21BT33
Biochemistry

Reading materials

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Module 1: Chemical foundation of Biology

The Chemical Foundation of Life Introduction

Elements in various combinations comprise all matter, including living things. Some of the most abundant elements in living organisms include carbon, hydrogen, nitrogen, oxygen, sulphur, and phosphorus. These form the nucleic acids, proteins, carbohydrates, and lipids that are the fundamental components of living matter. Biologists must understand these important building blocks and the unique structures of the atoms that make up molecules, allowing for the formation of cells, tissues, organ systems, and entire organisms. All biological processes follow the laws of physics and chemistry, so in order to how biological systems work, it is important to understand the underlying physics and chemistry. For example, the flow of blood within the circulatory system follows the laws of physics that regulate the modes of fluid flow. The breakdown of the large, complex molecules of food into smaller molecules is a series of chemical reactions.

Water in biological system capillary action Water, like carbon, has a special role in living things. It is needed by all known forms of life. All organisms on Earth are made up mostly of water, thus water is the biological cornerstone of life. Water is a simple molecule, containing just three atoms, two hydrogen

and one oxygen. Nonetheless, water's structure gives it unique properties that help explain why it is vital to all living organisms. In fact, without water, life would not be possible. This simple fact is why scientists are constantly looking for water on other planets - the presence of water could indicate the presence of life.

The Molecular Make-up of Water

Water is the "Universal Solvent"

As a polar molecule, water interacts best with other polar molecules, such as itself. This is because of the phenomenon wherein opposite charges attract one another: because each individual water molecule has both a negative portion and a positive portion, each side is attracted to molecules of the opposite charge. This attraction allows water to form relatively strong connections, called bonds, with other polar molecules around it, including other water molecules. In this case, the positive hydrogen of one water molecule will bond with the negative oxygen of the adjacent molecule,

whose own hydrogens are attracted to the next oxygen, and so on. Importantly, this bonding makes water molecules stick together in a property called cohesion. The cohesion of water molecules helps plants take up water at their roots. Cohesion also contributes to water's high boiling point, which helps animals regulate body

Many of water's roles in supporting life are due to its molecular structure and a few special properties. Water is a simple molecule composed of two smalls, positively charged hydrogen atoms and one large negatively charged oxygen atom. When the hydrogens bind to the oxygen, it creates an asymmetrical molecule with positive charge on one side and negative charge on the other side. This charge differential is called polarity and dictates how water interacts with other molecules.

Furthermore, since most biological molecules have some electrical asymmetry, they too are polar and water molecules can form bonds with and surround both their positive and negative regions. In the act of surrounding the polar molecules of another substance, water wriggles its way into all the nooks and crannies between molecules, effectively breaking it apart are dissolving it. This is what happens when you put sugar crystals into water: both water and sugar are polar, allowing individual water molecules to surround individual sugar molecules, breaking apart the sugar and dissolving it. Similar to polarity, some molecules are made of ions, or oppositely charged particles. Water breaks apart these ionic molecules as well by interacting with both the positively and negatively charged particles. This is what happens when you put salt in water, because salt is composed of sodium and chloride ions.

Water's extensive capability to dissolve a variety of molecules has earned it the designation of "universal solvent," and it is this ability that makes water such an invaluable life-sustaining force. On a biological level, water's role as a solvent helps cells transport and use substances like oxygen or nutrients. Water-based solutions like blood help carry molecules to the necessary locations. Thus, water's role as a solvent facilitates the transport of molecules like oxygen for respiration and has a major impact on the ability of drugs to reach their targets in the body.

Physical Properties of Water

- Appearance:

As you are aware now, water is a colourless, odourless, and tasteless liquid in its natural state. The crystal structure of water in hexagonal.

• Boiling Point of Water:

The boiling point is defined as the temperature at which the vapor pressure of the liquid is equal to the pressure surrounding the liquid, and thus the liquid changes to vapor. It is known to us that the boiling point of water is 100°C.

• Freezing Point of Water:

The freezing point is the temperature at which the substance changes state from liquid to solid. So, for water, the point at which liquid state water turns to solid- state ice is the freezing point of water, which is 0°C or 32°F.

• Specific Heat Capacity:

Water has a high specific heat capacity of 4.2 joules per gram at 25°C. This is due to the extensive hydrogen bonding between the water molecules.

• Density of Water:

The density of water is about 1 gm/cc and it varies with temperature in an unusual pattern. The density of water in different states - solid and liquid. In solid-state, the density is 0.9gm/cc

• Viscosity of Water:

The viscosity is defined by the resistance to deformation at a given rate. In other words, the thickness of the liquid e.g., syrup and water. The viscosity of water is 0.89 cP(centi-poise)

• Surface Tension of Water:

Surface tension is the tendency of the fluid to shrink in a minimum surface area. Water has a high surface tension of 72m N/m at 25°C. Because of this high surface tension of water, insects can walk on the surface of the water without any discomfort.

• Refractive Index of Water:

In simpler words, the refractive index is the number that describes how fast the light reached the material. The refractive index of water is 1.333 at 20°C.

• Compressibility of Water:

Compressibility is defined as the function of temperature and pressure and its effect on the substance. For water, the compressibility at 0°C is 5.1 × 10 10Pa and it reduces to

4.4 x 10 10Pa till - 45°C. As the pressure is increases, the compressibility decreases further.

• Dielectric Constant of Water:

Dielectric constant is a measure of how easily the material is polarized by an electric field. The dielectric constant of water is very high, which is at 78.6. This constant plays a very important role in water, being a universal solvent.

Chemical Properties of water: -

Amphoteric nature of water:

The amphoteric nature of water implies that water can act as both an acid and a base. It means that water can be a proton donor as well as a proton acceptor. Few examples of reactions are illustrated below: -

H2O + NH3 OH-NH4+ (acid) (base) (conjugate base) (conjugate acid) H2O+ H2S H3O++ HS- (base) (acid) (conjugate acid) (conjugate base)

Auto-protolysis of water (Self- ionisation):

During auto-protolysis, the ionisation of water takes place where water molecule, i.e. H20 deprotonates (removal of protons) in order to form a hydroxide ion OH-. The hydrogen nucleus H+ immediately protonates (accepts proton) with another water molecule to form a hydronium ion, H30+.

Self-ionisation of water is illustrated below: -

Water is of two type

Hard water - It contains salts of Calcium (Ca) and Magnesium (Mg) as hydrogen carbonate, chlorides and sulphates.

Soft water - The water which is free from salts of calcium and magnesium.

The hardness of water can further be classified as: -

Temporary hardness:

Temporary hardness is due to the presence of magnesium bicarbonate Mg (HCO3)2(aq) and calcium bicarbonate Ca (HCO3)2(aq). This type of hardness can be removed by boiling, as boiling promotes the formation of carbonate precipitate from bicarbonates, which can be separated.

Permanent hardness:

Permanent hardness is due to the presence of calcium and magnesium chlorides, sulphates, and nitrates. It cannot be removed by boiling. That's why it is known as permanent hardness.

In order to remove permanent hardness, hard water is treated with washing soda (Na2CO3).

Fun fact: Soap doesn't form lather in hard water.

Conclusion for physical and chemical properties of water:

Water or H2O is a transparent and colourless chemical substance. Approximately 70.9% of the Earth's surface is covered with water. It is the primary fluid of the Earth's hydrosphere, which is indispensable for sustaining life on Earth. It exists in three different states: Solid, Liquid and Gas. We normally use the liquid state of water in our daily lives. The ice is the solid-state of water, and the vaporization while boiling is the Gas state of water. So, here we learned about the physical and chemical properties of water separately.

Weak Interactions in Aqueous Systems

Hydrogen Bonding Gives Water Its Unusual Properties ● Water Forms Hydrogen Bonds

with Polar Solutes.

● Water Interacts Electrostatically with Charged Solutes.

● Entropy Increases as Crystalline Substances Dissolve Nonpolar Gases Are Poorly Soluble in Water

● Nonpolar Compounds Force Energetically Unfavourable Changes in the Structure of Water.

- Van der Waals Interactions Are Weak Interatomic.

- Attractions Weak Interactions Are Crucial to Macromolecular.

- Structure and Function Solutes Affect the Colligative Properties of Aqueous Solutions.

lionisation of water:

lionisation is defined as the process by which an atom or molecule gains or loses a positive or negative charge as a result of chemical changes. An ion is an electrically charged atom or molecule that results. If the ion has a negative charge, it is called an anion; if it has a positive charge, it is called a cation.

The basic ionisation reaction can be represented as follows: $M \rightarrow M^+ + e^-$

lionisation can occur as a result of the loss of an electron in collisions with subatomic particles, collisions with other atoms, molecules, and ions, or interactions with electromagnetic radiation.

Amphiprotic Nature of Water

Due to its highly polar structure, liquid water can either act as an acid (by donating a proton to a base) or a base (by using a lone pair of electrons to accept a proton).

- As bases:

When a strong acid like HCI dissolves in water, it separates into chloride ions (CI) and protons (H+). In turn, the proton reacts with a water molecule to form the hydronium ion (H3O+):

HCI (aq) + H2O (1) H3O+(aq) + Cl(aq)

The acid in this reaction is HCI, and the base is water, which accepts an H+ ion.

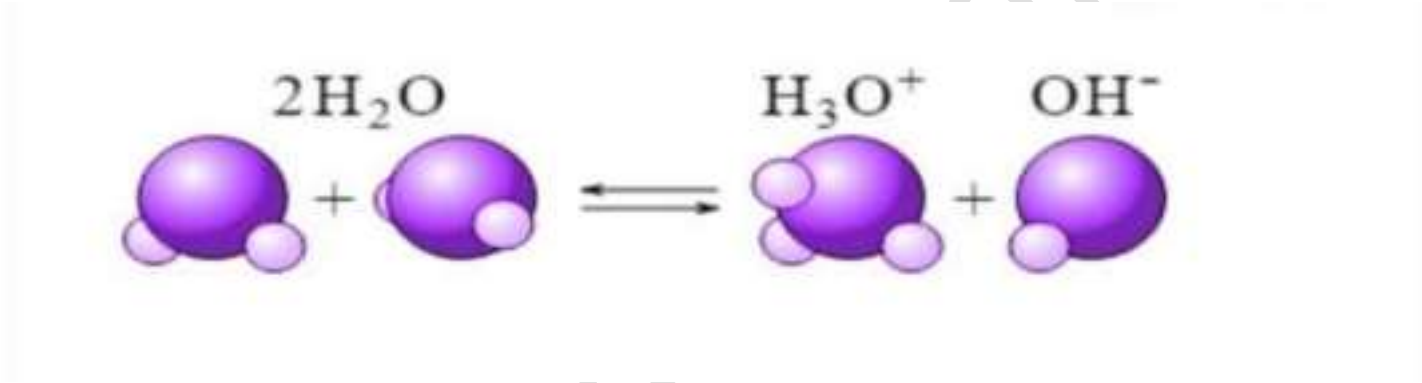
●As acids:

Water can also act as an acid. H2O donates a proton to NH3, which acts as a base, in this equilibrium reaction:

H2O (aq) + NH3(aq) (NH4) + + OH

Water is thus called amphiprotic because it can act as an acid or a base depending on the nature of the other reactant.

Self-ionisation of water:



Water will self-ionize to a very small extent under normal conditions. The reaction in which a water molecule donates one of its protons to a neighbouring water molecule, either in pure water or in an aqueous solution, is referred to as the self- ionization of water.

In an autoionization process, one water molecule can react with another to form an OH^- ion and an H3O+ ion:

2H2O (1) H3O+ (aq) + OH (aq)

Concentration of Solutions

There are three principal ways to express solution concentration in chemistry-percentage by mass, molarity, and molality.

The following table compares these three ways of stating solution con- centration. Examining the method of

preparation of the three types may help you understand the differences among them.

	Symbol	Meaning	How to prepare
Percentage	%	Grams solute per 100 g of solution	**5%:** Dissolve 5 g of solute in 95 g solvent.
Molarity	M	Moles solute per liter of solution	**5 M:** Dissolve 5 mol of solute in solvent and add solvent to make 1 L of solution.
Molality	*m*	Moles solute per kilogram of solvent	**5 *m*:** Dissolve 5 mol of solute in 1 kg of solvent.

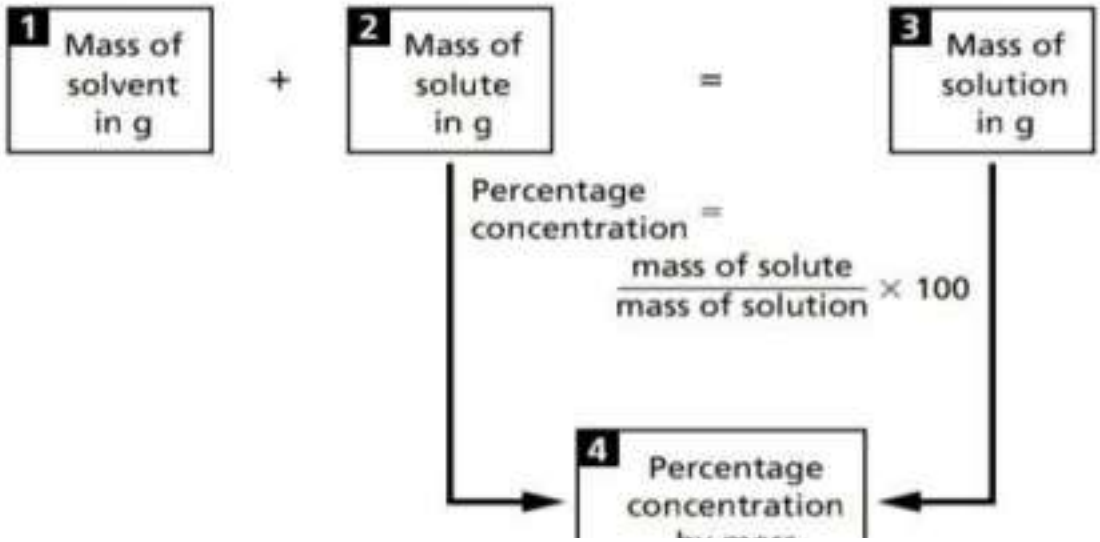
PERCENTAGE CONCENTRATION:

You will find percentages of solutes stated on the labels of many commercial products, such as household cleaners, liquid pesticide solutions, and shampoos. If your sink becomes clogged, you might buy a bottle of drain opener whose label states that it is a 2.4% sodium hydroxide solution. This means that the bottle contains 2.4 g of NaOH for every 100 g of solution

Computing percentage concentration is very much like computing per centage composition. Both involve finding the percentage of a single component of a multicomponent system. In each type of per- centage calculation, the mass of the important component (in percentage concentration, the solute) is divided by the total mass of the system and multiplied by 100 to yield a percentage.

In percentage concentration, the solute is the important component, and the total mass of the system is the mass of the solute plus the mass of the solvent.

General Plan for Solving Percentage Concentration Problems



PERCENT BY MASS OVER VOLUME (m/v)

Percent (m/v) is the mass of solute divided by the volume of the solution, multiplied by 100 %.

$$\text{Percent (m/v)} = \frac{\text{mass of solute}}{\text{volume of solution}} \times 100\ \%$$

EXAMPLE

If the density of the above solution is 0.857 g/mL, what is the percent (m/v) of rubbing alcohol?

Solution

$$\text{Volume of solution} = 500\ \text{g solution} \times \frac{1\text{mL solution}}{0.857\text{g solution}} = 583.4\ \text{mL solution}$$

(3 significant figures + 1 guard digit)

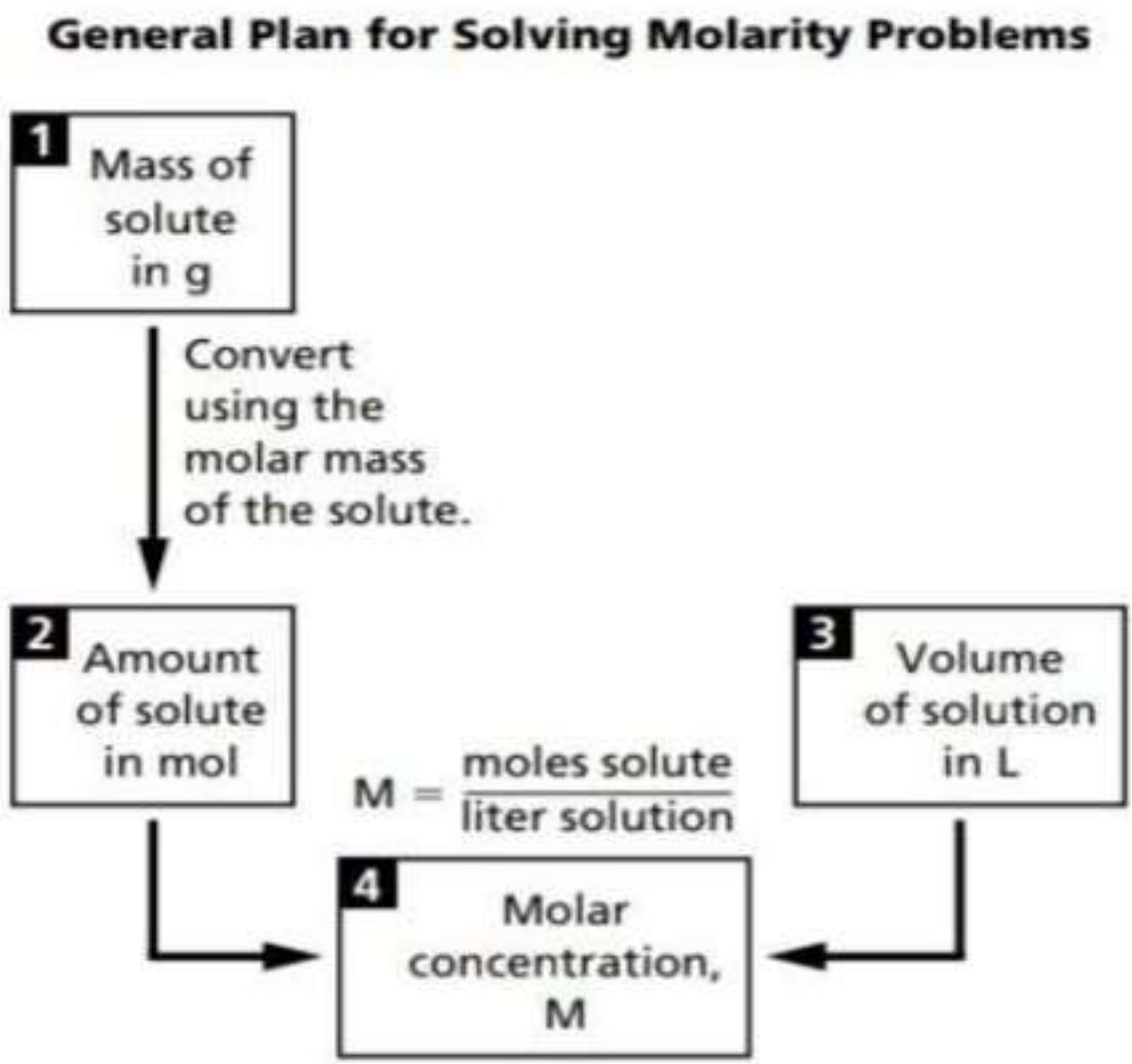
$$\text{Percent (m/v)} = \frac{\text{mass of rubbing alcohol}}{\text{volume of solution}} \times 100\ \% = \frac{275\text{g}}{583.4\text{mL}} \times$$

$100\ \% = 47.1\ \%$

MOLARITY:

Molarity is the most common way to express concentration in chemistry. Molarity is the number of moles of solute per litter of solution and is given as a number followed by a capital M. A 2 M solution of nitric acid contains 2 mol of HNO3 per litter of solution.

As you know, substances react in mole ratios. Knowing the molar concentration of a solution allows you to measure a number of moles of a dissolved substance by measuring the volume of solution.



$$\textbf{Molarity: } M = \frac{\text{moles of solute}}{\text{litres of solution}} \left(\frac{\text{mol}}{\text{L}}\right)$$

$$\textbf{Dilution problems: } M_1V_1 = M_2V_2$$

$$\textbf{Molar mass: } n = \frac{m}{MW}, \text{ where } n = \text{number of moles}$$

$$m = \text{mass}$$

$$MW = \text{molecular weight } \left(\frac{\text{g}}{\text{mol}}\right)$$

Example 1: Determine the molarity of 3.72 moles of NaBr in 575 mL of solution.

Solution: $[NaBr] = \frac{3.72\ \text{mol}}{0.575\ \text{L}} = 6.47\ \frac{\text{mol}}{\text{L}}$

Example 2: How many millilitres of concentrated H_2SO_4 (16.0 M) is required to prepa
250 mL of 6.00 M H_2SO_4 solution?

Solution: desired: $M_1 = 6.00$ M; $V_1 = 250$ mL
on hand: $M_2 = 16.0$ M; $V_2 = ?$

$$V_2 = \frac{M_1V_1}{M_2} = \frac{(6.00\ \text{M})(250\ \text{mL})}{16.0\ \text{M}} = 93.8\ \text{mL } H_2SO_4$$

Example 3: 15.32 mL of 0.5250 M HCℓ is required to titrate 17.50 mL of a NaOH solution. Determine the concentration of the NaOH solution.

Solution: Because we have a titration, we need the formula equation for the reacti

$$HC\ell + NaOH \rightarrow NaC\ell + H_2O$$

moles of acid: $0.5250\ \frac{\text{mol}}{\text{L}} \times (15.32 \times 10^{-3}\ \text{L}) = 8.043 \times 10^{-3}$ mol HCℓ

8.043×10^{-3} mol HCℓ reacts with 8.043×10^{-3} mol NaOH

concentration (molarity): $\frac{8.043 \times 10^{-3}\ \text{mol NaOH}}{1.750 \times 10^{-2}\ \text{L NaOH}} = 0.4596\ \frac{\text{mol}}{\text{L}}$

Calculate the molarity of $NaOH$ in the solution prepared by dissolving its 4 g in enough water to form 250 mL of the solution.

Solution

As per Molarity Formula

$$M = \frac{W}{M} \times \frac{1000}{\text{V ml}}$$

= (4 g / 40 g) × (1000/250) =0.1 mol/0.250 L

= 0.4 mol/L

= 0.4 M

Question 1

Calculate the molarity of $NaOH$ in the solution prepared by dissolving its 4 g in enough water to form 250 mL of the solution.

Solution

As per Molarity Formula

$$M = \frac{W}{M} \times \frac{1000}{\text{V ml}}$$

= (4 g / 40 g) × (1000/250) =0.1 mol/0.250 L

= 0.4 mol/L

= 0.4 M

Question 2

If 2 gm $NaOH$ is dissolute in water to make solution upto 250 cc. Give molarity of the solution:

Solution

NaOH = W = 2 g

Molar Mass of NaOH = 23 + 16 + 1 = 40

M = W/M × 1000/(V ml) = 2/40 × 1000/250=1/5 M

MOLALITY:

Molality is the amount in moles of solute per kilogram of solvent and is given by a number followed by an italic lowercase m. A 5 m aqueous solution of glucose contains 5 mol of C6H12O6 per kilogram of water. Molal concentration is important primarily in working with colligative proper- ties of solutiotion.

Problem #1: A solution of H_2SO_4 with a molal concentration of 8.010 m has a densit of 1.354 g/mL. What is the molar concentration of this solution?

Solution:

8.010 m means 8.010 mol / 1 kg of solvent

(8.010 mol) (98.0768 g/mol) = 785.6 g of solute

785.6 g + 1000 g = 1785.6 g total for solute and solvent in the 8.010 m solution.

1785.6 g / 1.354 g/mL = 1318.76 mL

8.01 moles / 1.31876 L = 6.0739 M

6.074 M (to four sig figs)

Problem #2: A sulfuric acid solution containing 571.4 g of H_2SO_4 per liter of solution has a density of 1.329 g/cm^3. Calculate the molality of H_2SO_4 in this solution

Solution:

1 L of solution = 1000 mL = 1000 cm^3

1.329 g/cm^3 times 1000 cm^3 = 1329 g (the mass of the entire solution)

1329 g minus 571.4 g = 757.6 g = 0.7576 kg (the mass of water in the solution)

571.4 g / 98.0768 g/mol = 5.826 mol of H_2SO_4

5.826 mol / 0.7576 kg = 7.690 m

21BT33

Problem #3: An aqueous solution is prepared by diluting 3.30 mL acetone (d = 0.789 g/mL) with water to a final volume of 75.0 mL. The density of the solution is 0.993 g/mL. What is the molarity, molality and mole fraction of acetone in this solution?

Solution:

1) Preliminary calculations:

mass of acetone: (3.30 mL) (0.789 g/mL) = 2.6037 g
moles of acetone: 2.6037 g / 58.0794 g/mol = 0.04483 mol <--- need to look up formula of acetone
mass of solution: (75.0 mL) (0.993 g/mL) = 74.475 g
mass of water in the solution: 74.475 g - 2.6037 g = 71.8713 g
moles of water: 71.8713 g / 18.015 g/mol = 3.9896 mol

2) Molarity:

0.04483 mol / 0.0750 L = 0.598 M

3) Molality:

0.04483 mol / 0.0718713 kg = 0.624 m

4) Mole fraction:

0.04483 mol / (0.04483 mol + 3.9896 mol) = 0.0111

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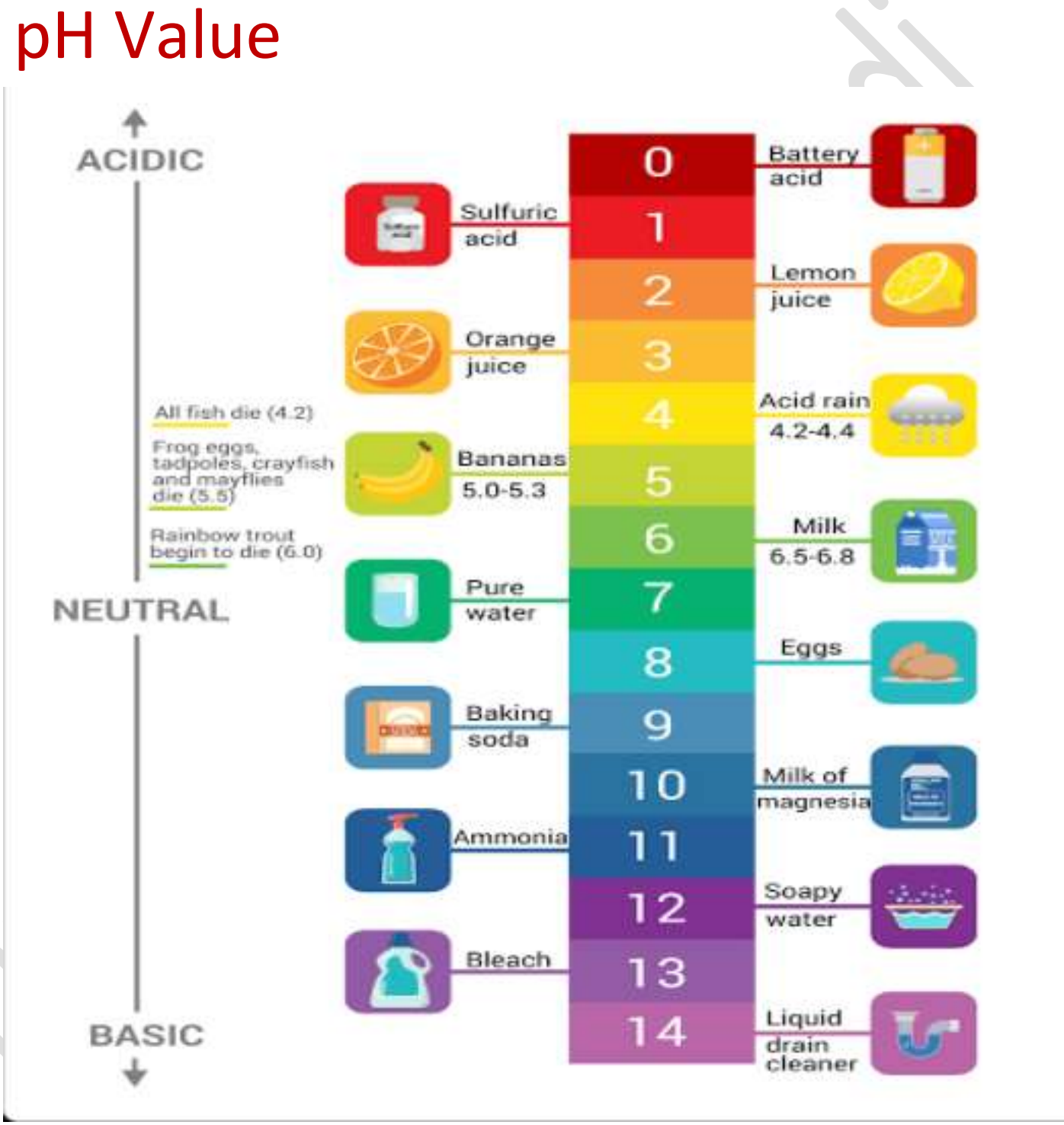
pH:

pH is a measure of how acidic/basic water is. The range goes from 0 to 14, with 7 being neutral. pH is less than 7 indicate acidity, whereas a pH of greater than 7 indicates a base. The pH of water is a very important measurement concerning water quality.



Henderson-Hasselbalch Equation:

The Henderson-Hasselbalch equation provides a relationship between the pH of acids (in aqueous solutions) and their pKa (acid dissociation constant). The pH of a buffer solution can be estimated with the help of this equation

when the concentration of the acid and its conjugate base, or the base and the corresponding conjugate acid, are known.

Equation of Henderson- Hasselbalch

The Henderson-Hasselbalch equation written as:

pH = pKa + log10 ([A-]/[HA])

Where [A] denotes the molar concentration of the conjugate base (of the acid) and [HA] denotes the molar concentration of the weak acid. Therefore, the Henderson-Hasselbalch equation can also be written as:

$$pH = pKa + \log \frac{[\text{conjugate base}]}{[\text{acid}]}$$

An equation that could calculate the pH value of a given buffer solution was first derived by the American chemist Lawrence Joseph Henderson. This equation was then re-expressed in logarithmic terms

by the Danish chemist Karl Albert Hasselbalch. The resulting equation was named the Henderson-Hasselbalch Equation.

Derivation of the Henderson- Hasselbalch Equation:

The ionisation constant of strong acids and strong bases can be easily calculated with the help of direct. However, the same methods cannot be used with acids and bases since the extent

Of ionization of these acids and bases methods is very low (weak acids and bases hardly ionize). Therefore, in order to approximate the pH of these types of solutions, the Henderson-Hasselbalch Equation is used.

Let us take an example of ionization of weak acid HA:

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

Acid dissociation constant, K_a can be given as:

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

Taking, negative log of RHS and LHS:

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

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

As we know,

$$-log\ [H^+] = pH and - log\ K_a = pKa$$

The equation above can also be written as,

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

Rearranging the equation,

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

The above equation is known as Henderson- Hasselbalch equation, popularly known as Henderson equation.

It is very useful for estimating the pH of a buffer solution and finding the equilibrium pH in acid-base reactions. From the equation, we can infer when concentration of both the species are same or in other words, acid will be half dissociation.

Preparation of buffers

A buffer is a solution that can resist pH change upon the addition of an acidic or basic component. It is able to neutralize small amounts of added acid or base, thus maintaining the pH of the solution relatively stable.

1. ACETIC ACID- SODIUM ACETATE BUFFER:

REAGENTS REQUIRED:

Acetic Acid 0.2M: 1.5 ml of glacial acetic acid is made up to 100ml with distilled water.

Sodium Acetate Solution: 0.64 gm of sodium acetate or 2.72gm of sodium acetate trihydrate is dissolved in 100ml Distilled water.

PROCEDURE:

Pipette out exactly 36.2ml of sodium acetate solution into 100ml of standard flask and add 14.8ml of glacial acetic acid, make the volume 100ml using distilled water using distilled water. This gives 0.2 M of acetic acid and sodium acetate buffer. The pH is measured with pH meter.

The pH meter is first standararised with pH buffer. Wash electrode with distilled water and introduced into 0.2M acetic acid-sodium acetate buffer prepared, the pH of solution is 4.6.

RESULT:

36.2ml Sodium acetate and 14.8 ml glacial acetic acid were mixed and buffer was prepared. pH was measured initial reading observed was 4 which made up to 4.6 with 5N NaOH.

2. BARBITONE BUFFER:

REAGENTS REQUIRED:

- Diethyl barbituric acid.

- Sodium diethyl barbiturate

PROCEDURE:

Dissolve 2.85gm of diethyl barbituric acid and 14.2gm of sodium diethyl barbiturate in distilled water and up to 1 litre. This gives the baritone buffer.

The pH meter is first standararised with pH buffer. Wash electrode with distilled water and introduced into baritone buffer prepared, the pH of solution is 6.8.

3. CITRATE BUFFER:

REAGENTS REQUIRED:

- Citric acid: Dissolve 2.101 gm of citric acid in 100ml distilled water.

Sodium citrate solution 0.1 M: Dissolved 2.941gm of sodium citrate in 100ml distilled water.

PROCEDURE:

46.5ml of citric acid with 3.5ml of sodium citrate solution and up to 100ml with distilled water. It corresponds to 0.1 M citrate buffer and standardised with pH meter and measures the pH of the prepared solution. This gives citrate buffer at pH 2.5.

RESULT:

Citrate buffer was prepared and the pH observed was 4.8 which was adjusted to 2.5 using 1N HCl and 5N NaOH.

4. CARBONATE- BICARBONATE BUFFER:

REAGENTS REQUIRED:

Sodium carbonate solution 0.2M: Dissolve 2.12gm of anhydrous sodium carbonate in 100ml Distilled water.

- Sodium bicarbonate solution: Dissolve 1.68gm of sodium bicarbonate in 100ml of distilled water.

PROCEDURE:

Pipette out exactly 27.5ml of sodium carbonate (NaCo) solution. To this add 22.5ml of sodium bicarbonate solution and made up to 100ml with distilled water which corresponds to 0.2 M sodium carbonate and bicarbonate buffer.

Standardise pH meter and measure the pH of required buffer. This gives the Carbonate- bicarbonate buffer pH 10.2.

RESULT:

Carbonate bicarbonate buffer was prepared and pH observed was 7.5 which was adjusted to 10.2 using IN Hel and 5N NaOH

5. PHOSPHATE BUFFER:

REAGENTS REQUIRED:

- Monobasic: Dissolve 2.78gm of sodium dihydrogen phosphate in 100ml of distilled water.

- Dibasic sodium phosphate (0.2M): Dissolve 5.3gm of disodium hydrogen phosphate or 7.17 gm sodium hydrogen phosphate in 100ml distilled water.

PROCEDURE:

39 ml of dihydrogen sodium phosphate is mixed with 61 ml of disodium hydrogen phosphate This made up to 200ml with distilled water This gives phosphate (Po); buffer of 0.2M.

Standardized pH meter with standard buffer. Washed electrode with distilled water and introduced it into phosphate buffer prepared. The pH of the solution is 6.8.

RESULT:

Phosphate buffer was prepared and pH was observed 8.5 which was made up to 6.8 using IN Hel and 5N NaOH

6. POTASSIUM PHOSPHATE BUFFER:

REAGENTS REQUIRED:

- Dipotassium hydrogen phosphate

- Potassium dihydrogen phosphate

PROCEDURE:

174.18 g/mol dipotassium hydrogen phosphate and 136.09 g/mol potassium dihydrogen phosphate was taken and made up to 200ml using distilled water. This gives the potassium buffer.

Standardised pH meter with standard buffer. Washed electrode with distilled water and introduced it into potassium buffer prepared. The pH of the solution is 6.5.

RESULT:

Dipotassium hydrogen phosphate (KH_2PO_4) and potassium dihydrogen phosphate (KH_2PO_4) solution were prepared and the pH was measured to be 9.87 and 4.23 respectively, the solution was made using IN Hel and 5N NaOH respectively and the pH was found to be 6.5.

Buffering against pH Changes in Biological Systems

Almost every biological process is pH dependent; a small change in pH produces a large change in the rate of the process.

Cells and organisms maintain a specific and constant cytosolic pH, keeping biomolecules in their optimal ionic state, usually near pH 7. In multicellular organisms, the pH of extracellular fluids is also tightly regulated. Constancy of pH is achieved primarily by biological buffers: mixtures of weak acids and their conjugate bases.

Biological buffering is illustrated by the phosphate and carbonate buffering systems of humans.

Blood plasma is buffered in part by the bicarbonate system, consisting of carbonic acid (H2CO3) as proton donor and bicarbonate (HCO3-) as proton acceptor

Buffering against pH changes in biological system

· The presence of a weak acid and associated salt (for example, acetic acid and sodium acetate) in the solution provides protection by maintaining equilibrium through ion transfer and neutralization.

· A mixture of two acid salts can provide the same effect; popular buffering agents include phosphate, carbonates, and ammonium salts. · The ability of most intact biological entities to prevent substantial changes in pH is an important trait.

· Excessive pH variation is not tolerated by the cytoplasmic fluid, which contains dissolved proteins, organic substrates, and inorganic salts. · The blood plasma is highly effective buffer solution that is almost perfectly engineered to maintain a pH range of 7.2 to 7.3 in the blood. · The circulating blood of animals contains a complex and important buffer system. CO_3 -HCO_3-: Na_2 HPO_4 ; oxygenated and deoxygenated forms of hemoglobin; and plasma proteins are the components of this system.

· To maintain their original strength, many commercial goods are roughly buffered.

Conclusion:

A buffer is an aqueous solution made up of a weak acid and its salt (acid buffer) or a weak base and its salt (base buffer) (basic buffer).

When a tiny amount of strong acid is given to it, its pH varies very little, and it is thus used to keep a solution's pH stable.

Buffer solutions are utilised in a variety of chemical processes. In nature, a buffer solution can be found in the form of blood.

The pH of human blood is 7.4 in its normal state. Many people suffer from extreme anxiety as well as alkalosis.

Alkalosis is a condition in which the blood pH is abnormally high.
Acidosis is a condition in which the pH of the blood is greater th

Module 2
Syllabus: Carbohydrates and Lipids

CARBOHYDRATES AND LIPIDS

There is a wide diversity in living organisms in our biosphere. Now a question that arises in our minds is: Are all living organisms made of the same chemicals, i.e., elements and compounds? We have learnt in chemistry how elemental analysis is performed. If we perform such an analysis on a plant tissue, animal tissue or a microbial paste, we obtain a list of elements like carbon, hydrogen, oxygen and several others and their respective content per unit mass of a living tissue. If the same analysis is performed on a piece of earth's crust as an example of non-living matter, we obtain a similar list. What are the differences between the two lists? In absolute terms, no such differences could be made out. All the elements present in a sample of earth's crust are also present in a sample of living tissue. However, a closer examination reveals that the relative abundance of carbon and hydrogen with respect to other elements is higher in any living organism than in earth's crust.

2.1 biomacromolecules

There is one feature common to all those compounds found in the acid soluble pool. They have molecular weights ranging from 18 to around 800 daltons (Da) approximately.
The acid insoluble fraction, has only four types of organic compounds i.e., proteins, nucleic acids, polysaccharides and lipids. These classes of compounds with the exception of lipids, have molecular weights in the range of ten thousand daltons and above. For this very reason, biomolecules, i.e., chemical compounds found in living organisms are of two types. One, those which have molecular weights less than one thousand dalton and are usually referred to as micromolecules or simply

Component	% of the total cellular mass
Water	70-90
Proteins	10-15
Carbohydrates	3
Lipids	2
Nucleic acids	5-7
Ions	1

biomolecules while those which are found in the acid insoluble fraction are called macromolecules or biomacromolecules.

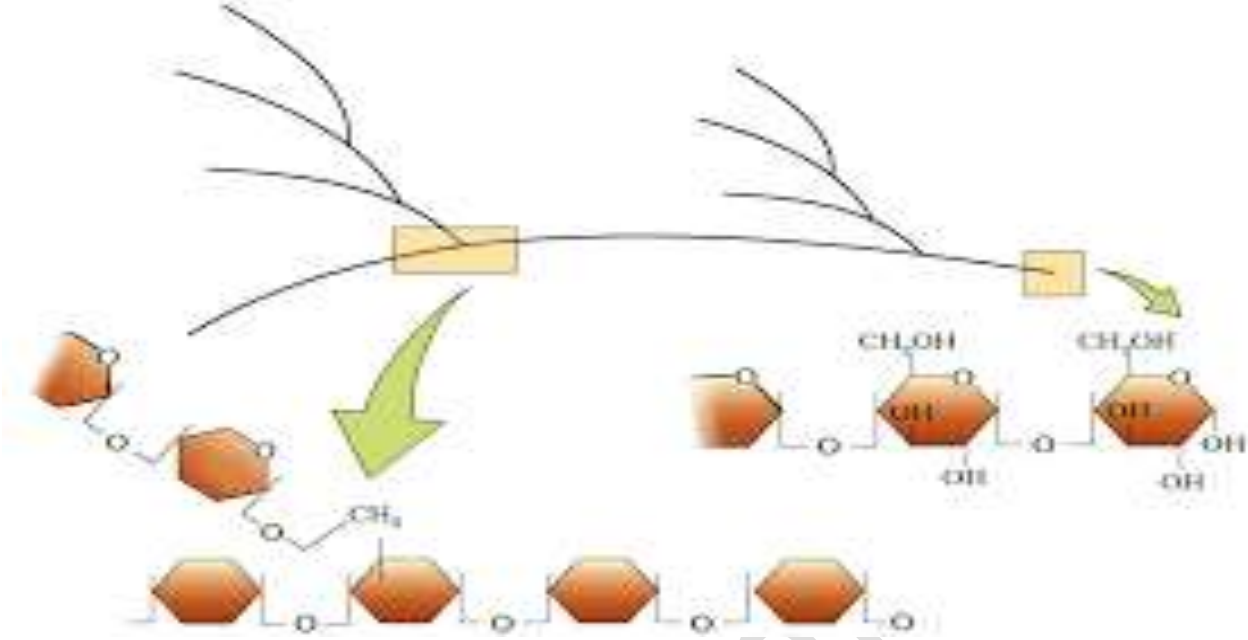
The molecules in the insoluble fraction with the exception of lipids are polymeric substances. Then why do lipids, whose molecular weights do not exceed 8oo Da, come under acid insoluble fraction, i.e.. macromolecular fraction? Lipids are indeed small molecular weightcompounds and are present not only as such but also arranged into structures like cell membrane and other membranes. When we grind a tissue, we are disrupting the cell structure. Cell membrane and other membranes are broken into pieces, and form vesicles which are not water soluble. Therefore, these membrane fragments in the form of vesicles get separated along with the acid insoluble pool and hence in the macromolecular fraction. Lipids are not strictly macromolecules.

The acid soluble pool represents roughly the cytoplasmic composition. The macromolecules from cytoplasm and organelles become the acid insoluble fraction. Together they represent the entire chemical composition of living tissues or organisms.

2.2 CARBOHYDRATES

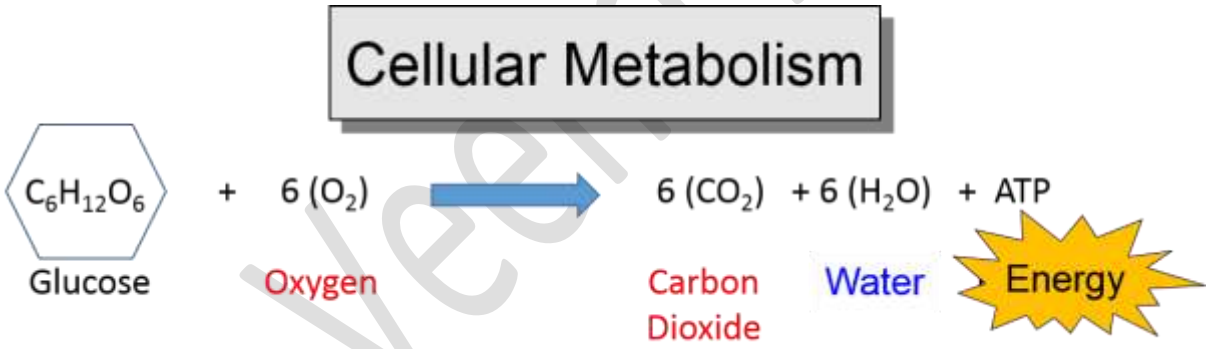
The acid insoluble pellet also have polysaccharides(carbohydrates) as another class of macromolecules. Polysaccharides are long chains of sugars. They are threads (literally a cotton thread) containing different monosaccharides as building blocks. For example, cellulose is a polymeric polysaccharide consisting of only one type of monosaccharide i.e., glucose. Cellulose is a homopolymer. Starch is a variant of this but present as a store house of energy in plant tissues. Animals have another variant called glycogen. Inulin is a polymer of fructose. In a polysaccharide chain (say glycogen), the right end is called the reducing end and the left end is called the non-reducing colour. It has branches as shown in the form of cartoon. Starch forms helical secondary

structures. The starch-I2 is blue in colour. Cellulose does not contain complex helices and hence cannot hold I2.



Diagrammatic representation of a portion of glycogen

Carbohydrates are polyhydroxy aldehydes or ketones. They are primarily produced by plants and form a very large group of naturally occurring organic substances. Some common examples are cane sugar, glucose, starch, etc. They have general molecular formulas that make them appear to be hydrates of carbon, C(H2O), from where the name carbohydrate was derived. Carbohydrates are formed in the plants by photosynthesis from carbon dioxide and water in the presence of sunlight.



2.2 Classification

Carbohydrates are classified into two main classes, sugars and polysaccharides.

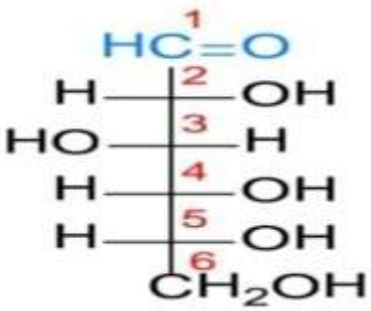
2.2.1 Sugars

Sugars are sweet crystalline substances that are soluble in water. These are further classified on the basis of their behaviour on hydrolysis.

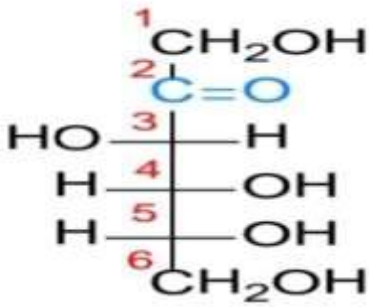
2.2.1.1 Monosaccharides

The simplest form of carbohydrate is the monosaccharide. 'Mono' means 'one' and 'saccharide' means 'sugar'. Monosaccharides are polyhydroxy aldehyde or ketone that cannot be hydrolyzed further to give simpler sugar. They may again be classified on the basis of the nature of carbonyl group.

- Polyhydroxy aldehydes are called aldoses. Example: Glucose
- Polyhydroxy ketones are called ketoses. Example: Fructose



Glucose
(Aldose)



Fructose
(Ketose)

The aldoses and ketoses are further divided based on the number of carbons present in their molecules, as trioses, tetroses, pentoses, hexoses etc. They are referred to as aldotrioses, aldotetroses, aldopentoses, aldohexoses etc.

Number of Carbons	General term	Aldehyde	Ketone
3	Triose	Aldotriose	Ketotriose
4	Tetrose	Aldotetrose	Ketotetrose
5	Pentose	Aldopentose	Ketopentose
6	Hexose	Aldohexose	Ketohexose
7	Heptose	Aldoheptose	Ketoheptose

Monosaccharides Structure

All monosaccharides have the same general formula of $(CH_2O)_n$, which designates a central carbon molecule bonded to two hydrogens and one oxygen. The oxygen will also bond to a hydrogen, creating a hydroxyl group. Because carbon can form 4 bonds, several of these carbon molecules can bond together. One of the carbons in the chain will form a double bond with oxygen, which is called a carbonyl group. If this carbonyl occurs at the end of the chain, the monosaccharide is in the aldose

family. If the carbonyl group is in the middle of the chain, the monosaccharides are in the ketose family.

Function of monosaccharides

Monosaccharides have many functions within cells.

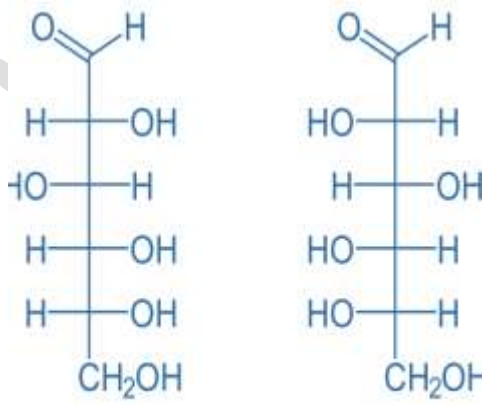
- First and foremost, monosaccharides are used to produce and store energy. Most organisms create energy by breaking down the monosaccharide glucose, and harvesting the energy released from the bonds.
- Other monosaccharides are used to form long fibers, which can be used as a form of cellular structure. Plants create cellulose to serve this function, while some bacteria can produce a similar cell wall from slightly different polysaccharides. Even animal cells surround themselves with a complex matrix of polysaccharides, all made from smaller monosaccharides.

Examples of monosaccharides

Glucose

Glucose is an important monosaccharide in that it provides both energy and structure to many organism. Glucose molecules can be broken down in glycolysis, providing energy and precursors for cellular respiration. If a cell does not need any more energy at the moment, glucose can be stored by combining it with other monosaccharides. Plants store these long chains as starch, which can be disassembled and used as energy later. Animals store chains of glucose in the polysaccharide glycogen, which can store a lot of energy.

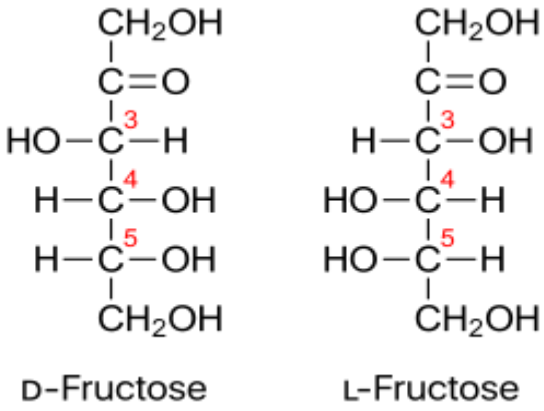
Glucose can also be connected in long strings of monosaccharides to form polysaccharides that resemble fibers. Plants typically produce this as cellulose. Cellulose is one of the most abundant molecules on the planet, and if we could weigh all of it at once it would weigh millions of tons. Each plant uses cellulose to surround each cell, creating rigid cell walls that help the plants stand tall and remain turgid. Without the ability of monosaccharides to combine into these long chains, plants would be flat and squishy.



D-Glucose L-Glucose

Fructose

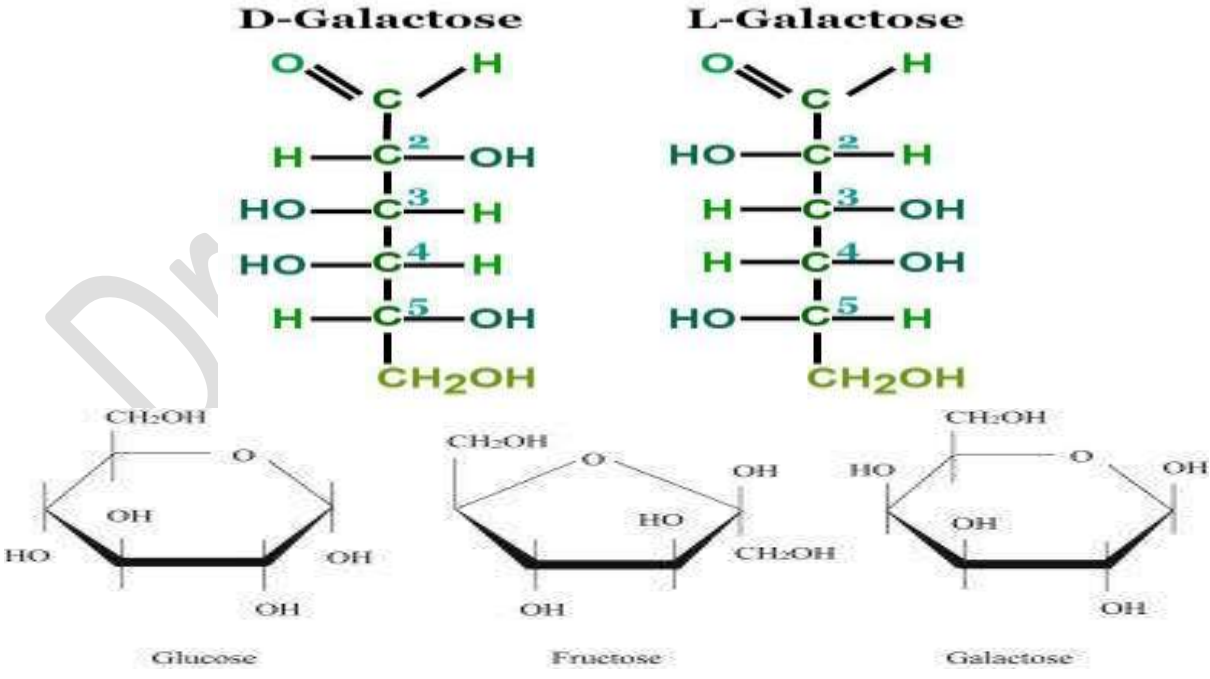
Although almost identical to glucose, fructose is a slightly different molecule. The formula ($(CH_2O)_6$) is the same, but the structure is much different. Below is an image of fructose:



Notice that instead of the carbonyl group being at the end of the molecule, as in glucose, it is the second carbon down. This makes fructose a ketose, instead of an aldose. Like glucose, fructose still has 6 carbons, each with a hydroxyl group attached. However, because the double bonded oxygen in fructose exists in a different place, a slightly different shaped ring is formed. In nature, this makes a big difference in how the sugar is processed. Most reactions in cells are catalyzed by specific enzymes. Different shaped monosaccharides each need a specific enzyme to be broken down.
Fructose, because it is a monosaccharide, can be combined with other monosaccharides to form oligosaccharides. A very common disaccharide made by plants is sucrose.

Galactose

Galactose is a monosaccharide produced in many organisms, especially mammals. Mammals use galactose in milk, to give energy to their offspring. Galactose is combined with glucose to form the disaccharide lactose. The bonds in lactose hold a lot of energy, and special enzymes are created by newborn mammals to break these bonds apart. Once being weaned of their mother's milk, the enzymes that break lactose down into glucose and galactose monosaccharides are lost.



2.2.1.2 Oligosaccharides

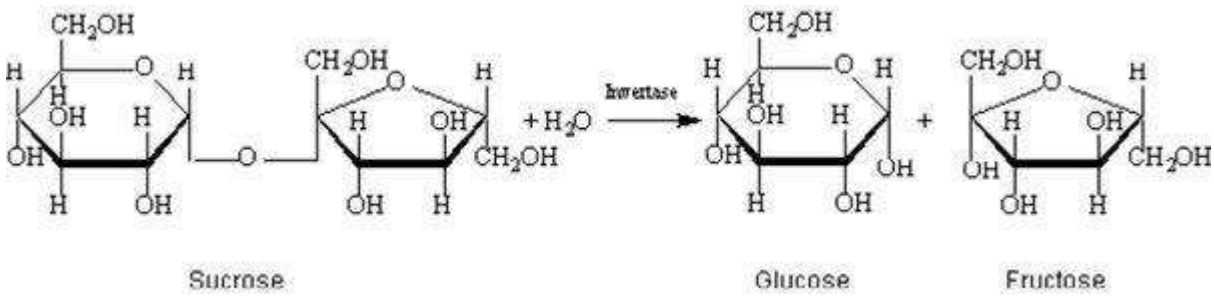
Carbohydrates that produce two to ten monosaccharide units during the hydrolysis are called oligosaccharides. They can be further classified based on the number of monosaccharide units formed on hydrolysis.

Disaccharides: They give two monosaccharide units on hydrolysis, which may be the same or different. For example, sucrose on hydrolysis gives one molecule each of glucose and fructose, whereas maltose gives two molecules of glucose. The oxide linkage is formed after the loss of the water molecule and then the two monosaccharides are formed by that linkage. When two monosaccharide units are joined via the oxygen atom then that linkage is called a glycosidic linkage.

Disaccharide structure

1.Sucrose

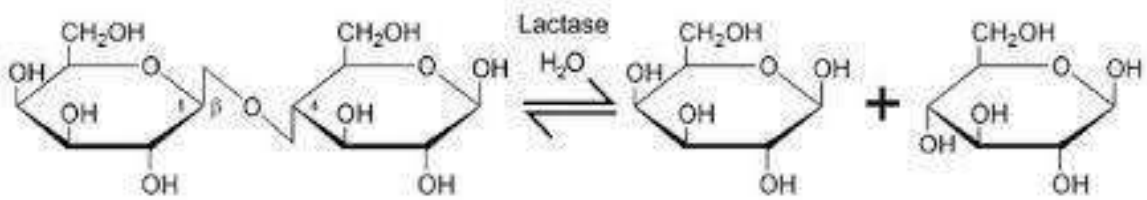
Sucrose being dextrorotatory in nature gives dextrorotatory glucose as well as laevorotatory fructose on hydrolysis. The overall mixture is laevorotatory and this is because the laevorotation of fructose (-92.4) is more than the dextrorotation of glucose(+52.5)



$C_{12}H_{22}O_{11}$ $C_6H_{12}O_6$ $C_6H_{12}O_6$

2.Lactose

Commonly it is called milk sugar as this disaccharide is found in milk. It is made up of ß-D-galactose and ß-D-glucose. The bond is between the first carbon of galactose and the fourth carbon of glucose. This is also a reducing sugar.

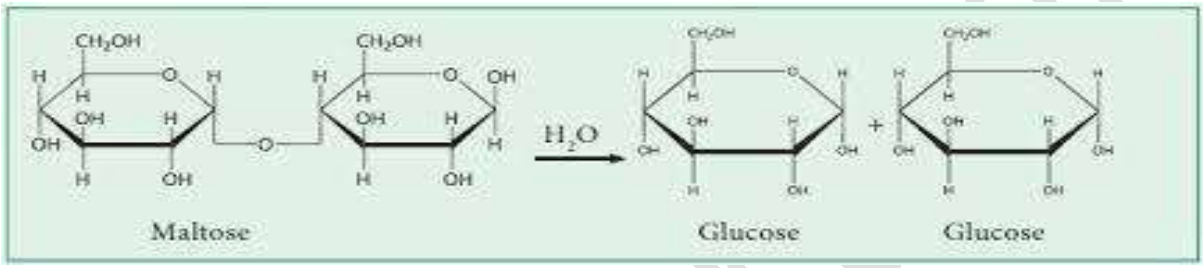


Lactose D-galactose D-glucose

$C_{12}H_{22}O_{11}$ $C_6H_{12}O_6$ $C_6H_{12}O_6$

3. Maltose

Maltose is also one of the disaccharide which have two α-D-glucose units which are connected by the first carbon of the glucose and also linked to the fourth carbon of another unit. In this solution, a free aldehyde can be produced at the first carbon of the second glucose of the solution and it is reducing sugar.



$C_{12}H_{22}O_{11}$ $C_6H_{12}O_6$ $C_6H_{12}O_6$

Isomers, Epimers, Enantiomers

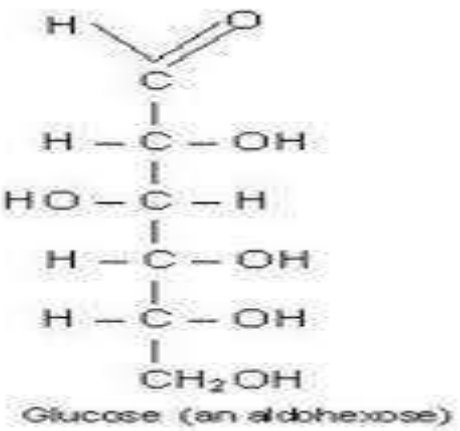
Isomerism: Isomerism is the phenomenon in which more than one compounds have the same chemical formula but different chemical structures.

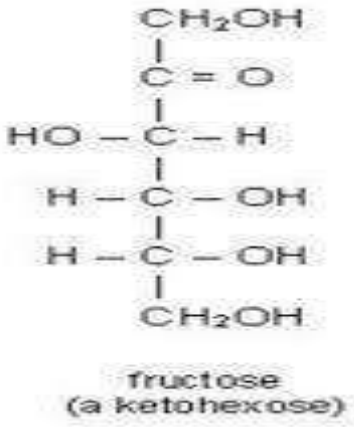
Isomers: Chemical compounds that have identical chemical formulae but differ in properties and the arrangement of atoms in the molecule are called isomers. Therefore, the compounds that exhibit isomerism are known as isomers.

Structural and Optical Isomerism in Carbohydrates

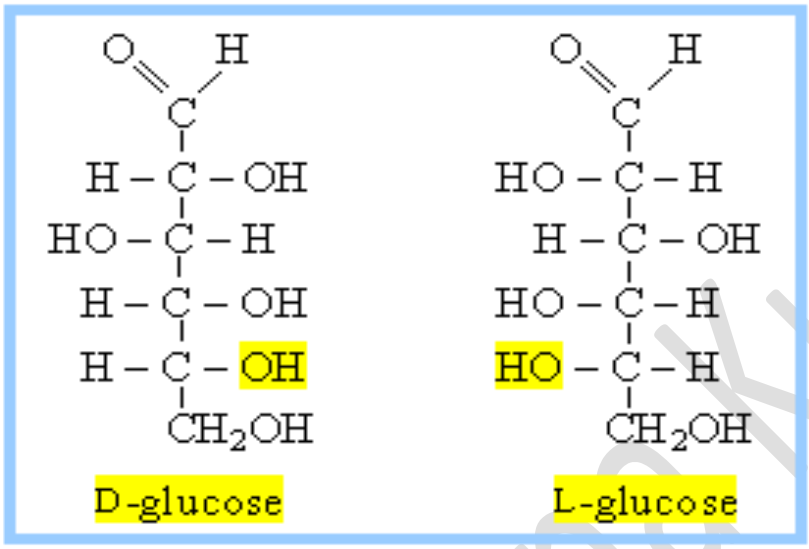
Structural and optical isomers are common in organic compounds such as carbohydrates.

Structural isomers of carbohydrates are the different structural forms of the same chemical formula; for example, Glucose and Fructose are structural isomers of each other. Chemical formula of both compounds is C6H12O6, but they have different structures, which leads to glucose having an aldehyde group and fructose having a ketone group.



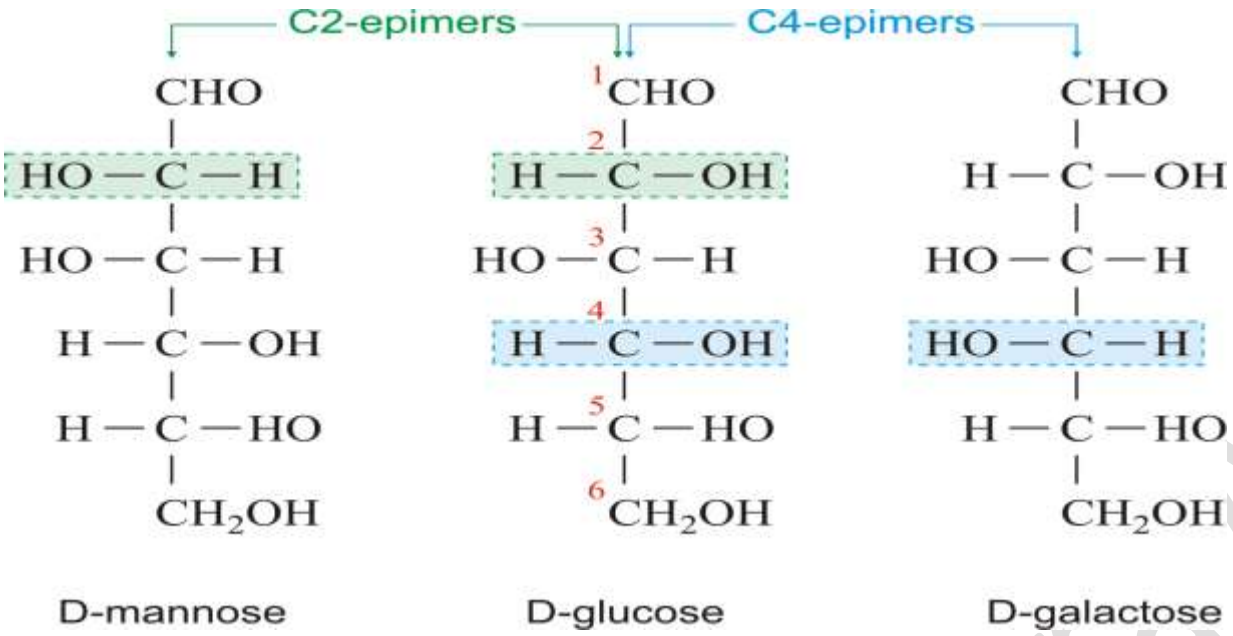


Optical isomers in carbohydrates are the different mirror images of the same structure. We name them as D and L isomers.



Epimerism: Optical isomerism in which isomers(epimers) can form about asymmetric atoms within the molecule especially carbohydrates.

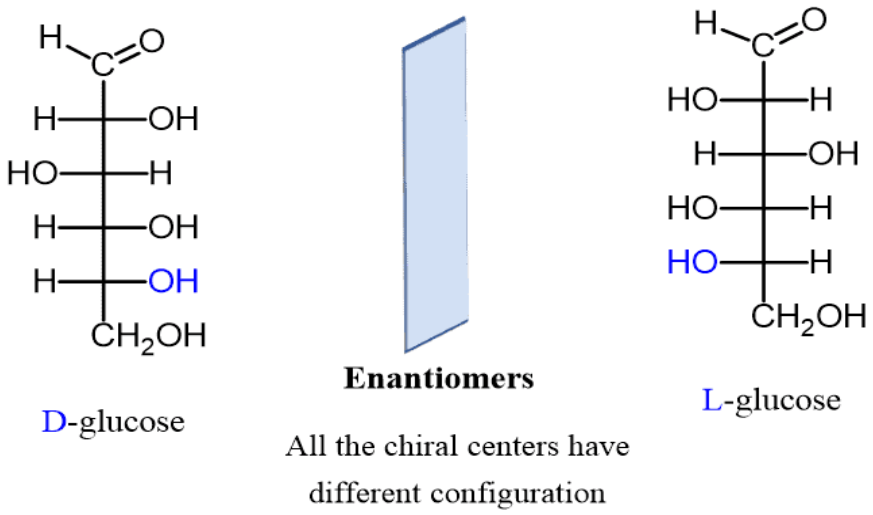
Epimers: Two sugars that differ only in the configuration around one carbon atom are called epimers.

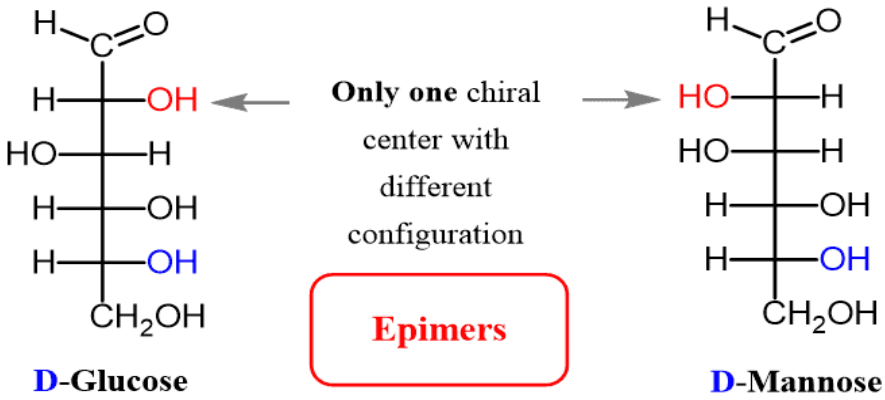


All epimers are isomers but not all isomers are epimers

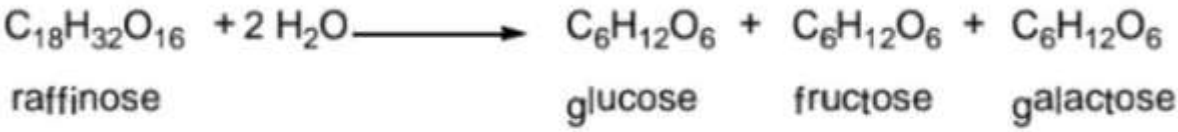
Enantiomers: Enantiomers are a pair of molecules that exist in two forms that are a mirror images of one another but cannot be superimposed one upon the other.

- The structural basis of enantiomerism is called chirality.
- Enantiomers are mirror images of each other.One such enantiomer contains C bound to 4 different molecules and is called a chiral molecule.
- Chiral molecules rotates the polarised light to the right(D form) or to the (L form) molecules.
  Examples: Amino acids(L form)
  Sugars(D form)
- Enantiomers are special types of stereoisomers





Trisaccharides: These carbohydrates yield three molecules of monosaccharides units on hydrolysis.



Function of disaccharide

The two main functions of disaccharides are to provide energy and to help with the absorption of nutrients.

- Disaccharides are broken down into two monosaccharides during digestion, and these monosaccharides are then used for energy or to help build other molecules like proteins and lipids.

- Disaccharides can also help in absorption of other nutrients like vitamins and minerals. For example, lactose, a disaccharide found in milk, helps in absorption of the nutrient calcium.
- Disaccharides are carbohydrates found in many foods and are often added as sweeteners. Sucrose, for example, is table sugar and Maltose is a sweetener that is often found in chocolates and other candies
- Lactose is found in breast milk and provides nutrition for infants.
- Disaccharides are used for transporting nutrients in the phloem.
- Plants also use disaccharides to transport monosaccharides as packaging monosaccharides into disaccharides makes the molecules less likely to break down during transport.

2.2.1.3 Polysaccharides

Polysaccharides are major classes of biomolecules. They are long chains of carbohydrate molecules, composed of several smaller monosaccharides. These complex bio-macromolecules functions as an important source of energy in animal cell and form a structural component of a plant cell. It can be a homopolysaccharide or a heteropolysaccharide depending upon the type of the monosaccharides. Polysaccharides can be a straight chain of monosaccharides known as linear polysaccharides and polysaccharides can be a branched chain of monosaccharides known as branched polysaccharides. These carbohydrates give a large number of monosaccharide units on hydrolysis. These monosaccharide units are joined together by oxide bridges. These linkages are called glycosidic linkages. The common and widely distributed polysaccharides correspond to the general formula $(C_6H_{10}O_5)_n$ are not sweet in taste, so they are called non-sugars. Some common examples are starch, cellulose, glycogen, etc.

Types of polysaccharides

Polysaccharides are categorised into :

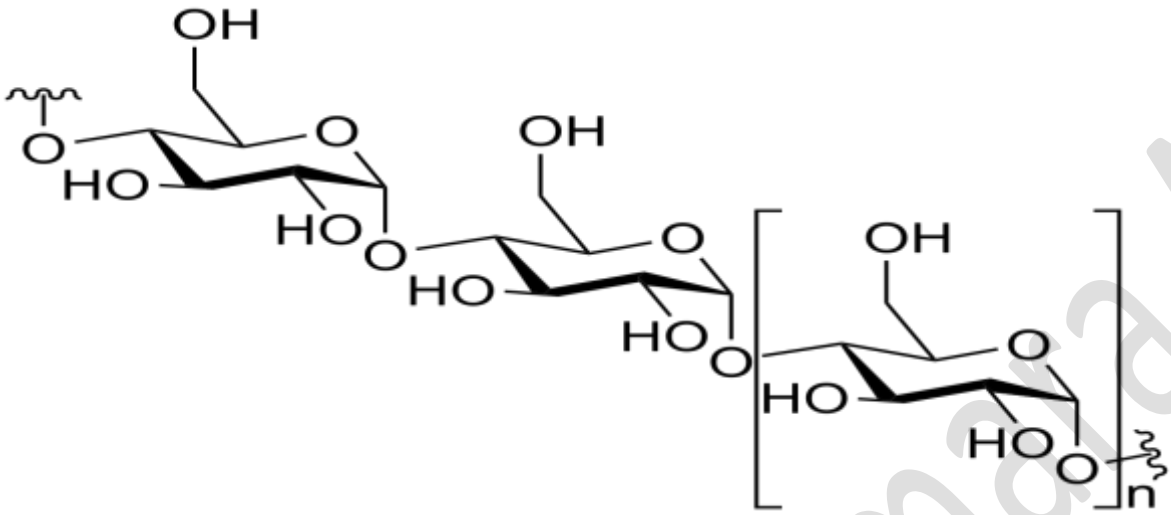
1. **Homopolysaccharides:** These are made up of one type of monosaccharide unit.

   Ex: cellulose, starch, glycogen.
2. **Heteropolysaccharides:** These are made up of two or more types of monosaccharide units. Ex: hyaluronic acid and they provide extracellular support for organisms.

Structure of polysaccharides

All polysaccharides are formed by the same basic process where monosaccharides are connected via glycosidic bonds. These glycosidic bonds consist of an oxygen molecule bridging two carbon rings. The bond is formed when a hydroxyl group is lost from the carbon of one molecule, while the hydrogen is lost by the hydroxyl group of another monosaccharide. Because two molecules of

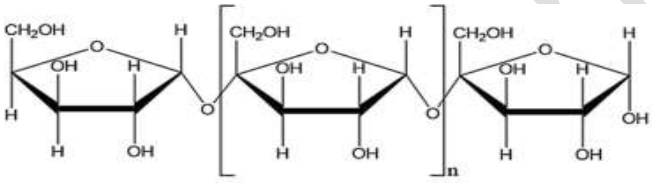
hydrogen and one of oxygen are expelled, the reaction is a dehydration reaction. The structure of the molecules being combined determines the structures and properties of the resulting polysaccharide. A polysaccharide used for energy storage will give easy access to the constituent monosaccharides whereas a polysaccharide used for support is usually a long chain of monosaccharides that form fibrous structures.



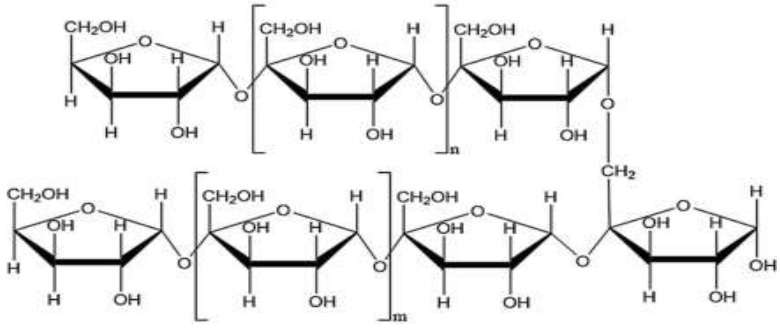
Some important Polysaccharides

Homopolysaccharides

1. **Starch:** It is the storage polysaccharide found in plant cells and exists in two forms: amylose is the helical form of starch comprised only of alpha-1,4 linkages and amylopectin that has a structure like glycogen except that the branched alpha-1,6 linkages are present on only about one in 30 monomers.



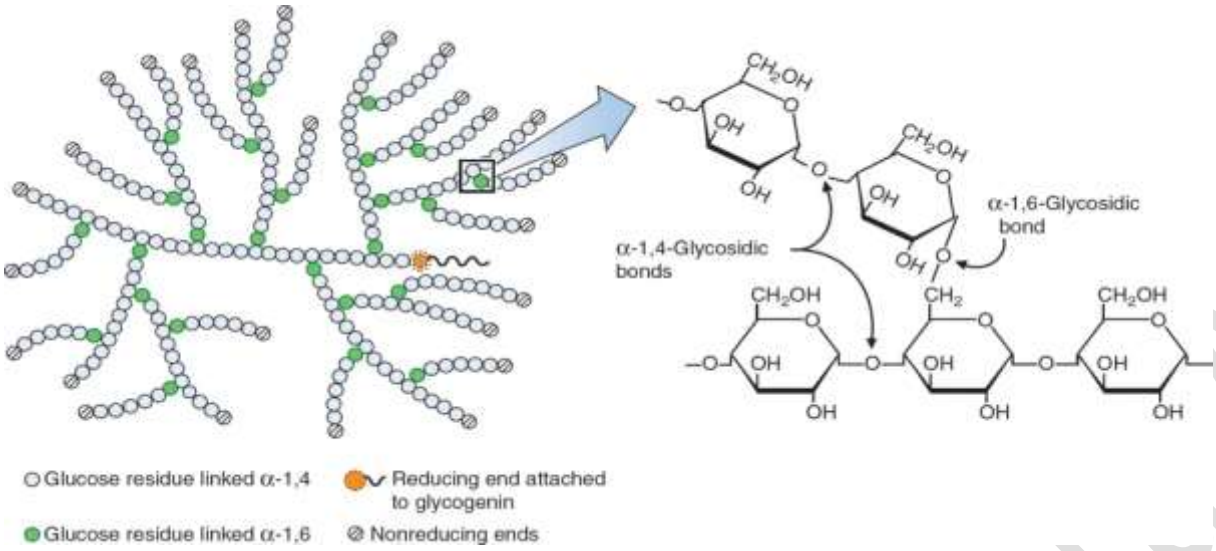
Amylose



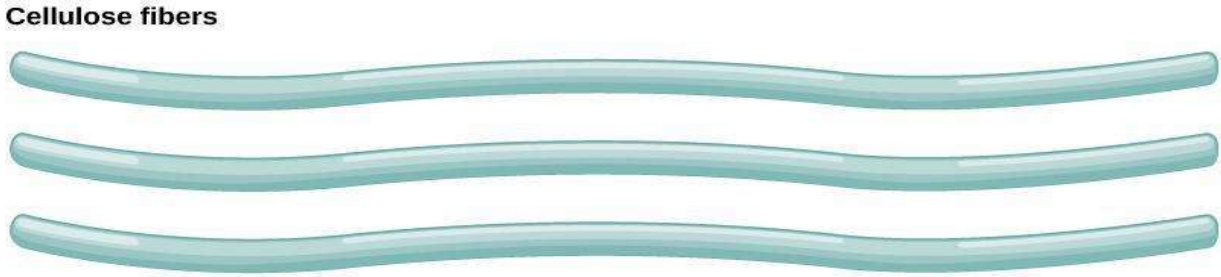
Amylopectin

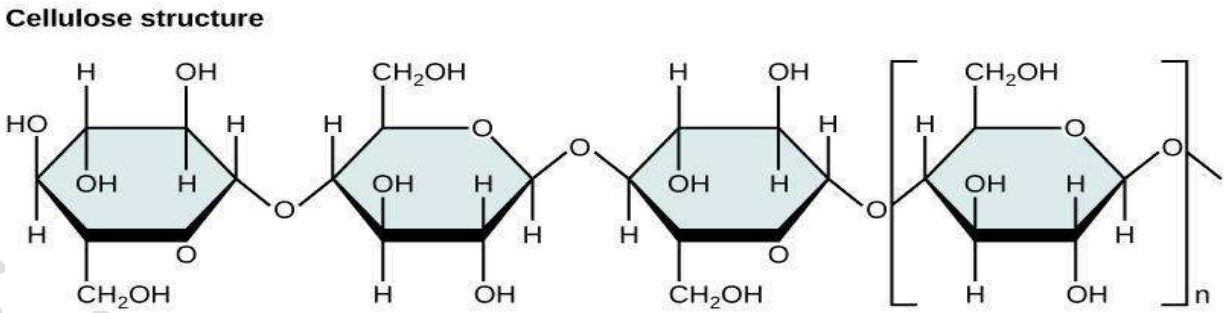
2. **Glycogen:** This polysaccharide is the polysaccharide found in animals to store energy and is composed of alpha-1,4-glycosidic bonds with branched alpha-1,6 bonds present at about

every tenth monomer. It is mainly produced by the liver and muscles, but it can also be made during a process called glycogenesis.



3. **Cellulose:** Cellulose is the main polysaccharide used for structural function in plants. This is one of the most common organic compounds found on the planet, obviously. Cellulose is an unbranched glucose residue polymer put together via beta-1,4 connections, which enables the molecule to form long, straight chains.





Heteropolysacchrides

These are found in different structural and functional roles in the human body.

• **Hyaluronic Acid:** Acts as a lubricant in the synovial fluid of joints

• **Chondroitin Sulfate:** It contributes to tensile strength and elasticity of cartilages, tendons, ligaments, and walls of the aorta.

• **Dermatan sulfate:** It is found mainly in the skin, and also is in vessels, heart, lungs. It may be related to coagulation and vascular diseases and other conditions.

• **Keratan sulfate:** Present in the cornea, cartilage bone and a variety of other structures as nails and hair.
• **Heparin:** Is present as an anticoagulant in the blood.

Note: Another type of polysaccharides that are found in the human body is glycosaminoglycans or mucopolysaccharides that are formed by the endoplasmic reticulum. These mature in the Golgi apparatus. They form important components of connective tissues and are found in collagen and elastin.

Functions of polysaccharides

The three main functions of polysaccharides are providing structural support, storing energy, and sending cellular communication signals.

- The carbohydrate structure largely determines its function. Linear molecules, like cellulose and chitin, are strong and rigid. Cellulose is the primary support molecule in plants, while fungi and insects rely on chitin.
- Polysaccharides used for energy storage tend to be branched and folded upon themselves. Because they are rich in hydrogen bonds, they are usually insoluble in water. Examples of storage polysaccharides are starch in plants and glycogen in animals.
- Polysaccharides used for cellular communication are often covalently bonded to lipids or proteins, forming glycoconjugates. The carbohydrate serves as a tag to help the signal reach the proper target. Categories of glycoconjugates include glycoproteins, peptidoglycans, glycosides, and glycolipids. Plasma proteins, for example, are actually glycoproteins.

REFER: PDB structures from rcsb.org

2.3Reducing and non reducing sugars

Sugars can be classified as reducing or non-reducing; this classification is dependent on their ability to donate electrons. **Reducing sugar**

- They can donate electrons (the carbonyl group becomes oxidised), the sugars become the reducing agent
- Thus reducing sugars can be detected using the Benedict's test as they reduce the soluble copper sulphate to insoluble brick red copper oxide.
- Hemiacetals or hemiketals are in equilibrium with the open- chain sugars in aqueous solution. These compounds can reduce an oxidizing agent (eg. Br_2), thus, they are classified as a reducing sugar.
- Examples: Glucose, Fructose, Maltose.

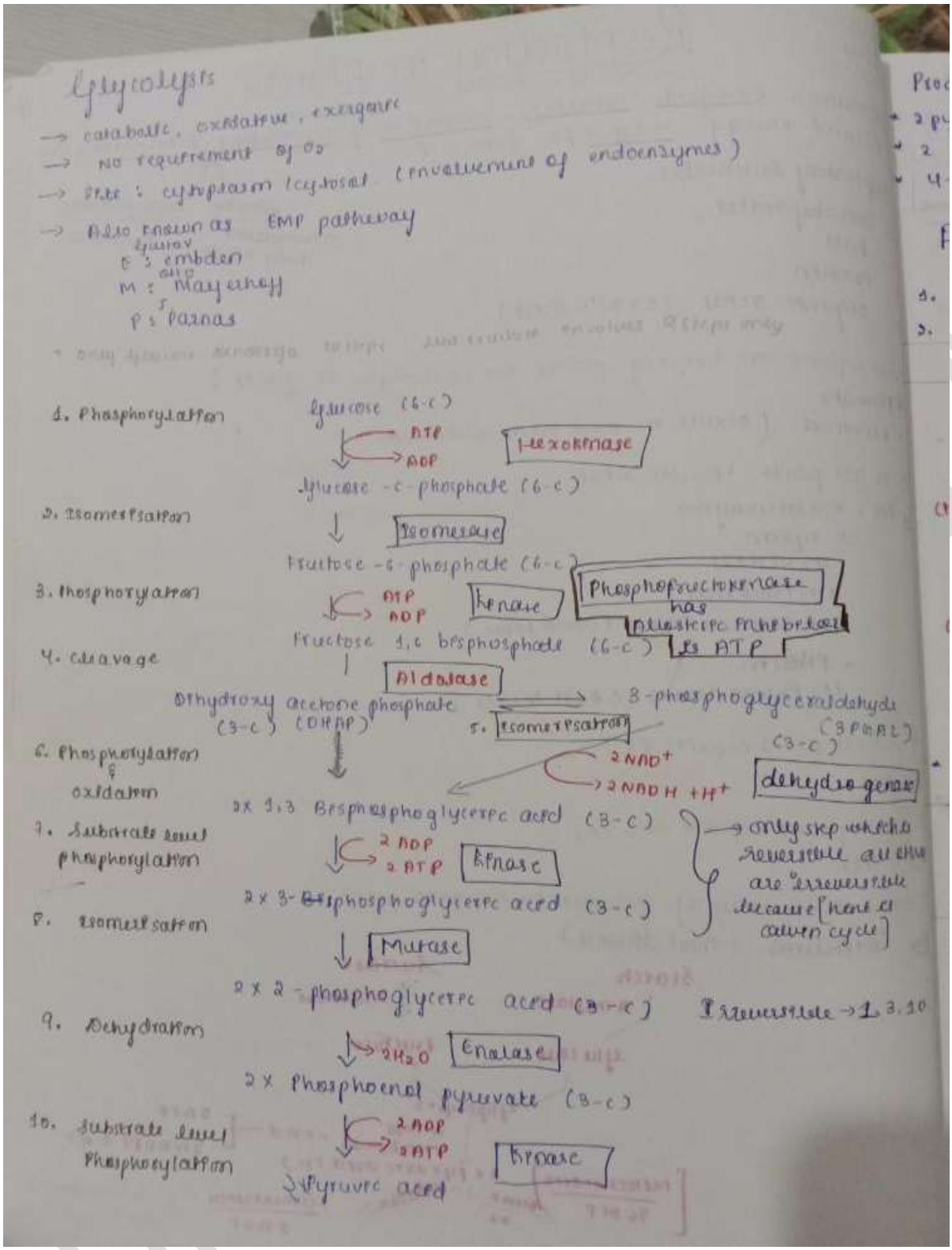
Non Reducing sugar

- Non-reducing sugars cannot donate electrons, therefore they cannot be oxidised
- To be detected non-reducing sugars must first be hydrolysed to break the disaccharide into its two monosaccharides before a Benedict's test can be carried out
- Cyclic acetals or ketals are not in equilibrium with their open chain carbonyl group containing forms in neutral or basic aqueous solutions. They cannot be oxidized by reagents such as Tollen's reagent (Ag^+, NH_3, OH) or Br_2. So, these are referred as non-reducing sugars.
- Example: sucrose.

2.4 Carbohydrate Metabolism

2.4.1 Glycolysis

Glycolysis is a metabolic pathway and an anaerobic source of energy that has evolved in nearly all types of organisms. The process entails the oxidation of glucose molecules, the single most important organic fuel in plants, mirobes, and animals. Most cells prefer glucose (there are exceptions, such as acetic acid bacteria which prefer ethanol). In glycolysis, per molecule of glucose, 2 ATP molecules are utilized, while 4 ATP, 2 NADH, and 2 pyruvates are produced. The pyruvate can be used in the citric acid cycle, or serve as a precursor for other reactions.
Glucose is a hexose sugar, which means that it is a monosaccharide with 6 carbon atoms and 6 oxygen atoms. The first carbon consists of an aldehyde group, and the other 5 carbons have 1 hydroxyl group each. In glycolysis, glucose is broken down ultimately into pyruvate and energy, a total of 2 ATP, is derived in the process (Glucose + 2 NAD++ 2 ADP + 2 Pi --> 2 Pyruvate + 2 NADH + 2 H+ + 2 ATP + 2 H2O). The hydroxyl groups allow for phosphorylation. The specific form of glucose used in glycolysis is glucose 6-phosphate.
Glucokinase is a subtype of hexokinase found in humans. Glucokinase has a lower affinity for glucose and is found only in the pancreas and liver, whereas hexokinase is found in all cells.



Function

Glycolysis occurs in the cytosol of the cell. It is metabolic pathway which creates ATP without the use of oxygen but can occur in the presence of oxygen as well. In cells which use aerobic respiration as the primary source of energy, the pyruvate formed from the pathway can be used in the citric acid cycle and go through oxidative phosphorylation to be oxidized into carbon dioxide and water. Even if cells primarily use oxidative phosphorylation, glycolysis can serve as an emergency backup for energy or serve as the preparation step before oxidative phosphorylation. In highly oxidative

tissue, such as the heart, the production of pyruvate is important for acetyl-CoA synthesis and L-malate synthesis and serves as a precursor to many molecules, such as lactate, alanine, and oxaloacetate.

Glycolysis precedes lactic acid fermentation; the pyruvate made in the former process serves as the prerequisite for the lactate made in the latter process. Lactic acid fermentation is the main source of ATP in animal tissues with low metabolic requirements and with low mitochondrial levels. In erythrocytes, lactic acid fermentation is the sole source of ATP for these cells have no mitochondria, and once mature, the red blood cells have little demand for ATP. Another part of the body which relies entirely or almost entirely on anaerobic glycolysis is the lens of the eye, which is devoid of mitochondria to prevent light scattering.

Though skeletal muscles prefer to catalyze glucose into carbon dioxide and water during heavy exercise where the amount of oxygen is inadequate, the muscles simultaneously undergo anaerobic glycolysis along with oxidative phosphorylation.

2.4.2 Fate of pyruvate

- To understand fate of pyruvate under different conditions.
- Pyruvate has 3 fates- depending on availability of oxygen.
- In the presence of oxygen (aerobic conditions): enter into the tricarboxylic acid (TCA)cycle-PDH.
- Under the anaerobic conditions: results in formation of lactic acid with help of lactate dehydrogenase or ethanol fermentation- pyruvate decarboxylase, alcohol dehydogenase.

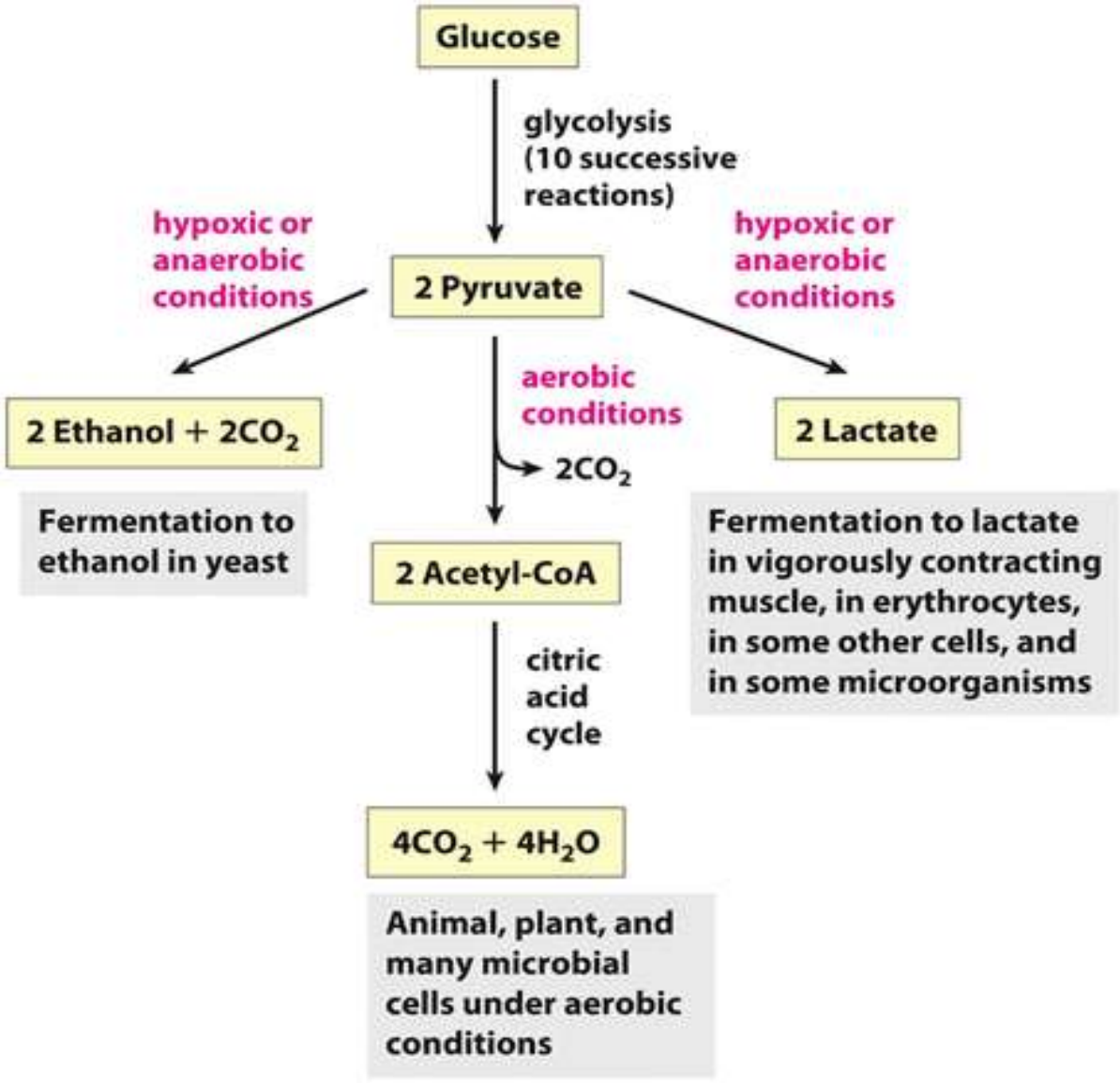
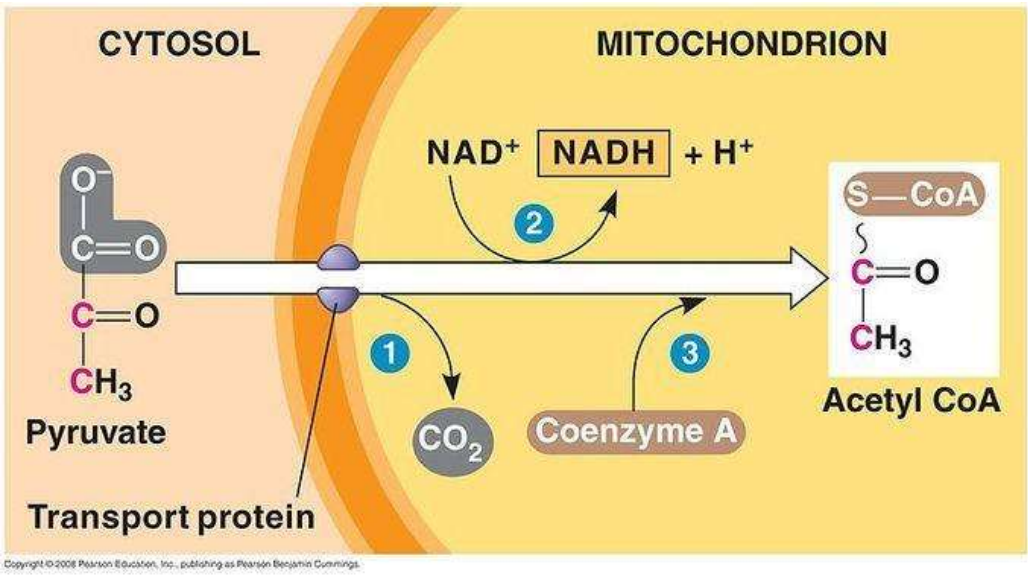


Figure 14-4
Lehninger Principles of Biochemistry, Sixth Edition

- Pyruvate, a key molecule in metabolism of eukaryotic and human and its fate differs depending upon presence and absence of oxygen.
- It is the end-product of glycolysis and is eventually transported into mitochondria as a major energy and participates in the TCA cycle.
- In the glycolysis, glucose is converted into two molecules of pyruvate with the generation of ATP. However, if reactions stops at pyruvate, due to imbalance redox, it would not proceed for long.
- The enzymatic activity of glyceraldehyde 3-phosphate dehydrogenase produces a molecule containing high phosphoryl-transfer potential and reduces NAD NADH. However, NAD molecule is present in very limited amount in the cell and it must be regenerated for glycolysis to proceed. This is achieved by the metabolism of pyruvate.
- Pyruvate are mainly converted into ethanol, lactic acid, or carbon dioxide.

- In the presence of oxygen, molecules like glucose and other sugars, fatty acids, and most amino acids are eventually oxidized to CO_2 and H_2O via the TCA cycle and the respiratory chain.
- The carbon skeletons of sugars and fatty acids are converted into the acetyl group of acetyl-CoA and enters into the TCA cycle, the form in which the cycle accepts most of its fuel input.
- In the matrix of the mitochondria, first pyruvate is converted to Acetyl-CoA by the enzyme pyruvate dehydrogenase complex (PDC) because former cannot enter the TCA cycle



- PDC holds a key position in connecting the glycolytic and oxidative pathway of the TCA cycle.
- This catalysis is sequential process which involves the oxidative decarboxylation of pyruvate and the formation of acetyl- COA, CO_2 and NADH (H). This reaction needs five co-factors namely Co-A, TPP, lipoate, FAD and NAD*.
- PDC are made up of several copies of three catalytic enzymes namely pyruvate dehydrogenase (El), dihydrolipoamide acetyltransferase (E2), and dihydrolipoamide dehydrogenase (E3) (Figure 3). They are found in prokaryotes as well as eukaryotes.

2.4.3 Tricarboxylic acid cycle

The tricarboxylic acid (TCA) cycle, also known as the Krebs or citric acid cycle, is the main source of energy for cells and an important part of aerobic respiration. The cycle harnesses the available chemical energy of acetyl coenzyme A (acetyl CoA) into the reducing power of nicotinamide adenine dinucleotide (NADH).

Named for its metabolism of the conjugate bases (citrate, isocitrate, and cis-aconitate) of three tricarboxylic acids in the early steps of the pathway, the Tricarboxylic Acid Cycle (or TCA cycle) is known simply as the Citric Acid Cycle. The term "cycle" refers to the fact that the initial six-carbon substrate (citrate) is oxidatively decarboxylated twice to form the pathway's ultimate four-carbon product (oxaloacetate), which then combines with two-carbon units (as acetyl-CoA) to regenerate citrate, thereby allowing the cycle to begin anew. This same pathway is also known as the Krebs Cycle in honor of Sir Hans Krebs, the German- born British biochemist, who proposed the pathway in the late 1930s and later earned the Nobel Prize for demonstrating its key properties.

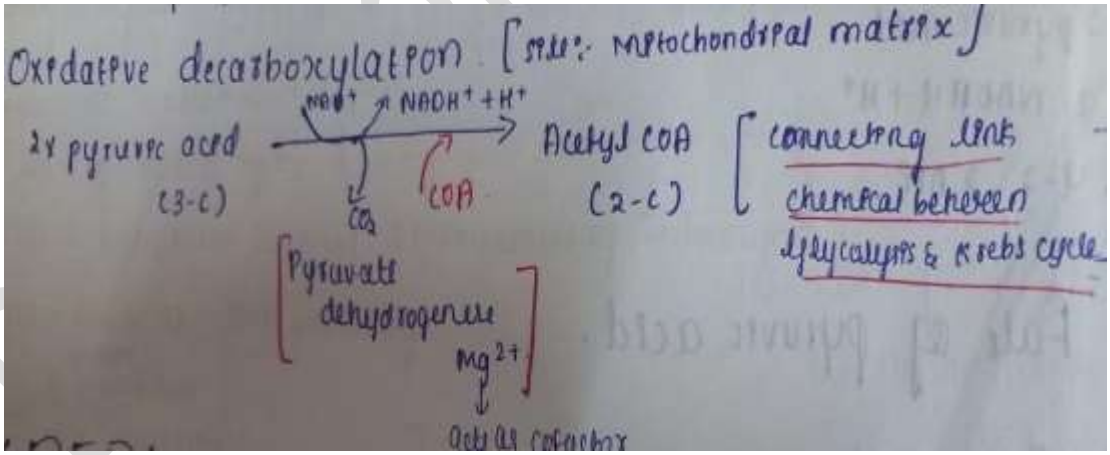
Aerobic respiration

Site: Mitochondria

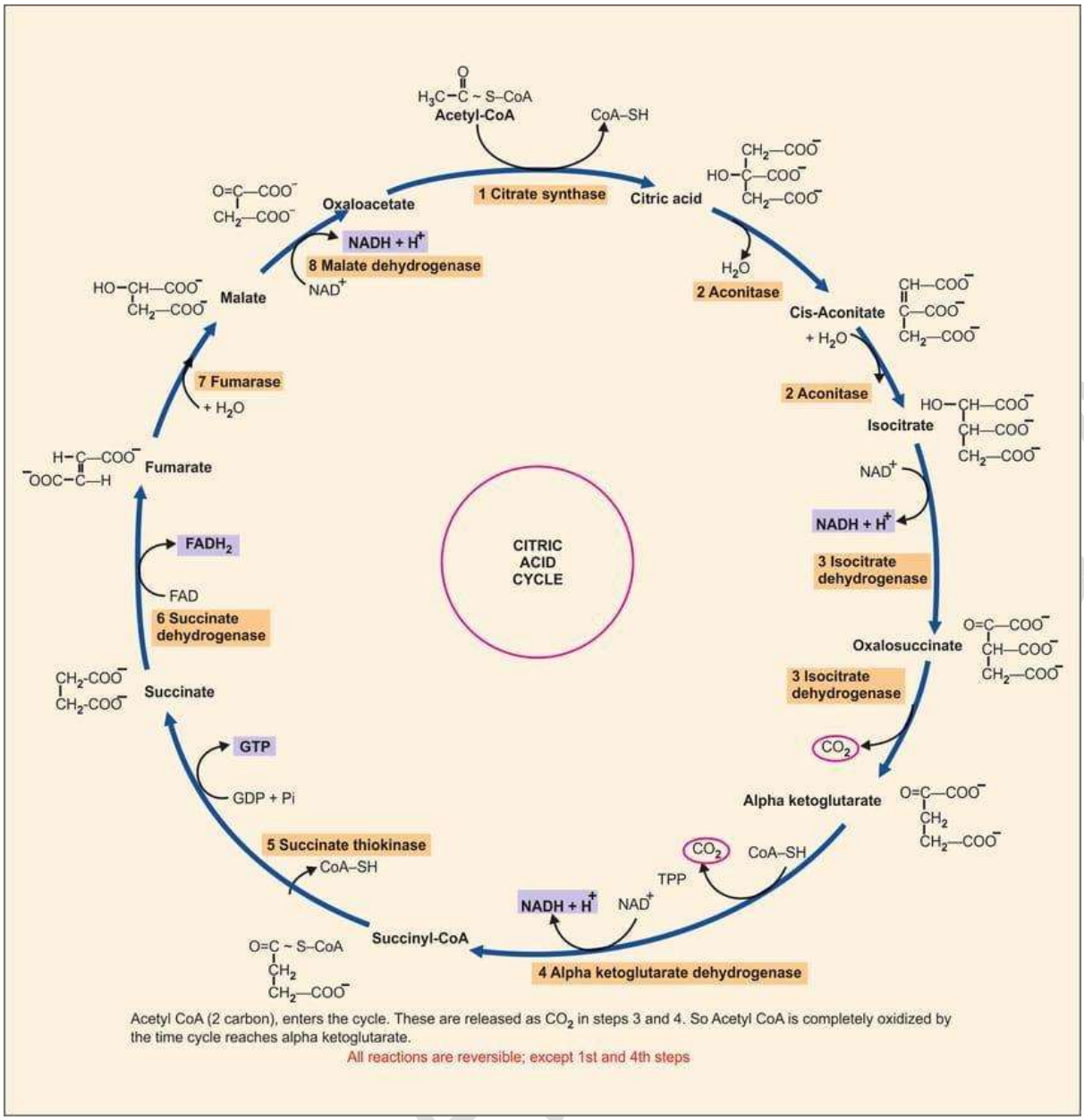
Events are:

1. **Oxidative decarboxylation**
2. **Tricarboxylic cycle**
3. **Oxidative phosphorylation**

1 Oxidative decarboxylation



2 Tricarboxylic acid



Acetyl-CoA enters the cycle, and is completely oxidized. During this process, energy is trapped. The sources of acetyl-CoA are shown in figure . Pyruvate derived from glycolysis is oxidatively decarboxylated to acetyl- CoA by the pyruvate dehydrogenase . This is the link between the TCA cycle and glycolysis. The acetyl-CoA is also derived from beta-oxidation of fatty acids. All the enzymes of citric acid cycle are located inside the mitochondria.

First Step: Formation of Citric Acid

The 4 carbon, oxaloacetate condenses with 2 carbon, acetyl-CoA to form 6 carbon compound, the citrate(tricarboxylic acid). The enzyme is citrate

Synthase. The hydolysis of the thioester bond in acetyl-CoA drives the reaction forward. This is an irreversible step. However, body can reverse this step by another enzyme, ATP citrate lyase.

Second step: Formation of Isocitrate

Citrate is isomerized to isocitrate by aconitase. The reaction takes place in two steps, with cis-aconitate as the intermediary.

Third step: Formation of Alpha Ketoglutarate

This reaction is catalysed by the enzyme, isocitrate dehydrogenase. First isocitrate is dehydrogenated to form oxalosuccinate. It undergoes spontaneous decarboxylation to form alpha ketoglutarate. The NADH generated in this step is later oxidized in electron transport chain(ETC) to generate ATPs. Isocitrate(6 carbons) undergoes oxidative decarboxylation to form alpha ketoglutarate (5 carbons). In this reaction, one molecule of CO_2 is liberated.

Fourth step: Formation of Succinyl-CoA

Next, alpha ketoglutarate is oxidatively deacarboxylated to form succinyl-CoA by the enzyme alpha ketoglutarate dehydrogenase. The NADH thus generated enters into ETC to generate ATPs. Another molecule CO_2 is removed in this step. This is the only irreversible step in this whole reaction cycle. The enzyme alpha ketoglutarate dehydogenase is a multienzyme complex having 3 enzyme proteins and 5 coenzymes. This is similar to the pyruvate dehydrogenase reaction.

Fifth step: Generation of Succinate

The next reaction involves a substrate level phosphorylation whereby a high energy phosphate is generated from the energy trapped in the thioester bond of succinyl CoA.The enzyme is succinate thiokinase. A molecule of GDP is phosphorylated to GTP and succinate is formed.The GTP can be converted to ATP by reacting with an ADP molecule:

GTP+ADP—GDP+ATP

Sixth step: Formation of Fumarate

Succinate is dehydrogenated to fumarate, an unsaturated dicarboxylic acid, by succinate dehydrogenase. The hydrogen atoms are accepted by FAD. The $FADH_2$ then enters into ETC to generate ATPs. The succinate dehydrogenase is competitively inhibited by malonate.

Seventh step: Formation of Malate

The formation of malate from fumarate is catalyzed by fumarase. The reaction involves the addition of a water molecule.

Eighth step: Regeneration of Oxaloacetate

Finally malate is oxidised to oxaloacetate by malate dehydrogenase. The coenzyme is NAD+. The NADH is generated in this step, which enters the electron transport chain, when ATPs are produced. The oxaloacetate can further condense with another acetyl-CoA molecule and the cycle continues.

Regulation of the TCA cycle

- **Metabolites:** Products of the cycle provide negative feed back on the enzymes that catalyse it. For example, NADH inhibits the majority of the enzymes found in the TCA cycle.
- **Citrate:** Inhibits phosphofructokinase, a key enzyme in glycolysis. This reduces the rate of production of pyruvate and therefore of acetyl CoA.
- **Calcium:** Accelerates the TCA cycle by stimulating the link reaction.

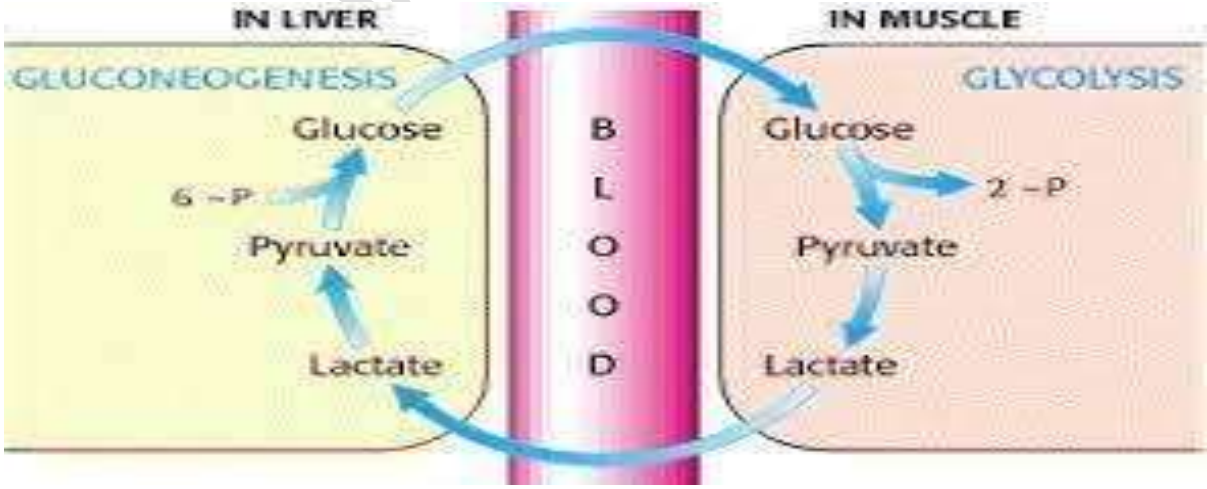
Function of the Tricarboxylic Acid Cycle

- The final common oxidative pathway that oxidizes acetyl CoA to CO_2.
- The source of reduced coenzymes that provide the substrate for the respiratory chain.
- The link between catabolic and anabolic pathways(amphibolic role).
- Provides precursors for synthesis of amino acids and nucleotides.
- Components of the cycle have a direct or indirect controlling effects on key enzymes of other pathways.

2.4.4 Gluconeogenesis

- Gluconeogenesis is defined as the biosynthetic pathway for formation of glucose de-novo (ie. not glucose from glycogen a regular stored form in most animals).
- Gluconeogenesis is a metabolic pathway that is actually responsible for the generating glucose from non-carbohydrate carbon containing substrates such as pyruvate, lactate, glycerol, and glucogenic amino acids.
- Gluconeogenesis is a ubiquitous process, observed in all of living kingdom including plants, animals, fungi and bacteria. This process is also referred to as an endogenous glucose (EGP).
- The formation of glucose molecules from various carbon skeletons is often necessary since the vital organs viz. testes, kidney (renal cortex) exclusively utilize glucose ATP production.
- Erythrocytes and human brain also heavily dependent on glucose formed from gluconeogenesis for energy requirements and utilize large amounts of glucose consumed as well as produced daily via gluconeogenesis.
- Gluconeogenesis it is the process that occurs chiefly in liver. While a very limited extent of the reactions occurs in kidney as well conditions in small intestine, but that requires specific physiological conditions.
- However, in addition to glucose, the brain derives its energy from ketone bodies via acetyl-CoA and shunted into the TCA cycle. The glucose requirement of the brain in an adult human being is approx 120 g, which accounts for majority of glucose needed by body (160 g) on day-to-day basis. The amount of glucose in body fluids is about 20 g, and that readily available from glycogen is approx 190 g. These glucose reserves are sufficient to meet day to day glucose requirements.
- But under conditions of longer period of starvation, glucose must compulsorily be formed from non carbohydrate sources.

- The preliminary carbon skeletons in gluconeogenesis is mainly from pyruvate, lactate, glycerol, and the amino acids alanine and glutamine
- Gluconeogenesis and glycogenolysis are the two mechanism that help in maintaining blood glucose levels in the body.
- In few ruminants, this is a continuous process. While in many other animals, the process mainly occurs during fasting, starvation, low-carbohydrate foods, or intense physical activity. The process is highly endergonic but due to coupling of ATP/GTP hydrolysis it ends up to be exergonic
- For gluconeogenesis from non-carbohydrate precursors of glucose they are first converted into pyruvate or enter the pathway at later stages of glucose metabolic pathways such as oxaloacetate (OAA) and dihydroxyacetone phosphate (DHAP)
- Lactate is primarily formed by skeletal muscles when the rate of glycolysis outnumbers the oxidative metabolism. Conversion of lactate into pyruvate is catalysed by lactate dehydrogenase. During starvation the skeletal muscles breakdown the proteins and amino acids are derived from dietary proteins.
- The reactions constitutes the Cori cycle where in pyruvate is synthesised from lactate in muscle tissues and in another reaction of transamination in muscles, alanine is formed from pyruvate. The amino group released is reduced in the form of urea. The reaction are popularly called Alanine cycle. Both of these Cori cycle and alanine cycle reactions allow generation of pyruvate and thereby favour entry into gluconeogenesis.
- The hydrolysis of triacylglycerols in adipocytes yields fatty acids and glycerol. Glycerol acts as a precursor of glucose, but animals are unable to transform fatty acid residues to glucose. Glycerol can either enter glycolysis or glyconeogenesis through dihydroxyacetone phosphate.
-

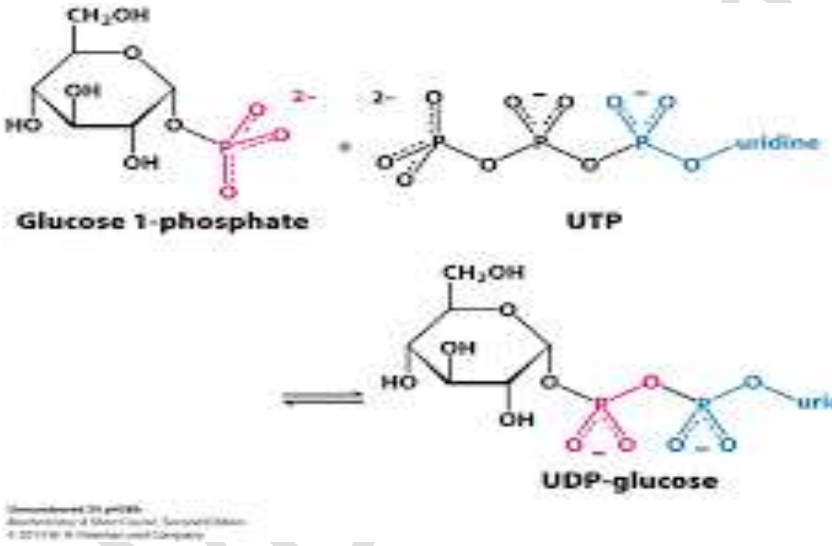


2.4.5 Glycogenesis

- It is the formation of glycogen from glucose,which occurs in all tissues of the body,but in large amount in liver and muscles.
- There are very small amount of glycogen synthesis and storage in the CNS; this is why it is completely dependent on blood glucose as a source of energy.

Glycogen synthesis

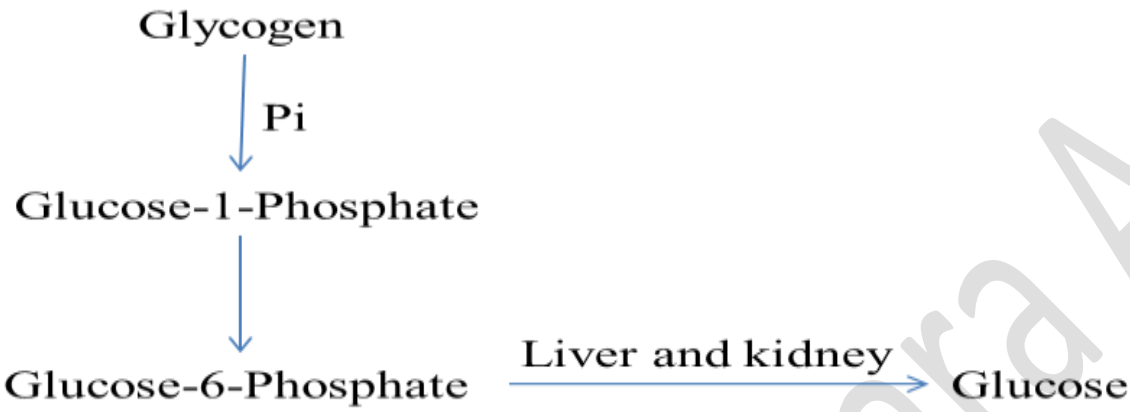
- A distinct system of enzymes exists for endergonic glycogen synthesis, coupled ultimately to the hydrolysis of ATP.
- Glucose 6-phosphate isomerizes to **glucose 1-phosphate** by the action of phosphoglucomatase.
- Synthesis of an activated form of glucose(UDP-glucose) : from glucose 1-phosphate and UTP(uridine phosphate) in a reaction catalysed by UDP-glucose pyrophosphorylase.



2.4.6. Glycogenolysis

- Glycogenolysis is the biochemical pathway in which glycogen breaks down into glucose -1-phosphate and glucose.
- The reaction in hepatocytes and the myocytes.
- The process is under the regulation of two key enzymes: phosphorylase kinase and glycogen phosphorylase.
- Glycogen is a branched polysaccharide consisting of glucose units. In humans, it is principal storage form of glucose.
- During times of need, the body breaks down glycogen to produce glucose.

- Glycogenolysis, along with glycolysis, plays a central role in carbohydrate metabolism. It is the principal route of glycogen utilization.



2.4.7. Pentose phosphate pathway

- The pentose phosphate pathway (also called the phosphogluconate pathway and the hexose monophosphate shunt and the HMP Shunt) is a metabolic pathway parallel to glycolysis.
- It generates NADPH and pentoses (5-carbon sugars) as well as ribose 5-phosphate, a precursor for the synthesis of nucleotides.While the pentose phosphate pathway does involve oxidation of glucose, its primary role is anabolic rather than catabolic. The pathway is especially important in red blood cells (erythrocytes).
- There are two distinct phases in the pathway. The first is the oxidative phase, in which NADPH is generated, and the second is the non- oxidative synthesis of 5-carbon sugars. For most organisms, the pentose phosphate pathway takes place in the cytosol; in plants, most steps take place in plastids.
- Like glycolysis, the pentose phosphate pathway appears to have a very ancient evolutionary origin.
- The reactions of this pathway are mostly enzyme-catalyzed in modern cells, however, they also occur non-enzymatically under conditions that replicate those of the Archean ocean, and are catalyzed by metal ions, particularly ferrous ions (Fe(II)).This suggests that the origins of

the pathway could date back to the prebiotic world.

Pentose Phosphate Pathway

The pentose phosphate pathway (also called the phosphogluconate pathway and the hexose monophosphate shunt) is a metabolic pathway parallel to glycolysis.

It generates NADPH and pentoses (5-carbon sugars) as well as ribose 5-phosphate, the last one a precursor for the bio synthesis of nucleotides.

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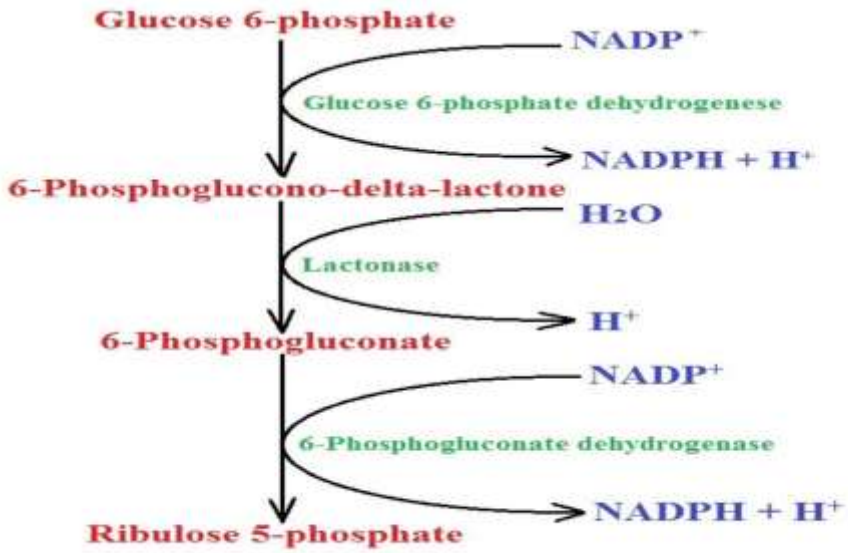
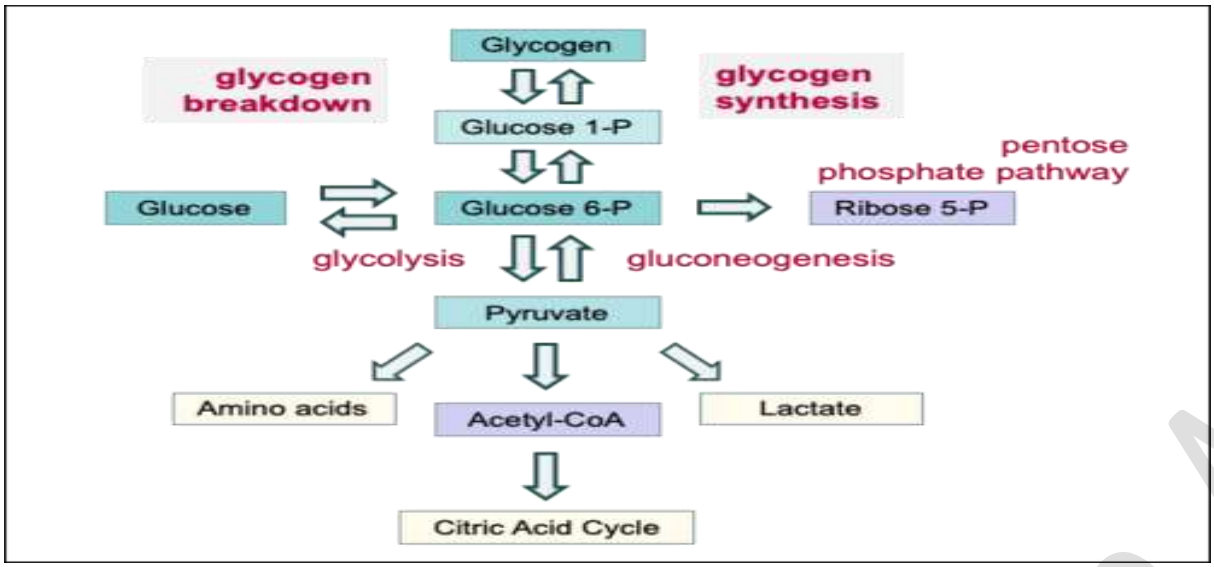
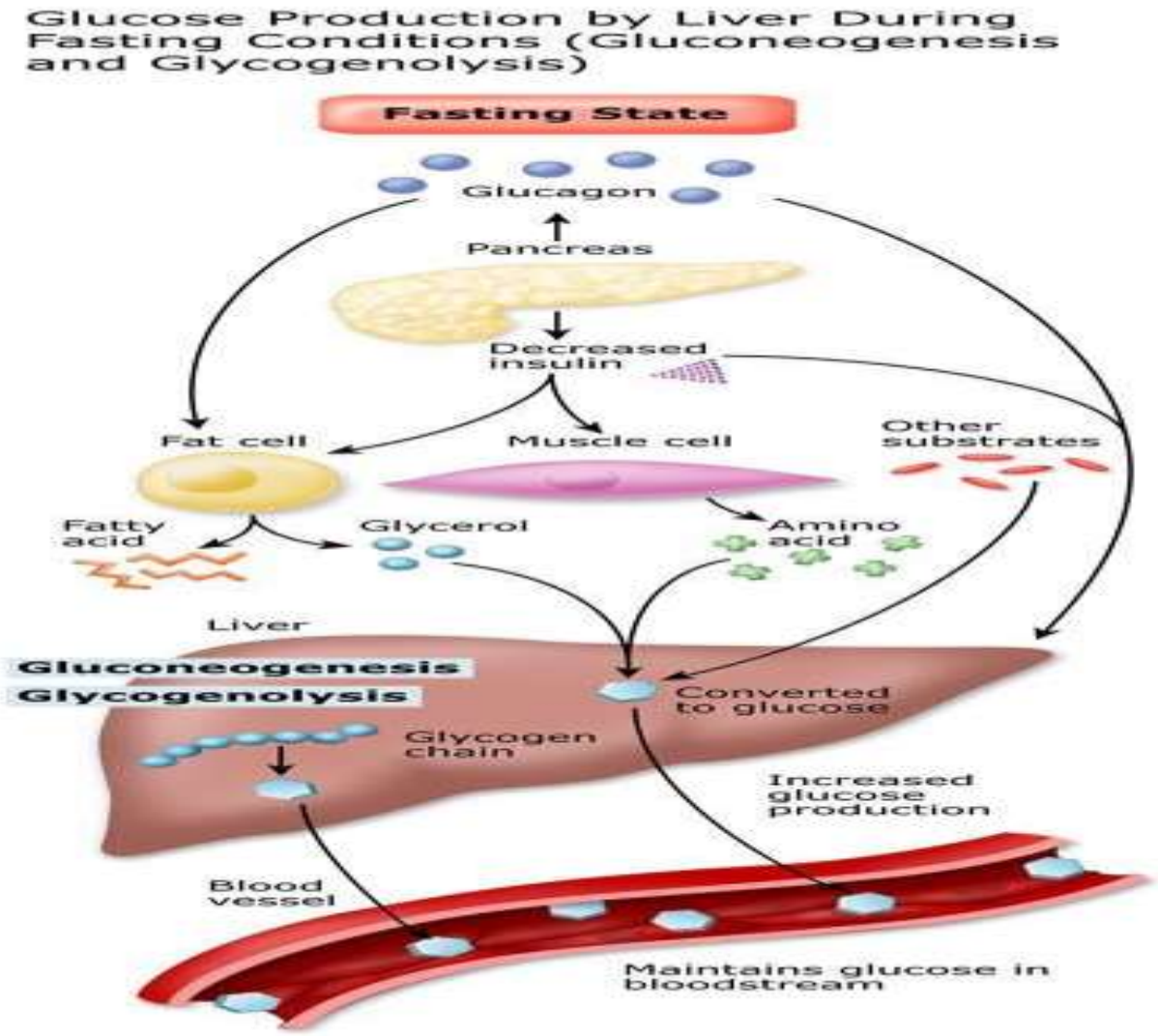


CARBOHYDRATE METABOLISM

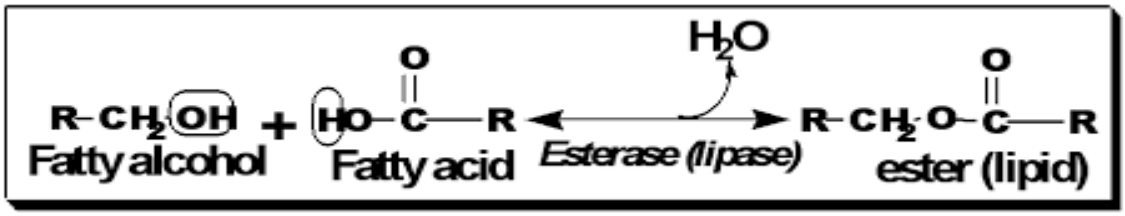


FASTING STATE



2.5 Lipids

Lipids are organic compounds that are found in living organisms.They have variety of structures and functions, and soluble in organic solvents due to their hydrocarbon component.
They are hydrophobic in nature. They are insoluble in polar solvents like water.
They are isolated using non polar solvents.
Lipids are organic compounds formed mainly from alcohol and fatty acids combined together by ester linkages.



2.5Classification of lipids

Lipids are broadly classified into simple and complex lipids

Simple lipids:

- Fatty Acids
- Triacylglycerols
- Steroids(cholesterol)

Complex lipids:

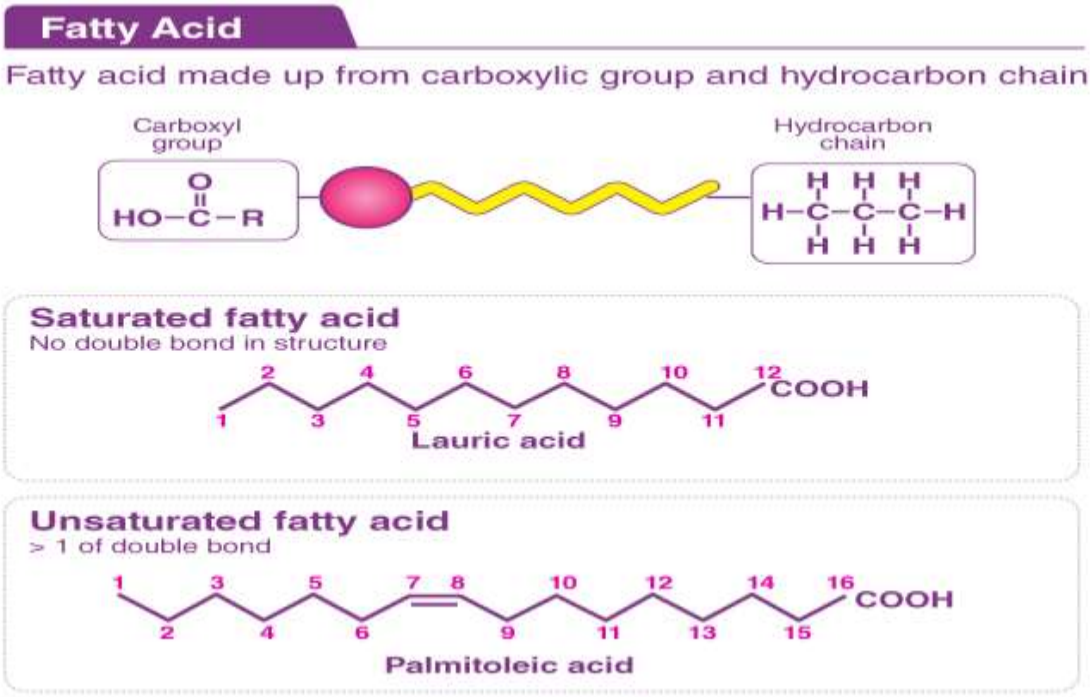
- Phospholipids
- Sphingolipids
- Glycolipids

2.5.1 Fatty Acid

Classification of fatty acid based on saturation

- Saturated
- Monounsaturated
- Polyunsaturated

If there is no double bond, the fatty acid is saturated
If there is one double bond, the fatty acid is monosaturated
If there are two or more double bonds, the fatty acids is polysaturated

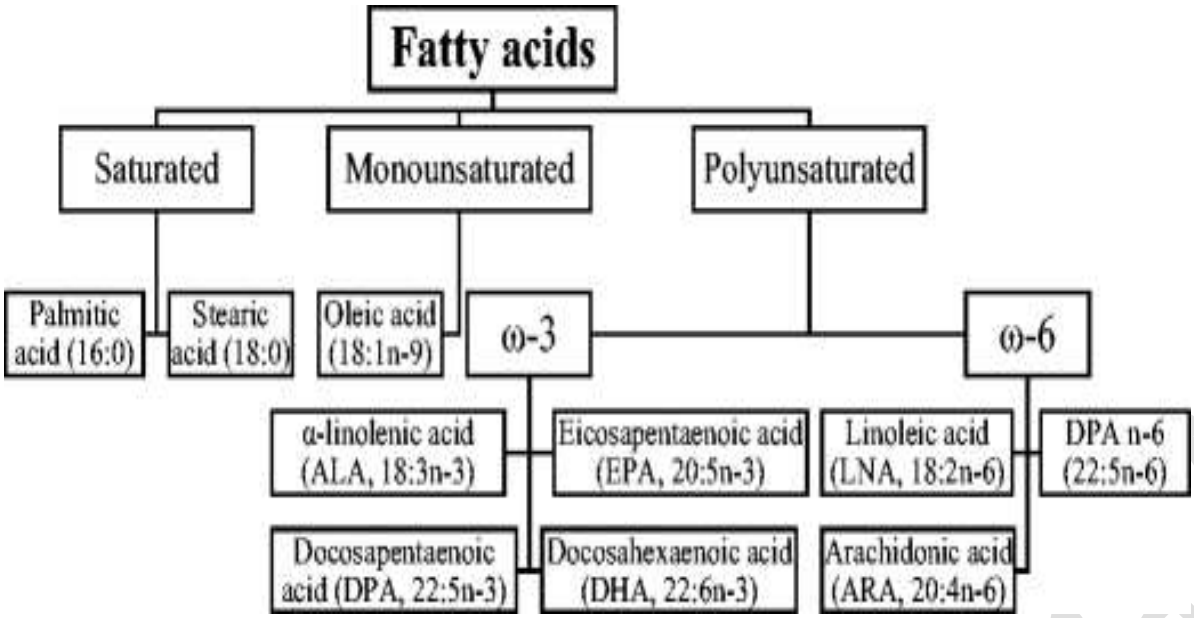


Nomenclature

- Saturated acids ends in "-anoic" acid.
  Ex: Octanoic acid
- Unsaturated acids with double ends in "-enoic" acid.
  Ex: Octadecenoic acid

Basic rules of nomenclature in lipids

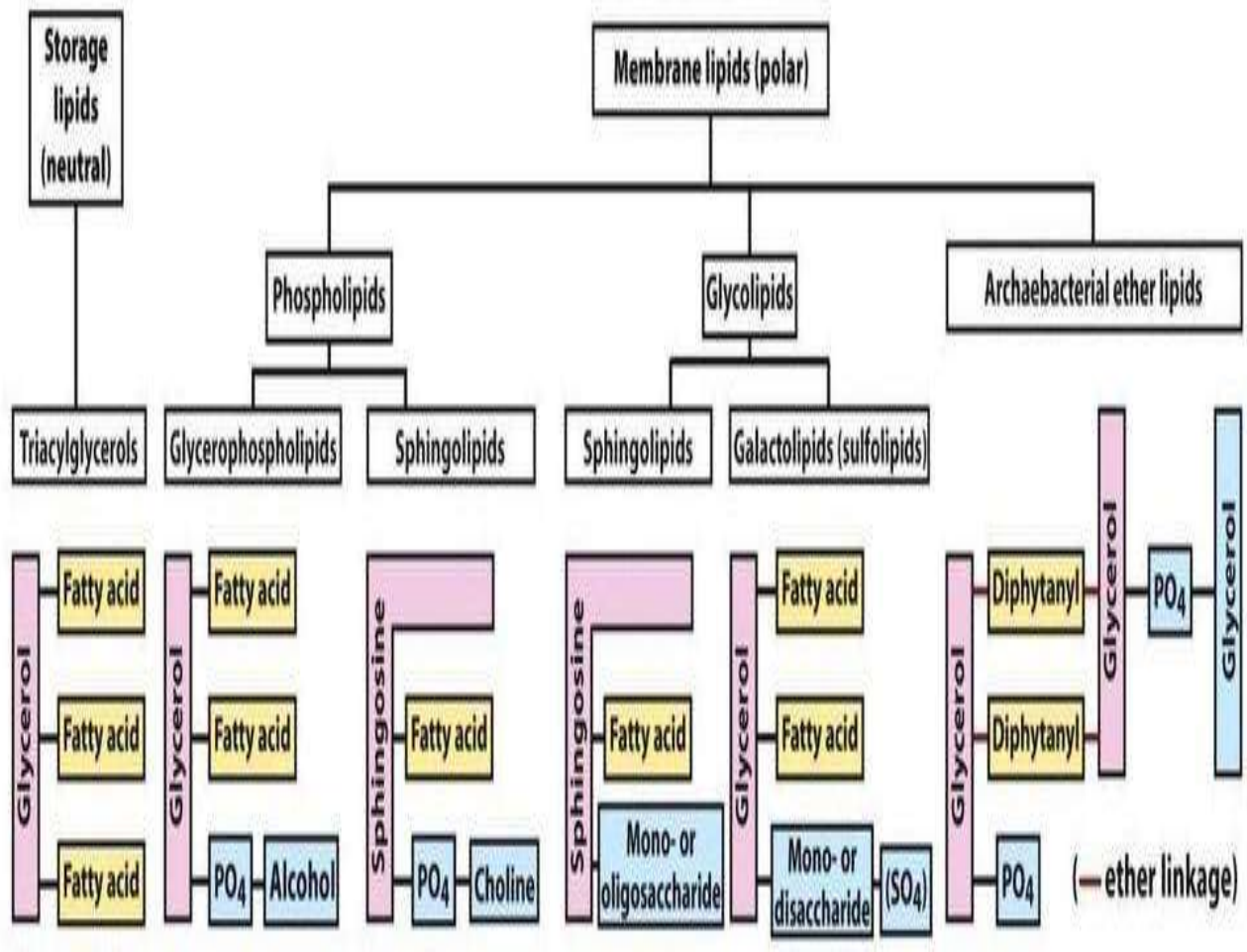
1. Carbon atoms are named from the carboxylic carbon (carbon no 1).
2. Rest of the carbon following are named as :2,3,4,.....and so on also known as α, ß and so on.
3. For polyunsaturated: The number of carbon is named from the opposite side of the carboxyl carbon and given the number and called omega.
4. Delta is used for indicating the position and number of double bonds.



List of fatty acids

Shorthand name	Trivial or (systemic) name	Abbreviation
Polyunsaturated fatty acids (PUFA)		
Omega-3		
18:3n3	Alpha-linolenic	ALA
20:5n3	(Eicosapentaenoic)	EPA
22:5n3	(Docosapentaenoic)	DPA
22:6n3	(Docosahexaenoic)	DHA
Omega-6		
18:2n6	Linoleic	LA
18:3n6	Gamma-linolenic	GLA
20:2n6	(Eicosadienoic)	–
20:3n6	Dihomo-gamma-linolenic	dGLA
20:4n6	Arachidonic	AA
22:4n6	(Docosatetraenoic)	–
22:5n6	(Docosapentaenoic)	–
Monounsaturated fatty acids (MUFA)		
16:1n7	Palmitoleic	–
18:1n9	Oleic	–
20:1n11	Gadoleic	–
22:1n9	Erucic	–
24:1n9	Nervonic	–
Saturated fatty acids (SFA)		
14:0	Myristic	–
16:0	Palmitic	–
18:0	Stearic	–
20:0	Arachidic	–
22:0	Behenic	–
24:0	Lignoceric	–

2.5.1.1 Storage and Membrane Lipids



2.5.2 Triglycerides:

An ester formed from glycerol and three fatty acid groups.Triglycerides are the main constituents of natural fats and oils.

2.5.3 Phospholipids:

1.Glycerophospholipids

- Glycerol 3-Phosphate is bonded to two fatty acid chains.
- The Phosphate group is linked to a hydrophilic group
- Amphipathic in nature
  Hydrophobic tail
  Hydrophilic phosphoryl heads

2.Sphingophospholipids

Any phospholipid that is derived from sphingosine or one of its derivates.

2.5.4 Steroids

- Derivatives of cyclopentanoperhydrophenanthrene ring.
- Consists of 4 fused rings called steroid nucleus with an 8-carbon chain.

- Steroids with-OH are called sterols
- Cholestrol is a major sterol in humans and aanimals
- Cholestrol in plasma is bound to fatty acids called cholesteryl esters.

2.5.5 Glycolipids

Glycolipids are lipids with a carbohydrate attached by a glycosidic bond.The glycolipids are an essential part of cell membranes. Glycolipids also help determine the blood group of an individual.

2.6

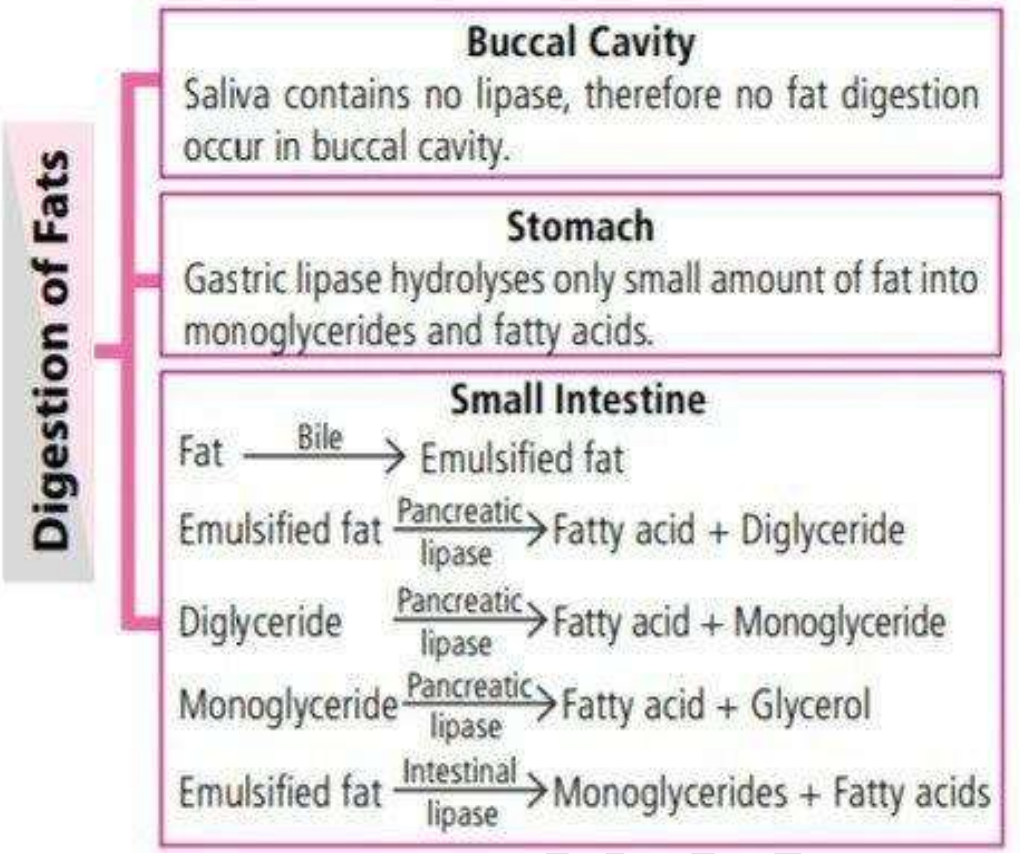
2.7 Lipid metabolism

Lipid metabolism is the synthesis and degradation of lipids in cells, involving the breakdown and storage of fats for energy and the synthesis of structural and functional lipids, such as those involved in the construction of cell membranes.
In animals, these fats are obtained from food and are synthesized by the liver. Majority of lipids in humans are triglycerides and cholesterol.
Lipid metabolism is often considered as the digestion and absorption process of dietary fat.
In plants, the second step after the hydrolysis is the absorption of the fatty acids into the epithelial cells of the intestinal wall. In the epithelial cells, fatty acids are packaged and transported to the rest of the body.

2.7.1 Digestion

- The primary digestion action occurring in the mouth is mechanical. Foods are broken up into smaller particles through chewing and moistened for passage into the stomach.
- Little, if any, chemical fat digestion takes place in the stomach
- Small intestine
  --Fat digestion occurs here
  --Enzyme from the pancreas activates other enzymes and act on fats
  --Bile helps in the emulsification of fats i.e., breaking down of the fats into very small micelles. Bike also activates lipases
  --The intestinal mucosal epithelium has goblet cells which secrete mucus. The secretions of the brush border cells of the mucosa alongwith the secretions of the goblet cells constitute the intestinal juice or succus entericus.
  --This juice contains a variety of enzymes like lipases, dipeptidases etc
  --Fats are broken down by lipases with the help of bile into di and monoglycerides.

- Absorption takes place in large intestine.



2.7.2 Absorption and Transportation

- Fatty acids and glycerol being insoluble, cannot be absorbed into the blood
- They are first incorporated into small droplets called micelles which move into the intestinal mucosa.
- They are re-formed into very small protein coated fat globules called the chylomicrons which are transported into the lymph vessels(lacteals) in the villi.
- These lymph vessels ultimately release the absorbed substances into the blood stream.

2.8 Biosynthesis of Palmitic acid

De Nova Synthesis: Formation of complex structures from a simpler ones.

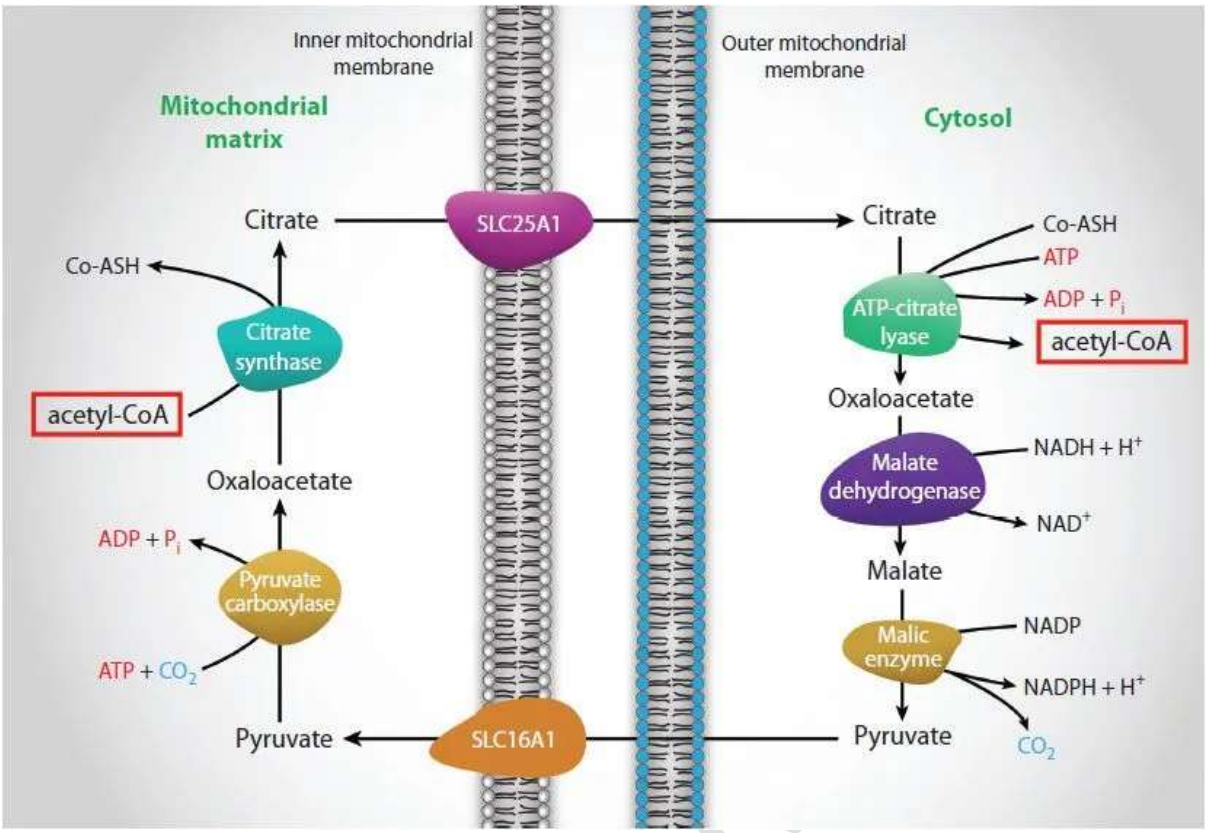
Liver, Kidney, Adipose tissue and Lactating mammary glands are the organs where de nova synthesis of fatty acid occurs.

Site of fatty acid biosynthesis is cytosol.

Prerequisite for fatty acid biosynthesis include:

1.Acetyl CoA

2.NADPH
3.ATP
4.Mn(Manganese)
5. CO2



Fatty acid biosynthesis can be understand in 3 stages:

1. **Production of Acetyl CoA and NADPH**
2. **Conversion of Acetyl CoA to Malonyl CoA**
3. **Reactions which are catalysed by fatty acid synthase complex**

Fatty acid synthase: It is a multifunctional enzyme whixh is made up of dimer with two identical subunits including ACP(Acyl Carrier Protein).

1. Production of Acetyl CoA and NADPH

- Acetyl CoA is produced in mitochondria by oxidation of pyruvate (PDH complex) and oxidation of fatty acid.
- Oxidation of fatty acid gives 8 molecules of acetyl CoA.
- But the problem is that acetyl CoA cannot permeable to mitochondria as it has to go cytosol for FAB.
- So, in mitochondria, acetyl CoA condense with OAA to form citrate. Citrate can pass through mitochondrial membrane and comes to cytosol where an enzyme called citrate lyase cleaved citrate into OAA and Acetyl CoA.

- OAA is converted into malate in cytosol and malate into pyruvate by malic enzyme with NADPH formation which is a reducing equivalent and required for fatty acid biosynthesis.

2. Formation of Malonyl CoA

It is formed by carboxylation of acetyl CoA by an enzyme called acetyl CoA carboxylase.

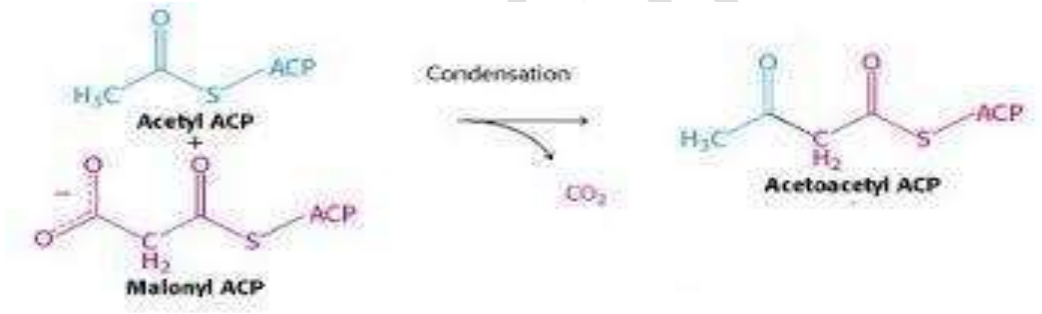
This enzyme is a ATP dependent and requires biotin as a cofactor.

Reactions which are catalyzed by Fatty Acid Synthase complex to form long chain fatty acid called Palmitic acid/Palmitate.

Acyl group from acetyl CoA and malonyl group from malonyl CoA is transferred to fatty acid synthase complex by Acetyl CoA-ACP transacylase and Malonyl CoA-ACP transacylase.

Step 1. Condensation Reaction

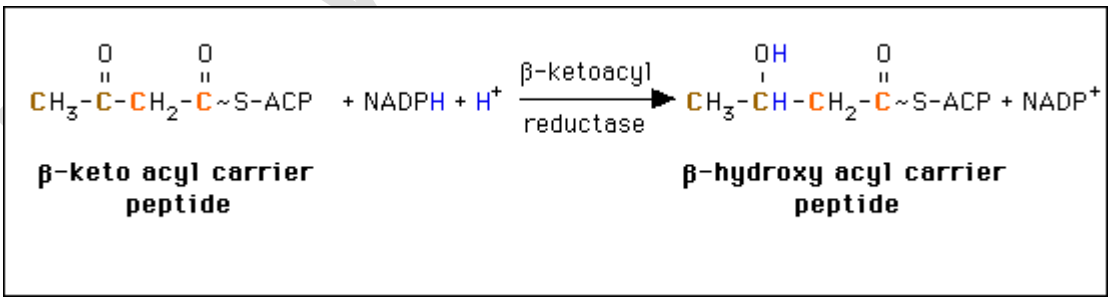
Acyl (from acetyl CoA) which is the first acyl group and 2 carbon derived from malonyl extends the acyl chain by 2 carbons so this condensation of both molecules is associated with decarboxylation and product form is Beta ketobutyryl-ACP. Enzyme is Beta ketoacyl-ACP synthase.



Step 2. Reduction Reaction

Beta ketobutyryl-ACP is then undergo reduction and ketoacyl group is turned into hydroxyl group. Electron donar is NADPH. Enzyme is beta ketoacyl-ACP reductase.

Final product is Beta hydroxybutyryl-ACP

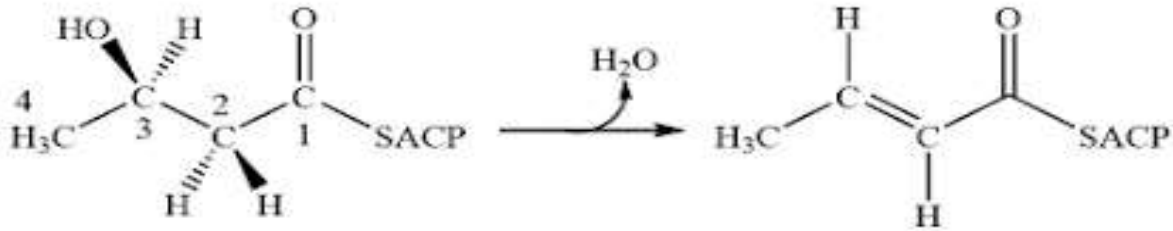


Step 3. Dehydration reaction

Beta hydroxybutyryl ACP undergoes dehydration and forms enoyl ACP. Enzyme is Beta hydroxyacyl-ACP dehydratase.

Elimination of water takes place during dehydration reaction.

Product formed will be butenoyl-ACP.



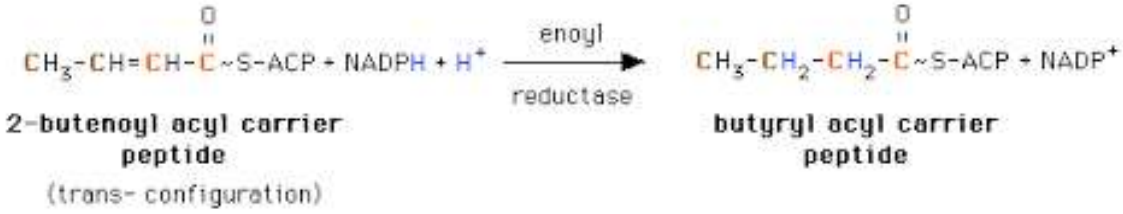
(R)-3-Hydroxybutyryl trans-Crotonyl ACP

Step 4. Reduction reaction

Enoyl ACP reductase catalyses this reaction using NADPH as a reducing equivalent and forms Acyl ACP.

The 4 carbon unit attached to ACP is butyryl. ACP is the carrier molecule which has to transfer carbon chain to cysteine part of Fatty acid synthase enzyme complex and in this way the above mentioned reactions repeated 6 more times.

Note-total 7 times reactions repeated to form 16 Carbon palmitate molecule. In each time, chain is elongated by 2 carbon unit. Then finally, palmitoyl thioesterase separates palmitate from ACP. In this way a fully saturated 16 carbon compound is formed which is called Palmitate.



Overall reaction will be :-

Firstpart

7 Acetyl-CoA + 7CO2 + 7ATP →7 malonyl-CoA + 7ADP + 7P*i*

Second part (7 cycles of condensation and reduction)

Acetyl-CoA + 7 malonyl-CoA + 14NADPH + 14H+ palmitate + 7CO2 + 8 CoA + 14NADP+ + 6H2O

Regulation of Fatty acid synthesis:

1. Controlled by hormones, enzyme and metabolites and end products.

2. Acetyl CoA carboxylase:- This enzyme is active in polymeric form and inactive when exist in monomeric form.

Citrate promotes polymeric form whereas palmitoyl CoA and malonyl CoA promotes its inactivation.

3. Hormonal control - it includes CAMP dependent phosphorylation for inactivation and vice versa for activation. Insulin promotes fatty acid synthesis and glucagon inhibits.

4. Availability of NADPH- It is provided by citrate (Acetyl CoA) or PPP/HMS (Hexose monophosphate shunt pathway) which significantly influences Fatty acid synthesis.

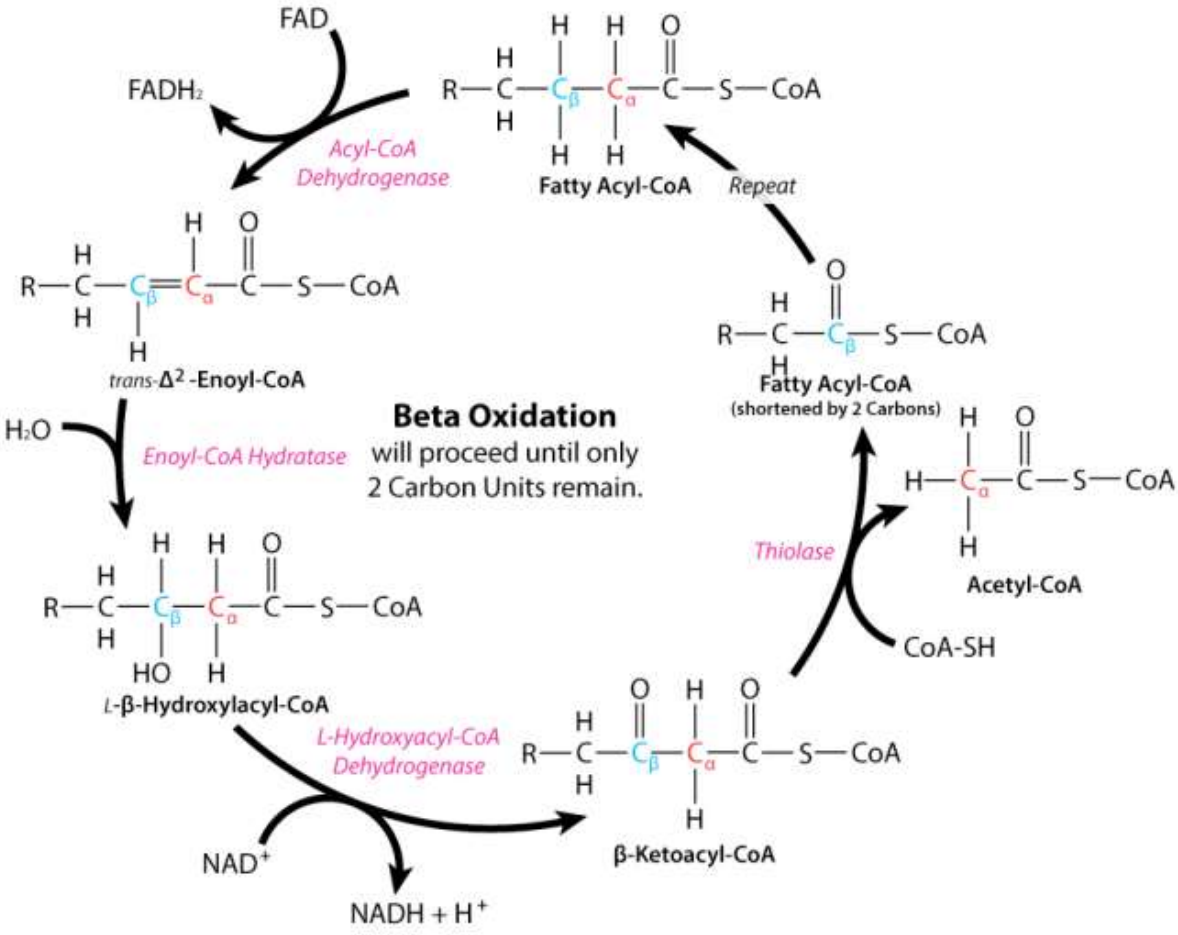
2.8 Biodegradation of fatty acid(beta oxidation)

After activation by ATP, once inside the mitochondria, the Beta-oxidation of a fatty acids occurs via four recurring steps:

1. Oxidation by FAD
2. Hydration
3. Oxidation by NAD+
4. Thiolysis
5. Production of acyl-CoA and acetyl-CoA

ß-oxidation occurs to convert fatty acids into 2-carbon acetyl CoA units. Acetyl CoA enters into TCA cycle to yield generate reduced NADH and reduced FADH2 . Reduced cofactors NADH and FADH2 participate in the electron transport chain in the mitochondria to yield ATP. There is no direct participation of the fatty acid.

The final product of B-oxidation of an even-numbered fatty acid is acetyl-CoA, the entry molecule for the citric acid cycle. If the fatty acid is an odd-numbered chain, the final product of ß-oxidation will be propionyl-CoA. This propionyl-CoA will be converted into intermediate methylmalonyl-CoA and eventually succinyl-CoA. which also enters the TCA cvcle.



MODULE 3
PROTEINS AND NUCLEIC ACIDS

Proteins:
A molecule made up of amino acids. Proteins are needed for the body to function properly. A protein is **a naturally occurring, extremely complex substance that consists of amino acid residues joined by peptide bonds**. Proteins are present in all living organisms and include many essential biological compounds such as enzymes, hormones, and antibodies.

Proteins are polypeptide structures consisting of one or more long chains of amino acid residues. They carry out a wide variety of organism functions, including DNA replication, transporting molecules, catalyzing metabolic reactions, and providing structural support to cells. A protein can be identified based on each level of its structure. Every protein at least contains a primary, secondary, and tertiary structure. Only some proteins have a quaternary structure as well. The primary structure is comprised of a linear chain of amino acids.

The secondary structure contains regions of amino acid chains that are stabilized by hydrogen bonds from the polypeptide backbone. These hydrogen bonds create alpha-helix and beta-pleated sheets of the secondary structure. The three-dimensional shape of a protein, its tertiary structure, is determined by the interactions of side chains from the polypeptide backbone. The quaternary structure also influences the three-dimensional shape of the protein and is formed through the side-chain interactions between two or more polypeptides. Each protein at least contains a primary, secondary, and tertiary structure. Only some proteins have a quaternary structure as well.

the primary structure of a protein is defined as the sequence of amino acids linked together to form a polypeptide chain. Each amino acid is linked to the next amino acid through peptide bonds created during the protein biosynthesis process. The two ends of each polypeptide chain are known as the amino terminus (N-terminus) and the carboxyl terminus (C-terminus). Twenty different amino acids can be used multiple times in the same polypeptide to create a specific primary protein structure sequence.

FUNCTION OF PROTEINS:

Protein helps repair and build your body's tissues. It drives metabolic reactions, maintains pH and fluid balance, and keeps the immune system strong. It also transports and stores nutrients and can act as an energy source. Protein is crucial to good health.In fact, the name comes from the Greek word *proteos*, meaning "primary" or "first place."Proteins are made up of amino acids that join together to form long chains. You can think of a protein as a string of beads in which each bead is an amino acid.There are 20 amino acids that help form the thousands of different proteins in your body.Proteins do most of their work in the cell and perform various jobs.Here are 9 important functions of protein in your body.

Growth and Maintenance:

Protein is required for the growth and maintenance of tissues. Your body's protein needs are dependent upon your health and activity level.

Causes Biochemical Reactions

- Enzymes are proteins that aid the thousands of biochemical reactions that take place within and outside of your cells catalyze chemical reactions
- provide structural support
- regulate the passage of substances across the cell membrane
- protect against disease
- coordinate cell signaling pathways
- Oxygen Transport.
- Proteins as Enzymes.
- Lysozyme - A Defensive Enzyme.
- Antibodies are Proteins.
- Structural Proteins.
- Contractile Proteins.

CLASSIFICATION OF AMINO ACIDS:

"Amino Acids are the organic compounds that combine to form proteins, hence they are referred to as the building components of proteins. These biomolecules are involved in several biological and chemical functions in the human body and are the necessary ingredients for the growth and **development of human beings. There are about 300 amino acids that occur in nature."**

Amino acids are organic compounds containing the basic amino groups (-NH2) and carboxyl groups (-COOH). The ingredients present in proteins are amino acids. Both peptides and proteins are long chains of amino acids. Altogether, there are twenty amino acids, which are involved in the construction of proteins.

TYPES OF AMINO ACIDS:

1. **DISPENSABLE AMINO ACIDS:**
    - the amino acids which can be produced in the body
    - 11 of the 20 amino acids are non-essential
    - Are produced within the body from other amino acids and other components

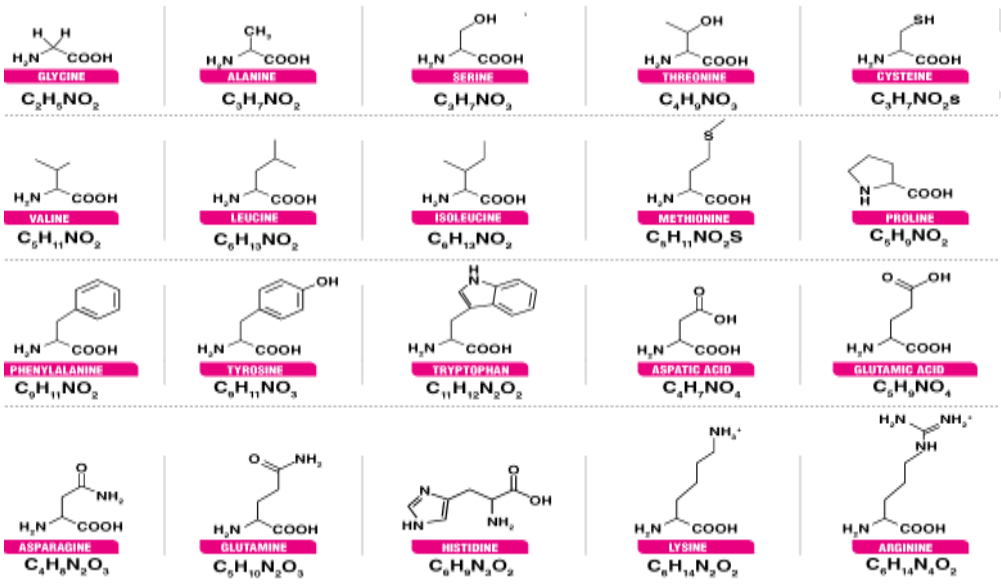
- Removal of toxins, integral in the synthesis of RBC and WBC, promotes brain function and many more.
- Probability of deficiency is rare, but can still occur due to starvation or illness.
- It is also called as non essential amino acids

2.IN DISPENSABLE AMINO ACIDS:

Essential amino acids are the amino acids which have to be taken in through diet as they "CAN NOT" be produced by the body9 amino acids out of 20 are thought to be essential

As the definition implies, essential amino acids have to be acquired through food – such as soy, quinoa, egg, chicken, meat or vegetable protein

Serves to build and repair muscle tissues. Also, it forms precursor molecules for the f Highly probably as these amino acids are acquired through foodormation of neurotransmitters in the brain



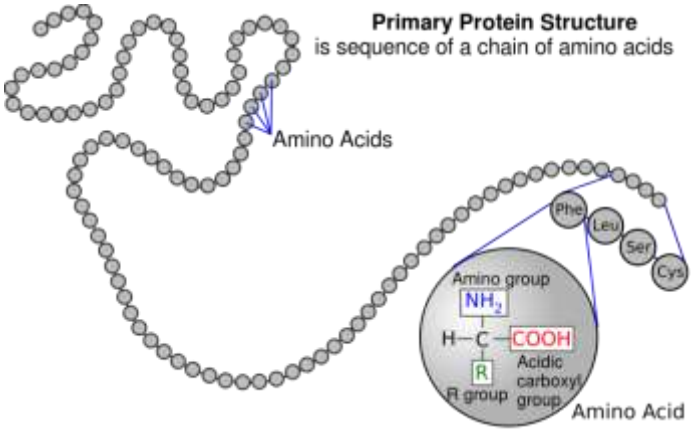
TYPES OF PROTEINS

- PRIMARY PROTEINS
- SECONDARD PROTEINS
- TERTIARY PROTEINS
- QUATERNARY PROTEINS

PRIMARY PROTEINS:

the primary structure of a protein is defined as **the sequence of amino acids linked together to form a polypeptide chain**.

Each amino acid is linked to the next amino acid through peptide bonds created during the protein biosynthesis process.

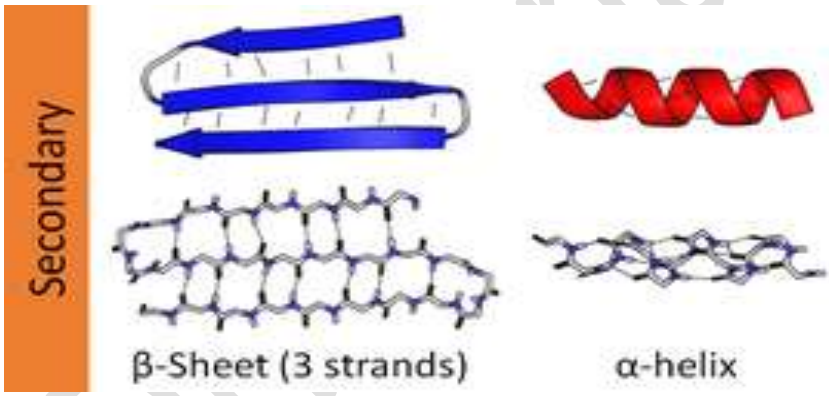


SECONDARY PROTEINS:

The secondary structure **arises from the hydrogen bonds formed between atoms of the polypeptide backbone**.
The hydrogen bonds form between the partially negative oxygen atom and the partially positive nitrogen atom.
The two main types of secondary structure are the α-helix and the ß-sheet.

The *α-helix* is a right-handed coiled strand. The side-chain substituents of the amino acid groups in an α-helix extend to the outside. Hydrogen bonds form between the oxygen of each C=O bond in the strand and the hydrogen of each N-H group four amino acids below it in the helix. The hydrogen bonds make this structure especially stable. The side-chain substituents of the amino acids fit in beside the N-H groups.

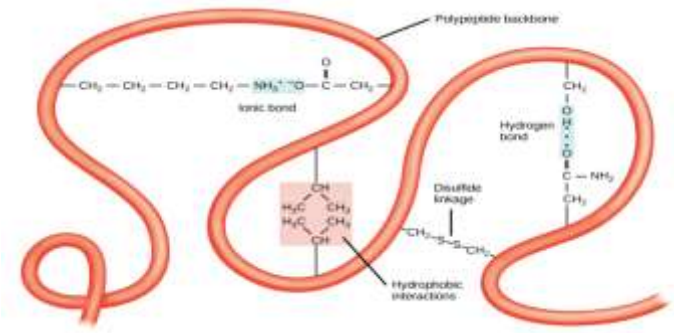
The hydrogen bonding in a *ß-sheet* is between strands (inter-strand) rather than within strands (intra-strand). The sheet conformation consists of pairs of strands lying side-by-side. The carbonyl oxygens in one strand bonds with the amino hydrogens of the adjacent strand. The two strands can be either parallel or anti-parallel depending on whether the strand directions (N-terminus to C-terminus) are the same or opposite. The anti-parallel ß-sheet is more stable due to the more well-aligned hydrogen bonds.



Tertiary protein structure:

The tertiary structure of a protein refers to **the overall three-dimensional arrangement of its polypeptide chain in space**. It is generally stabilized by outside polar hydrophilic hydrogen and ionic bond interactions, and internal hydrophobic interactions between nonpolar amino acid side chains.
The overall three-dimensional structure of a polypeptide is called its **tertiary structure**. The tertiary structure is primarily due to interactions between the R groups of the amino acids that make up the protein.
R group interactions that contribute to tertiary structure include hydrogen bonding, ionic bonding, dipole-dipole interactions, and London dispersion forces – basically, the whole gamut of non-covalent bonds. For example, R groups with like charges repel one another, while those with opposite charges can form an ionic bond. Similarly, polar R

groups can form hydrogen bonds and other dipole-dipole interactions. Also important to tertiary structure are **hydrophobic interactions**, in which amino acids with nonpolar, hydrophobic R groups cluster together on the inside of the protein, leaving hydrophilic amino acids on the outside to interact with surrounding water molecules.

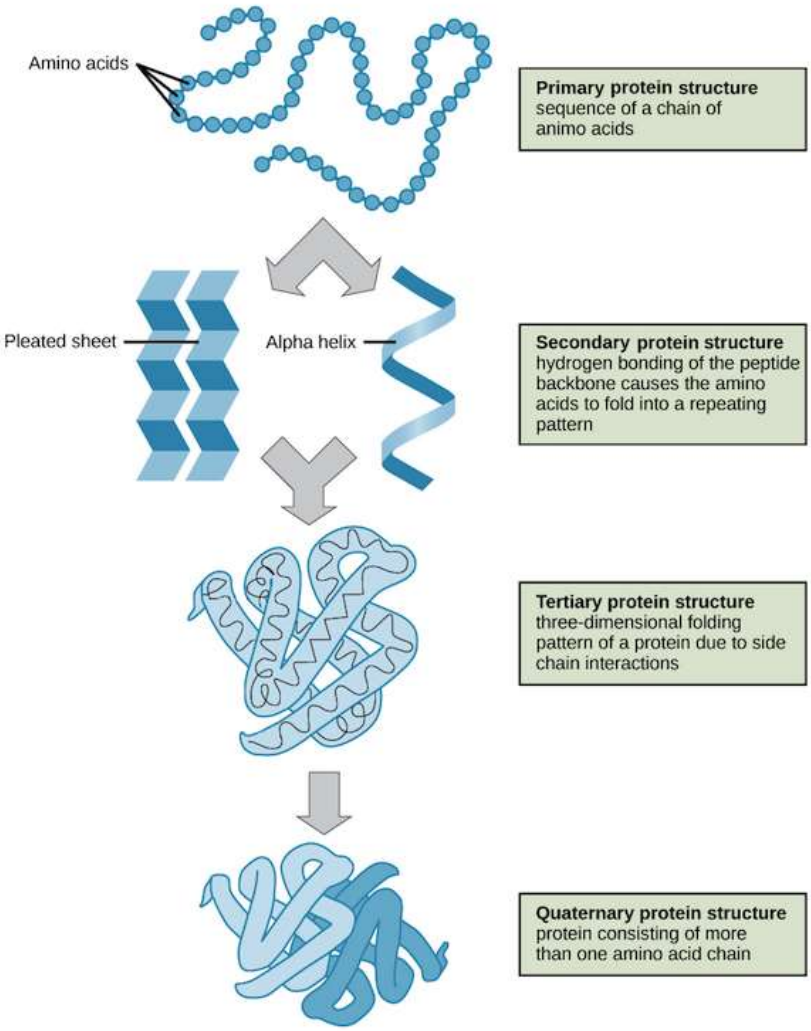


Quaternary structure

Many proteins are made up of a single polypeptide chain and have only three levels of structure (the ones we've just discussed). However, some proteins are made up of multiple polypeptide chains, also known as subunits. When these subunits come together, they give the protein its **quaternary structure**.

We've already encountered one example of a protein with quaternary structure: hemoglobin. As mentioned earlier, hemoglobin carries oxygen in the blood and is made up of four subunits, two each of the α and β types. Another example is DNA polymerase, an enzyme that synthesizes new strands of DNA and is composed of ten subunits start superscriptend superscript.

In general, the same types of interactions that contribute to tertiary structure (mostly weak interactions, such as hydrogen bonding and London dispersion forces) also hold the subunits together to give quaternary structure.



TYPES OF HELIX:

1. ALPHA HELIX
2. BETA HELIX

ALPHA HELIX:

Alpha-Helix and Beta-Pleated sheets are types of the secondary structure of the protein.

They both are shaped by hydrogen bonding between the carbonyl O of one amino acid and the amino H of another.

This section will discuss the protein, types of protein, and the primary and secondary protein structures, i.e. alpha-helix and beta-pleated sheets.

- Amino acids exist in the right-handed coiled rod-like structure.
- Intramolecular hydrogen bonding forms within the polypeptide chain to create a spiral structure.
- 3.6 amino acid residues are winded to form an alpha-helix polypeptide.
- Alpha-Helix can **Alpha-Helix can be a single chain polypeptide.**
- be a single chain polypeptide.
- Alkyl groups of alpha-helix are oriented outside of the helix.

Example: Keratin, Myoglobin and Haemoglobin.

Beta-Pleated Sheets of Protein

The second essential type of secondary structure of a protein is the Beta-Pleated Sheets of Protein. It consists of various beta strands linked by hydrogen bonds between adjacent strands. Three to ten amino acids are combined to create a beta-strand polypeptide.

Beta sheets are involved in forming the fibrils and protein aggregates observed in amyloidosis.

Alike alpha-helix, the residue hydrogen bond between the adjacent strands is separate from each other.

S No.	Alpha-Helix	Beta-Sheet
1	Amino acids exist in the right-handed coiled rod-like structure.	Amino acids exist in an almost entirely extended conformation, i.e. linear or sheet-like structure.
2	Intramolecular hydrogen bonding forms within the polypeptide chain to create a spiral structure.	Beta sheets are formed by linking two or more beta strands by intermolecular hydrogen bonds.
3	3.6 amino acid residues are winded to form an alpha-helix polypeptide.	Three to ten amino acids are combined to form a beta-strand polypeptide.
4	Alpha-Helix can be a single chain polypeptide.	Beta-Sheet cannot be in a single chain Polypeptide. There must be two or more beta-strands.
5	Alkyl groups of alpha-helix are oriented outside of the helix.	Alkyl groups are oriented both inside and outside of the sheet.
6	Example: Keratin, Myoglobin and Haemoglobin.	Example: Skin Fibres or Fibroin.

Biodegradation of amino acids:

The first step in amino acid degradation is **removal of the α-amino group**. Key steps in amino acid degradation include deamination, catalysed by pyridoxal phosphate-dependent transaminases, oxidoreductases or carbon–oxygen lyases, decarboxylase reactions and carbon skeleton rearrangements catalysed by isomerases.

Decarboxylation

Decarboxylation is **the reduction of carbon**, while transamination is the exchange within the amino group of an amino acid to a keto acid (the introduction or removal of nitrogen).

The term decarboxylation means the removal of a carboxyl group (-COOH) from any reactant molecule. The chemical reaction where a carboxyl group (-COOH) gets eliminated and carbon dioxide (
CO2CO2
) is released at the product end is called Decarboxylation. The liberation of
CO2CO2
makes the reaction almost irreversible in many cases. However, the reverse process i.e. Carboxylation is the addition of
CO2CO2
. Carboxylation accounts for the very first step of Photosynthesis after the intake of
CO2CO2
. Carboxylation results in the formation of Carboxylic acid. Most Decarboxylation Reactions involve carboxylic acids, where a carbon atom is broken off from the carbon chain. This carbon atom is released in the form of
CO2CO2

UREA CLYCLE:

The urea cycle begins in the mitochondria of hepatocytes and ends in the cytoplasm. The following reactions will be shown schematically below each described step. Note that the enzyme responsible for each respective step will not be shown but can be found in the preceding text.

Step 1

- The first step, which is also rate-limiting, involves the conversion of CO and ammonia into carbamoyl phosphate via the enzyme carbamoyl phosphate synthetase I (CPS I). Ammonia is the source of the first amine group of urea. What is unique about this step is that CPS I requires an obligate activator, N-acetyl-glutamate (NAG). NAG arises from glutamate + acetyl-CoA via the enzyme NAG synthase, which can be upregulated by arginine. Of note, some sources may use NH and HCO+ as the initial reactants, but these are equivalent to CO+ HO + NH.

- CO+ NH+ 2ATP produces carbamoyl phosphate +2ADP + P
    - Synthesis of NAG: glutamate + acetyl-CoA NAG

Step 2

- Carbamoyl phosphate and ornithine combine to form citrulline via ornithine transcarbamoylase (OTC). Citrulline is then transported from hepatocyte mitochondria into the cytoplasm by ornithine translocase.

- Carbamoyl phosphate + ornithine citrulline
    - Citrulline in mitochondria citrulline in the cytoplasm

Step 3

- Citrulline reacts with aspartate to form argininosuccinate. This reaction is carried out by the enzyme argininosuccinate synthetase, which requires ATP. Aspartate is the source of the second amine group on urea. Recall that aspartate results from the transamination of oxaloacetate and glutamate via aspartate transaminase, which requires vitamin B.

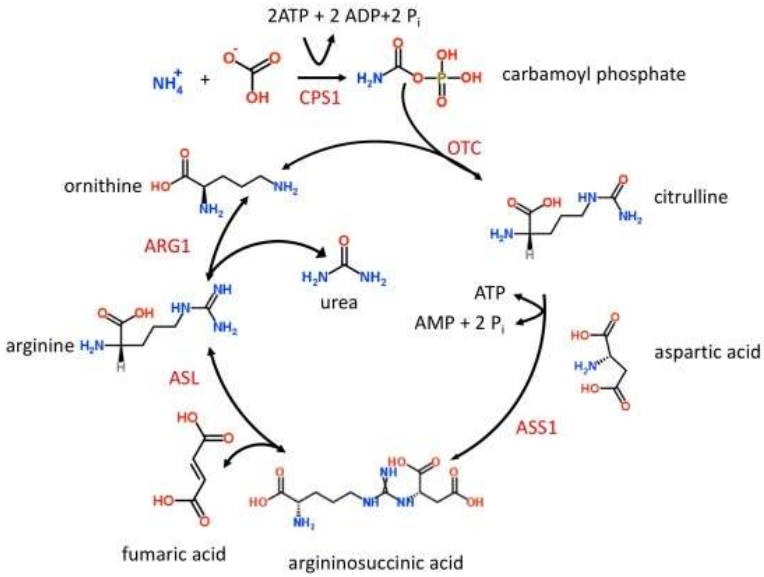
- Citrulline + aspartate + ATP argininosuccinate
    - Oxaloacetate + glutamate à aspartate + alpha-ketoglutarate

Step 4

- Argininosuccinate is converted into arginine via argininosuccinate lyase. This reaction also gives off fumarate, which is involved in the mitochondrial generation of NADH in the TCA cycle, as well as tyrosine catabolism.
- Argininosuccinate à arginine + fumarate

Step 5

- Arginine undergoes hydrolysis via arginase to form urea and ornithine. Take a moment to review step 2. Note that the regeneration of ornithine in step 5 is involved in step 2.
- Arginine + HO urea + ornithine



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NUCLEIC ACIDS:

nucleic acid, naturally occurring chemical compound that is capable of being broken down to yield phosphoric acid, sugars, and a mixture of organic bases (purines and pyrimidines). Nucleic acids are the main information-carrying molecules of the cell, and, by directing the process of protein synthesis, they determine the inherited characteristics of every living thing.

TYPES OF NUCLEIC ACIDS:

1.DNA
2.RNA

- **DNA:**

Deoxyribonucleic Acid (DNA)

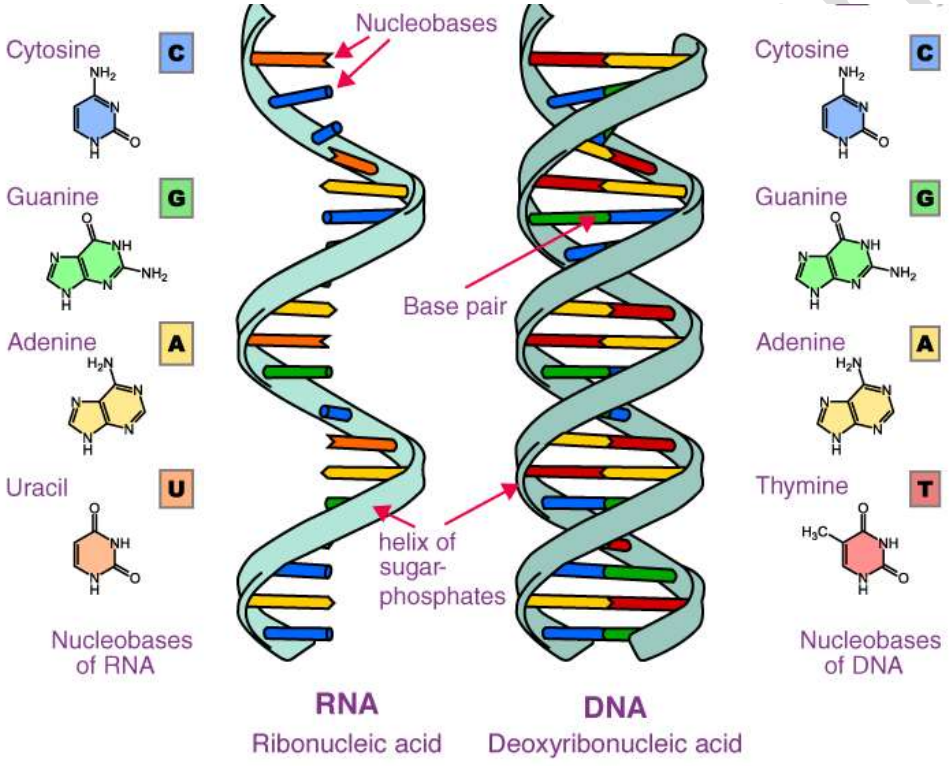
Chemically, DNA is composed of a pentose sugar, phosphoric acid and some cyclic bases containing nitrogen. The sugar moiety present in DNA molecules is β-D-2-deoxyribose. The cyclic bases that have nitrogen in them are adenine (A), guanine (G), cytosine(C) and thymine (T). These bases and their arrangement in the molecules of DNA play an important role in the storage of information from one generation to the next one. DNA has a double-strand helical structure in which the strands are complementary to each other.

- **RNA:**

Ribonucleic Acid (RNA)

The RNA molecule is also composed of phosphoric acid, a pentose sugar and some cyclic bases containing nitrogen. RNA has β-D-ribose in it as the sugar moiety. The heterocyclic bases present in RNA are adenine (A), guanine (G), cytosine(C) and uracil (U). In RNA the fourth base is different from that of DNA. The RNA generally consists of a single strand which sometimes folds back; that results in a double helix structure. There are three types of RNA molecules, each having a specific function:

-
    -
        1. **messenger RNA (m-RNA)**
        2. **ribosomal RNA (r-RNA)**
        3. **transfer RNA (t-RNA)**



The Functions of Nucleic Acids:

1. Nucleic acids are responsible for the transmission of inherent characters from parent to offspring.
2. They are responsible for the synthesis of protein in our body

3. DNA fingerprinting is a method used by forensic experts to determine paternity. It is also used for the identification of criminals. It has also played a major role in studies regarding biological evolution and genetics.

NUCLEOTIDE:

A nucleotide is an organic molecule with a basic composition of a nitrogenous base, pentose sugar and phosphate.

DNA and RNA are polynucleotides, which contain a chain of nucleotides monomers with different nitrogenous bases. Nucleotides are essential for carrying out metabolic and physiological activities.

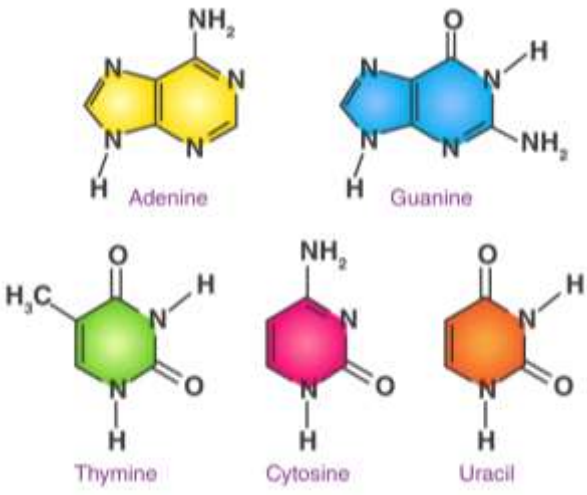
ATP (Adenosine triphosphate) acts as the energy currency of cells. Nucleotides form various coenzymes and cofactors, such as NAD, NADP, FAD, coenzyme A, etc. and are essential for many metabolic processes.

Nucleotide Structure –

A nucleotide consists of three units, which are covalently linked. They are:

1. Nitrogenous bases – Purine and Pyrimidine
2. Pentose Sugar – Ribose and Deoxyribose
3. Phosphate – monophosphate, diphosphate, triphosphate

1. **Nitrogenous Base:** They comprise pyrimidine or purine base. DNA contains adenine (A), guanine (G), cytosine (C) and thymine (T) whereas RNA contains adenine, guanine, cytosine and uracil (U).



2. Sugar: A nuc nucleic acid) contains deoxyribose sugar and RNA (Ribonucleic ac

A Nitrogenous base attached with the sugar is called **Nucleoside**.

3. Phosphate: Phosphate is associated with the sugar of nucleoside by an ester bond with the 5thC hydroxyl group. Nucleotides at least contain one phosphate group.

Phosphate of one nucleotide attaches to the 3rd C-OH group of the sugar of the 2nd nucleotide, thereby forming 5’ → 3’ linkage.

In DNA (double helix) there are two antiparallel strands of polynucleotides that are linked together by hydrogen bonds between nitrogenous bases. Purine pairs with pyrimidine base, A pairs with T and G pairs with C by two and three hydrogen bonds respectively.

In RNA instead of thymine (T), A pairs with U.

Phosphate group interlinks the sugar molecules of two nucleotides forming a chain. DNA and RNA are polynucleotides. Sugar phosphate chain forms the backbone of a polynucleotide chain.

When the phosphate group attaches to the hydroxyl group of the same sugar, it forms cyclic nucleotide, they are present as a single monomer, e.g. cAMP, cGMP used in intracellular signal transduction processes.

How do nucleotides and nucleosides differ?

Nucleoside = Nitrogenous base + Sugar

Nucleosides are named as Adenosine, Guanosine, Thymidine, Cytidine, Uridine

Nucleotide = Nucleoside + Phosphate

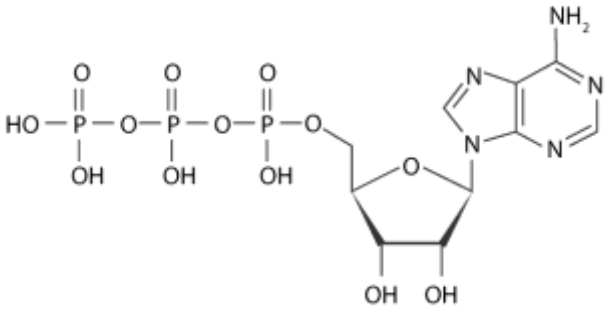
Nucleotides are named as Adenylic acid, Guanylic acid, Thymidylic acid, Cytidylic acid and Uridylic acid.

Nucleotides are also named as nucleoside mono, di or triphosphate, based on the number of phosphate groups attached to it, e.g. Adenosine monophosphate (AMP), Adenosine diphosphate (ADP) or Adenosine triphosphate (ATP).

DNA and RNA only contain nucleotides.

Other than polynucleotide chain of DNA and RNA, nucleotides are present in the body in various forms and are essential for life, e.g. ATP, cAMP, NAD^+, $NADP^+$, FAD, coenzyme A, etc.

Adenosine triphosphate (ATP): ATP is the energy currency of the cell. The energy required for metabolic processes is derived from ATP. It also acts as a coenzyme and is a precursor of DNA and RNA synthesis.



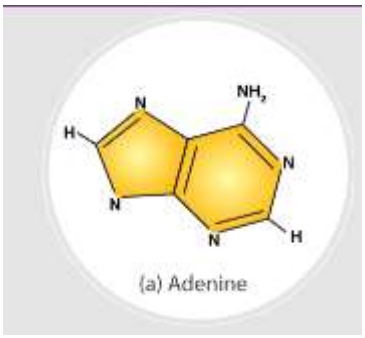
PURINES: Purine is a heterocyclic aromatic organic compound composed of a pyrimidine ring fused with imidazole ring.

Purine is a heterocyclic aromatic organic compound composed of a pyrimidine ring fused with imidazole ring.

It consists of two hydrogen-carbon rings and four nitrogen atoms

The melting point of purine is 214 °C

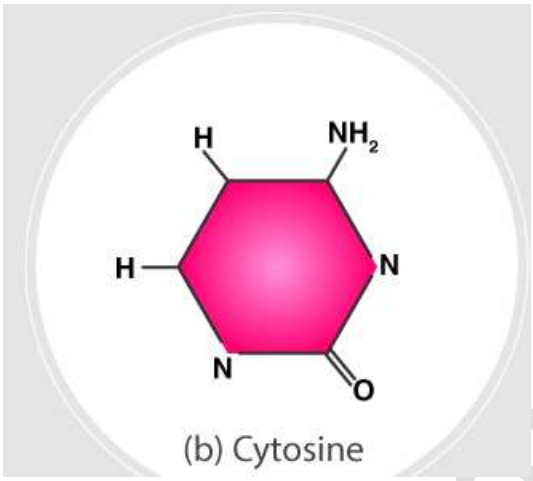
Catabolism results in the production of uric acid



(a) Adenine

PYRIMIDINE:

Pyrimidine is a heterocyclic aromatic organic compound that is composed of carbon and hydrogen.
It comprises cytosine, thymine, uracil as nucleobases
It consists of one hydrogen-carbon ring and two nitrogen atoms
The melting point of pyrimidine is 20-22 °C
Catabolism produces carbon dioxide, beta-amino acids and ammonia



(b) Cytosine

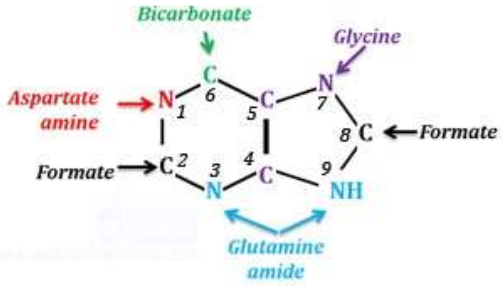
DNA and RNA Difference

DNA (Deoxyribonucleic acid)	RNA (Ribonucleic acid)
Definition	
It is a long polymer. It has a deoxyribose and phosphate backbone having four distinct bases: thymine, adenine, cytosine and guanine.	Is a polymer with a ribose and phosphate backbone with four varying bases: uracil, cytosine, adenine and guanine.
Location	
It is located in the nucleus of a cell and in the mitochondria.	It is found in the cytoplasm, nucleus and in the ribosome.
Sugar portion	
It has 2-deoxyribose.	It has Ribose.
Function	
The function of DNA is the transmission of genetic information. It acts as a medium for long-term storage.	RNA is critical for the transmission of the genetic code that is necessary for protein creation from the nucleus to the ribosome.
Predominant Structure	
DNA is a double-stranded molecule that has a long chain of nucleotides.	RNA is a single-stranded molecule which has a shorter chain of nucleotides.
Propagation	
DNA replicates on its own, it is self-replicating.	RNA does not replicate on its own. It is synthesized from DNA when required.
Nitrogenous Bases and Pairing	
The base pairing is as follows: GC (Guanine pairs with Cytosine) A-T (Adenine pairs with Thymine).	The base pairing is as follows: GC (Guanine pairs with Cytosine) A-U (Adenine pairs with Uracil).

I. De-novo synthesis of purines:

The purine nucleotides of nucleic acids are adenosine 5-monophosphate (AMP; adenylate) and guanosine 5-monophosphate (GMP; guanylate), containing the purine bases adenine and guanine respectively. The first idea about purine nucleotide biosynthesis in the cell was come from the study of John Buchanan (1948) by radioactive tracer studies

in birds by analyzing the biochemistry of uric acid (a purine present in the excreta of birds). The detailed biosynthetic pathways of the purine biosynthesis came latter in 1950 primarily by the works of Buchanan and G. Robert Greenberg.



Purine Nucleus
Showing the source of different atoms

Inosine monophosphate (IMP) is synthesized in 11 enzymatic steps from simple precursors as summarized below

Step-1: Ribose-5-phosphate activation and formation of PRPP):

Step-2: Acquisition of N9 atom of purine:

Step-3: Acquisition of C4, C5 & N7 atoms of purine:

Step-4: Acquisition of C8 atom of purine:

Step-5: Acquisition of N3 atom of purine:

Step-6: Purine imidazole ring formation:

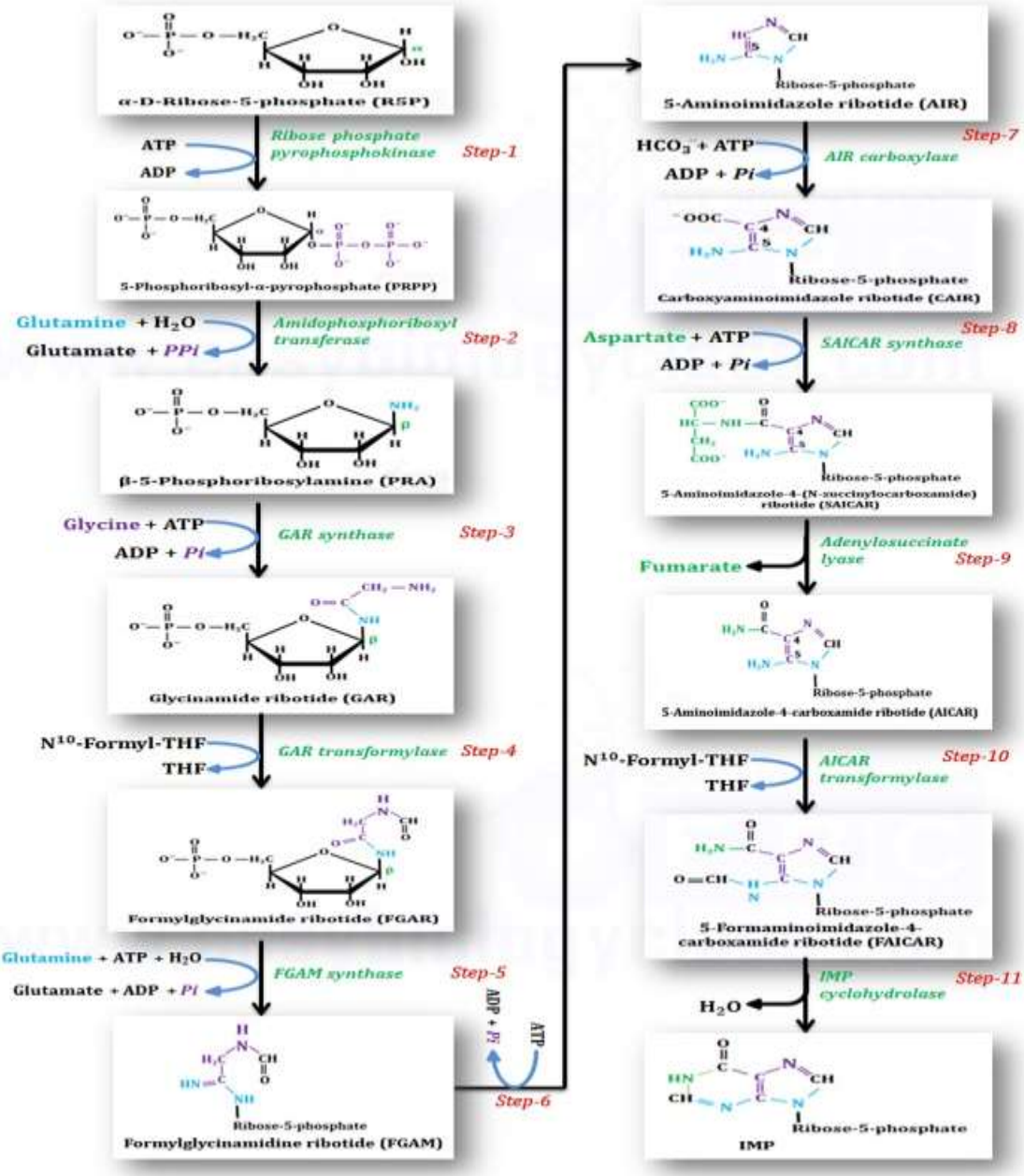
Step-7: Acquisition of C6 atom of purine:

Step-8: Acquisition of N1 atom of purine:

Step-9: Elimination of fumarate:

Step-10: Acquisition of C2 atom of purine:

Step-11: Cyclization to form IMP:



Inosine Monophosphate (IMP) Synthesis

Nucleotide Function

- Nucleotides are the building block of DNA and RNA. They contain genetic information
- Nucleotides act as coenzymes, which are required to catalyse many biochemical reactions by enzymes
- Energy is stored in our body as ATP. When there is a need for the energy they get converted to ADP or AMP. ATP also acts as a coenzyme
- NAD, NADP has an essential role to play in many redox reactions, they act as an electron carrier
- cAMP helps in transporting chemical signals and metabolic regulation
- Forms the constituents of DNA and RNA – They serve as building blocks of nucleic acids and are the carriers of activated metabolites for the process of biosynthesis
- Involved in storing chemical energy
- Required for DNA replication and RNA transcription in stages that rapidly divide
- Provides cellular energy sources and other metabolic functions
- Required for chemical associations in the response of cells to the hormones and other extracellular stimuli
- Serve as structural components of enzyme cofactors and other metabolic intermediates

B DNA

- Commonly occurring DNA form in normal physiological conditions, this form of DNA is a right-handed double helix
- The two strands of this DNA run in two different directions
- They show an asymmetrical structure, with the alternate presence of major and minor grooves. It is a result of the glycosidic bonds of a base pair not being diametrically opposed to one another
- Between the adjacent deoxyribonucleotides, there is a distance of 0.34 nm and each turn comprises 10.5 base pairs of length 3.4 nm
- The helical width of B-DNA is 2 nm and its backbone comprises sugar phosphates associated continuously through phosphodiester bonds. The core comprises nitrogenous bases

Z DNA

- Structurally differing, this form of DNA is a left-handed double helix
- The helical width of Z-DNA is 1.8 nm, making it the narrowest compared to the other DNA conformations
- Its distinguishing factor is its backbone appearing as though a zigzag
- Each turns comprises 12 base pairs, 4.56 nm long
- Two adjacent deoxyribonucleotides are 0.37 nm apart with the presence of hydrogen bonds between two strands

Key Difference between B DNA and Z DNA

The table below depicts the differences between B DNA and Z DNA.

B DNA	Z DNA
What it is	
One of the three common conformations of DNA helix, the chain twists up and to right around the front of the helical axis	One of the three common conformations of DNA helix, the chain twists up and to the left around the front of the helical axis
Helical type	
Right-handed	Left-handed
Occurrence	
Common	Less common comparatively
Description of major and minor groove	
Major groove – wide and deep Minor groove – narrow and deep	Major groove – narrow and deep Minor groove – wide and shallow
Condition for formation	
Normal physiological condition	High salt concentration
Repeating unit	
Mononucleotides (1 bp)	Dinucleotides (2 bp)
Arrangement of sugar residues	
Altering	Not altering
Helical diameter	
20 Å	18 Å
Glycosyl angle	
anti	C: anti, G: syn
Base pairs per turn	
10.5	12
Helical pitch	
34 Å	45 Å

Module 4:

Bioenergetics
Introduction:

Bioenergetics means study of the transformation of energy in living organisms.
The goal of bioenergetics is to describe how living organisms acquire and transform energy in order to perform biological work. The study of metabolic pathways is thus essential to bioenergetics.
In a living organism, chemical bonds are broken and made as part of the exchange and transformation of energy. Energy is available for work (such as mechanical work) or for other processes (such as chemical synthesis and anabolic processes in growth), when weak bonds are broken and stronger bonds are made. The production of stronger bonds allows release of usable energy.
Adenosine triphosphate (ATP) is the main "energy currency" for organisms; the goal of metabolic and catabolic processes are to synthesize ATP from available starting materials (from the environment), and to break- down ATP (into adenosine diphosphate (ADP) and inorganic phosphate) by utilizing it in biological processes.
In a cell, the ratio of ATP to ADP concentrations is known as the "energy charge" of the cell.
A cell can use this energy charge to relay information about cellular needs; if there is more ATP than ADP available, the cell can use ATP to do work, but if there is more ADP than ATP available, the cell must synthesize ATP via oxidative phosphorylation.
Living organisms produce ATP from energy sources via oxidative phosphorylation. The terminal phosphate bonds of ATP are relatively weak compared with the stronger bonds formed when ATP is hydrolyzed (broken down by water) to adenosine diphosphate and inorganic phosphate. Here it is the thermodynamically favorable free energy of hydrolysis that results in energy release; the phosphoanhydride bond between the terminal phosphate group and the rest of the ATP molecule does not itself contain this energy.
It is the study of energy changes accompanying biochemical reactions.
It describes the transfer and utilization of energy in biological systems.
It is concerned with the initial and final
energy states of reaction components and not the reaction mechanism or the time required for the Chemical reaction.

Types of Bioenergetics Reactions

1. Exergonic Reaction
Exergonic implies the release of energy from a spontaneous chemical reaction without any concomitant utilization of energy.
The reactions are significant in terms of biology as these reactions have an ability to perform work and include most of the catabolic reactions in cellular respiration.
Most of these reactions involve the breaking of bonds during the formation of reaction intermediates as is evidently observed during respiratory pathways. The bonds that are created during the formation of metabolites are stronger than the cleaved bonds of the substrate.
The release of free energy, G, in an exergonic reaction (at constant pressure and temperature) is denoted as
$\Delta G = G_{products} - G_{reactants} < 0$

2. Endergonic Reactions
Endergonic in turn is the opposite of exergonic in being non-spontaneous and requires an input of free energy.
Most of the anabolic reactions like photosynthesis and DNA and protein synthesis are endergonic in nature.
The release of free energy, G, in an exergonic reaction (at constant pressure and temperature) is denoted as
$\Delta G = G_{products} - G_{reactants} > 0$

3. Activation Energy

Activation energy is the energy which must be available to a chemical system with potential reactants to result in a chemical reaction. Activation energy may also be defined as the minimum energy required starting a chemical reaction.

Examples of Major Bioenergetics Processes

1. Glycolysis is the process of breaking down glucose into pyruvate, producing net eight molecules of ATP (per 1 molecule of glucose) in the process. Pyruvate is one product of glycolysis, and can be shuttled into other metabolic pathways (gluconeogenesis, etc.) as needed by the cell. Additionally, glycolysis produces equivalents in the form of NADH (nicotinamide adenine dinucleotide), which will ultimately be used to donate electrons to the electron transport chain.

2. Gluconeogenesis is the opposite of glycolysis; when the cell's energy charge is low (the concentration of ADP is
higher than that of ATP), the cell must synthesize glucose from carbon- containing biomolecules such as proteins, amino acids, fats, pyruvate, etc. For example, proteins can be broken down into amino acids, and these simpler carbon skeletons are used to build/ synthesize glucose.

3. The citric acid cycle is a process of cellular respiration in which acetyl coenzyme A, synthesized from pyruvate dehydrogenase, is first reacted with oxaloacetate to yield citrate. The remaining eight reactions produce other carbon- containing metabolites. These metabolites are successively oxidized, and the free energy of oxidation is conserved in the form of the reduced coenzymes FADH2 and NADH. These reduced electron carriers can then be re- oxidized when they transfer electrons to the electron transport chain.

4. Ketosis is a metabolic process whereby ketone bodies are used by the cell for energy (instead of using glucose). Cells often turn to ketosis as a source of energy when glucose levels are low; e.g., during starvation.

5. Oxidative phosphorylation and the electron transport chain is the process where reducing equivalents such as NADPH, FADH2 and NADH can be used to donate electrons to a series of redox reactions that take place in electron transport chain complexes. These redox reactions take place in enzyme complexes situated within the mitochondrial membrane. These redox reactions transfer electrons "down" the electron transport chain, which is coupled to the proton motive force. This difference in proton concentration between the mitochondrial matrix and inner membrane space is used to drive ATP synthesis via ATP synthase.

6. Photosynthesis, another major bioenergetic process, is the metabolic pathway used by plants in which solar energy is used to synthesize glucose from carbon dioxide and water. This reaction takes place in the chloroplast. After glucose is synthesized, the plant cell can undergo photophosphorylation to produce ATP.

Laws of thermodynamics:

1st law: Energy can neither be created nor be destroyed, but can be converted from one form to another.

2nd law: Total entropy of a system must increase if a process has to occur simultaneously.

Combining the two laws of thermodynamics, Gibbs in 1878, came up with the following equation

The relation between the change in free energy (∆G), enthalpy (∆H) and entropy (∆S) is expressed as-

$\Delta G = \Delta H - T\Delta S$

∆G= the change in free energy of a reacting system

T = the absolute temperature in kelvin at
which the process is taking place.

∆S = change in entropy

The Gibbs free energy (G) which is equal to the total amount of energy capable of doing work during a process at constant temperature and pressure.

o If ∆G is negative, then the process is spontaneous and termed exergonic.

o If ∆G is positive, then the process is nonspontaneous and termed endergonic.

o If ∆G is equal to zero, then the process has reached equilibrium.

The Enthalpy (H) which is the heat content of the system. Enthalpy is the amount of heat energy transferred (Heat absorbed or emitted) in a chemical process under constant pressure.

When ∆H is negative the process produces heat and is termed exothermic.

When ∆H is positive the process absorbs heat and is termed endothermic.

The Entropy (S) is a quantitative expression of the degree of randomness or disorder of the system. Entropy measures the amount of heat dispersed or transferred during a chemical process.

When ∆S is positive then the disorder of the system has increased.

When ∆S is negative then the disorder of the system has decreased.

Free energy

It is the energy actually available to do work

Change & free energy (∆G) predicts whether they are a chemical reaction feasible/favourable

Reaction can occur simultaneously if they are accompanied by decrease in free energy.

∆G approaches zero as reaction proceeds towards equilibrium.

During a chemical reaction, heat may be absorbed or released.

Enthalpy (∆H) is a measure of the change in heat content of the reactants, compared to products.

Entropy(∆S) represents a change in the randomness or disorder of reactants and products.

Entropy reaches a maximum as the reaction approaches equilibrium.

The reaction of biological system involves a temporary decrease in entropy.

$\Delta G = \Delta H - T\Delta S$

According to Boltzmann: $S=k \ln W$ where W is no. of states in the system.

Thus, Any reaction such as

$aA + bB \rightleftharpoons cC + dD$

in which a+b < c+d can be said to be driven by entropy.

If $\Delta G < 0$ then the reaction will be spontaneous.

The value of ΔG is directly related to the equilibrium constant.

$\Delta G0 = -RTlnkeq$

Actual free energy depends on the reactant & product.

$aA + bB \rightleftharpoons cC+dD$

$\Delta G=\Delta G0 + RTln [C]c[D]d /[A]a[B]b$

Free energies are additive, thus a favourable reaction ($\Delta G1<0$) can drive an unfavourable reaction ($\Delta G2>0$)

When $\Delta G1 + \Delta G2 <0$

(1) $A \rightarrow B$ $\Delta G101$

(2) $B \rightarrow C$ $\Delta G102$

Sum: $A \rightarrow C$ $\Delta G101+\Delta G102$

Negative & Positive ΔG

Consider the reactions: $A \rightleftharpoons B$

If ΔG is a negative number -- Net loss of energy-- reaction proceeds spontaneously --A is converted to B-Reaction is exergonic.

If ΔG is a positive number-- net gain of energy- does not proceed spontaneously from B to A--Reaction is endergonic -- Energy must be supplied to reactants.

Ex. Hydrolysis of ATP--Exergonic reaction

$ATP+H_2O \rightarrow ADP+ Pi$ ($\Delta G= - 7.3$ Cal/mol).

Reversal of the reaction ($ADP+Pi \rightarrow ATP$) is endergonic & occurs only when there is a supply of at least 7.3 Cal/mol (ΔG is positive).

$\Delta G=0$, reaction is in equilibrium free energy of the forward reaction ($A \rightarrow B$) is equal in magnitude but opposite in sign to that of backward

reaction ($B \rightarrow A$).

Energy Rich Compounds

High energy phosphates act as energy currency of cell.
Three major sources of high energy phosphates taking part in energy conservation or energy capture.
1. Oxidative phosphorylation (or OXPHOS in short)
In metabolic pathway, cells use enzymes to oxidize nutrients, thereby releasing energy which is used to produce adenosine triphosphate (ATP). In most eukaryotes, this takes place inside mitochondria. Almost all aerobic organisms carry out oxidative phosphorylation. This pathway is probably so pervasive because it is a highly efficient way of releasing energy, compared to alternative fermentation processes such as anaerobic glycolysis.
The process that accounts for the high ATP yield is known as oxidative phosphorylation.
In glycolysis and the citric-acid cycle generate other products besides ATP and GTP, namely NADH and FADH2. These products are molecules that are oxidized (i.e., give up electrons) spontaneously. The body uses these reducing agents (NADH and FADH2) in an oxidation-reduction reaction

2. Glycolysis:
Cells use the glycolysis pathway to extract energy from sugars, mainly glucose, and store it in molecules of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH). The end product of glycolysis is pyruvate, which can be used in other metabolic pathways to yield additional energy.
During glycolysis ATP molecules are used and formed in the following reactions (aerobic phase).

In the anaerobic phase oxidation of one glucose molecule produces 4 - 2 = 2 ATP.

3. TCA Cycle
The citric acid cycle (CAC) – also known as the tricarboxylic acid (TCA) cycle or the Krebs cycle is a series of chemical reactions used by all aerobic organisms to release stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins into carbon dioxide and chemical energy in the form of adenosine triphosphate (ATP).
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.

High energy compounds

Compounds in biological system which on hydrolysis yield free energy equal to or greater than yield energy less than -7.3 Kcal/mol are called low energy compounds.
Most of the High energy compounds contain Phosphate group hence they are also called High Energy phosphates. (Except acetyl).
These bonds are notated by the symbol '~' (squiggle).
Fritz Albert Lipmann invented this notation. The creation of energy rich compounds in cell carried out by 3 ways:

1. During oxidation and reduction of substrates (for instance Glucose) are formed intermediates with high-energy phosphate group.

Oxidation: Oxidation is referred as lose of electrons
Reduction: gain of electrons.

2. ATP- The most important high energy phosphate compound and its phosphoanhydride bonds are referred to as high energy bonds and are created in the process of oxidative phosphorylation in mitochondria.

The three process of ATP production includes glycolysis,
the TCA cycle, & oxidative phosphorylation.
In eukaryotic cells the latter two processes occur within mitochondria.

Electrons that are passed through ETC ultimately generates free energy capable of driving phosphorylation.

3. Some energy rich compounds are produced so phosphate is transferred from ATP to another molecule in the reaction which is catalysed by Kinase & high energy bond is preserved.
In the process of Glycolysis compounds like phosphoenol pyruvate & 1,3 bis - phosphoglycerate are ex. of high energy compounds.
Yield ATP from ADP called phosphorylation.
High Energy compounds are mainly classified into 5 group,
1) Pyrophosphate
2) Enol phosphates
3) Acyl phosphates
4) Thiol phosphates
5) Guanido phosphates

Pyrophosphate

Energy bond acid anhydride bonds. Bond formed by Condensation of acid groups (mainly phosphoric acid) or its derivative.

Phosphohydride

The Phosphoric anhydride bonds in ATP are "High energy" bonds.
On hydrolysis of 1 mol of this bond approximately 30.5KJ/mol energy is liberated.
ATP serve as principle immediate donor of free energy in biological systems.
Ex. of pyrophosphates is ATP; it has two high energy diphosphate bonds. phosphoanhydgide bond.

Enol Phosphates:

Enol phosphate bond is formed when phosphate group attaches to hydroxyl group which is bounded to a carbon atom having double bond.
Ex. phosphoenolpyruvate

Acyl phosphates
Ex. acyl phosphate is 1,3 -bisphosphoglycerate.
High energy bond is formed by the reaction between carboxylic acid group & phosphate group.

Thiol phosphate:

High energy phosphate bond is absent, instead high energy thioester bord is present.
This bond formed from the reaction between thiol & carboxylic acid group.
Ex. Acetyl CoA

Guanido phosphates:

Also known as phosphagens, guanidine phosphate bond is formed by the attachment of phosphate group to guanidine group.
Ex. phosphocreatine
(Present mostly in muscle cells where it acts as energy reserve)

Adenosine Triphosphate (ATP):

ATPase breaks ATP
ATP synthase forms ATP
When is ATP made in the Body? During a process called cellular Respiration that takes place in both plants & animals.

Biological significance of ATP:
The of ATP is associated with the release of large amount of energy.

ATP $+H_2O \rightarrow$ ADP +Pi+ 7.3 Cal

The energy liberated is utilized for various processes like muscle contraction, active transport etc.
ATP can also act as a donor or high-energy phosphate to low energy compounds, to make them energy rich.
ADP can accept high-energy phosphate from the compounds possessing higher free energy content to form ATP.
ATP is required for Synthesis of nucleoside biphosphates.

ATP + UDP ADP + UTP
ATP + GDP ADP + GTP

ATP + CDT ADP + CTP

The cyclic phosphodiester cAMP is formed from ATP in a reaction catalysed by adenylyl cyclase, cAMP, which is a secondary messenger participates in different regulatory functions of the cell.

ATP

Adenylyl cyclase.

Cyclic 3, 5-AMP

Functions of ATP:

- Metabolism
  synthesis
- Movement
  Muscle contraction
  Energy to allow muscle filament to slide.
- Active Transport:
  Changes the shape of carrier proteins.
- Secretion:

In the formation of the lysosomes necessary to exocytosis.

Chemical reactions:

A phosphate molecule from ATP can be transformed to another molecule.

Makes it more reactive

Lowers activation energy

CYCLIC ADENOSINE MONOPHOSPHATE

(cAMP, cyclic AMP or 3'-5'-cyclic adenosine monophosphate)

It is a second messenger important in many biological processes. cAMP is derived from adenosine triphosphate (ATP) and used for intracellular signal transduction in many different organisms, conveying the cAMP-dependent pathway.

cAMP is synthesized from ATP by adenylyl cyclase located on the inner side of the plasma membrane. Adenylyl cyclase is activated by a range of signaling molecules through the activation of adenylyl cyclase stimulatory G (Gs)-protein-coupled receptors and inhibited by agonists of adenylyl cyclase inhibitory G (Gi)-protein-coupled receptors. Liver adenylyl cyclase responds more strongly to glucagon, and muscle adenylyl cyclase responds more strongly to adrenaline.

cAMP decomposition into AMP is catalyzed by the enzyme phosphodiesterase.

Function: cAMP is a second messenger, used for intracellular signal transduction, such as transferring the effects of hormones like glucagon and adrenaline, which cannot pass through the cell membrane. It is involved in the activation of protein kinases and regulates the effects of adrenaline and glucagon. It also regulates the passage of Ca^{2+} through ion channels. cAMP and its associated kinases function in several biochemical processes, including the regulation of glycogen, sugar, and lipid metabolism by activating protein kinase

GUANOSINE TRIPHOSPHATE (GTP)

Guanosine-5'-triphosphate (GTP) is a purine nucleoside triphosphate. It can act as a substrate for both the synthesis of RNA during the transcription process and of DNA during DNA replication.

It also has the role of a source of energy or an activator of substrates in metabolic reactions, like that of ATP, but more specific. It is used as a source of energy for protein synthesis and gluconeogenesis.

GTP is essential to signal transduction, in particular with G-proteins, in second-messenger mechanisms where it is converted to Guanosine diphosphate (GDP) through the action of GTPases.

USES:

- Energy transfer

GTP is involved in energy transfer within the cell. For instance, a GTP molecule is generated by one of the enzymes in the citric acid cycle. This is tantamount to the generation of one molecule of ATP, since GTP is readily converted to ATP with nucleoside-diphosphate kinase (NDK).

- Genetic translation

During the elongation stage of translation, GTP is used as an energy source for the binding of a new amino-bound tRNA to the A site of the ribosome.

- Mitochondrial function

The translocation of proteins into the mitochondrial matrix involves the interactions of both GTP and ATP.

Synthesis of AMP and GMP from IMP.

Cyclic Guanosine Monophosphate

Cyclic guanosine monophosphate (cGMP) is a cyclic nucleotide derived from guanosine triphosphate (GTP).

cGMP acts as a second messenger much like cyclic AMP. Its most likely mechanism of action is activation of intracellular protein kinases in response to the binding of membrane-impermeable peptide hormones to the external cell surface.

Synthesis: Guanylate cyclase (GC) catalyzes cGMP synthesis. This enzyme converts GTP to cGMP.

Effects

- cGMP is a common regulator of ion channel conductance, glycogenolysis, and cellular apoptosis. It also relaxes smooth muscle tissues. In blood vessels, relaxation of vascular smooth muscles leads to vasodilation and increased blood flow.
- cGMP is a secondary messenger in phototransduction in the eye.
- cGMP is involved in the regulation of some protein-dependent kinases.

Biological Oxidation-Reduction Reactions:

The transfer of phosphoryl groups is a central feature of metabolism. Equally important is another kind of transfer, electron transfer in oxidation-reduction reactions. These reactions involve the loss of electrons by one chemical species, which is thereby oxidized, and the gain of electrons by another, which is reduced. The flow of electrons in oxidation-reduction reactions is responsible, directly or indirectly, for all work done by living organisms. In non-photosynthetic organisms, the sources of electrons are reduced compounds (foods); in photo- synthetic organisms, the initial electron donor is a chemical species excited by the absorption of light. The path of electron flow in metabolism is complex. Electrons move from various metabolic intermediates to specialized electron carriers in enzyme-catalysed reactions. The carriers in turn donate electrons to acceptors with higher electron affinities, with the release of energy. Cells contain a variety of molecular energy transducers, which convert the energy of electron flow into useful work.

In many organisms, a central energy conserving process is the stepwise oxidation of glucose to CO2 , in which some of the energy of oxidation is conserved in ATP as electrons are passed to O2.
Biological oxidation-reduction reactions can be described in terms of two half-reactions, each with a characteristic standard reduction potential, E0.

When two electrochemical half-cells, each containing the components of a half-reaction, are connected, electrons tend to flow to the half-cell with the higher reduction potential The strength of this tendency is proportional to the difference between the two reduction potentials (∆E) and is a function of the concentrations of oxidized and reduced species.

The standard free-energy change for an oxidation-reduction reaction is directly proportional to the difference in standard reduction potentials of the two half-cells:

$\Delta G0 = -nF \Delta E0$

Many biological oxidation reactions are dehydrogenations in which one or two hydrogen atoms (H++ e-) are transferred from a substrate to a hydrogen acceptor. Oxidation-reduction reactions in living cells involve specialized electron carriers. NAD and NADP are the freely diffusible coenzymes of many dehydrogenases. Both NAD+ and NADP+ accept two electrons and
one proton. NAD and NADP are bound to dehydrogenases in a widely conserved structural motif called the Rossmann fold.
FAD and FMN, the flavin nucleotides, serve as tightly bound prosthetic groups of flavoproteins. They can accept either one or
two electrons. Flavoproteins also serve as light receptors in cryptochromes and photolyases.

Electron transport chain:

The electron transport chain is a series of four protein complexes that couple redox reactions, creating an electrochemical gradient that leads to the creation of ATP in a complete system named oxidative phosphorylation. It occurs in mitochondria in both cellular respiration and photosynthesis. In the former, the electrons come from breaking down organic molecules, and energy is released. In the latter, the electrons enter the chain after being excited by light, and the energy released is used to build carbohydrates.
Fundamentals:
Aerobic cellular respiration is made up of three parts: glycolysis, the citric acid (Krebs) cycle, and oxidative phosphorylation. In glycolysis, glucose metabolizes into two molecules of pyruvate, with an output of ATP and nicotinamide adenine dinucleotide (NADH). Each pyruvate oxidizes into acetyl CoA and an additional molecule of NADH and carbon dioxide (CO2). The acetyl CoA is then used in the citric acid cycle, which is a chain of chemical reactions that produce CO2, NADH, flavin adenine dinucleotide (FADH2), and ATP. In the final step, the three NADH and one FADH2 amassed from the previous steps are used in oxidative phosphorylation, to make water and ATP.
Oxidative phosphorylation has two parts: the electron transport chain (ETC) and chemiosmosis. The ETC is a collection of proteins bound to the inner mitochondrial membrane and organic molecules, which electrons pass through in a series of redox reactions, and release energy. The energy released forms a proton gradient, which is used in chemiosmosis to make a large amount of ATP by the protein ATP-synthase.
Photosynthesis is a metabolic process that converts light energy into chemical energy to build sugars. In the light-dependent reactions, light energy and water are used to make ATP, NADPH, and oxygen (O2). The proton gradient used to make the ATP forms via an electron transport chain. In the light-independent reactions, sugar is made from the ATP and NADPH from the previous reactions.
Cellular:
In the electron transport chain (ETC), the electrons go through a chain of proteins that increases its reduction potential and causes a release in energy. Most of this energy is dissipated as heat or utilized to pump hydrogen ions (H+) from

the mitochondrial matrix to the intermembrane space and create a proton gradient. This gradient increases the acidity in the intermembrane space and creates an electrical difference with a positive charge outside and a negative charge inside. The ETC proteins in a general order are complex I, complex II, coenzyme Q, complex III, cytochrome C, and complex IV.

Complex I, also known as ubiquinone oxidoreductase, is made up of NADH dehydrogenase, flavin mononucleotide (FMN), and eight iron-sulfur (Fe-S) clusters. The NADH donated from glycolysis, and the citric acid cycle is oxidized here, transferring 2 electrons from NADH to FMN. Then they are transferred to the Fe-S clusters and finally from Fe-S to coenzyme Q. During this process, 4 hydrogen ions pass from the mitochondrial matrix to the intermembrane space, contributing to the electrochemical gradient. Complex I may also play an important role in causing apoptosis in programmed cell death.

(NADH + H+) + CoQ + 4 H+(matrix) -> NAD+ + CoQH2 + 4 H+(intermembrane)

Complex II, also known as succinate dehydrogenase, accepts electrons from succinate (an intermediate in the citric acid cycle) and acts as a second entry point to the ETC. When succinate oxidizes to fumarate, 2 electrons are accepted by FAD within complex II. FAD passes them to Fe-S clusters and then to coenzyme Q, similar to complex I. However; no protons are translocated across the membrane by complex II, therefore less ATP is produced with this pathway.

Succinate + FAD -> Fumarate + 2 H+(matrix) + FADH2

FADH2 + CoQ -> FAD + CoQH2

Glycerol-3-Phosphate dehydrogenase and Acyl-CoA dehydrogenase also accept electrons from glycerol-3-P and fatty acyl-CoA, respectively. Inclusion of these protein complexes allows for the donation to the ETC by cytosolic NADH (glycerol-3-P acts as a shuttle to regenerate cytosolic NAD from NADH) and fatty acids undergoing beta-oxidation within the mitochondria (acyl-CoA is oxidized to enoyl-CoA in the first step, producing FADH2).

Coenzyme Q, also known as ubiquinone (CoQ), is made up of quinone and a hydrophobic tail. Its purpose is to function as an electron carrier and transfer electrons to complex III. Coenzyme Q undergoes reduction to semiquinone (partially reduced, radical form CoQH-) and ubiquinol (fully reduced CoQH2) through the Q cycle. This process receives further elaboration under Complex III.

Complex III, also known as cytochrome c reductase, is made up of cytochrome b, Rieske subunits (containing two Fe-S clusters), and cytochrome c proteins. A cytochrome is a protein involved in electron transfer that contains a heme group. The heme groups alternate between ferrous (Fe2+) and ferric (Fe3+) states during the electron transfer. Because cytochrome c can only accept a single electron at a time, this process occurs in two steps (the Q cycle), in contrast to the single-step complex I and II pathways. Complex III also releases 4 protons into the intermembrane space at the end of a full Q cycle, contributing to the gradient. Cytochrome c then transfers the electrons one at a time to complex IV.

Q Cycle:

Step 1 in the Q cycle involves ubiquinol (CoQH2) and ubiquinone (CoQ) binding to two separate sites on complex III. CoQH2 transfers each electron to a different path. One electron goes to Fe-S and then cytochrome c, while the second electron is transferred to cytochrome b and then to CoQ bound at the other site. While this occurs, 2 H+ ions are released into the intermembrane space, contributing to the proton gradient. CoQH2 is now oxidized to ubiquinone and dissociates from the complex. The CoQ bound at the second site enters a transitional CoQH- radical state from accepting one of the electrons.

The second step of the cycle involves a repeat of the first: a new CoQH2 binds to the first site and transfers two electrons like before (and 2 more H+ ions released). Again, one electron passes to cytochrome c and one to cytochrome b, which this time works to reduce CoQH- to CoQH2 before it dissociates from complex III and can be recycled. In this way, one full cycle appears as follows:

2 CoQH2(site 1) + CoQ (site 2) + 2 Cyt c(ox) + 2 H+(matrix) -> 2 CoQ (site 1) + CoQH2(site 2) + 2 Cyt c(red) + 4 H+(intermembrane)

Complex IV, also known as cytochrome c oxidase, oxidizes cytochrome c and transfers the electrons to oxygen, the final electron carrier in aerobic cellular respiration. The cytochrome proteins a and a3, in addition to heme and copper groups in complex IV transfer the donated electrons to the bound dioxygen species, converting it into molecules of water. The free energy from the electron transfer causes 4 protons to move into the intermembrane space contributing to the proton gradient. Oxygen reduces via the following reaction:

2 cytochrome c(red) + ½O2 + 4 H+(matrix) -> 2 cytochrome c(ox) + 1 H2O + 2 H+(intermembrane)

ATP synthase, also called complex V, uses the ETC generated proton gradient across the inner mitochondrial membrane to form ATP. ATP-synthase contains up of F0 and F1 subunits, which act as a rotational motor system. F0 is hydrophobic and embedded in the inner mitochondrial membrane. It contains a proton corridor that is protonated and deprotonated repeatedly as H+ ions flow down the gradient from intermembrane space to matrix. The alternating ionization of F0 causes rotation, which alters the orientation of the F1 subunits. F1 is hydrophilic and faces the mitochondrial matrix. Conformational changes in F1 subunits catalyze the formation of ATP from ADP and Pi. For every 4 H+ ions, 1 ATP is produced. ATP-synthase can also be forced to run in reverse, consuming ATP to produce a hydrogen gradient, as is seen in some bacteria.

Molecular:
Nicotinamide adenine dinucleotide has two forms: NAD+ (oxidized) and NADH (reduced). It is a dinucleotide connected by phosphate groups. One nucleoside has an adenine base and the other nicotinamide. When involved in metabolic redox reactions, the mechanism is as shown in Reaction 1.
Reaction 1: RH2 + NAD+ -> R + H+ + NADH
R is the reactant, for example, sugar.
NADH enters the ETC at complex I and produces a total of 10 H+ ions through the ETC (4 from complex I, 4 from complex III, and 2 from complex IV). ATP-synthase synthesizes 1 ATP for 4 H+ ions. Therefore, 1 NADH = 10 H+, and 10/4 H+ per ATP = 2.5 ATP per NADH (**some sources round up**). When NADH is oxidized, it breaks into NAD+, H+, and 2 e- as shown in Reaction 2.
Reaction 2: NADH -> H+ + NAD+ + 2 e-
Flavin adenine dinucleotide has 4 redox states, 3 of them being FAD (quinone, fully oxidized form), FADH- (semiquinone, partially oxidized), and FADH2 (hydroquinone, fully reduced). FAD is made up of an adenine nucleotide and a flavin mononucleotide (FMN), connected by phosphate groups. FMN is synthesized in part from vitamin B2 (riboflavin). FAD contains a highly stable aromatic ring, and FADH2 does not. When FADH2 oxidizes, it becomes aromatic and releases energy, as seen in Reaction 3. This state makes FAD a potent oxidizing agent, with an even more positive reduction potential than NAD. FADH2 enters the ETC at complex II and creates a total of 1.5 ATP (4 H+ from complex III, and 2 H+ from complex IV; 6/4 H+ per ATP = 1.5 ATP per FADH2 **some sources round up**).
Reaction 3: FADH2 -> FAD + 2 H+ + 2 e-
FAD also functions in several metabolic pathways outside of the ETC, including DNA repair (MTHF repair of UV damage), fatty acid beta-oxidation (acyl-CoA dehydrogenase), and synthesis of coenzymes (CoA, CoQ, heme).
KEY POINTS ABOUT THE ELECTRON TRANSPORT CHAIN:

The electron transport chain is located in the mitochondrial inner membrane and contains several different kinds of electron carriers: flavin mononucleotide, iron-sulfur proteins, coenzyme Q, heme-containing cytochromes, and copper ions.
Three large multiprotein complexes serve as proton pumps by harnessing the energy from electron flow through the ETC to oxygen; in turn, the chemiosmotic energy in the proton gradient that is created by the pumps is coupled to the synthesis of ATP by the ATP synthase complex.
ATP regulates its own synthesis and the flow of electrons through respiratory control; if ATP synthesis slows down, electron transport slows down and vice versa.
Cytosolic NADH cannot pass through the mitochondrial membrane, so it shuttles its electrons through the glycerol phosphate shuttle and the malate-aspartate shuttle.

ATP and ADP are transported in exchange for each other by the ATP/ADP translocase.

ATP synthesis:

Since 1929, when it was discovered that ATP is a substrate for muscle contraction, the knowledge about this purine nucleotide has been greatly expanded. Many aspects of cell metabolism revolve around ATP production and consumption. It is important to understand the concepts of glucose and oxygen consumption in aerobic and anaerobic life and to link bioenergetics with the vast number of reactions occurring within cells. ATP is universally seen as the energy exchange factor that connects anabolism and catabolism but also fuels processes such as motile contraction, phosphorylations, and active transport. It is also a signaling molecule in the purinergic signaling mechanisms. In this review, we will discuss all the main mechanisms of ATP production linked to ADP phosphorylation as well the regulation of these mechanisms during stress conditions and in connection with calcium signaling events.

Within cells, energy is provided by oxidation of “metabolic fuels” such as carbohydrates, lipids, and proteins. It is then used to sustain energy-dependent processes, such as the synthesis of macromolecules, muscle contraction, active ion transport, or thermogenesis. The oxidation process results in free energy production that can be stored in phosphoanhydrine “high-energy bonds” within molecules such as nucleoside diphosphate and nucleoside triphosphate (i.e., adenosine 5′ diphosphate and adenosine 5′ trisphosphate, ADP, and ATP, respectively), phosphoenolpyruvate, carbamoyl phosphate, 2,3-bisphosphoglycerate, and other phosphagens like phosphoarginine, or phosphocreatine. Among them, ATP is the effective central link—the exchange coin—between energy-producing and the energy-demanding processes that effectively involve formation, hydrolysis, or transfer of the terminal phosphate group.

In general, the main energy source for cellular metabolism is glucose, which is catabolized in the three subsequent processes—glycolysis, tricarboxylic acid cycle (TCA or Krebs cycle), and finally oxidative phosphorylation—to produce ATP. In the first process, when glucose is converted into pyruvate, the amount of ATP produced is low. Subsequently, pyruvate is converted to acetyl coenzyme A (acetyl-CoA) which enters the TCA cycle, enabling the production of NADH. Finally, NADH is used by the respiratory chain complexes to generate a proton gradient across the inner mitochondrial membrane, necessary for the production of large amounts of ATP by mitochondrial ATP synthase. In addition, it should be mentioned that acetyl-CoA can be generated also by lipid and protein catabolism.

Basic principles of ATP producing pathways:

Glycolysis

Glycolysis is a process by which glucose is partially converted through a series of enzyme-catalyzed reactions into two molecules of pyruvate. Some mammalian cell types (erythrocytes, sperm) and tissues (brain, renal medulla) are able to survive only (or mostly) on the energy derived from glycolysis. The steps comprising the processes leading to the breakdown of the six-carbon glucose into two three-carbon pyruvate molecules can be divided into two phases: the preparatory phase and the so-called “payoff” phase.

In the first phase, glucose is phosphorylated at the hydroxyl group on C-6 by hexokinase (HK) generating glucose 6-phosphate. This event is fundamental to “trap” the hexose within the cell. In fact, the existence of a transporter of phosphorylated hexose has not been reported in mammalian cells. In this way, the phosphorylation of glucose shifts the equilibrium of glucose concentration, preventing its escape. Several types of HKs have been found, each with specific features. In the case of HK IV (glucokinase), known to be liver-specific, it is the insensitivity to glucose 6-phosphate inhibition that allows its direct regulation by the levels of glucose in the blood. Recently, there has been increased interest in the mitochondria-associated HK (mtHK). mtHK is able to promote cell survival through an AKT-mediated pathway. This was one of the first mechanisms suggested to couple metabolism to cell fate because of its ability to participate in mitochondrial dynamics during apoptosis and especially due to its involvement in the formation of the mitochondrial permeability transition pore.

Subsequently, glucose 6-phosphate is converted to fructose 6-phosphate by glucose 6-phosphate isomerase. This isomerization is fundamental for the subsequent step in which C-1 is once again phosphorylated, resulting in the formation of fructose 1,6-bisphosphate. Aldolase is then able to split fructose 1,6-bisphosphate into two three-carbon

molecules: dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP). This step represents the real "lysis" phase.

Until now, the glycolytic pathway consumed ATP instead of producing it. This should be interpreted as an investment raising the free-energy content of the intermediates, and the real yield of the process starts from here, with the beginning of the second phase.

DHAP is isomerized by triosephosphate isomerase to form a second molecule of GAP. The carbon chain of the entire glucose is thus converted into two molecules of GAP. Each of these molecules is oxidized and phosphorylated by inorganic phosphate to form 1,3-bisphosphoglycerate. During this process, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) uses nicotinamide adenine dinucleotide (NAD+) as cofactor and releases NADH for each molecule of GAP. The resulting NADH will directly feed into the respiratory chain to propel mitochondrial ATP synthesis. It is noteworthy that GAPDH is also able to regulate several processes which are not part of the glycolytic pathway. These include the regulation of apoptosis, membrane fusion, microtubule bundling, RNA export, DNA replication, and repair.

Some energy is released through the conversion of 1,3-bisphosphoglycerate into two molecules of pyruvate by the sequential steps performed by phosphoglycerate kinase (PGK), phosphoglycerate mutase, enolase, and pyruvate kinase. The conversions of 1,3-bisphosphoglycerate to 3-phosphoglycerate (by PGK) and phosphoenolpyruvate to pyruvate (by pyruvate kinase) are the steps that promote ATP synthesis from ADP in glycolysis. The last step is also a fundamental regulator of the whole process. Pyruvate kinase (PK) undergoes allosteric regulation by fructose 1,6-bisphosphate that promotes PK activity and boosts the rate of glycolysis. Allosteric regulation and tissue expression characterize several isoforms of the PK enzyme, i.e., the isoform M2, usually expressed during embryogenesis, has been found as a special promoter of tumorigenesis. This isoform is characterized by a high affinity to phosphoenolpyruvate, and it has been associated with favoring the conversion of pyruvate to lactate instead of its entry in the TCA cycle.

Thus, the second phase of glycolysis provides four molecules of ATP and two of NADH per molecule of glucose, paying the investment of the preparatory phase. The final balance of this process is then: two molecules of ATP, two of NADH (that could directly feed into the respiratory chain), and two of pyruvate. The latter enters the TCA cycle and undergoes complete oxidation in aerobic conditions.

During anaerobic conditions (such as what occurs in muscles during a burst of extreme activity, when oxygen is not obtained fast enough from the blood), the low oxygen amounts do not allow the complete and efficient oxidation of pyruvate. During these conditions, NADH (produced in large amounts from the citric acid cycle; see next section) cannot be reoxidized to NAD, thus limiting the activity of GAPDH and glucose consumption. Pyruvate is then reduced to lactate with the consumption of one NADH in a process called lactic fermentation catalyzed by lactate dehydrogenase. In this way, the two molecules of NADH produced in glycolysis are consumed in lactic fermentation to restore the NAD reservoir, and the final balance of one glucose degradation is two molecules of ATP. This condition occurs also in aerobic conditions in erythrocytes (that have no mitochondria) or in many cancer cells as was originally observed by doctor Otto Warburg in 1930, and which led to the widely accepted Warburg effect theory .

Citric acid cycle

The TCA, also known as the citric acid cycle, was elucidated by Sir Hans Krebs in 1940 when he concluded, "the oxidation of a triose equivalent involves one complete citric acid cycle" . The "triose" deriving from glycolysis is completely oxidized into three molecules of CO_2 during a sequence of reactions that allow the reduction of cofactors NAD and flavin adenine nucleotide (FAD), providing energy for the respiratory chain in the form of electrons. In 1949, it was demonstrated by Kennedy and Lehningher that the entire cycle occurs inside mitochondria .

The starting material for the citric acid cycle is directly provided by the pyruvate coming from glycolysis through the activity of the pyruvate dehydrogenase complex. This enzymatic complex, composed of multiple copies of the three enzymes pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3), oxidizes pyruvate to acetyl-CoA and CO_2 in an irreversible reaction in which the carboxyl group is removed from pyruvate as a molecule of CO_2. This reaction is strictly related to the cycle, even if is not comprised in it. The acetyl group introduces two carbons in each turn of the cycle; these carbons will then leave the cycle as CO_2.

The first reaction of the citric acid cycle is the condensation of one acetyl-CoA and a molecule of citrate to generate oxaloacetate and is catalyzed by citrate synthase. Citrate is then transformed into isocitrate by aconitase through the formation of cis-aconitate. This step is reversible and could lead to the formation of both citrate and isocitrate. Only the fast consumption of isocitrate by its dehydrogenase can force the reaction to the proper direction. Isocitrate dehydrogenase catalyzes the first irreversible oxidation leading to the decarboxylation of isocitrate, generating CO2 and α-ketoglutarate. The second carbon leaves the cycle in the following step, when the newly generated α-ketoglutarate is immediately decarboxylated by the α-ketoglutarate dehydrogenase complex in a reaction similar to the pyruvate decarboxylation. In fact, both these complexes share high similarities in enzyme amino acid composition and in the organization of the different subunits. Energy released from both oxidations is used to generate NADH from NAD that directly feeds into the respiratory chain.

The following step is catalyzed by succinyl–CoA synthetase and utilizes the energy derived from the CoA removal to phosphorylate GDP (or ADP) to GTP (or ATP). Selectivity for the nucleotide is determined by the isozyme involved. It has been well established that at least two isozymes of succinyl–CoA synthetase are expressed in animal tissues , and the proportion between them seems to be tissue-specific.

The succinate generated in the previous step is the four-carbon compound that is then converted, by three sequential reactions, to oxaloacetate to conclude the cycle. The first of these steps is the oxidation of succinate to fumarate by succinate dehydrogenase. This enzyme, tightly bound to the inner mitochondrial membrane (IMM), catalyzes FAD reduction to FADH2 that provides electrons for the respiratory chain. Fumarate is then hydrated by fumarate hydratase to L-malate. It is particularly interesting that both succinate dehydrogenase and fumarate hydratase are oncosuppressor genes. It has been demonstrated that inactivation of these oncosuppressors leads to the accumulation of succinate and fumarate that spread in the cytosol and promote hypoxia-inducible factor 1α (HIF1α) accumulation by inactivating prolyl hydroxylase enzymes (promoter of HIF1α degradation); HIF1α in turn promotes a pseudo-hypoxic condition that favors tumor development. The last event that completes the citric acid cycle is the oxidation of L-malate to oxaloacetate. This reaction is performed by L-malate dehydrogenase which induces the reduction of another molecule of NAD to NADH. The resulting molecule of oxaloacetate is suitable for starting another cycle through condensation with an acetyl group. During all these processes, only one molecule of ATP (or GTP) is produced, but three molecules of NADH and one of FADH2 (plus one molecule of NADH from pyruvate dehydrogenase), which provide electrons for respiratory chain, are also generated and subsequently result in the production of large amounts of ATP (discussed later).

Respiratory chain and oxidative phosphorylation

Respiratory chain comprises a series of components (complexes) conducting electron transfer across the membrane and involved in oxidative phosphorylation (OXPHOS), a process which occurs in aerobic conditions. In eukaryotic cells, electron transport occurs in mitochondria and chloroplasts, whereas in bacteria it is carried out across the plasma membrane. As mentioned, the electron transfer is considered a part OXPHOS, the process through which ADP is phosphorylated into ATP by dint of energy derived from the oxidation of nutrients.

Four protein complexes and ATP synthase, all bound to the IMM, as well as two shuttles are the known players of one of the trickiest mechanisms resolved in biochemistry. The first of these complexes is the NADH/ubiquinone oxidoreductase (complex I) which removes electrons from NADH (produced in the citric acid cycle) and passes them on to the first shuttle, ubiquinone, a liposoluble cofactor located within the phospholipid bilayer of the IMM. Succinate dehydrogenase (or complex II) is another entrance site for electrons into the respiratory chain. In this case, electrons derived from the oxidation of succinate are passed through FAD to ubiquinone. Once ubiquinone is reduced to ubiquinol, it is able to pass electrons to the third complex, ubiquinone/cytochrome c oxidoreductase. Here, electrons are moved through several heme groups from the liposoluble shuttle ubiquinone to the water-soluble shuttle cytochrome c. Cytochrome c is a small protein (about 12.5 kDa), located in the intermembrane space (IMS), which can accommodate one electron in its heme group. Despite its water solubility, cytochrome c is usually bound to the external surface of the

IMM due to the interaction with the cardiolipin. This interaction (crucial in the determination of the cell fate) helps the shuttle to reach its electron acceptor, complex IV. Cytochrome c oxidase is the last complex of the electron transport. Electrons from cytochrome C are accumulated in copper centers and passed to oxygen through heme groups. Oxygen is then reduced to water. This constitutes the bulk of oxygen consumption in all aerobic life. Electron transport through complexes I, III, and IV induces the pumping of protons from the matrix to the IMS. Specifically, for every two electrons coming from one molecule of NADH, four H+ are moved by complex I, four by complex III, and two by complex IV. The second respiratory complex does not generate any proton movement. The respiratory chain in active mitochondria generates a large difference in [H+] across the IMM, resulting in the generation of an electrical potential (about −180 to −200 mV) and variation in the pH of about 0.75. A constant proton motive force drives the ATP synthesis through the last step of OXPHOS, the ATP synthase. Understanding the activity and organization of this enzyme won researchers more than one Nobel Prize. First, Peter Mitchell in 1978 received his prize for the formulation of the chemiosmotic theory. Initially, he hypothesized how an enzymatic activity could at the same time involve ion transport (proton transport through the IMM) and a chemical reaction (ATP phosphorylation). Almost two decades later, in 1997, the Nobel Prize was awarded to Paul Boyer and John Walker who elucidated the mechanism of action of ATP synthase, here briefly reviewed. ATP synthase could be divided in two main components: F0 that allows the channeling of protons and F1 that catalyzes ATP phosphorylation. The F0 is embedded in the IMM, while the F1 resides in the mitochondrial matrix and is bound to the F0 through a γ subunit (which drives conformational changes) and a b2δ dimer (that holds F0 and F1 together). The protons flow from the intermembrane space to the matrix through the F0 inducing its rotation; the movement is transmitted from the γ subunit to the F1 causing conformational rearrangements. The F1 has a trimeric structure consisting of αβ dimers. This structure allows three different conformational states which is able to bind ADP + Pi, ATP, or remain unbound. The sequential changes are linked to the binding of substrates, phosphorylation, and release of ATP. The three available dimers are never in the same conformational state, and, what is more, the conformational changes in one dimer drive rearrangements in the other (for a more detailed explanation, refer to). It has been calculated that, for the synthesis of one ATP molecule, four protons are required (three for the ATP synthase rearrangements and one for ATP, ADP, and Pi transport). Once synthesized, ATP can locate inside mitochondrial matrix or be transported into the IMS by the nucleotide exchanger adenine nucleotide translocase (ANT) which passively exchanges ATP with ADP. Once in the IMS, ATP can freely pass the OMM through the voltage-dependent anion channel (VDAC).

ATP production is strongly regulated upon environmental stresses:

Phosphorylation of ATP is strongly modulated by environmental stresses, such as hypoxia or heat shock. It has also been demonstrated, both in vitro and in vivo, that intracellular ATP levels are implicated in the regulation of fundamental cellular processes, such as growth, development, and death/survival decisions.

Calcium dependent regulation:

New experimental tools introduced in the last years have enormously expanded our ability to monitor the dynamics of mitochondrial events in the living cell. These organelles have been recognized as fascinating structures, involved in many aspects of mammalian physiology and pathophysiology. They play subtle roles in glucose homeostasis, act as

oxygen-sensors in the regulation of respiration, and are pivotal in the pathways to both necrotic and apoptotic cell death. Mitochondria also take up calcium, impacting the spatiotemporal dynamics of intracellular calcium signals, but their central and ubiquitous task is clearly the production of ATP.

Oxidative phosphorylation:

Oxidative phosphorylation is the principle purpose of oxygen respiration and the principle use of breathed in oxygen to generate energy in the body.

Oxidative phosphorylation (OXPHOS)is defined as an electron transfer chain driven by substrate oxidation that is coupled to the synthesis of ATP through an electrochemical transmembrane gradient. Historically, bovine heart mitochondria have been the system of choice for the structural characterization of eukaryotic OXPHOS complexes, because they can be purified in relatively large quantities. The yeast Saccharomyces cerevisiae, which is amenable to a large variety of molecular genetic tools, has become the model organism to study the biogenesis of mitochondrial complexes and the effect of mutations on OXPHOS components. By contrast, mitochondria of photosynthetic organisms have been poorly characterized from a biochemical point of view, mainly due to the difficulties in obtaining preparations free of chloroplast contaminants. Nevertheless, the characterization of Arabidopsis mitochondrial components through proteomic approaches has advanced significantly.

Oxidative phosphorylation is the process by which ATP synthesis is coupled to the movement of electrons through the mitochondrial electron transport chain and the associated consumption of oxygen. This process is the most efficient for ATP synthesis, generating approximately 36 ATP molecules per glucose molecule, compared to the two molecules of ATP generated during glycolysis.

The free energy released by stepwise oxidation reactions between NADH, FADH2, and ubiquinol pumps protons from the mitochondrial matrix, across the mitochondrial inner membrane, and into the intermembrane space.This pumping action creates a tremendous proton concentration imbalance between the intermembrane space and the matrix. The potential energy stored in this proton gradient is then used to power ATP synthase phosphorylating ADP to generate ATP.

The electron transport chain involves the transfer of electrons from NADH and FADH2 to ubiquinone (also called Coenzyme Q) through a series of four large protein complexes that reside in the mitochondrial inner membrane. Because the electrons begin the process at a high energy state and end the process in a low energy state, the electron transport chain entails the stepwise release of energy, which the protein complexes harness in order to pump protons from the mitochondrial matrix into the intermembrane space; each reaction in the electron transport chain represents a slight decrease in the energy of the electrons as they pass from complex to complex. An oxygen molecule sits at the end of the electron transport chain as the final electron acceptor, where it joins with two free protons to become water in a highly exothermic reaction. Without oxygen, the electrons in the electron transport chain cannot continue to fall down their potential energy gradient, and progression of electrons through the transport chain stops.

Photosystems and photophosphorylation:

Photosystems are the functional units for photosynthesis, defined by a particular pigment organization and association patterns, whose work is the absorption and transfer of light energy, which implies transfer of electrons. Physically, photosystems are found in the thylakoid membranes. There are two kinds of photosystems: photosystem I (PSI) and photosystem II (PSII). PSII acts first during the light transformation process in photosynthesis, but it was named PSII because it was discovered second.

Each photosystem consists of two closely linked components: the first is the antenna complex formed by hundreds of pigment molecules that capture photons and transfer the harvested light energy to the second component named the reaction center, which possesses Chl A molecules in a matrix of protein. When excitation energy reaches chlorophyll a at the reaction center, electron transfer is initiated through an electron transport chain.

PSI is located at the outer surface of the thylakoid membrane, and contains chlorophyll b; chlorophyll a (in the forms: a-670, a-680, a-695, a-700), and carotenoids; and one particular chlorophyll a-700 form (named Chl A-P700) is the active reaction center. PSII is located at the inner surface of the thylakoid membrane, and contains chlorophyll b;

chlorophyll a (forms a-660, a-670, a-680, a-695, a-700), phycobillins, and xanthophylls; and a Chl A-P680 form is the active reaction center.

Photosystems are pigment-containing protein complexes that contain reaction centers that convert radiant energy (hv) into chemical energy. Upon excitation, the pigment (P) becomes a strong reducing agent (P+) that allows it to pass an electron to a primary acceptor (A), which then becomes reduced (A−). This process, which takes place within the reaction center, is known as charge separation and is represented in the following reaction:

$P+A+hv \Rightarrow P^{+}+A^{-}$

This reaction is irreversible as a consequence of the rapid re-reduction of P+, which occurs as a result of the acquisition of an electron from an electron donor, as well as the rapid reoxidation of A− that results from the reduction of the next electron acceptor. Both PS I and PS II are oriented in the thylakoid membrane such that the excited electron in the reaction center moves from the lumen side of the membrane to the stromal side of the membrane in an electrogenic manner.

The two photosystems, PSII and PSI, share a common ancestor and therefore have basic similarities, but they are now comprised of distinct populations of proteins (albeit with features shared between the photosystems) and cofactors, most of which are now specific to each photosystem. Some cofactors, chlorophylls a and b, and carotenoids (most importantly lutein, β-carotene, and neoxanthin) are pigments so are key to photosynthetic light-absorption and are bound to specific sites in the pigment binding proteins of PSI and PSII. The amount of PSII or PSI reaction centres in a typical leaf is about 1 μmol m− 2 per photosystem type while total leaf chlorophyll content is approximately 0.5 mmol m− 2. Note that these are approximate values. The absorption of a photon in the range of PAR by a photosynthetic pigment result in an electron being excited from the ground state to a higher energy level; if this energy level is higher than the first excited state (the S1 state), it will relax to the S1 state within approximately 10− 14 s. These excited states can migrate from one pigment molecule to another, though in the case of the carotenoids and chlorophyll b migration to Chl A is energetically downhill so excited states formed on carotenoids and Chl B end up on Chl A. Once on Chl A, the excited state is free to migrate from Chl A to Chl A via either Forster resonance or as a delocalized exciton.

Within each PSII and PSI, there is a specialized structures called reaction centres that have the ability to convert the some of the energy of the excited state on Chl A to chemical energy; reaction centres of PSII and PSI differ in their energetics and the detail of the operation, but they share many features, consistent with them having a common ancestor. Despite the complexities of both photosystems, they have a quite basic function; in both cases, they take an electron from a relatively weaker reducing agent and donate it to a relatively stronger reducing agent. This is would normally be an endergonic process, but it is driven (i.e., made exergonic) by the energy of the excited state of Chl A. This process of charge separation leading the generation of reductants and oxidants in the reactions centres of photosystems I and II provides the energetic impulse that drives most of the life in the biosphere. The quantum efficiencies of the photosystems are high; for PSI it thought to be about 0.99, while for PSII, it is probably around 0.9, which is higher than that typically measured using chlorophyll fluorescence (about 0.82), the difference being due to the presence of weak fluorescence from PSI in the total fluorescence signal.

The two types of photosystems are connected to differently to the photosynthetic electron (and proton) transport network. Photosynthetic electron transport pathways are divided into two main classes: the first is linear electron transport through PSII and PSI (LET), while the second is comprised of variants of cyclic electron transport passing through PSI (CET). All types of CET involve the plastoquinol pool, the cytochrome b6/f complex, PSI, and ferredoxin, while all types of LET involve PSII (with its oxygen evolving complex; OEC), the plastoquinol pool, the cytochrome b6/f complex, PSI, and ferredoxin. The cytochrome b6/f complex and ferredoxin are important nodes in electron transport as a whole. Photosynthetic electron transport is associated with proton release into the aqueous thylakoid lumen. The lumen is physically separated from the stroma by a membrane that is impermeable to the passage of protons, so the addition of these protons to the lumen creates a proton potential (a proton energy difference, or proton motive force (ΔμH +; in kJ mol− 1)) between the lumen and the stroma, which is comprised of voltage (FΔψ where F is the Faraday; protons carry a positive charge) and concentration (2.3RTΔpH; the lumen becomes more acidic)
components.

Electron transport chain and photophosphorylation by photosystem:
Recently, a number of techniques, some of them relatively new and many often used in combination, have given a clearer picture of the dynamic role of electron transport in Photosystem I of photosynthesis and of coupled cyclic photophosphorylation. For example, the photoacoustic technique has detected cyclic electron transport in vivo in all the major algal groups and in leaves of higher plants. Spectroscopic measurements of the Photosystem I reaction center and of the changes in light scattering associated with thylakoid membrane energization also indicate that cyclic photophosphorylation occurs in living plants and cyanobacteria, particularly under stressful conditions. In cyanobacteria, the path of cyclic electron transport has recently been proposed to include an NAD(P)H dehydrogenase, a complex that may also participate in respiratory electron transport. Photosynthesis and respiration may share common electron carriers in eukaryotes also. Chlororespiration, the uptake of O2 in the dark by chloroplasts, is inhibited by excitation of Photosystem I, which diverts electrons away from the chlororespiratory chain into the photosynthetic electron transport chain. Chlororespiration in N-starved Chlamydomonas increases tenfold over that of the control, perhaps because carbohydrates and NAD(P)H are oxidized and ATP produced by this process. The regulation of energy distribution to the photosystems and of cyclic and non-cyclic phosphorylation via state 1 to state 2 transitions may involve the cytochrome b 6-f complex. An increased demand for ATP lowers the trans thylakoid pH gradient, activates the b 6-f complex, stimulates phosphorylation of the light-harvesting chlorophyll-protein complex of Photosystem II and decreases energy input to Photosystem II upon induction of state 2. The resulting increase in the absorption by Photosystem I favors cyclic electron flow and ATP production over linear electron flow to NADP and 'poises' the system by slowing down the flow of electrons originating in Photosystem II. Cyclic electron transport may function to prevent photoinhibition to the photosynthetic apparatus as well as to provide ATP. Thus, under high light intensities where CO2 can limit photosynthesis, especially when stomates are closed as a result of water stress, the proton gradient established by coupled cyclic electron transport can prevent over-reduction of the electron transport system by increasing thermal de-excitation in Photosystem II (Weis and Berry 1987). Increased cyclic photophosphorylation may also serve to drive ion uptake in nutrient-deprived cells or ion export in salt-stressed cells. There is evidence in some plants for a specialization of Photosystem I. For example, in the red alga Porphyra about one third of the total Photosystem I units are engaged in linear electron transfer from Photosystem II and the remaining two thirds of the Photosystem I units are specialized for cyclic electron flow. Other organisms show evidence of similar specialization. Improved understanding of the biological role of cyclic photophosphorylation will depend on experiments made on living cells and measurements of cyclic photophosphorylation in vivo.

The formation of ATP from ADP and inorganic phosphate using light energy in photosynthesis (compare oxidative phosphorylation). There are two pathways, noncyclic and cyclic photophosphorylation, which occur in the thylakoid membranes of the chloroplasts. In noncyclic photophosphorylation electrons derived from the photolysis of water are raised to higher energy levels in photosystems I and II and pass along an electron transport chain of carrier molecules (see ferredoxin; plastocyanin; plastoquinone) to NADP reductase. This enzyme transfers electrons to NADP+ to make NADPH, which provides reducing power for the light-independent reactions of photosynthesis. In cyclic photophosphorylation the electrons from photosystem I that are raised to a higher energy level are recycled through the electron carrier system back to photosystem I. Both pathways of electron flow cause H+ ions to be pumped by a group of cytochromes, the cytochrome b6–f complex, across the thylakoid membrane. This creates a proton gradient that drives the phosphorylation of ADP to ATP by the enzyme ATP synthetase

The third type of phosphorylation to make ATP is found only in cells that carry out photosynthesis. This process is similar to oxidative phosphorylation in several ways. A primary difference is the ultimate source of the energy for ATP synthesis. In oxidative phosphorylation, the energy comes from electrons produced by oxidation of biological molecules. In photosynthesis, the energy comes from the light of the sun. Photons from the sun interact with chlorophyll molecules in reaction centers in the chloroplasts of plants or membranes of photosynthetic bacteria.

The similarities of photophosphorylation to oxidative phosphorylation include:

- a membrane associated electron transport chain
- creation of a proton gradient
- harvesting energy of the proton gradient by making ATP with the help of an ATP synthase.

Some of the differences include:

- the source of the electrons – H2O for photosynthesis versus NADH/FADH2 for oxidative phosphorylation
- direction of proton pumping – into the thylakoid space of the chloroplasts versus outside the matrix of the mitochondrion
- movement of protons during ATP synthesis – out of the thylakoid space in photosynthesis versus into the mitochondrial matrix in oxidative phosphorylation
- nature of the terminal electron acceptor – NADP+ in photosynthesis versus O2 in oxidative phosphorylation.

Electron transport: chloroplasts vs mitochondria

In some ways, the movement of electrons in chloroplasts during photosynthesis is opposite that of electron transport in mitochondria. In photosynthesis, water is the source of electrons and their final destination is NADP+ to make NADPH. In mitochondria, NADH/FADH2 are electron sources and H2O are their final destination. How do biological systems get electrons to go both ways? It would seem to be the equivalent of going to and from a particular place while always going downhill, since electrons will move according to potential.

The answer is the captured energy of the photons from the sun, which elevates electrons to an energy where they move “downhill” to their NADPH destination in a Z-shaped scheme. The movement of electrons through this scheme in plants requires energy from photons in two places to “lift” the energy of the electrons sufficiently.

Last, it should be noted that photosynthesis actually has two phases, referred to as the light cycle (described above) and the dark cycle, which is a set of chemical reactions that captures CO2 from the atmosphere and “fixes” it, ultimately into glucose. The dark cycle is also referred to as the Calvin Cycle.

Photosynthesis:

Photosynthesis is an energy capture process found in plants and other organisms to harvest light energy and convert it into chemical energy. This photochemical energy is stored ultimately in carbohydrates which are made using ATP (from the energy harvesting), carbon dioxide and water. In most cases, a byproduct of the process is oxygen, which is released from water in the capture process. Photosynthesis is responsible for most of the oxygen in the atmosphere and it supplies the organic materials and most of the energy used by life on Earth.

Steps

The steps in the photosynthesis process varies slightly between organisms. In a broad overview, it always starts with energy capture from light by protein complexes, containing chlorophyll pigments, called reaction centers. Plants sequester these proteins in chloroplasts, but bacteria, which don't have organelles, embed them in their plasma membranes.

Energy from the light is used to strip electrons away from electron donors (usually water) and leave a byproduct (oxygen, if water was used). Electrons are donated to a carrier and ultimately are accepted by NADP+, to become NADPH. As electrons travel towards NADP+, they generate a proton gradient across the thylakoid membrane, which is used to drive synthesis of ATP. Thus NADPH, ATP, and oxygen are the products of the first phase of photosynthesis called the light reactions. Energy from ATP and electrons from NADPH are used to reduce CO2 and build sugars, which are the ultimate energy storage directly arising from photosynthesis.

Chloroplasts

Chloroplasts are found in almost all aboveground plant cells, but are primarily concentrated in leaves. The interior of a leaf, below the epidermis is made up of photosynthesis tissue called mesophyll, which can contain up to 800,000 chloroplasts per square millimeter.

The chloroplast's membrane has a phospholipid inner membrane, a phospholipid outer membrane, and a region between them called the intermembrane space (Figure 5.61). Within the inner chloroplast membrane is the stroma, in which the chloroplast DNA and the enzymes of the Calvin cycle are located. Also, within the stroma are stacked, flattened disks known as thylakoids which are defined by their thylakoid membranes. The space within the thylakoid membranes is termed the thylakoid spaces or thylakoid lumen. The protein complexes containing the light-absorbing pigments, known as photosystems, are located on the thylakoid membrane. Besides chlorophylls, carotenes and xanthophylls are also present, allowing for absorption of light energy over a wider range. The same pigments are used by green algae and land plants.

Brown algae and diatoms add fucoxanthin (a xanthophyll) and red algae add phycoerythrin to the mix. In plants and algae, the pigments are held in a very organized fashion complexes called antenna proteins that help funnel energy, through resonance energy transfer, to the reaction center chlorophylls. A system so organized is called a light harvesting complex. The electron transport complexes of photosynthesis are also located on the thylakoid membranes.

Light reactions of photosynthesis

In chloroplasts, the light reactions of photosynthesis involving electron transfer occur in the thylakoid membranes. Separate biochemical reactions involving the assimilation of carbon dioxide to make glucose are referred to as the Calvin cycle, also sometimes referred to as the "dark reactions". This will be discussed elsewhere in the section on metabolism.

The chloroplasts are where the energy of light is captured, electrons are stripped from water, oxygen is liberated, electron transport occurs, NADPH is formed, and ATP is generated. The thylakoid membrane corresponds to the inner membrane of the mitochondrion for transport of electrons and proton pumping.

The thylakoid membrane does its magic using four major protein complexes. These include Photosystem II (PS II), Cytochrome b6f complex (Cb6f), Photosystem I (PS I), and ATP synthase. The roles of these complexes, respectively, are to capture light energy, create a proton gradient from electron movement, capture light energy (again), and use proton gradient energy from the overall process to synthesize ATP.

Light harvesting

Harvesting the energy of light begins in PS II with the absorption of a photon of light at a reaction center. PS II performs this duty best with light at a wavelength of 680 nm and it readily loses an electron to excitation when this occurs, leaving PS II with a positive charge. This electron must be replaced. The ultimate replacement source of electrons is water, but water must lose four electrons and PS II can only accept one at a time.

Manganese centers

An intermediate Oxygen Evolving Complex (OEC) contains four manganese centers that provide the immediate replacement electron that PSII requires. After four electrons have been donated by the OEC to PS II, the OEC extracts four electrons from two water molecules, liberating oxygen and dumping four protons into the thylakoid space, thus contributing to the proton gradient. The excited electron from PS II must be passed to another carrier very quickly, lest it decay back to its original state. It does this, giving its electron within picoseconds to pheophytin.

Pheophytin passes the electron on to protein-bound plastoquinones. The first is known as PQA. PQA hands the electron off to a second plastoquinone (PQB), which waits for a second electron and collects two protons to become PQH2, also known as plastoquinol. PQH2 passes these to the Cytochrome b6f complex (Cb6f) which uses passage of electrons through it to pump protons into the thylakoid space. ATP synthase makes ATP from the proton gradient created in this way. Cb6f drops the electron off at plastocyanin, which holds it until the next excitation process begins with absorption of another photon of light at 700 nm by PS I.

Absorption of light at PS I:

With absorption of a photon of light by PS I, a process begins, that is similar to the process in PS II. PS I gain a positive charge as a result of the loss of an excited electron and pulls the electron in plastocyanin away from it. Meanwhile, the excited electron from PS I passes through an iron-sulfur protein, which gives the electron to ferredoxin (another iron sulfur protein). Ferredoxin then passes the electron off to the last protein in the system known as Ferredoxin: NADP+ oxidoreductase, which gives the electron and a proton to NADP+, creating NADPH.

Note that reduction of NADP+ to NADPH requires two electrons and one proton, so the four electrons and two protons from oxidation of water will result in production of two molecules of NADPH. At this point, the light cycle is complete - water has been oxidized, ATP has been created, and NADPH has been made. The electrons have made their way from water to NADPH via carriers in the thylakoid membrane and their movement has released sufficient energy to make ATP. Energy for the entire process came from four photons of light.

The two photosystems performing all of this magic are protein complexes that are similar in structure and means of operation. They absorb photons with high efficiency so that whenever a pigment in the photosynthetic reaction center absorbs a photon, an electron from the pigment is excited and transferred to another molecule almost instantaneously. This reaction is called photo-induced charge separation and it is a unique means of transforming light energy into chemical forms.

Cyclic photophosphorylation

Besides the path described above for movement of electrons through PS I, plants have an alternative route that electrons can take. Instead of electrons going through ferredoxin to form NADPH, they instead take a backwards path through the the proton-pumping b6f complex. This system, called cyclic photophosphorylation which generates more ATP and no NADPH, is similar to a system found in green sulfur bacteria. The ability of plants to switch between non-cyclic and cyclic photosystems allows them to make the proper ratio of ATP and NADPH they need for assimilation of carbon in the dark phase of photosynthesis. This ratio turns out to be 3 ATPs to 2 NADPHs.

Oxidative Phosphorylation Inhibitors:

Certain poisons can inhibit cellular oxidative phosphorylation such as rotenone, carboxin, antimycin A, cyanide, carbon monoxide (CO), sodium azide, and oligomycin. Rotenone inhibits complex I, carboxin inhibits complex II, antimycin A inhibits complex III, and cyanide and CO inhibit complex IV. Oligomycin inhibits ATP synthase.

Rotenone (and some barbiturates) – inhibits complex I (coenzyme Q binding site)

Rotenone is a broadly used pesticide, but more often in the US as a piscicide (fish). Rotenone blocks complex I from passing electrons from the Fe-S clusters to ubiquinone. It is poorly absorbed through the skin, but rarely deadly as poisoning can cause vomiting and removal of the substance. However, purposeful ingestion can be fatal.

Carboxin – inhibits complex II (coenzyme Q binding site)

Carboxin is a fungicide that is no longer in use because of newer, more broad-spectrum agents. Similar to rotenone, carboxin interferes with ubiquinone at the binding site.

Doxorubicin – coenzyme Q (theoretical)

Doxorubicin is used in cancer chemotherapy, typically breast and bladder carcinomas, and lymphoma. A well-known side effect of doxorubicin is dilated cardiomyopathy. One proposed mechanism of causation is the generation of reactive oxygen species within myocardial tissue as the drug interferes with electron transfer by coenzyme Q.

Antimycin A – inhibits complex III (cytochrome c reductase)

Antimycin A is a piscicide that binds to cytochrome c reductase at the Qi binding site. This activity prevents ubiquinone from binding and accepting an electron, thereby blocking the recycling of ubiquinol (CoQH2) by the Q cycle.

Carbon Monoxide (CO) – inhibits complex IV (cytochrome c oxidase)

Carbon monoxide binds to and inhibits cytochrome c oxidase (complex IV). In addition to the disruption of the ETC, carbon monoxide also binds to hemoglobin at an oxygen-binding site converting it to carboxyhemoglobin. In this state, oxygen is displaced from hemoglobin, effectively blocking delivery to body tissues. The cardiac and central nervous systems, both organ systems which are highly dependent on oxygen consumption, manifest the common signs of CO poisoning. Symptoms such as tachycardia, hypotension, or arrhythmias may couple with fatigue, headache, nausea, vomiting, and changes in vision. More serious cases may display seizure, coma, retinal hemorrhages, or a characteristic cherry-red blood hue of the skin, though more often useful on autopsy (caution is critical: some patients may appear "normal" rather than pale/dusky because of inadequate tissue oxygenation).

Sources of CO are paint strippers, house fires, wood-burning stoves, automobile exhaust, and other gasoline- or propane-fueled equipment. A CO saturation monitor can detect CO levels. Ratios of carboxyhemoglobin to hemoglobin greater than 10% are likely to show as symptomatic. Regular pulse oximetry devices read the percent of bound hemoglobin, irrespective of what is bound. Therefore, when CO is bound rather than O2, a patient's pulse Ox may still appear normal and cannot be used reliably. Instead, a co-oximeter should be used. Treatment for CO poisoning is to dissociate the bound CO with O2. Providing 100% supplemental oxygen via non-rebreather or administering hyperbaric oxygen are options.

Cyanide (CN) – inhibits complex IV (cytochrome c oxidase)

Cyanide also binds to and inhibits cytochrome c oxidase (complex IV). Similar symptoms as a result of tissue hypoxia can present in affected patients. In contrast, these patients tend to have hypoxia that is not responsive to supplemental O2 and an almond breath odor. Typical sources of cyanide include house fires (furniture or rugs), jewelry cleaning solutions, plastic or rubber manufacturing, iatrogenic from prescribed nitroprusside, or even some fruit seeds (apricots, peaches, apples).

Treatment can include nitrites to oxidize hemoglobin iron from Fe2+ to Fe3+, also known as methemoglobin, a conformation that binds cyanide, preventing it from contacting the ETC. However, this prevents blood cells from transporting oxygen, therefore requiring further treatment with methylene blue to reduce Fe3+ back to Fe2+. Another option is administering hydroxocobalamin, a form of vitamin B12, or thiosulfate, although thiosulfate is not time efficient and typically requires combination therapy with nitrites.

Oligomycin – inhibits ATP-synthase (complex V)

Oligomycin is a macrolide antibiotic synthesized by Streptomyces species that inhibits the F0 subunit of ATP-synthase, preventing ATP production. Its predominant use is for research purposes.

Energy shuttles:

i. NADH:

An energy shuttle which delivers high energy electrons to the electron transport chain where they will eventually power the production of 2 to 3 ATP molecules. When this electron shuttle is not carrying high energy electrons, meaning it has been oxidized (lost its electrons), it is left with a positive charge and is called NAD+.

ii. FADH2:

Another energy shuttle that carries high energy electrons to the electron transport chain, where they will ultimately drive production of 1 to 2 ATP molecules. The oxidized form of FADH2 is FAD and happens just like in NADH. High energy molecules:

iii. ATP:
The basic energy currency of the cell. It's a form of energy that cells can use right away.

iv. GTP:
Similar to ATP, GTP can be easily converted to ATP in the cell.

Shuttle pathway is a pathway that helps in the transfer of electrons from NADH to the electron transport chain.
The two main shuttle system in humans are:
l. Glycerol phosphate shuttle
2. Malate-aspartate shuttle

Glycerol phosphate shuttle:

This shuttle system is not much common to be used in humans. It is present in insect flight muscle and in white muscle. This alternative means of moving reducing equivalents from the Cytosol to the respiratory chain operates in skeletal muscle and the brain.
• It delivers the reducing equivalents from NADH through FAD in glycerol 3-phosphate Dehydrogenase to ubiquinone and thus into Complex Ill, not Complex I
Cytosolic glycerol 3-phosphate dehydrogenase oxidizes NADH to NAD+.
• The reducing equivalents are transported through glycerol 3-phosphate into the mitochondria.
• An isozyme of Glycerol 3-phosphate dehydrogenase—present on the outer surface of the inner mitochondrial membrane—reduces FAD to
FADH2.
Dihydroxyacetone phosphate escapes into the cytosol and the shuttling continues.
FADH2 gets oxidized via ETC to generate 2 ATE
• Note that this shuttle does not involve membrane transport systems.

What does Glycerol phosphate do?

The glycerol phosphate shuttle is an important pathway for delivery of cytosolic reducing equivalents into mitochondrial oxidative phosphorylation and plays essential physiological roles in yeast, plants and animals.

How many ATPs are produced by glycerol phosphate shuttle?

In eukaryotes, glycerol phosphate shuttle is present which consumes 2 ATPs for entry of glycolytic NADH into Mitochondria. So, 36 ATPs are generated in Aerobic respiration.

1. The glycerol 3 – phosphate shuttle differs from the malate aspartate shuttle in that its deliverers the reducing equilents from NADH to ubiquinone and thus into complex not I.

2. The mitochondria of plants have an extremally oriented NADH dehydrogenase that can transfer directly from cytosolic NADH into the respiratory chain at the level of ubiquinone.
3. Because this pathway bypasses the NADH dehydrogenase of complex and the associated proton movement.

4. The yield of ATP from cytosolic NADH is less than that from NADH generated in the matrix.

5. This pathway is alternative means of ironing reducing equivalents from the cystols to the mitochondria matrix operates in skeleton muscle and the brain.

6. In the cystol, dihydroxyacetone phorphente accepts 2 reducing equivalents from NADH in a catalysed by cystolic of 3p dehydrogenises.

7. An isozyme of 3p dehydrogenates bound to the outer face of the inner membrane then transfer reducing equivalents from of 3p in the inter membrane space to ubiquinone.

8. Note that this shuttle does not involve membrane transport system.

Malate - Aspartate shuttle

The Malate - aspartate shuttle provides an important mechanism to regulate glycolysis and lactate metabolism in the heart by
transferring reducing equivalents from cytosol into mitochondria.
The malate–aspartate shuttle translocate electrons produced during glycolysis into mitochondria across the inner mitochondrial membrane. Shuttle defects can thus disrupt oxidative phosphorylation. The manifestations of shuttle deficiency include exercise-induced myalgia, pigmentary, and elevated serum creatine kinase.56 No neurological impairment has been noted. Nevertheless, one of the components of the malate–aspartate shuttle, the mitochondrial aspartate–glutamate carrier isoform 1 (AGC1), has been associated with global cerebral hypomyelination.57 This component is specific to neurons and muscle, where it supplies aspartate to the cytosol thereby contributing to the provision of acetyl groups (via n-acetyl aspartate) necessary for myelin lipid synthesis. Patients with shuttle deficiency exhibit arrested psychomotor development, hypotonia, and seizures. A ketogenic diet has been used with the goal of ameliorating seizures and hypomyelination.

Module 5

Disorders of Metabolism

Introduction:

Metabolism is the process your body uses to make energy from the food you eat. Food is made up of proteins, carbohydrates, and fats. Chemicals in your digestive system (enzymes) break the food parts down into sugars and acids, your body's fuel. Your body can use this fuel right away, or it can store the energy in your body tissues. If you have a metabolic disorder, something goes wrong with this process.

Carbohydrate metabolism disorders are a group of metabolic disorders. Normally your enzymes break carbohydrates down into glucose (a type of sugar). If you have one of these disorders, you may not have enough enzymes to break down the carbohydrates. Or the enzymes may not work properly. This causes a harmful amount of sugar to build up in your body. That can lead to health problems, some of which can be serious. Some of the disorders are fatal.

Lactose intolerance:

• Lactose intolerance, also called lactase deficiency or hypolactasia, is the inability to digest and metabolize lactose, a sugar found in milk.

• Lactose intolerance is not an allergy because it is not an immune response but caused by lactase deficiency.

Symptoms

• Abdominal bloating and cramps
• Flatulence
• Diarrhoea
• Nausea

Borborygmi (rumbling stomach)
Vomiting (particularly in adolescents)

Types of lactase deficiency:

• Primary lactase deficiency is a genetically determined absence or decrease in the enzyme is noted.
• In non-caucasian groups, primary lactase deficiency is abnormal in adulthood.

Primary lactase deficiency develops over time and begins after about age 2 when the body begins to produce less lactase. Most children who have lactase deficiency do not experience symptoms of lactose intolerance until late adolescence or adulthood.

Risks:

Lactose intolerance is a common condition that is more likely to occur in adulthood, with a higher incidence in older adults. Some ethnic and racial populations are more affected than others, including African Americans, Hispanic Americans, American Indians, and Asian Americans. The condition is least common among Americans of northern European descent.

Researchers have identified a possible genetic link to primary lactase deficiency. Some people inherit a gene from their parents that makes it likely they will develop primary lactase deficiency. This discovery may be useful in developing future genetic tests to identify people at risk for lactose intolerance.

The cause of lactose intolerance is best explained by describing how a person develops lactase deficiency.

The amount of change needed in the diet depends on how much lactose a person can consume without symptoms. For example, one person may have severe symptoms after drinking a small glass of milk, while another can drink a large glass without symptoms. Others can easily consume yogurt and hard cheeses such as cheddar and Swiss but not milk or other milk products.

Although the body's ability to produce lactase cannot be changed, the symptoms of lactose intolerance can be managed with dietary changes. Most people with lactose intolerance can tolerate some amount of lactose in their diet. Gradually introducing small amounts of milk or milk products may help some people adapt to them with fewer symptoms. Often, people can better tolerate milk or milk products by taking them with meals.

Management:

• Avoiding lactose-containing products

• Alternative products such as Plant- based milks and derivatives are inherently lactose free: soy milk, rice milk, almond milk, hazelnut milk, oat milk, hemp milk, peanut milk, horchata.

Diagnosis

• Dietary history of the patient who are complaining flatulance, abdominal pain, diarrhoea.

• Familial history of the patient.

• Check whether the patient has underwent partial gastrectomy and other related procedures.

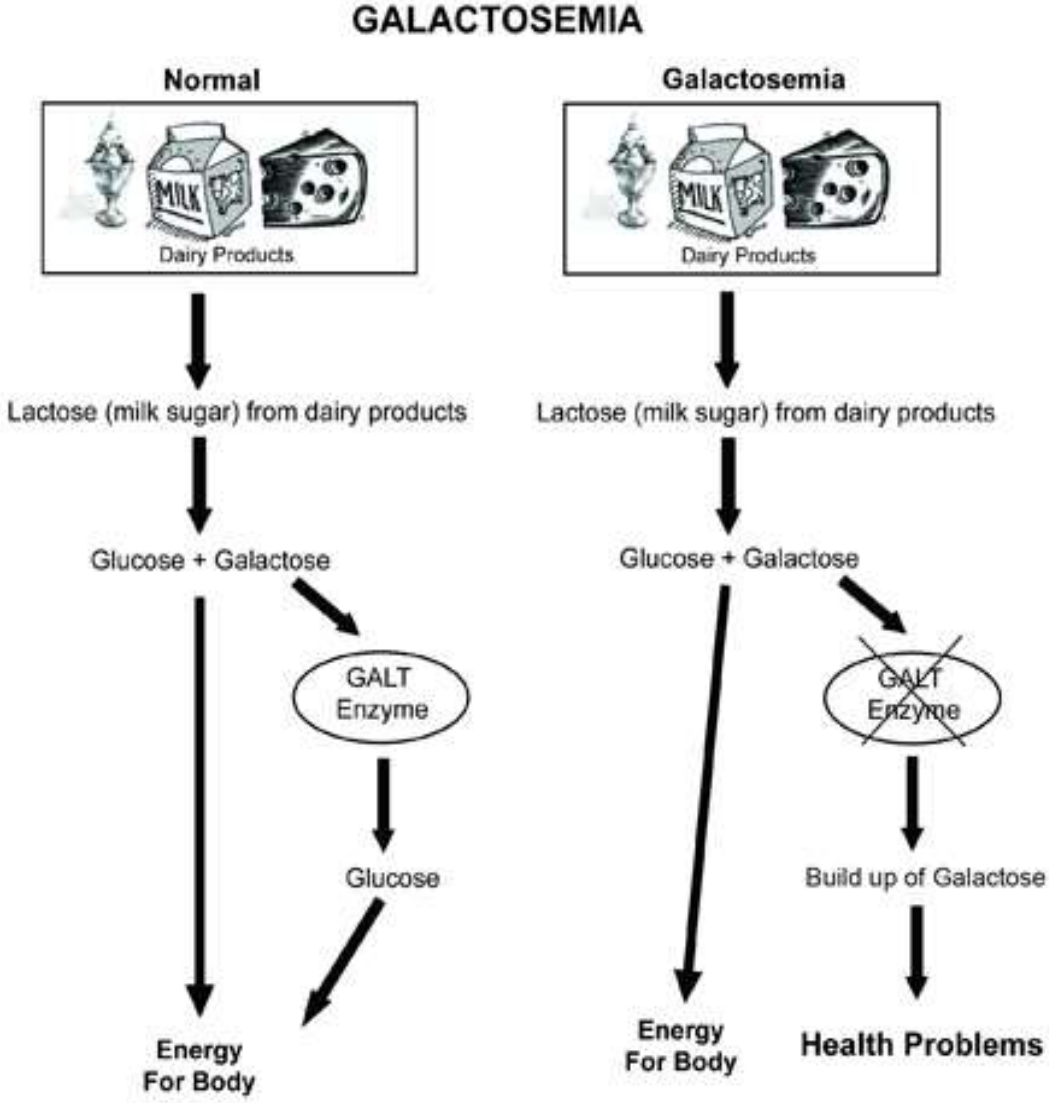
• Tests - stool acidity test, hydrogen breath tests.

Galactosemia:

Galactosemia is a disorder that affects how the human body processes a simple sugar called GALACTOSE.

Galactosemia is a gentic metabolic disease, in which babies are not able to metabolized the galactose sugar that found in breast milk, cow’s milk & dairy product.

DEFICIENCY OF GALT ENZYME CAUSE GALACTOSEMIA:



GALT – GLUCOSE-1-PHOSPHATE URIDYL TRANSFERASE CLINICAL GALACTOSEMIA(GALT)s.

- Galactosemia is due to defiency of the enzyme called Galactose-1-phosphate uridyltransferase
- Mutation in GALT gene is located on chromosome 9 is responsible for this disorder.

1.Galactose metabolism is impaired leading to increased galactose level in blood (galactosemia) & urine (galactsurial).

2. Accumulated galactose is diverted for production of galactitol by the enzyme aldol reductase. Galactitol has been implicated in the development of cataract.

3. The accumulation of galactose 1-phosphate andgalactitol in various tissues like liver, nervous tissue,lens and kidney leads to impairment in their function.

4. The accumulation of galactose 1-phosphate in liver results in the depletion of inorganic phosphate further metabolic functions.

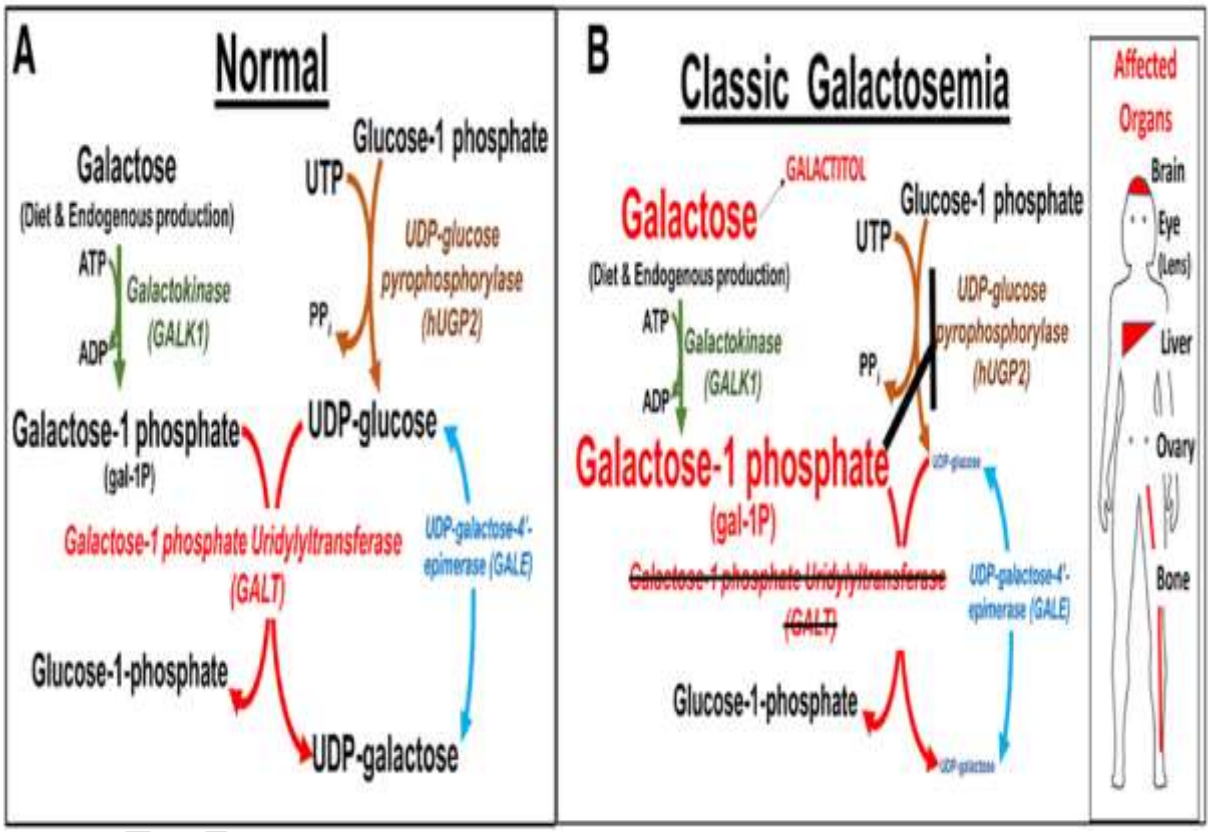
1. Type-1 Galactosemia

* It occurs due to mutation or missing or non-function of "GALACTOSE-1-PHOSPHATE URIDYLTRANSFERASE" [GALT].
* This is most common type of galactosemia.
* When galactose cannot be changed by glucose, its by-product such as galactose-1- phosphate builds up in tissue & Blood, that affects many parts of body.

2.Type-2 galctosemia

* Children in this type may get catract or clouding in their eyes, do not have any other issues .
* This type of case is fewer than type-1.

3. Type-3 Galactosemia



* Milder than type-1 but worse than type-2.
* Children with this type can have symptoms like catract, delayed development, kidney issues, liver problem or intellectual disabilities.

Symptoms [EARLY STAGE]:

* Refuse to eat or feeding.

* splitting up or vomiting.
* yellowing of skin, eyes (jaundice) because of increase of galctose level that damage the liver.
* Cataract.

LATE SYMPTOMS

* Learning disability because of excessive galactose damage in brain.
* Neurological impairment like ataxia etc.

Treatment for galactosemia:

Galctosemia patient should avoid dairy product.

* Avoid any type of dairy product.
* Eliminate breast feeding.
* Eliminate lactose & galactose from food.

Alternative product of galactose

* Soy formula.
* Meat based formula.
* Calcium supliment.

Early diagnosis

* Affected individuals who are diagnosed early and avoid all forms of dairy products lead a normal life but there have been a few cases of mild form of intellectual dysfunction in some people.

Glycogen storage disease:

GLYCOGEN STORAGE DISEASE: Glycogen storage disease (GSD) is a genetic condition in which the body has an enzyme problem and is not able to store or break down the complex sugar glycogen properly. The body's cells need a steady supply of glucose in order to function in the right way. The body uses as much glucose as it needs to function and stores the rest to use later. Before it can be stored, the body must combine the simple glucose units into a new, complex sugar called glycogen.

Special proteins called enzymes help both make and break down the glycogen in a process called glycogen metabolism. Sometimes a person is born missing an enzyme needed for this process or it may not work right. The muscles and organs need a certain level of glucose in the blood to work properly. When the body is missing an enzyme or has a flawed enzyme and is not able to use glycogen the right way, it leads to a condition called glycogen storage disease (GSD). • • • glycogen stoage disease is an autosomal recessive diorder causd by a deficiency of phosphofructokinase(PFK) This enzyme catalyzes

the first step in glycolysis , converting Fructose-6-phosphate to fructose-1-6-bisphosphate It plays an important role as a key regulatory enzyme of glycolysis.

What are the types of GSD?

Each type of GSD centers on a certain enzyme or set of enzymes involved in glycogen storage or break down. There are at least 13 types of glycogen storage disease. Type 0 (Lewis' disease) – Liver. Type I (von Gierke's disease) Type Ia – Liver, kidneys, intestines; Type Ib – Liver, kidneys, intestines, blood cells. Type II (Pompe's disease) – Muscles, heart, liver, nervous system, blood vessels. Type III (Forbes-Cori disease) – Liver, heart, skeletal muscles, blood cells. Type IV (Andersen's disease) – Liver, brain, heart, muscles, skin, nervous system. Type V (McArdle's disease) – Skeletal muscles. Type VI (Hers' disease) – Liver, blood cells. Type VII (Tarui's disease) – Skeletal muscles, blood cells. Type IX – Liver. Type XI (Fanconi-Bickel syndrome) – Liver, kidneys, intestines.

How common are they?

A glycogen storage disorder occurs in about one in 20,000 to 25,000 babies. The most common types of GSD are types I, II, III, and IV, with type I being the most common. What are the symptoms of(GSD): Symptoms vary based on the type of GSD. Some GSDs affect mostly the liver. These include Types 0, I, III, IV, VI and IX. However, they may sometimes have overlapping symptoms affecting muscle and heart. These types (except for GSD type 0)

Other symptoms :

Tiredness. Very slow growth. Obesity (being very overweight). Problems with bleeding and blood clotting. Kidney problems. Low resistance to infections. Breathing problems. Heart problems. Mouth sores. Gout.

What causes GSDs?

GSDs occur when there is a problem with the gene that has the instructions for making the enzyme that is missing or not working right. The gene is passed down from parents to children. In most cases, in order to have the GSD, a child must get a bad gene from both parents.

How GSDs ARE DETECETED:

There are four symptoms that might cause the doctor to suspect a type of GSD that affects the liver. These include: A low blood glucose level. An enlarged liver. Lagging growth. Abnormal blood tests.

How is glycogen storage disease (GSD) treated?

Treatment varies depending on the type of GSD. For types of GSD that involve the liver, treatment is aimed at keeping the right level of glucose in the blood. Treatment consists of taking regular doses of uncooked cornstarch and/or nutrition supplements. Type IV GSDs with progressive liver disease may have to be considered for liver transplantation after a thorough evaluation. • •

Can glycogen storage disease (GSD) be prevented?

GSDs are handed down from parents to children through their genes. Therefore, they cannot be prevented. Parents can find out through genetic testing if they carry a gene for a GSD. Both parents must have a gene for the same type of GSD for a child to inherit the disorder.

Diabetes:

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin produced.

Insulin is a hormone that helps to regulate the amount of sugars like glucose in blood.

Diabetes is one of the disorders carbohydrates.

Chemistry of insulin

Insulin is a polypeptide hormone composed of 51 amino acids arranged in 2 chains, A and B chains.

A chain contains 21 and B chain contains 30 amino acids.

These 2 chains are connected by peptide bonds.

Synthesis of insulin

Insulin is synthesized as larger precursor polypeptide chain, preproinsulin. It has 109 amino acids.

It is converted into proinsulin with 86 amino acids in endoplasmic reticulum.

Proinsulin Is transported to golgi apparatus then converted to insulin with 51 amino acids and C peptide chain with 35 amino acids

Insulin regulates the metabolic activity of carbohydrates, fats and proteins.

Insulin is the first hormone to be produced by DNA technology.

It was first engineered by eli lilly company in1978.

Insulin production by DNA technology

First the DNA encoding human insulin is extracted from human pancreatic cell using restriction enzyme.

Then plasmid obtained from bacteria is cut open by same restriction enzyme.

The plasmid and the DNA are binded using DNA ligase forming recombinant plasmid.

Then the recombinant plasmid is induced into E.coli bacteria.
The E.coli cells are then cultured .
The E.coli bacteria with recombinant plasmid induces the gene expression by producing polypeptides.
The poly peptides are extracted from bacteria and processed into functional human insulin.

There are two types of diabetes based on variation of insulin supply.
Type 1 diabetes
Type 2 diabetes
Type 1 diabetes
It is a chronic condition in which the pancreas produce little or no insulin.Due to this,sugar levels in the blood increases
Type 1 diabetes is an autoimmune disorder in which the immune system attacks and destroys bets cells.

Causes:

Family history: anyone with a parent or sibling with type 1 diabetes has a slight higher risk of developing the condition.
Geography
Old age
Type 2 diabetes
Type 2 diabetes is a disease that occurs when your blood glucose is too high.
The insulin produced is not enough or it resists the insulin.
It is the most common type of the diabetes.

Treatment:

There is no cure for type 2 diabetes but losing weight , eating well and exercising can help you manage.
Quitting smoking , dietary fiber also helps to reduse the blood sugar level.
Also require frequent blood sugar monitering and take insulin shots according to the sugar levels.

Atherosclerosis:

Atherosclerosis is the buildup of fats, cholesterol and other substances in and on the artery walls.
A build up of cholesterol plaque in the walls of arteries, causing obstruction of blood flow.

- The plaque can cause arteries to narrow, blocking blood flow.

Symptoms:

*If you have atherosclerosis in your heart arteries, you may have chest pain or pressure.
*If you have atherosclerosis in the arteries leading to your brain.
*If you have atherosclerosis in the arteries in your arms and legs.

Causes:

- *High cholesterol.

- *Insulin resistance.
- *Diabetes.
- *Smoking or chewing tobacco.
- *Obesity.
- *High triglycerides.

Test:

*Blood test.
*Electrocardiogram (ECG or EKG).
*Exercise stress test.
*Echocardiogram.
* Coronary calcium scan.
* Ankle-brachial index (ABI).

Treatment:

Medications:

*Statins and other cholesterol drugs.
*Aspirin.
*Blood pressure medications.
*Other medications.

Surgery or other procedures:

*Angioplasty and stent placement.
*Endarterectomy.
*Fibrinolytic therapy.
*Coronary artery bypass graft (CABG) surgery.

Lifestyle and home remedies:

*Don't smoke.
*Exercise most days of the week.
*Maintain a healthy weight.
*Eat healthy foods.

Alternative medicine:

*Barley.
*Blond psyllium.

*Calcium.
*Fish oil.
*Cocoa.
*Green tea.

Acidosis-kesosis:

- Ketoacidosis is a metabolic state caused by uncontrolled production of ketone bodies that cause a metabolic acidosis. While ketosis refers to any elevation of blood ketones, ketoacidosis is a specific pathologic condition that results in changes in blood pH and requires medical attention. The most common cause of ketoacidosis is diabetic ketoacidosis but can also be caused by alcohol, medications, toxins, and rarely, starvation.

Causes:

- Ketoacidosis is caused by the uncontrolled production of ketone bodies. Usually the production of ketones is carefully controlled by several hormones, most importantly insulin. If the mechanisms that control ketone production fail, ketone levels may become dramatically elevated and cause dangerous changes in physiology such as a metabolic acidosis.

Diabetic ketoacidosis:

- The most common cause of ketoacidosis is a deficiency of insulin in type 1 diabetes or late-stage type 2 diabetes. This is called diabetic ketoacidosis and is characterized by hyperglycemia, dehydration and metabolic acidosis. Other electrolyte disturbances such as hyperkalemia and hyponatremia may also be present. A lack of insulin in the bloodstream allows unregulated fatty acid release from adipose tissue which increases fatty acid oxidation to acetyl CoA, some of which is diverted to ketogenesis. This raises ketone levels significantly above what is seen in normal physiology.

Starvation:

- Starvation is a rare cause of ketoacidosis, usually instead causing physiologic ketosis without ketoacidosis. Ketoacidosis from starvation most commonly occurs in the setting of an additional metabolic stressor such as pregnancy, lactation, or acute illness.

SIGNS AND SYMPTOMS

- Being very thirsty
- Urinating often
- Feeling a need to throw up and throwing up
- Having stomach pain
- Being weak or tired
- Being short of breath
- Having fruity-scented breath
- Being confused

Exams and Tests

- These tests can help diagnose acidosis. They can also determine whether the cause is a breathing problem or a metabolic problem. Tests may include:
- Arterial or venous blood gas
- Basic metabolic panel, (a group of blood tests that measure your sodium and potassium levels, kidney function, and other chemicals and functions)
- Blood ketones
- Lactic acid test
- Urine ketones
- Urine pH
- Other tests may be needed to determine the cause of the acidosis.

Treatment:

- Treatment is aimed at the health problem causing the acidosis. In some cases, sodium bicarbonate (the chemical in baking soda) may be given to reduce the acidity of the blood. Often, you will receive lots of fluids through your vein.

Gaucher diseases:

Gaucher disease belongs to a group of Sphingolipidoses or lysosomal storage disease

Spingolipids→sphingasine+fa-(ceramide)

Cerebroside

cerebroside→ceramide+monosacharide(Glucose)

Glucocerebrocide

Glucocerebrosidase

ß-Glucocerebrosidasealso called as(acid ß-glucoside,D-glucosyl-N-acylsphingosine glucohydrolase,or Gcase)

It is an enzyme with glucosylceramidase activity by hydrolysis
The beta-glycosidic linkage of the chemical glucocerebroside
An intermediate in glycolipid metabolism that is abundant in cell membranes
It is localized in the lysosome,where it associated with the lysosomal membrane
Beta-Glucocerebrosidase is 497 amino acids in length
Gaucher disease is a rare genetic disorder where a person lacks an enzyme called Glucocerebrosidase
Deficiency of this enzyme causes harmful substances to build up in the liver, spleen , bones , bone marrow and brain
These substances prevent the organs and cells from working properly.

Three types of Gaucher disease

- Type 1
- Type 2
- Type 3

Gaucher disease type 1:

- The most common type in the U.S., Gaucher disease type 1 affects the spleen ,liver and bones.
- It does not affect the brain or spinal cord.
- Gaucher disease type 1 is treatable, but there is no cure .
- For some people , symptoms are mild .
- Other people experience severe bruising, fatigue and pain , especially in the bones and belly.

- Symptoms can appear at any age, from childhood to adulthood.

Gaucher disease type 2:

A rare form of the disorder, type 2 appears in babies under six months old.
It cause an enlarged spleen, movement problems and severe brain damage . There is no treatment for Gaucher disease type 2 .
Babies with this condition pass away within two to three years.

Gaucher disease type 3:

Worldwide, Gaucher disease type 3 is the most common form, but it's rare in the United States. It appears before age 10 and causes bone and organ abnormalities and neurological (brain) problems. Treatments can help many people with Gaucher disease type 3 live into their 20s or 30s.

Symptoms:

- Enlarged spleen.
- Enlarged liver.
- Eye movement disorders.
- Yellow spots in the eyes.
- Not having enough healthy red blood cells (anemia)
- Extreme tiredness (fatigue)
- Bruising.
- Lung problems

Treatment

- Enzyme replacement therapy, which is effective for types 1 and 3.
- Medicines.
- Regular physical exams and bone density screening to check your disease.
- Bone marrow transplant.

- Surgery to remove all or part of your spleen.
- Joint replacement surgery.
- Blood transfusions.

Tay Sachs disease:

- Tay-Sachs disease is a rare genetic disorder passed from parents to children.
- This is an inborn error of metabolism due to failure of degradation of gangliosides GM2.
- The enzyme hexosaminidase a is deficient in this condition.

CAUSES:

- It occurs when a child inherits a flaw (mutation) in the hexa gene from both parents.
- The genetic change that causes Tay-Sachs disease results in a deficiency of the enzyme beta-hexosaminidase A.
- This enzyme is required to break down the fatty substance GM2 ganglioside.
- The buildup of fatty substances damages nerve cells in the brain and spinal cord.
- These fatty substances, called gangliosides, build up to toxic levels in the brain and spinal cord and affect the function of the nerve cells. A child can only have it if both parents have this faulty gene. The parents themselves don't usually have any symptoms – this is known as being a "carrier".

SYMPTOMS:

- Exaggerated response when the baby hears loud noises
- "Cherry-red" spots in the eyes
- Muscle weakness, progressing to paralysis

- Movement problems
- Vision loss and blindness
- Hearing loss and deafness
- Problems swallowing
- Loss of mental functions and a lack of response to surroundings.

Diagnosis:

- To confirm that a child has Tay-Sachs disease, the health care provider will ask about symptoms and any family hereditary disorders.
- They will also do a physical exam.
- The child may need to see a neurologist and an ophthalmologist for nervous system and eye examinations.
- Diagnostic blood test: the blood test checks the levels of hexosaminidase A enzyme in the blood. The levels are low or absent in Tay-Sachs disease.
- Genetic testing: this test can examine the hexa gene to identify whether there are changes that indicate Tay-Sachs disease.
- Eye exam: during an eye exam, the health care provider may see a cherry-red spot in the back of the eyes, which is a sign of the disease.
- Prenatal testing for Tay Sachs disease can be done during pregnancy by removing a tiny piece of the placenta (chorionic villi sampling) or by removing a small sample of the amniotic fluid around the baby (amniocentesis).

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Treatment:

- There is no cure for Tay-Sachs disease, and no treatments are currently proved to slow progression of the disease. Some treatments can help in managing symptoms and preventing complications. The goal of treatment is support and comfort.

10.LDL-hypercholesterolemia:

Hypercholesterolemia is a disorder known for an excess of low-density lipoprotein (LDL) in your blood. Many people can treat it by making changes to their diet and adding exercise to their lifestyles. Others need to take medicine to bring their LDL level down to a normal level. These treatments lower your risk of heart attacks and strokes.

Symptoms:

There are no symptoms of hypercholesterolemia in most people.

However, if you have severe hypercholesterolemia, you may have cholesterol deposits on your eyelid skin (xanthelasma) or connective tissue (xanthoma). Also, you may have cholesterol in your eye. This is called a corneal arcus.

How is hypercholesterolemia diagnosed?

Usually at your annual physical exam, your healthcare provider will:

Ask you about your medical history and your family's health.

• Do a physical exam.

• Order a lipid panel blood test, which you usually do after a period of fasting.

How is hypercholesterolemia treated?

Hypercholesterolemia treatment involves bringing down your LDL level to prevent heart disease.

You can do this in several ways:

• Exercising more.

Staying at a healthy weight. Eating foods low in saturated fat.

Lowering your stress level.

• Taking cholesterol-lowering medications.

• Avoiding tobacco products.

• Controlling high blood pressure and blood sugar.

• Having lipoprotein apheresis (using a device to take lipoproteins out of your blood and then putting your blood back into your body).

What tests will be done to diagnose hypercholesterolemia?

Once your provider rules out other causes of your hypercholesterolemia, they can do genetic testing. If you have pure hypercholesterolemia (familial hypercholesterolemia), your provider may suggest genetic testing for your family.

Hypercholesterolemia causes include:

• Your genes (pure or familial hypercholesterolemia).

A diet that includes a lot of saturated and/or trans fats.

• A lack of exercise.

Tobacco products.

Obstructive liver disease.

• Diabetes.

• Hypothyroidism.
• Anorexia nervosa.
Chronic kidney failure.
• Nephrotic syndrome.
Amiodarone
• Rosiglitazone.
Cyclosporine
Hydrochlorothiazide

ALKAPTONURIA:

- Defect in the enzyme homogentisate oxidase, that catalyzes oxidation of homogentisate to maleylactoactate
- Homogentisate accumulates in blood and body tissues and is excreted in large amounts in urine.
- The urine of alkaptonuric patients becomes dark after being exposed to air
- The alkapton imparts a characteristic black-brown color to urine.
- Alkaptonuria is a harmless condition.
- Later in life deposition of dark colored alkapton pigments in connective tissues and bones occur.
- This results in black pigmentation of the sclera, ear, nose and cheeks and the clinical condition is known as ochronosis.
- Ochronosis leads to tissue damage and may develop joint pain, arthritis and backache.

DIAGNOSIS:

- The urine sample of patients of alkaptonuria turns dark on standing in air.
- The urine gives positive test with ferric chloride and silver nitrate due to reducing activity of homogentisate.

TREATMENT:

Since alkaptonuria is not considered life threatening, this condition is not treated.
Later in life, the symptoms of arthritis may be treated but the condition itself is not.

HOMOCYSTINURA:

- Homocystinuria is an autosomal recessive disorder caused by
- loss-of-function mutations in the CBS gene, which encodes
- cystathionine B-synthase. The enzyme deficiency causes
- accumulation of homocysteine and methionine in the blood.
- Many cases of homocystinuria are diagnosed through newborn screening programmes
- Homocystinuria II: N 5-N10 Methylene THF reductase.
- Homocystinuria III: N5N 10-Methyl THF- homocysteine methyltransferase. This is mostly due to impairment in the synthesis of methyl- cobalamin.
- Homocystinuria IV: N5-Methyl THF homo- cysteine methyl transferase. This is primarily due to a defect in the intestinal absorption of vitamin B12
- mbbs

TREATMENT:

- Treatment is dietary, involving a methionine- restricted, cysteine supplemented
- diet, as well as large doses of pyridoxine Vit B6
- Cysteine Non essential amino acid (we can make it)
- • contains sulfur
- Help Makes proteins
- • It's found in beta-keratin.
- • This is the main protein in nails, skin, and hair. Cysteine is important for making collagen.

PHENYLKETONURI:

Phenylketonuria (PKU) is an autosomal recessive metabolic genetic disorder characterized by a mutation in the gene for the hepatic enzyme phenylalanine hydroxylase (PAH), rendering it nonfunctional. This enzyme is necessary to metabolize the amino acid phenylalanine (Phe) to the amino acid tyrosine. When PAH activity is reduced, phenylalanine accumulates and is converted into phenylpyruvate (also known as phenylketone), which can be detected in the urine.

- The enzyme phenylalanine hydroxylase (in the presence of co-factor Tetrahydrobiopterin BH) normally converts the amino acid phenylalanine into the amino acid tyrosine. If this reaction does not take place, phenylalanine accumulates and tyrosine is deficient. Excessive phenylalanine can be metabolized into phenylketones through the minor route, a transaminase pathway with glutamate. Metabolites include phenylacetate, phenylpyruvate and phenethylamine. Elevated levels of phenylalanine in the blood and detection of phenylketones in the urine is diagnostic, however most patients are diagnosed via newborn screening.

TREATMENT:

No cure

- A strictly controlled phenylalanine free diet up to the age of about 14 years old.
- Phenylalanine is itself an essential amino acid small doses must be supplied.
- After 14 years, the growth and development of the brain is not affected by high levels of phenylalanine in the body.

TYROSINEMIA:

There are three types of tyrosinemia:

1.Tyrosinemia type-I (tyrosinosis/hepatorenal tyrosinemia)

2. Tyrosinemia type-II (Richner-Hanhart syndrome)

3. Tyrosinemia type-III (neonatal tyrosinemia).

TYPE:1

Tyrosinemia type-l, also called tyrosinosis

Caused by a genetic deficiency of fumarylacetoacetate hydroxylase.

Results in accumulation and excretion of tyrosine and its metabolites:

P-hydroxyphenyl-pyruvate,

P-hydroxyphenyl-lactate
P-hydroxyphenyl-acetate,
N-acetyltyrosin.

Tyramine:

The deficiency of enzyme fumarylacetoacetate hydroxylase causes liver failure, kidney dysfunction, polyneuropathy, and vitamin D- resistant rickets.
Results in accumulation and excretion of tyrosine and its metabolites:
P-hydroxyphenyl-pyruvate,
P-hydroxyphenyl-lactate
P-hydroxyphenyl-acetate,
N-acetyltyrosin
Tyramine.
The deficiency of enzyme fumarylacetoacetate hydroxylase causes liver failure, kidney dysfunction, polyneuropathy, and vitamin D- resistant rickets.

DIAGNOSIS:

A diagnosis of tyrosinemia type I may be suspected in infants who display failure to thrive and an enlarged liver (hepatomegaly) during the first three months of life.
The diagnosis is expected when tyrosine metabolites and succinylacetone are detected in the urine.
It is also possible to make the diagnosis based on decreased activity of fumarylacetoacetate hydroxylase (FAH) in liver tissue but this test is not readily available.

TREATMENT:

The patient should be kept on diet low in phenylalanine and tyrosine.

TYPE:2

Tyrosinemia type-ll is caused by genetic deficiency of hepatic enzyme tyrosine aminotransferase (tyrosine transaminase).

■ Tyrosine and its toxic metabolites accumulates in blood and tissues and appears in urine.
■ The accumulation of tyrosine produces lesions in eye and skin and
causes mental retardation

TREATMENT

• Diet low in tyrosine and phenylalanine is recommended.
• Diet with vitamin C may benefit the corneal and skin lesions of tyrosine aminotransferase deficiency, but not the mental retardation.

TYPE:3

- Caused by absence of the enzyme P- hydroxyphenyl-pyruvate Hydroxylase
- Serum tyrosine levels are high in premature infants resulting from an immature liver and its limited ability to synthesize the enzyme, p-hydroxyphenyl-pyruvate hydroxylase.

- As the liver matures, the accumulated tyrosine is metabolized and serum levels decrease within 4 to 8 weeks of age.
- It is benign condition and responds well to ascorbic acid.

Lesh-Nyhn Syndrome:

It is inherited disorder caused by a deficiency of the enzyme Hypoxanthine-Guanine Phosphoribosyl Transferase(HGPRT)

► This deficiency occurs due to mutations in the HPRT 1 gene located on the X chromosome.

► LNS is a X linked recessive disease carried by the female parent and passed on to a male child.

How common is LNS ?

LNS affects about 1 in 380,000 live births.

Is LNS curable ?

NO , There is no cure for LNS and the prognosis is poor.

(Prognosis means : an opinion , based on medical experience ,of the likely development of a disease or illness in the future)

► Children with LNS experience a severe painful form of arthritis is called gout.

► They have also poor muscle control (dystonia).

Symptoms :

❑ LNS in children affects

- ➢ Mental ability , movement and behaviour.
- ➢ Poor muscle control and developmental delays are common early signs of the disorder.
- ➢ Babies may have orange-coloured crystals in their diapers if they have too much uric acid

- But most children with the disease do not show

symptoms until they are about 4 months.

Injuring self and others :

- Compulsive (uncontrollable)self-injury is a noticeable symptom of LNS after a child's teeth come in. This behaviour usually involves:
  - ► Banging the head or limbs.
  - ► Biting lips ,fingers and cheeks.
  - ► Poking the eyes.

In some cases , children with the disorder may try to hurt others. The may use verbal abuse or grab , hit , pinch or spit.

How is it diagnosed ?

► Physical and chemical abnormalities present from infancy biochemical confirmation-

i.e Azathioprine testing .

► Excess uric acid through a blood or urine test .

► LNS may be confirmed by a through clinical evaluation,

including patient history and specialized blood tests.

GOUT :

What is gout ?

► Accumulation of uric acid crystals – especially in joints.

Why it is called Disease of king ?

► Due to its association with rich foods and alcohol consumption.

Diagnosis :

► X-rays.

► Ultrasound.

► Magnetic resonance imaging (MRI)

► CT (computed tomography) sacn

► Blood tests to measure the uric acid in blood.

► Joint aspiration – using a needle to remove a sample of fluid from inside a joint.

►

Symptoms :

Intense joint pain.

► Lingering discomfort.

► Inflammation and redness.

► Limited range of motion .

Hyperuricemia:

- URICEMIA: Presence of uric acid in blood
- Hypercemia is an excess of uric acid in the blood. Uric acid passes through the liver, and enters to the bloodstream. Most of it is excreted in the urine, or passes through intestines to regulate "normal" levels.
- Plasma urate concentration > 7.0 mg/dL.

- Also important to blood uric acid levels are purines. Purines are nitrogen containing compounds, which are made inside the cells of our body(endogenous), or come from outside of our body, from foods containing purines(exogenous). Purine break down into uric acid.
- Increased levels of uric acid from excess purines may accumulate in tissues, and form crystals. This may cause high acid levels in the blood
- The amount of urate in the body depends on the balance between the amount of purines eaten in food, the amount of urate synthesised within the body(e.g., through the cell turnover), and the amount of urate that is excreted in urine or through the gastrointestinal tract.

WHAT CAUSES HYPERURICEMIA?

- Normal uric acid blood levels varies between genders, 2-5 mg/dL for females and 3-7 mg/dL for men.
- The upper limit lies at 7.0 mg/dL where any higher puts us at risk for developing hyperuricemia.
- The cause of hyperuricemia is divided into three categories:

➢ Increased production of uric acid

➢ Decreased excretion of uric acid

➢ Combination of the two processes.

SYMPTOMS

The two most common complaints associated with hyperuricemia are gout and uric acid nephrolithiasis.

GOUT: A patient will complain of red hot swollen joint, most commonly in the big toe.

NEPHROLITHIASIS: Patients will complain of flank pain, hematuria, nausea/vomiting and colicky pain

INCREASED PRODUCTION OF URIC ACID

Overproduction occurs in a minority of patients who have hyperuricemia. Hyperuricemia induced by overproduction may be caused by external factors like high purine diet or internal factors like increased purine breakdown. Overproduction of uric acid can be caused by the following:

- DIET: A diet majorly consists of high purine meats, organ foods and legumes can contribute to the overproduction of uric acid.
- LESCH NYHAN SYNDROME and KELLY SEEGMILLER SYNDROME: Patients with this syndrome are also more likely to develop gout.

- INCREASED NUCLEIC ACID SYNDROME: Patients with this syndrome are also likely to develop gout and kidney stones.
- TUMOR LYSIS SYNDROME: This syndrome is known to produce the most complications of hyperuricemia.

DECREASED EXCRETION OF URIC ACID

Underexcretion makes up most cases of hyperuricemia. Urate, a salt of uric acid, is filtered by the kidneys and undergoes a process that ends in the urate leaving the body through the urine. One misstep in this process can result in renal insufficiency and subsequently a poor rate of uric acid excretion.

Under excretion or poor kidney function can be caused by the following:

- RENAL INSUFFICIENCY: In the case of renal failure, uric acid remains in the blood as the kidneys are unable to properly perform the excretion process or other organic compounds compete for clearance.
- METABOLIC SYNDROME: Other health complications such as hypertension, obesity and insulin resistance can contribute to the decreased excretion of urate by the kidneys.

MEDICATIONS:

- Diuretics, low dose salicylates, cyclosporine and nicotinic acid have been linked to causing poor excretion performance by the kidneys.
- GENETICS: While considered rare, patients with this condition experience progressive renal failure that results in a lower rate of uric acid excretion.
- HYPERTENSION: High blood pressure and the associated medications have been known to cause hyperuricemia.
- LEAD EXPOSURE(CHRONIC): Clinical studies have shown that occupational or prolonged exposure to lead can affect kidney function
- OTHER CONDITIONS: Hyperthyroidism, sarcoidosis, trisomy 21, hyperparathyroidism, pre-eclampsia and eclampsia.

PREVENTION:

Dietary measures that may help prevent hyperuricemia include the following

- Avoidance or restricted consumption of high purine foods (eg, organ meals, sardines).
- Avoidance of excess ingestion of alcoholic drinks, particularly beer.

HYPOURICEMIA:

- Hypouricemia is a level of uric acid in blood serum that is below normal. Hypouricemia is arbitrarily defined as a serum urate concentration of less than 2 mg/dL(119 micromole/L).
- It occurs in approximately 2% of hospitalized patients and less than 0.5% percent of the normal population.
- Hypouricemia has been thought of as a biochemical disorder with no clinical signifance other than as a marker of underlying disease. However, individuals with renal tubular urate wasting may have an increased incidence of acute omkidney injury(AKI; previously called acute renal failure).

- Hypouricemia may be caused by:

➢ Decreased uric acid production.

➢ Uric acid oxidation due to treatment with uricase, or decreased renal tubular reabsorption due to inherited or acquired disorders

- There are no known abnormalities of intestinal uricolysis that produce hypouricemia.

SYMPTOMS

- Some people with this condition develop kidney problems.
- After strenuous exercise, they can develop exersice induced acute kidney injury, which causes pain in their sides and lower back as well as nausea and vomiting that can last several hours.

It is important to distinguish:

- Primary : Genetic defect - hereditary xanthinuria

  Transport defect – primary renal hypouricemia
  (RHUC1, RHUC2).
- Secondary : Increased renal secretion(Fanconi sy.,Wilson's disease)

  severe liver disease, thyrotoxicosis, diabetes mellitus,
  acute respiratory sy.

PRIMARY:

- Genetic defect : Hereditary xanthinuria

Hereditary xanthinuria is a condition that most often affects the kidneys. It is characterised by high levels of a compound called xanthine and very low levels of another compound called uric acid in the blood and urine. The excess xanthine can accumulate in the kidneys and other tissues

- Primary renal hypouricemia

It is a genetic disorder characterised by defective renal uric acid(UA) reabsorption with complications such as nephrolithiasis and exercise induced acute renalfailure.

SECONDARY:

- Increased renal secretion (Fanconi sy., Wilson's disease)

The renal manifestation of Wilson's disease are nephrolithiasis and vitamin D resistant rickets. Fanconi's syndrome may occur rarely. This case was detected to have severe osteoporosis due to distal RTA.

Thyrotoxicosis:

Thyrotoxicosis is the clinical syndrome that results from the exposition of tissues to an excess of circulating thyroid hormone. In most instances, thyrotoxicosis is due to an active overproduction of thyroid hormone by the thyroid follicular epithelial cells (hyperthyroidism).

TREATMENT:

- Idiopathic hypouricemia usually requires no treatment.
- In some cases, hypouricemia is a medical sign of an underlying condition that does require treatment. For example, if hypouricemia reflects high excretionof uric acid into the urine (hyperuricosuria) with its risk of uric acid nephrolithiasis, the hyperuricosuria may require treatment.
- Drugs and dietary supplements that may be helpful are:

Insoitol
Antiuricosurics

ADENOSINE DEAMINASE DEFICIENCY:

Adenosine deaminase(ADA)deficiency is an inherited disorder that damages the immune system. It causes severe combined immunodeficiency(SCID). It is one of the enzymes in the purine metabolism. IMPORTANCE OF ADA • • • • Deficiency of ADA in human manifest primarily as a sever lymphopenia. In this review, we discuss phenotypical, biochemical and metabolic hallmark of the disease. It can be biochemically and genetically manipulated. Purine catabolic enzyme affects lymphopoiesis.

SYMPTOMS OF ADA:

Symptoms usually appear in the first months of life. Its common for babies to get infection. Children with ADA often have diarrhea and widspread skin rashes. If the disease does'nt appear until later in childhood or adulthood, the symptoms may be mild at first. First symptoms may be an ear or upperrespiratory system.

CAUSES OF ADA:

ADA is caused by mutation in the ADA gene. The enzyme is found throughout the body but is most active in specialized white blood cells is called lymphocytes. It is caused by harmful bacteria and viruses.

AUTOSOMAL RECESSIVE PATTERN

INHERITANCE OF ADA

It is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations. The parents of an individual with an autosomal recessive condition each carry one copy of the mutated gene.

How is ADA diagnosed ?

Diagnosis of ADA deficiency is established by biochemical and molecular genetic testing. ADA deficiency has a recent blood transfusion, analysis of ADA activity can be measured.

How is ADA deficiency is cured permanently?

A permanent cure of ADA deficiency is gene therapy at early embryonic stages.

What are the steps involved in treatment of ADA gene therapy?

Infection of lymphocytes by genetically engineered retrovirus. Culture of lymphocytes. Isolation of lymphocytes from ADA deficient patient. Genetically modified lymphocytes are injected back to patient.