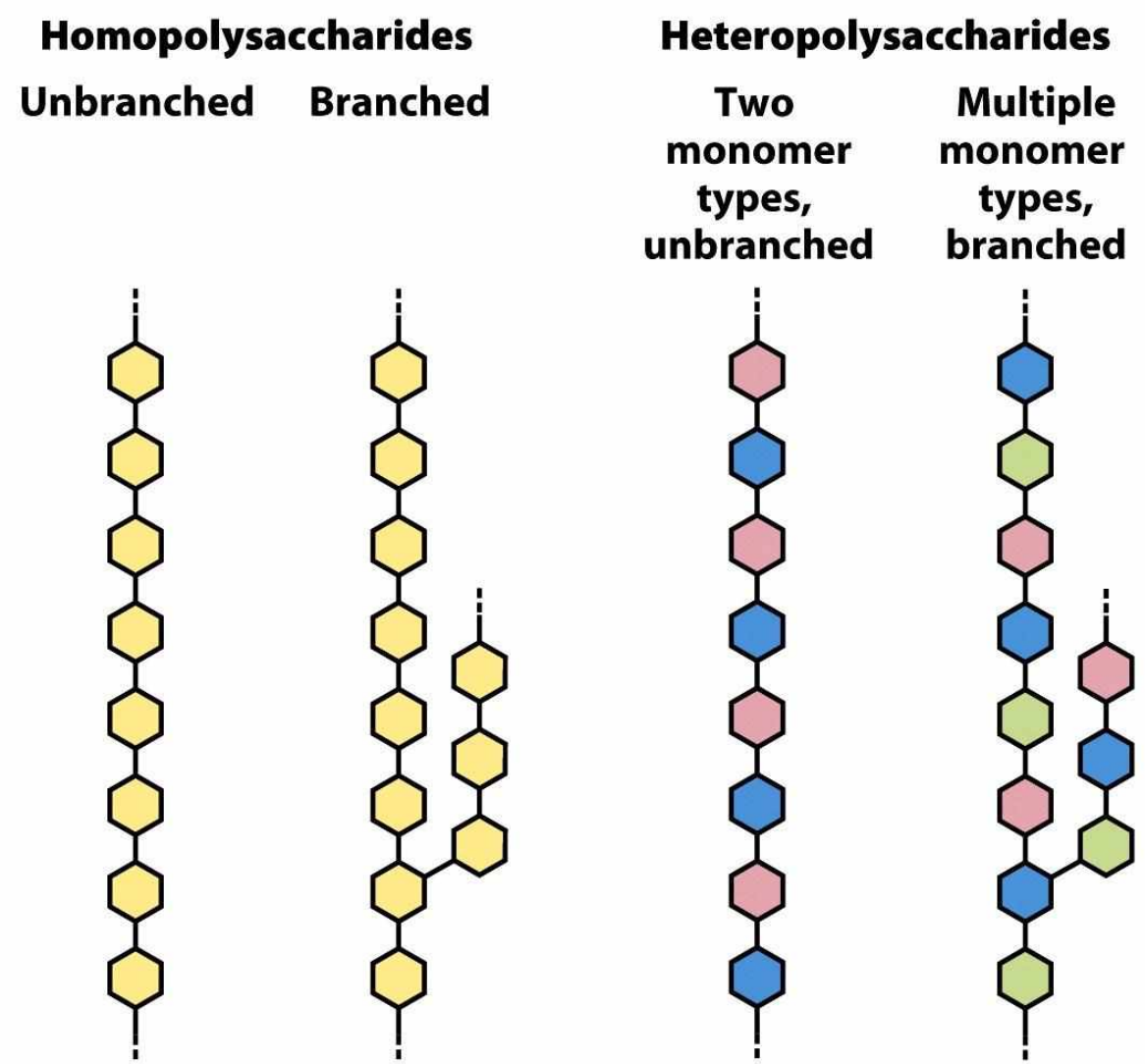
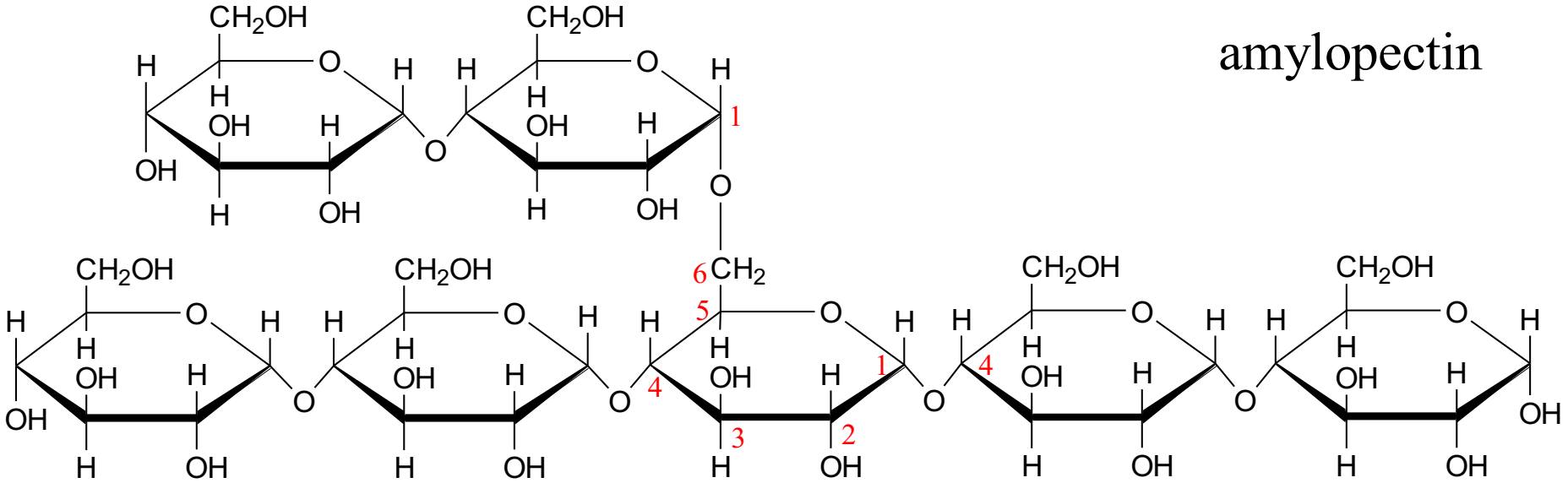
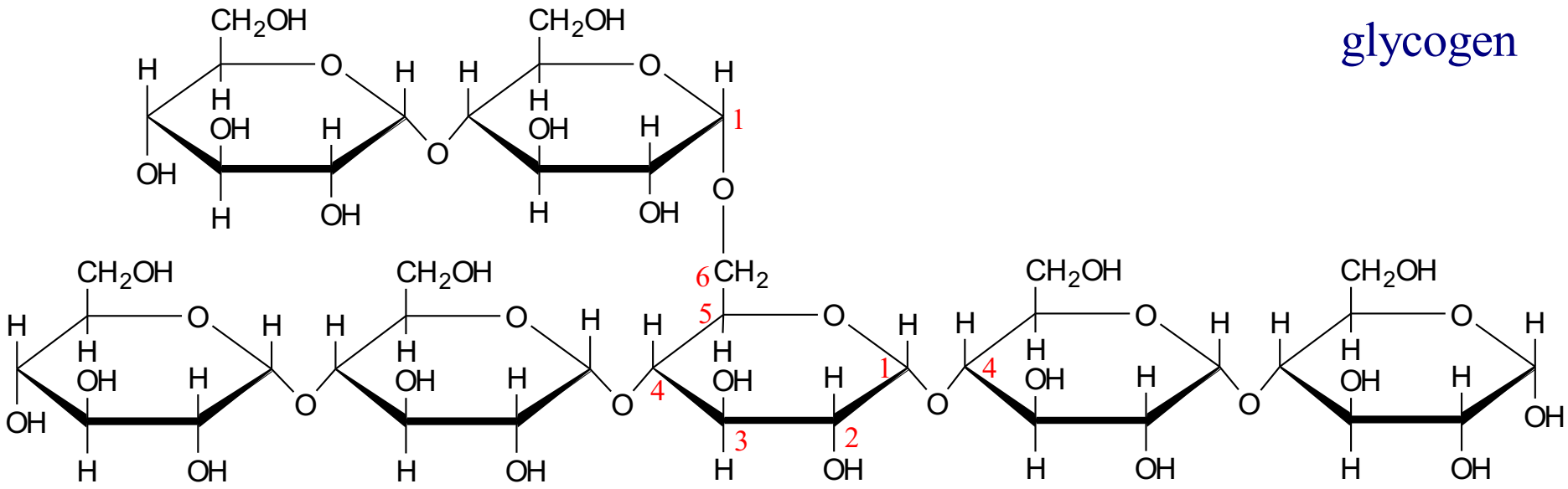


Figure 7-13
Lehninger Principles of Biochemistry, Fifth Edition



Amylopectin is a glucose polymer with mainly α(1→4) linkages, but it also has **branches** formed by α(1→6) linkages. Branches are generally longer than shown above.

The branches produce a compact structure & provide multiple chain ends at which enzymatic cleavage can occur.

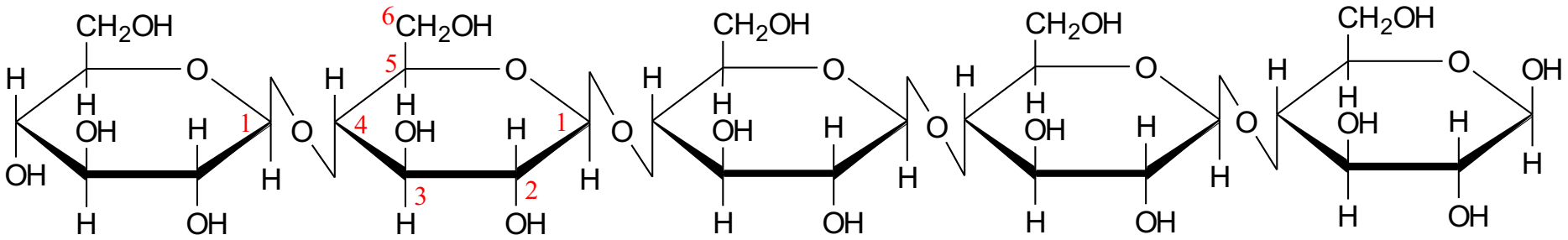


Glycogen, the glucose storage polymer in **animals**, is similar in structure to amylopectin.

But glycogen has **more** $\alpha(1\rightarrow6)$ **branches**.

The highly branched structure permits rapid glucose release from glycogen stores, e.g., in muscle during exercise.

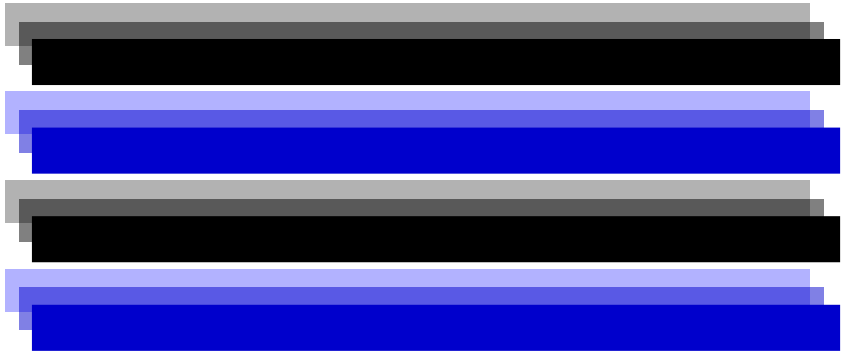
The ability to rapidly mobilize glucose is more essential to animals than to plants.



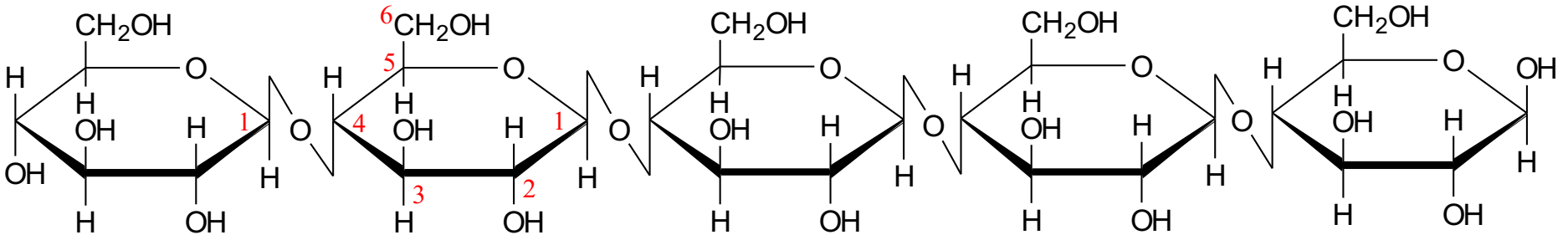
cellulose

Cellulose, a major constituent of plant cell walls, consists of long linear chains of glucose with β(1→4) linkages.

Every other glucose is flipped over, due to β linkages. This promotes intra-chain and inter-chain H-bonds and van der Waals interactions, that cause cellulose chains to be straight & rigid, and pack with a crystalline arrangement in thick bundles - microfibrils.



Schematic of arrangement of cellulose chains in a microfibril.

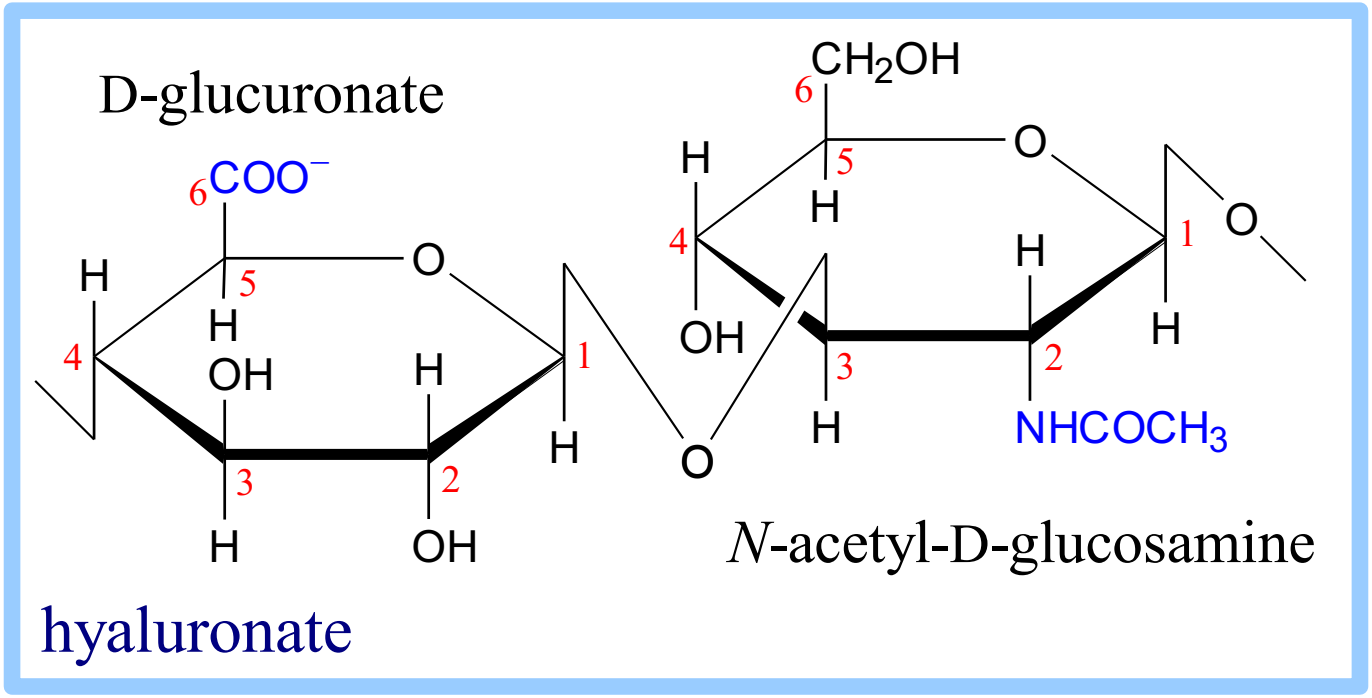


Multisubunit **Cellulose Synthase** complexes in the plasma membrane spin out from the cell surface microfibrils consisting of 36 parallel, interacting cellulose chains.

These **microfibrils** are very **strong**.

The **role** of cellulose is to impart strength and rigidity to plant cell walls, which can withstand high hydrostatic pressure gradients. Osmotic swelling is prevented.

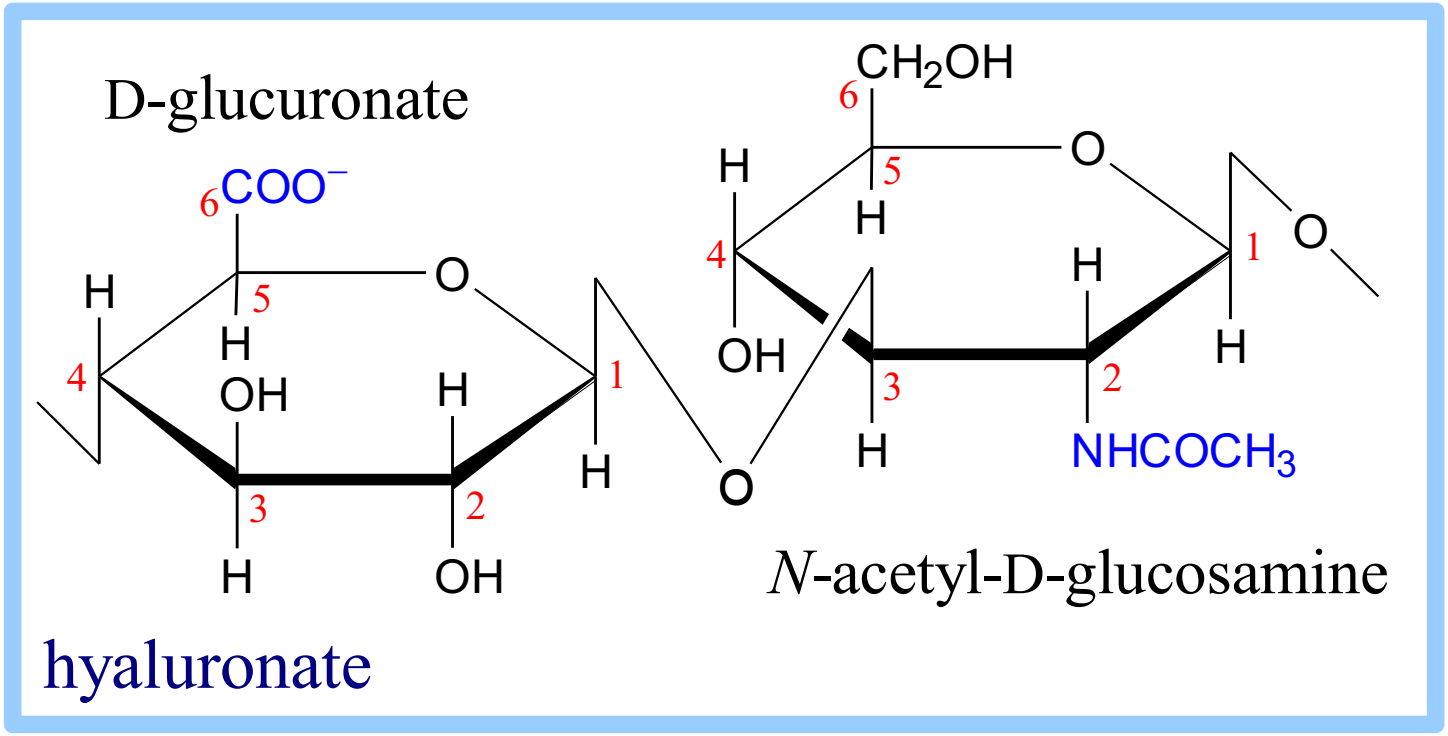
Explore and compare structures of amylose & cellulose using Chime.



Glycosaminoglycans (mucopolysaccharides) are linear polymers of **repeating disaccharides**.

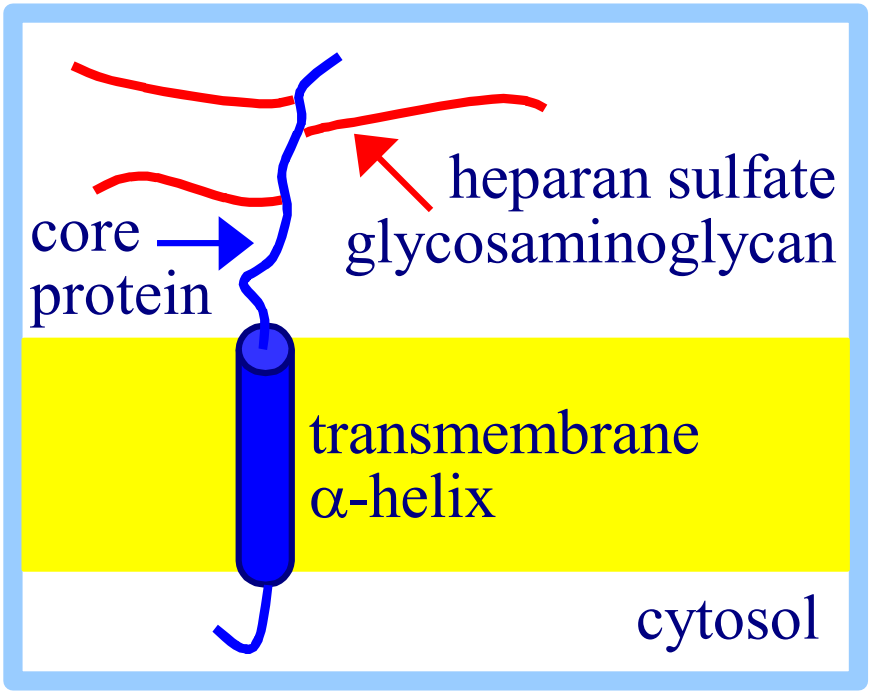
The constituent monosaccharides tend to be **modified**, with acidic groups, amino groups, sulfated hydroxyl and amino groups, etc.

Glycosaminoglycans tend to be **negatively charged**, because of the prevalence of acidic groups.



Hyaluronate (hyaluronan) is a **glycosaminoglycan** with a repeating disaccharide consisting of 2 glucose derivatives, glucuronate (glucuronic acid) & *N*-acetyl-glucosamine.

The glycosidic linkages are β(1→3) & β(1→4).

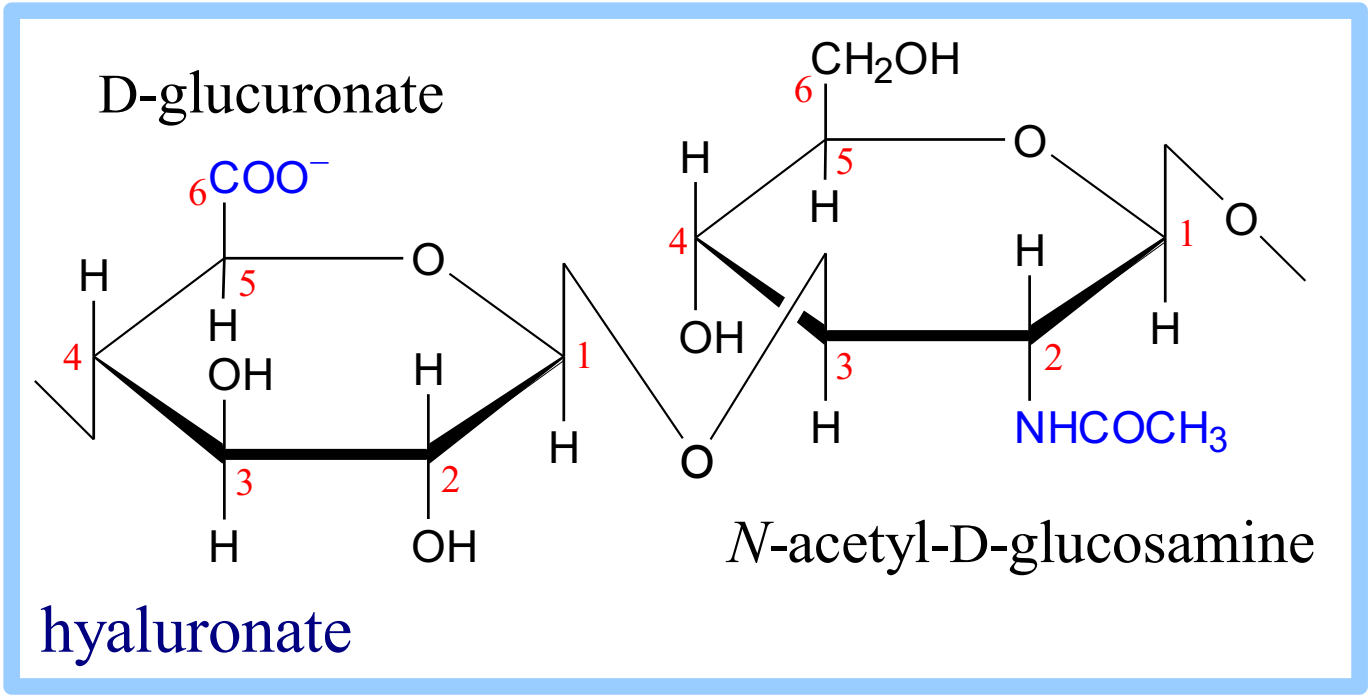


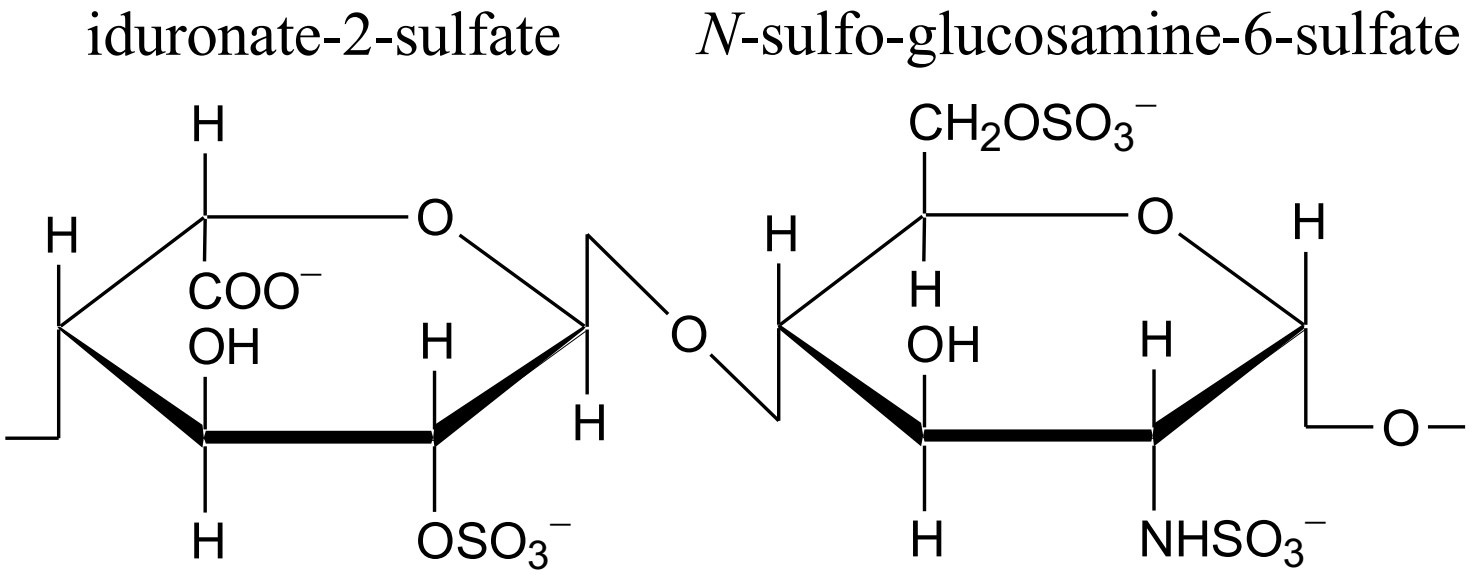
Proteoglycans are **glycosaminoglycans** that are covalently linked to serine residues of specific **core proteins**.

The glycosaminoglycan chain is synthesized by sequential addition of sugar residues to the core protein.

Some proteoglycans of the extracellular matrix **bind** non-covalently to **hyaluronate** via protein domains called **link modules**. E.g.:

- Multiple copies of the **aggrecan** proteoglycan associate with hyaluronate in cartilage to form large complexes.
- **Versican**, another proteoglycan, binds hyaluronate in the extracellular matrix of loose connective tissues.





heparin or heparan sulfate - examples of residues

Heparan sulfate is initially synthesized on a membrane-embedded core protein as a polymer of alternating ***N*-acetylglucosamine** and **glucuronate** residues.

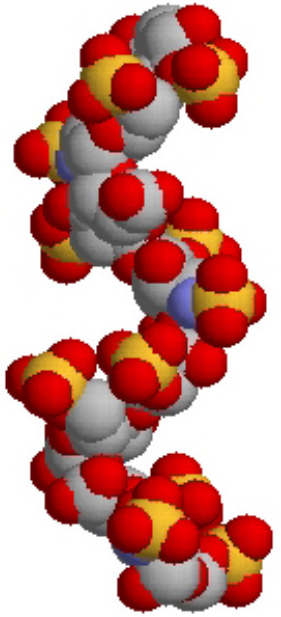
Later, in segments of the polymer, glucuronate residues may be converted to the sulfated sugar **iduronic acid**, while *N*-acetylglucosamine residues may be deacetylated and/or sulfated.

Heparin, a soluble glycosaminoglycan found in granules of mast cells, has a structure similar to that of heparan sulfates, but is more **highly sulfated**.

When released into the blood, it inhibits clot formation by interacting with the protein antithrombin.

Heparin has an **extended helical conformation**.

PDB 1RID



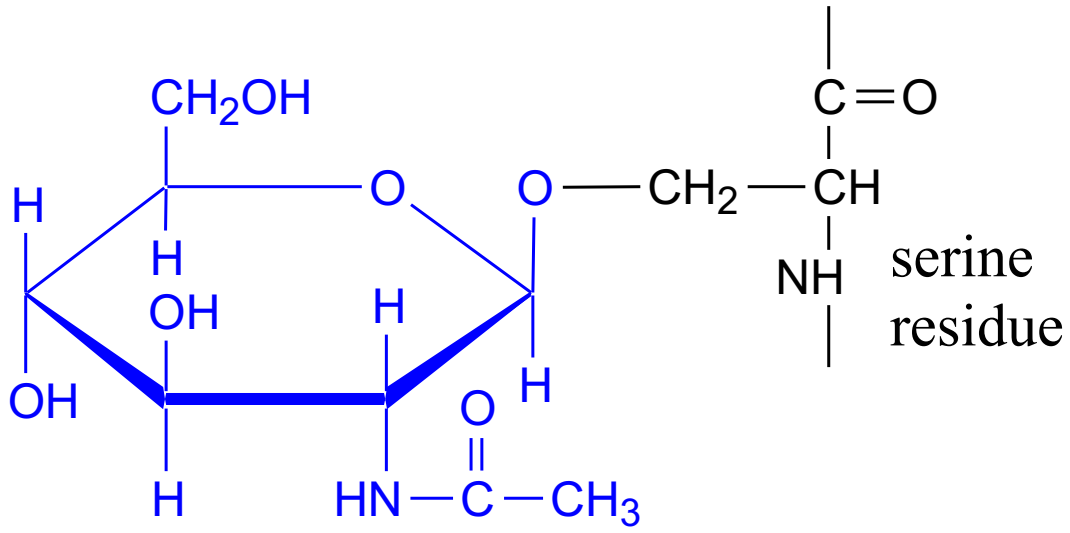
heparin: $(IDS\text{-}SGN)_5$

C O N S

Charge repulsion by the many negatively charged groups may contribute to this conformation.

Heparin shown has 10 residues, alternating IDS (iduronate-2-sulfate) & SGN (N-sulfo-glucosamine-6-sulfate).

Oligosaccharides that are covalently attached to proteins or to membrane lipids may be linear or branched chains.

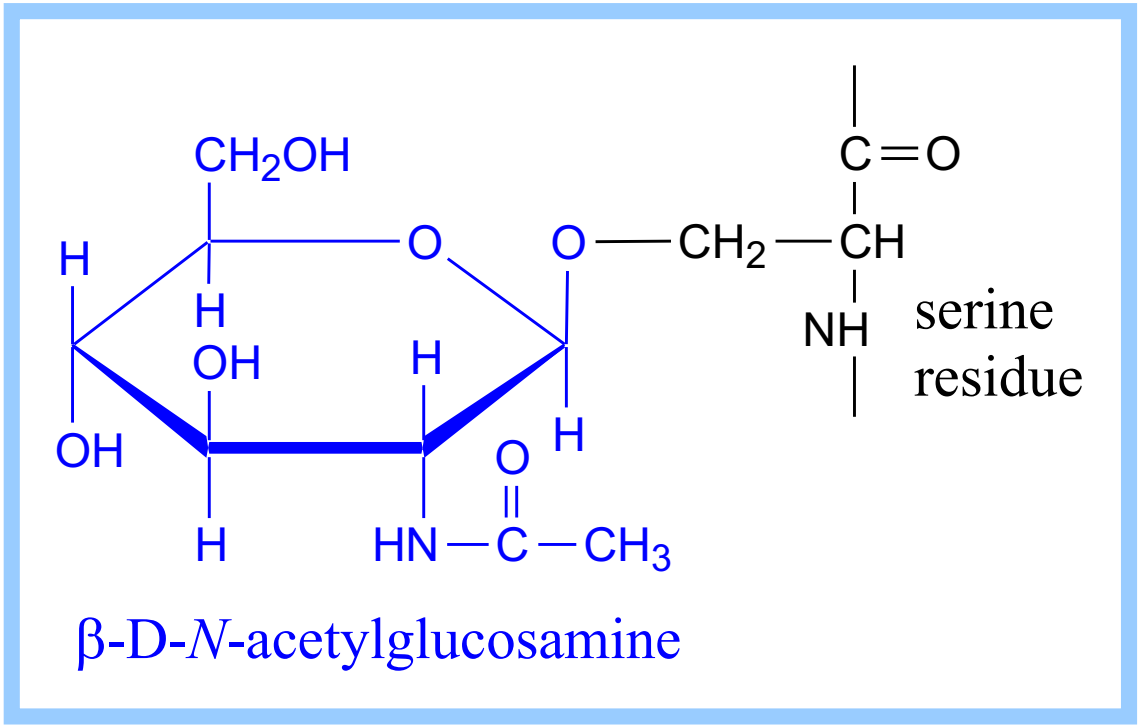


β-D-*N*-acetylglucosamine

O-linked oligosaccharide chains of glycoproteins vary in complexity.

They link to a protein via a glycosidic bond between a sugar residue & a **serine or threonine OH**.

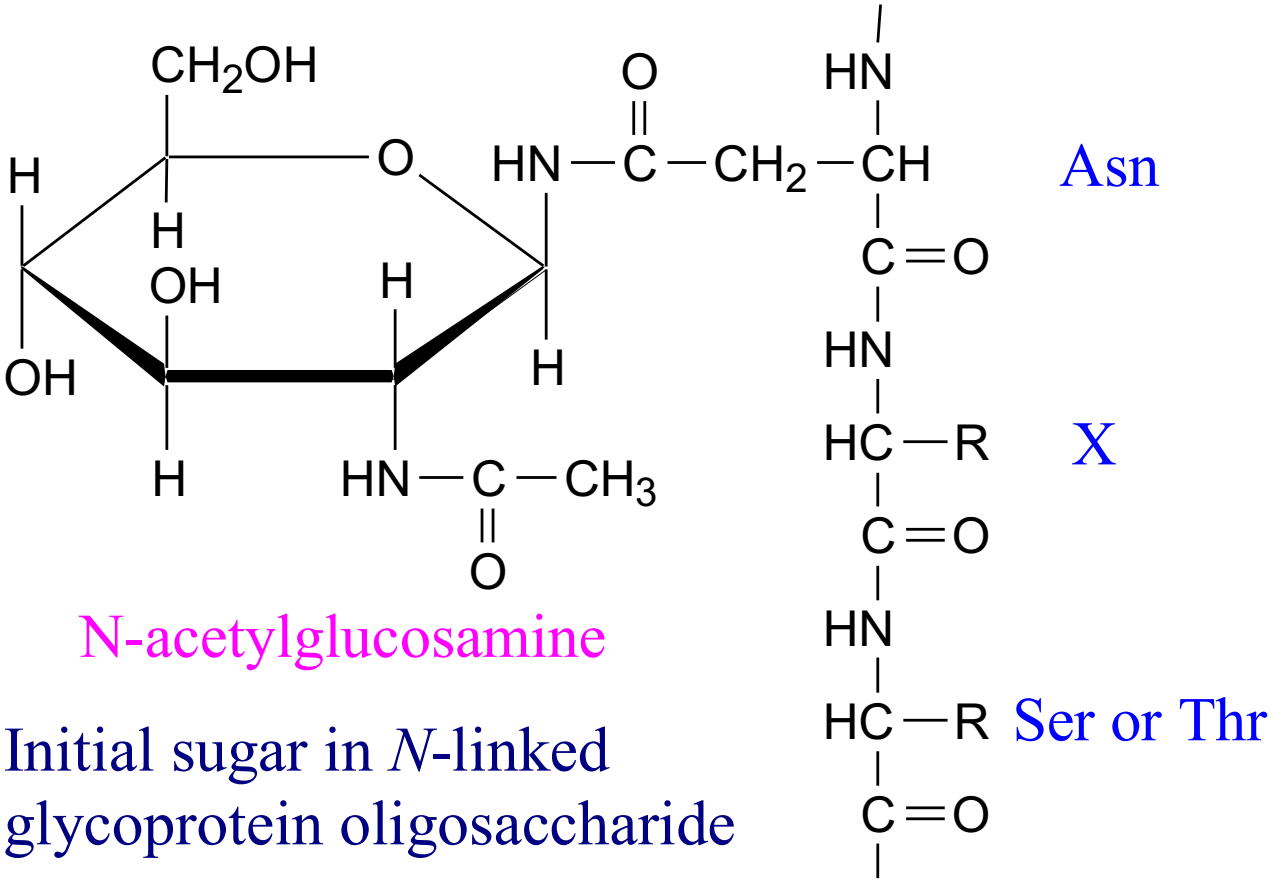
O-linked oligosaccharides have roles in **recognition**, **interaction**, and **enzyme regulation**.



***N*-acetylglucosamine (GlcNAc)** is a common O-linked glycosylation of protein serine or threonine residues.

Many cellular proteins, including enzymes & transcription factors, are **regulated** by reversible GlcNAc attachment.

Often attachment of GlcNAc to a protein OH **alternates with phosphorylation**, with these 2 modifications having opposite regulatory effects (stimulation or inhibition).



***N*-linked oligosaccharides** of glycoproteins tend to be complex and branched.

First ***N*-acetylglucosamine** is linked to a protein via the side-chain N of an asparagine residue in a particular 3-amino acid sequence.

Many proteins secreted by cells have attached N-linked oligosaccharide chains.

Genetic diseases have been attributed to deficiency of particular enzymes involved in synthesizing or modifying oligosaccharide chains of these glycoproteins.

Such diseases, and gene knockout studies in mice, have been used to define pathways of modification of oligosaccharide chains of glycoproteins and glycolipids.

Carbohydrate chains of plasma membrane glycoproteins and glycolipids usually face the outside of the cell.

They have roles in cell-cell interaction and signaling, and in forming a protective layer on the surface of some cells.

Lectins are glycoproteins that **recognize** and **bind** to specific **oligosaccharides**.

Concanavalin A & **wheat germ agglutinin** are plant lectins that have been useful research tools.

The **C-type lectin-like domain** is a **Ca^{++}-binding** carbohydrate recognition domain in many **animal lectins**.

Recognition/binding of CHO moieties of glycoproteins, glycolipids & proteoglycans **by** animal **lectins** is a factor in:

- cell-cell recognition
- adhesion of cells to the extracellular matrix
- interaction of cells with chemokines and growth factors
- recognition of disease-causing microorganisms
- initiation and control of inflammation.

- Questions:
- 1. Define: Epimer , Superimpossible , Anomer, Haworth projection. Enantiomers , Aldoses ,Ketoses ,Hemiacetal, Hemiketal Glycogene , N – shape glycosidic linkage, D and L ISOMERS, Mulish test
- 2. What are the differences between:
- A. Starch and Glycogen
- B. Amylopectin and cellulose
- C. Amylose and Glycogen
- D. Fischer and Haworth projection in monosaccharides
- E. Alpha and beta D – Glucose
- 3. Draw the following: Aldotriose , Aldopentose , ketotetrose , ketohexose ,ketotrios , ketohexose ,Aldohexose Aldotetrose ,,and explain how many stereoisomer in each one.
- 4. Draw Haworth projection of Glucose , fructose , sucrose , maltose cellubiose , ribose , deoxyribose
- 5. write the acidic hydrolysis product of:
- a. amylose ,b. amylopectin ,c. cellulose d. glycogene , e. sucrose f. Molsh test

Madenet Al elm University College
Department of Biology
2nd Year Biochemistry I 2021 – 2022

CARBOHYDRATE METABOLISM

Dr. Hamza Yaseen Isa
Lecture 4 I 2

CELL RESPIRATION

THE CONCEPT OF 'RESPIRATION' IS CENTRAL TO ALL LIVING PROCESSES

All living cells are made up of **chemical substances**

The processes of living involve **reactions** between the substances.

For example, a reaction between carbon and oxygen (such as burning coal in air) changes the carbon in the coal, and oxygen in the air into carbon dioxide

The reaction between carbon and oxygen also releases **energy** in the form of heat and light (flames)

Living organisms get their energy from reactions like this (but not reactions which are violent enough to produce flames)

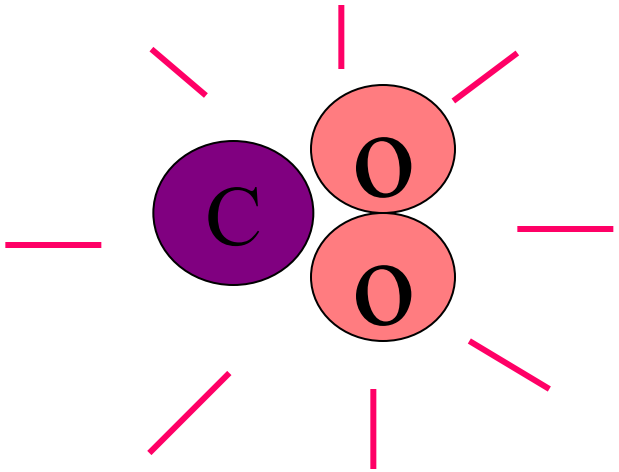
One of the energy-producing reactions is called **respiration**(Respiration is not the same thing as breathing)

The chemical reactions of respiration take place in all living cells

This reaction can be represented by the equation

$$C + O_2 \longrightarrow CO_2$$

an atom of carbon **C** plus a molecule of oxygen **O_2**



combine to form a molecule of carbon dioxide **CO_2**

The reaction takes place between oxygen and a substance which contains carbon. The reaction produces carbon dioxide and water, and releases energy

The carbon-containing substances come from **FOOD**

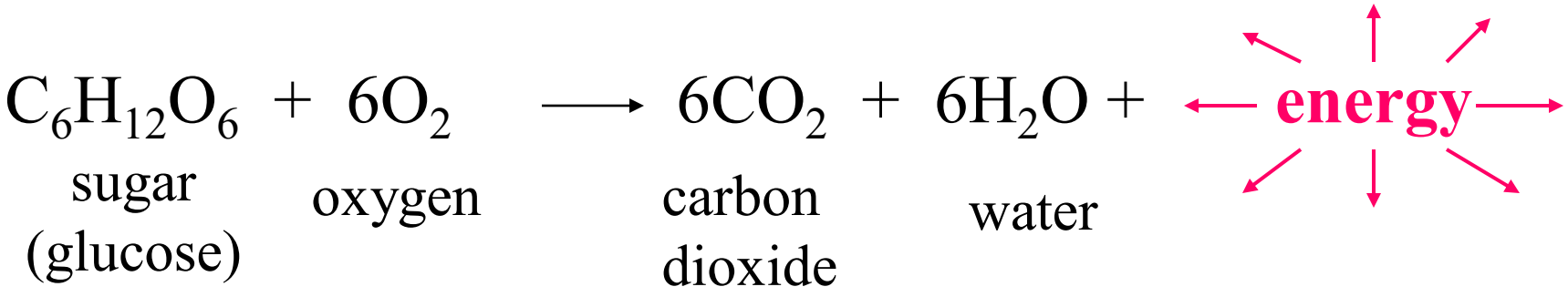
The oxygen comes from the **AIR** (or water)

The **energy** is used to drive other chemical reactions taking place in cells

One example of this is the release of energy in muscle cells to make them contract and produce movement

One example of an energy-producing reaction in cells is the breakdown of sugar when it combines with oxygen

This can be represented by the equation

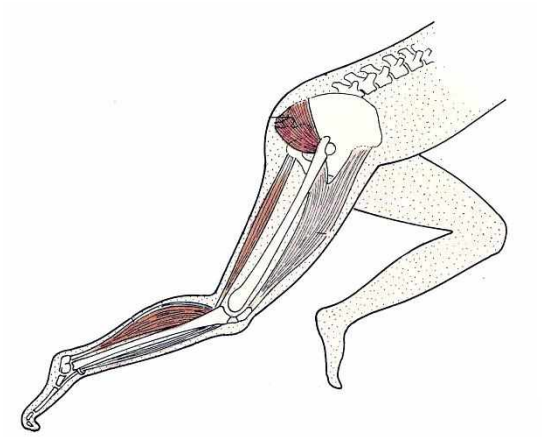


This means that one molecule of sugar reacts with six molecules of oxygen to produce six molecules of carbon dioxide and six molecules of water.

Energy is released during this process

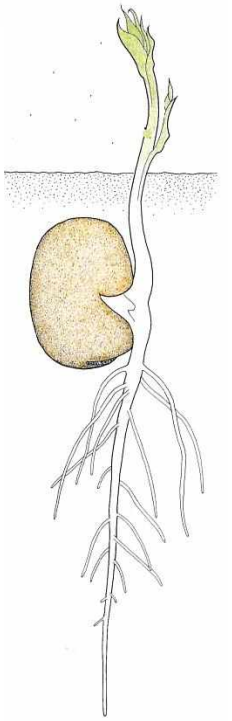
Some examples of the use of energy in organisms

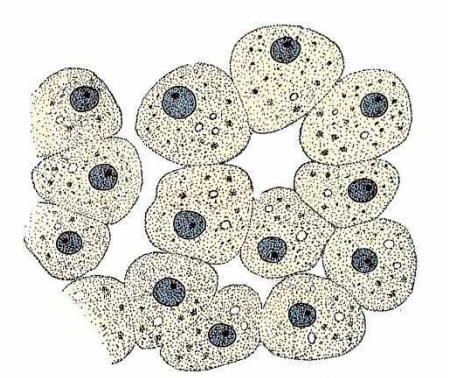
Respiration supplies the energy for



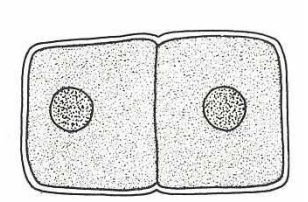
muscle contraction

germination





chemical changes in cells

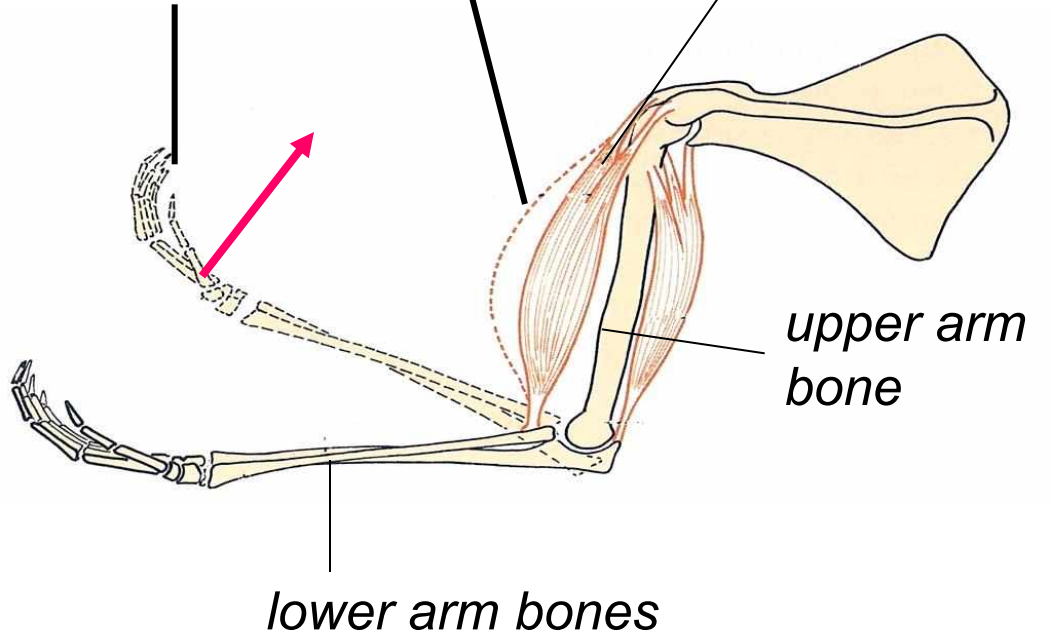


cell division

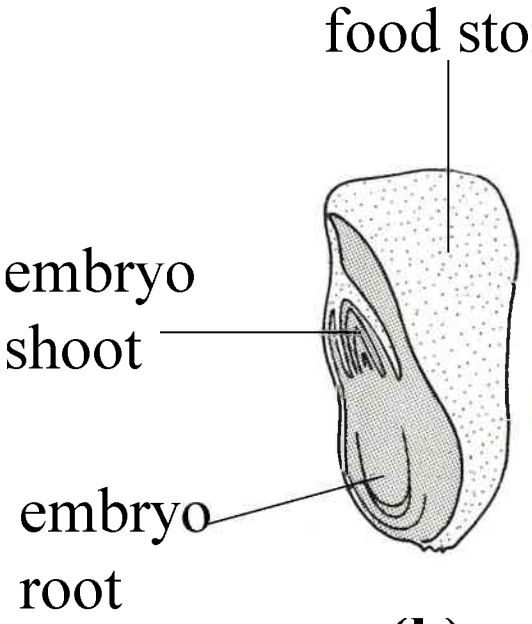
The blood stream brings food and oxygen to the muscle cells. Respiration occurs in the cells and releases energy which……

…….makes the muscle contract

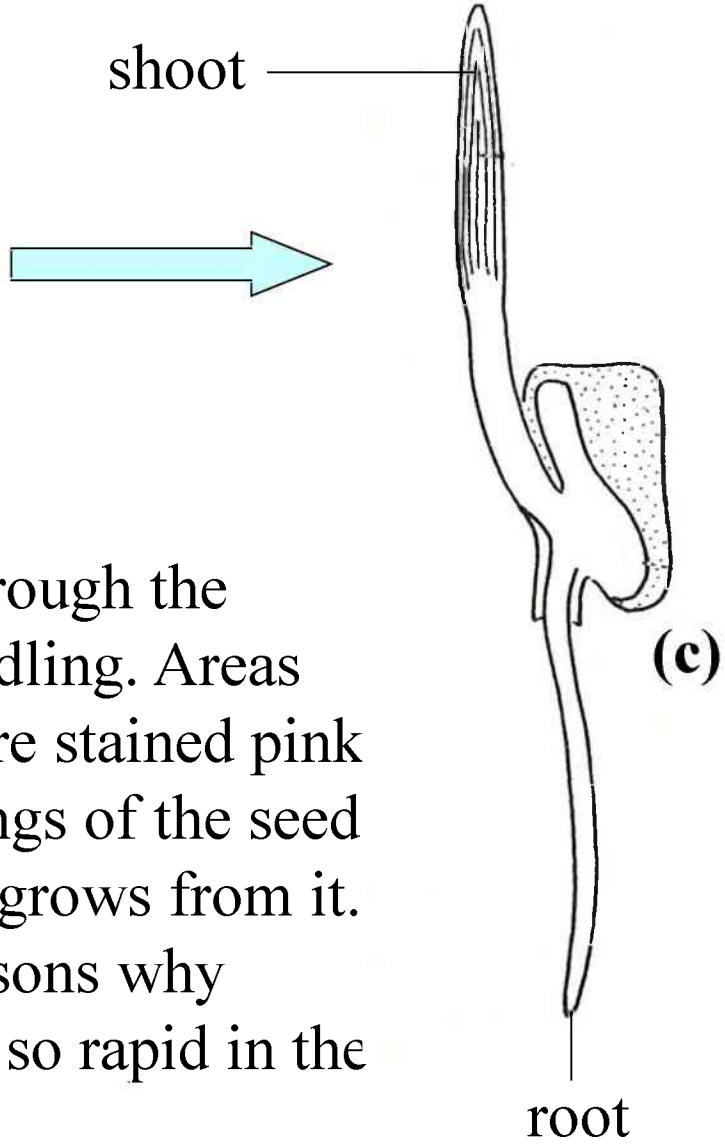
and pull the lower arm up



(a)



(b)



(c)

(a) is a section cut through the length of a maize seedling. Areas of rapid respiration are stained pink (b) and (c) are drawings of the seed and the seedling that grows from it. Can you suggest reasons why respiration should be so rapid in the stained regions?

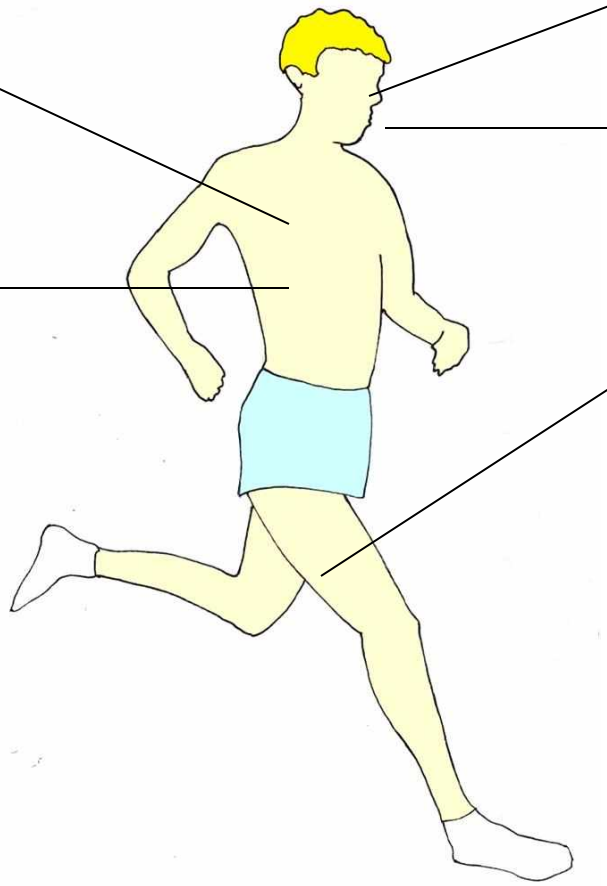
One example of respiration in ourselves

2. The lungs absorb oxygen from the air

1. Air taken in

1.Food taken in

2.The stomach and intestine digest food. One of the products is glucose



3.The blood stream carries glucose and oxygen to the muscles

4 RESPIRATION

Glucose and oxygen react to produce energy for muscle contraction

5 Carbon dioxide is carried to the lungs by the blood

Anaerobic Respiration

The process of respiration described so far has been defined as the release of **energy** when foodstuffs such as glucose react with oxygen to produce carbon dioxide and water.

This form of respiration, which needs oxygen, is called **aerobic** respiration.

There is another form of respiration which does not need oxygen and is called **anaerobic** respiration.

In anaerobic respiration, glucose is still broken down to carbon dioxide with the release of **energy**, but without the involvement of oxygen

The glucose is not completely broken down to CO_2 and H_2O but to CO_2 and alcohol (ethanol).

Anaerobic respiration can be represented by the equation

$$C_6H_{12}O_6 \xrightarrow{\text{energy}} 2C_2H_5OH + 2CO_2$$

glucose — alcohol

The energy released by anaerobic respiration is considerably less than the energy from aerobic respiration.

Anaerobic respiration takes place at some stage in the cells of most living organisms.

For example, our own muscles resort to anaerobic respiration when oxygen is not delivered to them fast enough.

Micro-organisms

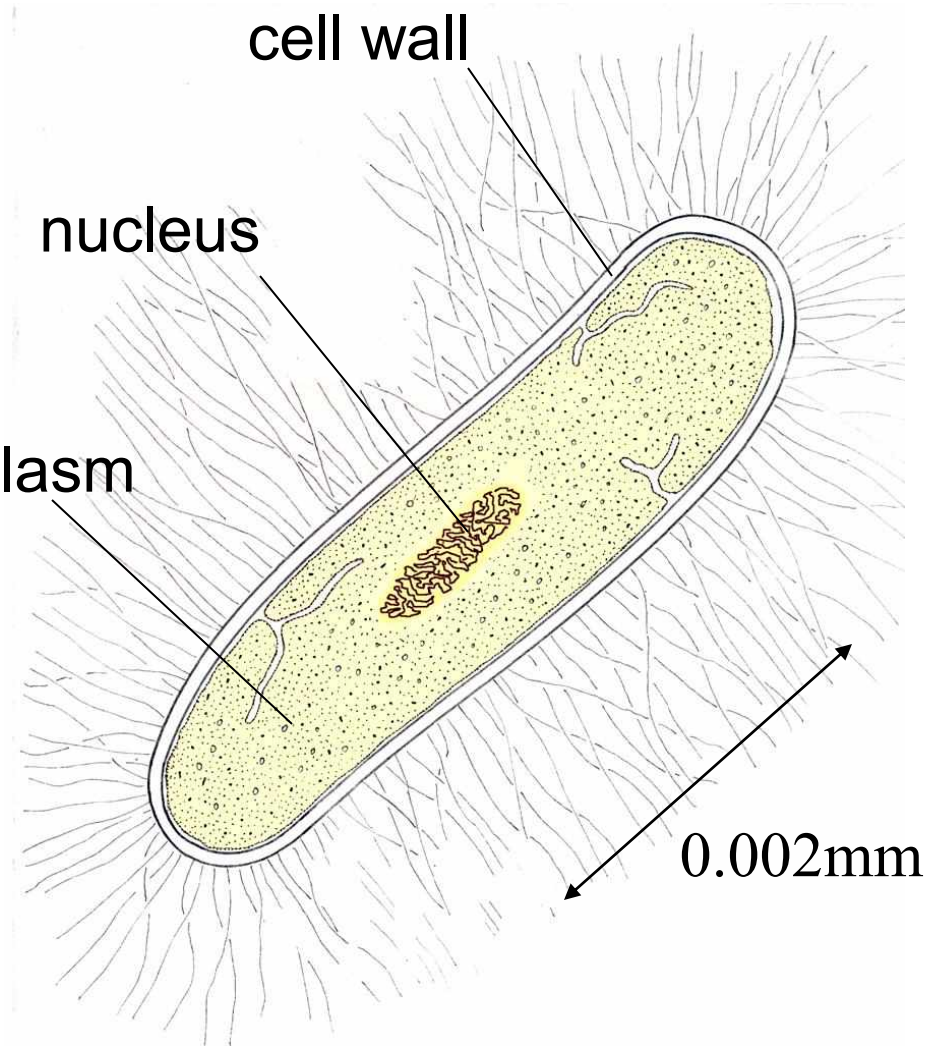
Anaerobic respiration is widely used by many micro-organisms such as **bacteria** and **yeasts**.

Bacteria and yeasts are microscopic single-celled organisms.

Bacteria are to be found everywhere, in or on organisms, in water, air and soil

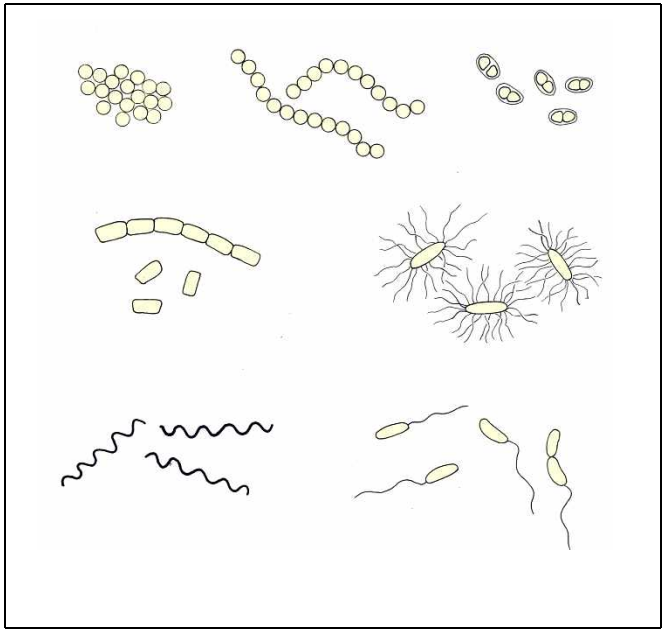
Yeasts are usually found in close association with vegetable matter such as fruit

Bacteria



a single bacterium

there are many species of bacteria and they have different shapes and sizes



Aerobic and anaerobic bacteria

Bacteria which need oxygen in order to respire are called **aerobic bacteria**.

Aerobic bacteria are likely to be found in the air, water and soil where oxygen is available

Bacteria which can respire without needing oxygen are called anaerobic bacteria

Anaerobic bacteria are to be found in situations where oxygen is lacking, such as in stagnant water, waterlogged soils or the intestines of animals

Fermentation

One form of anaerobic respiration in bacteria and yeasts is called fermentation.

During fermentation, sugar is broken down to alcohol and carbon dioxide

The reaction described in previous slide is an example of fermentation

Fermentation is involved in brewing and wine-making

The conversion of food into a form that can be absorbed by the body is called digestion. It describes how the body breaks down food and uses it for energy, cell repair and growth. It starts in the mouth, continues in the stomach and small intestine and is completed in the large intestine.
The liver and pancreas add enzymes and juices that aid in this process.

1. Digestion of Food:

Digestion in Mouth

The major carbohydrates present in our diet are starch, glycogen, sucrose, lactose , maltose and very little concentrations of fructose and pentose. Milk and other fluid items like juices escape digestion in the mouth as they do not reside in the mouth for a longer time, whereas, starch and glycogen containing solid foods are masticated with saliva thoroughly.

Saliva contains ptyalin, an alpha amylase, which attacks the alpha 1-4 linkages resulting in the formation of monosaccharide glucose, disaccharide maltose and Trisaccharide maltotriose. The optimum pH for salivary amylase is pH 6.7. Ptyalin needs chloride ions for their effective action

Digestion in Stomach

Ptyalin is inactivated due to low pH. There are no enzymes to act upon carbohydrates in the stomach. Dietary sucrose may be hydrolyzed to equimolar quantities of glucose and fructose by the HCl present.

Digestion in duodenum

When the food bolus reaches the duodenum, it is mixed with the pancreatic juice, which contains amylase. Its action is similar to that of the ptyalin, but it is more powerful . The optimum pH of pancreatic amylase ranges between 6.9 – 7.1 and it needs chloride ions for its action

Digestion in small intestine

Five enzymes are present in small intestine to hydrolyze the carbohydrates completely to mono saccharides.Only monosaccharides can be absorbed by the intestinal mucosa. The absorption rate of the monosaccharides is in the following order: Galactose > Glucose > Fructose > Mannose > Xylose > Arabinose

2. Carbohydrate as a source of energy

The major function of carbohydrate in metabolism is to serve as fuel and get oxidized to provide energy for other metabolic processes. The metabolic intermediates are used for various biosynthetic reactions. For this purpose, carbohydrate is utilized by the cells mainly in the form of glucose. A major part of dietary glucose is converted to glycogen for storage in liver. Glucose is degraded in the cell by way of a series of phosphorylated intermediates mainly via two metabolic pathways.

2.1. Glycolysis

2.2. Tricarboxylic acid cycle

2.3.HMP shunt

2.1- Glycolysis

Oxidation of glucose to pyruvate is called glycolysis. It was first described by Embden-Meyerhof and Parnas. Hence it is also called as Embden-Meyerhof pathway.
Glycolysis occurs virtually in all tissues. Erythrocytes and nervous tissues derive the energy mainly from glycolysis. This pathway is unique in the sense that it can proceed in both aerobic (presence of O2) and anaerobic (absence of O2) conditions.

All the enzymes of glycolysis are found in the extra mitochondrial soluble fraction of the cell, the cytosol.

2.1.1 Reactions of glycolytic pathway(Aerobic Glycolysis):

Series of reactions of glycolytic pathway which degrades glucose to pyruvate are represented below. The sequence of reactions occurring in glycolysis may be considered under four stages.

2.1.1.1 Stages of Reactions:

Stage I

This is a *preparatory phase*. Before the glucose molecule can be split, the rather asymmetric glucose molecule is converted to almost symmetrical form, fructose 1,6-diphosphate by donation of two phosphate groups from ATP.

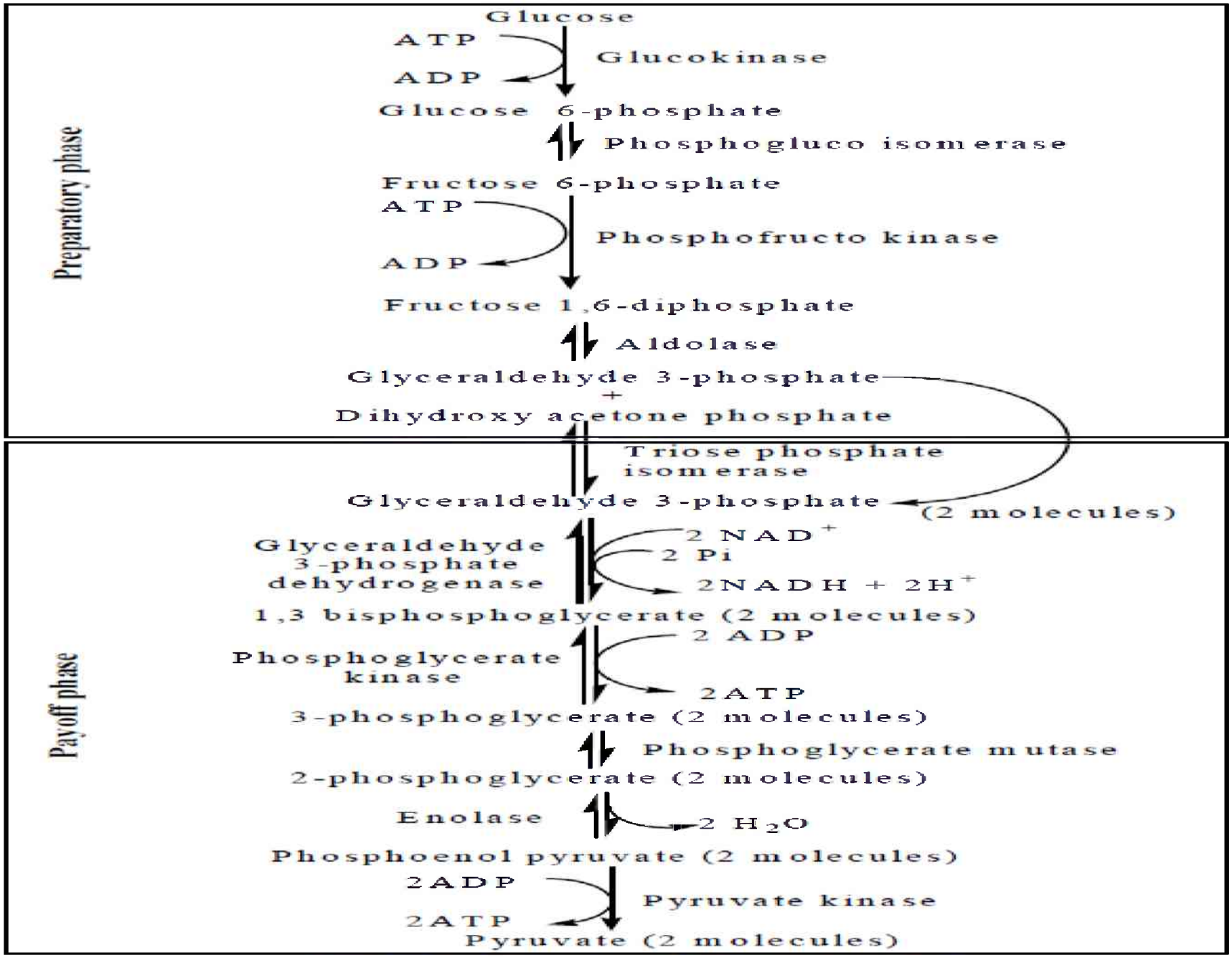
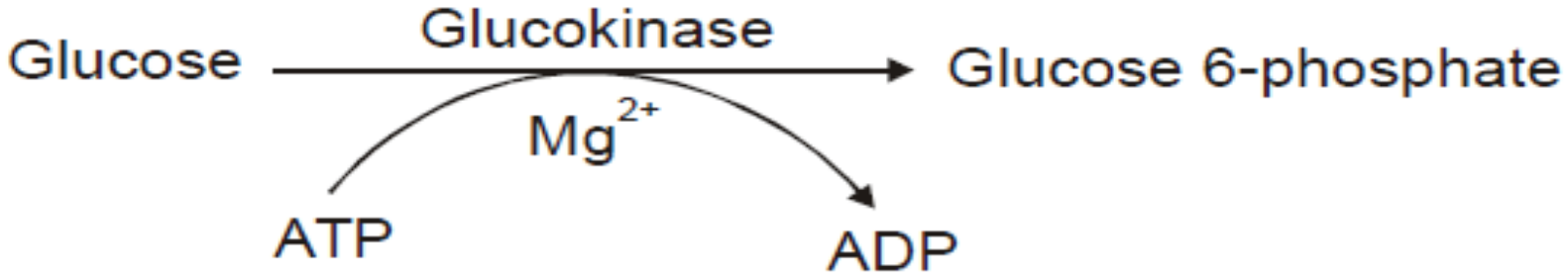


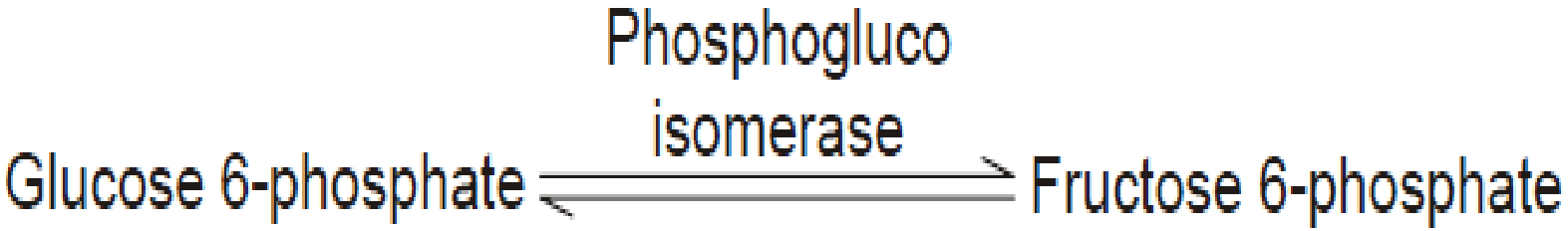
Fig.3.1 The glycolytic pathway

1. Uptake of glucose by cells and its phosphorylation

Glucose is freely permeable to liver cells, intestinal mucosa and kidney tubules where glucose is taken up by 'active' transport. In other tissues insulin facilitates the uptake of glucose. Glucose is phosphorylated to form glucose 6-phosphate. The enzyme involved in this reaction is glucokinase. This reaction is irreversible.

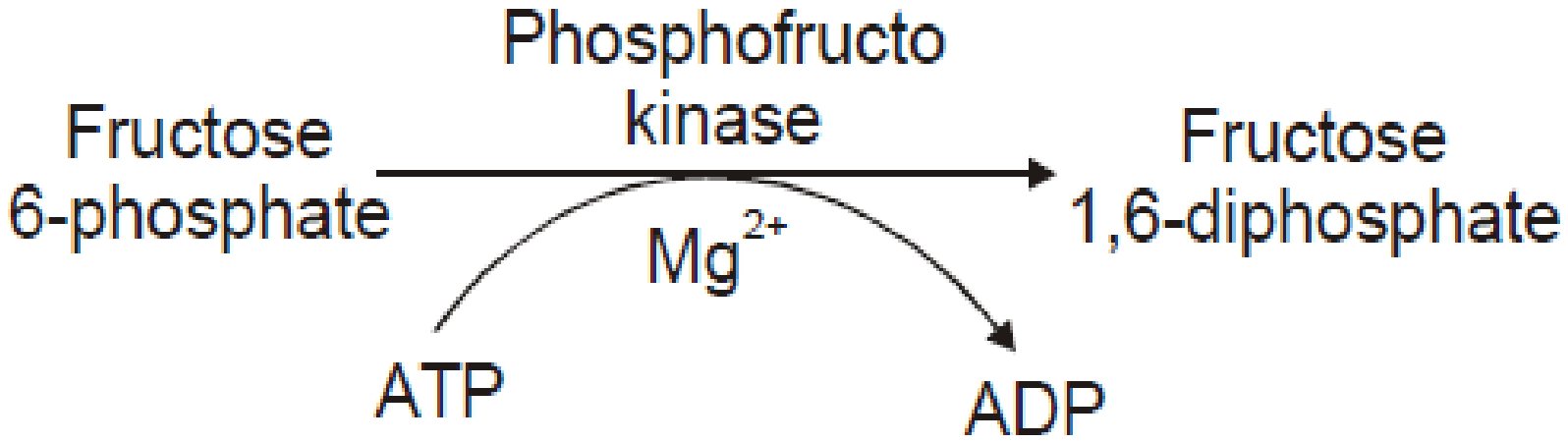


2. Conversion of glucose 6-phosphate to fructose 6-phosphate



3. Conversion of fructose 6-phosphate to fructose 1,6 diphosphate.

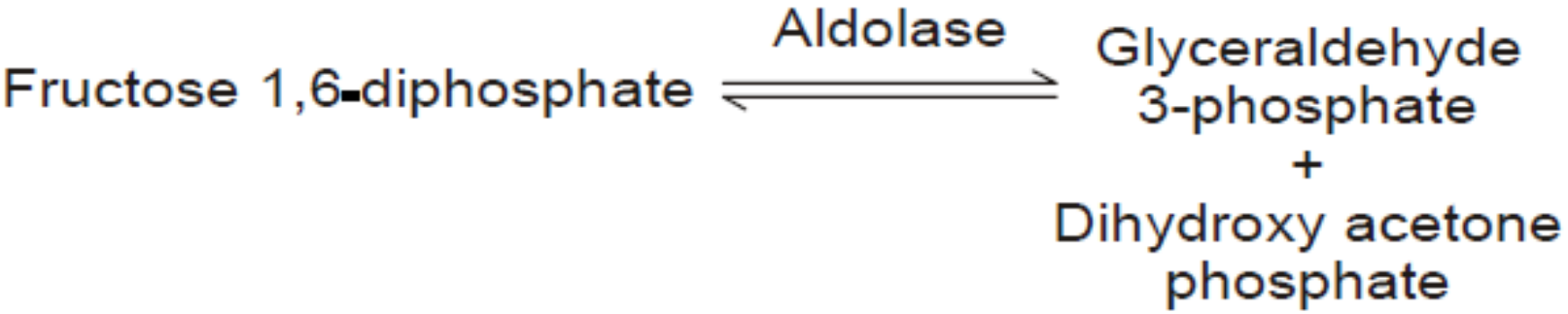
Fructose 6-phosphate is phosphorylated irreversibly at 1 position catalyzed by the enzyme phosphofructokinase to produce fructose 1,6-diphosphate



Stage II

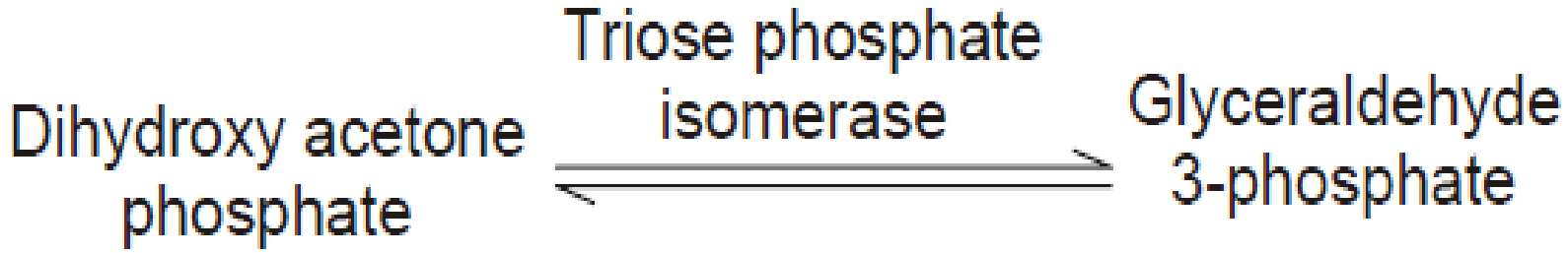
1. Actual splitting of fructose 1,6 diphosphate

Fructose 1,6 diphosphate is split by the enzyme aldolase into two molecules of triose phosphates, an aldotriose-glyceraldehyde 3-phosphate and one ketotriose - dihydroxy acetone phosphate. The reaction is reversible. There is neither expenditure of energy nor formation of ATP.



2. Interconvertion of triose phosphates

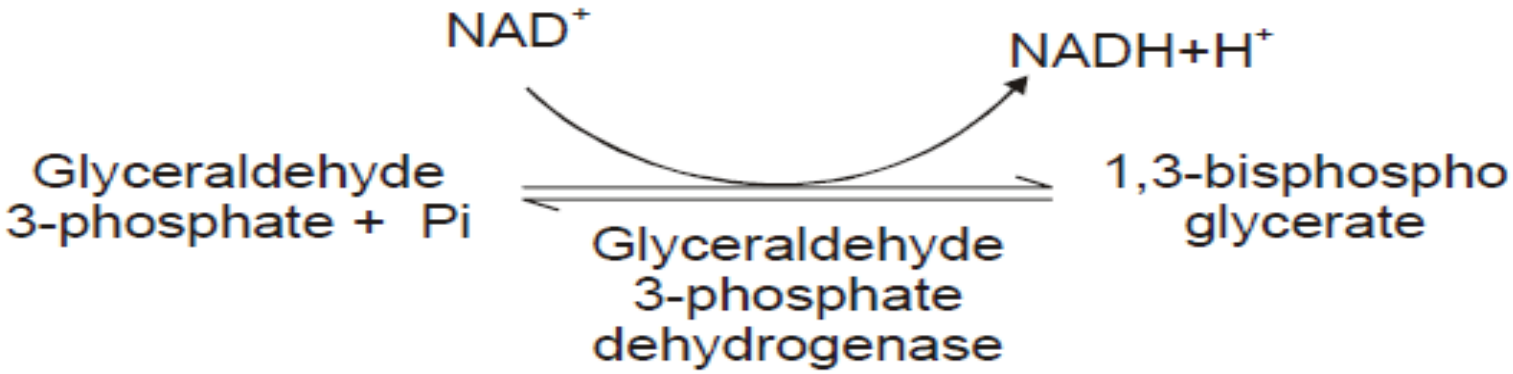
Both triose phosphates are interconvertible



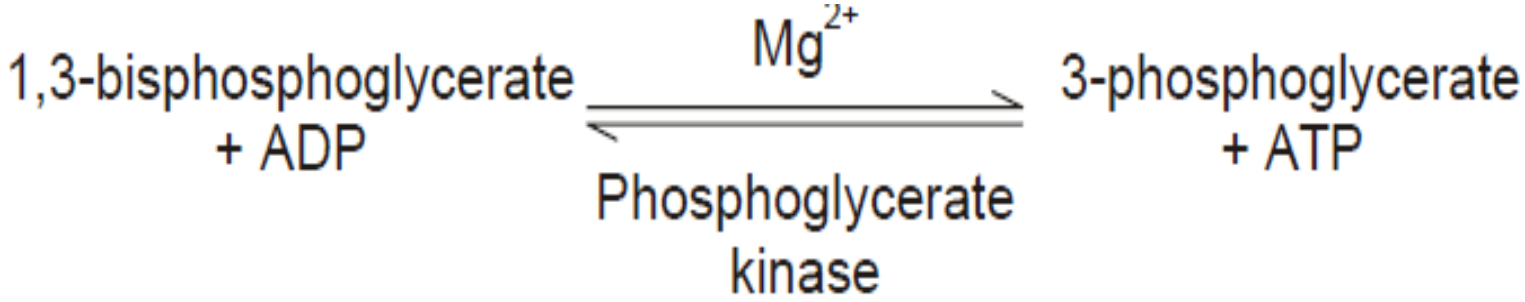
Stage III

It is the energy yielding stage. Reactions of this type in which a aldehyde group is oxidised to an acid are accompanied by liberation of large amounts of potentially useful energy.

1. Oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate



2. Conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate



Stage IV

It is the recovery of the phosphate group from 3-phosphoglycerate..

1. Conversion of 3-phosphoglycerate to 2-phosphoglycerate.

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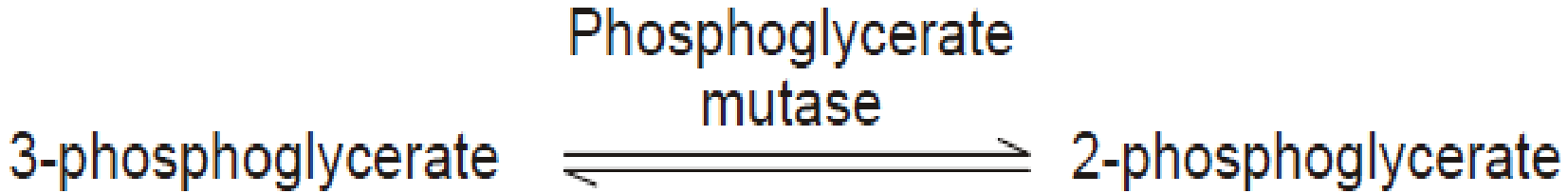
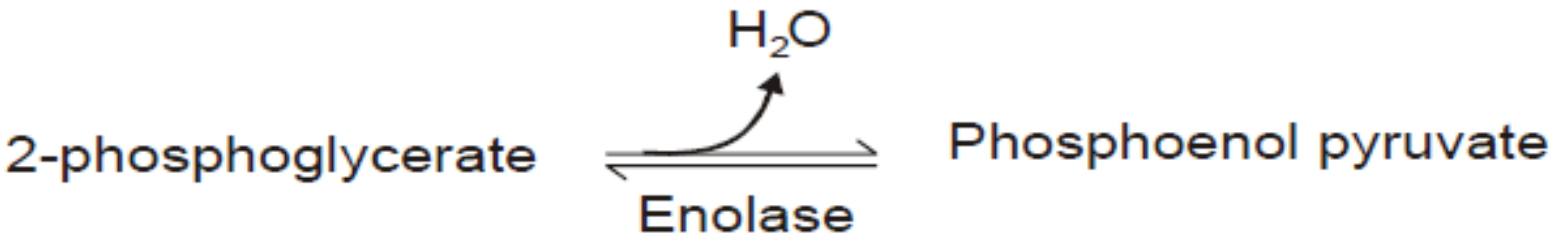
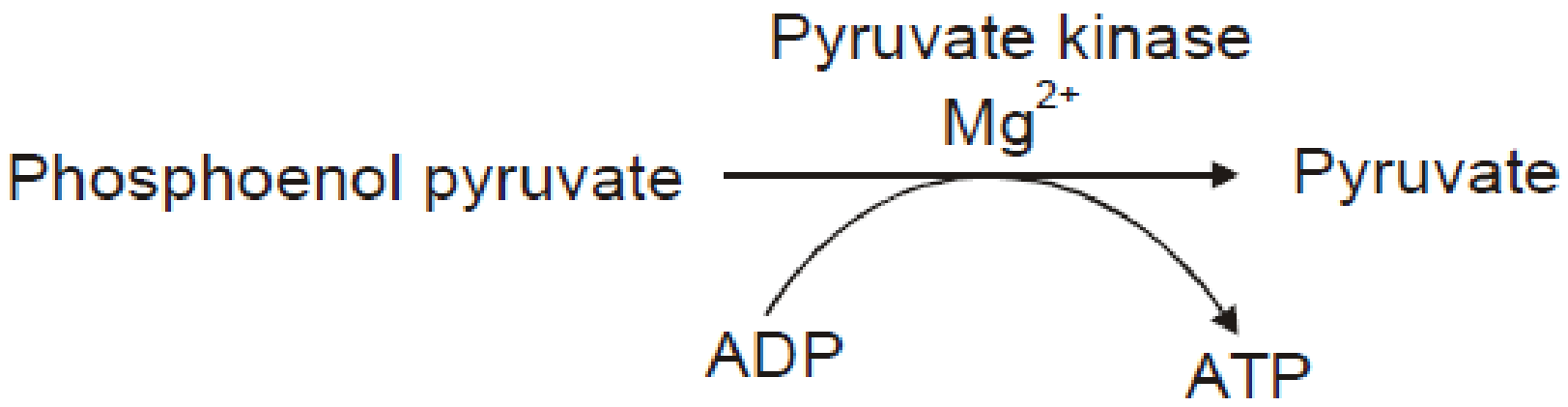


2. Conversion of 2-phosphoglycerate to phosphoenol pyruvate



3. Conversion of phosphoenol pyruvate to pyruvate

Phosphoenol pyruvate is converted to pyruvate, the reaction is catalysed by the enzyme pyruvate kinase. The high energy phosphate group of phosphoenol pyruvate is directly transferred to ADP, producing ATP.
The reaction is irreversible.



2.1.1.2 Energy yield per glucose molecule oxidation:

During glycolysis ATP molecules are used and formed in the following reactions (aerobic phase)

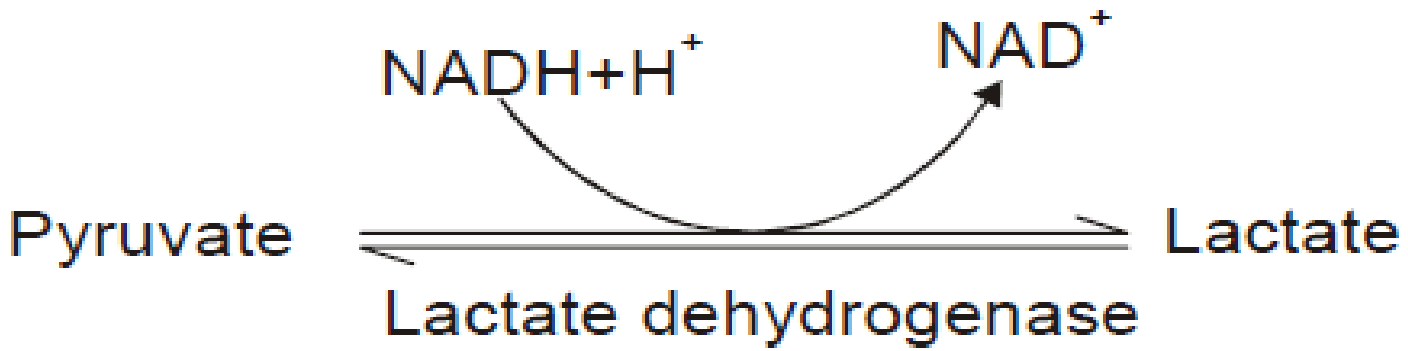
Table 3.1

Reactions Catalyzed	ATP used	ATP formed
Stage I 1. Glucokinase (for phosphorylation)	1	
2. Phosphofructokinase I (for phosphorylation)	1	
Stage II 3. Glyceraldehyde 3-phosphate dehydrogenase (oxidation of 2 NADH in respiratory chain)		6
4. Phosphoglycerate kinase (substrate level phosphorylation)		2
Stage IV 5. Pyruvate kinase (substrate level phosphorylation)		2
Total	2	10

Net gain = 8 ATP

2.1.2 Anaerobic phase

In the absence of O2, reoxidation of NADH at glyceraldehydes 3-phosphate dehydrogenase stage cannot take place in respiratory chain. But the cells have limited coenzyme. Hence to continue the glycolysis, NADH must be reoxidized to NAD+. This is achieved by reoxidation of NADH by conversion of pyruvate to lactate (without producing ATP).



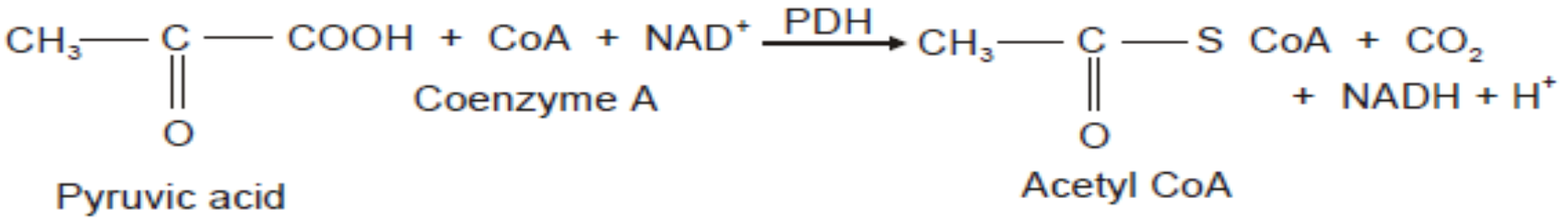
It is to be noted that in the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase, therefore, no ATP produced.
In the anaerobic phase oxidation of one glucose molecule produces 4 - 2 = 2 ATP.

2.2. Tricarboxylic acid cycle (TCA cycle)

This cycle is the aerobic phase of carbohydrate metabolism and follows the anaerobic pathway from the stage of pyruvate and is called as citric acid cycle or TCA cycle. The name citric acid cycle stems from citric acid which is formed in the first step of this cycle. This cycle is also named "Krebs cycle" after H.A. Krebs, an English biochemist who worked on it.

Under aerobic conditions, pyruvate is oxidatively decarboxylated to acetyl coenzyme A (active acetate) before entering the citric acid cycle.

This occurs in the mitochondrial matrix and forms a link between glycolysis and TCA cycle

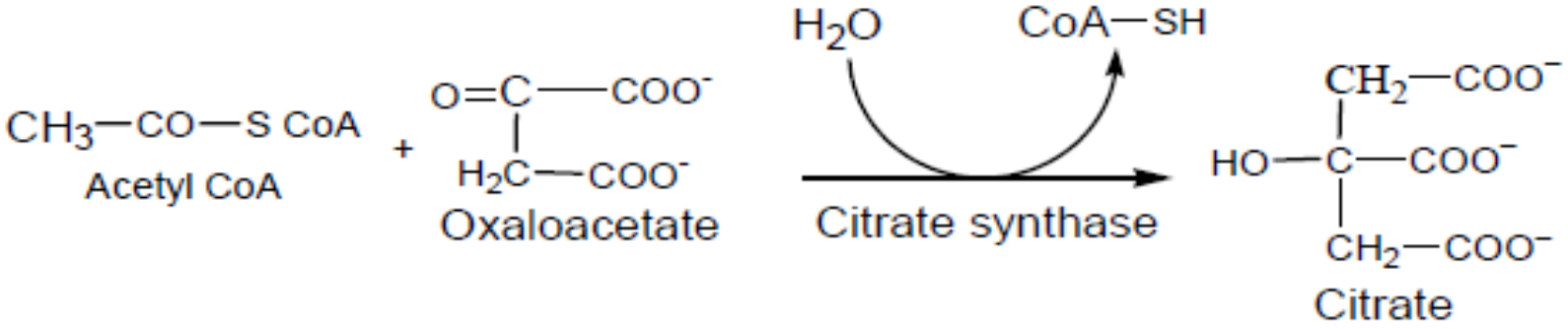


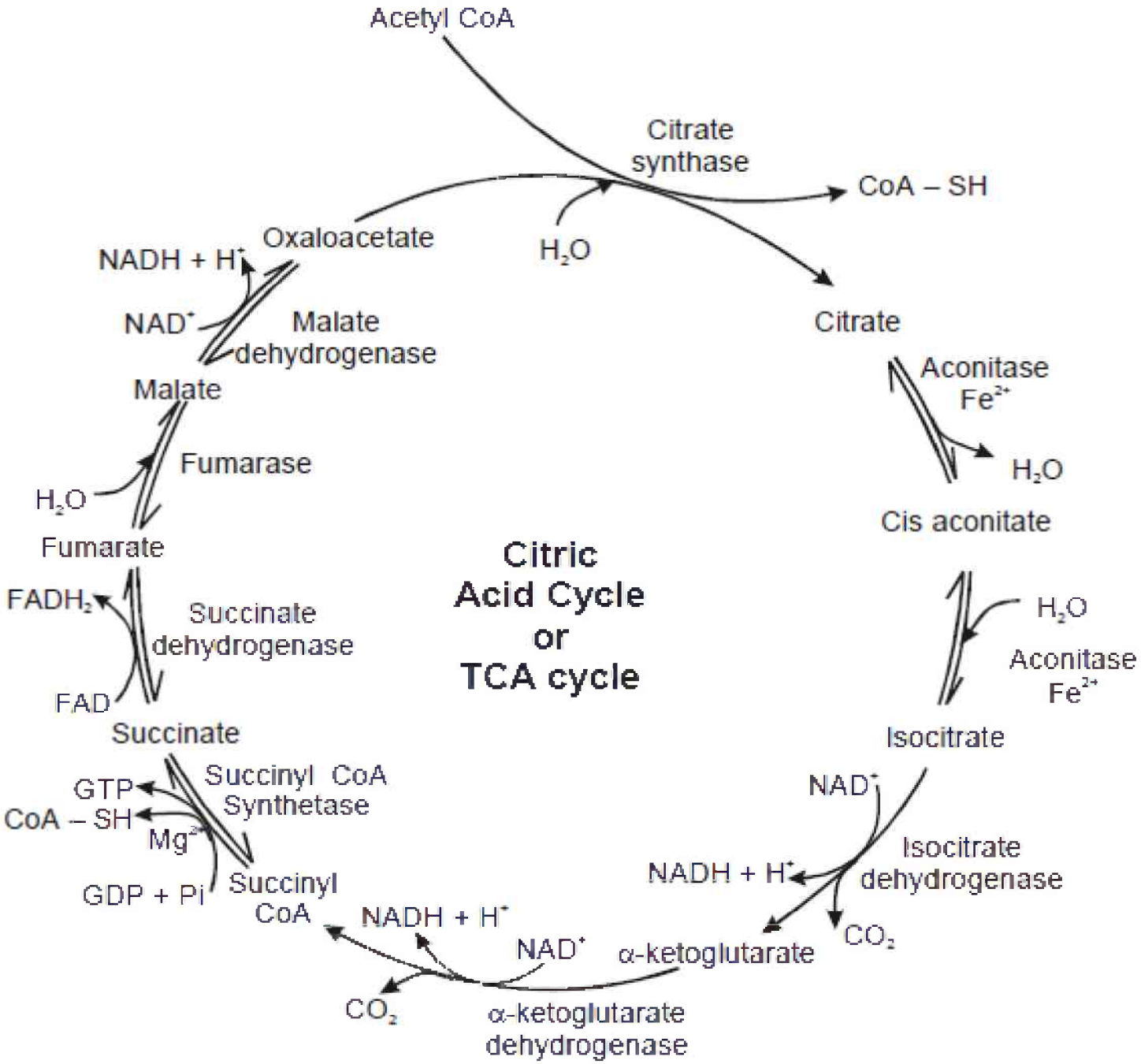
2.2.1 Reactions of the citric acid cycle:

There are eight steps in the cycle and the reactions are as follows:

1. Formation of citrate

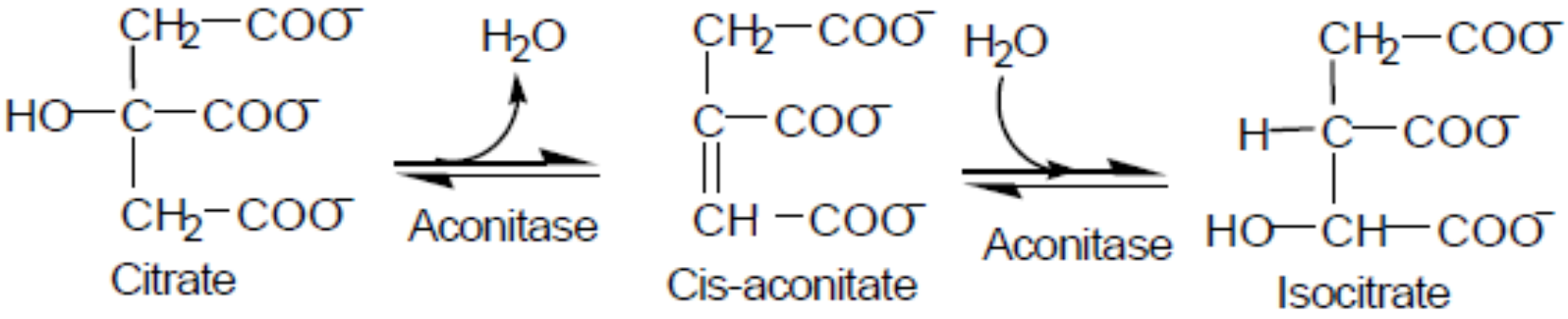
The first reaction of the cycle is the condensation of acetyl CoA with oxaloacetate to form citrate, catalyzed by citrate synthase. This is an irreversible reaction.




Acetyl CoA
Citrate synthase
CoA – SH
H_2O
Oxaloacetate
Citrate
NADH + H^+
NAD^+
Malate dehydrogenase
Malate
Aconitase Fe^{2+}
H_2O
Cis aconitate
Fumarase
H_2O
Fumarate
Citric Acid Cycle or TCA cycle
$FADH_2$
Succinate dehydrogenase
FAD
H_2O
Aconitase Fe^{2+}
Succinate
Isocitrate
Succinyl CoA Synthetase
GTP
CoA – SH
Mg^{2+}
GDP + Pi
NAD^+
Isocitrate dehydrogenase
NADH + H^+
CO_2
Succinyl CoA
NADH + H^+
NAD^+
α-ketoglutarate
CO_2
α-ketoglutarate dehydrogenase

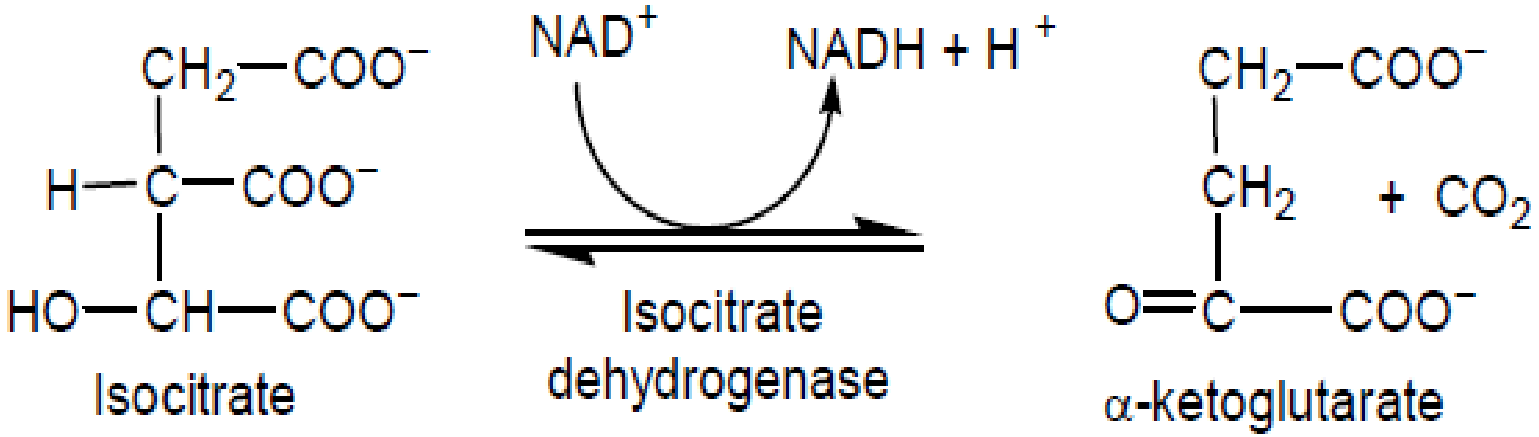
2. Formation of isocitrate via cis aconitate

The enzyme aconitase catalyzes the reversible transformation of citrate to isocitrate, through the intermediary formation of cis aconitate



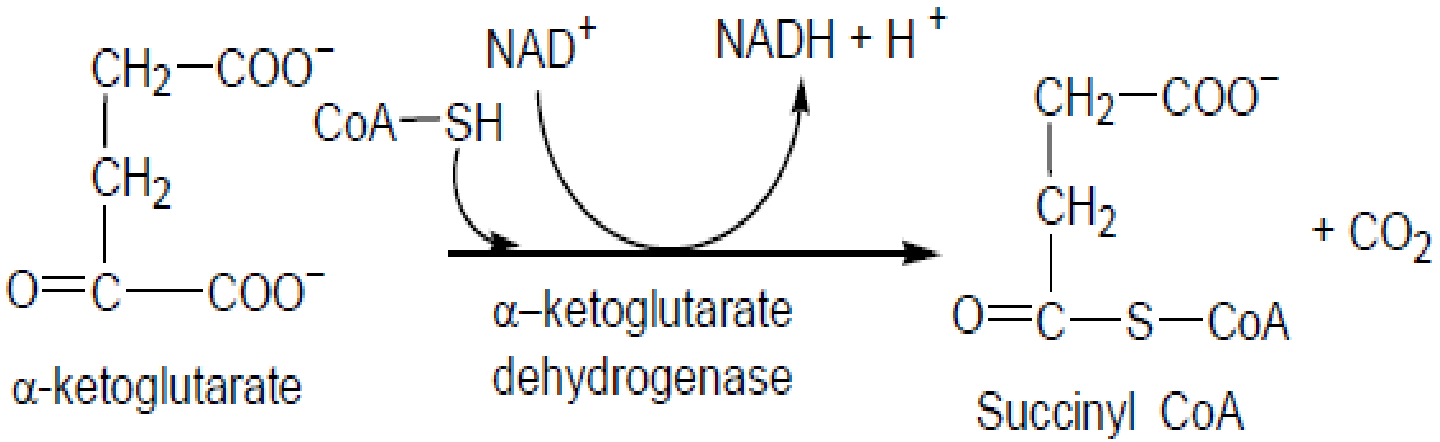
3. Oxidation of isocitrate to a-ketoglutarate and CO2

In the next step, isocitrate dehydrogenase catalyzes oxidative decarboxylation of isocitrate to forma-ketoglutarate.



4. Oxidation of a-keto glutarate to succinyl CoA and CO2

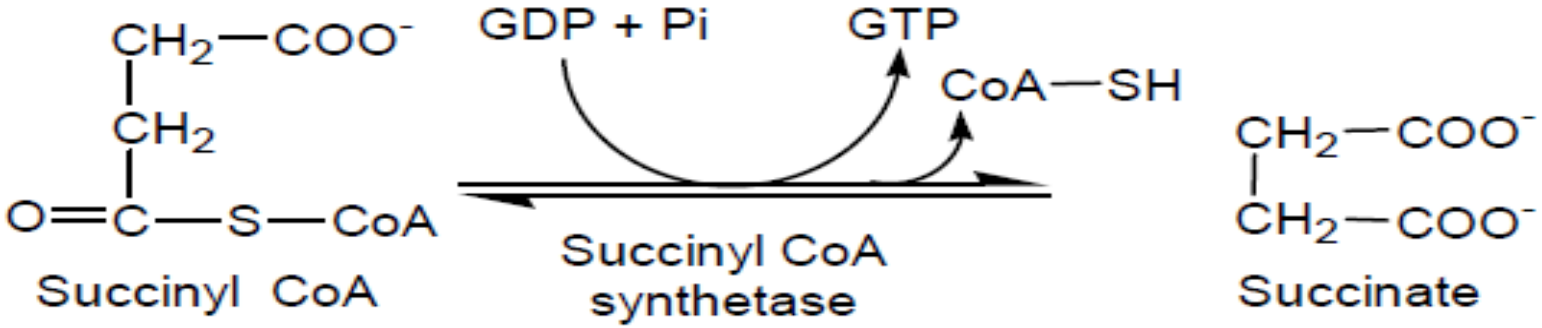
The next step is another oxidative decarboxylation, in which a-ketoglutarate is converted to succinyl CoA and CO2 by the action of the a-ketoglutarate dehydrogenase complex. The reaction is irreversible



5. Conversion of succinyl CoA to succinate

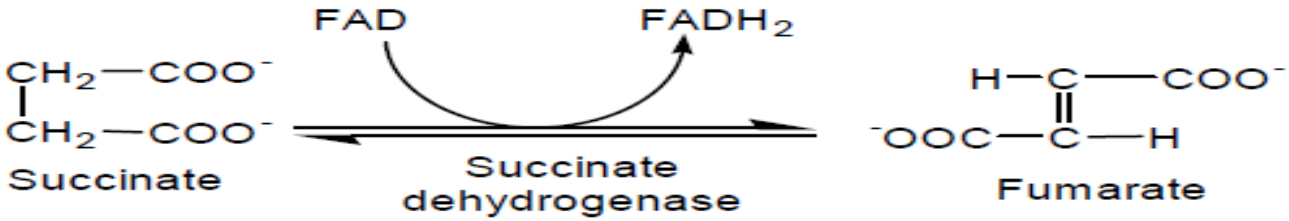
The product of the preceding step, succinyl CoA is converted to succinate to continue the cycle. GTP is formed in this step (substrate level phosphorylation).

The enzyme that catalyzes this reversible reaction is called succinyl CoA synthetase or succinic thiokinase.



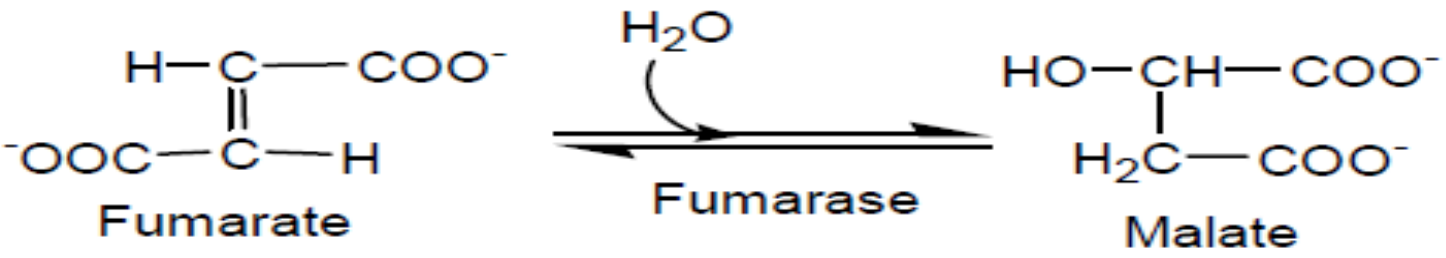
6. <u>Oxidation of succinate to fumarate</u>

The succinate formed from succinyl CoA is oxidized to fumarate by the enzyme succinate dehydrogenase

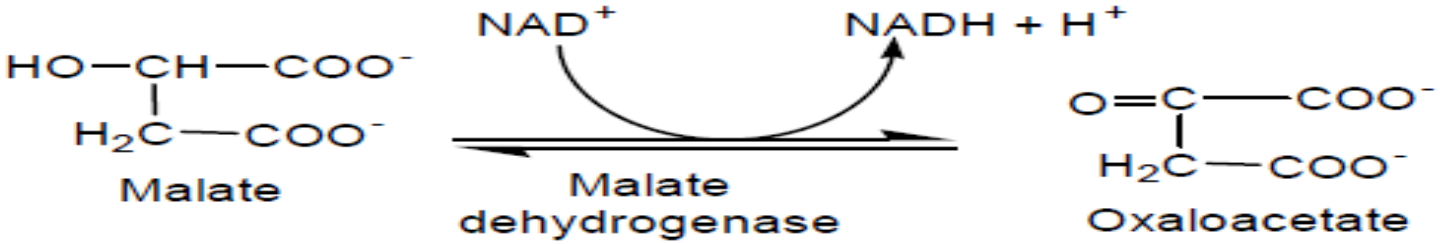


7. Hydration of fumarate to malate

The reversible hydration of fumarate to malate is catalyzed by fumarase.



8. Oxidation of malate to oxaloacetateThe last reaction of the citric acid cycle is, NAD linked malate - dehydrogenase which catalyses the oxidation of malate to oxaloacetate.



2.2.2 Energy yield from TCA cycle

If one molecule of the substrate is oxidized through NADH in the electron transport chain three molecules of ATP will be formed and through FADH2, two ATP molecules will be generated.

As one molecule of glucose gives rise to two molecules of pyruvate by glycolysis, intermediates of citric acid cycle also result as two molecules.

Reactions	No.of ATP formed
1. 2 isocitrate → 2 α-ketoglutarate	
(2 NADH + 2H$^+$) (2 × 3)	6
2. 2 α-ketoglutarate → 2 succinyl CoA	
(2 NADH + 2H$^+$) (2 × 3)	6
3. 2 succinyl CoA → 2 succinate	
(2 GTP = 2ATP)	2
4. 2 succinate → 2 Fumarate	
(2 $FADH_2$) (2 × 2)	4
5. 2 malate → 2 oxaloacetate	
(2 NADH + 2H$^+$) (2 × 3)	6
Total No.of ATP formed	24

Madenet Al elm University College
Department of Biology
2nd Year Biochemistry I 2021– 2022

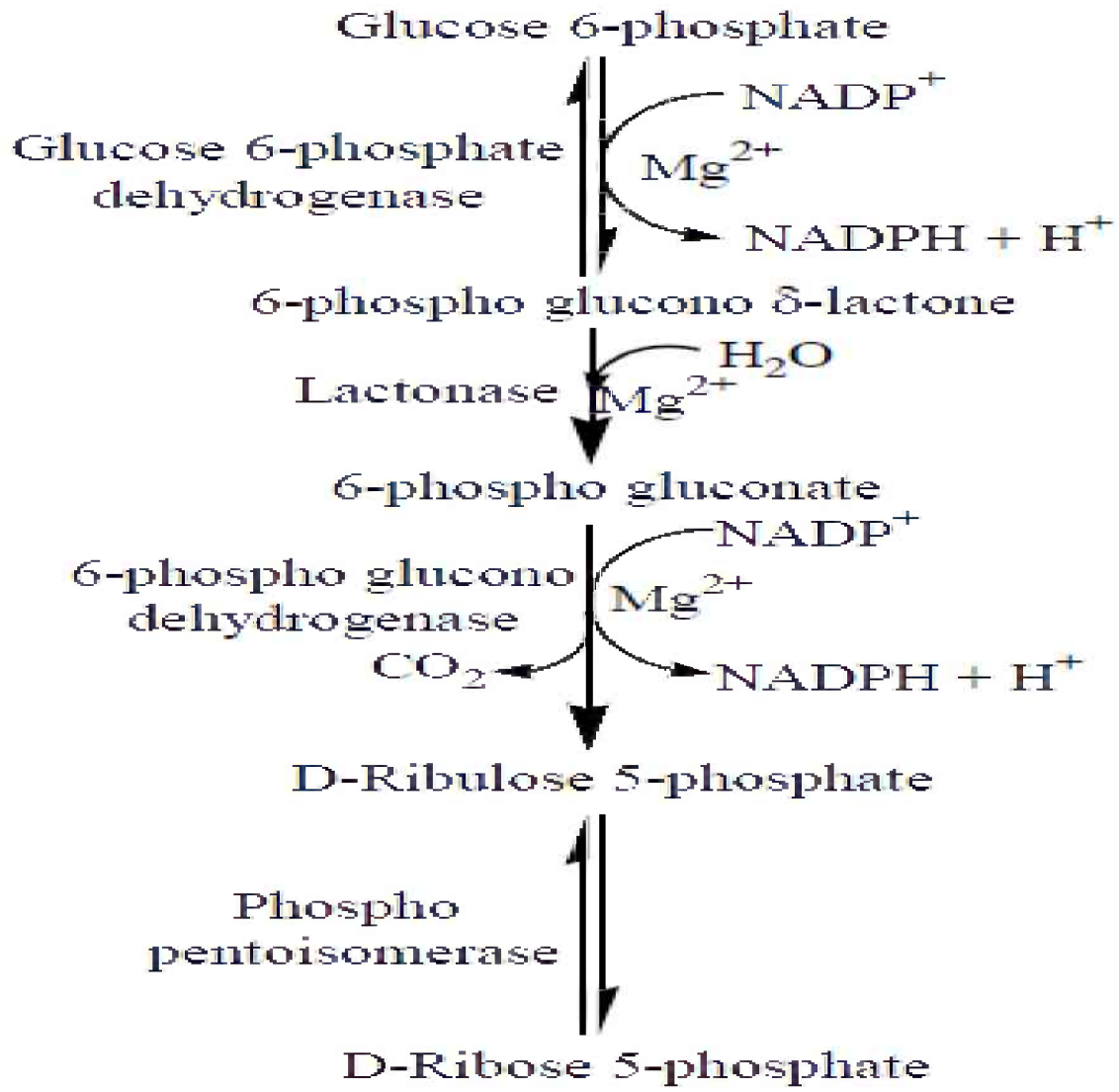
CARBOHYDRATE METABOLISM (continued…….)

Dr. Hamza Yaseen Isa
Lecture 5 I 2

2.3. HMP shunt pathway of metabolism

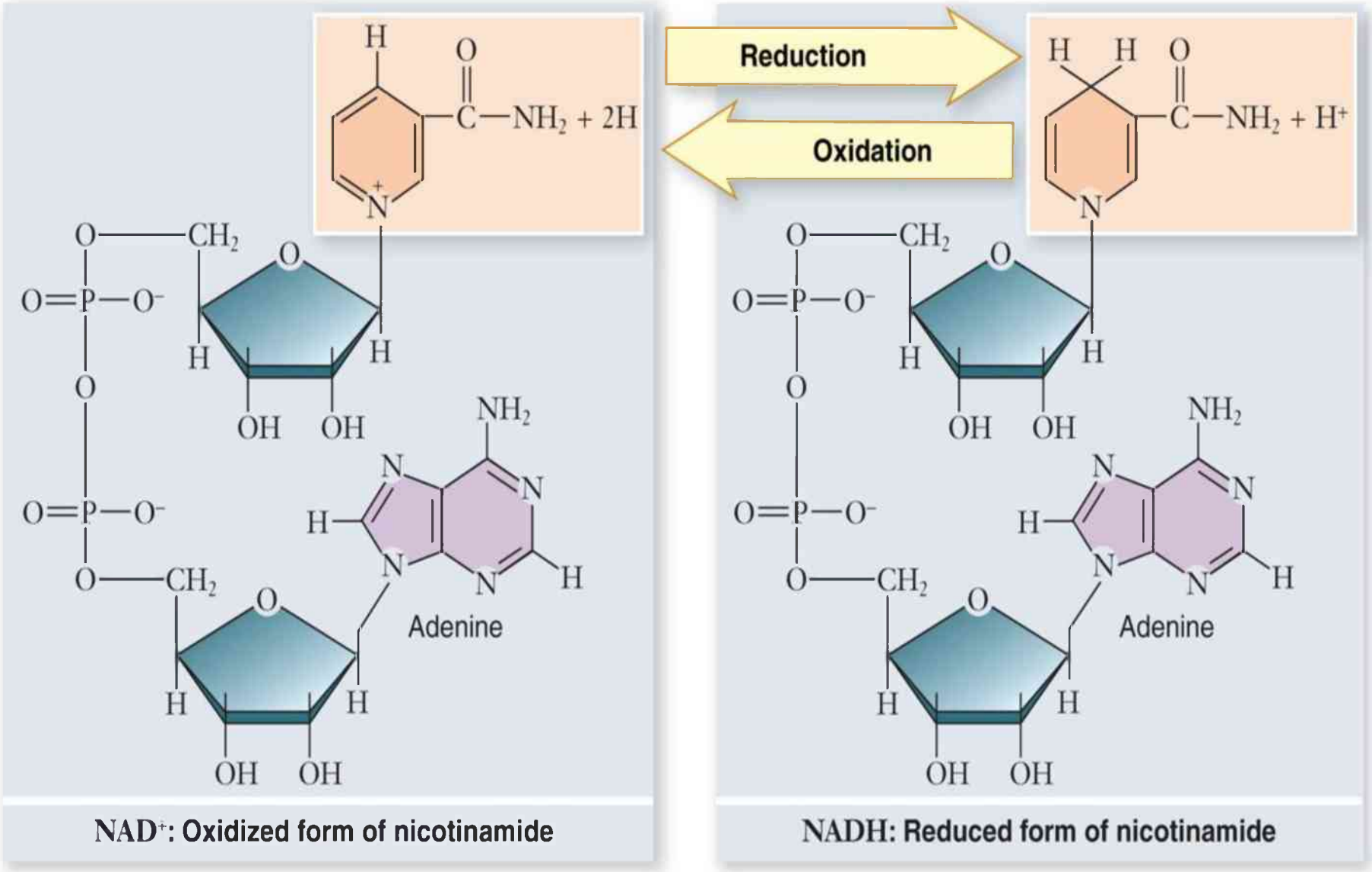
Although glycolysis and citric acid cycle are the common pathways by which animal tissues oxidize glucose to CO2 and H2O with the liberation of energy in the form of ATP, a number of alternative pathways are also discovered. The most important one is Hexose Monophosphate Shunt Pathway (HMP shunt). The pathway occurs in the extra mitochondrial soluble portion of the cells-Cytosol.

Unlike glycolysis and Krebs cycle which are primarily concerned with the generation of ATP, HMP shunt generates a different type of metabolic energy - the reducing power. Some of the electrons and hydrogen atoms of fuel molecules are conserved for biosynthetic purposes rather than ATP formation. This reducing power of cells is NADPH (reduced nicotinamide adenine dinucleotide phosphate).



Oxidative reactions of the hexose mono-phosphate pathway

The fundamental difference between NADPH and NADH
(reduced nicotinamide adenine dinucleotide) is that NADH is oxidized by the respiratory chain to generate ATP whereas NADPH serves as a hydrogen and electron donor in reductive biosynthesis, for example, in the biosynthesis of fatty acids and steroids.
The first reaction of the pentose phosphate pathway is the dehydrogenation of glucose 6-phosphate by glucose 6-phosphate dehydrogenase to form 6-phosphoglucono d-lactone

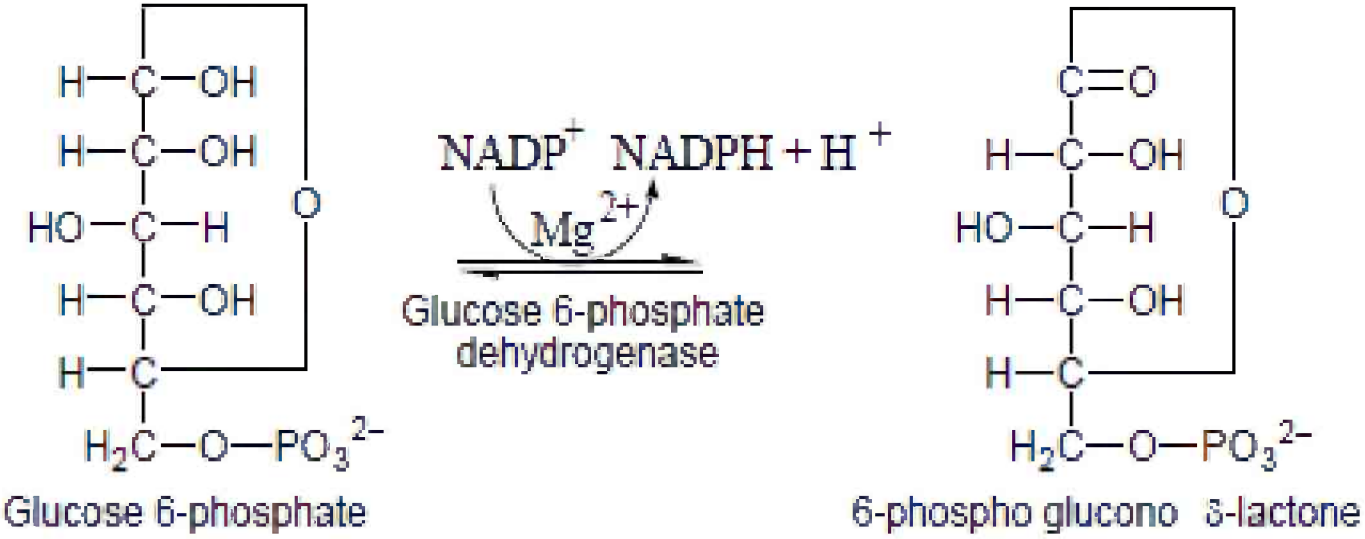


NAD^+: Oxidized form of nicotinamide

NADH: Reduced form of nicotinamide

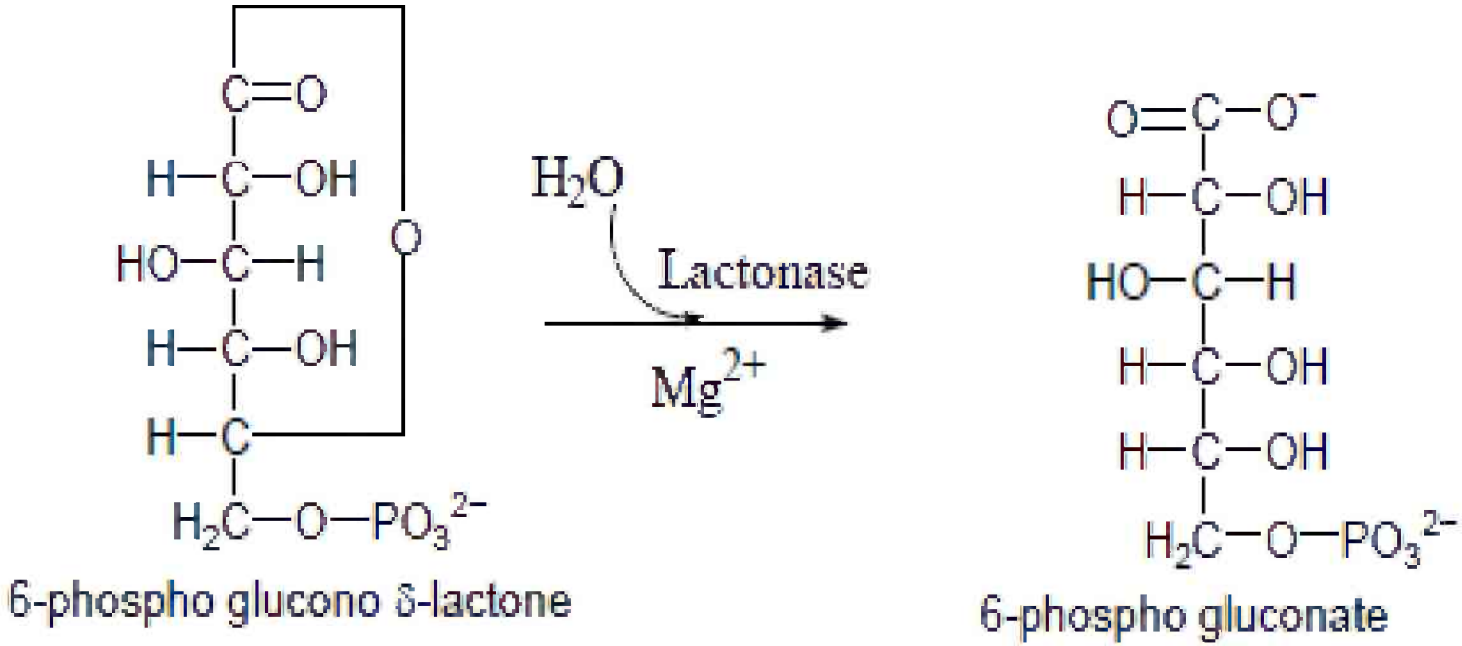
Step 1

Glucose 6-phosphate in the presence of NADP and the enzym glucose 6-phosphate dehydrogenase, forms 6-phospho glucono-δ-lactone The first molecule of NADPH is produced in this step.



Step 2

The 6-phospho glucono δ-lactone is unstable and the est spontaneously hydrolyses to 6-phosphogluconate. The enzyme th catalyses the reaction is lactonase

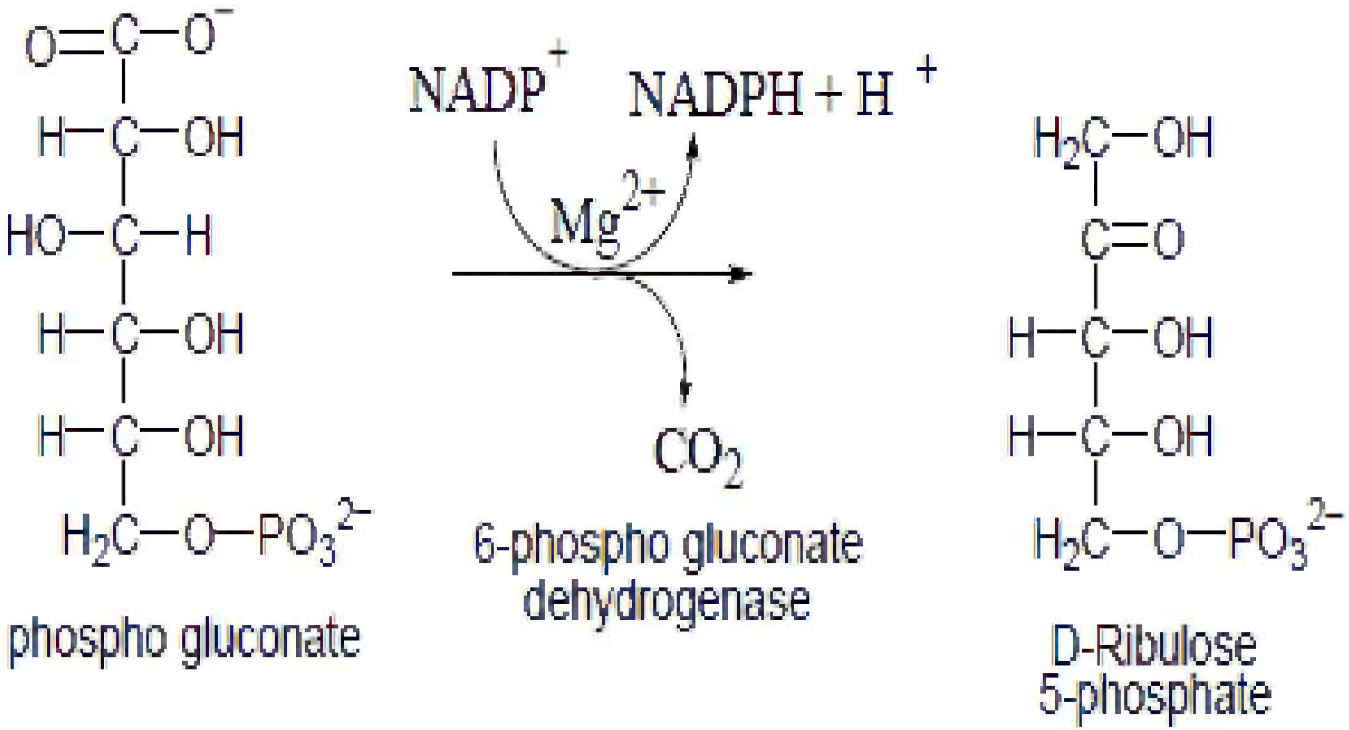


6-phospho glucono δ-lactone

6-phospho gluconate

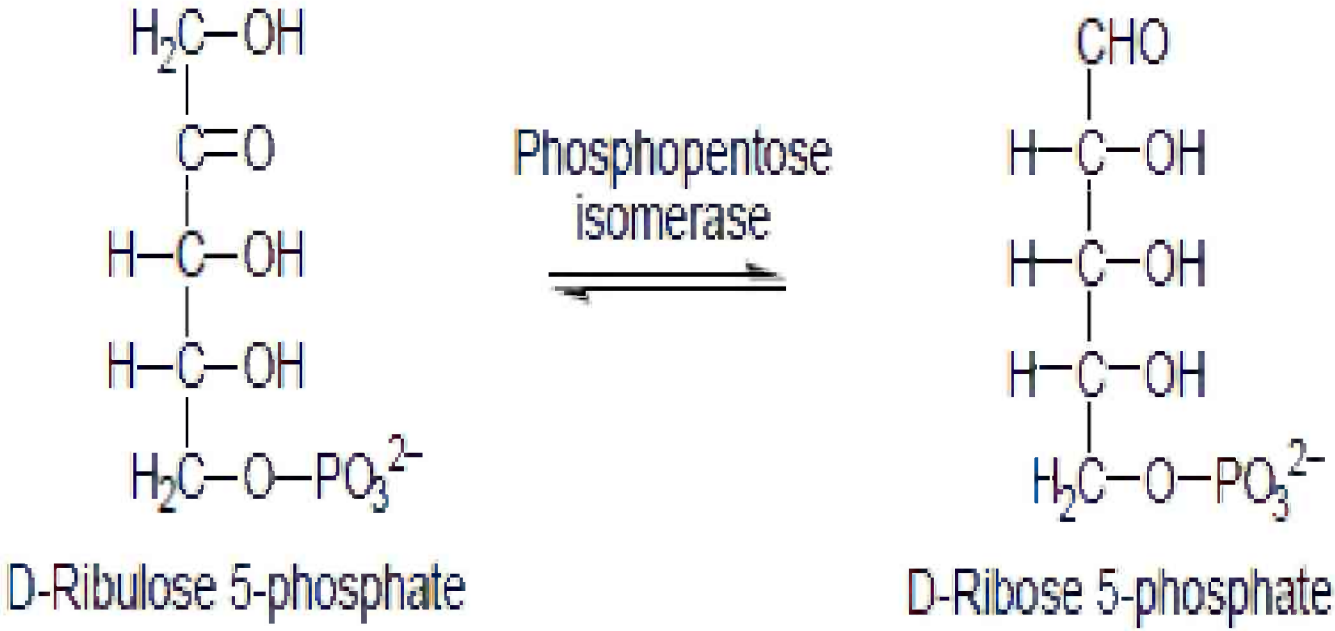
Step 3

6-phospho gluconate further undergoes dehydrogenation and decarboxylation by 6-phosphogluconate dehydrogenase to form the ketopentose, D-ribulose 5-phosphate. This reaction generates the second molecule of NADPH.



Step 4

The enzyme phosphopentose isomerase converts ribulose 5-phosphate to its aldose isomer, D-ribose 5-phosphate.



In some tissues, the hexose phosphate pathway ends at this point, and its overall equation is

$$\text{Glucose 6-phosphate} + 2NADP^{+} + H_2O \longrightarrow \text{Ribose 5-phosphate} + CO_2 + 2NADPH + 2H^{+}$$

The net result is the production of NADPH, a reductant for biosynthetic reactions, and ribose 5-phosphate, a precursor for nucleotide synthesis.

Glycogen:

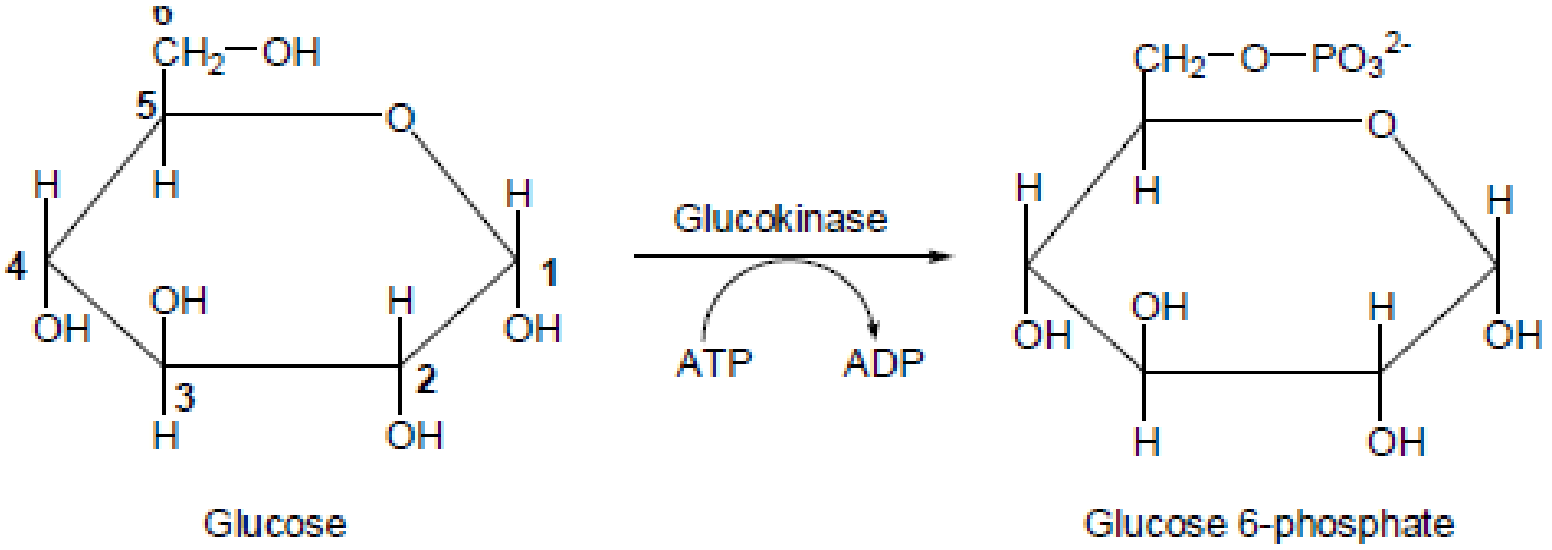
Glycogen is the major storage form of carbohydrate in animals and corresponds to starch in plants. It occurs mainly in liver.

Glycogen biosynthesis:

The process of biosynthesis of glycogen from glucose is known as glycogenesis. This occurs in all the tissues of the body but the major sites are liver and muscles. A considerable amount is synthesized in kidney also. Glycogenesis is a very essential process since the excess of glucose is converted and stored up as glycogen which could be utilized at the time of requirement. In the absence of this process the tissues are exposed to excess of glucose immediately after a meal and they are starved of it at other times. The following are the various reactions of glycogenesis.

Step 1

Glucose is phosphorylated to glucose 6-phosphate, a reaction that is common to the first reaction in the pathway of glycolysis from glucose. This reaction is catalyzed by hexokinase in muscle and glucokinase in liver in the presence of ATP.

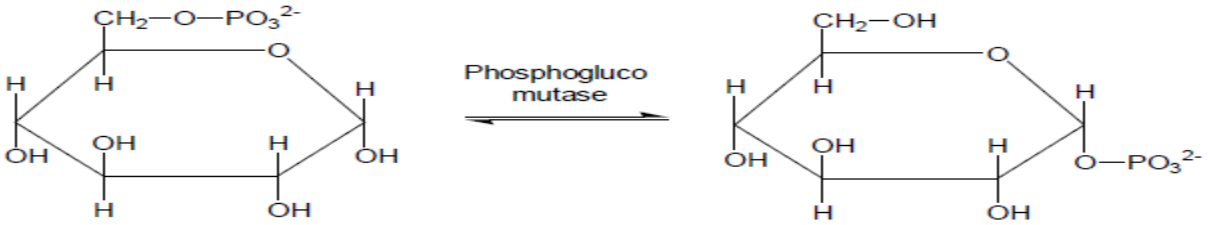


Glucose

Glucose 6-phosphate

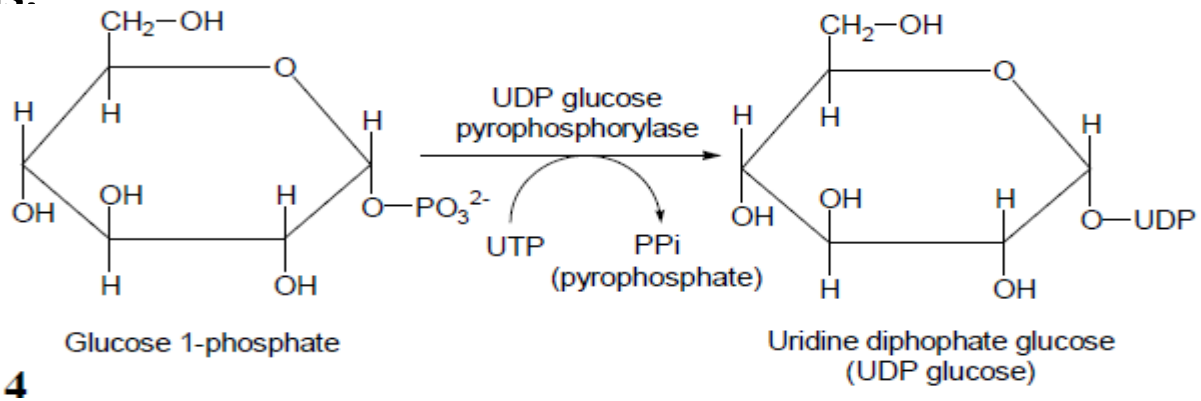
Step 2

Glucose 6-phosphate is then reversibly converted to glucose 1-phosphate in a reaction catalyzed by enzyme phosphoglucomutase. This process requires Mg2+ and a small amount of glucose 1,6-diphosphate as coenzyme.



Step 3

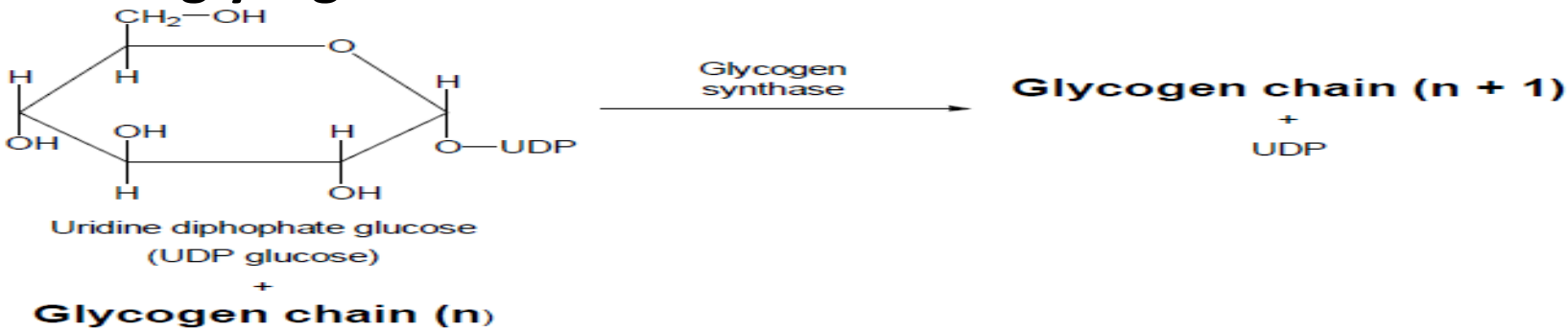
The glucose 1-phosphate is then activated by the energy produced by the hydrolysis of uridine triphosphate (UTP) in the presence of uridine diphosphate glucose pyrophophosrylase. This is a key reaction in glycogen biosynthesis.



tep 4

Step 4

UDP-glucose is the immediate donor of glucose residues in the reaction catalyzed by glycogen synthase, which promotes the transfer of the glucose residue from UDP-glucose to a non reducing end of a branched glycogen chain.

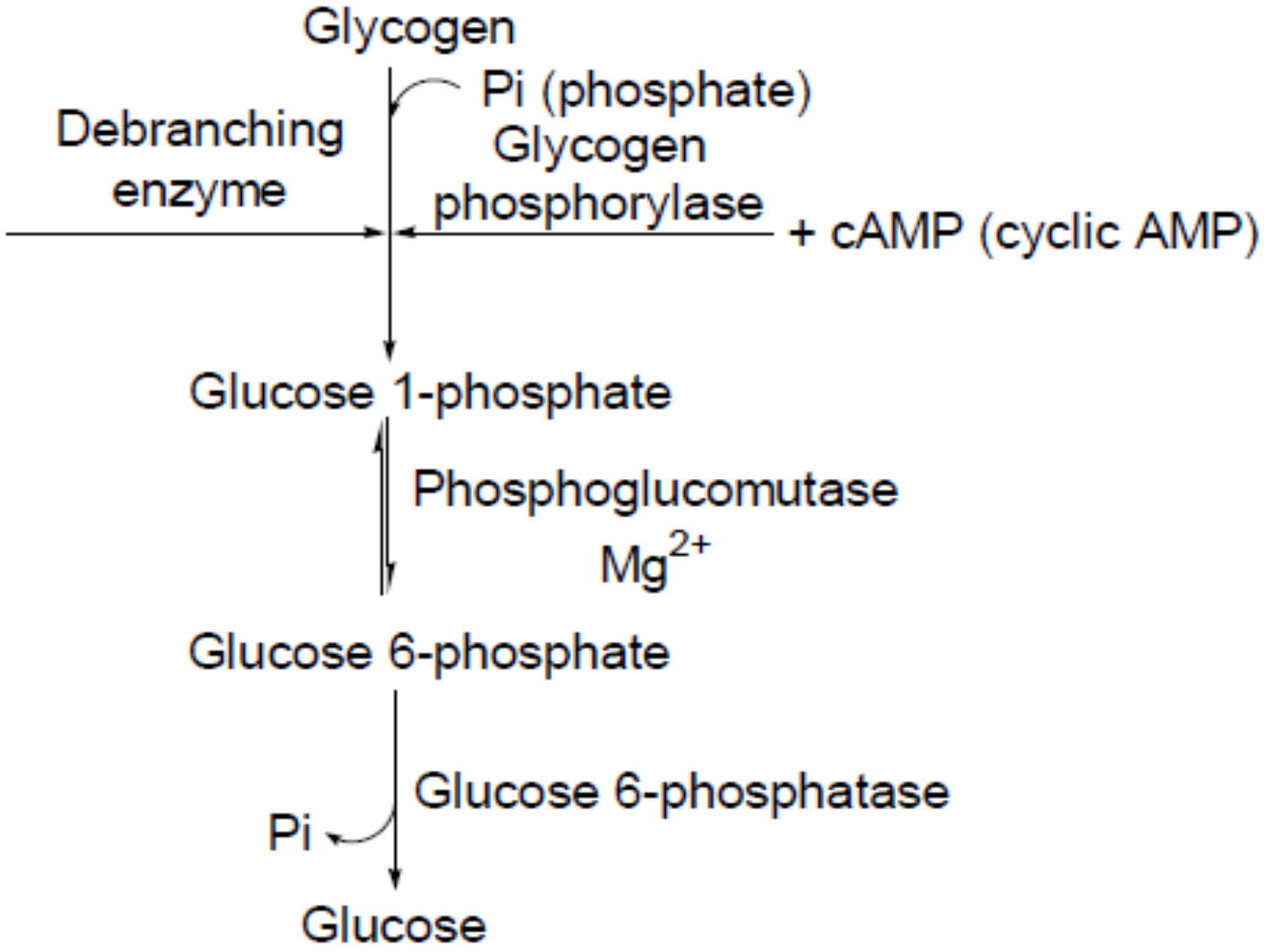


Step 5

When the chain has become long with more than 8 glucose units, a second enzyme, namely branching enzyme amylo 1-4 to 1-6 Trans glycosylase acts on the glycogen and helps in joining of 1,4 glycogen chain with a similar neighboring chain to forma 1-6 linkage, thus forming a branching point in the molecule. Glycogen thus formed may be stored in liver, muscles and tissues.

Degradation of glycogen (Glycogenolysis)

When the blood sugar level falls (Hypoglycemia), glycogen stored in the tissues specially glycogen of liver and muscles may be broken down and this process of breakdown of glycogen is called glycogenolysis



Gluconeogenesis:

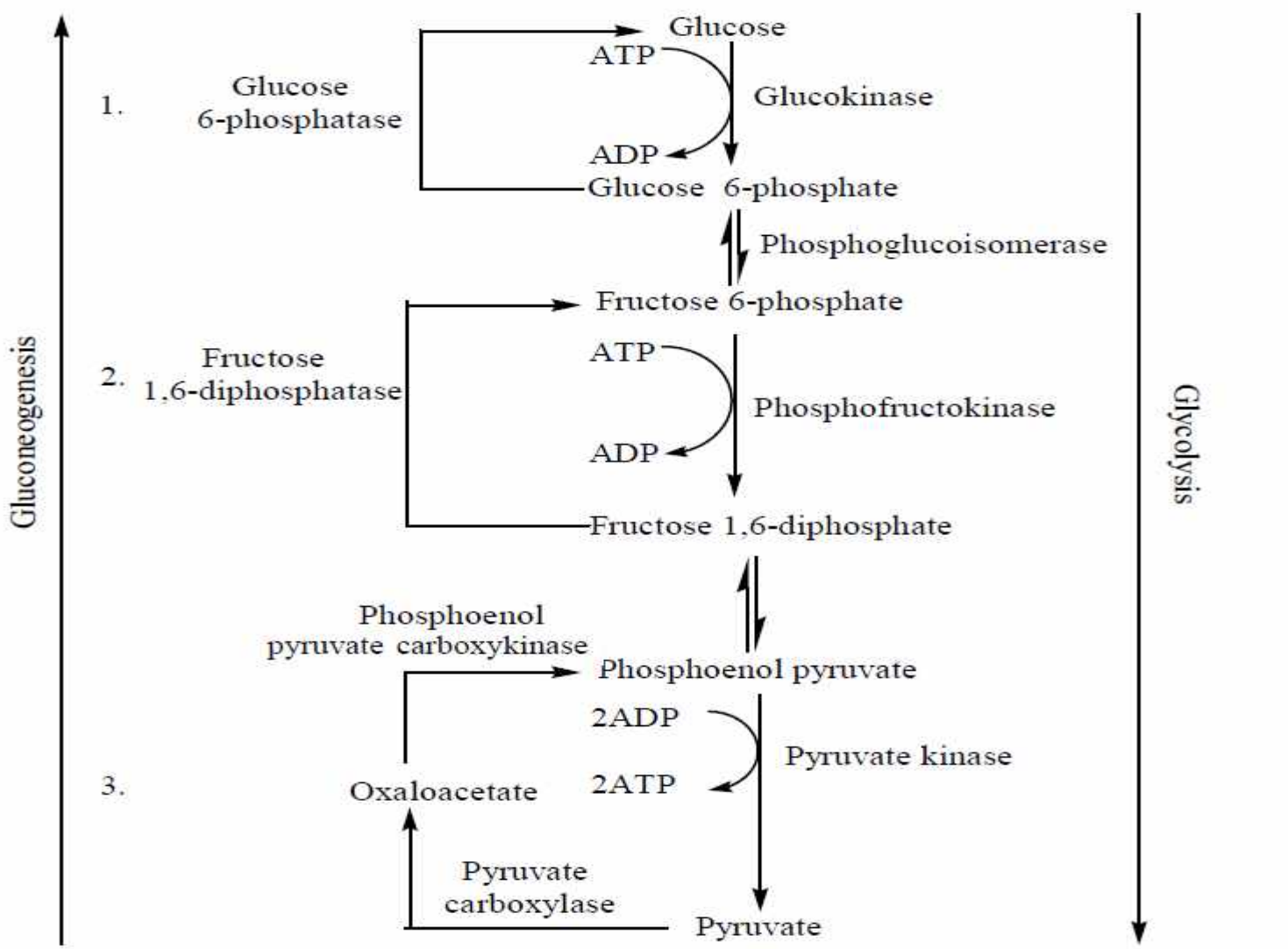
The synthesis of glucose from non-carbohydrate precursors is known as gluconeogenesis. The major site of gluconeogenesis is liver. It usually occurs when the carbohydrate in the diet is insufficient to meet the demand in the body, with the intake of protein rich diet and at the time of starvation, when tissue proteins are broken down to amino acids.

Gluconeogenesis and glycolysis

Gluconeogenesis and glycolysis are opposing metabolic pathways and share a number of enzymes. In glycolysis, glucose is converted to pyruvate and in gluconeogenesis pyruvate is converted to glucose. However gluconeogenesis is not exact reversal of glycolysis. There are three essentially irreversible steps in glycolysis which are:

1. Glucose + ATP $\xrightarrow{\text{Glucokinase}}$ Glucose 6-phosphate + ADP
2. Fructose 6-phosphate + ATP $\xrightarrow{\text{Phosphofructo kinase}}$ Fructose 1,6-diphosphate + ADP
3. Phosphoenol pyruvate + ADP $\xrightarrow{\text{Pyruvate kinase}}$ Pyruvate + ATP

In gluconeogenesis these three reactions are bypassed or substituted by the following new ones.



Diabetes Mellitus

Diabetes mellitus is an important disorder of carbohydrate metabolism. However, fat and protein metabolism are also affected in diabetic condition. Diabetes means excretion of excessive volume of urine and mellitus means sweet. So the word diabetes milletus refers to chronic excretion of large volume of urine containing glucose.

Diabetes mellitus, caused by a deficiency in the secretion or action of insulin, is a relatively common disease. Insulin is an endocrine hormone which is secreted by b-cells of islets of Langerhans of pancreas. The abnormality in glucose metabolism is indicative of diabetes or a tendency towards the condition. Diabetes mellitus is really a group of diseases in which the regulatory activity of insulin is defective.

There are two major clinical classes of the disease :
1. Type-I or insulin dependent diabetes mellitus (IDDM)
The disease begins early in the life and quickly becomes severe.
2. Type - II or non-insulin dépendent diabetes mellitus (NIDDM)

The disease is slow to develop, milder and often goes unrecognized. Type one requires insulin therapy and careful, life long control of the balance between glucose intake and insulin dose. The decreased or defective production of insulin is characterised by the following symptoms.
1. Decreased permeability of the cell membrane for glucose resulting in the accumulation of glucose in the blood. This condition is known as hyperglycemia. Glucose concentration increases as high as 500 mg/100 ml of blood.

2. Polyuria: This means excretion of increased quantity of urine. This is to excrete the additional quantity of glucose in urine (glycosuria).

3. Polydipsia: The excessive thirst which leads to increased consumption of water. This condition is known as polydipsia. This is to replace the volume of water excreted due to polyuria.

4. Polyphagia: Excessive appetite leads to polyphagia and increased intake of food. This is to replace the lost nourishment. The diabetic has voracious appetite, but inspite of over eating, they lose weight and become lean and emaciated.

5. As glucose is not enough for energy production, increased mobilisation of fat from adipose tissue occurs. But the metabolism of fat is incomplete resulting in the production of large amounts of the intermediary products of fat metabolism namely ketone bodies (eg. Acetoacetate and b-hydroxybutarate). This condition is known as 'ketosis' and excess ketone bodies cause severe acidosis, ultimately resulting in 'coma'.

6. Deposition of lipids in the walls of the blood vessels resulting "atherosclerosis".

Biochemical measurements on the blood and urine are essential in the diagnosis and treatment of diabetes, which causes profound changes in metabolism. A sensitive diagnostic criterion is provided by the glucose tolerance test (GTT).

Glucose Tolerance Test (GTT)

After a night without food, the patient drinks a test dose of 100 g of glucose dissolved in a glass of water. The blood glucose concentration is measured before the test dose and at 30 min intervals for several hours thereafter. A normal individual assimilates the glucose readily, the blood glucose rising to no more than about 80 to 120 mg/100 ml; little or no glucose appears in the urine. Diabetic individuals assimilate the test dose of glucose poorly; their blood glucose level far exceeds the kidney threshold (about 180 mg/100ml), causing glucose to appear in their urine.

Exercises

I. Choose the correct answer from the given four alternatives

a. Blood sugar is i) sucrose ii) lactose iii) glucose iv) fructose

b. Glycolysis occurs in i) mitochondria ii) cytosol iii) nucleus iv) ribosome

c. How many ATP molecules are generated during glycolysis i) 2 ii) 10 iii) 6 iv) 8

d. The end product of glycolysis is i) pyruvate ii) citrate iii) acetyl CoA iv) lactate

e. The important reducing power produced in HMP shunt pathway is i) NADH ii) NADPH iii) FAD iv) FADH2

f. Lactate is converted to glucose in i) skeletal muscle ii) liver iii) kidney iv) lung

g. Insulin is secreted by i) liver ii) kidney iii) pancreas iv) thyroid

II. Fill up the blanks

a . Glucokinase acts on glucose to form ——————————

.b.. In the anaerobic phase one molecule of glucose produces ——————— molecules of ATP

c.. Tricarboxylic acid cycle occurs in ——————————

d. —————————— is precursor for nucleotide synthesis

e. Glycogen biosynthesis is known as ——————————

f. The major source of glucose in ruminants is ————————

VI. Answer the following

1. What are the reaction sequences of glycolysis?

2. Explain the HMP shunt pathway

Madenet Alem University College
Department of Biology
2nd Year Biochemistry I 2021– 2022

Nucleic Acids

DNA & RNA

Dr. Hamza Yaseen Isa

Lecture 6 I 2

The distribution of nucleic acids in the eukaryotic cell

- DNA is found in the nucleus
  with small amounts in mitochondria and chloroplasts
- RNA is found throughout the cell

DNA substance is present in the nucleus of all cells in all living organisms

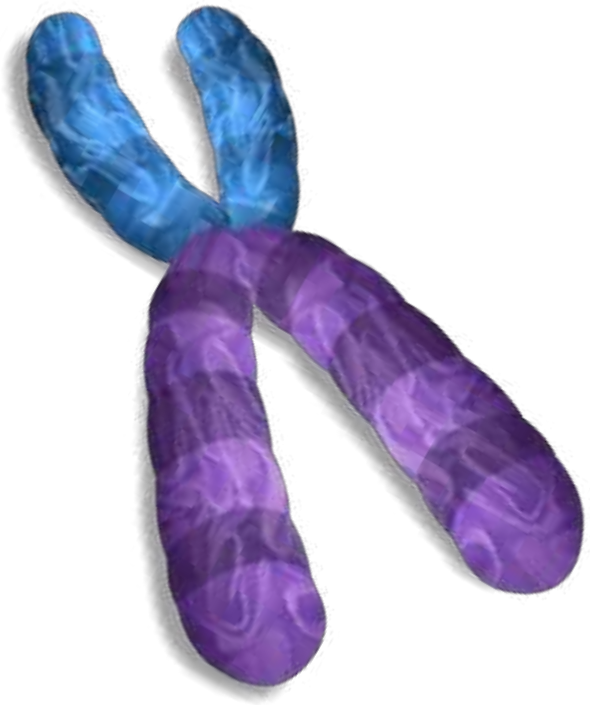
DNA controls all the chemical changes which take place in cells

The kind of cell which is formed, (muscle, blood, nerve etc) is controlled by DNA

How is DNA packaged?

In eukaryotic cells, DNA is packaged as chromosomes in the nucleus.

There is around 2 m of DNA in a cell, so to fit it needs to be tightly coiled and folded.



Eukaryotic DNA is associated with proteins called histones. Together, these form chromatin – the substance from which chromosomes are made. Chromosomes can only be seen under a light microscope during cell division.

In prokaryotic cells, DNA is loose in the cytoplasm – there are no histones or chromosomes.

DNA as genetic material: The circumstantial evidence

1. **Present in all cells and virtually restricted to the nucleus**
2. **The amount of DNA in somatic cells (body cells) of any given species is constant (like the number of chromosomes)**
3. **The DNA content of gametes (sex cells) is half that of somatic cells.**
   In cases of polyploidy (multiple sets of chromosomes) the DNA content increases by a proportional factor
4. **The mutagenic effect of UV light peaks at 253.7nm. The peak for the absorption of UV light by DNA**

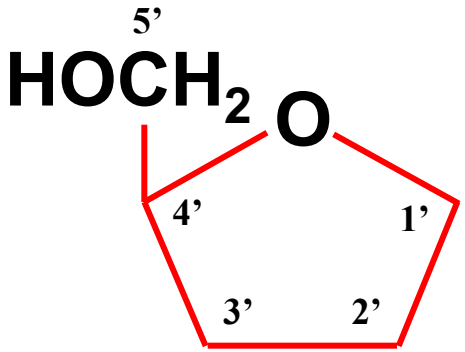
NUCLEIC ACIDS (DNA and RNA) Notes

DNA – **Deoxyribonucleic Acid**

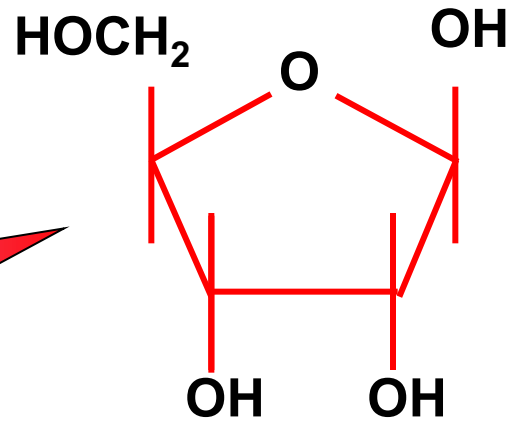
- DNA controls all living processes including production of new cells – **cell division**
- DNA carries the genetic code – **stores** and **transmits** genetic information from one **generation** to the next
- Chromosomes are made of **DNA and histones**
- DNA is located in the **nucleus** of the cell

Nucleotide Structure
1- Sugars

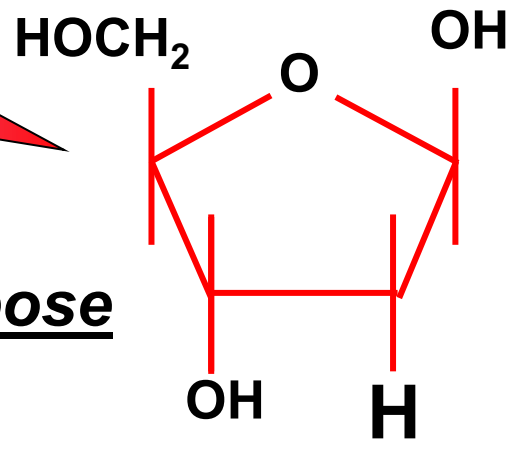
Generic Ribose Structure



Ribose

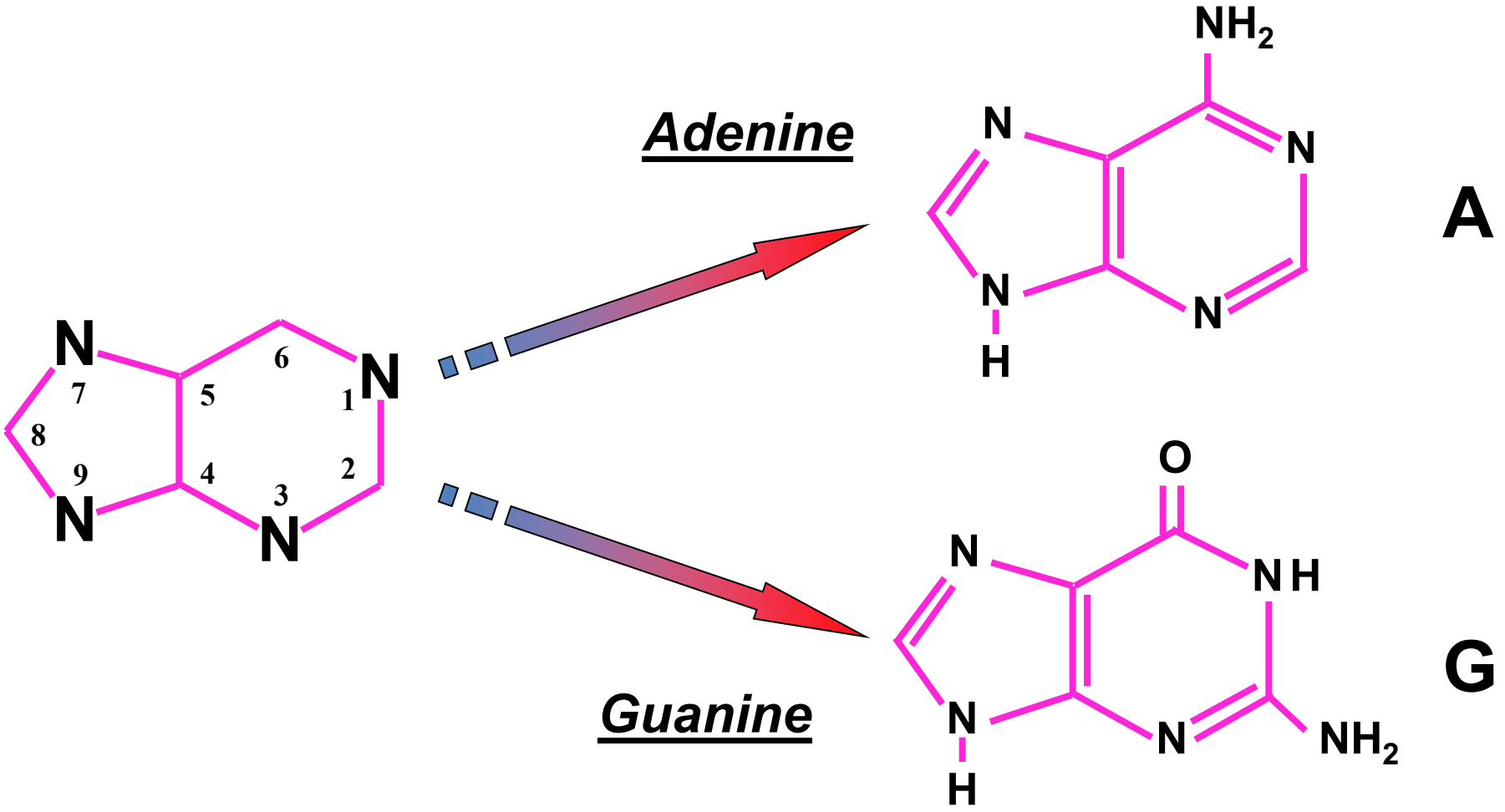


Deoxyribose



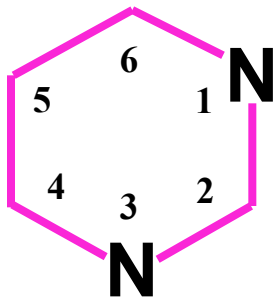
N.B. Carbons are given numberings as a prime

Nucleotide Structure
2- Bases - Purines

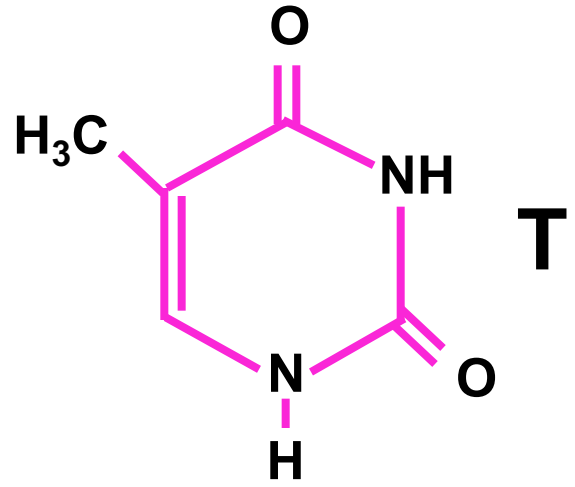


Nucleotide Structure

3- Bases - Pyrimidines

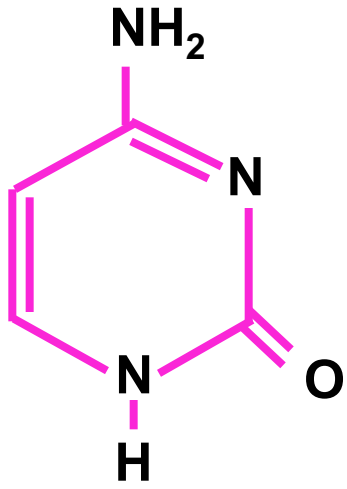


Thymine



T

Cytosine



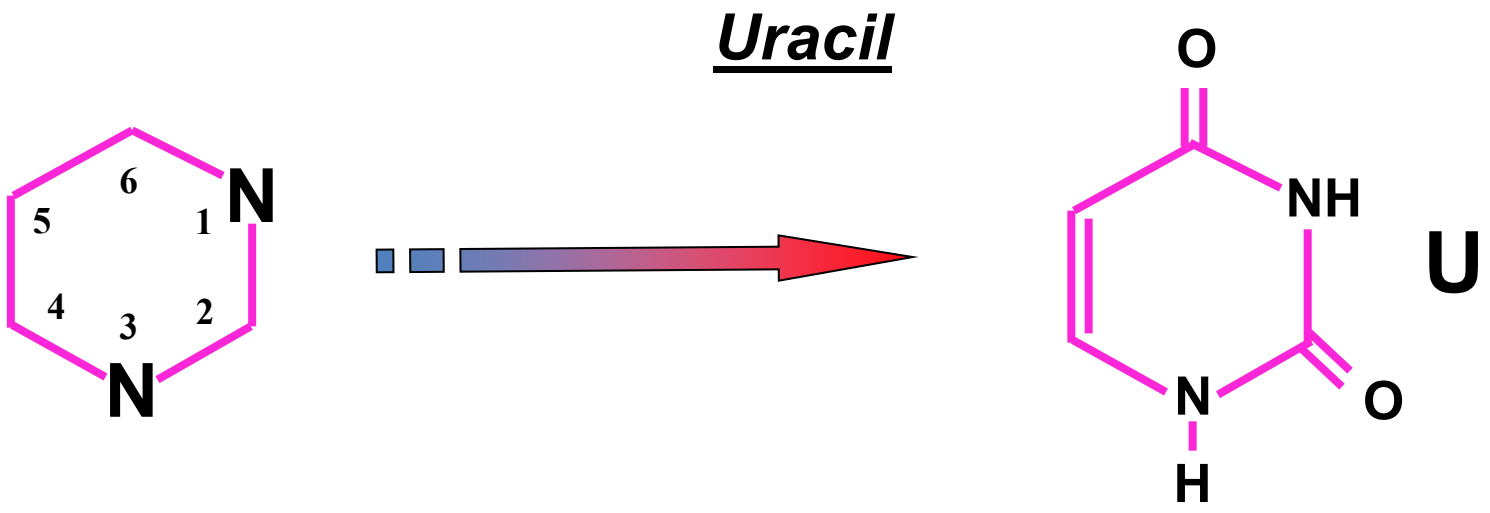
C

Nucleotide Structure
3-Bases - Pyrimidines

Thymine is found ONLY in DNA.

In RNA, thymine is replaced by uracil

Uracil and Thymine are structurally similar

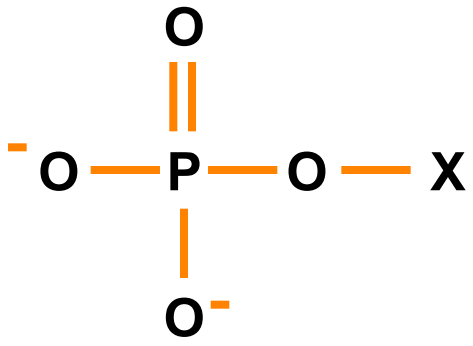


Nucleotide Structure
4- Phosphate Group

Phosphate groups are what makes a nucleoside a nucleotide

Phosphate groups are essential for nucleotide polymerization

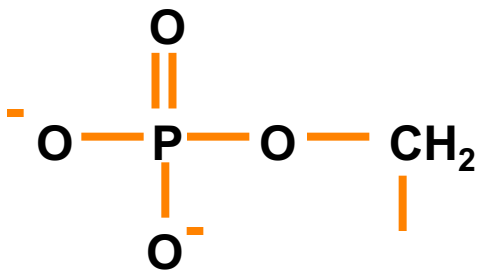
Basic structure:



Nucleotide Structure
4- Phosphate Groups

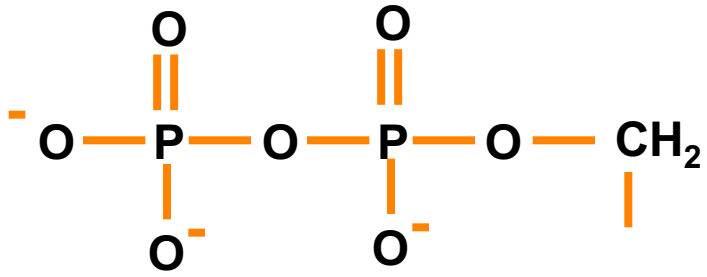
Number of phosphate groups determines nomenclature

Monophosphate
e.g. AMP

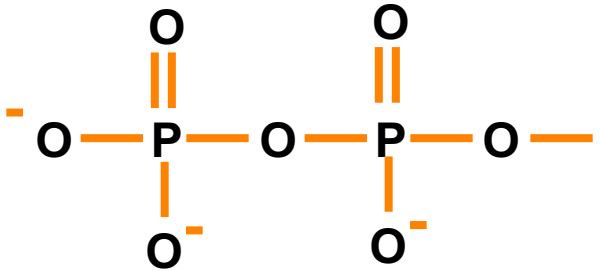


Free = inorganic phosphate (Pi)

Diphosphate
e.g. ADP



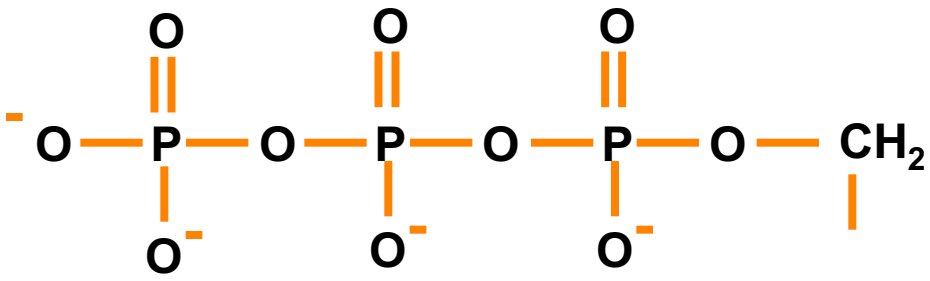
Free = Pyro-phosphate (PPi)



Nucleotide Structure
4- Phosphate Groups

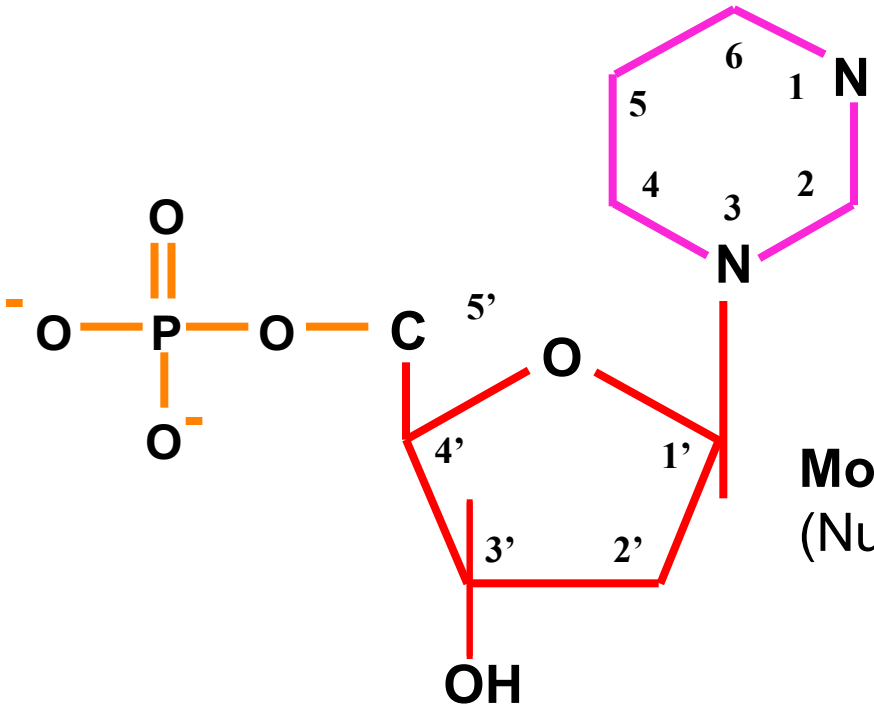
Triphosphate
e.g. ATP

No Free form exists



Nucleotide Structure

Base-Sugar-PO_4^{2-}



Monophosphate
(Nucleotide)

N. B. Nucleotide without phosphate group is Nucleoside

TABLE 8–1 Nucleotide and Nucleic Acid Nomenclature

Base	Nucleoside	Nucleotide	Nucleic
Purines			
Adenine	Adenosine Deoxyadenosine	Adenylate Deoxyadenylate	RNA DNA
Guanine	Guanosine Deoxyguanosine	Guanylate Deoxyguanylate	RNA DNA
Pyrimidines			
Cytosine	Cytidine Deoxycytidine	Cytidylate Deoxycytidylate	RNA DNA
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA
Uracil	Uridine	Uridylate	RNA

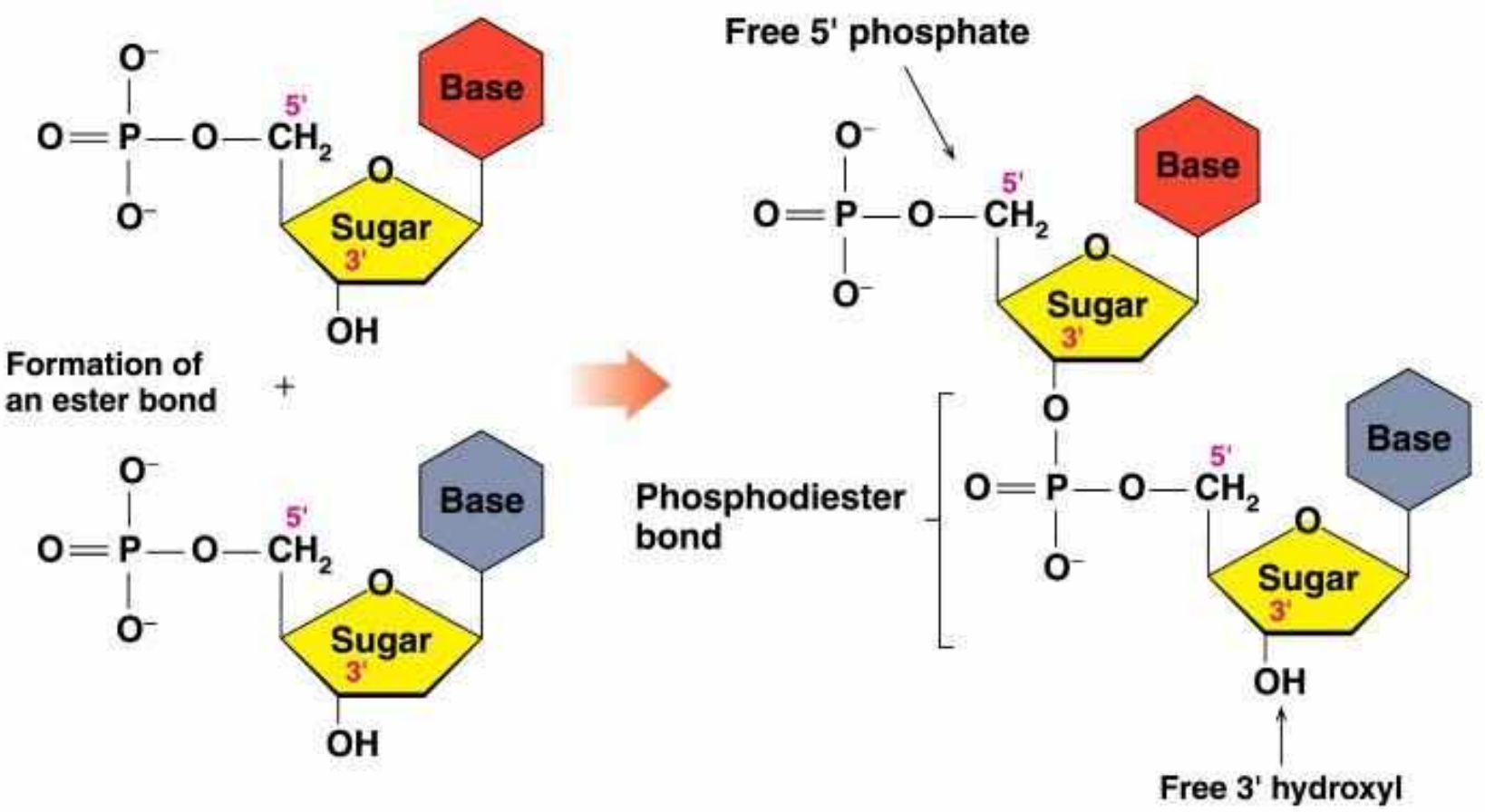
Note: "Nucleoside" and "nucleotide" are generic terms that include both ribo- and deoxyribo- forms. Also, ribonucleosides and ri
cleotides are here designated simply as nucleosides and nucleotides (e.g., riboadenosine as adenosine), and deoxyribonucleosic
deoxyribonucleotides as deoxynucleosides and deoxynucleotides (e.g., deoxyriboadenosine as deoxyadenosine). Both forms of
are acceptable, but the shortened names are more commonly used. Thymine is an exception; "ribothymidine" is used to describe
usual occurrence in RNA.

Table 8-1

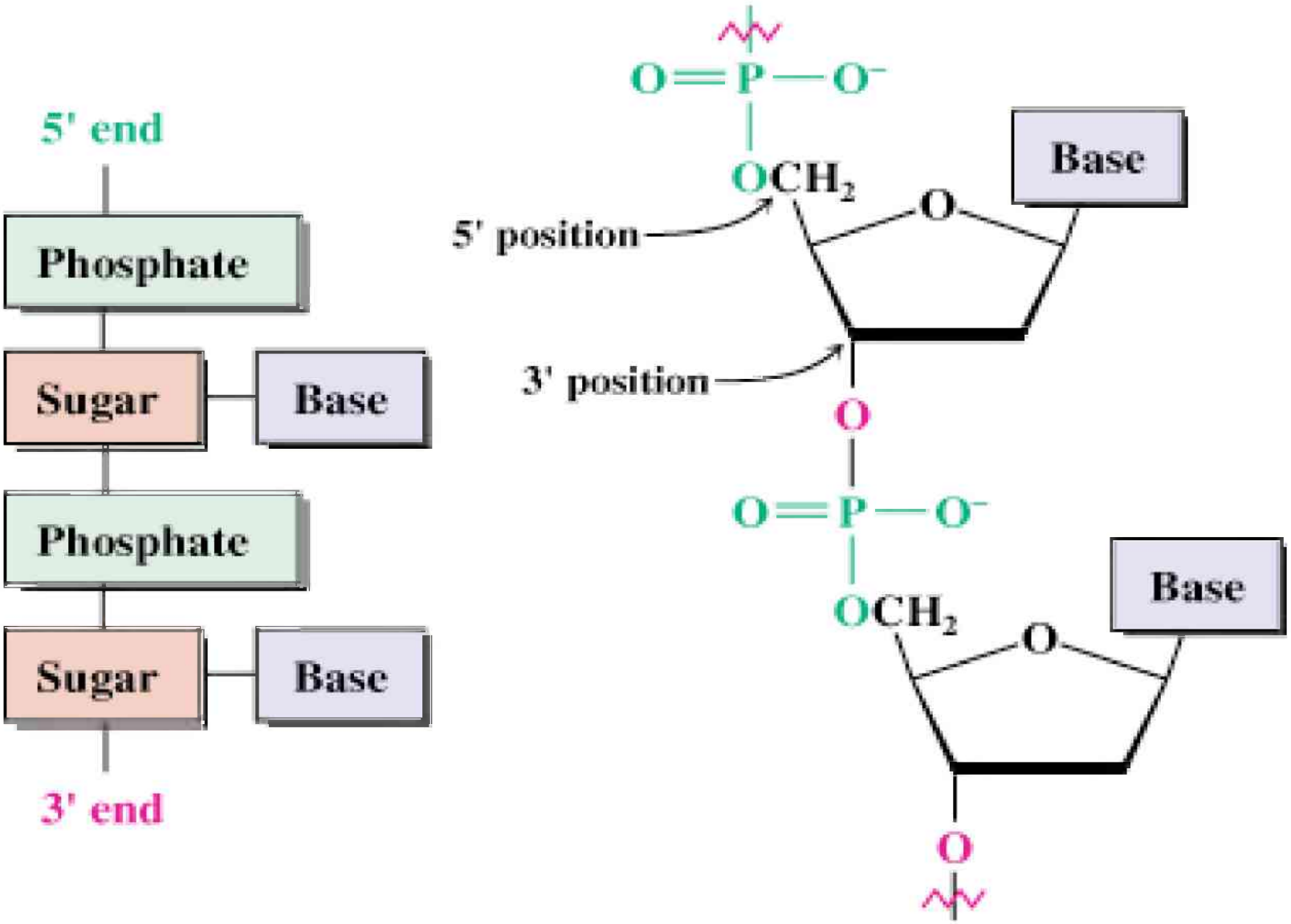
Lehninger Principles of Biochemistry, Fifth Edition

Primary Structure of Nucleic Acids

- The primary structure of a nucleic acid is the nucleotide sequence
- The nucleotides in nucleic acids are joined by phosphodiester bonds
- The 3’-OH group of the sugar in one nucleotide forms an ester bond to the phosphate group on the 5’-carbon of the sugar of the next nucleotide

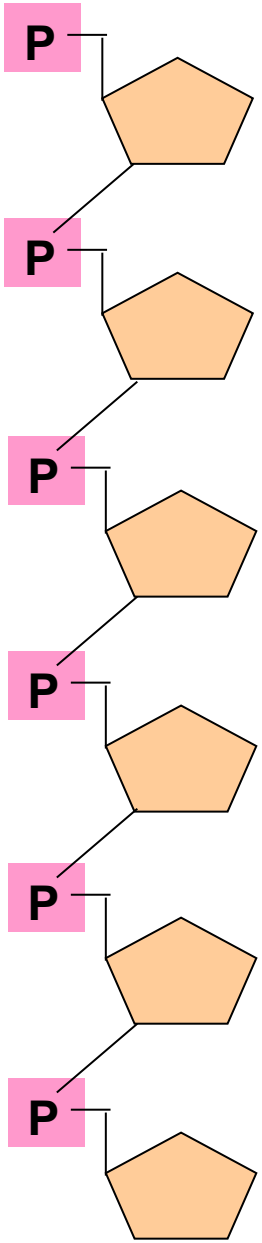


Generalized Structure of DNA



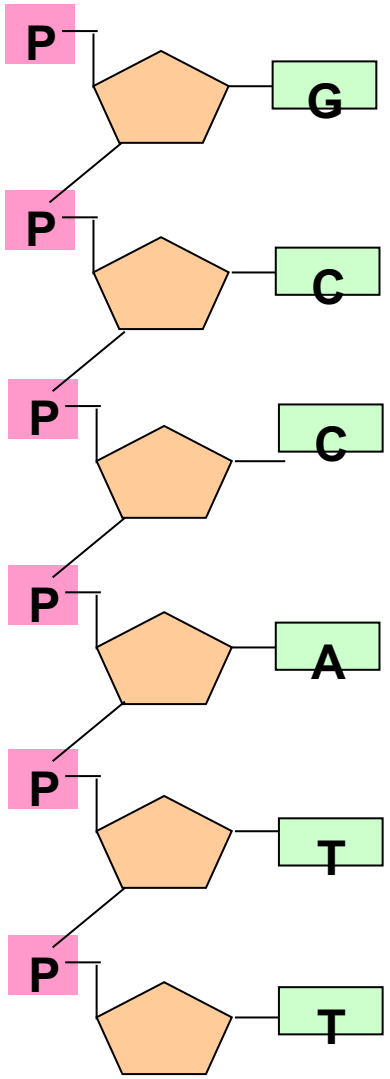
THE SUGAR-PHOSPHATE BACKBONE

- **The nucleotides are all orientated in the same direction**
- **The phosphate group joins the 3rd Carbon of one sugar to the 5th Carbon of the next in line.**

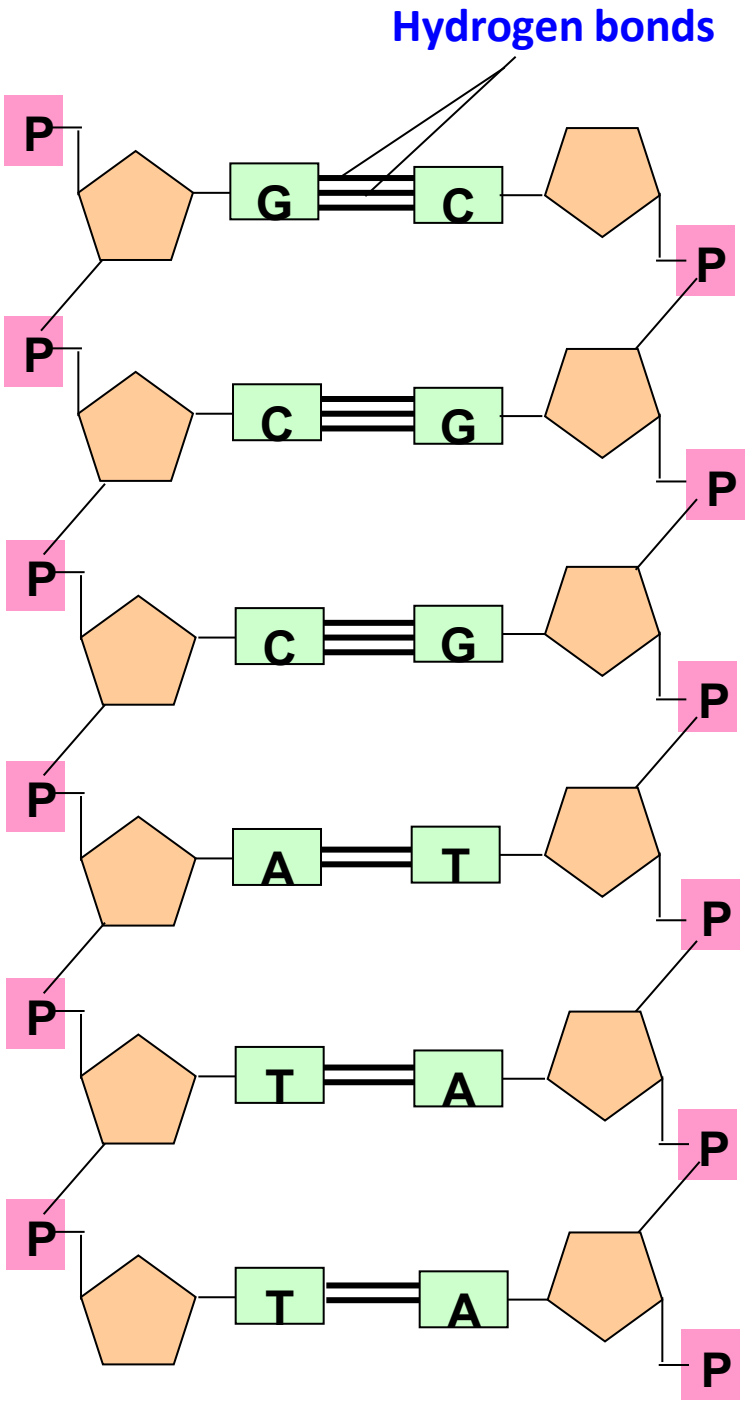


ADDING IN THE BASES

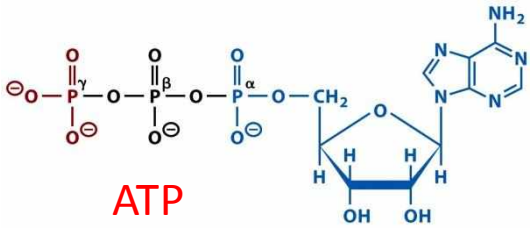
- The bases are attached to the 1st Carbon
- Their order is important
  It determines the genetic information of the molecule



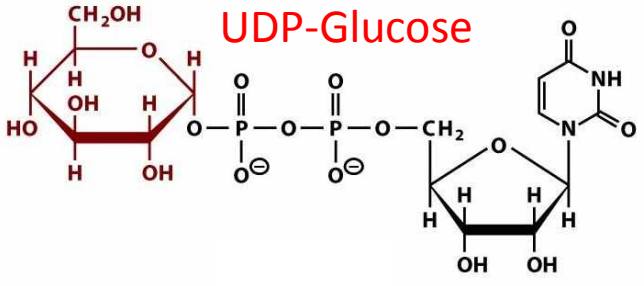
DNA IS MADE OF TWO STRANDS OF POLYNUCLEOTIDE



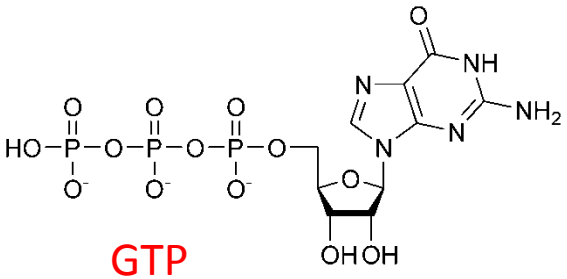
Some Examples of Nucleotides



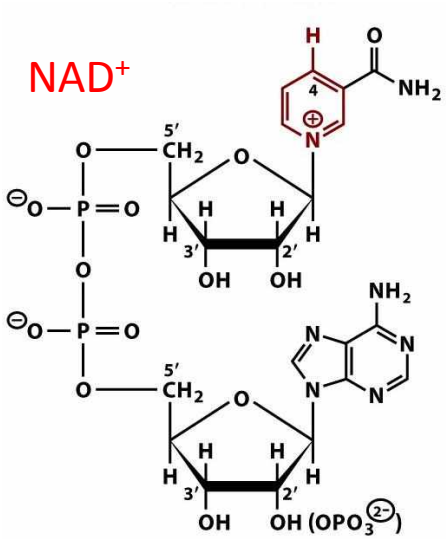
ATP



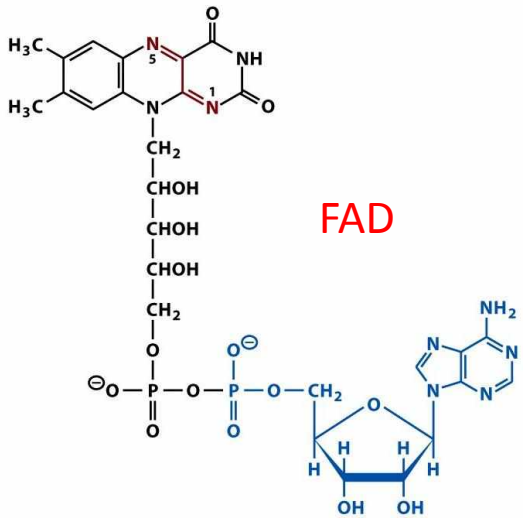
UDP-Glucose



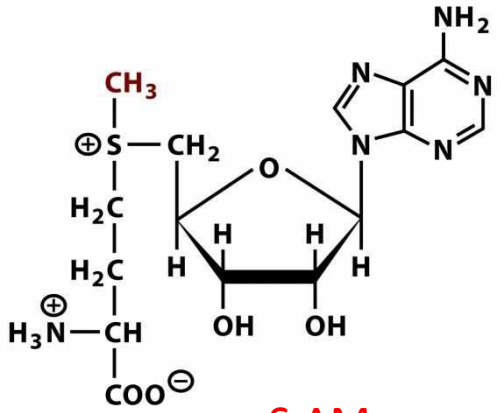
GTP



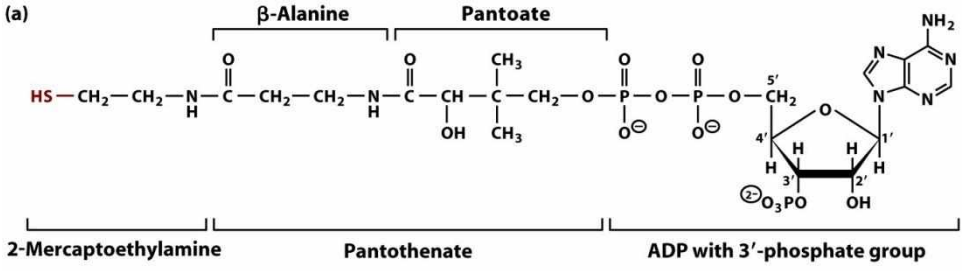
NAD^+



FAD



S-AM



CoA

DNA IS MADE OF TWO STRANDS OF POLYNUCLEOTIDE

- **The sister strands of the DNA molecule run in opposite directions (antiparallel)**
- **They are joined by the bases**
- **Each base is paired with a specific partner:**

A is always paired with T

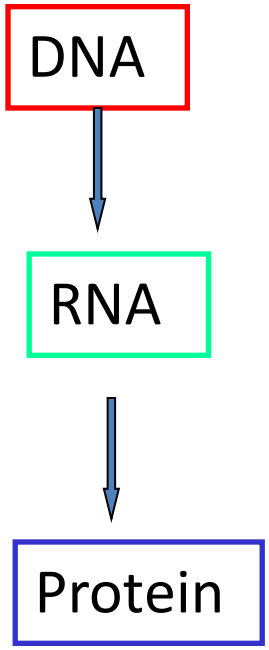
G is always paired with C

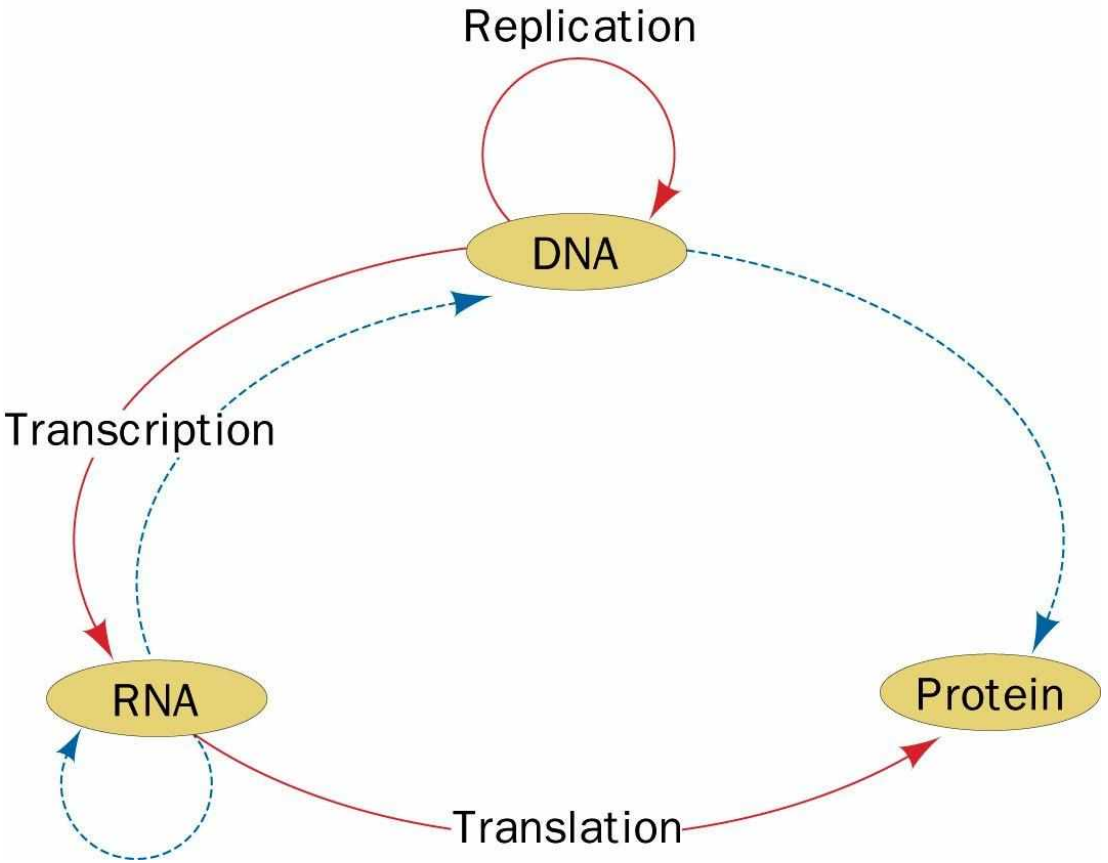
Purine with Pyrimidine

- **Thus the sister strands are complementary but not identical**
- **The bases are joined by hydrogen bonds, individually weak but collectively strong.**

The central dogma of molecular biology.

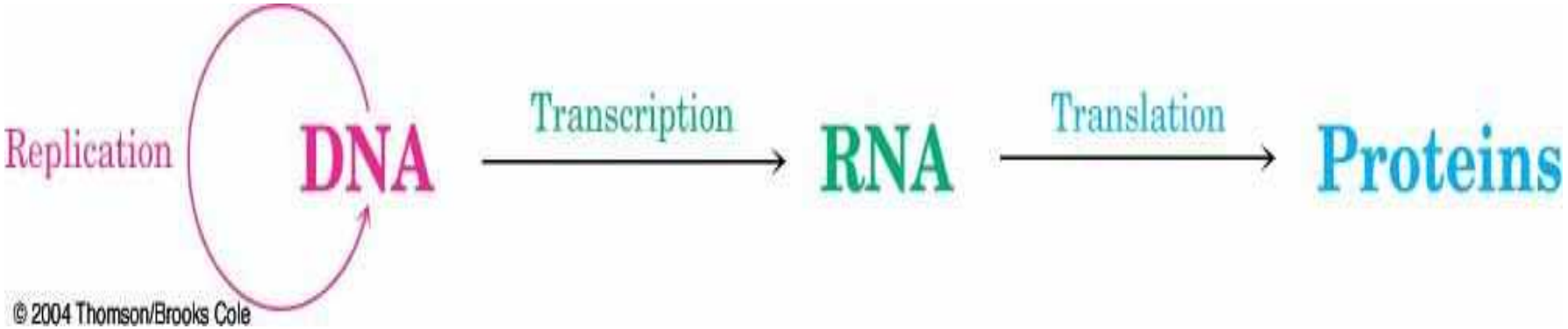
Transfer of Information from DNA to Protein





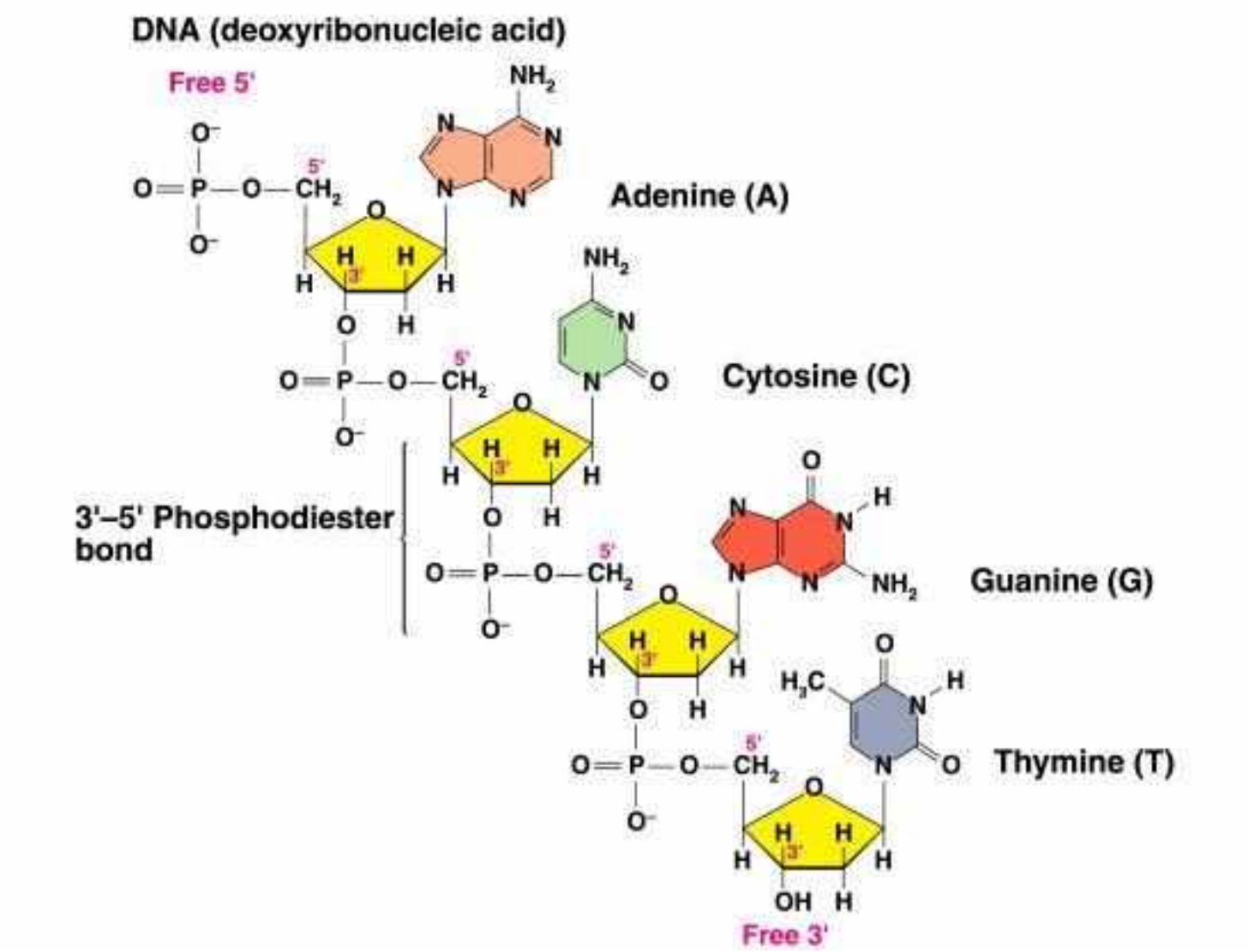
Nucleic Acids and Heredity

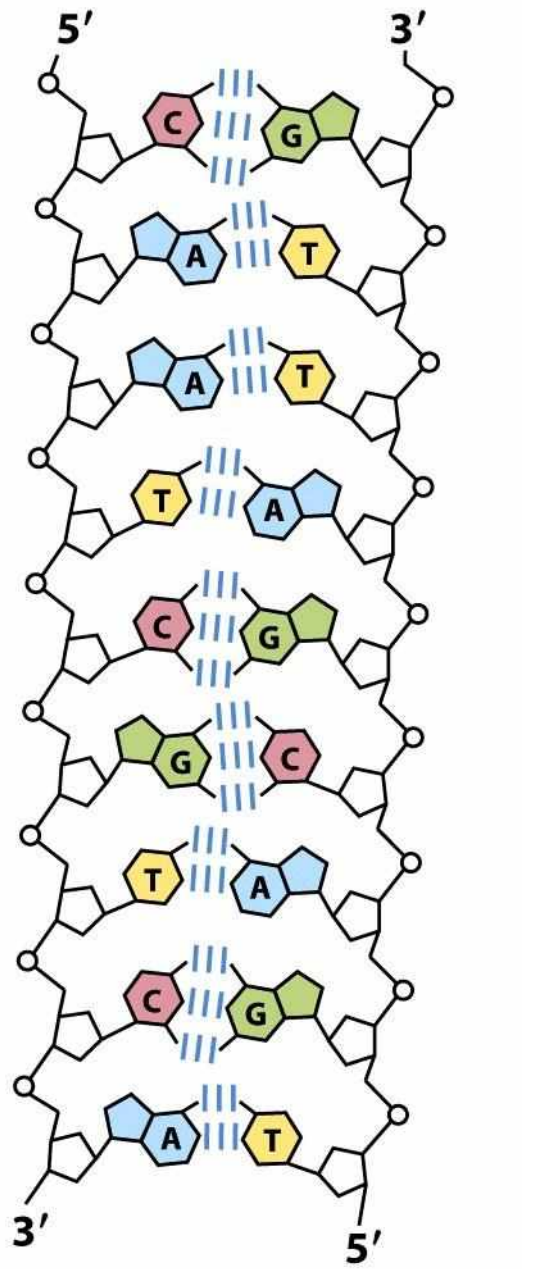
- **Processes in the transfer of genetic information:**
- **Replication: identical copies of DNA are made**
- **Transcription: genetic messages are read and carried out of the cell nucleus to the ribosomes, where protein synthesis occurs.**
- **Translation: genetic messages are decoded to make proteins.**



Example of DNA Primary Structure

- **In DNA, A, C, G, and T are linked by 3'-5' ester bonds between deoxyribose and phosphate**





Properties of a DNA double helix

The strands of DNA are antiparallel

The strands are complimentary

There are Hydrogen bond forces

There are base stacking interactions

There are 10 base pairs per turn

Secondary Structure: DNA Double Helix

- **In DNA there are two strands of nucleotides that wind together in a double helix**

  - the strands run in opposite directions

  - the bases are arranged in step-like pairs

  - the base pairs are held together by hydrogen bonding
- **The pairing of the bases from the two strands is very specific**
- **The complimentary base pairs are A-T and G-C**

  - two hydrogen bonds form between A and T

  - three hydrogen bonds form between G and C
- **Each pair consists of a purine and a pyrimidine, so they are the same width, keeping the two strands at equal distances from each other**

replication

Before a cell divides, the DNA strands unwind and separate

Each strand makes a new partner by adding the appropriate nucleotides

The result is that there are now two double-stranded DNA molecules in the nucleus

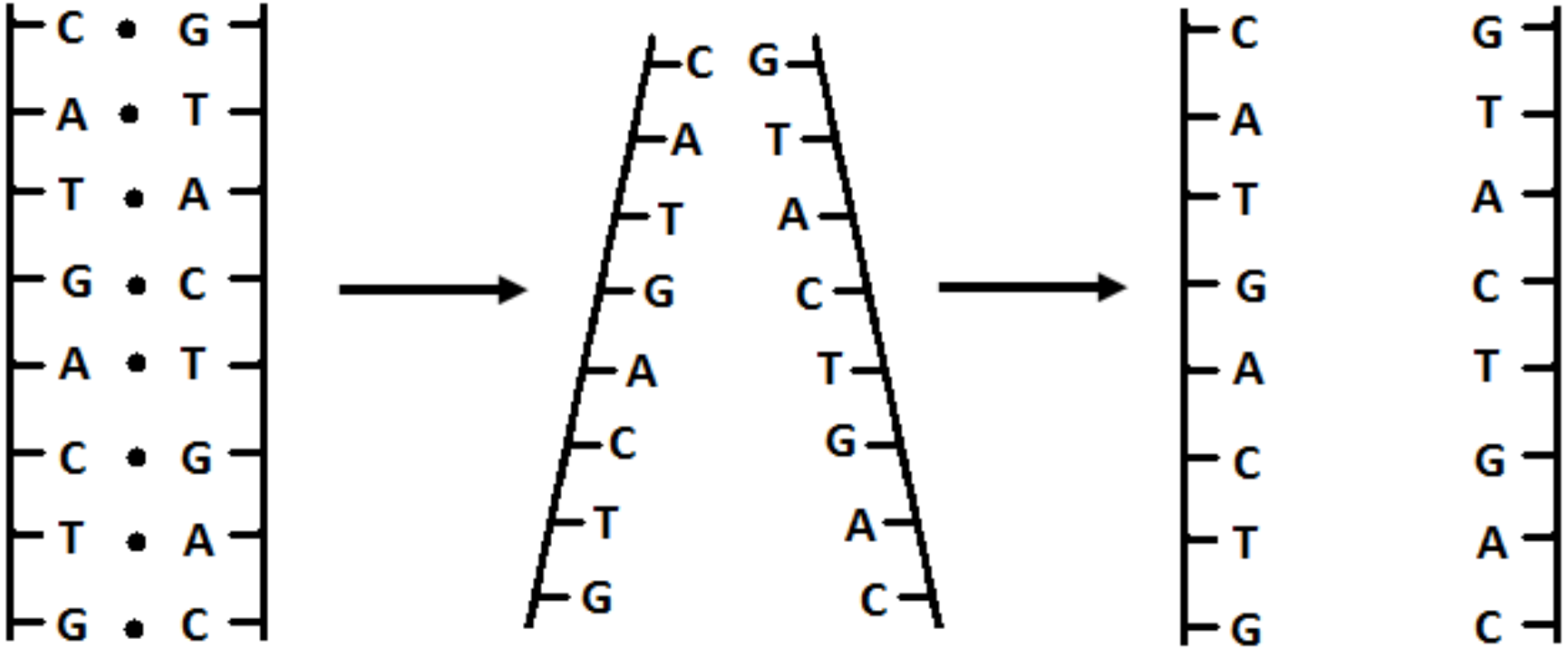
So that when the cell divides, each nucleus contains identical DNA

This process is called replication

STEP 1

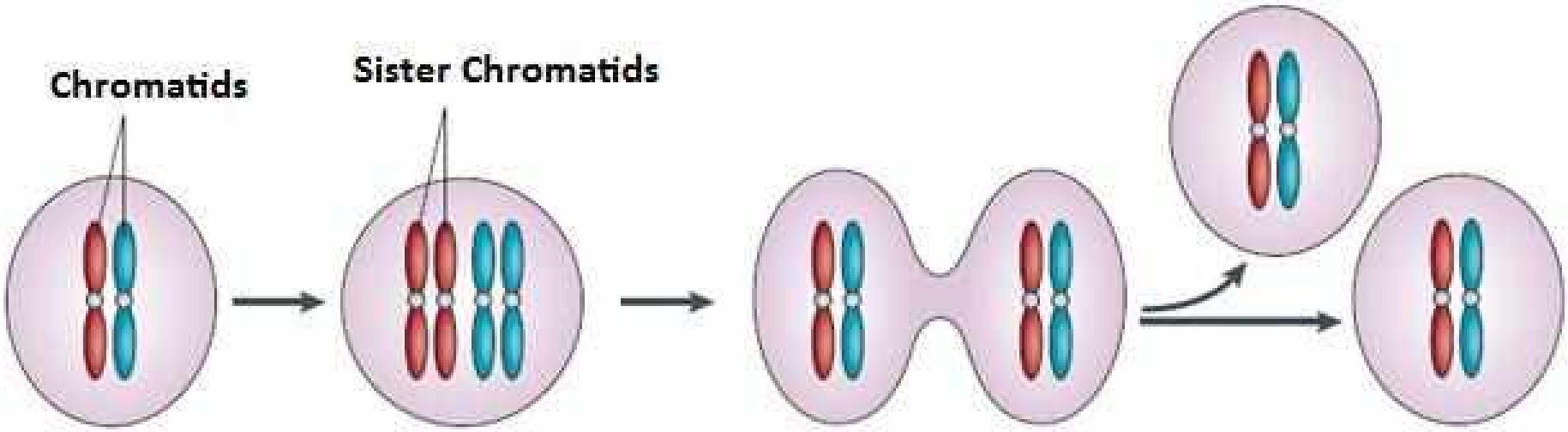
Hydrogen bonds between base pairs are **broken** by the enzyme **Helicase** and DNA molecule **unzips**

DNA molecule separates into **complementary halves**



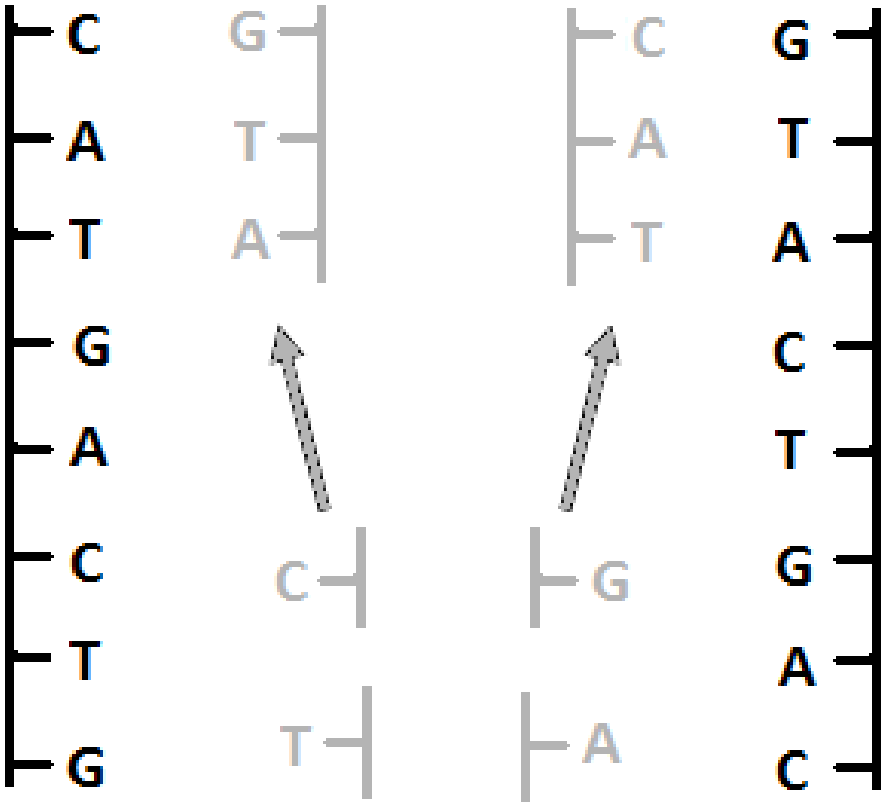
DNA Replication

- Cell division involving **mitosis** produces 2 **daughter** cells that are genetically **identical** to each other and genetically identical to the **parent** cell
- Remember that for this to happen, DNA in the parent cell must be **replicated** (copied) **before** the cell divides – this process occurs during **Interphase** in the cell cycle



STEP 2

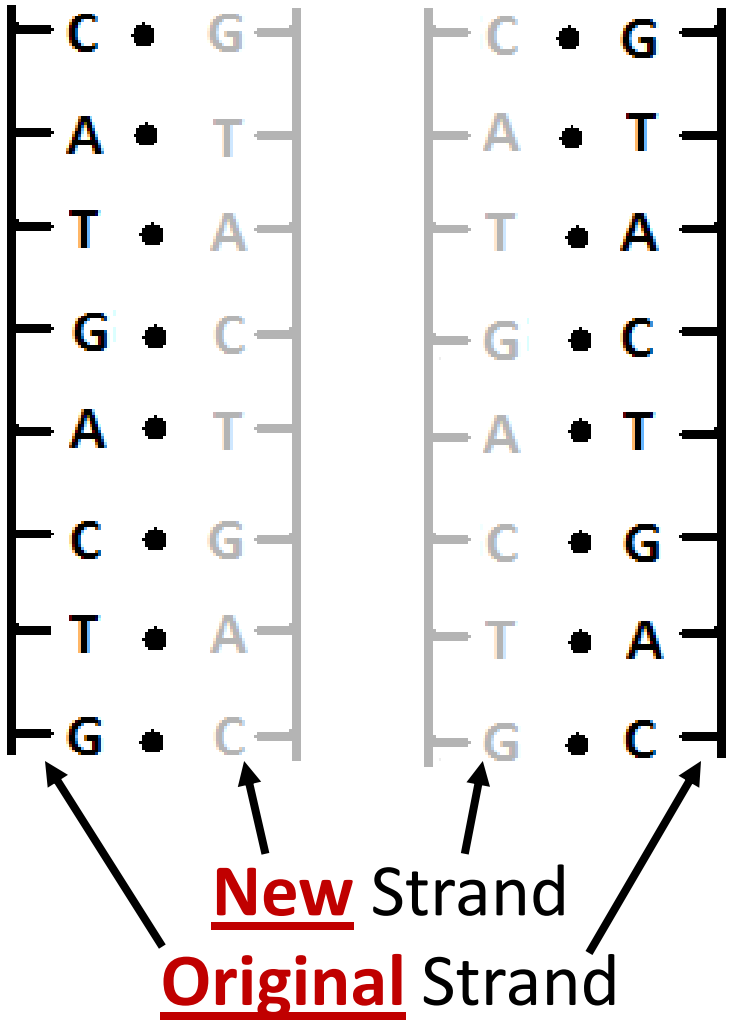
Nucleotides match up with complementary bases



Free nucleotides abundant in **nucleus**

STEP 3

Nucleotides are linked into 2 new strands of DNA by the enzyme, **polymerase**—DNA polymerase also **proofreads** for copying errors



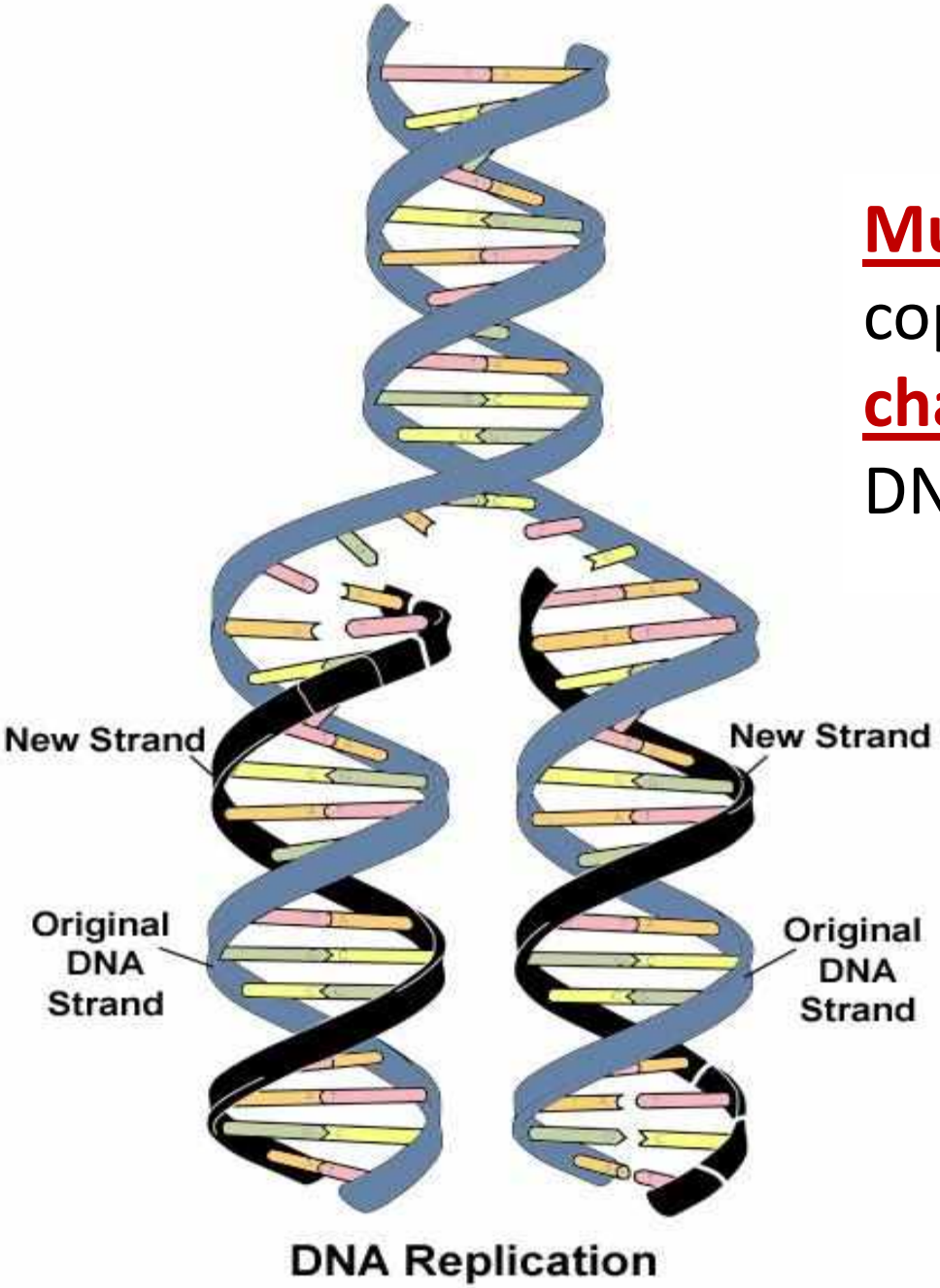
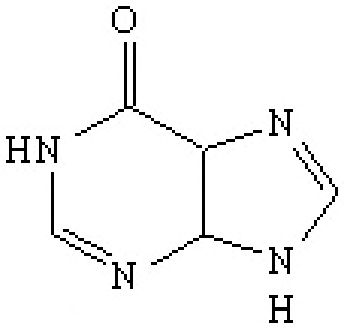


Image adapted from: National Human Genome Research Institute.

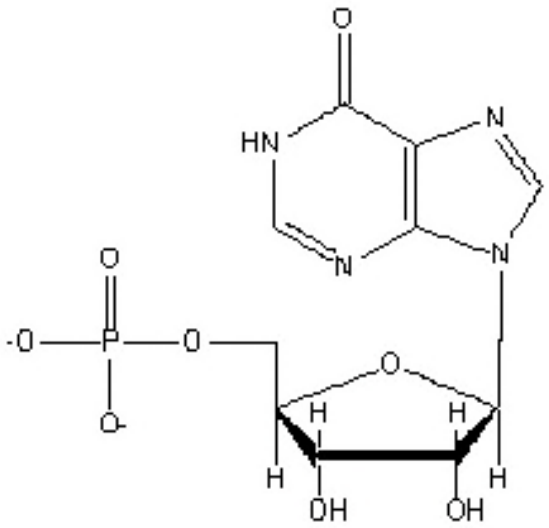
Mutations occur when copying **errors** cause a **change** in the **sequence** of DNA nucleotide bases

Nucleotide Metabolism

- PURINE RIBONUCLEOTIDES: formed *de novo*
  - i.e., purines are not initially synthesized as free bases
  - First purine derivative formed is Inosine Mono-phosphate (IMP)
    - **The purine base is hypoxanthine**
    - **AMP and GMP are formed from IMP**



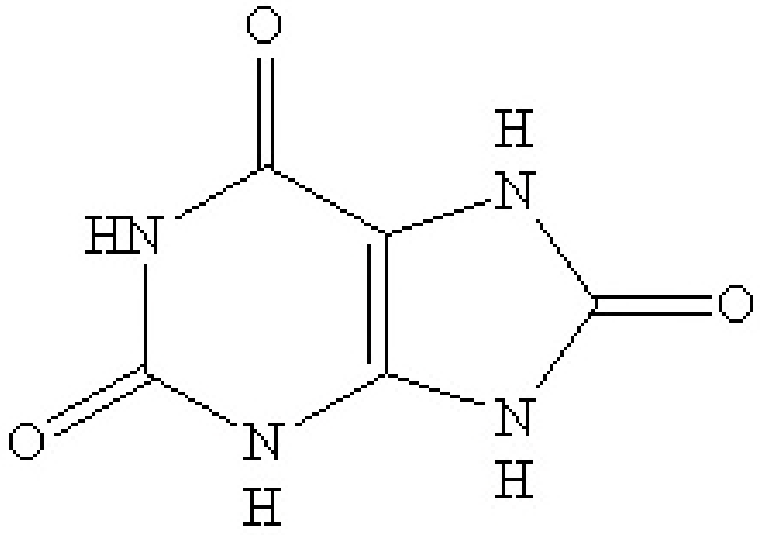
Hypoxanthine



IMP

Purine Nucleotides

- Get broken down into Uric Acid (a purine) Buchanan (mid 1900s) showed where purine ring components came from:



Uric Acid

N_1: Aspartate Amine
C_2, C_8: Formate
N_3, N_9: Glutamine
C_4, C_5, N_7: Glycine
C_6: Bicarbonate Ion

A CASE STUDY : GOUT

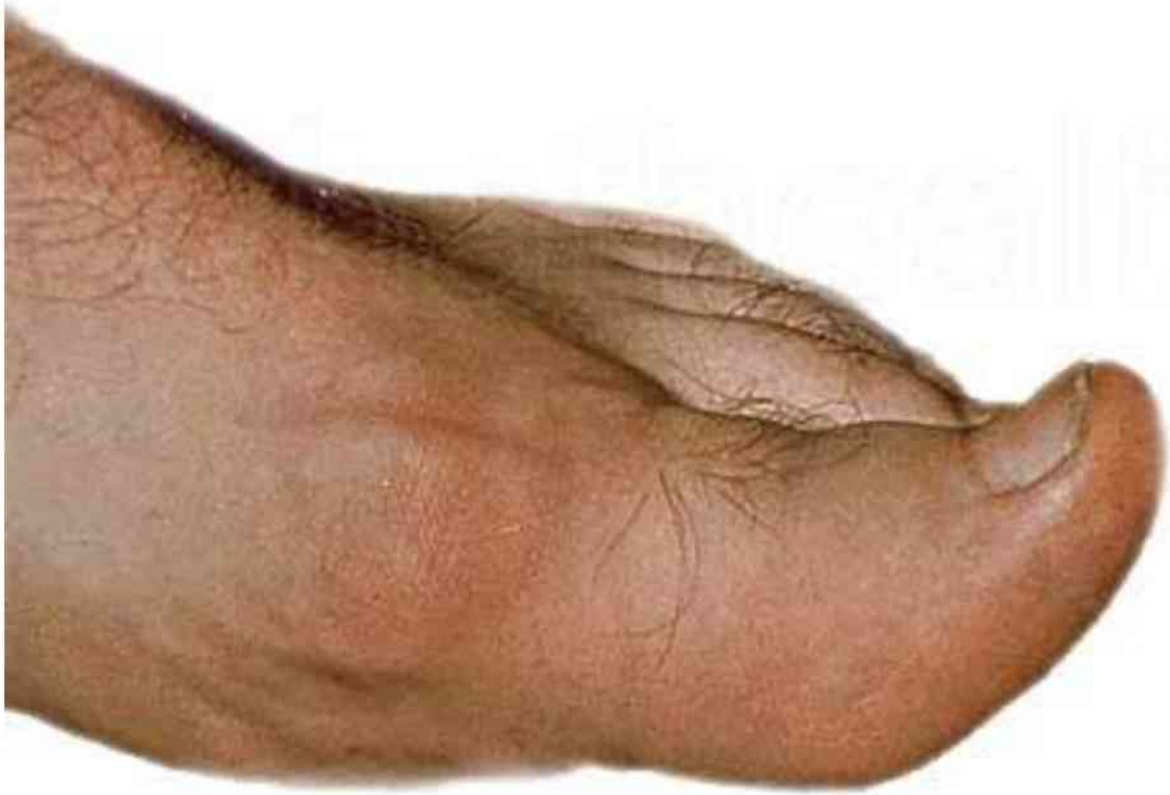
- **A 45 YEAR OLD MAN AWOKE FROM SLEEP WITH A PAINFUL AND SWOLLEN RIGHT GREAT TOE. ON THE PREVIOUS NIGHT HE HAD EATEN A MEAL OF FRIED LIVER AND ONIONS, AFTER WHICH HE MET WITH HIS POKER GROUP AND DRANK A NUMBER OF BEERS.**
- **HE SAW HIS DOCTOR THAT MORNING, "GOUTY ARTHRITIS" WAS DIAGNOSED, AND SOME TESTS WERE ORDERED. HIS SERUM URIC ACID LEVEL WAS ELEVATED AT 8.0 mg/dL (NL < 7.0 mg/dL).**
- **THE MAN RECALLED THAT HIS FATHER AND HIS GRANDFATHER, BOTH OF WHOM WERE ALCOHOLICS, OFTEN COMPLAINED OF JOINT PAIN AND SWELLING IN THEIR FEET.**

A CASE STUDY : GOUT

- **THE DOCTOR RECOMMENDED THAT THE MAN USE NSAIDS FOR PAIN AND SWELLING, INCREASE HIS FLUID INTAKE (BUT NOT WITH ALCOHOL) AND REST AND ELEVATE HIS FOOT. HE ALSO PRESCRIBED ALLOPURINOL.**
- **A FEW DAYS LATER THE CONDITION HAD RESOLVED AND ALLOPURINOL HAD BEEN STOPPED. A REPEAT URIC ACID LEVEL WAS OBTAINED (7.1 mg/dL). THE DOCTOR GAVE THE MAN SOME ADVICE REGARDING LIFE STYLE CHANGES.**

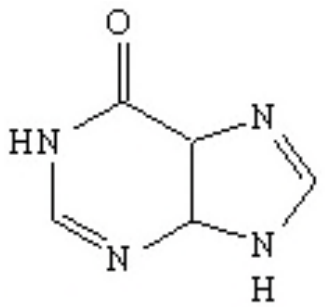
Gout

- **Impaired excretion or overproduction of uric acid**
- **Uric acid crystals precipitate into joints (Gouty Arthritis), kidneys, ureters (stones)**
- **Lead impairs uric acid excretion – lead poisoning from pewter drinking goblets**
  - **Fall of Roman Empire?**
- **Xanthine oxidase inhibitors inhibit production of uric acid, and treat gout**
- **Allopurinol treatment – hypoxanthine analog that binds to Xanthine Oxidase to decrease uric acid production**

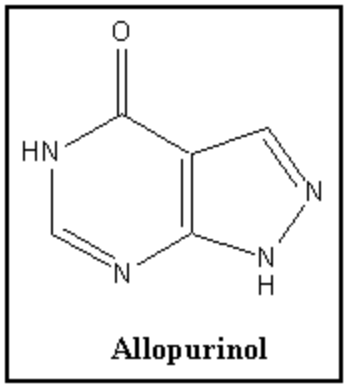


ALLOPURINOL IS A XANTHINE OXIDASE INHIBITOR

A SUBSTRATE ANALOG IS CONVERTED TO AN INHIBITOR, IN THIS CASE A “SUICIDE-INHIBITOR”



Hypoxanthine



Allopurinol

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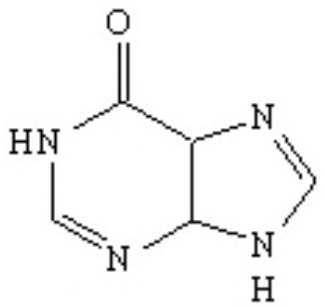
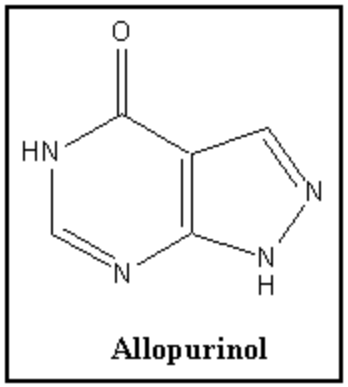


Hypoxanthine



Allopurinol

Madenet Al elm University College
Department of Biology
2nd Year Biochemistry I 2021 – 2022

Enzymes

Structure, Classification and Mechanism of Action

Dr. Hamza Yaseen Isa
Lecture 7 I 2

Objectives:

- Concept for enzymes.
- Mechanism of enzyme action.
- Factors affect rate of enzyme action.
- Enzyme specificity.
- Enzyme kinetics (Km & Vmax).
- Enzyme inhibition.
- Regulation of enzyme activity.
- Clinical uses of enzymes in diagnosis and prognosis of different diseases.
- Classes of enzymes.
- Coenzymes.

Importance

- Enzymes play an important role in Metabolism, Diagnosis, and Therapeutics.
- All biochemical reactions are enzyme catalyzed in the living organism.
- Level of enzyme in blood are of diagnostic importance e.g. it is a good indicator in disease such as myocardial infarction.
- Enzyme can be used therapeutically such as digestive enzymes.

Define enzymes
(Enzymes as Biological Catalysts)

- **Enzymes** are proteins that increase the rate of reaction by lowering the energy of activation

- They catalyze nearly all the chemical reactions taking place in the cells of the body.

- Not altered or consumed during reaction.

Reusable

What is the difference between an enzyme and a protein?

Protein enzymes are classified into **2 types:**

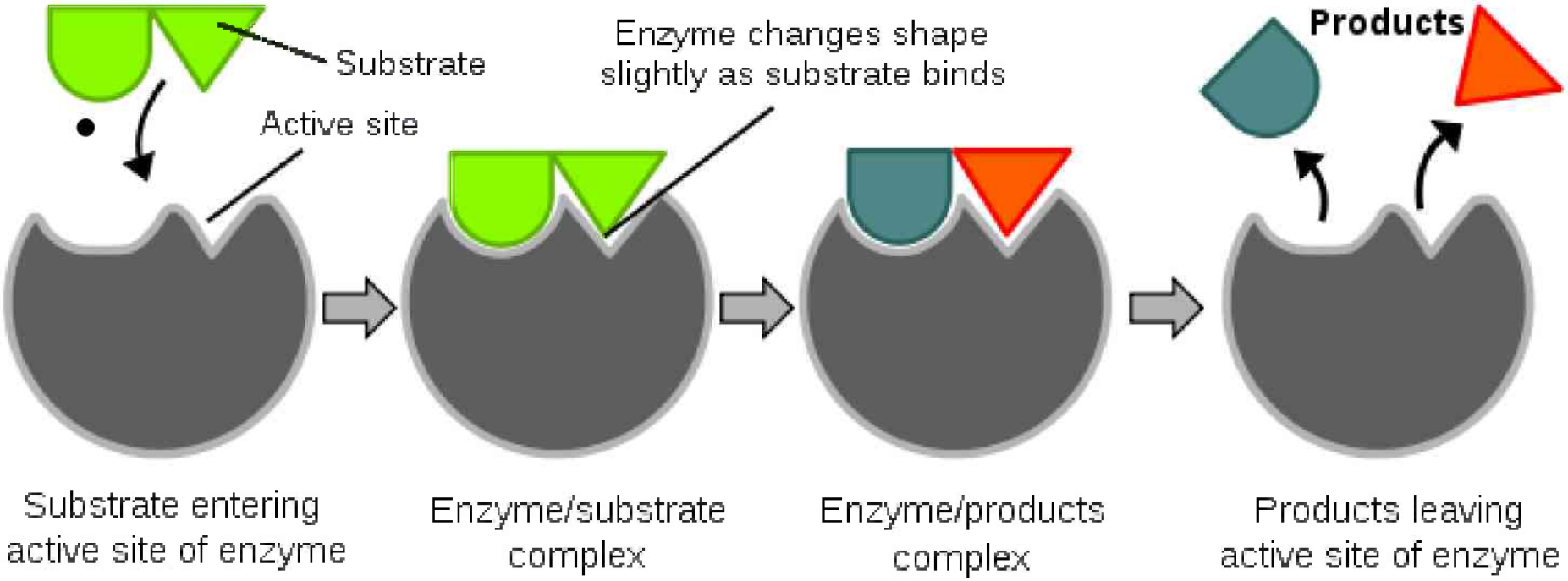
1- Simple Protein enzymes: They are formed of protein only.

2- Complex (conjugated) Protein : They are formed of protein part and non protein part.

- **All enzymes are proteins except some RNAs**
- **not all proteins are enzymes**

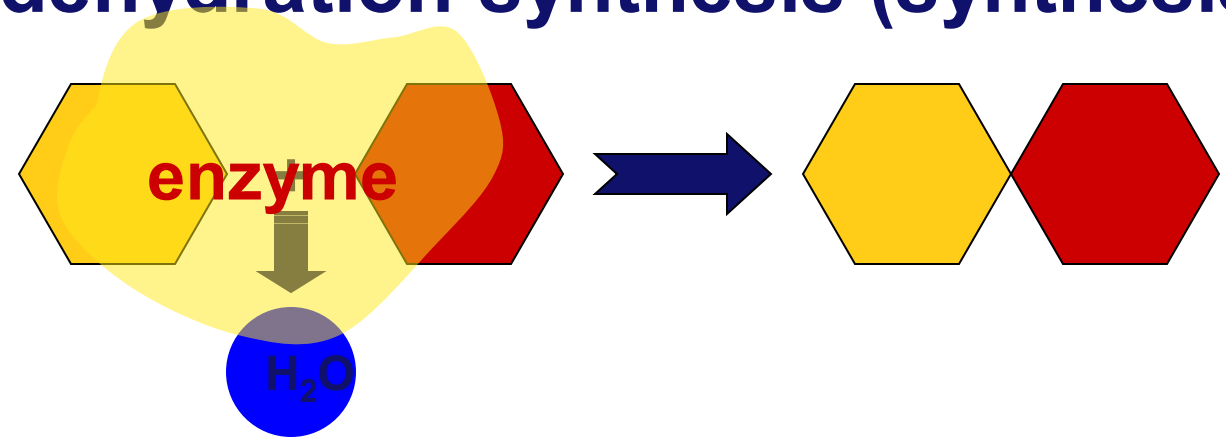
ACTIVE SITES

- **Enzyme molecules contain a special pocket or cleft called the active sites. The area on the enzyme where the substrate or substrates attach to is called the active site.**
- **Enzymes are usually very large proteins and the active site is just a small region of the enzyme molecule.**

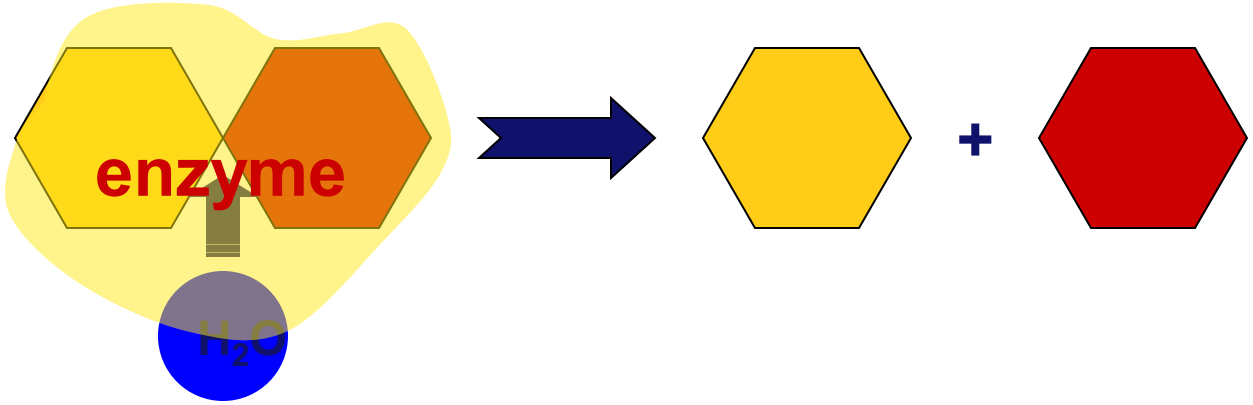


Examples

- **dehydration synthesis (synthesis)**

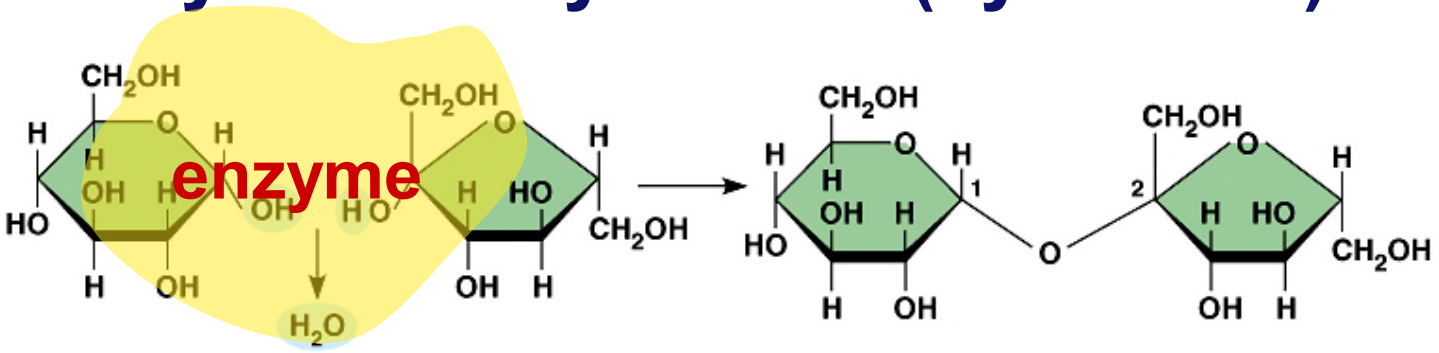


- **hydrolysis (digestion)**



Examples

- **dehydration synthesis (synthesis)**



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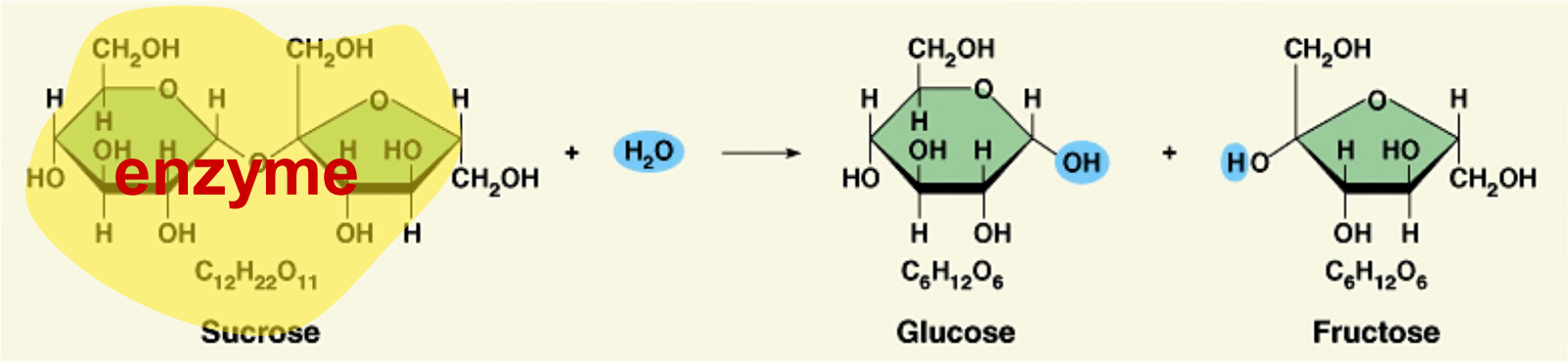


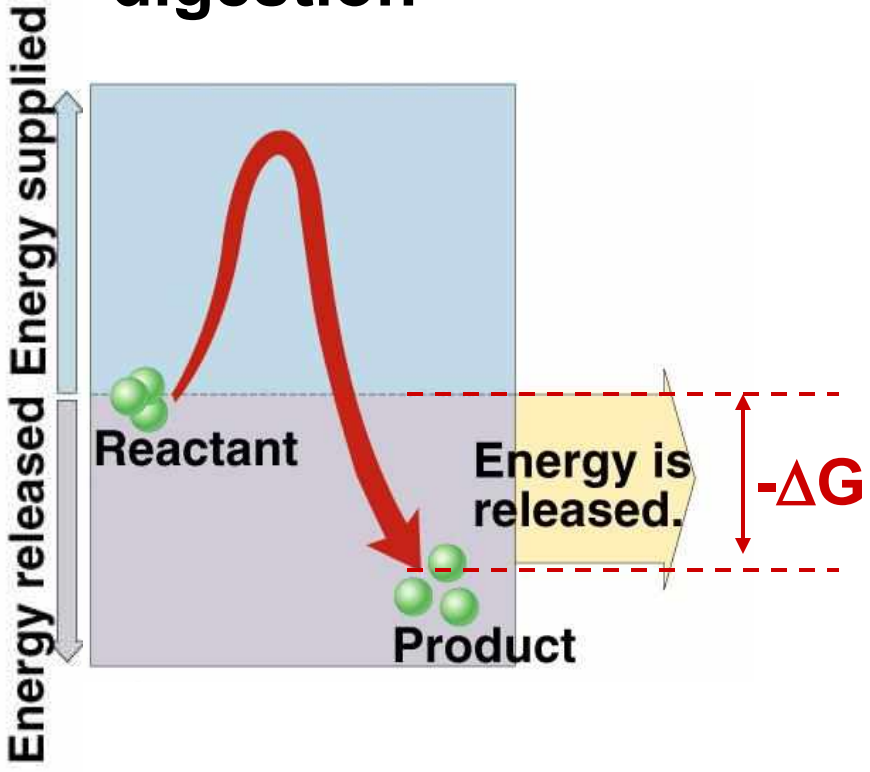
Chemical reactions & energy

- Some chemical reactions release energy
  - **Exergonic (catabolism)**
  - digesting polymers
  - hydrolysis = catabolism
- Some chemical reactions require input of energy
  - **Endergonic(anabolism)**
  - building polymers
  - dehydration synthesis = anabolism

Endergonic vs. exergonic reactions

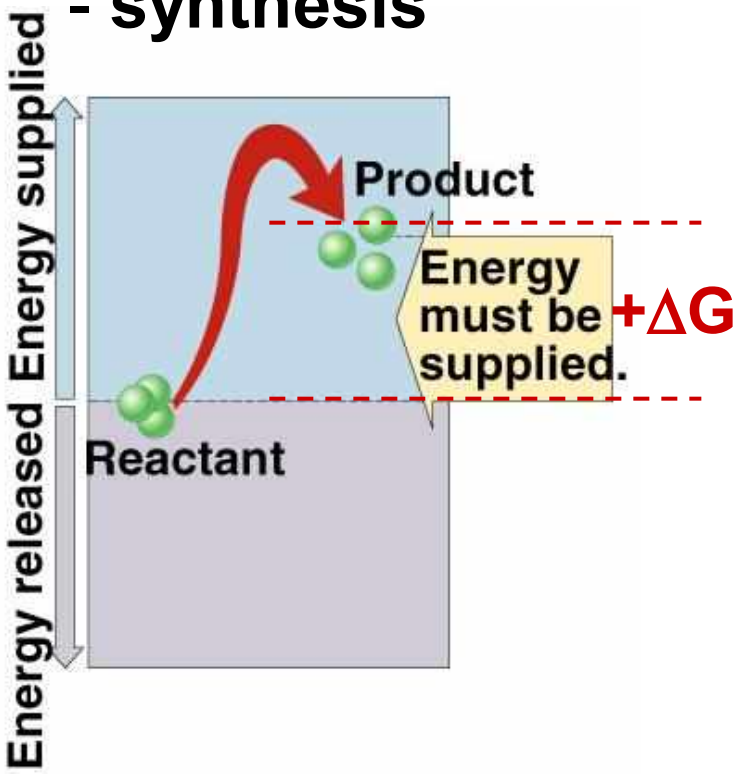
exergonic

- energy released
- digestion



endergonic

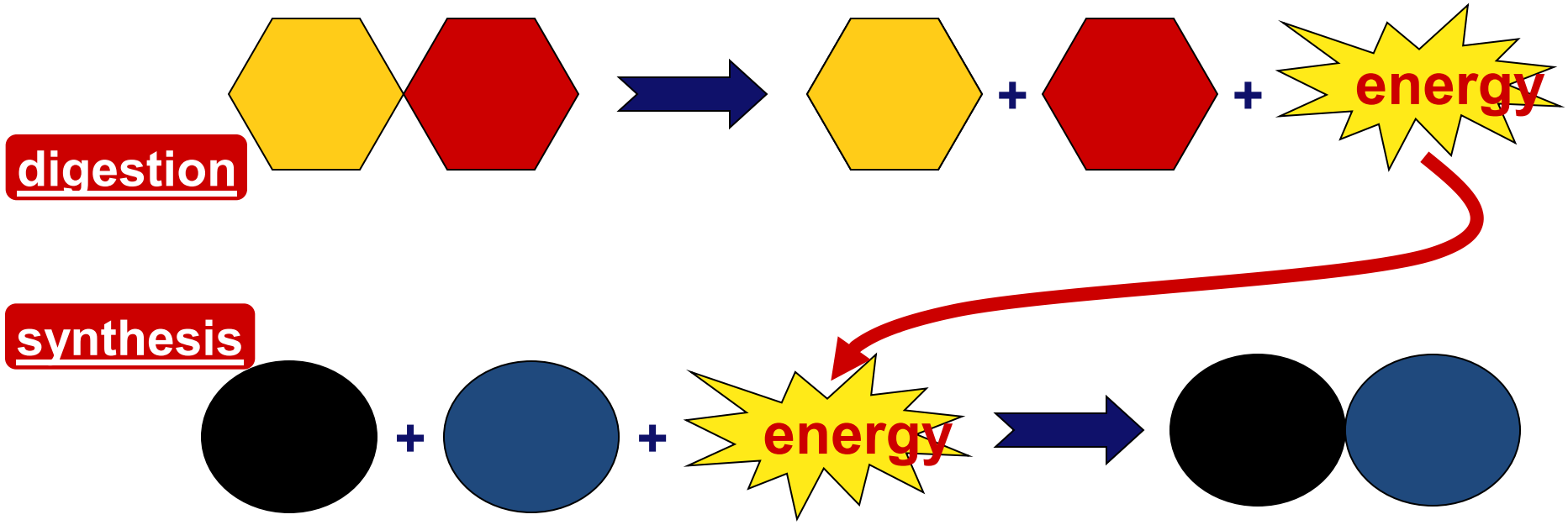
- energy invested
- synthesis



ΔG = change in free energy = ability to do work

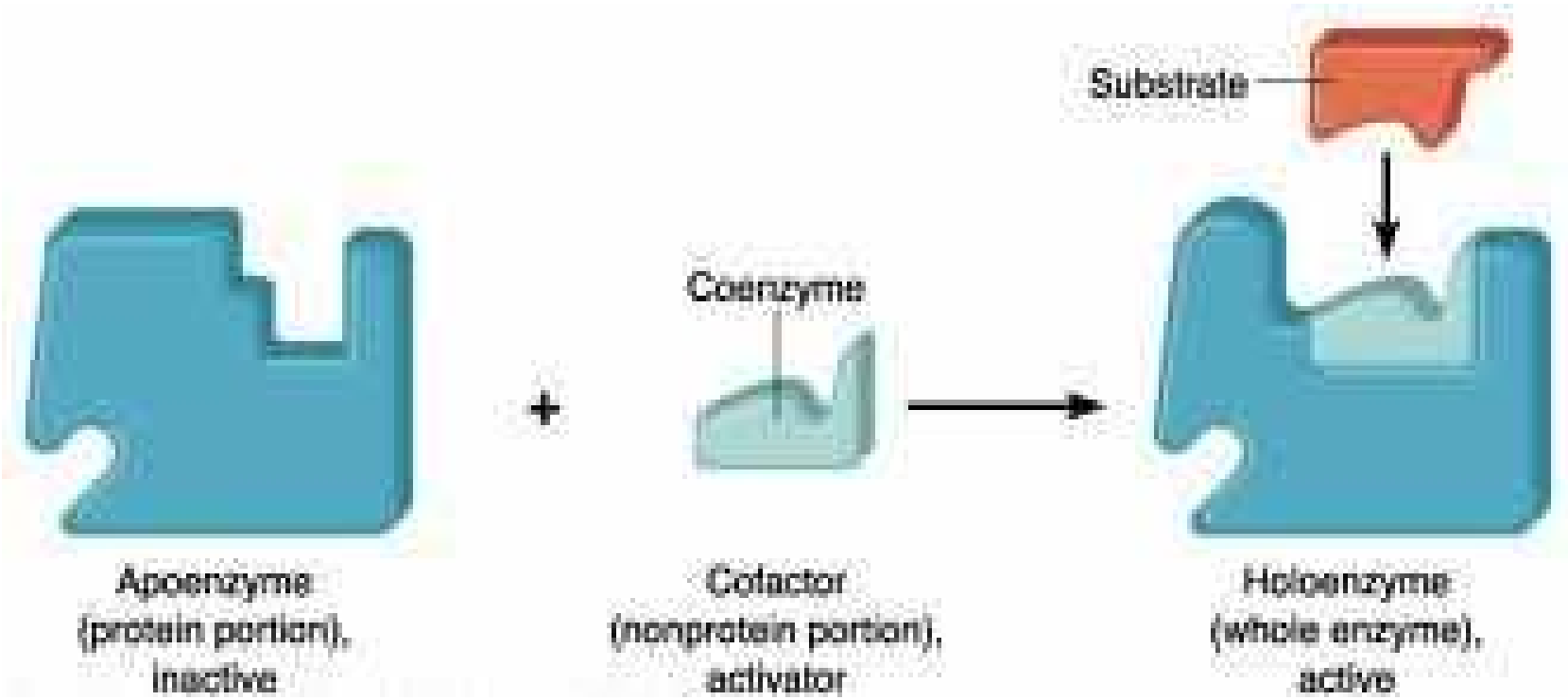
Energy & life

- Organisms require energy to live
  - where does that energy come from?
    - **coupling exergonic reactions (releasing energy) with endergonic reactions (needing energy)**



APOENZYME and HOLOENZYME

- The enzyme without its non protein moiety is termed as Apo enzyme and it is inactive.
- Holoenzyme is an active enzyme with its non protein moiety component.



Important Terms to Understand Biochemical Nature And Activity of Enzymes

- **Cofactor:**
  - A cofactor is a non-protein chemical compound that is bound (either tightly or loosely) to an enzyme and is required for catalysis.
  - Types of Cofactors:
    - Coenzymes.
    - Prosthetic groups.

Types of Cofactors

- **Coenzyme:**
  The non-protein component, loosely bound to apoenzyme by non-covalent bond.
- **Examples : vitamins or compound derived from vitamins ,ex : Vit B derivatives ; NAD and FAD.**
- **Prosthetic group**
  The non-protein component, tightly bound to the apoenzyme by covalent bonds is called a Prosthetic group.

Enzyme Specificity

- Enzymes have varying degrees of **specificity** for substrates
- Enzymes may recognize and catalyze:
  - a single substrate
  - a group of similar substrates
  - a particular type of bond

Activation energy or Energy of Activation:

All chemical reactions require some amount of energy to get them started.

OR

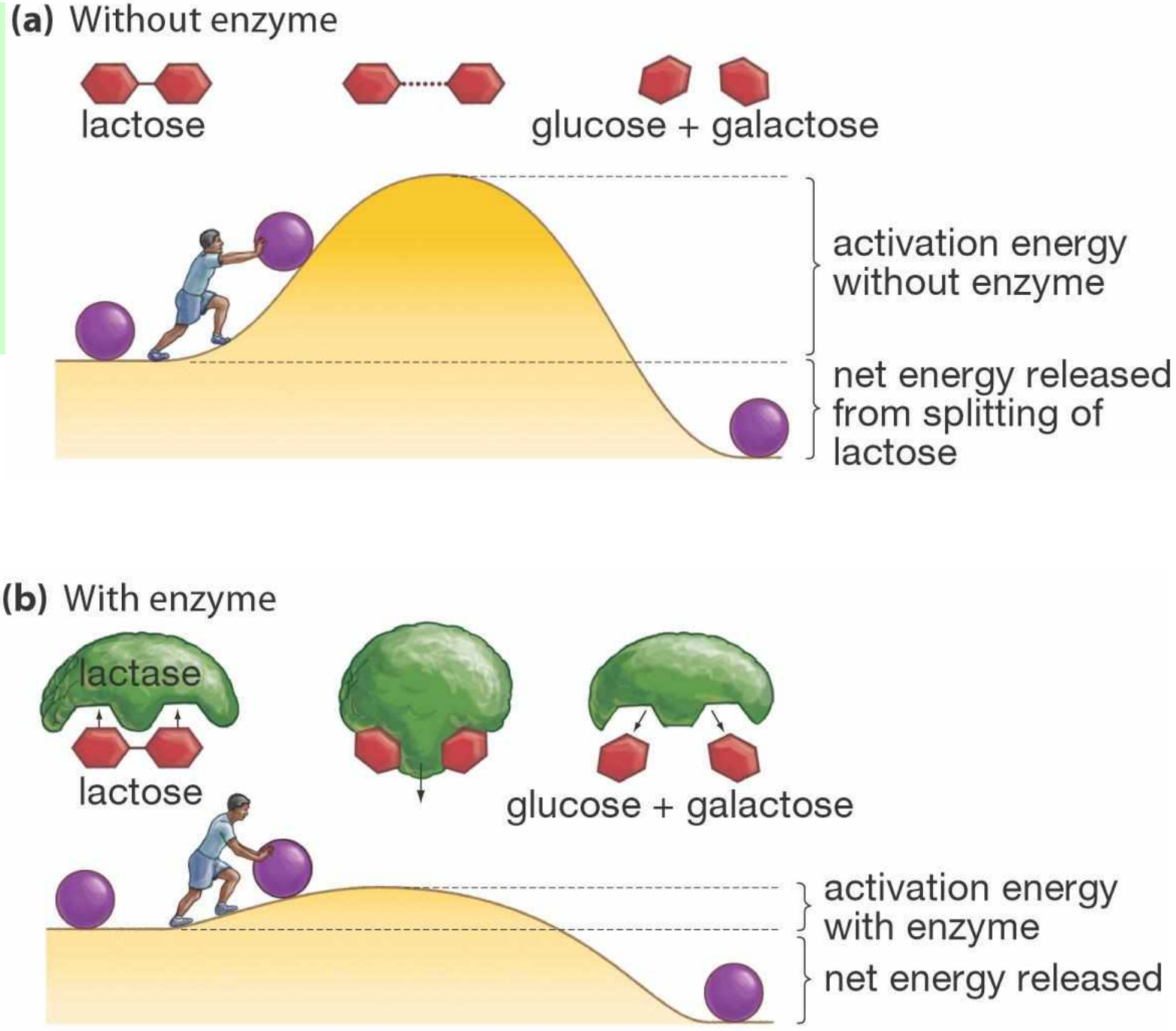
- **It is First push to start reaction.**

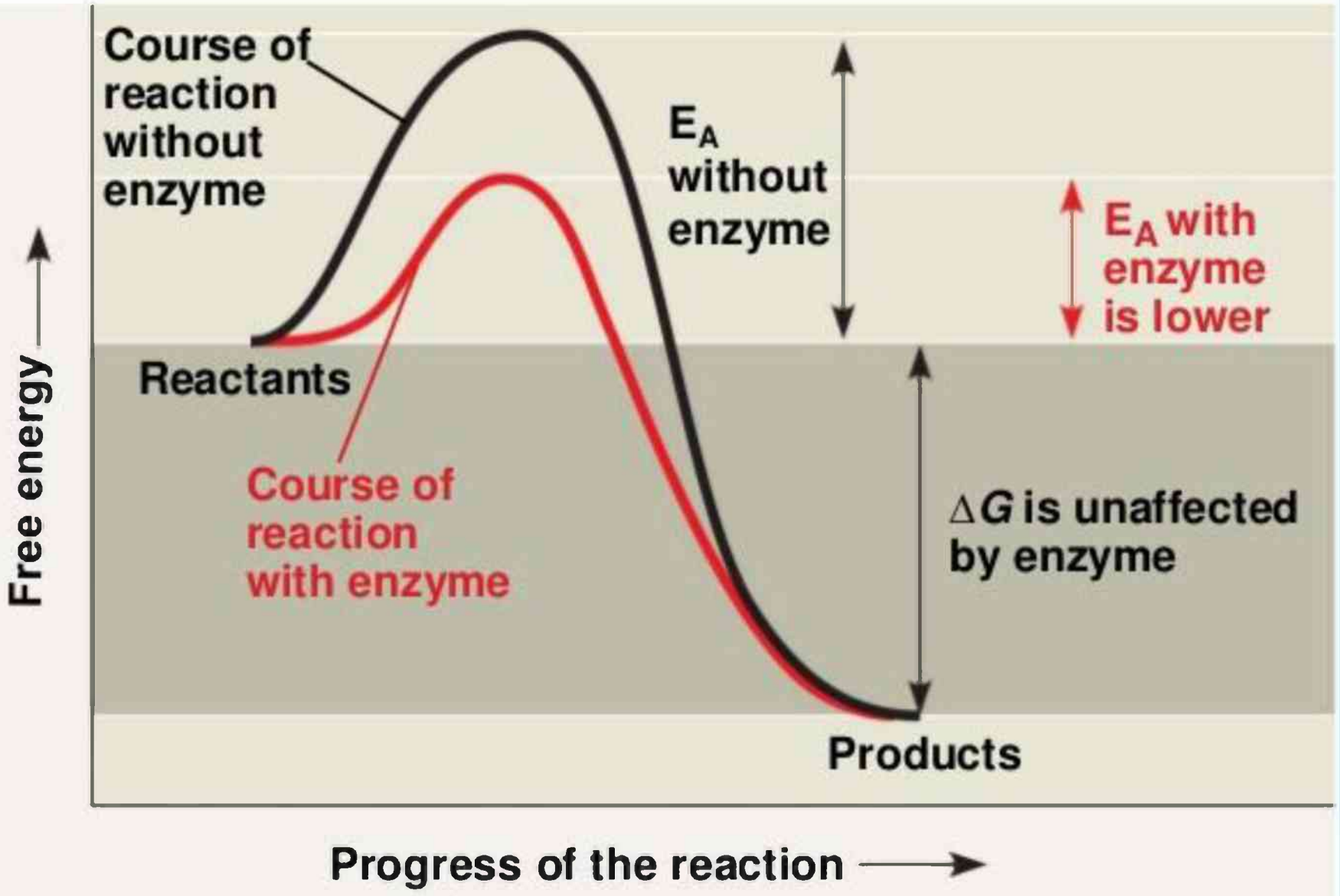
This energy is called activation energy.

Mechanism of Action of Enzymes

- **Enzymes increase reaction rates by decreasing the Activation energy:**
- **Enzyme-Substrate Interactions:**
  - **Formation of Enzyme substrate complex by:**
  - Lock-and-Key Model
  - Induced Fit Model

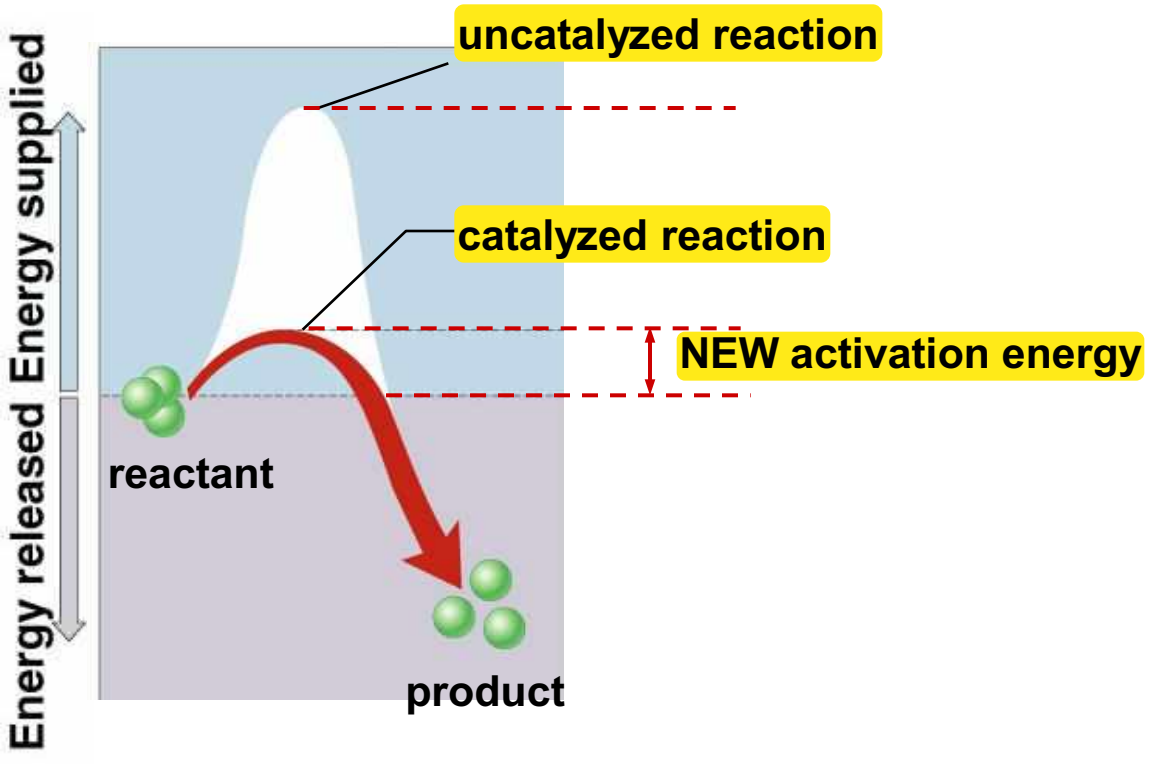
Enzymes Lower a Reaction's Activation Energy




Course of reaction without enzyme
E_A without enzyme
E_A with enzyme is lower
Reactants
Course of reaction with enzyme
ΔG is unaffected by enzyme
Products
Free energy
Progress of the reaction

Reducing Activation energy

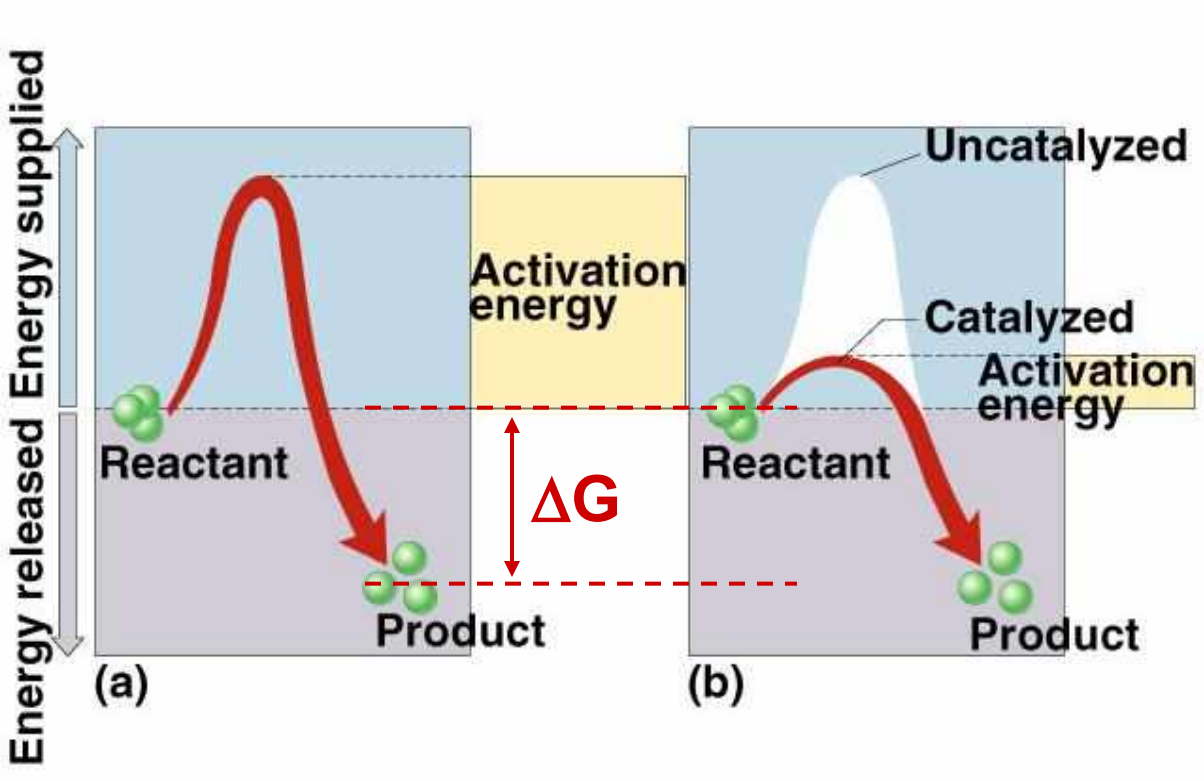
- Catalysts
  - reducing the amount of energy to start a reaction



Catalysts

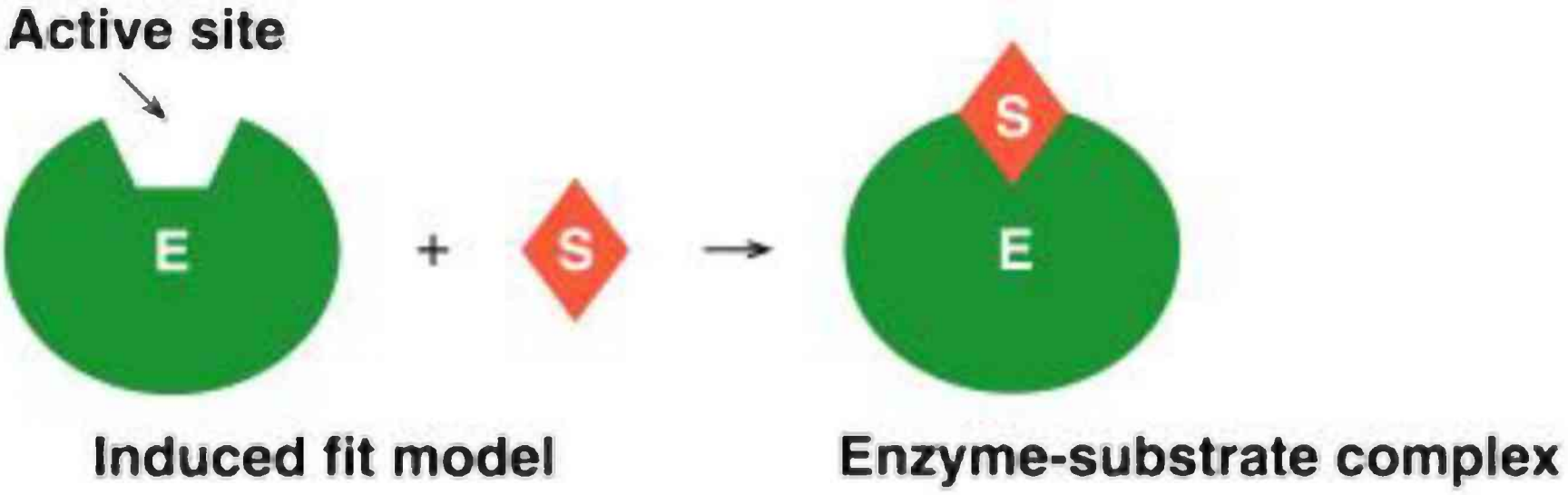
- So what's a cell got to do to reduce activation energy?
  - get help! … chemical help…

ENZYMES



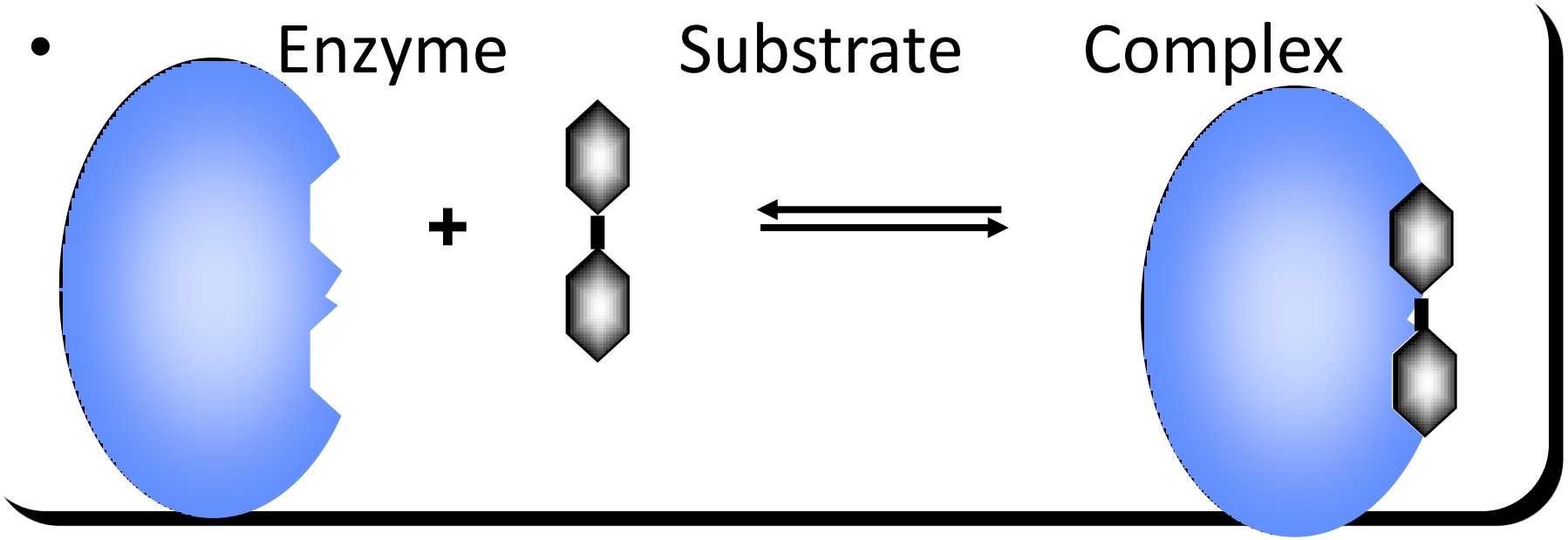
Induced Fit Model

- In the **induced-fit model** of enzyme action:
  - the active site is flexible, not rigid
  - the shapes of the enzyme, active site, and substrate adjust to maximumize the fit, which improves catalysis
  - there is a greater range of substrate specificity
- This model is more consistent with a wider range of



Enzyme-substrate complex

- Step 1:
- Enzyme and substrate combine to form complex
- E + S ⇌ ES
- Enzyme Substrate Complex

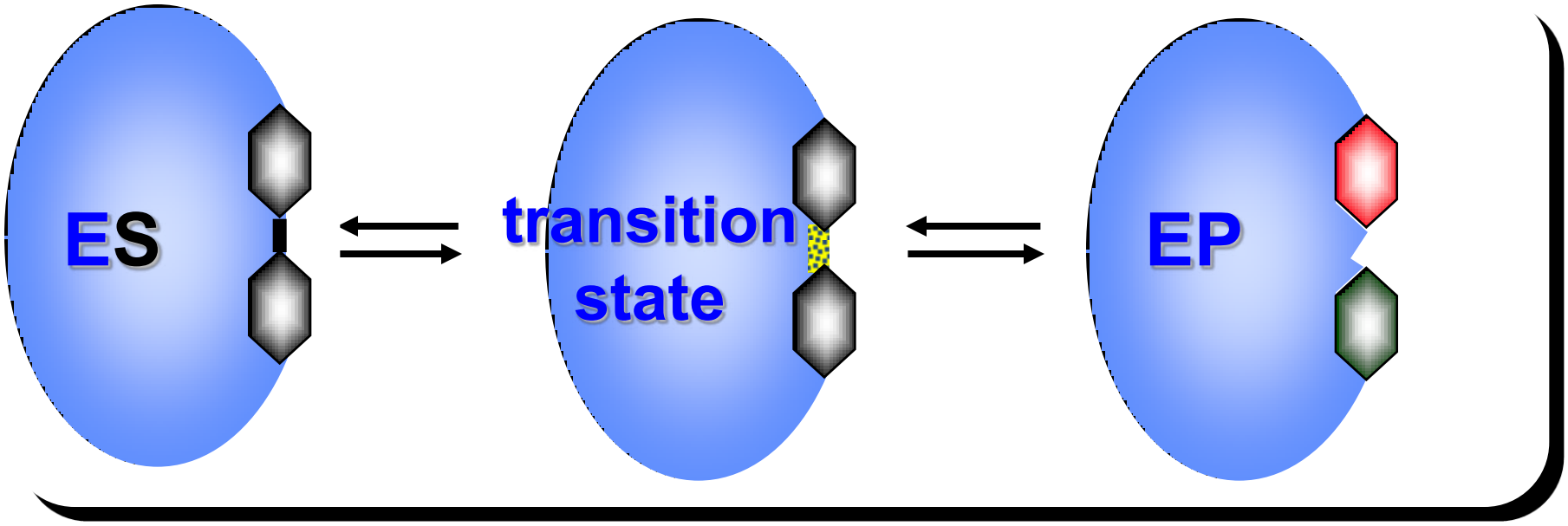


Enzyme-product complex

- Step 2: Within the active site of the ES complex, the reaction occurs to convert substrate to product (P)

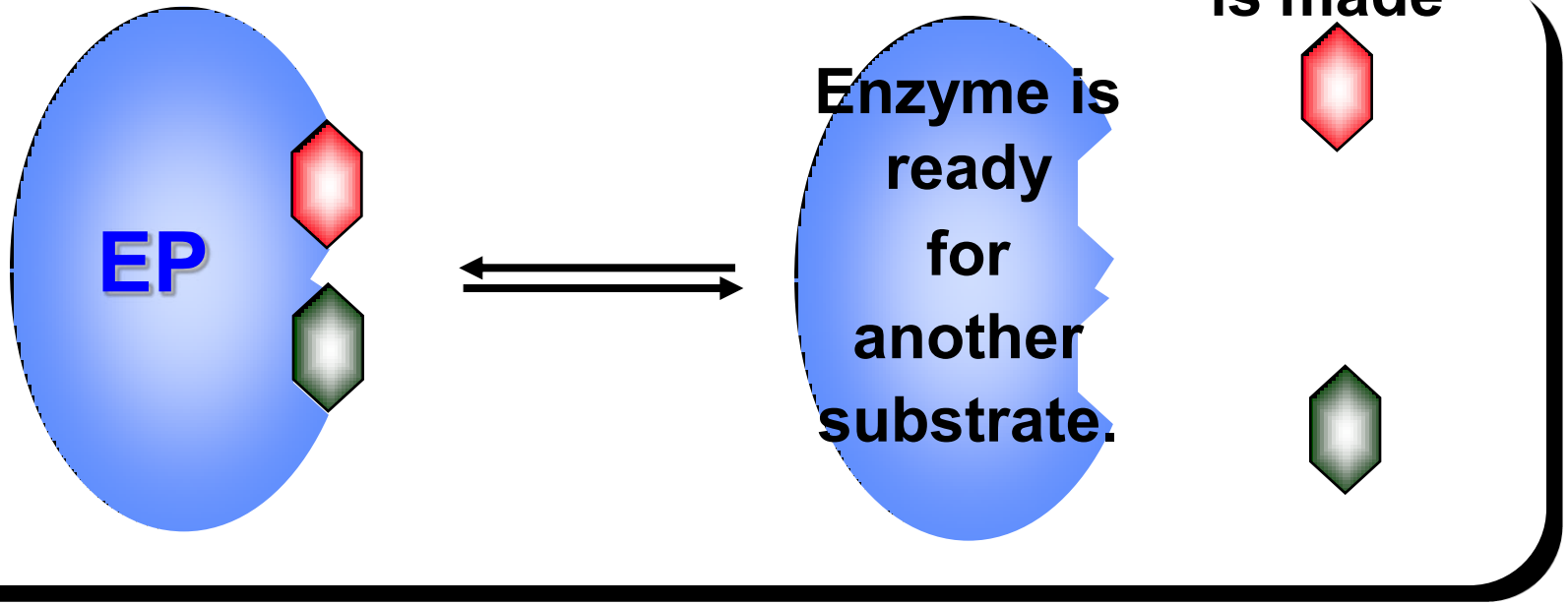
An enzyme-product complex is formed.

ES ⇌ EP



Product

- The enzyme and product separate
  - EP ⇌ E + P



What Affects Enzyme Activity?

- **Three factors:**
  1. **Environmental Conditions**
  2. **Cofactors and Coenzymes**
  3. **Enzyme Inhibitors**

1. Environmental Conditions

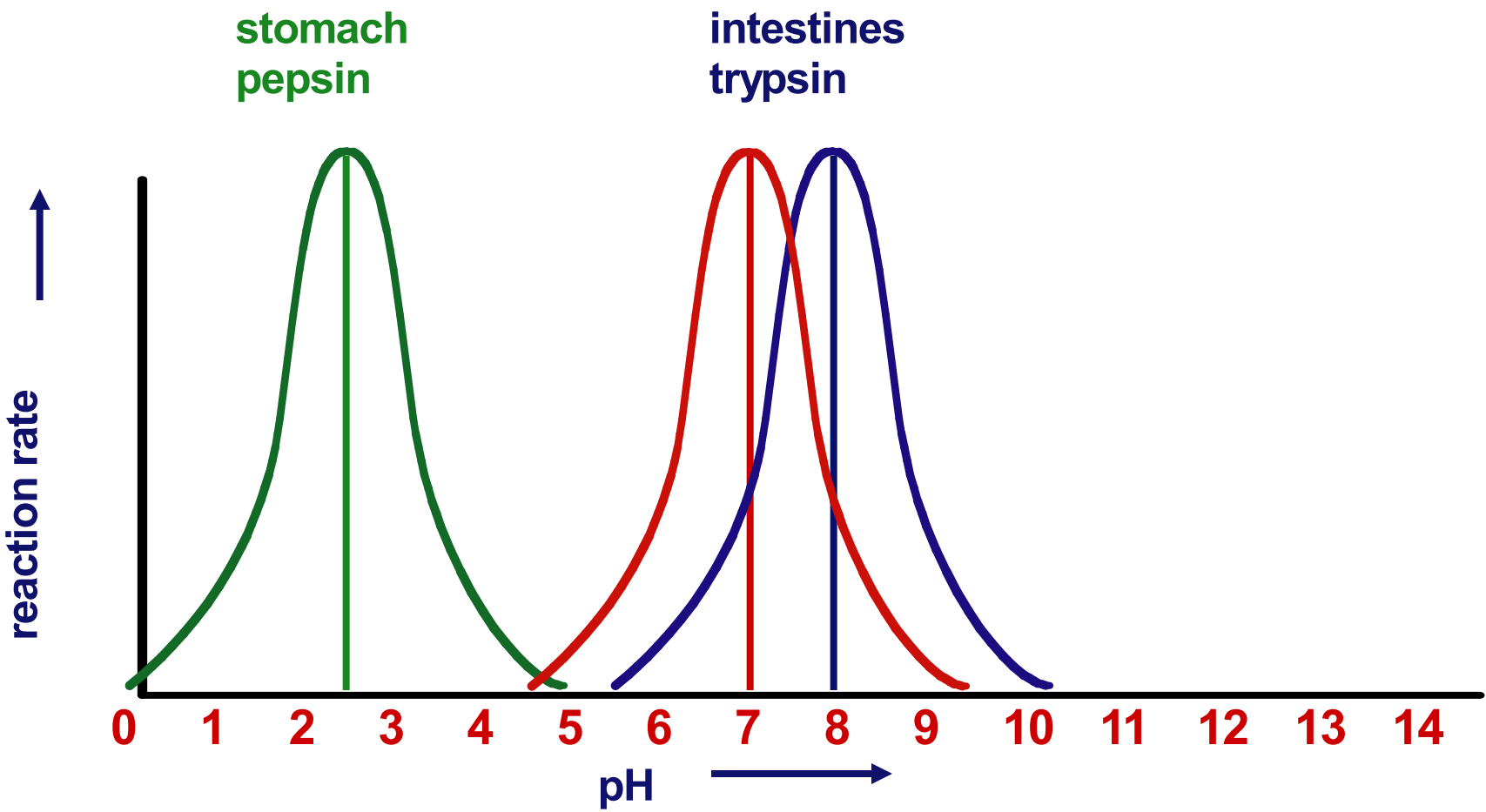
1. Extreme Temperature are the most dangerous

- high temps may denature (unfold) the enzyme.

2. pH (most like 6 - 8 pH near neutral)

3. substrate concentration .

pH


stomach
pepsin
intestines
trypsin
reaction rate
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
pH

2. Cofactors and Coenzymes

- **Inorganic substances (zinc, iron) and vitamins (respectively) are sometimes needed for proper enzymatic activity.**

- **Example:**

  Iron must be present in the quaternary structure - hemoglobin in order for it to pick up oxygen.

Naming Enzymes

- **The name of an enzyme in many cases end in *–ase***
- **For example, *sucrase* catalyzes the hydrolysis of sucrose**

- **The name describes the function of the enzyme**
  For example, *oxidases* catalyze oxidation reactions

- **Sometimes common names are used, particularly for the digestion enzymes such as *pepsin* and *trypsin***

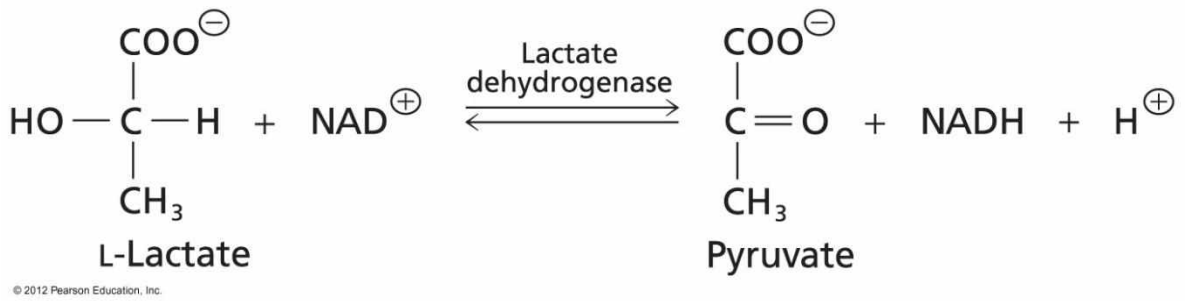
- **Some names describe both the substrate and the function**
- **For example, *alcohol dehydrogenase* oxides ethanol**

Enzymes Are Classified into six functional Classes (EC number Classification) by the International Union of Biochemists (I.U.B.). on the Basis of the Types of Reactions That They Catalyze

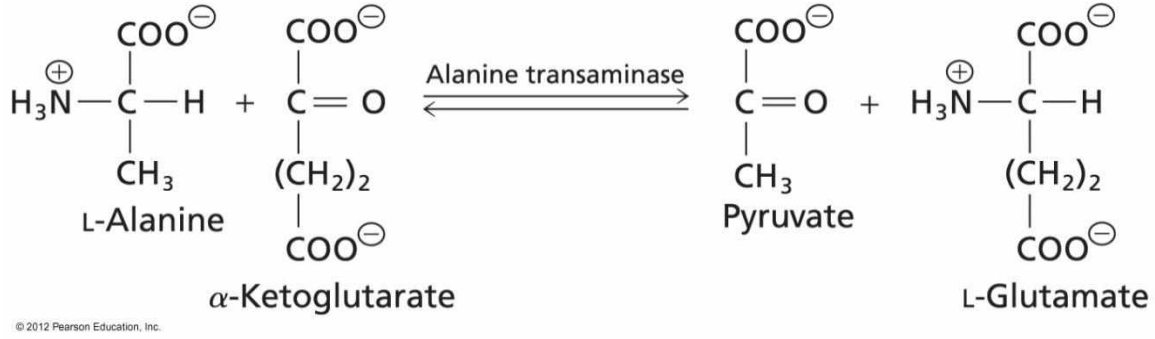
- **EC 1. Oxidoreductases**
- **EC 2. Transferases**
- **EC 3. Hydrolases**
- **EC 4. Lyases**
- **EC 5. Isomerases**
- **EC 6. Ligases**

Enzyme Classes

1. Oxidoreductase

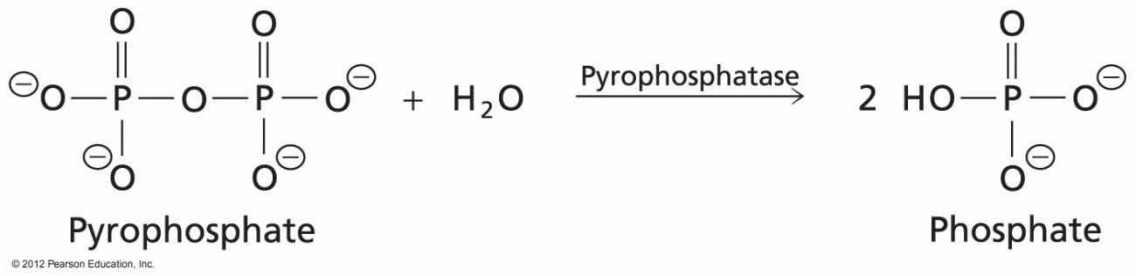


2. Transferase

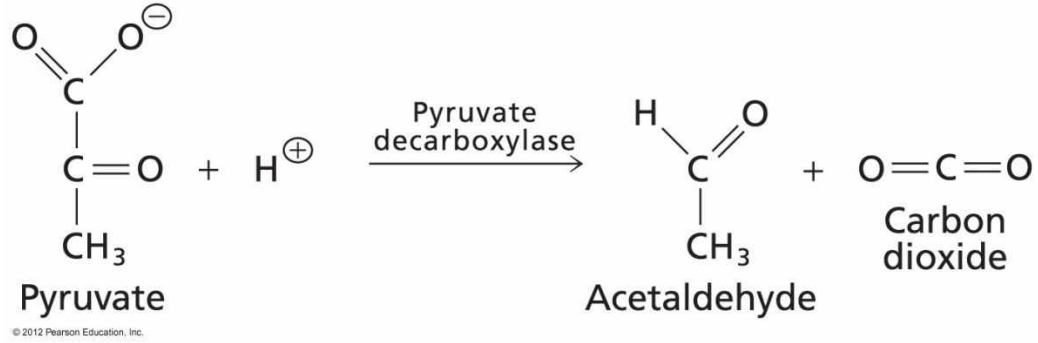


Enzyme Classes

3. Hydrolase



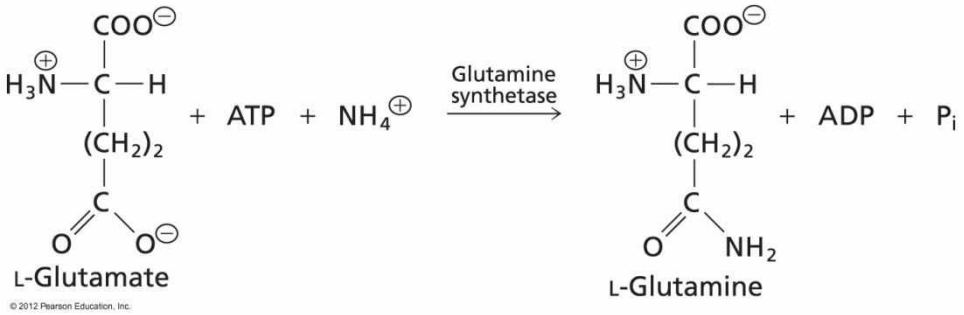
4. Lyase



Enzyme Classes

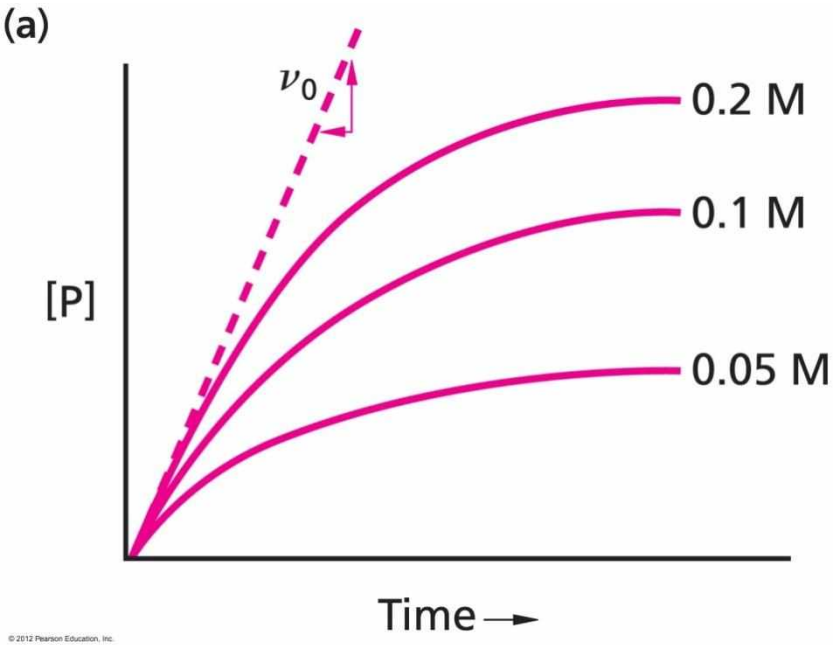
5. Isomerase

6. Ligase



Chemical Kinetics

- Rate: measure product formed per second
- Rate slows as reactant disappears
- Measure initial rate
- Do a second experiment with more starting material, and the initial rate is faster



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Department of Biology
2nd Year Biochemistry I 2021 – 2022

BIOENERGETICS

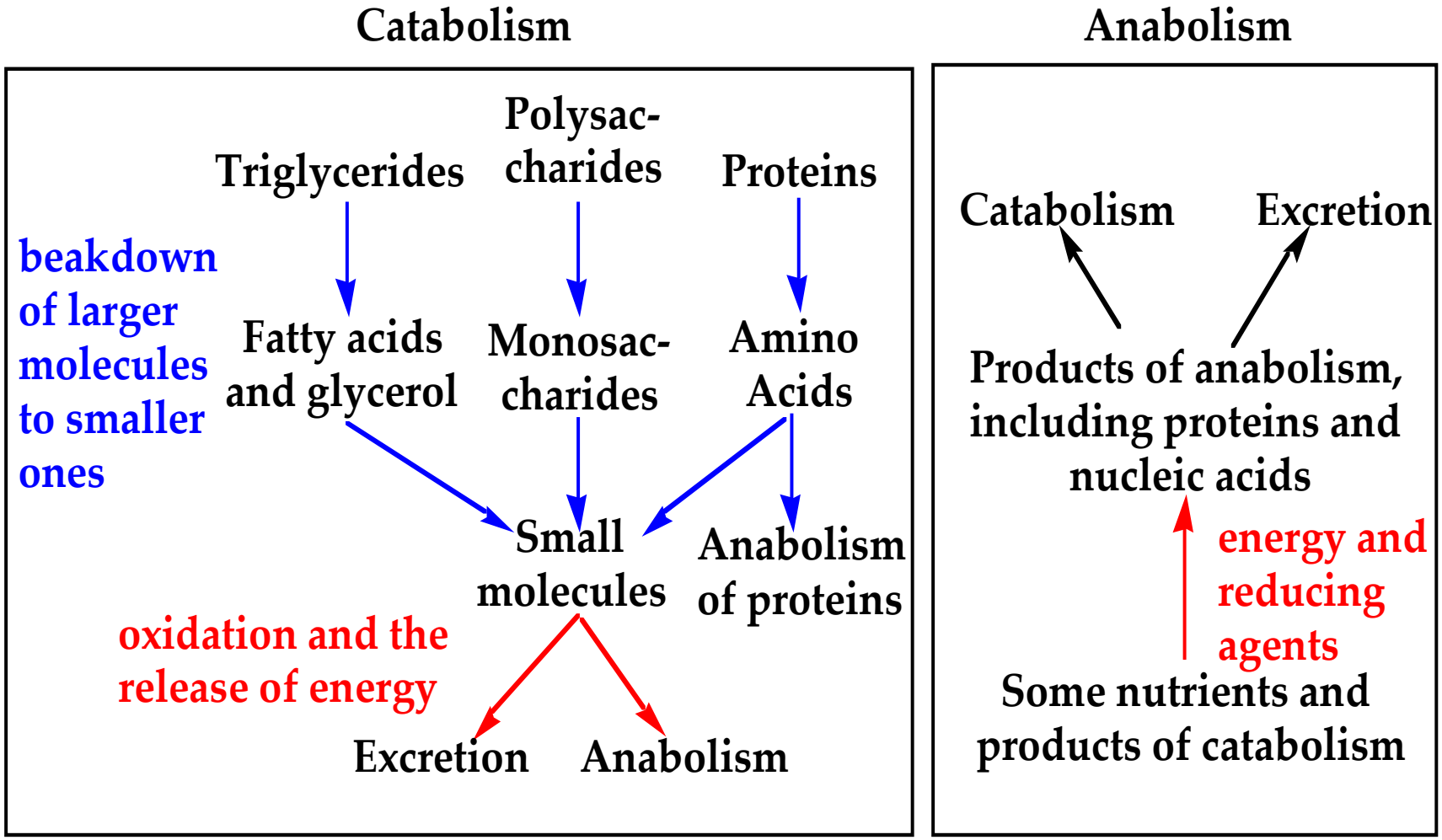
Dr. Hamza Yaseen Isa
Lecture 81 2

Metabolism

- **Metabolism:** the sum of all chemical reactions involved in maintaining the dynamic state of a cell or organism.
  - **Pathway:** a series of biochemical reactions.
  - **Catabolism:** the biochemical pathways that are involved in generating energy by breaking down large nutrient molecules into smaller molecules with the concurrent production of energy.
  - **Anabolism:** the pathways by which biomolecules are synthesized.

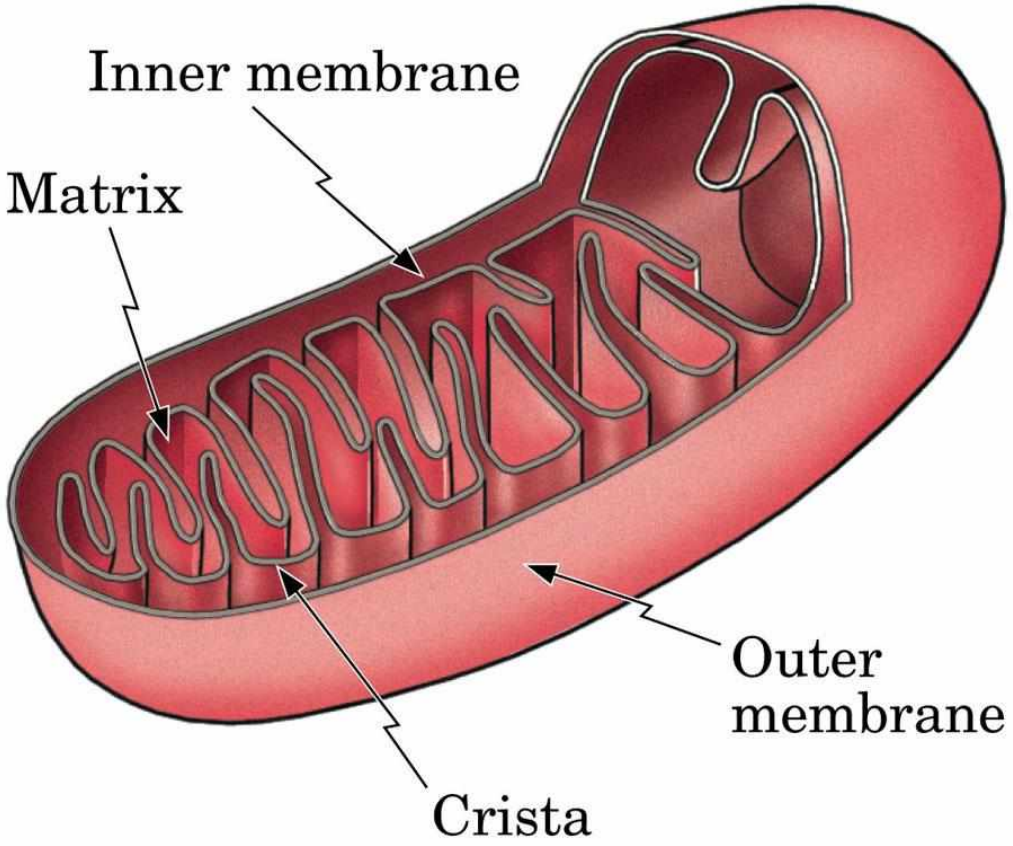
Metabolism

– **Metabolism is the sum of catabolism and anabolism.**



A Mitochondrion

organelles in which the common catabolic pathway takes place in higher organisms; the purpose of this catabolic pathway is to convert the energy stored in food molecules into energy stored in molecules of ATP.

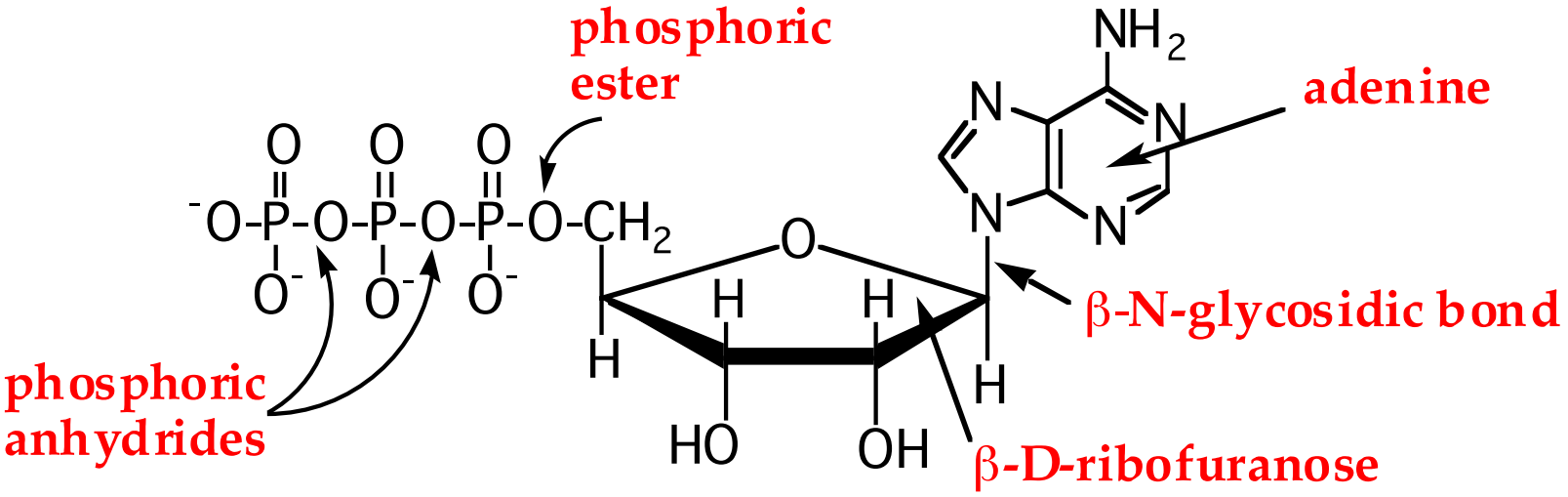


Common Catabolic Pathway

- The two parts to the common catabolic pathway:
  - The glycolysis and citric acid cycle, also called the tricarboxylic acid (TCA) or Krebs cycle.
  - **Electron transport chain and phosphorylation, together called oxidative phosphorylation**
  - .
- Four principal compounds participating in the common catabolic pathway are:
  - AMP, ADP, and ATP
  - NAD^+/NADH
  - FAD/$FADH_2$
  - coenzyme A; abbreviated CoA or CoA-SH

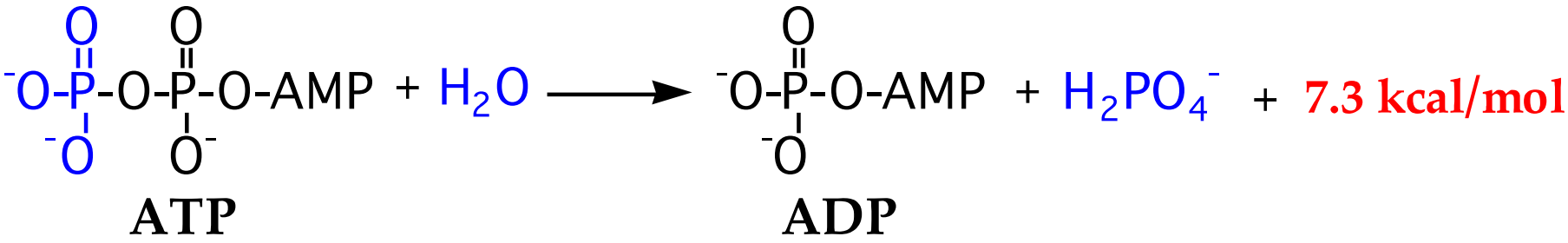
Adenosine Triphosphate

- **ATP** is the most important compound involved in the transfer of phosphate groups.
  - ATP contains two phosphoric anhydride bonds and one phosphoric ester bond.



ATP

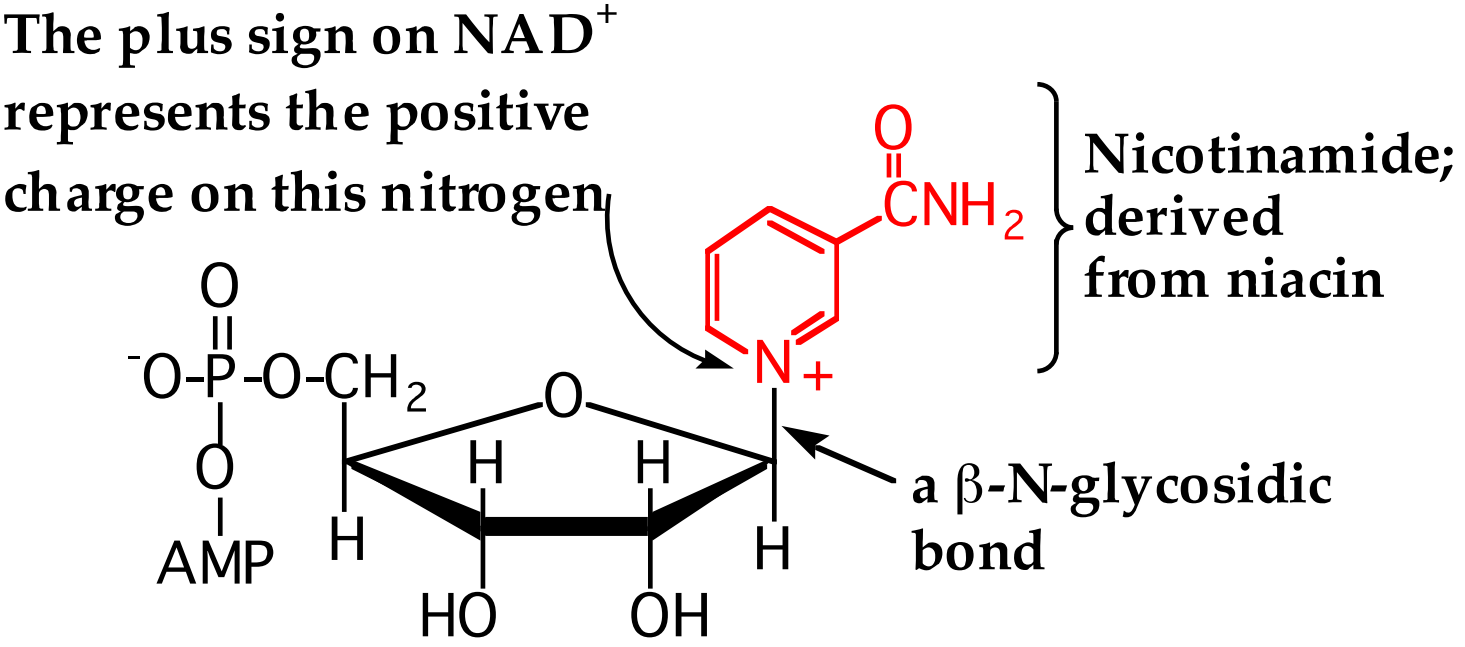
– Hydrolysis of the terminal phosphate (anhydride) of ATP gives ADP, phosphate ion, and energy.



– Hydrolysis of a phosphoric anhydride liberates more energy than hydrolysis of a phosphoric ester.
– We say that ATP and ADP contain two high-energy phosphoric anhydride bonds.
– ATP is a universal carrier of phosphate groups.
– ATP is also a common currency for the storage and transfer of energy.

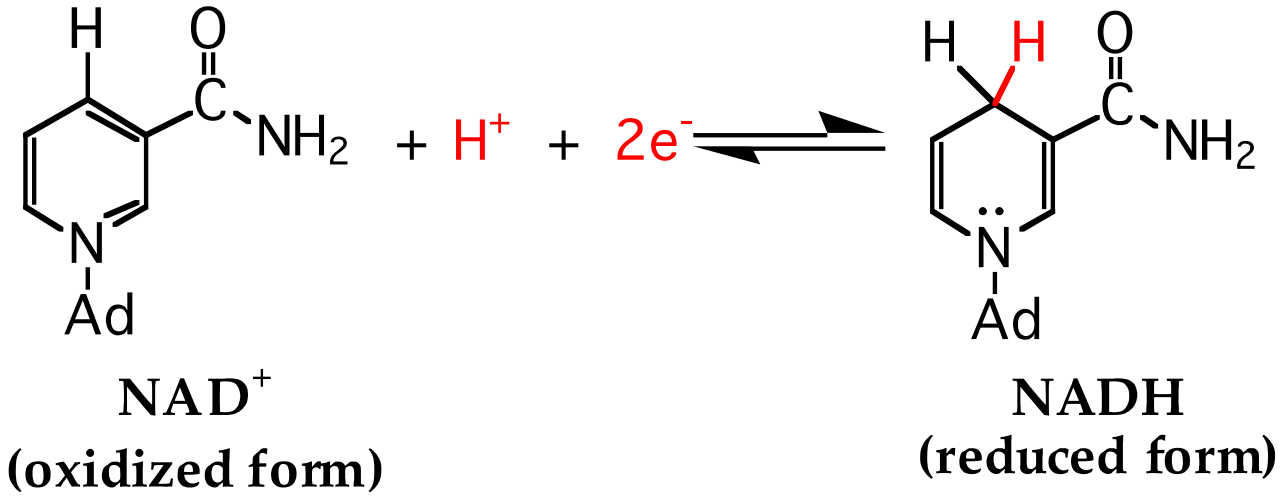
$NAD^+/NADH_2$

- **Nicotinamide adenine dinucleotide (NAD^+)** is a biological oxidizing agent.



$NAD^+/NADH$

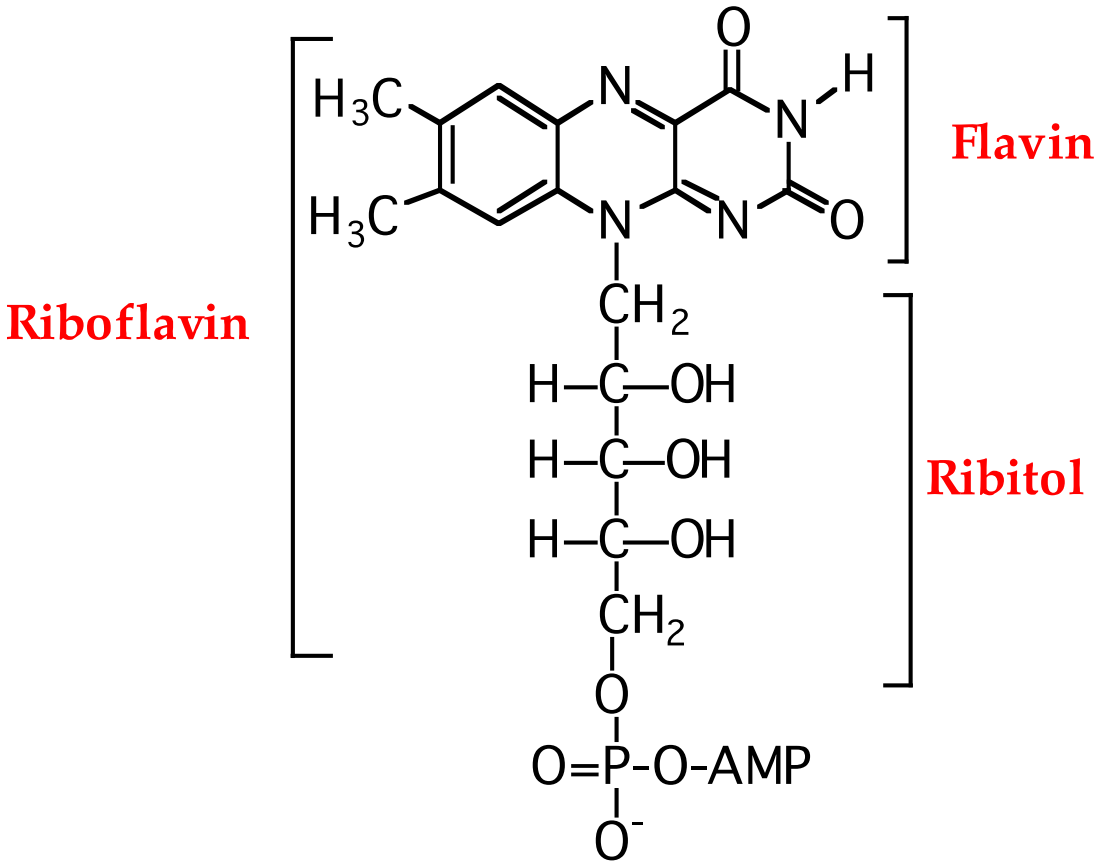
- NAD^+ is a two-electron oxidizing agent, and is reduced to NADH.
- NADH is a two-electron reducing agent, and is oxidized to NAD^+.



- **NADH is an electron and hydrogen ion transporting molecule.**

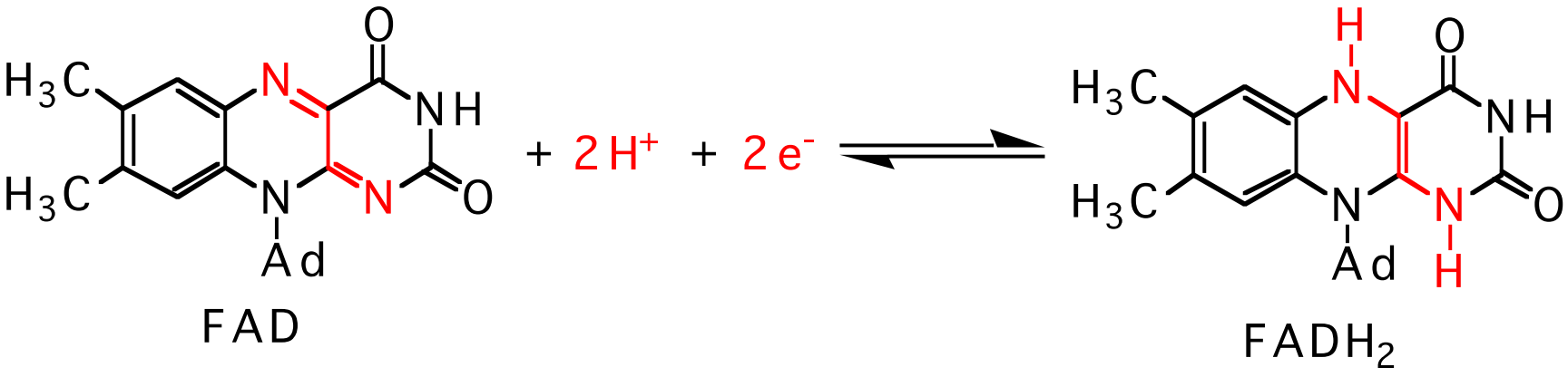
$FAD/FADH_2$

- **Flavin adenine dinucleotide (FAD)** is also a biological oxidizing agent.



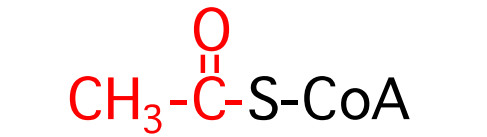
$FAD/FADH_2$

- FAD is a two-electron oxidizing agent, and is reduced to $FADH_2$.
- $FADH_2$ is a two-electron reducing agent, and is oxidized to FAD.



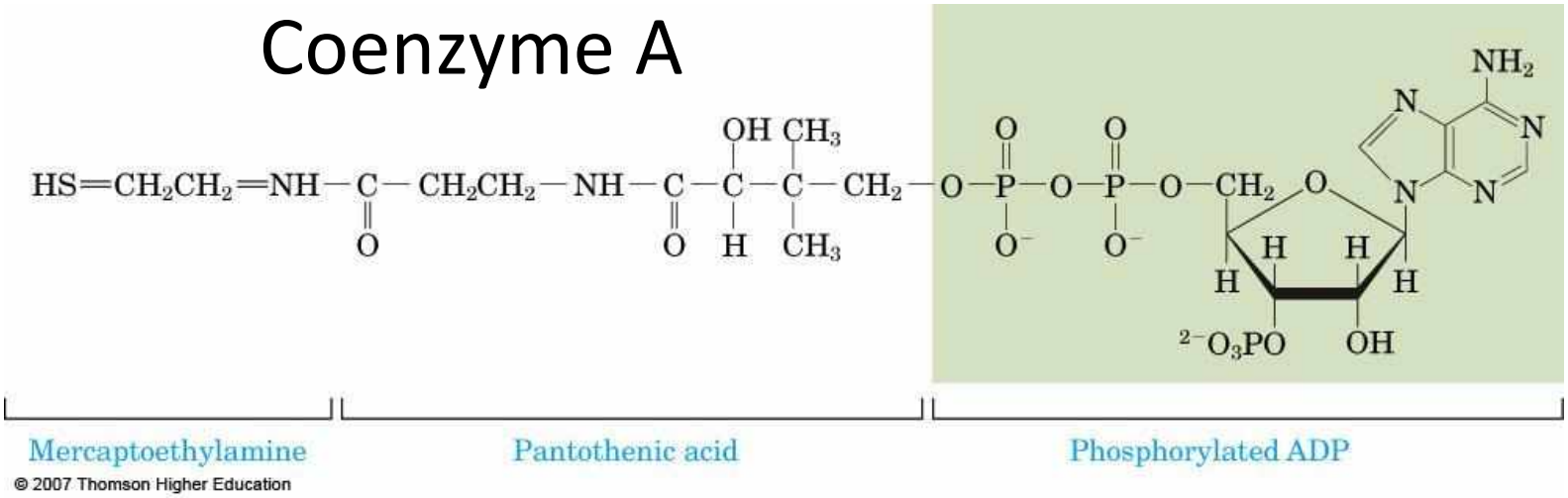
Coenzyme A

- **Coenzyme A (CoA)** is an acetyl-carrying group.
  - Like NAD^+ and FAD, coenzyme A contains a unit of ADP
  - CoA is often written **CoA-SH** to emphasize the fact that it contains a sulfhydryl group.
  - The vitamin part of coenzyme A is pantothenic acid.
  - The acetyl group of acetyl CoA is bound as a high-energy thioester.

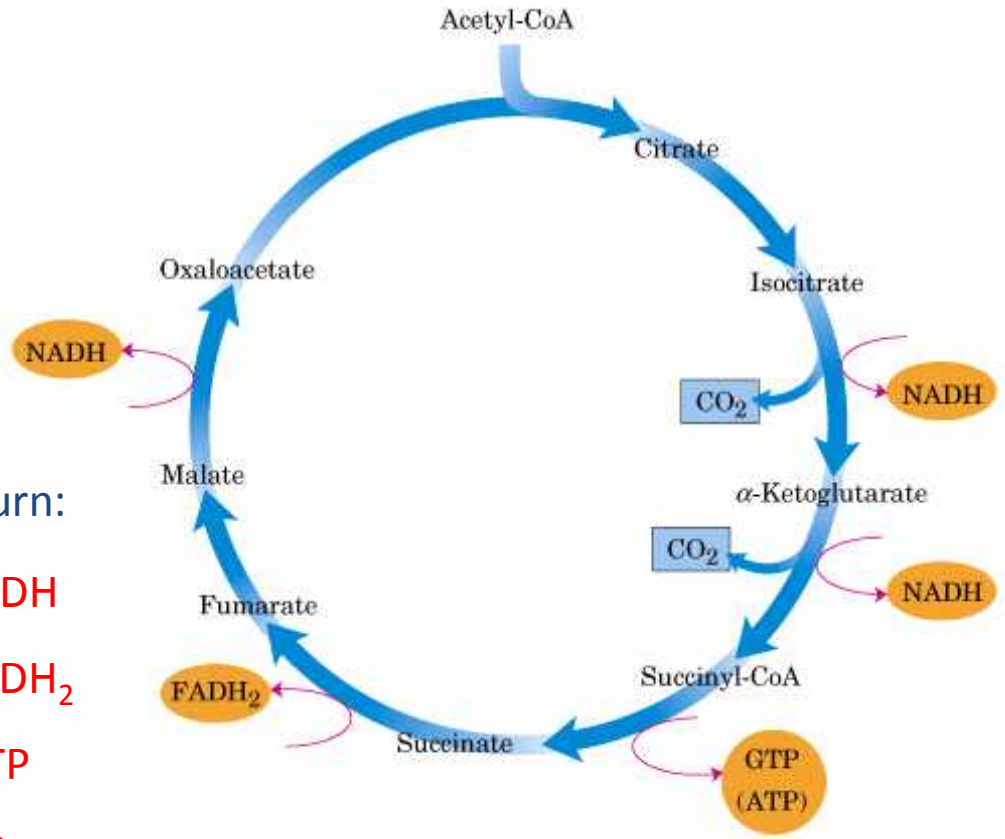


Acetyl coenzyme A
(An acyl CoA)

Coenzyme A



- Citric Acid Cycle
- Overview: the two carbon acetyl group of acetyl CoA is fed into the cycle and two CO_2 are given off.
- There are four oxidation steps in the cycle.

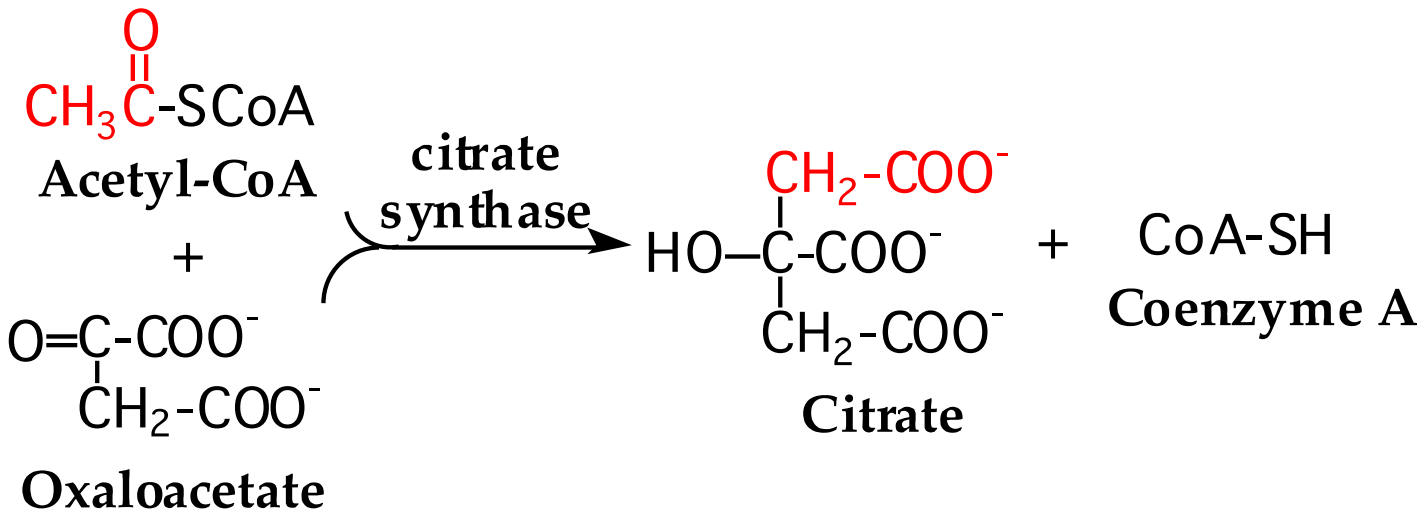


Per turn:

- 3 NADH
- 1 $FADH_2$
- 1 GTP
- 2 CO_2

Citric Acid Cycle

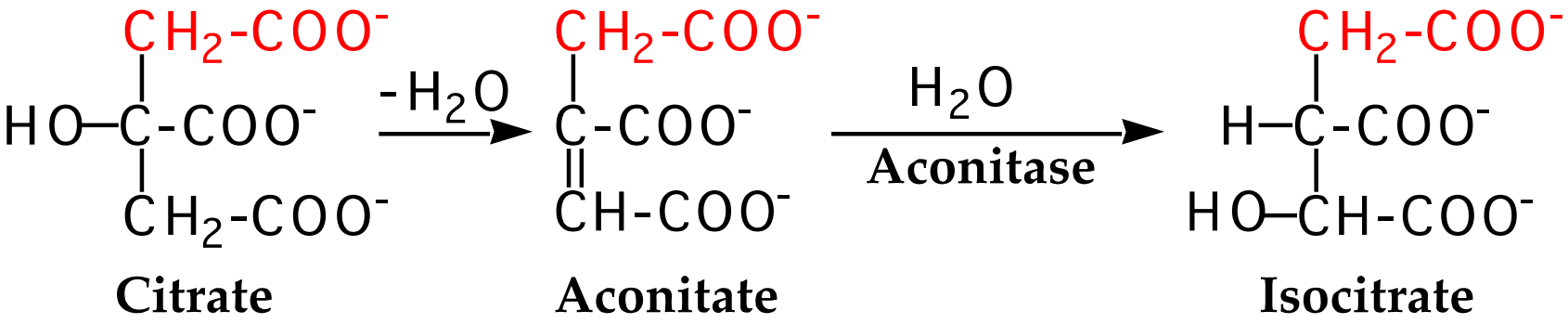
- Step 1: condensation of acetyl CoA with oxaloacetate:
  - The high-energy thioester of acetyl CoA is hydrolyzed.
  - This hydrolysis provides the energy to drive Step 1.



  - Citrate synthase, an allosteric enzyme, is inhibited by NADH, ATP, and succinyl-CoA.

Citric Acid Cycle

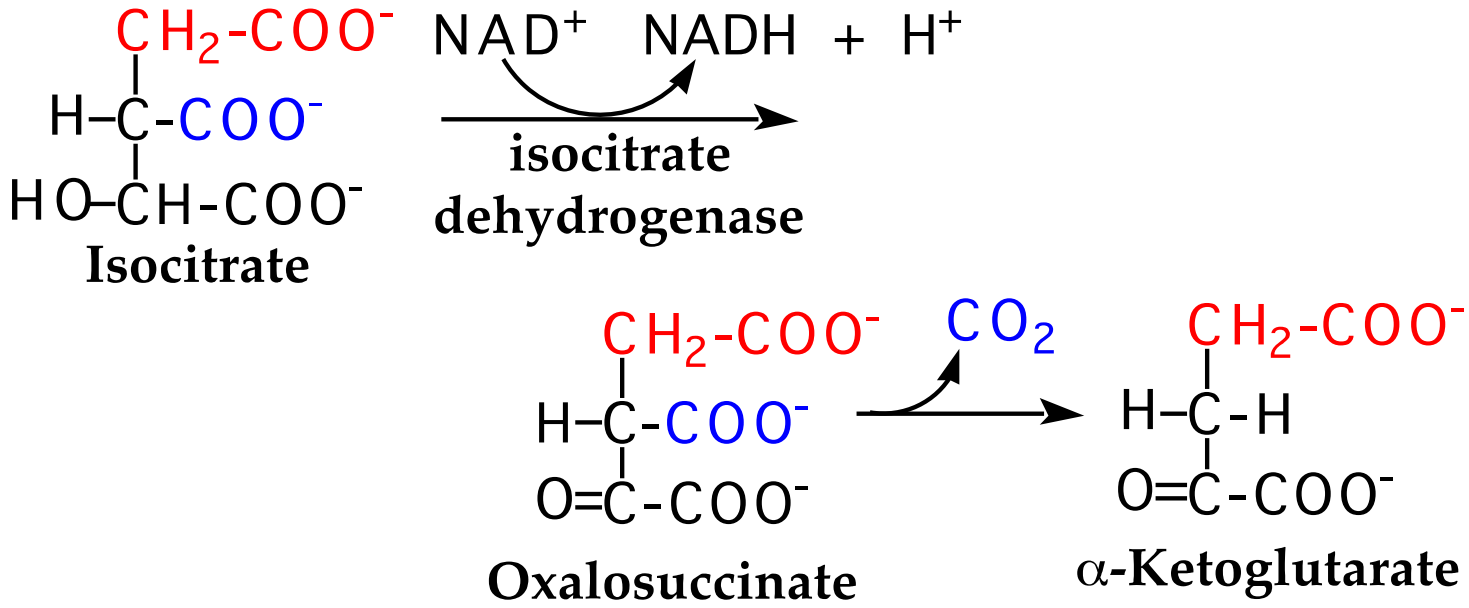
- Step 2: dehydration and rehydration, catalyzed by aconitase, gives isocitrate.



  - Citrate and aconitate are achiral; neither has a stereocenter.
  - Isocitrate is chiral; it has 2 stereocenters and 4 stereoisomers are possible.
  - Only one of the 4 possible stereoisomers is formed in the cycle.

Citric Acid Cycle

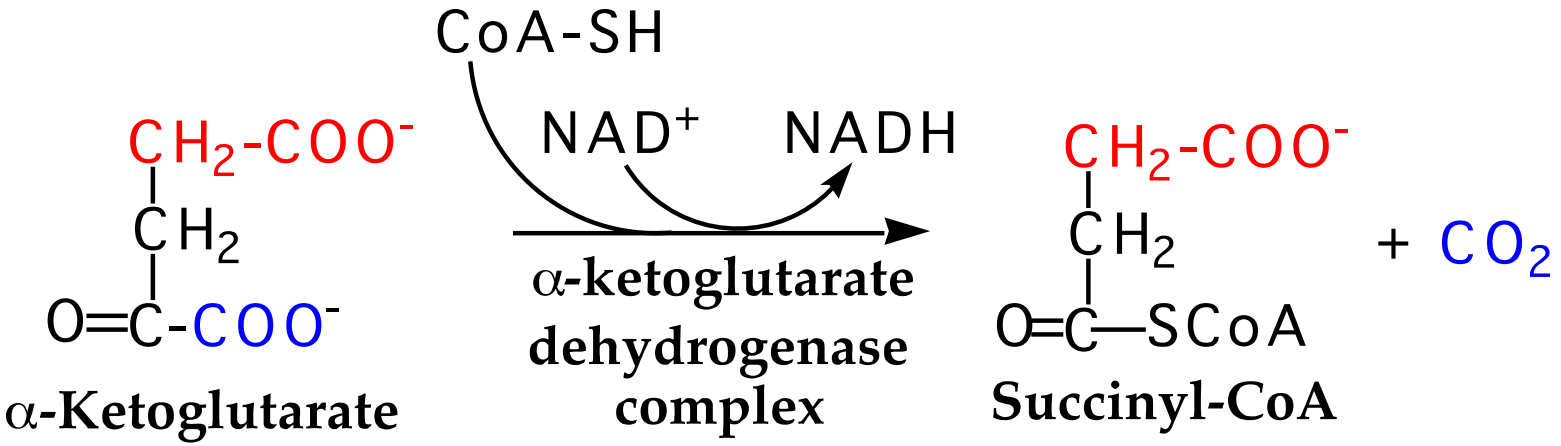
- Step 3: oxidation of isocitrate followed by decarboxylation gives α-ketoglutarate.



  - Isocitrate dehydrogenase is an allosteric enzyme; it is inhibited by ATP and NADH, and activated by ADP and NAD^+.

Citric Acid Cycle

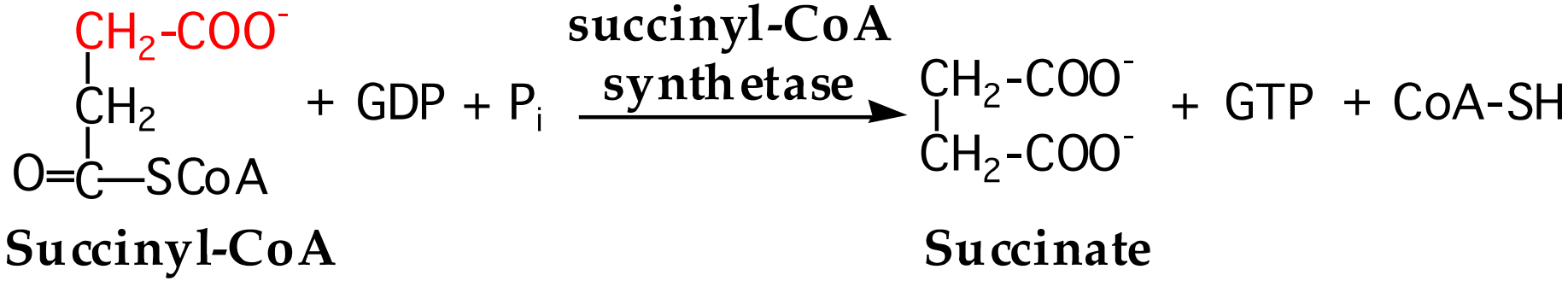
- Step 4: oxidative decarboxylation of α-ketoglutarate to succinyl-CoA.



  - The two carbons of the acetyl group of acetyl CoA are still present in succinyl CoA.
  - This multienzyme complex is inhibited by ATP, NADH, and succinyl CoA; it is activated by ADP and NAD^+.

Citric Acid Cycle

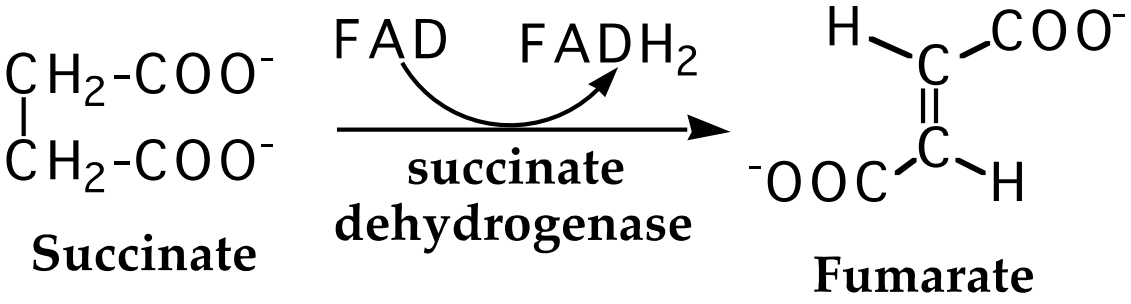
- Step 5: formation of succinate.



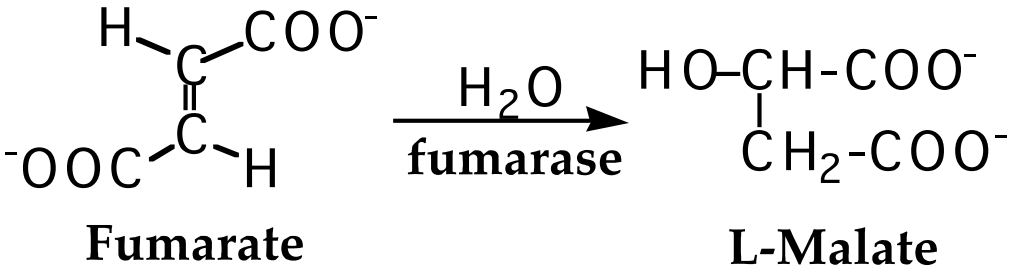
  - The two CH_2-COO^- groups of succinate are now equivalent.
  - This is the first, and only, energy-yielding step of the cycle; a molecule of GTP is produced.

Citric Acid Cycle

- Step 6: oxidation of succinate to fumarate.



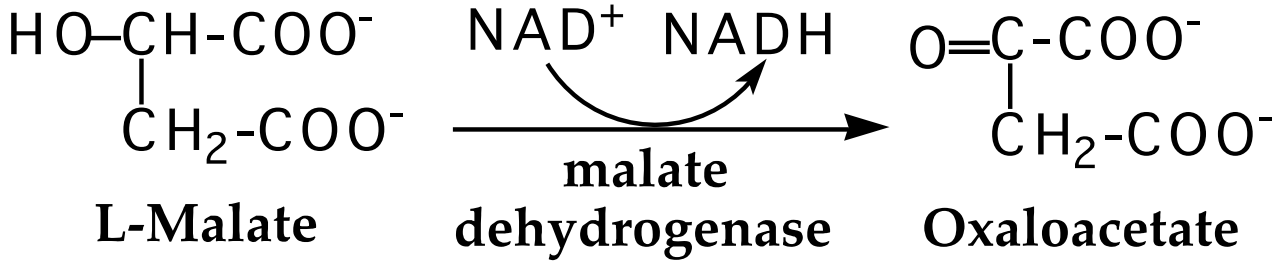
- Step 7: hydration of fumarate to L-malate.



  - Malate is chiral and can exist as a pair of enantiomers; It is produced in the cycle as a single stereoisomer.

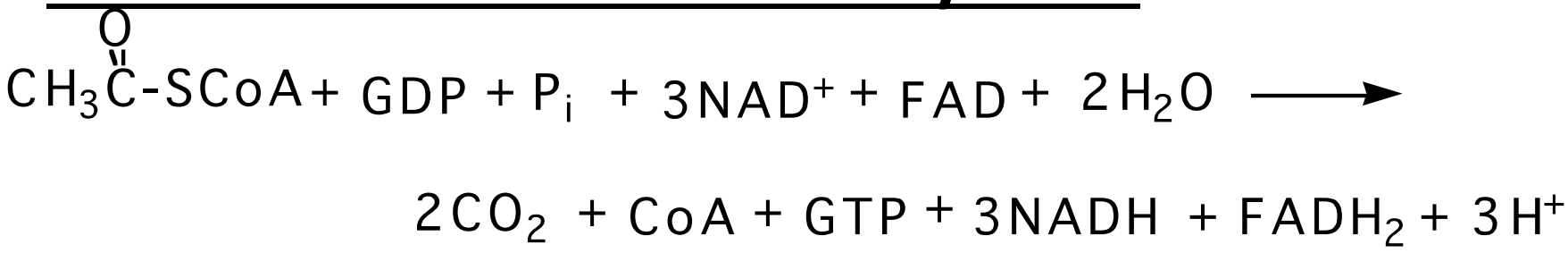
Citric Acid Cycle

- Step 8: oxidation of malate.



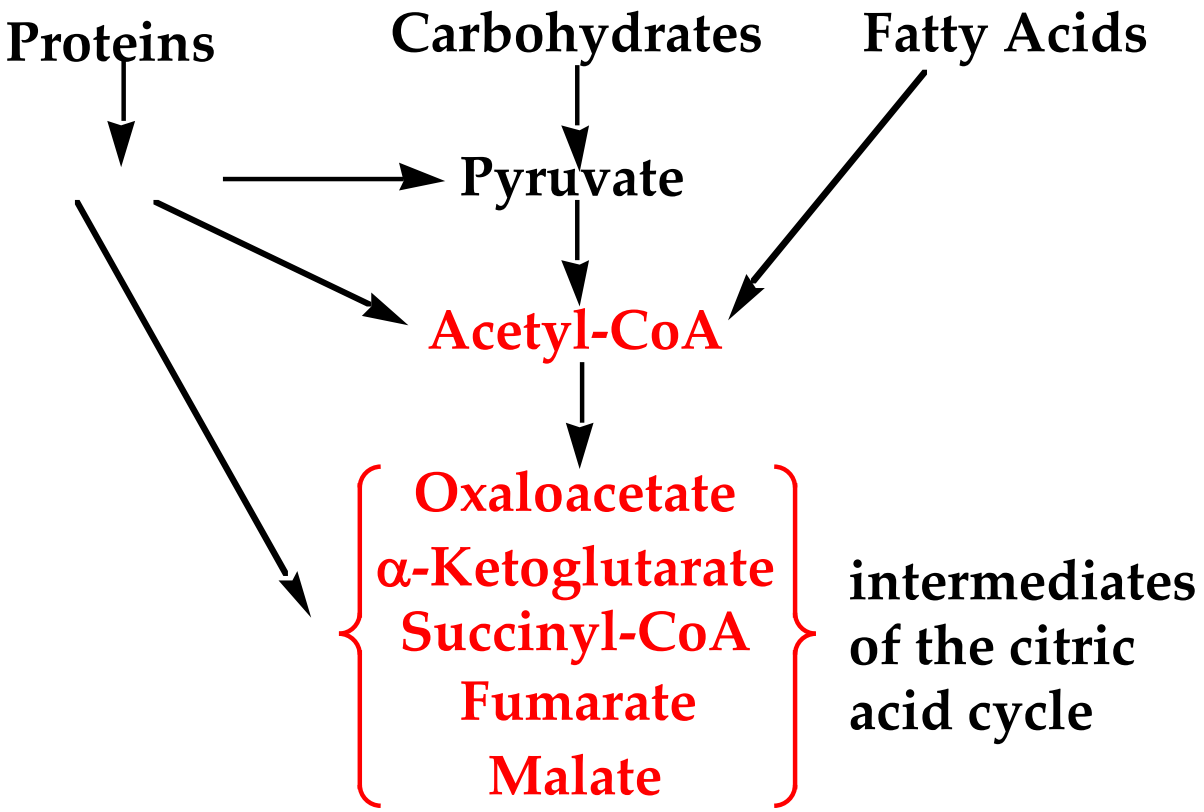
  - Oxaloacetate now can react with acetyl CoA to start another round of the cycle by repeating Step 1.

- **<u>The overall reaction of the cycle is:</u>**



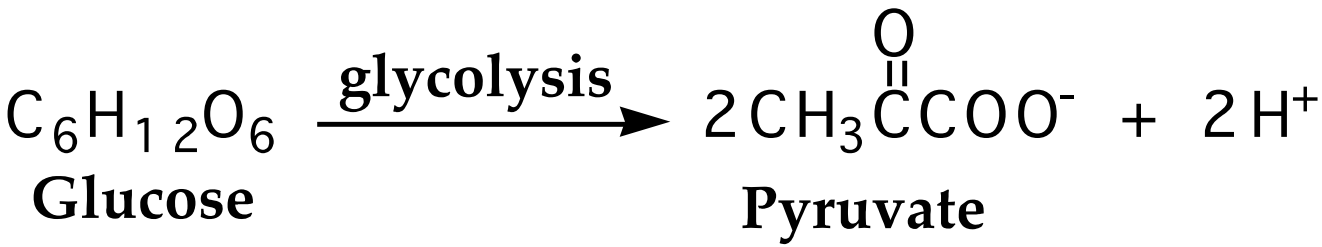
TCA Cycle in Catabolism

- The catabolism of proteins, carbohydrates, and fatty acids all feed into the citric acid cycle at one or more points:



Glycolysis

- **Glycolysis:** a series of 10 enzyme-catalyzed reactions by which glucose is oxidized to two molecules of pyruvate.



Irreversible

-- irreversible means
acetyl-CoA
cannot be converted
backward
to pyruvate;

hence “fat cannot be converted to carbohydrate”

Madenet Al elm University College
Department of Biology
2nd Year Biochemistry I 2021 – 2022

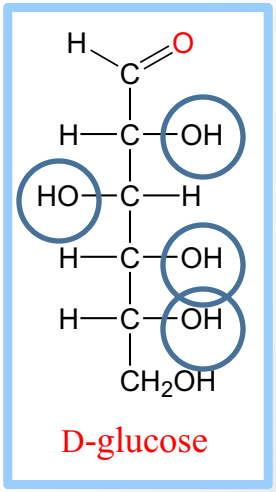
Qualitative tests of Carbohydrate

Dr. Hamza Yaseen Isa
Lecture 9 I 2

Introduction

- **Carbohydrates are the key source of energy used by living things.**
- **Also serve as extracellular structural elements as in cell wall of bacteria and plant.**
- **Carbohydrates are defined as the polyhydroxy aldehydes or polyhydroxy ketones.**

- **Most , but not all carbohydrate have a formula**
- **$(CH_2O)_n$ (hence the name hydrate of carbon)**
- **In human body, the D-glucose is used.**
- **Simple sugars ends with –ose**



D-glucose

Classification

1-Simple sugar: (one unit)

Monosaccharides contain one monosaccharide unit.

2-Complex sugar (more than one):

Disaccharides contain two monosaccharide units.

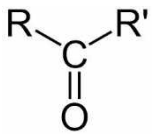
- Oligosaccharides contain 3-9 monosaccharide units.

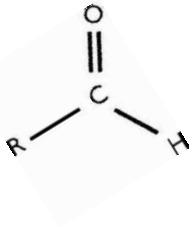
- Polysaccharides can contain more than 9 monosaccharide units.

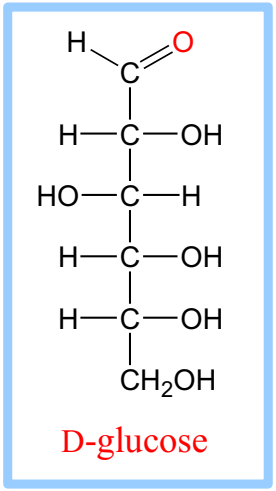
- **Complex carbohydrates can be broken down into smaller sugar units through a process known as hydrolysis.**

Monosaccharide

- They can be classified by the number of carbon atoms
- trioses (C-3)
- tetroses (C-4)
- pentoses (C-5)
- hexoses (C-6)
- heptoses (C-7)







- also be classified as ketoses or aldoses.
- A ketose contains a carbonyl group attached to two R groups having one or more hydroxyl groups.

- An aldose contains terminal aldehyde group in addition to R group containing -OH.

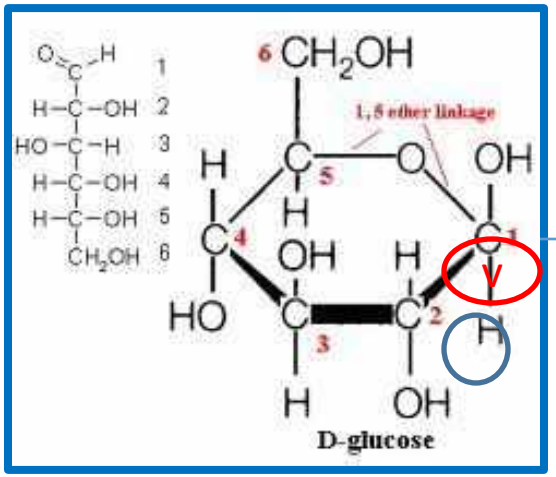
aldose

Solubility

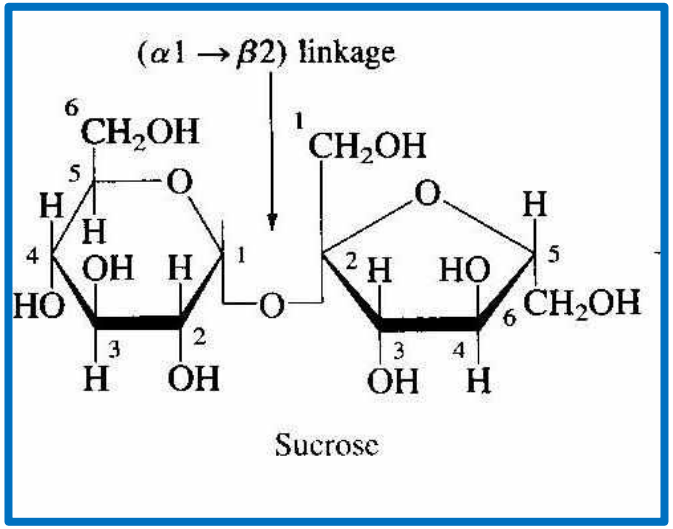
- Monosaccharide and disaccharide can be dissolved freely in water because water is a polar substance, while polysaccharide cannot be dissolved easily in water, because, it has high molecular weight , which give colloidal solutions in water soluble.

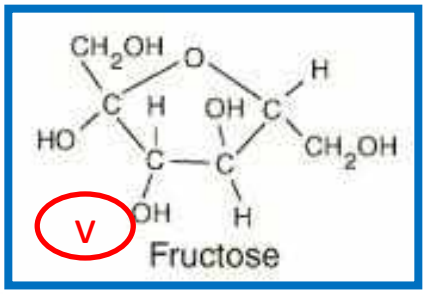
Reducing and non reducing sugars

- **Reducing and non reducing sugar :If the oxygen on the anomeric carbon of a sugar is not attached to any other structure, that sugar can act as a reducing agent and is termed a reducing sugar.**



Anomeric carbon





reducing

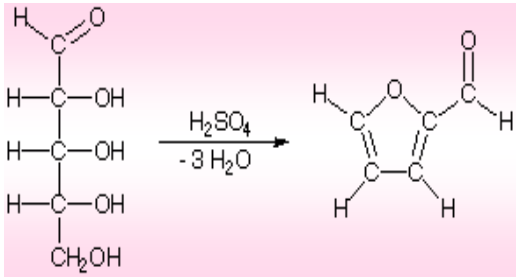
Non-reducing

Molisch test

- This test is specific for all carbohydrates. Monosaccharide gives a rapid positive test, Disaccharides and polysaccharides react slower.

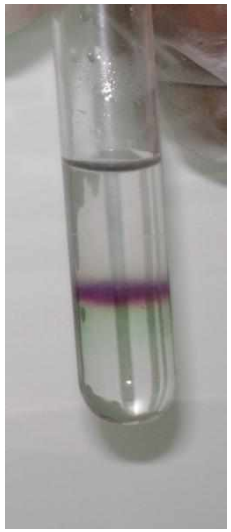
- Objective: To identify the carbohydrate from other macromolecules lipids and proteins.

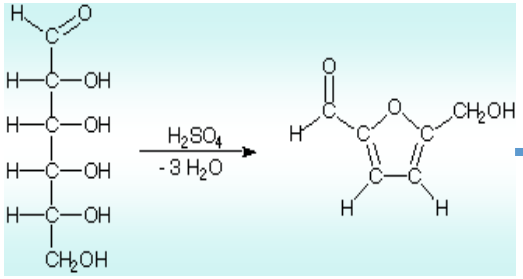
- **Principle: The test reagent(H2SO4) dehydrates pentose to form furfural and dehydrates hexoses to form 5- hydroxymethyl furfural.**
- **The furfural and 5- hydroxymethyl furfural further react with α-naphthol present in the test reagent to produce a purple product.**



furfural

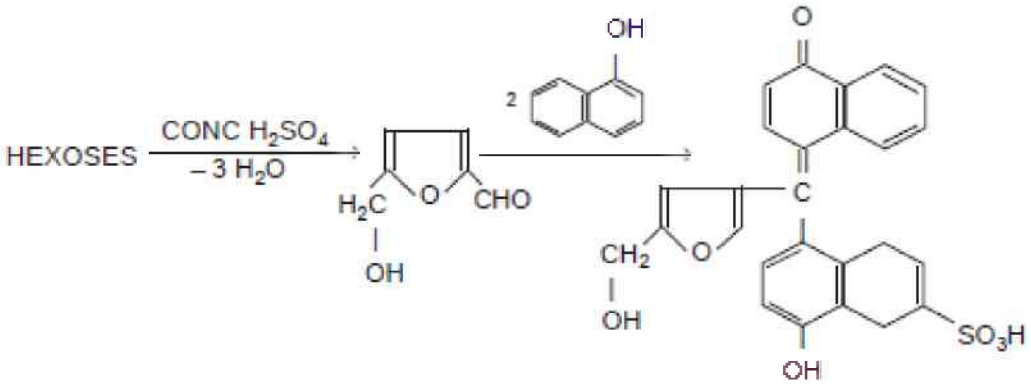
α-naphthol → Purple color



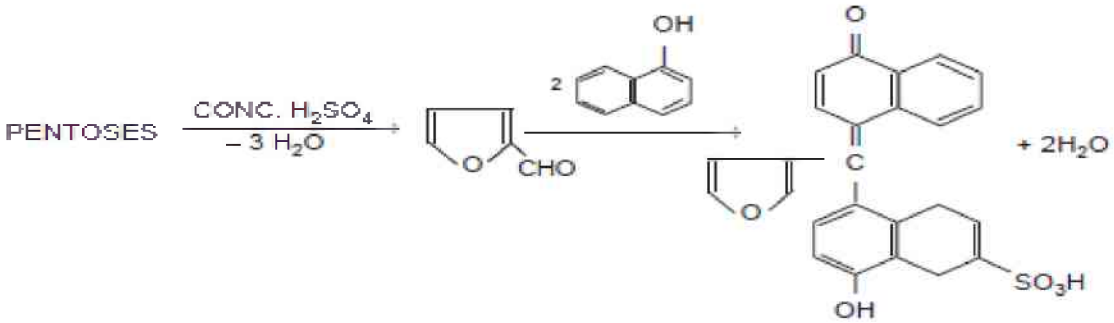


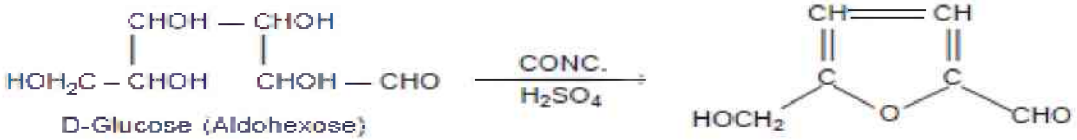
5- hydroxy methyl furfural

α- naphthol → Purple color



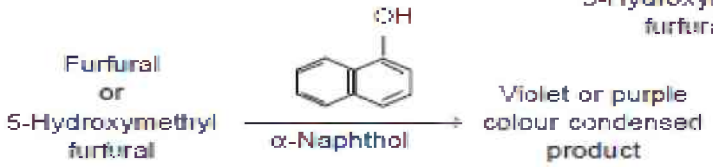
Molisch reagent: α-naphthol in ethanol (ethanolic a-naphthol)





D-Glucose (Aldohexose)

5-Hydroxymethyl furfural



Method

1-Two ml of a sample solution is placed in a test tube.
2-Two drops of the Molisch reagent (which α-napthol in 95% ethanol) is added.
3-The solution is then poured slowly into a tube containing two ml of concentrated sulfuric acid so that two layers form, producing violet ring appear as liaison between the surface separations.

Tube	observation
1-glucose	
2-ribose	
3-sucrose	
4-starch	

Benedict's test

- **Benedict's reagent is used as a test for the presence of reducing sugars.**
- **All monosaccharides are reducing sugars; they all have a free reactive carbonyl group.**

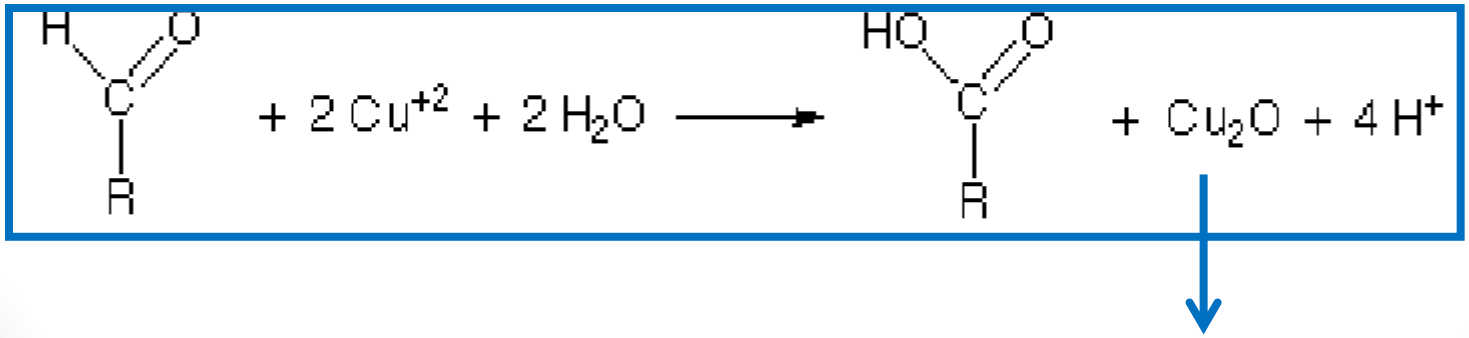
Some disaccharides have exposed carbonyl groups and are also reducing sugars. Other disaccharides such as sucrose are non-reducing sugars and will not react with Benedict's solution.

Large polymers of glucose, such as starch, are not reducing sugars

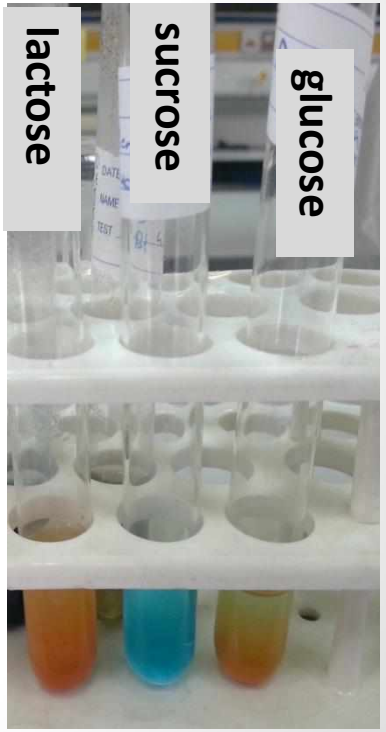
- **Objective: To distinguish between the reducing and non-reducing sugars.**

Benedict's test

- **Principle**: The copper sulfate (CuSO4) present in Benedict's solution reacts with electrons from the aldehyde or ketone group of the reducing sugar in **alkaline medium**.
- Reducing sugars are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper oxide.



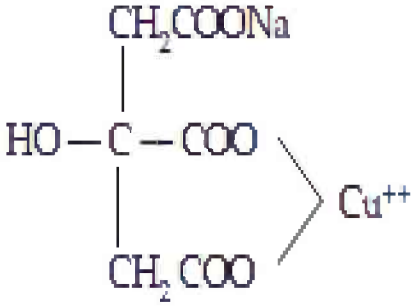
reddish precipitate of copper



Reactions

$$CuSO_4 \longrightarrow Cu^{++} + SO_4^{--}$$

Cu^{++} + Sodium Citrate $\longrightarrow$ Cupric: Sodium citrate complex



Reducing sugar $\xrightarrow{Na_2CO_3}$ Enediols forms of reducing sugar.

Enediols + Cupric: Sodium citrate complex $\xrightarrow{\text{Boiling}}$ Cu^+ + Mixture of sugar acids.

$$Cu^+ + OH^- \longrightarrow CuOH$$

$$2CuOH \xrightarrow{\text{Heat}} Cu_2O\downarrow + H_2O$$

Cuprous oxide.

- **Method:**
- **One ml of a sample solution is placed in a test tube.**
- **Two ml of Benedict's reagent is added.**
- **The solution is then heated in a boiling water bath for five minutes.**
- **A positive test is indicated by: The formation of a reddish precipitate.**

Tube	observation
1-glucose	
2-sucrose	
3-lactose	

Barfoed's Test

- This test is performed to distinguish between reducing mono saccharides, reducing disaccharides and non reducing disaccharides.
- **Objective:** To distinguish between mono- , di- and poly saccharides.
- Principle: Barfoed's test used copper (II) ions in a slightly acidic medium
- Reducing mono saccharides are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide within three minutes. Reducing disaccharides undergo the same reaction, but do so at a slower rate.
- The non reducing sugars give negative result.

- Barfoed's reagent, cupric acetate in acetic acid , so in acidic medium , disacchride is a weaker reducing agent than monosacchride, so mono sacchride will reduce the copper in less time.

$$(CH_3COO)_2Cu + 2H_2O \rightarrow 2CH_3COOH + Cu(OH)_2$$

Cupric hydroxide

$$Cu(OH)_2 \xrightarrow{\Delta} CuO + H_2O$$

$$\text{D-glucose} + 2CuO \rightarrow \text{D-gluconic acid} + Cu_2O \downarrow$$

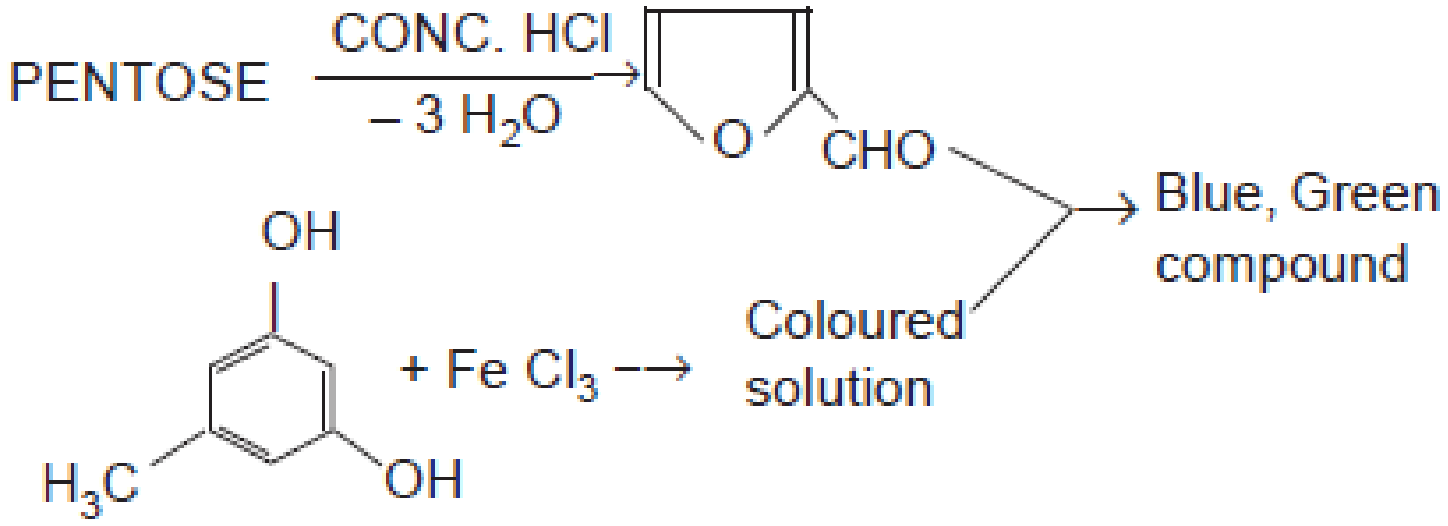
Cuprous oxide
(Red ppt.)

- **Place one ml of a sample solution in a test tube.**
- **Add 3 ml of Barfoed's reagent (a solution of cupric acetate and acetic acid.**
- **Heat the solution in a boiling water bath for 6 minutes(after the 3 min check the tubes).**

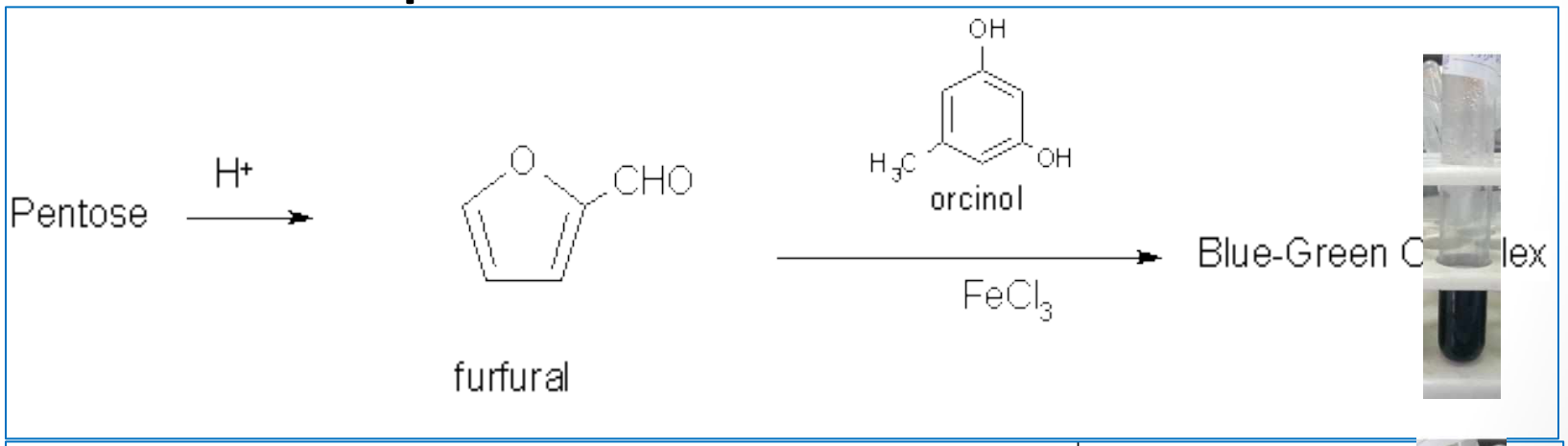
Tube	observation
1-glucose	
2-sucrose	
3-lactose	

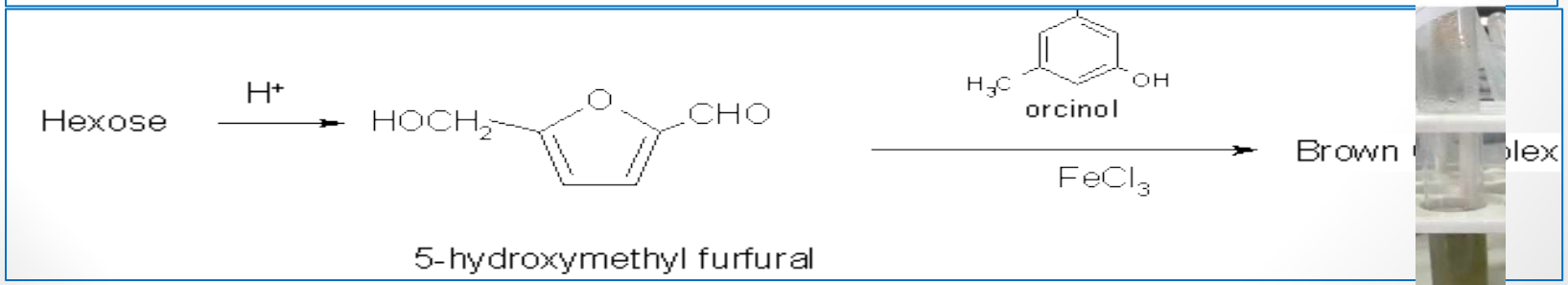
Bial's Test

- **This test is used to distinguish between pentose and hexose mono saccharides.**
- **Objective: To distinguish between pentose monosaccharide and hexose monosaccharide**



- **Principle: Bial's test uses concentrated HCl as a dehydrating acid and orcinol + traces of ferric chloride as condensation reagent. The test reagent dehydrates pentoses to form furfural. Furfural further reacts with orcinol and the iron ion present in the test reagent to produce a bluish or green product, while hexoses yield muddy-brown to grey condensation product.**





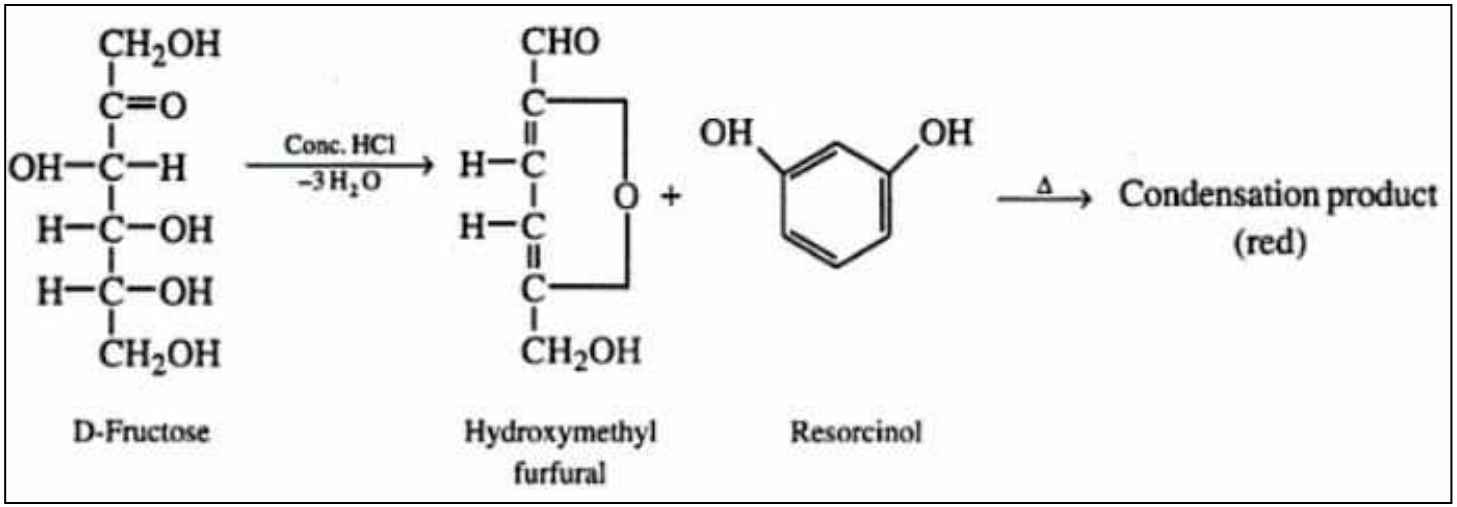
- **Put 2 ml of a sample solution in a test tube.**
- **Add 2 ml of Bial's reagent (a solution of orcinol, HCl and ferric chloride) to each tube.**
- **Heat the tubes gently in hot water bath.**
- **If the color is not obvious, more water can be added to the tube.**

Tube	observation
1-glucose	
2-ribose	
3-fructose	

Seliwanoff's Test

- **This test is used to distinguish between aldoses (like glucose) and ketoses (like fructose).**
- **Objective: To distinguish between aldose and ketose saccharides.**

- **Principle: Seliwanoff's Test uses 6M HCl as dehydrating agent and resorcinol as condensation reagent. The test reagent dehydrates ketohexoses to form 5-hydroxymethylfurfural. 5-hydroxymethylfurfural further condenses with resorcinol present in the test reagent to produce a cherry red product within two minutes. Aldohexoses react to form the same product, but do so more slowly giving yellow to faint pink color.**



Method:

- **One half ml of a sample solution is placed in a test tube.**
- **Two ml of Seliwanoff's reagent (a solution of resorcinol and HCl) is added.**
- **The solution is then heated in a boiling water bath for two minutes.**

Tube	observation
1-glucose	
2-fructose	

Test	objective
Molisch test	To identify the carbohydrate from other macromolecules lipids and proteins
Benedict's test	Benedict's reagent is used as a test for the presence of reducing sugars.
Barfoed's Test	to distinguish between reducing monosaccharides, reducing disaccharides and non reducing disaccharides.
Bial's Test	To distinguish between pentose monosaccharide and hexose monosaccharide
Seliwanoff's Test	To distinguish between aldose and ketone sugars

Iodine test:

This test is used for polysaccharides detection and differentiation.

Iodine forms a coordination complex between the helically coiled polysaccharides chain and the iodine centrally located with in the helix due to adsorption. The iodine colour obtained with the polysaccharides depends upon the length of the unbranched or linear (α1,4 linkage) chain available for complex formation. Amylose a linear chain component of starch gives a deep blue colour. Amylopectin, a branched chain component of starch gives a purple colour.

Glycogen gives a reddish brown colour.

Colour with iodine
Blue colour
Blue colour
Purple colour
Brown red colour
Brown to colourless
No colour
No colour

Polysaccharides
Starch
Amylose
Amylopectin
Glycogen
Dextrins
Cellulose or inulin
Disaccharides or monosaccharides