Cambridge Advanced Subsidiary Level Notes
9700 Biology

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Contents

1 Cell structure

1.1 The microscope in cell studies

Make temporary preparations of cellular material suitable for viewing with a light microscope

Part of Paper 3.

Draw cells from microscope slides and photomicrographs

The drawings made must have *continuous* outlines. Any structures clearly visible in the photomicrograph must also be drawn on the drawing.

Calculate magnifications of images and actual sizes of specimens from drawings, photomicrographs and electron micrographs (scanning and transmission)

The definition of magnification is as follows

$$\text{magnification} = \frac{\text{image size}}{\text{object size}}$$

which we can use as a formula to find any unknown variable when two other variables are given.

Use an eyepiece graticule and stage micrometer scale to make measurements and use the appropriate units, millimetre (mm), micrometre (µm) and nanometre (nm)

The *eyepiece graticule* is a transparent piece of glass, with a hundred divisions that can be added to the end of a microscope lens, such that the hundred markings of length can be overlain on top of the slide being observed. We can use these markings to determine the length of a cell being observed, in terms of *eyepiece graticule units*, the equivalent of which we are to deduce after calibrating it.

To calibrate the eyepiece graticule, we bring into focus a piece similar to the eyepiece graticule, called a *stage micrometer*, on which there are markings with real world units. We align those markings with those of the graticule, hence finding the equivalent of one graticule unit.

Because the objects viewed with microscopes are very small, measuring them in terms of millimetres (mm) is not convenient enough, we must use smaller units, namely micrometres (µm) and nanometres (nm). The conversions are as follows

$$1\,\text{mm} = 1 \times 10^{-3}\,\text{µm}$$

$$1\,\text{mm} = 1 \times 10^{-6}\,\text{nm}$$

Define resolution and magnification and explain the differences between these terms, with reference to light microscopy and electron microscopy

The *resolution* of an image is the ability to distinguish between to very closely located objects; the higher the resolution of an image, the greater the detail that can be observed. Electron micrographs tend to have a higher resolution than light micrographs at the same magnification. *The light microscope has a maximum resolution of 200 nm.*

The reason behind this is the fact that visible light has a minimum wavelength of 400 nm, and half of a light wave can collide and reflect off of an object.

Electron microscopes can give a resolution of 0.5 nm. Electrons are shot at the specimen to be observed, through which the electrons pass and hit a fluorescent screen, which gives an overall black and white image. However, if the subject being observed is stained beforehand, a greater clarity can be achieved.

1.2 Cells as the basic units of living organisms

Recognise organelles and other cell structures found in eukaryotic cells and outline their structures and functions, limited to:

- *cell surface membrane*
- *nucleus, nuclear envelope and nucleolus*
- *rough endoplasmic reticulum*
- *smooth endoplasmic reticulum*
- *Golgi body (Golgi apparatus or Golgi complex)*

- *mitochondria (including the presence of small circular DNA)*
- *ribosomes (80S in the cytoplasm and 70S in chloroplasts and mitochondria)*
- *lysosomes*
- *centrioles and microtubules*
- *cilia*
- *microvilli*
- *chloroplasts (including the presence of small circular DNA)*
- *cell wall*
- *plasmodesmata*
- *large permanent vacuole and tonoplast of plant cells*

The *cell surface membrane* is present in both plant and animal cells. It is about 7 nm thick and has three layers which are visible at high magnification. The membrane is *partially permeable* and controls exchange between the cells and its environment.

The *nucleus* consists of the *nuclear envelope* and the *nucleolus*. The nuclear envelope is made up of two membranes, the outer of which is continuous with the endoplasmic reticulum. This envelope has many small pores called *nuclear pores* which allow and control exchange of substances between the nucleus and the cytoplasm. Substances such as messenger RNA (mRNA), transfer RNA (tRNA) and ribosomes are substances that leave the nucleus. Proteins, nucleotides, ATP and some hormones are examples of substances that enter the nucleus. The nucleus contains the chromosomes, which contain DNA, the genetic material that encodes genetic information. The average cell contains almost two metres of DNA, which is folded up into a very compact shape. This is done with the help of histone proteins. This combination of DNA and proteins is called *chromatin*, which also contains some RNA. The cell may also contain one or more structures called *nucleoli* (singlar: nucleolus). It has its own DNA, the instructions of which it uses to synthesise ribosomes. This DNA is one or two chromosomes which contain the genetic code for ribosomal RNA (rRNA), which is the form of RNA used in the manufacture of ribosomes. It also contains the genes for making tRNA. Around the core of the nucleolus are less dense units where the ribosomal subunits are assembled, combining rRNA and ribosomal proteins. The size of a cell is related to the amount of ribosomes it makes. The structures making up the nucleolus are only together when ribosomes need to be synthesised, during nuclear division, ribosomes need not be synthesised, as a result the nucleolus does not exist during cell division.

The *endoplasmic reticulum (ER)* is of two types: *smooth endoplasmic reticulum (SER)* and *rough endoplasmic reticulum (RER)*. Both of these structures are continuous with the outer layer of the nuclear membrane. The membranes of the ER form flattened compartments called *sacs/cisternae*. Processes take place inside these cisternae separate from the cytoplasm. Transport of molecules can also occur through the ER separate from the rest of the cytoplasm. The RER is so called because it is covered with numerous ribosomes, which are the sites of protein synthesis. They are found free in the cytoplasm as well as part of the RER. The SER lacks ribosomes, thus appearing smooth. Its function is to produce lipids, steroids, such as cholesterol and hormones such as testosterone and oestrogen. The SER stores calcium ions which are involved in muscle contraction and hence muscle cells are seen to be abundant in muscle cells.

The *Golgi body/apparatus/complex* is a stack of flattened sacs called cisternae. There may be multiple of these in a single cell, and the stack is constantly forming at one end from vesicles which bud off of the ER, and are broken down again at the other end to form *Golgi vesicles*. It is responsible for collecting and processing certain molecules, especially proteins from the RER. It contains hundreds of enzymes for this purpose, and after processing the molecules are transported away in Golgi vesicles. The release of these molecules is called secretion and the pathway taken by these molecules is called the secretion pathway. Some of the functions of the Golgi apparatus are as follows:

- Making lysosomes.

- Sugars are added to proteins making molecules called glycoproteins.
- Sugars are added to lipids making glycolipids.
- Golgi enzymes are involved in the synthesis of new cell walls during plant cell division.
- Mucin in the respiratory tract is released by the Golgi apparatus.

The *mitochondrion* (plural: mitochondria), is bound by two membranes, where the inner one forms various finger-like projections called *cristae* (singular: crista), which stick into the inside of the mitochondrion, which is called the matrix. The number of mitochondria in a cell directly corelates to how much energy its processes require. Mitochondria perform aerobic respiration, releasing energy from high-energy molecules, transferring it to molecules of *ATP (adenosine triphosphate)*, which is known as the universal energy carrier, which carries the energy in all living cells. The ATP produced spreads to any part of the cell where it is needed, being a soluble molecule, where it is broken down to adenosine diphosphate (ADP), releasing energy.

Ribosomes are structures that are only visible under an electron microscope. They are seen to consist of two subunits: one large and one small. They are measured in S units, which are a measure of how rapidly substances centrifuge, where the the faster the sedimentation, the larger the S number. *Eukaryotic ribosomes are 80S whereas prokaryotic ribosomes are 70S.* Mitochondria and chloroplasts contain 70S ribosomes, showing that they were once prokaryotic.

Lysosomes are sacs bound by a single membrane. In plant cells, the large central vacuole *may* act as the lysosome. They contain digestive enzymes which can engulf and destroy unwanted cell components such as molecules or organelles. They play a major role in endocytosis which is the process that occurs when a cell takes in or engulfs an external body. Lysosomal enzymes may be released from the cell for extracellular digestion, which is called *exocytosis*. Lysosomal enzymes may even be released into the cell itself, resulting in the digestion of the whole cell, called *autolysis*.

Microtubules are long, rigid and hollow tubes found in the cytoplasm. They make up the cytoskeleton, which is the structural component of cells that determines cell shape. They are made of tubulin, which is of two forms: α-tubulin and β-tubulin which combine to form dimers. These dimers join end to end forming protofilaments, thirteen of which line up alongside each other in a ring to form a cylinder with a hollow centre, which is the microtubule. Microtubules also:

- Secretory vesicles and other organelles and cell components are transported along the exterior of microtubules, forming an intracellular transport system.
- A spindle of microtubules is used for the separation of chromosomes during nuclear division.
- Microtubules form part of the structure of centrioles.
- Microtubules are essential in the beating movements of cilia and flagella.

Microtubules are assembled at special locations within the cell called *microtubule organising centres (MTOCs)*.

A *centriole* is a hollow cylinder about 500 nm long, formed from a ring of nine triplets of microtubules. Just outside the animal nucleus, there are two centrioles, perpendicular to one another, in a region known as the *centrosome*[1]. The centrosome is the MTOC, where centrioles are needed for the production of cilia. Centrioles are found at the bases of cilia and flagella, where they are known as basal bodies acting as MTOCs.

Cilia are whip-like beating extensions of eukaryotic cells. Cilia have two central microtubules and a ring of nine microtubule doublets (MTDs) around the outside. This is referred to as the $9 + 2$ structure. Each MTD has an A and a B microtubule, which have a rings of of 13 protofilaments and 10 protofilaments respectively. The A microtubules have arm-like structures, made of the protein dynein which connect with the B molecules of neighbouring MTDs during beating. There exists a basal body at the base of the cilia,

[1] Extension content, out of syllabus.

identical to centrioles, from which cilia grow. The beating motion of the cilia is caused by the dynein arms making contact with and sliding along neighbouring MTDs. This sliding motion is then converted to bending. This can cause liquid along the cell to move, or the cell to move through the liquid. Single celled organisms use these structures for locomotion. They are present lining the respiratory tract.

Microvilli (singular: microvillus) are finger-like extensions of the cell surface membrane. They are typical of certain animal cells such as the epithelial cells. They increase the surface area of the cell.

Chloroplasts are structures found in plant cells that are the site of photosynthesis in plants. Light energy is absorbed by photosynthetic pigments, chlorophyll, that are found on the membranes of the chloroplast. The membrane consists of fluid-filled sacs called *thylakoids* which are flattened, membrane-bound, and stack up like piles of coins, forming structures called grana (meaning granular). This absorption of light is called the *light-dependent* stage of photosynthesis. The *light-independent* stage uses the energy and reducing power generated during the first stage to convert carbon dioxide into sugars, a process that takes place in the *stroma*, where the sugars formed are stored in the form of starch grains. Lipid droplets are also found here, which are reserves to make membranes or are the residues of membranes broken down. Chloroplasts also have 70S ribosomes and circular DNA.

2 Biological molecules

2.1 Testing for biological molecules

Describe and carry out the Benedict's test for reducing sugars, the iodine test for starch, the emulsion test for lipids and the biuret test for proteins

Benedict's test for reducing sugars

We are required to mix equal volumes of given sample and Benedict's solution (copper (II) sulphate) and heat for a period of time. The solution is initially blue in colour, but it changes colour after heating, which is usually done in a water bath. Following are the colours gotten and their implications:

colour	implication
blue	reducing sugar absent
green	low concentration of reducing sugar
orange	medium concentration of reducing sugar
red	high concentration of reducing sugar

Iodine test for starch

Adding aqueous iodine to sample will cause the colour to change from dark brown to blue-black in presence of starch in the sample. Otherwise the aqueous iodine remains dark brown.

Biuret test for proteins

Adding biuret solution to a sample changes its colour to mauve (a shade of purple) in presence of protein. Otherwise colour remains unchanged as blue.

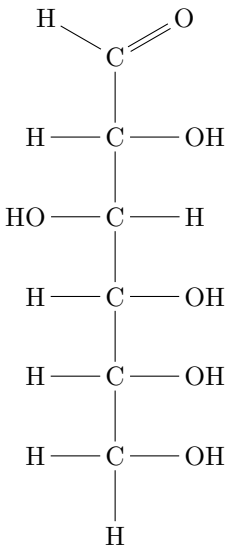
Describe and carry out a test to identify the presence of non-reducing sugars, using acid hydrolysis and Benedict's solution

For concentrations very close together in value, the above results for Benedict's solution are not enough. For this, we observe the solution as it is being heated and record the time required for it to first change colour.

Describe and carry out a test to identify the presence of non-reducing sugars, using acid hydrolysis and Benedict's solution

2.2 Carbohydrates and lipids

Describe and draw the ring forms of α-glucose and β-glucose



Define the terms monomer, polymer, macromolecule, monosaccharide, disaccharide and polysaccharide

Monomers are small single subunits which bond with many repeating subunits to form large molecules called *polymers*.

A *monosaccharide* is a molecule consisting of a single sugar unit with the general formula $(CH_2O)_n$. A *disaccharide* is a sugar molecule consisting of two monosaccharides joined together by a glycosidic bond. A *polysaccharide* is a polymer whose subunits are monosaccharides joined together by glycosidic bonds.

State the role of covalent bonds in joining smaller molecules together to form polymers

State that glucose, fructose and maltose are reducing sugars and that sucrose is a non-reducing sugar

Describe the formation of a glycosidic bond by condensation, with reference to disaccharides, including sucrose, and polysaccharides

3 Enzymes

3.1 Mode of action of enzymes

State that enzymes are globular proteins that catalyse reactions inside cells (intracellular enzymes) or are secreted to catalyse reactions outside cells (extracellular enzymes)

Explain the mode of action of enzymes in terms of an active site, enzyme–substrate complex, lowering of activation energy and enzyme specificity, including the lock-and-key hypothesis and the induced-fit hypothesis

Enzyme molecules have a special feature called an *active site*. This is where the *substrate* binds to the enzyme.

The concept where the active site and substrate have very specific complementary shapes is called the *lock-and-key hypothesis*. The substrate is held in place by temporary bonds which form between the substrate and R groups in the active site of the enzyme's amino acids. Due to the specificity in this hypothesis, each enzyme only acts on one type of substrate.

New evidence paved way for the *induced-fit hypothesis*. This adds the idea that the enzyme, and sometimes the substrate, can change shape slightly as the substrate molecules enters the enzyme in order to ensure a perfect fit.

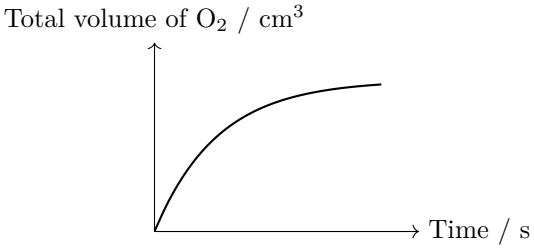
An anzyme may catalyse a reaction where the substrate is split into two molecules, or where two substrates are joined into one molecule. The substrate(s) bind to the active site, temporarily forming the *enzyme-substrate complex*, where after the reaction is done, the products leave the active site.

An enzyme catalysed reaction reduces the *activation energy* of a reaction, hence increasing the rate of the reaction.

Investigate the progress of enzyme-catalysed reactions by measuring rates of formation of products using catalase and rates of disappearance of substrate using amylase

Catalase is the enzyme that catalyses the breakdown of hydrogen peroxide into water and oxygen. We may collect the gaseous product, oxygen to investigate the rate of this reaction.

A graph is drawn of total volume of oxygen collected against time at which it is collected.



Notice that, at the beginning of the reaction the rate of reaction is higher and it decreases sa the reaction progresses due to decrease in susbtrate concentration.

We may also do so by measring the rate of disappearance of starch by using amylase to measure rate of amylase activity.

Outline the use of a colorimeter for measuring the progress of enzyme-catalysed reactions that involve colour changes

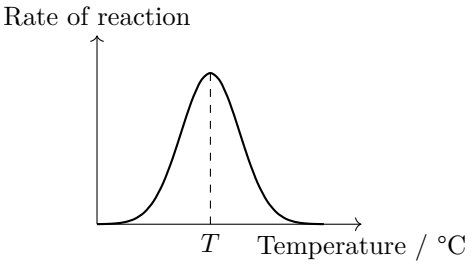
Colorimeters can give quantitative readings for colors. As it does, we can investigate colour changes using it.

3.2 Factors that affect enzyme action

Investigate and explain the effects of the following factors on the rate of enzyme-catalysed reactions:

- *temperature*
- *pH (using buffer solutions)*
- *enzyme concentration*
- *substrate concentration*
- *inhibitor concentration*

Temperature

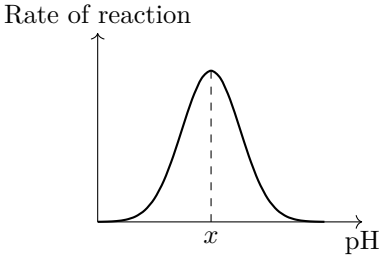


The above graph shows the trend of all enzyme-catalysed reactions with changes in temperature.

At low temperatures, the reaction takes place very slowly as the molecules involved have very little kinetic energies, which means the substrate and enzyme collide very infrequently. With greater energy, collisions are more frequent and more reactions happen. Above a certain temperature however, the enzyme molecule gains so much kinetic energy that the molecule vibrates to the extent that some of the bonds holding the molecule in its shape break apart. It becomes impossible for the enzyme-substrate complex to form as a result.

The temperature at which enzymes have their highest rate of reaction (T in the given diagram) is called their *optimum temperature*.

pH

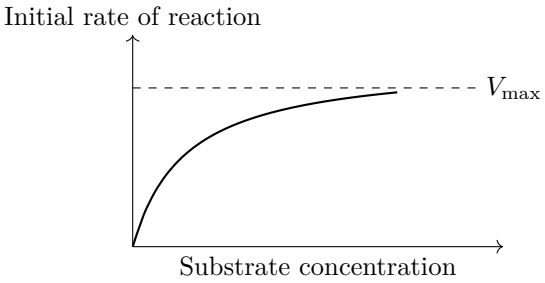


pH is a measure of the concentration of hydrogen ions in a solution, where low pH shows high number of hydrogen ions and vice versa. Ions in amino acids are affected by the presence of hydrogen and hydroxide ions due to charges, and as a result pH affects enzyme shapes. As such there is an *optimum pH* at which the enzyme has its highest rate of reaction, where the enzyme's shape is unscathed.

Enzyme concentration

Enzyme concentration is directly related to rate of reaction. As enzyme concentration increases, rate of reaction increases and vice versa.

Substrate concentration



If you go on increasing substrate concentration, keeping the enzyme concentration constant, there comes a point when every enzyme active site is full. If more substrate is added, the enzyme simply cannot work faster.

The maximum possible rate is represented as $V_{\max}$, which stands for maximum velocity.

Inhibitor concentration

With increase in inhibitor concentration, rate of reaction decreases and vice versa.

Explain that the maximum rate of reaction (V_{max}) is used to derive the Michaelis–Menten constant (K_m), which is used to compare the affinity of different enzymes for their substrates

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For $\frac{1}{2}V_{\max}$, the corresponding substrate concentration is called the Michaelis-Menten constant K_m. As such, we can assume that, the lower the K_m, the faster the rate of reaction reaches $\frac{1}{2}V_{\max}$ and hence $V_{\max}$. This shows that a lower K_m value corresponds to a greater enzyme activity and vice versa.

Explain the effects of reversible inhibitors, both competitive and non-competitive, on enzyme activity

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Competitive inhibitors have a similar shape to an enzymes substrate and hence bind to the active site,

blocking the substrate itself to bind, slowing down the rate of the reaction.

Non-competitive inhibitors do the same, but these molecules bind to a different part of the enzyme, altering its shape. As a result, the enzyme cannot carry out its reactions.

Investigate the difference in activity between an enzyme immobilised in alginate and the same enzyme free in solution, and state the advantages of using immobilised enzymes

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Enzymes are immobilised when used industrially, in alginate. Substrates are made to run *over* these immobilised enzymes, rather than having the enzymes diffuse freely in a solution.

Immobilised enzymes can be reused, and the products may also be enzyme free. These enzymes do not denature as easily under pH or temperature changes.

4 Cell membranes and transport

4.1 Fluid mosaic membranes

Describe the fluid mosaic model of membrane structure with reference to the hydrophobic and hydrophilic interactions that account for the formation of the phospholipid bilayer and the arrangement of proteins

Phospholipids are polar molecules with hydrophobic tails and hydrophillic heads. Such molecules, interact with water to form ball like structures called *micelles* or sheet like structures called *bilayers*.

The basic structure of cell membranes consist of phospholipids. In this base structure, there are multiple other molecules embedded. The combination of these molecules with the phospholipid bilayer is what gives the *mosaic* structure. The individual layers of the bilayer itself can move (slide) about as well as the protein molecules embedded, giving *fluidity* to the structure.

Describe the arrangement of cholesterol, glycolipids and glycoproteins in cell surface membranes

Cholestorol, glycolipids, and glycoproteins are the molecules embedded into the cell surface membranes.

Describe the roles of phospholipids, cholesterol, glycolipids, proteins and glycoproteins in cell surface membranes, with reference to stability, fluidity, permeability, transport (carrier proteins and channel proteins), cell signalling (cell surface receptors) and cell recognition (cell surface antigens – see 11.1.2)

Phospholipids

Phospholipid tails may be unsaturated or saturated. Unsaturated tails are bent, and so phospholipids with such tails are more fluid, having more freedom to move about. Plus, the longer the tail is, the less fluid the bilayer is too.

Under higher temperatures, the bilayer loses fluidity, microorganisms respond by decreasing saturation of the phospholipid tails.

Cholesterol

Cholesterol is a relatively small molecule with hydrophilic heads and hydrophobic tails. They fit into the phospholipid bilayer with the same orientation as the phospholipids themselves. These molecules reduce fluidity of the bilayer by getting between the phospholipid molecules. So the greater the proportion of cholesterol, the less fluid the bilayer. Cholesterol also prevents the decrease of fluidity of the phospholipid bilayer at low temperatures. The polarity of these molecules also prevents the passage of ions and polar molecules across the membrane.

Glycolipids, glycoproteins and proteins

Glycolipids and glycoproteins are lipid and protein molecules combined with portions of carbohydrate.

The carbohydrate chains of glycoproteins help them act as *receptor molecules*. This allows these molecules to bind with particular substances at the cell membrane. Certain *signalling receptors* recognise messenger molecules such as hormones and neurotransmitters. Upon binding of the receptor with such molecules, a series of chemical reactions begins inside the cell.

Glycolipids and glycoproteins can act as cell markers, *antigens*, allowing cells to recognise each other. These interactions are important in growth, development and immune responses.

Certain proteins are *transport proteins*. These provide hydrophillic channels for ions and polar molecules to pass through the membrane. They are *channel proteins* and *carrier proteins*.

Some proteins are enzymes embedded into the cell membrane itself.

Certain proteins on the interior of the cell surface membrane are attached to a system of protein filaments known as the cytoskeleton. This controls the shape of the cell.

Outline the main stages in the process of cell signalling leading to specific responses:

- *secretion of specific chemicals (ligands) from cells*
- *transport of ligands to target cells*
- *binding of ligands to cell surface receptors on target cells*

In cell signalling, following occurs.

1. A stimulus causes release of a *cell signalling molecule* or *ligand*. Glucagon, insulin are molecules which are hormones, are examples of ligands.
2. This ligand is transported to the target cells, which, in case of hormones, is by means of the circulatory system via blood.
3. The ligand then binds to cell surface receptors on the target cells. The receptors here, are shaped complementarily to the signalling molecules in question.

4.2 Movement into and out of cells

Describe and explain the processes of simple diffusion, facilitated diffusion, osmosis, active transport, endocytosis and exocytosis

Simple diffusion is the net, passive movement of a substance from a region of its higher concentration to that of its lower concentration as a result of random motion of its molecules. Across the cell surface membrane, non-polar molecules such as oxygen and carbon dioxide, move via this process. Water molecules too, even though they are polar, move by this same process as they are small enough. *Hydrophobic molecules can cross membranes because the interior of the membrane is hydrophobic.* Factors that affect diffusion are as follows:

- Steepness of concentration gradient: The difference between the regions of high and low concentration is the concentration gradient. The higher the difference between these concentrations, the steeper the concentration gradient. The steeper the concentration gradient, the faster the diffusion.
- Temperature: Particles at high temperatures have greater kinetic energy, so they move and diffuse faster.
- Nature of the molecule: Large molecules diffuse slower than smaller molecules. Polar molecules also diffuse slower than non polar molecules.
- Surface area to volume ratio: The greater the surface area to volume ratio of the cell, the faster the rate of diffusion. Note that, the greater the 3-dimensional size of the cell, the lower its surface area to volume ratio.

We may say that it is due to diffusion that the size of the cell is what it is. Diffusion may only take place across very small distances, so cells must accomodate those very small distances.

Diffusion of large polar molecules and ions across the membrane occurs only with the help of certain protein molecules. Diffusion that requires the help of such proteins is known as *facilitated diffusion*. Described below are the types of proteins involved:

- Channel proteins: These are water filled pores. These are gated structures that allow charged substances such as ions to diffuse through. They are gated in the sense that part of the protein molecule on the interior may move to open or close the pore, allowing control over ion exchange. Some of these proteins require energy in the form of ATP to change shape.
- Carrier proteins: These are proteins that may flip between two shapes, where there is a binding site open alternatively on one side of the membrane. When the molecule binds to this carrier protein, it changes shape, expelling the molecule to the other side. Carrier proteins that require energy to change shape are known as pumps. They are involved in active transport.

The rate of facilitated diffusion depends on the proportion of transport proteins present on the membrane.

Osmosis is the net diffusion of water molecules from a region of higher water potential to a region of lower water potential, through a partially permeable membrane.

Water potential may be thought of as the concentration of water. It is measured in kPa. We consider water, which is a solution with the highest possible water potential, to have water potential, $\psi = 0\,\text{kPa}$. Thus, any solution with water potential lower than this, is negative, and the more negative the solution, the lower the water potential.

Osmosis is a specific type of diffusion, and is hence affected in the same way by the same factors as diffusion.

Active transport is the movement of molecules or ions through transport proteins across a cell membrane, against their concentration gradient, using energy from ATP. The energy is used to make the carrier protein involved change shape.

Investigate simple diffusion and osmosis using plant tissue and non-living materials, including dialysis (Visking) tubing and agar

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Figure it out.

Illustrate the principle that surface area to volume ratios decrease with increasing size by calculating surface areas and volumes of simple 3-D shapes (as shown in the Mathematical requirements)

Try it.

Investigate the effect of changing surface area to volume ratio on diffusion using agar blocks of different sizes

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Figure it out.

Explain the movement of water between cells and solutions in terms of water potential and explain the different effects of the movement of water on plant cells and animal cells (knowledge of solute potential and pressure potential is not expected)

Excessive osmosis into an animal cell may cause a buildup of pressure on the interior of the cell, causing it to burst. Excessive osmosis out of an animal cell causes it to lose its shape and shrink. In case of plant cells, excessive inward osmosis causes the cell to become turgid, as the cell wall prevents bursting. In case of excessive outward osmosis, the decrease in interior pressure causes the cell to shrink, and the cell membrane to tear away from the cell wall. In this condition, it is said that the cell is *plasmolysed*.

5 The mitotic cell cycle

5.1 Replication and division of nuclei and cells

Describe the structure of a chromosome, limited to:

- *DNA*
- *histone proteins*
- *sister chromatids*
- *centromere*
- *telomeres*

Chromosomes are structures observable right before eukaryotic cells divide. It is made of two identical structures called *chromatids*, and the two identical chromatids of one chromosome are called *sister chromatids*. The entire structure of the chromosome is made from molecules called *DNA*, wrapped around special proteins called *histone proteins* and the mixture of DNA and proteins such as histones is called chromatin. *Histones are basic, and thus can easily interact with the acidic DNA.* These two sister chromatids are held together by a narrow region called the *centromere*, and the position of this centromere along the chromosome is characteristic for a particular chromosome. *Telomeres* are regions of repeating nucleotide sequences present at either end of a chromatid.

Explain the importance of mitosis in the production of genetically identical daughter cells during:

- *growth of multicellular organisms*
- *replacement of damaged or dead cells*
- *repair of tissues by cell replacement*
- *asexual reproduction*

Mitosis is the division of a nucleus into two so that the two daughter cells have exactly the same number and type of chromosomes as the parent cell. As such, it is imporant in the following cases

- Growth of multicellular organisms: The production of genetically identical cells allows the growth of multicellular organisms from unicellular zygotes. This may be across the entire body (as in the case of animals) or specific growing regions (as in the case for plants).
- Replacement of damaged or dead cells and *repair of tissues by cell replacement*: Cells are constantly dying and being replaced by identical cells, which are produced from neighbouring or stem cells via mitosis.
- Asexual reproduction: The production of new individuals of a species by a single parent organism is known as *asexual reproduction*. For unicellular organisms, cell division results in reproduction. However, in plants, it may take the form of "budding off" where the parent plant has plants that bud off from its body, forming separate organisms down the line.

Outline the mitotic cell cycle, including:

- *interphase (growth in* G_1 *and* G_2 *phases and DNA replication in S phase)*
- *mitosis*
- *cytokinesis*

In *interphase* the cell grows after cell division and carries out its normal functions. The DNA in the nucleus replicates so that each chromosome consists of two identical sister chromatids. This phase of the cell cycle is called S phase (which stands for synthesis), which is relatively short. The gap after cell division and before the S phase is called the G_1 phase (standing for first phase of growth). In this phase, the centrosomes also replicate.

The gap after S phase and before cell division is called the G_2 phase.

During G_1, cells make the RNA, enzymes and other proteins needed for growth.

During G_2, the cell continues to grow and the new DNA that was made during the S phase is checked and repaired. There is a sharp increase in the production of the protein tubulin, which is needed to make microtubules for the mitotic spindle.

Mitosis, or M phase follows interphase. Mitosis itself consists of form phases: prophase, metaphase,

anaphase and telophase (in that sequence, the abbreviation PMAT can be used to remember this sequence).

Cytokinesis begins in telophase, which is the division of the cytoplasm and cell into two by constriction from the edges of the cell.

Outline the role of telomeres in preventing the loss of genes from the ends of chromosomes during DNA replication

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The main function of telomeres is to ensure that the ends of the molecule are included in the replication and not left out when DNA is replicated. This is because, the copying enzyme cannot run to the end of a strand of DNA and complete the replication, it stops a little short of the end. The telomeres themselves consist of *multiple repeat sequences*. As long as extra bases are added to the telomere during each cell cycle to replace those that are not copied, no vital information will be lost. The enzymes that does this is called *telomerase*

Fully differentiated or specialised cells have their telomeres get a little shorter, as the mechanism that tops up telomeres is absent, until the vital DNA is no longer protected and the cell dies. This is one of the mechanisms of aging.

Outline the role of stem cells in cell replacement and tissue repair by mitosis

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A *stem cell* is a relatively unspecialised cell that retains the ability to divide an unlimited number of times, and which has the potential to become a specialised cell (such as a blood cell or muscle cell). So, as per required, for a damaged cell or dead cell, these stem cells produce the specialised cell.

Explain how uncontrolled cell division can result in the formation of a tumour

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Cancers start when changes occur in the genes that control cell division. The term for a mutated gene that causes cancer is an *oncogene*. These mutations cause uncontrolled cell growth, which cause the production of a mass of cells known as a *tumour*. Tumours that spread from their site of origin are known as *malignant tumours*, and those that do not are called *benign tumours*.

5.2 Chromosome behaviour in mitosis

Describe the behaviour of chromosomes in plant and animal cells during the mitotic cell cycle and the associated behaviour of the nuclear envelope, the cell surface membrane and the spindle (names of the main stages of mitosis are expected: prophase, metaphase, anaphase and telophase)

Prophase

In early prophase, chromosomes start to appear as the chromatin coils up, becoming shorter and thicker. In late prophase, the nuclear envelope breaks up into small vesicles and essentially disappears, as does the nucleolus which forms part of several chromosomes. The chromosomes are now seen to consist of two identical chromatids, where each chromatid contains one DNA molecule. The centrosomes move to opposite ends of the nucleus where they form poles of a *spindle* and the spindle itself is formed at the end of prophase.

Metaphase

Centrosomes, now at the poles, help to organise production of the spindle microtubules. Chromosomes line up across the equator of the spindle, which are attached by their centromeres to the spindle.

Anaphase

Chromatids start moving to opposite poles, centromeres first, pulled by the microtubules.

Telophase

Nucleoli begin reforming, as do nuclear envelopes. The centrosomes that were at either pole now separate two either cell. Chromatids, having reached the poles of the spindle, will now uncoil again, which now contains one DNA molecule each, which will replicate itself during interphase before the next division.

The above describes the case for animal cells. The only differences in the case of plants cells is that plant cells do not contain centrosomes and that after division of a plant cell, a new cell wall must form between the daughter nuclei.

Interpret photomicrographs, diagrams and microscope slides of cells in different stages of the mitotic cell cycle and identify the main stages of mitosis

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Look it up.

6 Nucleic acids and protein synthesis

6.1 Structure of nucleic acids and replication of DNA

Describe the structure of nucleotides, including the phosphorylated nucleotide ATP (structural formulae are not expected)

Nucleotides are molecules consisting of a nitrogen containing base, a pentose sugar and a phosphate group.

There are four different nitrogen containing bases: adenine, guanine, thymine and cytosine (abbreviated and referred to as A, G, T and C, respectively).

The phosphate group present is what gives nucleic acids their acidic nature.

State that the bases adenine and guanine are purines with a double ring structure, and that the bases cytosine, thymine and uracil are pyrimidines with a single ring structure (structural formulae for bases are not expected)

We may remember the above with pyrimidine – the longer name, has smaller structures and hence only one ring, and purine – the smaller name has larger structures with two rings.

Purines – double ring – A, G. Pyrimidines – single ring – U, C and T.

Describe the structure of a DNA molecule as a double helix, including:

- *the importance of complementary base pairing between the 5′ to 3′ strand and the 3′ to 5′ strand (antiparallel strands)*
- *differences in hydrogen bonding between C–G and A–T base pairs*
- *linking of nucleotides by phosphodiester bonds*

The two ends of a DNA strand are called the 5′ end and the 3′ end. At the 5′ end is phosphate and at the 3′ end is sugar. Each DNA molecule is made up of two polynucleotide chains, and these two chains are right handed helices. For these two strands to be held together, the bases of either molecule must be held together. This is done by the means of hydrogen bonding between "complementary base pairs".

Adenine pairs with thymine (A–T) and cytosine pairs with guanine (C–G). *A links with T by two hydrogen bonds; G links with C by three hydrogen bonds.* A purine always binds with a pyrimidine.

In each polynucleotide strand, the nucleotides themselves are bound by *phosphodiester bonds*, as the phosphate group is bound to two sugar groups.

Some miscellaneous information about the structure of DNA follows:

- The two chains coil around each other to form a double helix.
- Each chain has a sugar-phosphate backbone with bases projecting at right angles.
- The bases in one chain are attracted to the bases in the other by means of hydrogen bonding, which holds the two chains together.
- Purines are two rings wide and pyrimidines are one ring wide, and since purines always bind with pyrimidines, the distance between two strands of a DNA molecule is always 3 rings.
- A complete turn of the double helix takes place every 10 base pairs.

Describe the semi-conservative replication of DNA during the S phase of the cell cycle, including:

- *the roles of DNA polymerase and DNA ligase (knowledge of other enzymes in DNA replication in cells and different types of DNA polymerase is not expected)*
- *the differences between leading strand and lagging strand replication as a consequence of DNA polymerase adding nucleotides only in a 5′ to 3′ direction*

DNA polymerase is an enzyme that copies DNA. Note that, it can only do so in the 5′ to 3′ direction. The process of DNA replication follows:

1. The two DNA strands are first "unzipped" and partially separated.
2. A molecule of DNA polymerase attaches to each of the single strands. It adds one new nucleotide at a time, which is held by hydrogen bonding to the strand being copied.
3. For the strand that runs $3'$ to $5'$, the copying process produces a continuous new strand of DNA. This new strand is called the *leading strand*.
4. For the strand that runs $5'$ to $3'$, the polymerase cannot copy continuously, so the copying is done in short fragments called *Okazaki fragments*. This new strand is called the *lagging strand*.
5. DNA polymerase attaches nucleotides of the new strands by means of hydrogen bonding to the template strand. Another enzyme *DNA ligase*, connects the neighbouring nucleotides with phosphodiester bonds.

This method of DNA replication is said to be *semi-conservative*, because upon replication of each DNA molecule, half the original molecule remains (is conserved) in each of the new molecules.

Describe the structure of an RNA molecule, using the example of messenger RNA (mRNA)

An RNA molecule is a single polynucleotide strand. Here, the base thymine is replaced by uracil.

6.2 Protein synthesis

State that a polypeptide is coded for by a gene and that a gene is a sequence of nucleotides that forms part of a DNA molecule

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Self explanatory.

Describe the principle of the universal genetic code in which different triplets of DNA bases either code for specific amino acids or correspond to start and stop codons

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Each amino acid is coded for by a sequence of three subsequent DNA bases. Given the four bases, there are 64 possible combinations of these "triplets". But there are only 20 amino acids to code for. So multiple triplet sequences can code for the same amino acid. The triplets are also called *codons*. There are codongs that correspond to signals to start or stop transcribing called start codons or stop codons.

Describe how the information in DNA is used during transcription and translation to construct polypeptides, including the roles of:

- *RNA polymerase*
- *messenger RNA (mRNA)*
- *codons*
- *transfer RNA (tRNA)*
- *anticodons*
- *ribosomes*

The process by which mRNA is made from DNA is called *transcription*. The process by which the message carried by mRNA is decoded to make protein is called *translation*.

Transcription

The enzyme responsible for this process is *RNA polymerase*. The enzyme attaches to the beginning of the gene to be copied, it starts to unwind the DNA of the gene and another enzyme breaks the hydrogen bonds between the two strands. This creates two single stranded sections of DNA with the normal double helical structure either side of this unzipped section. Only one of the exposed strands is copied and it is known as the *template strand* or *transcribed strand* whereas the other is known as the *non template strand* or *non transcribed strand*. *Note that, uracil is present in RNA instead of thymine.*

As RNA polymerase moves along the gene, nucleotides appraoch and hydrogen bond with their complementary nucleotides in the DNA, and the new nucleotides that neighbour each other are bonded to each other by means of phosphodiester bonds by the RNA polymerase itself, forming the mRNA strand.

Once the phosphodiester bond is formed, the hydrogen bond to the template strand is unnecessary and breaks off. Once a stop codon is reached, the RNA polymerase leaves the DNA and releases the mRNA strand.

Translation

This is the process by which the sequence of codons in mRNA is converted to a sequence of amino acids in a polypeptide. It involves another type of rna called tRNA (transfer RNA, and mRNA stands for messenger RNA). Each amino acid has a different tRNA molecule to carry it. The amino acid is attached at one end of the molecule. At the other end of the molecule three projecting bases form an anticodon. This is complementary to the codon for the amino acid carried by that tRNA. Enzymes are responsible for making sure that each tRNA carries the correct amino acid.

When mRNA molecule arrives, molecule arrives at a ribosome, it enters a groove between the two subunits of the ribosome where it is held ready to recieve the first tRNA molecule. The tRNA with the anticodon complementary to the first codon on the mRNA enters the ribosome and attaches to the codon by hydrogen bonding. Two tRNA molecules can fit into the ribosome at any time, so the second tRNA enters the ribosome, which has anticodon which matches the second codon on the mRNA strand. The amino acids carried by these two tRNA are now side to side and bond via peptide bond. The first tRNA now leaves, the ribosome clicks forward one codon and the third tRNA enters, carrying the next amino acid. This process repeats until a stop codon is reached.

State that the strand of a DNA molecule that is used in transcription is called the transcribed or template strand and that the other strand is called the non-transcribed strand

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Self explanatory and done above.

Explain that, in eukaryotes, the RNA molecule formed following transcription (primary transcript) is modified by the removal of non-coding sequences (introns) and the joining together of coding sequences (exons) to form mRNA

In eukaryotes, the mRNA is modified before it leaves the nucleus, this is called RNA processing. RNA splicing occurs here, which is the removal of sections from the primary transcript (the initial strand of mRNA formed is called the primary transcript). The sections removed are called introns, the bits that remain are called exons, which have to be and are joined together. The introns that remain in the nuclear cytoplasm are then used to form other, new mRNA strands. The process of splicing allows one gene to code for several proteins or different forms of the same protein.

State that a gene mutation is a change in the sequence of base pairs in a DNA molecule that may result in an altered polypeptide

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It makes sense that the change in sequence of nucleotides in a codon may result in the change in the amino acids, and hence, polypeptides coded for. However, that may not always be the case because of the fact that many nucleotide sequences code for the same amino acid, so a change in base sequence may end up coding for the same nucleotide sequence.

Explain that a gene mutation is a result of substitution or deletion or insertion of nucleotides in DNA and outline how each of these types of mutation may affect the polypeptide produced

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The sequence may change by means of substitution, which only changes one base. However, insertion or deletion of a base, called *frame shift* mutation, will end up changing the entire sequence of the rest of the gene, and may cause significant change in the polypeptide.

7 Transport in plants