

Answer Key for FYBSc Paper I SEM I Paper Code 0055222

Q. 1 Q. 1. A) Fill in the blanks: (07)

1. A ____ is a solution containing either a weak acid and its salt or weak base and its salt, which is resistant to changes in pH. Ans. Buffer
2. _____ is a molecule or ion having separate positively and negatively charged groups. Ans. Zwitterions
3. Amino acids are building blocks of _____. Ans. Proteins
4. Methionine and Cysteine are _____ containing amino acids. Ans. Sulphur
5. Maltose is composed of two molecules of _____. Ans. Glucose
6. Phenyl isothiocyanate reagent is used in _____ reaction. Ans. Edman's reaction
7. Polysaccharides are composed of 10-1000 units of monosaccharides bonded through _____ linkages. Ans. glycosidic bonds

B. Match the column

(07)

- a. iv
- b. vii
- c. v
- d. i
- e. ii
- f. iii
- g. vi

1. C) Define / Explain the following terms:

(06)

1. Nucleosome. It is the basic unit of chromatin fibre, with histone octamer with DNA would around it.
2. Microtubule. It is a cytoskeletal element, about 20 nm diameter, made up of 11 protofilaments of tubulin protein.
3. Polytene chromosome. It is a giant chromosome, by about 10 rounds of DNA replication, which are close to each other with dark and light bands.
4. Axoneme. It is the central core of Flagellum/cilium which shows 9+2 arrangement of microtubules
5. Karyolymph. It is the ground substance of nucleus, also known as nuclear sap.

6. Centromere. It is the point at which the 2 sister chromatids are joint with each other.

Q.2. A) Explain any one of the following: (10)

1. Derive Henderson and Hasselbalch equation. What is its use?

Derivation of the Henderson-Hasselbalch (H-H) Equation

From: $K_a = \frac{[H^+][A^-]}{[HA]}$, Solve for $[H^+]$, $[H^+] = K_a \frac{[HA]}{[A^-]}$

Negative log of each side:

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

Convert to p scale: $pH = pK_a - \log\left(\frac{[HA]}{[A^-]}\right)$

Invert log: $pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right)$

$$pH = pK_a + \log \frac{[\text{proton acceptor}]}{[\text{proton donor}]}$$

Calculate the pH of a 2 L solution containing 10 mL of 5 M acetic acid (CH_3COOH) and 10 mL of 1 M sodium acetate (CH_3COONa). pK_a of $CH_3COOH = 4.76$.

Henderson-Hasselbalch Equation

Wednesday, April 18, 2018 12:23 PM

- Henderson-Hasselbalch Eq.
 - **Henderson-Hasselbalch equation:** A mathematical relationship between:
 - pH
 - pK_a of the weak acid, HA
 - The concentrations of HA and its conjugate base A⁻.
 - It is derived in the following way:
 - Remember K_a...
$$\text{HA} + \text{H}_2\text{O} \rightleftharpoons \text{A}^- + \text{H}_3\text{O}^+$$
$$K_a = \frac{[\text{A}^-][\text{H}_3\text{O}^+]}{[\text{HA}]}$$
 - Take the -log...
$$\log K_a = \log [\text{H}_3\text{O}^+] + \log \frac{[\text{A}^-]}{[\text{HA}]}$$
 - Substitute pK_a and pH...
$$\text{pK}_a = \text{pH} - \log \frac{[\text{A}^-]}{[\text{HA}]}$$
 - Rearrange terms...
$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]} \quad \text{Henderson-Hasselbalch Equation}$$
 - A BUFFER: WEAK ACID + salt of WEAK ACID
 - RATIO of weak acid and its salt

2. Chemical properties and biological role of Carbohydrates.

A carbohydrate is a simple sugar. Its basic **structure** is composed of the **elements carbon**, hydrogen and oxygen, with generally twice the hydrogen as carbon and oxygen. In its simplest form, a carbohydrate is a chain of **sugar molecules** called **monosaccharides**.

Structure and Function of Carbohydrates

Most people are familiar with carbohydrates, one type of macromolecule, especially when it comes to what we eat. To lose weight, some individuals adhere to “low-carb” diets. Athletes, in contrast, often “carb-load” before important competitions to ensure that they have enough energy to compete at a high level. Carbohydrates are, in fact, an essential part of our diet; grains, fruits, and vegetables are all natural sources of carbohydrates. Carbohydrates provide energy to the body, particularly through glucose, a simple sugar that is a component of starch and an

ingredient in many staple foods. Carbohydrates also have other important functions in humans, animals, and plants.

Molecular Structures

Carbohydrates can be represented by the stoichiometric formula $(\text{CH}_2\text{O})_n$, where n is the number of carbons in the molecule. In other words, the ratio of carbon to hydrogen to oxygen is 1:2:1 in carbohydrate molecules. This formula also explains the origin of the term “carbohydrate”: the components are carbon (“carbo”) and the components of water (hence, “hydrate”). Carbohydrates are classified into three subtypes: monosaccharides, disaccharides, and polysaccharides.

Monosaccharides

Monosaccharides (*mono-* = “one”; *sacchar-* = “sweet”) are simple sugars, the most common of which is glucose. In monosaccharides, the number of carbons usually ranges from three to seven. Most monosaccharide names end with the suffix *-ose*. If the sugar has an aldehyde group (the functional group with the structure R-CHO), it is known as an aldose, and if it has a ketone group (the functional group with the structure RC(=O)R'), it is known as a ketose. Depending on the number of carbons in the sugar, they also may be known as trioses (three carbons), pentoses (five carbons), and or hexoses (six carbons). See Figure 1 for an illustration of the monosaccharides.

MONOSACCHARIDES

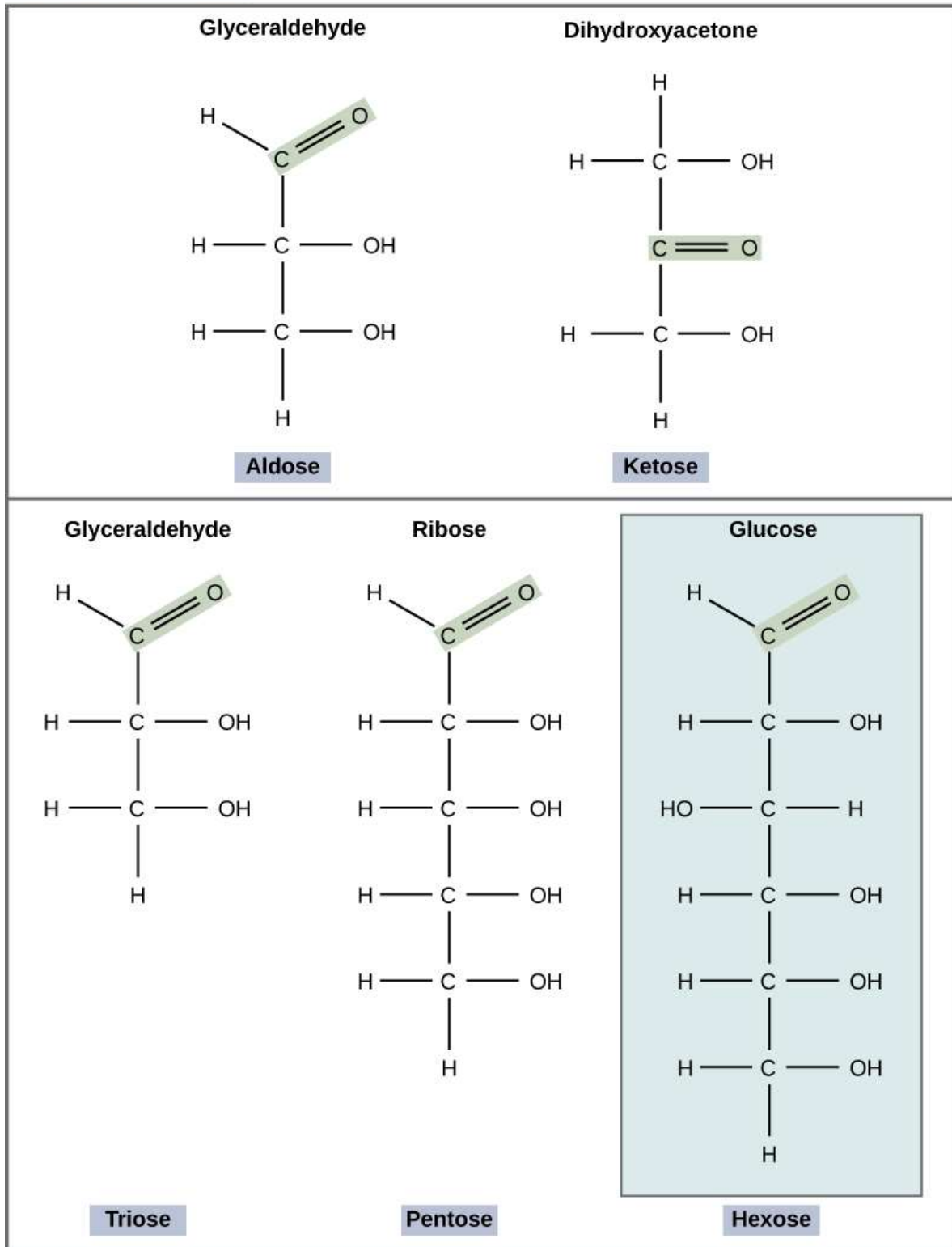


Figure 1. Monosaccharides are classified based on the position of their carbonyl group and the number of carbons in the backbone. Aldoses have a carbonyl group (indicated in green) at the

end of the carbon chain, and ketoses have a carbonyl group in the middle of the carbon chain. Trioses, pentoses, and hexoses have three, five, and six carbon backbones, respectively.

The chemical formula for glucose is $C_6H_{12}O_6$. In humans, glucose is an important source of energy. During cellular respiration, energy is released from glucose, and that energy is used to help make adenosine triphosphate (ATP). Plants synthesize glucose using carbon dioxide and water, and glucose in turn is used for energy requirements for the plant. Excess glucose is often stored as starch that is catabolized (the breakdown of larger molecules by cells) by humans and other animals that feed on plants.

Galactose and fructose are other common monosaccharides — galactose is found in milk sugars and fructose is found in fruit sugars. Although glucose, galactose, and fructose all have the same chemical formula ($C_6H_{12}O_6$), they differ structurally and chemically (and are known as isomers) because of the different arrangement of functional groups around the asymmetric carbon; all of these monosaccharides have more than one asymmetric carbon (Figure 2).

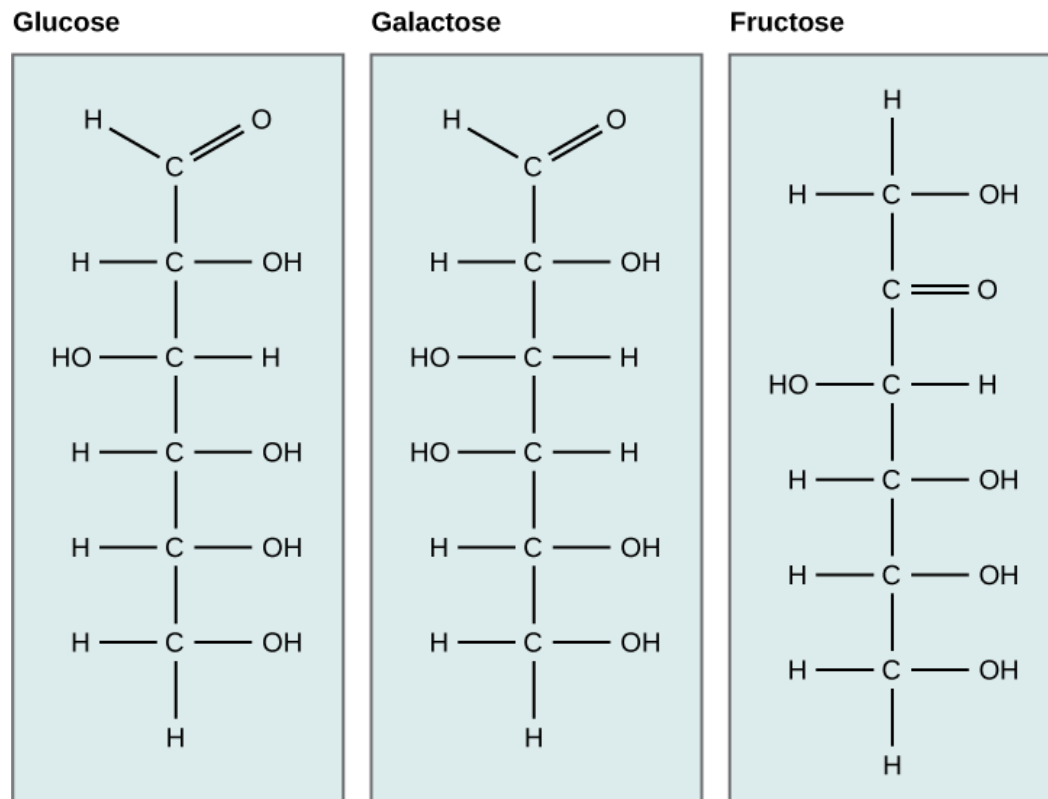


Figure 2. Glucose, galactose, and fructose are all hexoses. They are structural isomers, meaning they have the same chemical formula ($C_6H_{12}O_6$) but a different arrangement of atoms.

Monosaccharides can exist as a linear chain or as ring-shaped molecules; in aqueous solutions they are usually found in ring forms (Figure 3). Glucose in a ring form can have two different arrangements of the hydroxyl group ($-OH$) around the anomeric carbon (carbon 1 that becomes asymmetric in the process of ring formation). If the hydroxyl group is below carbon number 1 in

the sugar, it is said to be in the alpha (α) position, and if it is above the plane, it is said to be in the beta (β) position.

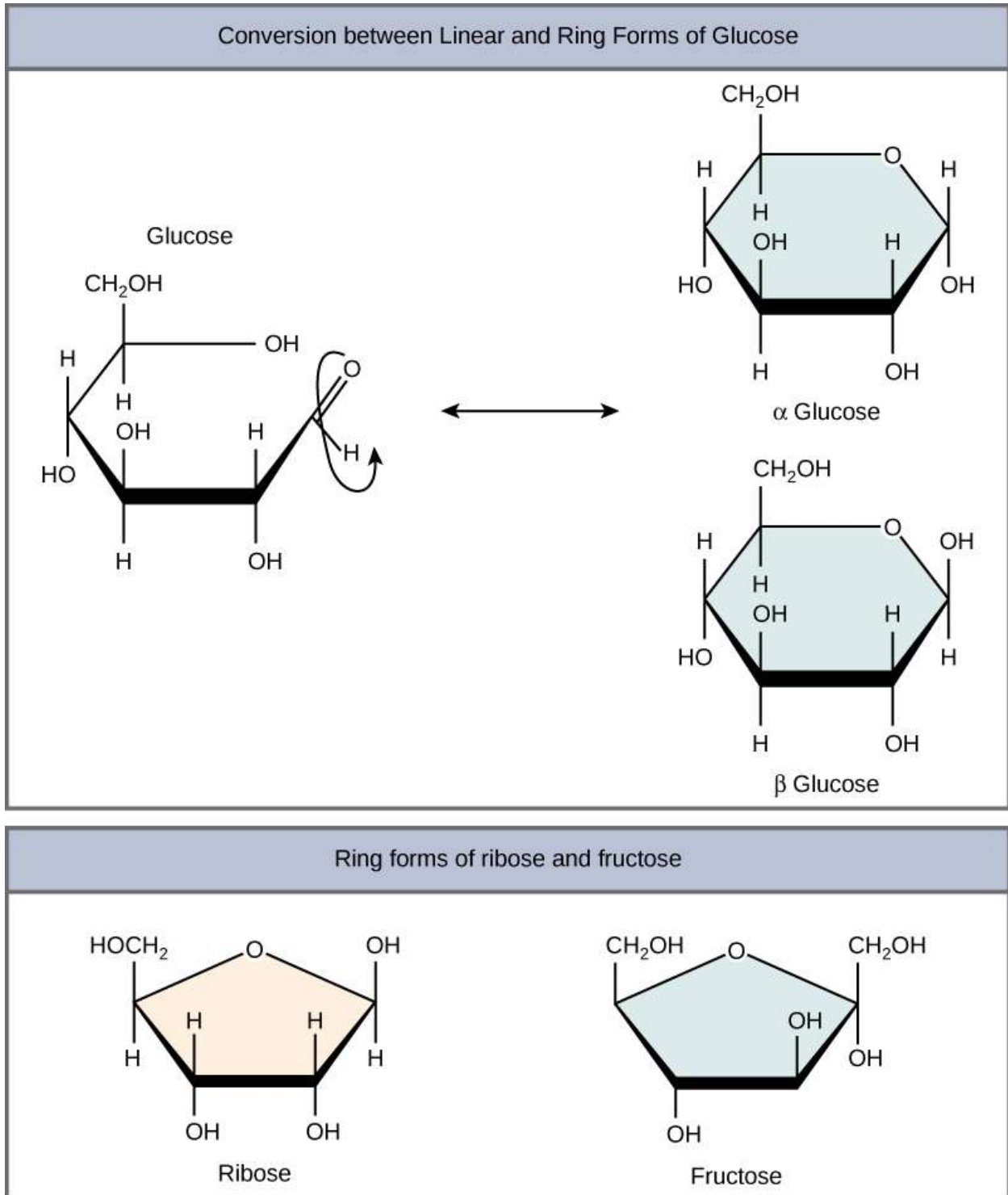


Figure 3. Five and six carbon monosaccharides exist in equilibrium between linear and ring forms. When the ring forms, the side chain it closes on is locked into an α or β position. Fructose

and ribose also form rings, although they form five-membered rings as opposed to the six-membered ring of glucose.

Disaccharides

Disaccharides (*di-* = “two”) form when two monosaccharides undergo a dehydration reaction (also known as a condensation reaction or dehydration synthesis). During this process, the hydroxyl group of one monosaccharide combines with the hydrogen of another monosaccharide, releasing a molecule of water and forming a covalent bond. A covalent bond formed between a carbohydrate molecule and another molecule (in this case, between two monosaccharides) is known as a **glycosidic bond** (Figure 4). Glycosidic bonds (also called glycosidic linkages) can be of the alpha or the beta type.

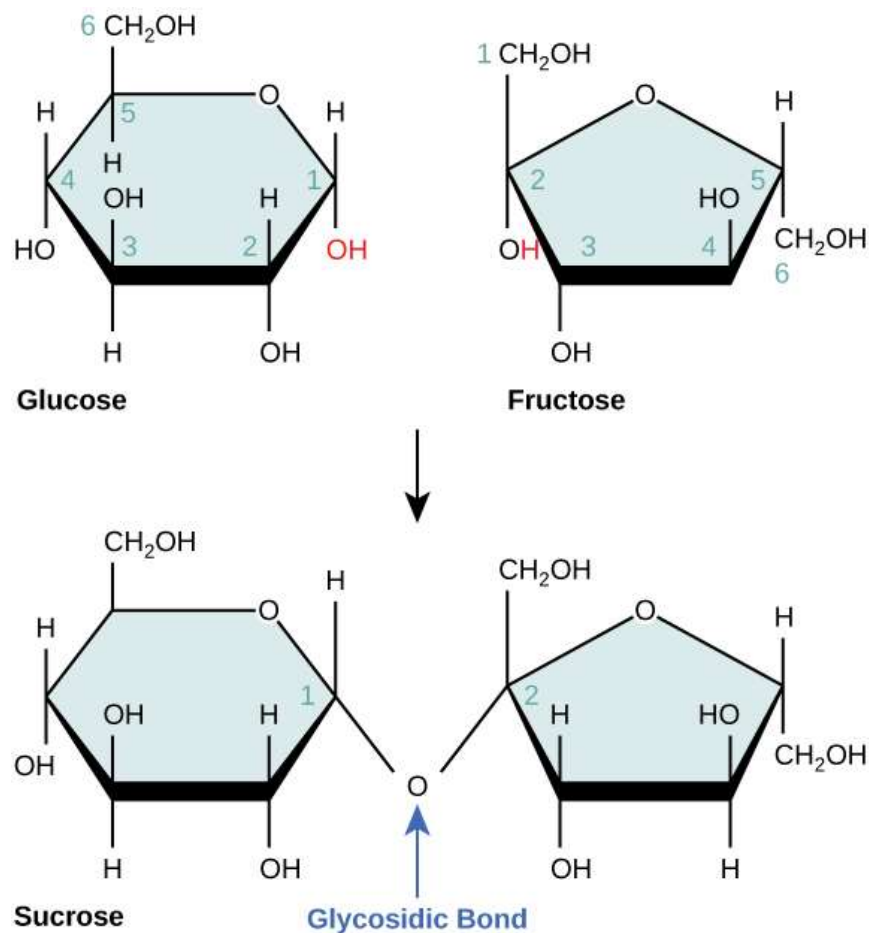


Figure 4. Sucrose is formed when a monomer of glucose and a monomer of fructose are joined in a dehydration reaction to form a glycosidic bond. In the process, a water molecule is lost. By convention, the carbon atoms in a monosaccharide are numbered from the terminal carbon closest to the carbonyl group. In sucrose, a glycosidic linkage is formed between carbon 1 in glucose and carbon 2 in fructose.

Common disaccharides include lactose, maltose, and sucrose (Figure 5). Lactose is a disaccharide consisting of the monomers glucose and galactose. It is found naturally in milk. Maltose, or malt sugar, is a disaccharide formed by a dehydration reaction between two glucose molecules. The most common disaccharide is sucrose, or table sugar, which is composed of the monomers glucose and fructose.

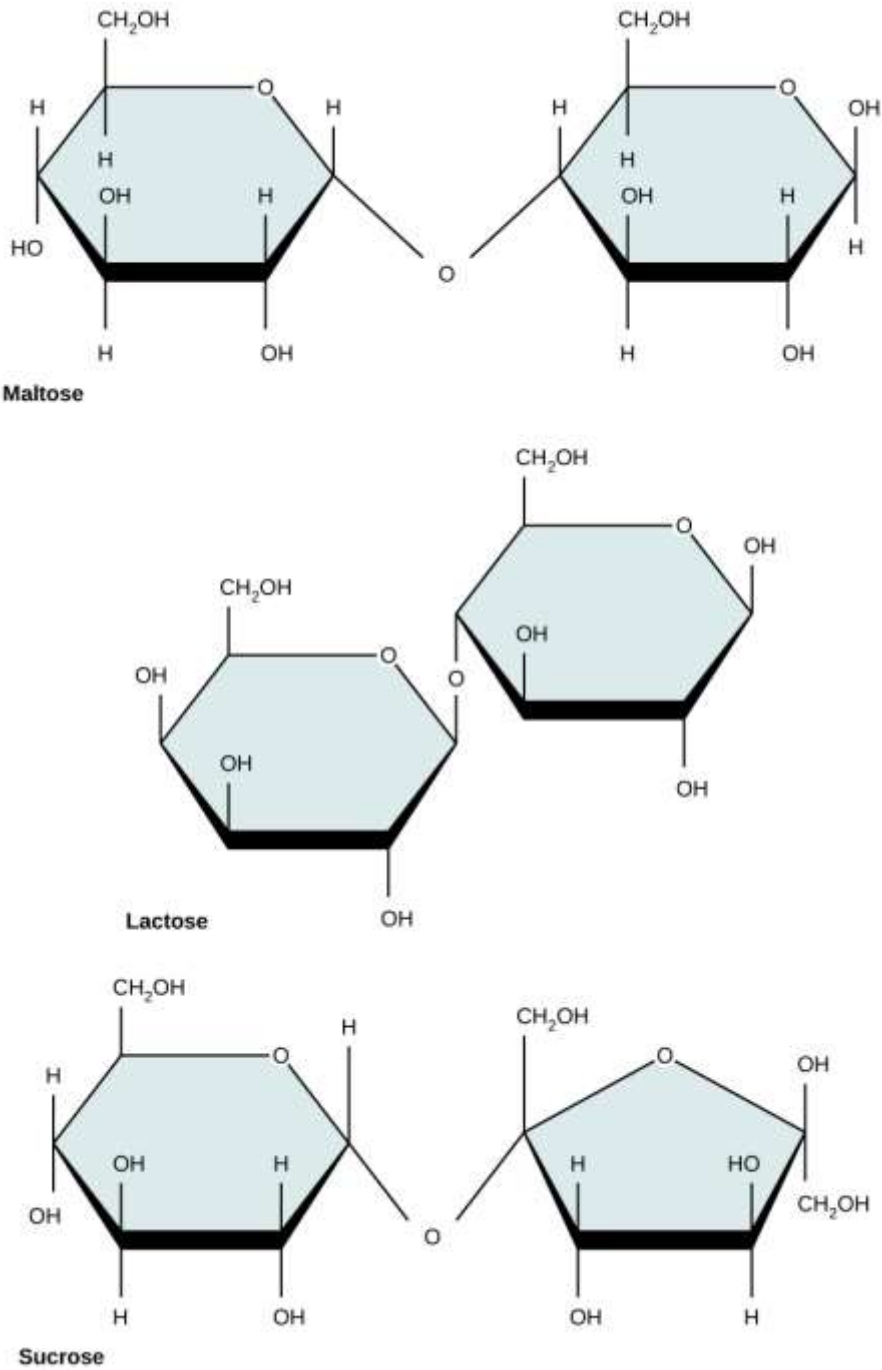


Figure 5. Common disaccharides include maltose (grain sugar), lactose (milk sugar), and sucrose (table sugar).

Polysaccharides

A long chain of monosaccharides linked by glycosidic bonds is known as a **polysaccharide** (*poly-* = “many”). The chain may be branched or unbranched, and it may contain different types of monosaccharides. The molecular weight may be 100,000 daltons or more depending on the number of monomers joined. Starch, glycogen, cellulose, and chitin are primary examples of polysaccharides.

Starch is the stored form of sugars in plants and is made up of a mixture of amylose and amylopectin (both polymers of glucose). Plants are able to synthesize glucose, and the excess glucose, beyond the plant’s immediate energy needs, is stored as starch in different plant parts, including roots and seeds. The starch in the seeds provides food for the embryo as it germinates and can also act as a source of food for humans and animals. The starch that is consumed by humans is broken down by enzymes, such as salivary amylases, into smaller molecules, such as maltose and glucose. The cells can then absorb the glucose.

Starch is made up of glucose monomers that are joined by α 1-4 or α 1-6 glycosidic bonds. The numbers 1-4 and 1-6 refer to the carbon number of the two residues that have joined to form the bond. As illustrated in Figure 6, amylose is starch formed by unbranched chains of glucose monomers (only α 1-4 linkages), whereas amylopectin is a branched polysaccharide (α 1-6 linkages at the branch points).

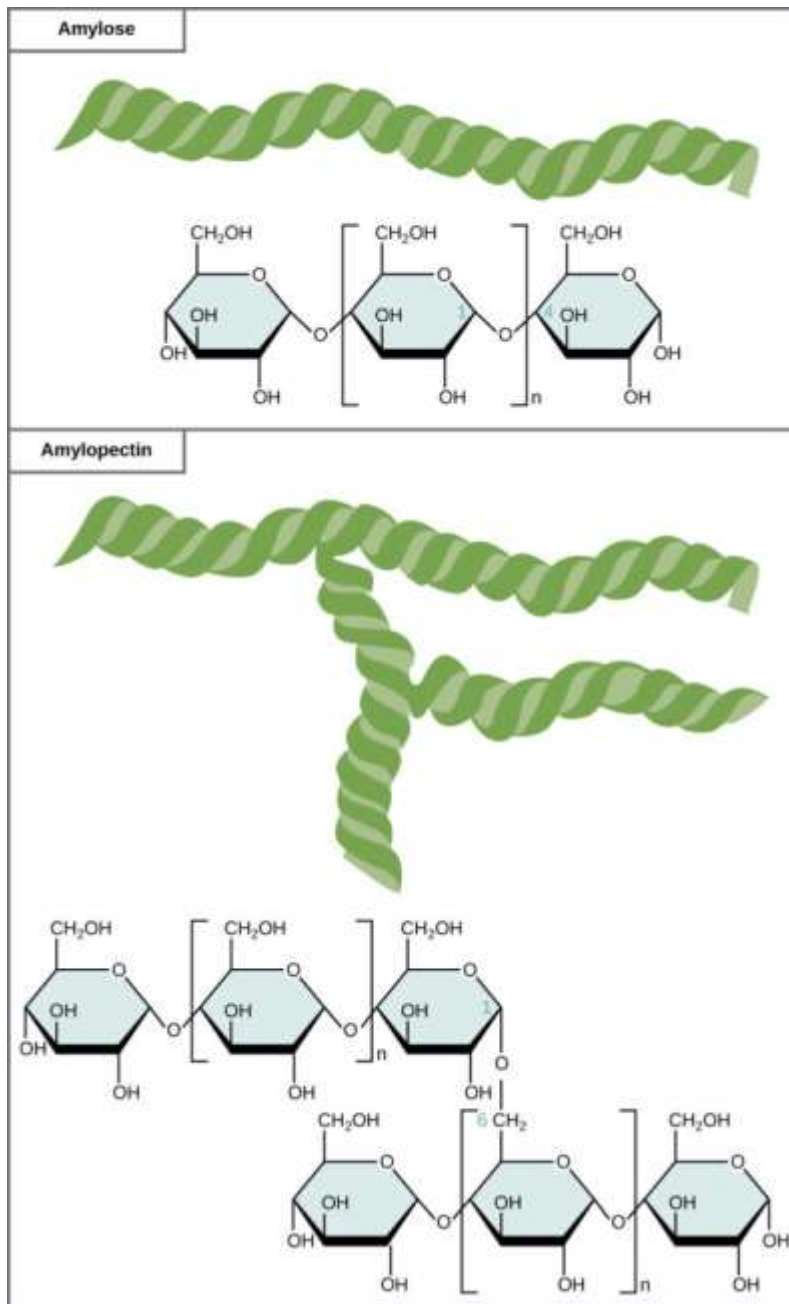
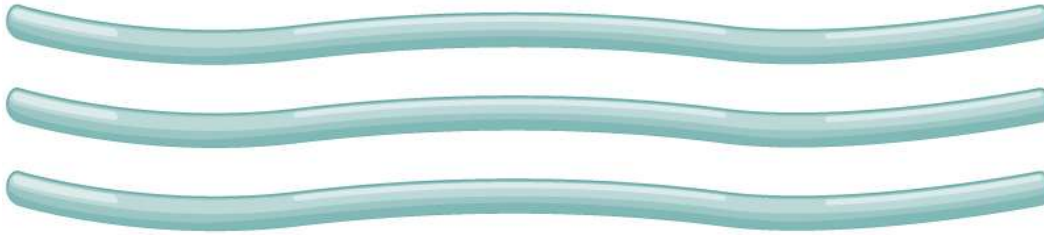


Figure 6. Amylose and amylopectin are two different forms of starch. Amylose is composed of unbranched chains of glucose monomers connected by α 1,4 glycosidic linkages. Amylopectin is composed of branched chains of glucose monomers connected by α 1,4 and α 1,6 glycosidic linkages. Because of the way the subunits are joined, the glucose chains have a helical structure. Glycogen (not shown) is similar in structure to amylopectin but more highly branched.

Glycogen is the storage form of glucose in humans and other vertebrates and is made up of monomers of glucose. Glycogen is the animal equivalent of starch and is a highly branched molecule usually stored in liver and muscle cells. Whenever blood glucose levels decrease, glycogen is broken down to release glucose in a process known as glycogenolysis.

Cellulose is the most abundant natural biopolymer. The cell wall of plants is mostly made of cellulose; this provides structural support to the cell. Wood and paper are mostly cellulosic in nature. Cellulose is made up of glucose monomers that are linked by β 1-4 glycosidic bonds (Figure 7).

Cellulose fibers



Cellulose structure

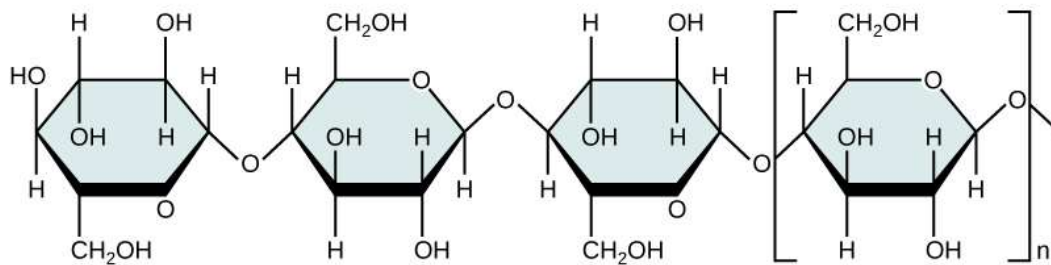


Figure 7. In cellulose, glucose monomers are linked in unbranched chains by β 1-4 glycosidic linkages. Because of the way the glucose subunits are joined, every glucose monomer is flipped relative to the next one resulting in a linear, fibrous structure.

As shown in Figure 7, every other glucose monomer in cellulose is flipped over, and the monomers are packed tightly as extended long chains. This gives cellulose its rigidity and high tensile strength—which is so important to plant cells. While the β 1-4 linkage cannot be broken down by human digestive enzymes, herbivores such as cows, koalas, buffalos, and horses are able, with the help of the specialized flora in their stomach, to digest plant material that is rich in cellulose and use it as a food source. In these animals, certain species of bacteria and protists reside in the rumen (part of the digestive system of herbivores) and secrete the enzyme cellulase. The appendix of grazing animals also contains bacteria that digest cellulose, giving it an important role in the digestive systems of ruminants. Cellulases can break down cellulose into glucose monomers that can be used as an energy source by the animal. Termites are also able to break down cellulose because of the presence of other organisms in their bodies that secrete cellulases.

Carbohydrates serve various functions in different animals. Arthropods (insects, crustaceans, and others) have an outer skeleton, called the exoskeleton, which protects their internal body parts (as seen in the bee in Figure 8).

This exoskeleton is made of the biological macromolecule chitin, which is a polysaccharide-containing nitrogen. It is made of repeating units of N-acetyl- β -d-glucosamine, a modified sugar.

Chitin is also a major component of fungal cell walls; fungi are neither animals nor plants and form a kingdom of their own in the domain Eukarya.

Carbohydrates are a group of macromolecules that are a vital energy source for the cell and provide structural support to plant cells, fungi, and all of the arthropods that include lobsters, crabs, shrimp, insects, and spiders. Carbohydrates are classified as monosaccharides, disaccharides, and polysaccharides depending on the number of monomers in the molecule. Monosaccharides are linked by glycosidic bonds that are formed as a result of dehydration reactions, forming disaccharides and polysaccharides with the elimination of a water molecule for each bond formed. Glucose, galactose, and fructose are common monosaccharides, whereas common disaccharides include lactose, maltose, and sucrose. Starch and glycogen, examples of polysaccharides, are the storage forms of glucose in plants and animals, respectively. The long polysaccharide chains may be branched or unbranched. Cellulose is an example of an unbranched polysaccharide, whereas amylopectin, a constituent of starch, is a highly branched molecule. Storage of glucose, in the form of polymers like starch or glycogen, makes it slightly less accessible for metabolism; however, this prevents it from leaking out of the cell or creating a high osmotic pressure that could cause excessive water uptake by the cell.

Q. 2. B) Describe any two of the following: (10)

1. Chemical properties of water.

Chemical Properties of Water

Water has many unique characteristics that make it ideal for nurturing life. Learn about them with this tutorial.

Hydrogen Bonds

Water – a [polar molecule](#) – tends to be slightly positive on the hydrogen side and slight negative on the oxygen side. (See the illustration in the tutorial.) The electrostatic bond between the positive hydrogen side of this molecule and other negative ions or polar molecules is called a **hydrogen bond**.

Molecules and ions with which water forms hydrogen bonds (such as sodium chloride) are **hydrophilic**. On the other hand, ions and molecules that do not form hydrogen bonds with water are **hydrophobic**.

Liquidity at Room Temperature

At room temperature, most compounds with low molecular weights take gaseous form. With water, however, hydrogen bonding helps to keep it a liquid at room temperature.

Kept relatively close together, the molecules at room temperature are unable to dissipate sufficiently to form a gas. Temperatures of 212°F (or 100°C) are required to break the hydrogen bonds and convert liquid water into water vapor.

Chemical Reactions

When ionic compounds such as sodium chloride are added to water, hydrogen bonding will tend to pull those ionic compounds apart. This makes water a **natural solvent**.

Once ionic compounds dissolve, their anions and cations circulate through the water allowing further reactions to occur. Thus, water also sponsors and facilitates **chemical reactions**.

Stable Temperatures

Water takes more heat to raise its temperature than other common compounds, since much of that heat is required to first break the hydrogen bonds.

Water also retains heat, so its temperature falls slowly.

This means that larger systems of water (such as the ocean or a body) tend to maintain more or less constant temperatures, which in turn helps the earth (and us) to maintain relatively constant temperatures.

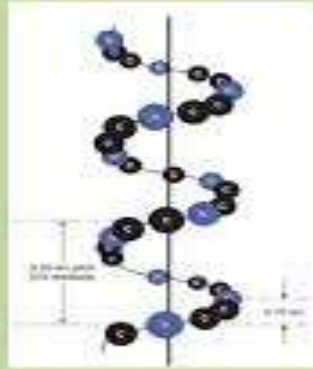
Freezing Point

At 32°F (or 0°C) and below, water molecules form hydrogen bonds in a crystalline lattice structure. This bonding spaces the molecules a bit farther apart than usual, causing water to expand when it freezes. This results in **ice being less dense** than liquid water, which is why ice floats

2. Secondary structure of Proteins.

SECONDARY STRUCTURE : Alpha Helix

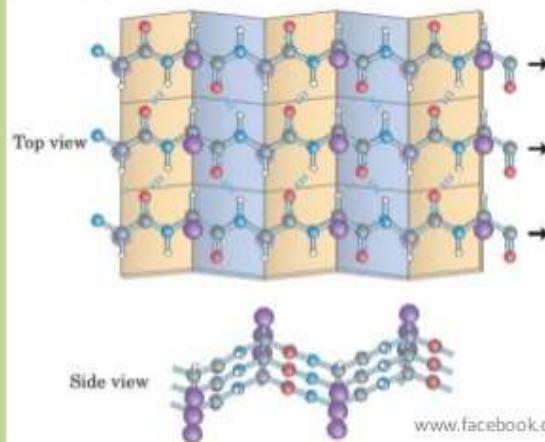
- α helix is twisted by an equal amount about each α -carbon
- With a phi angle of approx. -57° and a psi angle of approx -47°
- Complete turn of the helix contains an average of 3.6 aminoacyl residues
- Distance it rises per turn (**pitch**) is 0.54 nm
- **R groups** of each aminoacyl residue in an α helix **face outward**



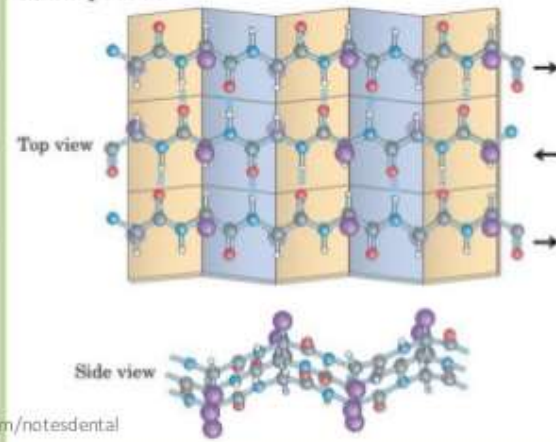
SECONDARY STRUCTURE : Beta Sheet

- **Parallel:** polypeptide chain proceed in the same direction amino to carboxyl
- **Antiparallel:** they proceed in opposite directions

(b) Parallel



(a) Antiparallel



3. Structure of α -glucose and β -glucose.

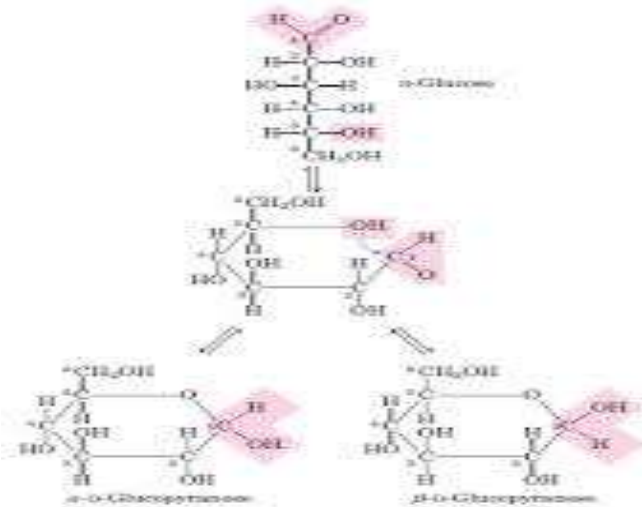


FIGURE 7-6 Formation of the two cyclic forms of α -glucose. Reaction between the aldehyde group of C-1 and the hydroxyl group at C-5 forms a hemiacetal linkage, producing either of two stereoisomers, the α and β anomers, which differ only in the stereochemistry around the hemiacetal carbon. The interconversion of α and β anomers is called mutarotation.

4. Compare between globular and fibrous protein.

Q. 3 A. Answer any one of the following:

(10)

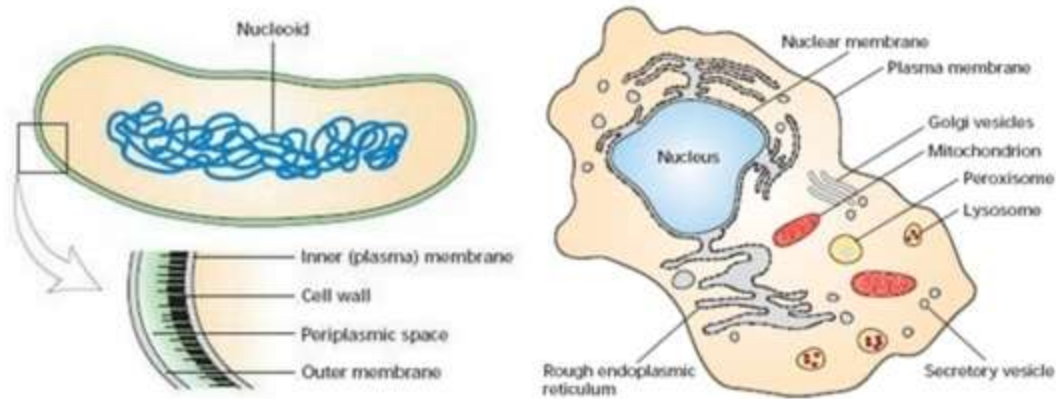
1. Compare and contrast between a Eukaryotic and Prokaryotic cell

	<u>Eukaryotic Cell</u>	<u>Prokaryotic Cell</u>
Nucleus	Present	Absent
Number of chromosomes	More than one	One--but not true chromosome: Plasmids
Cell Type	Usually multicellular	Usually unicellular (some cyanobacteria may be multicellular)
True Membrane bound Nucleus	Present	Absent
Example	Animals and Plants	Bacteria and Archaea
Genetic Recombination	Meiosis and fusion of gametes	Partial, unidirectional transfers <u>DNA</u>
Lysosomes and	Present	Absent

peroxisomes		
Microtubules	Present	Absent or rare
Endoplasmic reticulum	Present	Absent
Mitochondria	Present	Absent
Cytoskeleton	Present	May be absent
DNA wrapping on proteins.	Eukaryotes wrap their DNA around proteins called histones.	Multiple proteins act together to fold and condense prokaryotic DNA. Folded DNA is then organized into a variety of conformations that are supercoiled and wound around tetramers of the HU protein.
Ribosomes	Larger	Smaller
Vesicles	Present	Present
Golgi apparatus	Present	Absent
Chloroplasts	Present (in plants)	Absent; chlorophyll scattered in the cytoplasm
Flagella	Microscopic in size; membrane bound; usually arranged as nine doublets surrounding two singlets	Submicroscopic in size, composed of only one fiber
Permeability of Nuclear Membrane	Selective	not present
Plasma membrane with steroid	Yes	Usually no
Cell wall	Only in plant cells and fungi (chemically simpler)	Usually chemically complexed

Vacuoles	Present	Present
Cell size	10-100um	1-10um

SIMILARITIES BETWEEN PROKARYOTIC CELL AND EUKARYOTIC CELL



2. Principle of Compound Microscope and its parts

A compound microscope is an indispensable instrument in any biological laboratory. It is used for passive observation of structural details of a cell, tissue or organ in sections.

A modern compound microscope has following structural components.

Non-Optical Components:

1. Base (foot):

It is U or horseshoe-shaped metallic structure that supports the whole microscope.

2. Pillar:

It is a short upright part that connects to base as well as arm.

3. Arm (Limb):

It is a curved metallic handle that connects with the arm by inclination joint. It supports stage and body tube.

4. Inclination Joint:

It is used for tilting the microscope if required for observation in sitting position.

5. Stage:

It is a metallic platform with a central hole fitted to the lower part of the arm. Microscopic slides held on the stage by either simple side clips or by a mechanical stage clip.

6. Body tube:

It is meant for holding ocular and objective lenses at its two ends. The end holding ocular lens is called head while the end containing 3-4 objective lens is called nose piece. The body tube has an internal pathway for the passage of light rays which form the enlarged image or microscopic objects.

7. Draw tube:

It is a small tube that remains fixed at the upper end of the body tube. It holds eyepiece or ocular lens.

8. Rack and pinion:

The microscope has a rack and pinion attached either to body tube or the stage for bringing the object under focus.

9. Adjustment screws:

There are two pairs of screws for moving the body tube in relation to stage, larger for coarse adjustment and smaller for fine adjustment. In fine adjustment the body tube or stages moves for extremely short distances. In coarse adjustment the body tube or stage can move up and distance. In coarse adjustment is meant for briefly objective lens at a proper distance from the object so as to form image of the same at the ocular end. Fine adjustment is required to obtain sharp image.

10. Automatic Stop:

It is a small screw fitted at lower end or rack and pinion. It is meant for stopping the downward sliding of the body tube so as to prevent the damage of objective lens and the slide.

Optical Components:**11. Diaphragm:**

It is flitted just below the stage for regulating the amount of light failing on the object. Diaphragm is of two types, disc and iris.

12. Condenser:

It is attached below the diaphragm. Condenser can be moved up and down to focus light on the object.

13. Reflector (Mirror):

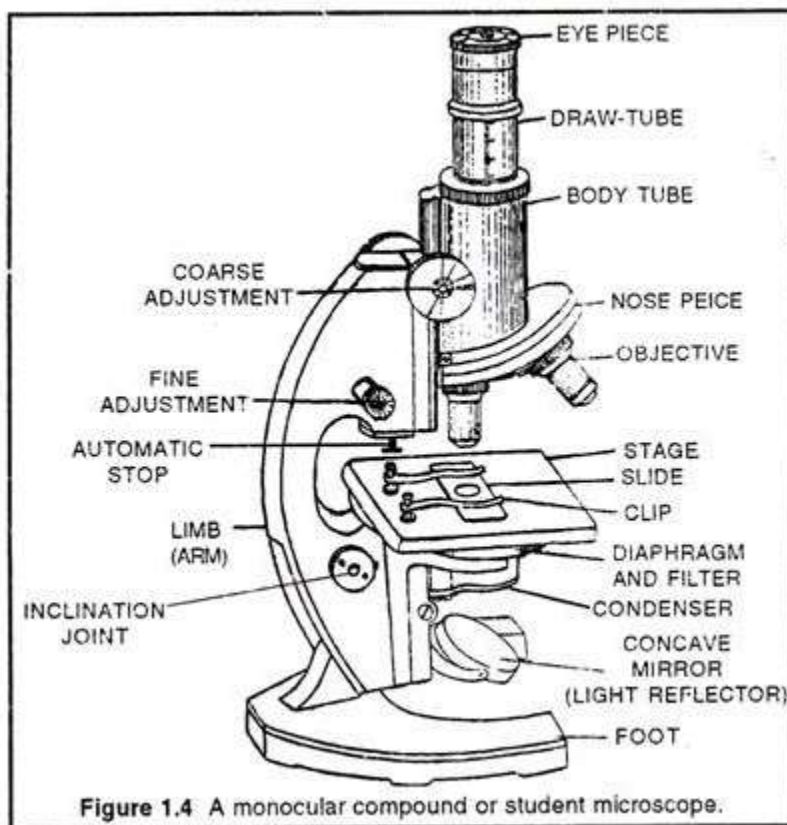
It is attached just above the base. Both its surface bear mirrors, plane on one side and concave on other side. Plane side is used in strong light and concave side in weak light. Reflector directs the light on the object through the condenser and diaphragm system.

14. Objective Lenses:

They are fitted over the nose piece. Objective lenses are of two to three types – low power (commonly 10X or 5X), high power (commonly 45X) and oil immersion (commonly 100X, can be more). An objective lens is not a simple lens but compound lens. It forms real inverted image of the object inside the body tube.

15. Ocular Lens or Eyepiece:

It is lens through which image of the microscopic object is observed. It also takes part in magnification. Depending upon magnification, the eye piece is of four types-5X, 10X, 15X, and 20 X. Advanced microscope has two eye pieces so that both the eyes can be used (Fig. 1.4). Microscope head having device for using two eye pieces is called binocular head. It contains a number of internal mirrors and prisms for the passage of light.

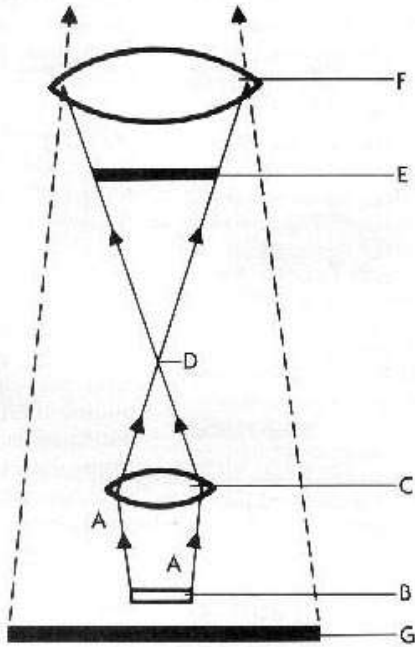


Working Principle of Compound Microscope:

The compound microscope is most commonly used in clinical and educational laboratories. It has a combination of lenses that enhances both magnifying power as well as the resolving power. The specimen or object, to be examined is usually mounted on a transparent glass slide and positioned on the specimen stage between the condenser lens and objective lens. A beam of visible light from the base is focused by a condenser lens onto the specimen. The objective lens picks up the light transmitted by the specimen and create a magnified image of the specimen called primary image inside the body tube. This image is again magnified by the ocular lens or eye piece. When higher magnification is required, the nose piece is rotated after low power focusing to bring the objective of higher power (generally 45X) in line with the illuminated part of the slide. The objective lens comes very near the cover slip but it does not touch the same. Only fine adjustment it moved for proper focusing. More light may be required. After observation under high power, the nose piece is rotated to bring back the slide under low power.

Occasionally very high magnification it required (e.g. for observing bacterial cell). In that case, oil immersion objective lens (usually 100X) is employed. After focusing under low power a drop of immersion oil (e.g. cedar oil, olive oil) placed over the illuminated part of the cover-slip.

The nose piece is rotated to bring the oil immersion lens in line with die specimen. It comes in contact with the oil. By using fine adjustment only, the specimen is brought under focus. Immersion oil increases the sharpness of the image. Soon after observation, both the lens and the slide are cleared of the oil by fine cotton cloth or lens paper. The common light microscope is also called bright field microscope because the image is produced amidst a brightly illuminated field. The image appears darker because the specimen or object is denser and somewhat opaque than the surroundings. Part of the light passing through or object is absorbed. Bright field microscope is used [or study or preserved and stained material as well as live and unstained object or material. However, differentiation is poor in case of live unstained specimen. Special microscopes for their study are dark field, phase contrast and differential interference contract microscopes.

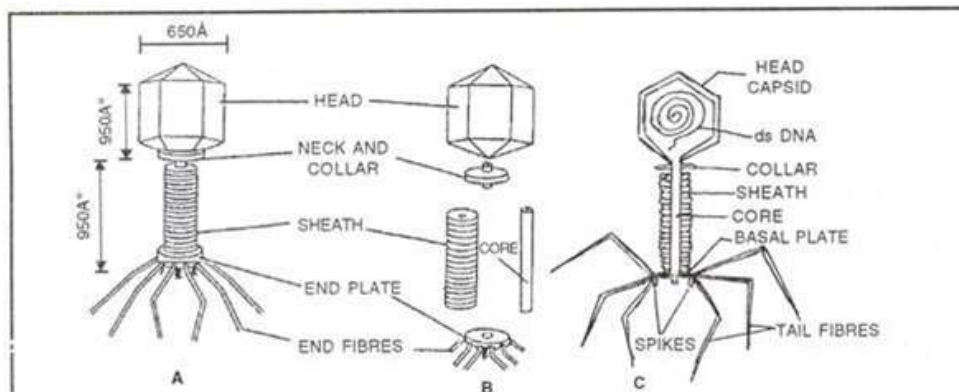


Q. 3. B. Describe Any Two of the following:

(10)

1. Structure of a Bacteriophage with suitable diagram.

The virion of T-even phage is binomial or tadpole like structure with a polyhedral head connected to a helical tail through a short collar. The head composed of about 2000 capsomeres and encloses a tightly packed dsDNA (50 nm long). The tail has an inner hollow tube called core, surrounded by a contractile sheath which consists of 24 annular rings. The distal end of the tube is connected to a hexagonal basal plate with spike or tail spin at each corner. Six long, flexible tail fibers also arise from the basal plate which helps in adsorption to bacteria.



2. Any two types of methods of Isolation of microorganisms.

Isolation of Microorganisms:

Microorganisms occur in natural environment like soil. They are mixed with several other forms of life. Many microbes are pathogenic. They cause a number of diseases with a variety of symptoms, depending on how they interact with the patient. The isolation and growth of suspected microbe in pure culture is essential for the identification and control the infectious agent. The primary culture from natural source will normally be a mixed culture containing microbes of different kinds. But in laboratory, the various species may be isolated from one another. A culture which contains just one species of microorganism is called a pure culture. The process of obtaining a pure culture by separating one species of microbe from a mixture of other species, is known as isolation of the organisms.

Methods of Isolation:

There are special techniques employed to obtain pure cultures of microorganisms. In few cases it is possible to secure pure culture by direct isolation or direct transfer. This can be done only in those situations in which pure culture occurs naturally. Kinds of specimens taken for culturing will depend on the nature and habitat of microbes.

Different pathogens can be isolated from body tissues and fluids such as blood, urine, sputum, pus, faces, spinal fluid, bile, pleural fluids, stomach fluids etc. In the blood stream of a patient suffering with typhoid fever, the bacteria *Salmonella typhosa* may be present.

A pure culture of this bacterium may be obtained by drawing blood sample using a sterilized hypodermic syringe and treating the blood with anticoagulant such as heparin and potassium oxalate. The presence of the anticoagulant prevents the pathogenic microbe from entrapping in fibrin clot. The sample of the blood may be inoculated into a suitable medium.

Following isolation methods are employed to isolate microbes from mixed cultures:

1. Streaking 2. Plating 3. Dilution 4. Enriched procedure, and 5. Single cell technique.

Streaking:

This is most widely used method of isolation. The technique consists of pouring a suitable sterile medium into sterile petriplate and allowing the medium to solidify. By means of a sterile loope or straight needle or a sterile bent glass-rod a small amount of growth preferably from a broth culture or bacterial suspension is streaked back and forth across the surface of agar until about one third of the diameter of the plate has been covered. The needle is then flamed and streaking in done at right angles to and across the first streak. This serves to drag bacteria out in a long line

from the initial streak. When this streaking is completed the needle is again flamed and streaking is done at right angles to the second streak and parallel to the first. It includes diluting of a mixture of microorganisms until only a few hundred bacteria are left in each millilitre of the suspension. A very small amount of the dilution is then placed in a sterile petriplate by means of a sterile loop or pipette. The melted agar medium is cooled to about 45°C and is poured into plate. The microorganism and agar are well mixed. When the agar is solidified the individual bacterium will be held in place and will grow to a visible colony.

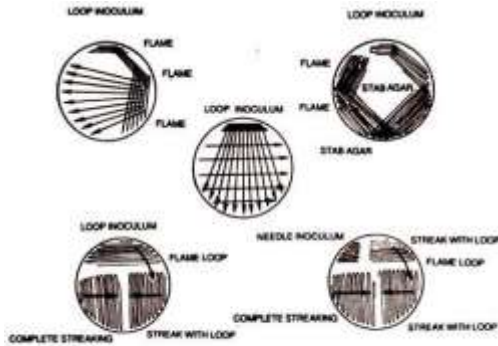


Fig. 2.1 Different patterns used for obtaining pure culture by streaking.

Dilution:

This method is used for the microorganisms which cannot be easily isolated by streaking or plating method. Sometimes when several organisms are present in a mixture, with one organism predominating, the predominating form may be isolated by this method. For example, when raw milk is allowed to sour at room temperature it will, at the time of curding, have a mixture of microorganisms with high percentage of *Streptococcus lactis*.

If 1 ml of the sour milk is taken into a tube containing 9 ml. of sterile milk (in which no organisms are present) then 1 ml. of this mixture is transferred with a sterile pipette into a second tube of sterile milk and the procedure is repeated i.e. from second to third tube, third to fourth tube until a series of about 10 tubes are inoculated. By this serial dilution, the chances are that a pure culture of *S. lactis* will be obtained.

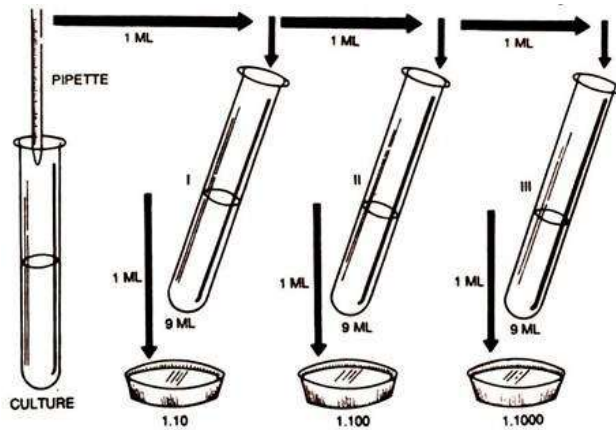
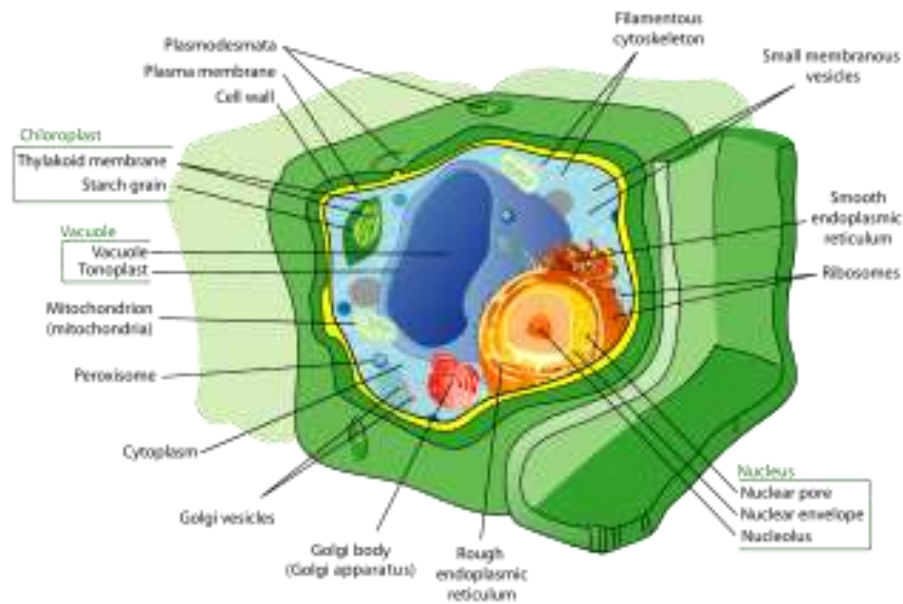


Fig. 2.2 Dilution method of isolation.

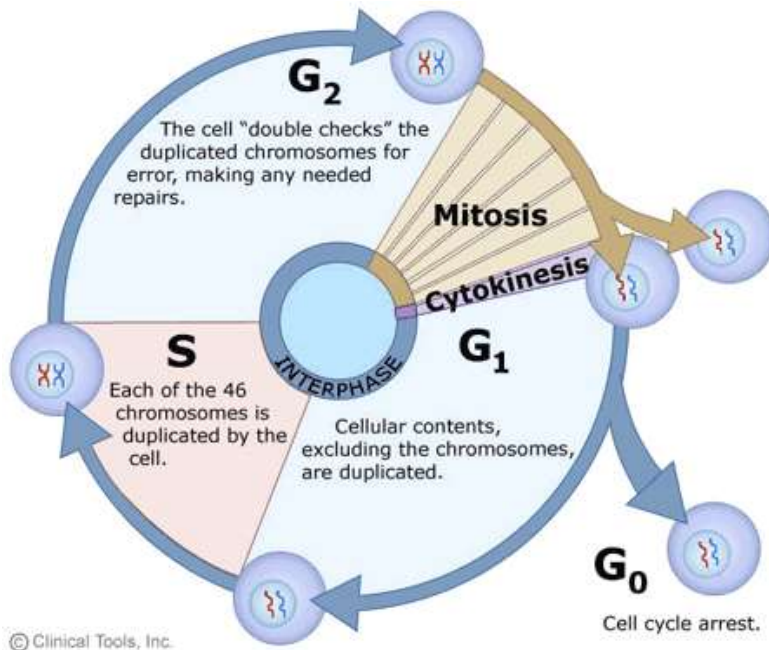
3. Ultrastructure of a Plant Cell.



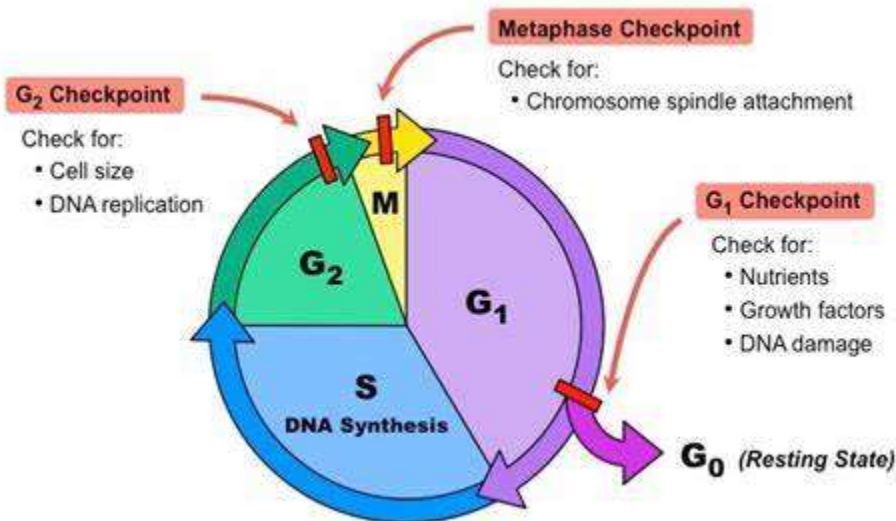
Plant cells are eukaryotic cells of the types present in green plants, photosynthetic eukaryotes of the kingdom Plantae. Their distinctive features include primary cell walls containing cellulose, hemicelluloses and pectin, the presence of plastids with the capability to perform photosynthesis and store starch, a large vacuole that regulates turgor pressure, the absence of flagellae or centrioles, except in the gametes, and a unique method of cell division involving the formation of a cell plate or phragmoplast that separates the new daughter cells.

- Plant primary cell walls are constructed on the outside of the cell membrane and are composed of cellulose and hemicelluloses and pectin. In many cases lignin, suberin or cutin are secreted by the protoplast as secondary wall layers inside the primary cell wall. This contrasts with the cell walls of fungi, which are made of chitin, of bacteria, which are made of peptidoglycan and of archaea, which are made of pseudopeptidoglycan. Cell walls perform many essential functions. They provide shape to form the tissue and organs of the plant, and play an important role in intercellular communication and plant-microbe interactions.
- Many types of plant cells contain a large central vacuole, a water-filled volume enclosed by a membrane known as the tonoplast that maintains the cell's turgor, controls movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.
- Specialized cell-to-cell communication pathways known as plasmodesmata, occur in the form of pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.
- Plant cells contain Plastids, the most notable being chloroplasts, which contain the green-colored pigment chlorophyll that absorbs sunlight, and allows the plant to make its own food in the process known as photosynthesis. Other types of plastids are the amyloplasts, specialized for starch storage, elaioplasts specialized for fat storage, and chromoplasts specialized for synthesis and storage of pigments. As in mitochondria, which have a genome encoding 37 genes, plastids have their own genomes of about 100–120 unique genes and, it is presumed, arose as prokaryotic endosymbionts living in the cells of an early eukaryotic ancestor of the land plants and algae.
- Plant cell division takes place by construction of a phragmoplast as a template for building a cell plate late in cytokinesis. This process is characteristic of land plants and a few groups of algae, notably the Charophytes and the Chlorophyte Order Trentepohliales.
- The motile, free-swimming sperm of bryophytes and pteridophytes, cycads and *Ginkgo* are the only cells of land plants to have flagella similar to those in animal cells, but the conifers and flowering plants do not have motile sperm and lack both flagella and centrioles.

4. Diagrammatic representation of Cell cycle with a note on its check points



Checkpoints of cell cycle



Q.4. A) Describe any one of the following:

(10)

1. Microfilaments and their structure and function in skeletal muscle fibres.

Ans. These are cytoskeletal elements with diameter 8-10 nm. They are present in the cytoplasm and nucleus. In the skeletal muscles, they are as actin and myosin microfilaments as A and B filaments. Description and detailed diagram.

2. The composition of bacterial cell wall and cell wall of Gram negative bacteria.

Ans. the cell wall in prokaryotic bacteria is composed of **peptidoglycan**. This molecule is unique to bacterial cell wall composition. Peptidoglycan is a polymer composed of double-sugars

and amino acids (protein subunits). This molecule gives the cell wall rigidity and helps to give bacteria shape. Peptidoglycan molecules form sheets which enclose and protect the bacterial plasma membrane.

In **gram-negative bacteria**, the cell wall is not as thick because it contains a much lower percentage of peptidoglycan. The gram-negative bacterial cell wall also contains an outer layer of lipopolysaccharides (LPS). The LPS layer surrounds the peptidoglycan layer and acts as an endotoxin (poison) in pathogenic bacteria (disease causing bacteria). The LPS layer also protects gram-negative bacteria against certain antibiotics, such as penicillins.

Bacterial cell wall is composed of peptidoglycan. Alternate molecules of N-acetyl Glucose amine and N-acetyl muramic acid and small peptide attached as side chains.

Cell wall of Gram negative bacteria description in details, has 2 layers and the outer layer is made of lipids.

Diagram for peptidoglycan structure and Gram negative bacterial cell wall is necessary.

Q. 4. B) Answer any two of the following: (10)

1. Describe the structure of nuclear membrane.

Ans. Double layered, with nuclear pores, perinuclear space, nuclear lamina all description. Diagram necessary.

2. Distinguish between Euchromatin and Heterochromatin.

Ans.

<i>Euchromatin:</i>	<i>Heterochromatin:</i>
1. It is narrower, 10-30 nm in diameter.	1. Heterochromatin is thicker, 100 nm in diameter.
2. Euchromatin is lightly stained.	<i>2. It is darkly stained</i>
3. It is somewhat diffused.	3. Heterochromatin is condensed.
4. Euchromatin is fibrous.	4. Heterochromatin is granular.
5. It forms the bulk of chromatin.	5. It is present at certain places in the chromatin.
6. It contains active genes.	6. Heterochromatin does not possess active genes.
7. Euchromatin takes part in transcription.	7. Transcription is absent in heterochromatin.
8. Euchromatin is affected by a number of	8. Heterochromatin is not influenced by

factors like pH, temperature and hormones.	these factors.
9. Crossing over is quite common.	9. Heterochromatin inhibits crossing over.
10. It replicates early.	10. It replicates late in the S-phase.
11. Nucleosome strand has minimum coiling.	11. Nucleosome strand has solenoid coiling.

3. Describe the structure of primary cell wall of Plant cell.

Ans. **Primary cell wall:** This layer is formed between the middle lamella and plasma membrane in growing plant cells. It is primarily composed of cellulose microfibrils contained within a gel-like matrix of hemicellulose fibers and pectin polysaccharides. The primary cell wall provides the strength and flexibility needed to allow for cell growth.

Primary wall composition and architecture

Primary walls isolated from higher plant tissues and cells are composed predominantly of polysaccharides together with lesser amounts of structural glycoproteins (hydroxyproline-rich extensins), phenolic esters (ferulic and coumaric acids), ionically and covalently bound minerals (e.g. calcium and boron), and enzymes. In addition walls contain proteins (expansins) that are believed to have a role in regulating wall expansion. Lignin, a macromolecule composed of highly cross-linked phenolic molecules, is a major component of secondary walls.

The major polysaccharides in the primary wall are:

Cellulose - a polysaccharide composed of 1,4-linked β -D-glucose residues

Hemicellulose - branched polysaccharides that are structurally homologous to cellulose because they have a backbone composed of 1,4-linked β -D-hexosyl residues. The predominant hemicellulose in many primary walls is xyloglucan. Other hemicelluloses found in primary and secondary walls include glucuronoxylan, arabinoxylan, glucomannan, and galactomannan.

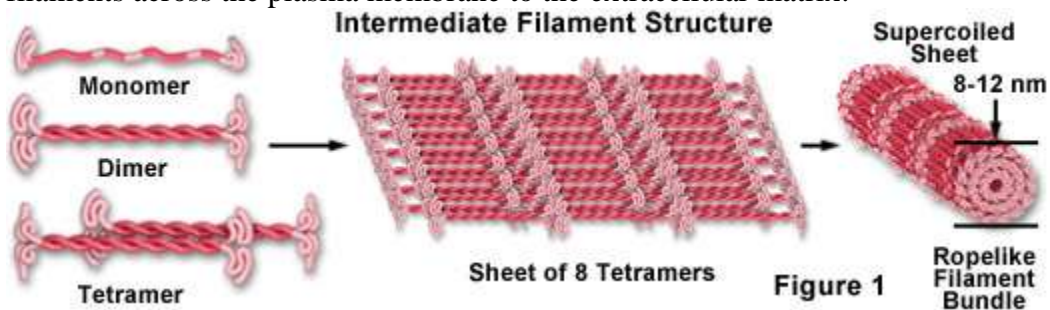
Pectin - a family of complex polysaccharides that all contain 1,4-linked α -D-galacturonic acid. To date three classes of pectic polysaccharides have been characterized: Homogalacturonans, rhamnogalacturonans, and substituted galacturonans.

The organization and interactions of wall components is not known with certainty and there is still considerable debate about how wall organization is modified to allow cells to expand and grow. Several models have been proposed to account for the mechanical properties of the wall:

4. Write a note on intermediate filaments.

Ans.

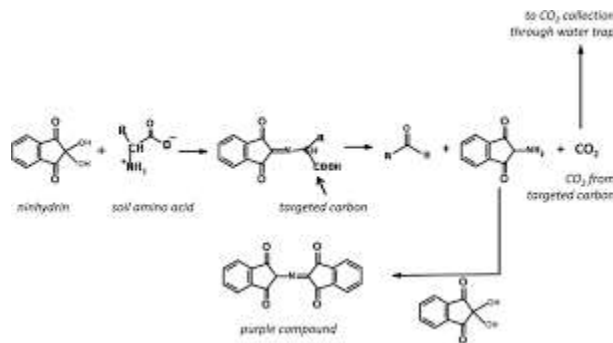
Intermediate filaments are strong but flexible polymers that provide mechanical support for metazoan cells. These filaments are composed of many different but homologous proteins. The filaments were named *intermediate*, because their 10-nm diameters are intermediate between those of the thick and thin filaments in striated muscles, where they were first recognized. They are not found in plants, fungi, or prokaryotes, although one bacterial species has a coiled-coil protein with some properties of intermediate filaments. Cytoplasmic intermediate filaments, in particular keratin filaments, tend to cluster into wavy bundles that vary in compactness, forming a branching network between the plasma membrane and the nucleus. Intercellular junctions called desmosomes anchor intermediate filaments to the plasma membrane and thereby transmit mechanical forces between adjacent cells. Hemidesmosomes connect intermediate filaments across the plasma membrane to the extracellular matrix.



Q. 5 Write Short notes on Any four

(20 mks)

1. Ninhydrin reaction



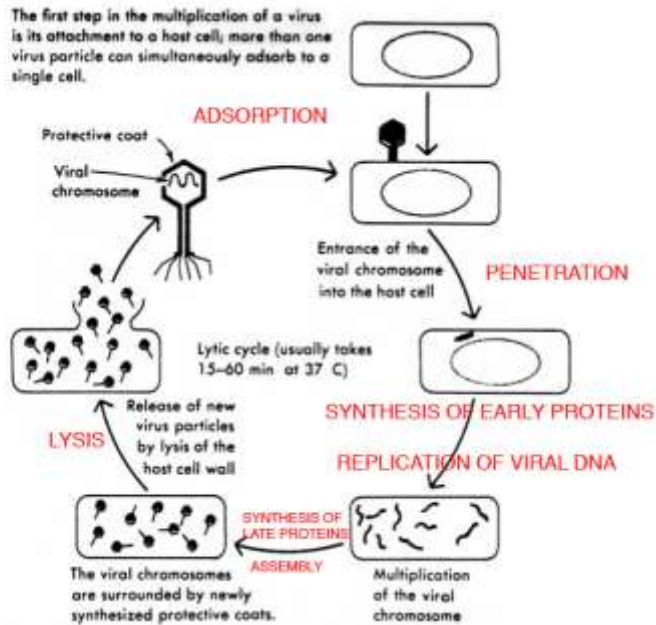
2. Differentiate between homo and hetero polysaccharides

Homopolysaccharides and heteropolysaccharides are polymer components. These polysaccharides are made out of monomers known as monosaccharides. Homopolysaccharides and heteropolysaccharides can be found as structural components in plant tissues and animal tissues. There are many commercially important polysaccharides as well. The main difference between homopolysaccharides and heteropolysaccharides is that **homopolysaccharides are composed of the same repeating unit whereas heteropolysaccharides are composed of different repeating units.**

3. Any two techniques of microbial culture preservation.

- (1) Periodic transfer to fresh media: Bacteria can be maintained by periodically preparing a fresh stock culture from the previous culture. The culture medium, the storage temperature, and the time interval at which the transfers are made vary from one bacteria to another. The temperature and the type of media chosen should support a slow rather than a rapid growth rate so that the time interval between transfers can be as long as possible.
- (2) Preservation by overlaying cultures with Mineral Oil: Many bacteria are successfully preserved by covering the growth on an agar slant with sterile mineral oil. The oil should cover the slant completely. To ensure this, the oil should be about half an inch above the tip of the slant.
- (3) Preservation by Lyophilization: Most bacteria die if cultures are allowed to become dry. However, freeze-drying can preserve many kinds of bacteria that would be killed by ordinary preservation techniques.
 - (i) Equipment used for Lyophilization: A lyophilizer consists of:
 - A vacuum chamber that contains shelves.
 - A vacuum pump.
 - A refrigeration unit.
 - (ii) Procedure:
 - The first step in the lyophilization process is to freeze a product to solidify all of its water molecules.
 - Once frozen, the product is placed in a vacuum and gradually heated without melting the product.
 - This process, called sublimation, transforms the ice directly into water vapor, without first passing through the liquid state.
 - The water vapor given off by the product in the sublimation phase condenses as ice in a condenser, within the lyophilizer's vacuum chamber.
 - The vials are now sealed off under vacuum and stored in a refrigerator.

4. Lytic cycle of a Virus



5. Nucleolus

Ans. The nucleolus is the nuclear subdomain that assembles ribosomal subunits in eukaryotic cells. The nucleolar organiser regions of chromosomes, which contain the genes for pre-ribosomal ribonucleic acid (rRNA), serve as the foundation for nucleolar structure. The nucleolus disassembles at the beginning of mitosis, its components disperse in various parts of the cell and reassembly occurs during telophase and early G1 phase. Ribosome assembly begins with transcription of pre-rRNA. During transcription, ribosomal and non-ribosomal proteins attach to the rRNA. Subsequently, there is modification and cleavage of pre-rRNA and incorporation of more ribosomal proteins and 5S rRNA into maturing pre-ribosomal complexes. The nucleolus also contains proteins and RNAs that are not related to ribosome assembly and a number of new functions for the nucleolus have been identified. **Diagram necessary.**

6. Fungal cell wall: The **fungal wall** is a sophisticated cell organelle. It defines the volumetric shape of the cell, provides osmotic and physical protection and, together with the plasma membrane and periplasmic space, influences and regulates the influx of materials into the cell. However, it is also able to control the environment in the immediate external vicinity of the cell membrane, and it represents the interface between the organism and the outside world. This is an **active interface**, since the interaction of the organism and the outside world (and the latter will include other cells) is subject to modulation and modification. The fungal cell wall is metabolically active, interactions between its components occur to give rise to the mature cell wall structure. So the wall must be understood to be a **dynamic structure** which is subject to modification at various times to suit various functions. Besides enclosing and supporting the cytoplasm, those functions include selective permeability, as a support for immobilised enzymes and cell-cell recognition and adhesion. The wall is a multilayered complex of polysaccharides, glycoproteins and proteins. The polysaccharides are glucans and mannans and include some very complex polysaccharides (like gluco-galacto-mannans). In hyphae the major component of the wall, and certainly the most important for its structural integrity, is **chitin** (a linear polymer of *N*-acetylglucosamine) though this is frequently cross linked to other wall constituents, particularly

a **$\beta(1\rightarrow3)$ -glucan**, the terminal reducing residue of a chitin chain being attached to the non-reducing end of a $\beta(1\rightarrow3)$ -glucan chain by a $(1\rightarrow4)$ linkage. Removal of the outer wall layers with lytic enzymes has revealed the architecture of the inner chitin wall to be composed of microfibrils formed by the aggregation of the chitin polymers by hydrogen bonding. The chitin inner wall is cross-linked to the outer β -glucan components and forms a major structural component of the walls of most true fungi. Synthesis of the cell wall occurs at the outer surface of the plasma membrane of the growing hyphal tip. **Chitin synthase** is the enzyme that catalyses formation of chitin from the precursor UDP-*N*-acetylglucosamine. Chitin synthase adds two molecules of UDP-*N*-acetylglucosamine (UDPGlcNAc) to the existing chitin chain in the reaction. It appears as an inactive **zymogen** requiring activation by cleavage of a peptide by an endogenous protease to generate the active enzyme

