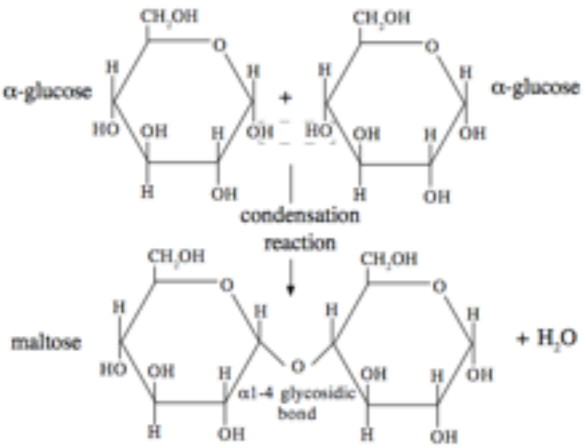
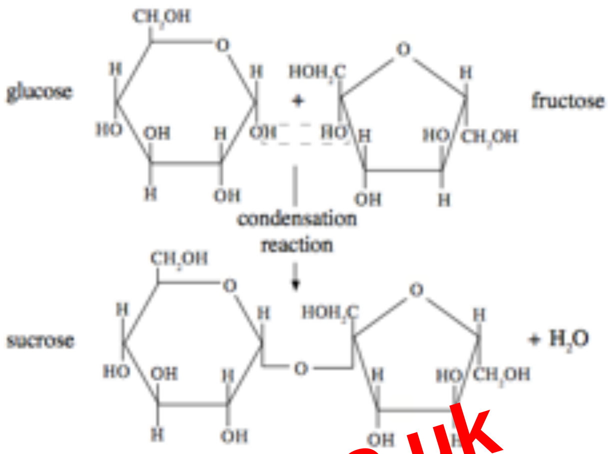
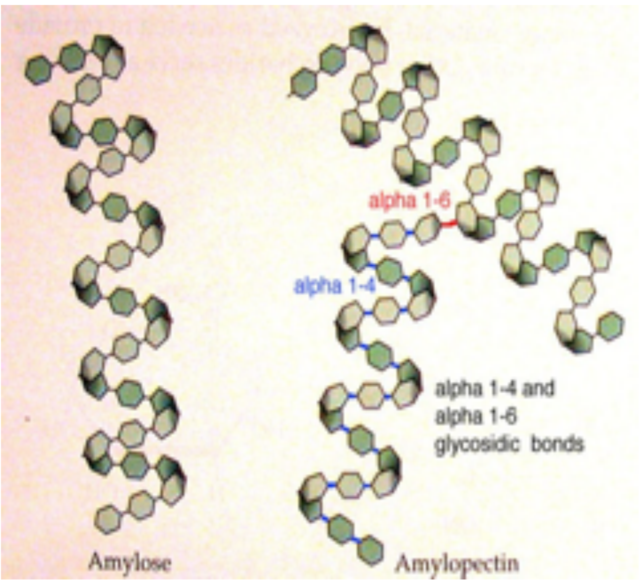
until alkaline. The original test is then repeated and should be positive, as the non-reducing sugars are hydrolysed into reducing sugars.

Disaccharides are two monosaccharides chemically joined by a glycosidic bond formed through a condensation reaction.
Two alpha-glucose units make maltose.
A glucose and fructose unit make fructose.
A glucose and galactose unit make lactose.
Under suitable conditions the addition of water to a disaccharide causes hydrolysis so that the glycosidic bond is broken releasing the constituent monosaccharides.





Polysaccharides are polymers formed by combining many monosaccharide molecules, illustrating the variety of different biological molecules that can be made from a few basic monomer units. These molecules are larger and more complex, making them insoluble. Insolubility in water means that they don't affect water potential of a cell and are good for storage. Also, their large size means that they cannot diffuse out of cells easily, again making them useful for storage.
Starch is a polysaccharide found in many parts of plants in the form of small granules and grains (e.g. within chloroplasts). Large amounts are found in seeds and storage organs of plants, so it is a major energy source in most diets. Iodine dissolved in potassium iodide solution turns from yellow to blue-black in the presence of starch.

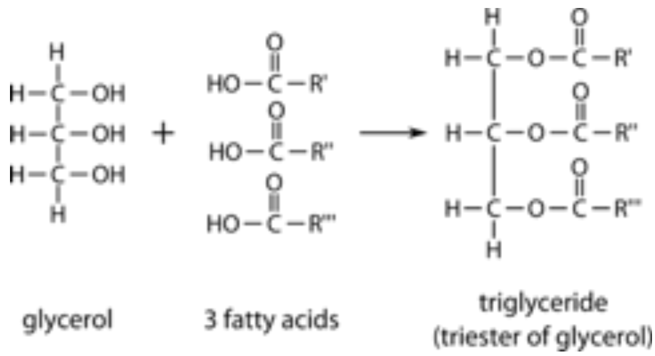


Starch is made up of around 200-100000 alpha glucose molecules joined by glycosidic bonds formed by a series of condensation reactions.
Starch is a heteropolysaccharide composed of amylose (unbranched chains of alpha glucose) and amylopectin (branched chains of alpha glucose). Amylose constitutes for around 20% of starch; it's alpha helical structure makes it compact, so lots can be stored in a small amount of space.
Amylopectin's branches can be acted on by enzymes simultaneously, increasing the rate at which glucose monomers are released by hydrolysis.
Also, starch is large and insoluble, doesn't affect water potential and is made up of alpha glucose which can be easily transported and readily used in respiration, making it ideal for energy storage.

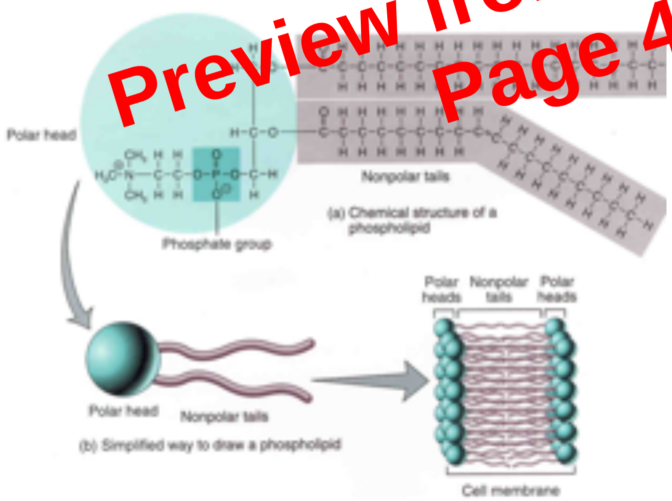
Lipids -

Lipids are organic compounds containing carbon, hydrogen and oxygen. The proportion of water they contain is less than that in other organic molecules such as carbohydrates. They are insoluble in water, but soluble in organic solvents such as alcohols and acetone.
Lipids are a valuable source of energy as they contain twice as much energy as the same mass of carbohydrates. They also play a role in insulating, as they slow conductors of heat and electricity (e.g. they help make up the myelin sheath around nerves). Their insolubility makes them useful for waterproofing (e.g. waxy cuticles) and they help provide flexibility to cell membranes. Finally, fat is used to protect delicate internal structure, such as the kidneys.

Triglycerides are a type of lipid consisting of three fatty acids combined to one glycerol molecule. Each fatty acids forms an ester bond with glycerol in a condensation reaction.
It is variations in the fatty acids that causes variations in properties of different fats and oils. A fatty acid is a carboxylic acid with a long aliphatic hydrocarbon chain. This hydrocarbon chain can be saturated (containing no double bonds between carbon atoms), unsaturated or polyunsaturated.



The high chemical strength of the C-H bond makes triglyceride molecules useful for energy storage, alongside their low mass to energy ratio. This makes them more compact and lighter to carry. Triglycerides are non-polar and therefore insoluble in water, so they do not affect water potential of cells, again making them useful for storage.
Finally, triglycerides release water when oxidised due to the high hydrogen to oxygen ration, making them a valuable source of water, particularly for organisms living in dry environments (e.g. camels).



Phospholipids are another type of lipid consisting of two fatty acids and one phosphate group attached to one glycerol molecule. Fatty acids are hydrophobic and phosphate groups are hydrophilic, so the phospholipid molecule has a hydrophobic tail and hydrophilic head. This makes the molecule polar, causing phospholipids to form micelles and bilayers in aqueous environments, such as the cell surface membrane. This helps to create a hydrophobic barrier.
Phospholipids are an essential part of the cell surface membrane, as they help provide flexibility and allow glycolipids to form - this is important for cell recognition.

The emulsion test can be used to test for the presence of lipids.

1. Take a dry sample and grease-free test-tube
2. Add $5cm^3$ ethanol to $2cm^3$ of sample
3. Shake to dissolve any lipid
4. Add $5cm^3$ of water and shake gently
5. A cloudy-white colour indicates the presence of a lipid. This is due to light refracting off oil droplets emulsified in water

[Using water instead of a sample can be used as a control measure]

Section 2: Nucleic Acids

<u>Nucleotides -</u>
Nucleic acids are polymers made up of many nucleotide monomers. Two important nucleic acids are RNA and DNA.
Nucleotides are made up of a pentose sugar, a phosphate group and a nitrogen containing organic base. These bases include cytosine, thymine, uracil, adenine and guanine.
The three components are joined through a condensation reaction to form a mono-nucleotide. When the pentose sugar of one mono-nucleotide and the phosphate group of another join through a condensation reaction, a phosphodiester bond is formed. This produces a dinucleotide. Many joined nucleotides form a polynucleotide.

Ribonucleic acid (RNA) is a single, relatively short polynucleotide chain in which the pentose sugar is always ribose and the possible bases are A, G, C and U.

<u>DNA -</u>
Deoxyribonucleic acid (DNA) contains a deoxyribose sugar, a phosphate group and the possible bases are A, G, C and T. The 5' carbon of the sugar is attached to the phosphate group, whilst the 3' carbon is attached to a hydroxyl group. DNA is made up of two long polynucleotide chains, joined by hydrogen bonds between base pairs. This gives DNA a helical structure, where the two strands run antiparallel to each other. The base pairing is specific; adenine always pairs with thymine and guanine always pairs with cytosine. The base pairs are said to be complementary to each other. The ratio of A to T is always the same, as is the ratio of C to G, but the proportion of the pairs varies within DNA.

DNA is a stable molecule because the phosphodiester backbone protects the chemically reactive bases within the helix. There is also base stacking, which occurs when there are interactive forces between the base pairs, helping to hold the DNA molecule together. Finally, the more C to G bonds there are, the more stable the DNA molecule, because there are 3 hydrogen bonds between the two organic bases.

DNA structure serves in aiding its function. DNA's main function is to pass on genetic information from cell to cell and generation to generation. The variety in the possibility of base sequences provides genetic diversity within organisms.
DNA is adapted to carry out this function by;

- Having a stable structure that passes between generations with little change
- The hydrogen bonds joining the two strands together can be separated during DNA replication and protein synthesis
- Base pairing allows for DNA to transfer information as mRNA
- It is a large, compact molecule so can carry lots of genetic information
- The sugar-phosphate back bond helps protect the genetic information from external forces that may corrupt it

DNA replication occurs before nuclear division and cytokinesis. The semi-conservative model of replication is universally accepted;

1. DNA helicase breaks the hydrogen bonds joining the complementary bases together
2. The DNA helix unwinds into two separate strands
3. Each exposed polynucleotide strand acts as template to which complementary free floating nucleotides bind to via specific base pairing
4. DNA polymerase catalyses the condensation reaction which form phosphodiester bonds between the newly bonded nucleotides
5. Each new identical molecule of DNA contains one of the original DNA strands, hence the name 'semi-conservative replication'

DNA polymerase's active site is only complementary to the 3' end of the new DNA strand, so nucleotides are added in a 5' to 3' direction and DNA polymerase works in a 3' to 5' direction. The up of a larger and smaller subunit that fit together. In eukaryotic cells ribosomes are 80S (25 nm in diameter) and in prokaryotes they are 70S, which is smaller.

- Cell wall
  The cell wall is an organelle found in plant cells, bacteria and fungi. In plant cells the cell wall is made up of the polysaccharide cellulose, cemented to other cells by a middle lamella made up of pectins. This provides functional and mechanical strength to the plant, prevent cells from bursting after making osmotic gains and allow for the passage of water throughout the plant. A plant cell wall also contains plasmodesmata; channels for exchanging substances between adjacent cells.
  The cell walls in algae are made up of cellulose and/or glycoproteins, where as the cell walls in fungi are made up of chitin, glycan and glycoproteins.

- Vacuoles
  Vacuoles are fluid-filled sacs surrounded by a single membrane called the tonoplast. The fluid contains mineral salts, sugars, amino acids and wastes. It may also contain pigments called anthocyanin, which attract pollinating insects.
  The cell vacuole increases the turgidity of a cell and provides a temporary food store.

Cell specialisation -
Unless they are a stem cell, all cells are specialised; the genes that they express help them to carry out a certain function. This affects the proteins they code for and therefore their ability to perform certain tasks.

Tissues - specialised cells that are aggregated to carry out a specific function. For example, epithelial tissues and xylem
Organs - Aggregates of tissues that are coordinated to perform a variety of functions. In mammals an example is the stomach; it contains muscle tissues to churn food, epithelium to produce secretions and connective tissues to hold the organ together.
Organ systems - Organs that work together as a single unit (e.g. the digestive system the respiratory system and the circulatory system).

Prokaryotic cell structure -
Prokaryotes cells are smaller than eukaryotes. They contain no membrane bound organelles and the genetic information is in the form of a free-floating single strand of circular DNA and plasmids, not attached to any histones. Prokaryotic cells are be less than 2 micrometers in diameter, whereas eukaryotic cells tend to be up to 50 times bigger.

Bacteria are a type of prokaryote ranging from 0.1 to 10 micrometers in length.
They possess a murein cell wall surrounded by a capsule of mucilaginous slime, helping bacteria to stick together. Within the cell wall there is a cell-surface membrane, 70S ribosomes, glycogen granules and oil droplets (for food reserves).
They do not have a nucleus or nuclear envelope, but a circular strand of free-floating DNA. There are also separate circular strands of DNA called plasmids, which can reproduce independently. It is the plasmids that can give rise to antibiotic resistance.
Some bacteria also have a flagellum for locomotion.
Prokaryotes like bacteria divide through binary fission. The circular DNA replicates and both copies attach to the cell membrane. The plasmids also replicate. The cell membrane grows between the two DNA molecules and pinches inwards. A new cell wall forms, creating two daughter cells with the same copy of circular DNA, but varying numbers of plasmids.

Viruses are another type of prokaryote.
They are acellular organisms ranging from 20-300 nanometers in length. A protein capsid with free-floating nucleic acids is contained within a lipid envelope covered in attachment proteins. Held within the capsid alongside the nucleic acids is reverse transcriptase, which is used by viruses for replication using the host cell's cell machinery.

Section 4: Transport Across Cell Membranes

Cell surface membrane -
The plasma membrane that surrounds a cell and forms the boundary between the cell cytoplasm and the environment. It allows for the cell's internal conditions to differ from those of the environment and controls the passage of substances

Components of the cell surface membrane:

- *Phospholipids*
  Phospholipids form a bilayer with the hydrophilic heads facing the outside of the cell membrane and the hydrophobic tails facing the centre of the cell membrane. It makes the cell membrane lipid-soluble, flexible and self-sealing. Water-soluble substances cannot pass through the phospholipid bilayer, only small non-polar molecules.
- *Proteins*
  Proteins are interspersed through the cell surface membrane, either at the surface of the bilayer or completely across. Proteins at the surface give mechanical support or may be part of glycolipids and receptors. Proteins that span across the bilayer tend to be protein channels or carrier proteins. Proteins can help cells adhere together.
  Protein channels allow water-soluble ions to diffuse across the membrane. Protein carriers bind to ions/molecules and move the molecules across the membrane through a conformational change in shape. Protein carriers are also used in active transport.
- *Cholesterol*
  Cholesterol molecules add strength to the membranes. Because cholesterol molecules are very hydrophobic, they prevent water loss and loss of ions from the cell. They increase rigidity of the cell membrane by binding to the fatty acid tails of the phospholipids, helping to reduce lateral movement of molecules. The membrane is less fluid at high temperatures.
- *Glycolipids*
  Glycolipids are formed of carbohydrates covalently bonded to lipids. The lipid is embedded in the bilayer of the cell surface membrane, whereas the carbohydrate extends outside the cell, acting as a recognition site. Glycolipids also help maintain the stability of the membrane and help cells attach to form tissues.
- *Glycoproteins*
  Glycoproteins are formed of carbohydrates attached to extrinsic proteins and act as cell-surface-receptors, usually to hormones. As well as acting as recognition sites for specific chemicals, glycoproteins also help cells attach together to form tissues. Glycoproteins are used by lymphocytes to recognise an organism's own cells.

<u>Fluid-mosiac model of the cell-surface membrane -</u>
The model is known as fluid because the individual phospholipid molecules can move relative to one another, providing flexibility to the structure. Because of the proteins embedded in the bilayer, the variety in sizes and shapes looks to the same as the tiles of a mosaic.
Most substances do not freely diffuse across the phospholipid bilayer of the cell-surface membrane because they are not lipid soluble, too large or they are polar molecules. Not only are polar molecules unable to pass through the non-polar hydrophobic tails of the bilayer, some proteins will repel molecules with the same charge.
At temperatures below 0 degrees Celsius the membrane is rigid, the transport proteins denature, so the permeability of the membrane increases. Ice crystals may form and pierce the membrane, it is highly permeable when it thaws.
At temperatures between 0 and 45 degrees Celsius the membrane is partially permeable because the phospholipids are mobile. As temperature increase, the phospholipids move more, increasing permeability.
At temperatures above 45 degrees Celsius the phospholipid bilayer begins to break down, increasing permeability immensely. Water within the cell expands, increasing the pressure on the membrane. Transport membranes again denature.

Transport in plants -
Water and mineral ions in solution move through the xylem vessels of a plant, from the roots to the leaves.
Xylem vessels are tube-like structures formed from dead cells (vessel elements), with no end plates to allow the uninterrupted passage of water.
The humidity of the air surrounding the stomata is greater than that of the atmosphere, so water vapour molecules diffuse out into the atmosphere. The water vapour lost by air space surrounding the stomata is replaced by water evaporating from the cell walls of the surrounding mesophyll cells. This is known as transpiration. Transpiration is a passive process, using energy from the sun.
This transpiration lowers the water potential of the mesophyll cells, creating a water potential gradient that pulls water up from the xylem. This is due to cohesion of water molecules and is called transpiration pull. This causes tension in the xylem vessel, giving rise to the name cohesion-tension theory.
Evidence for cohesion-tension theory:
- Transpiration is greatest during the day. The increased tension of the xylem causes the diameter of tree trunks to shrink
- If a xylem vessel is broken water can no longer be drawn up. Water isn't leaked out, but instead air is drawn in, which is consistent with the xylem being under tension rather than pressure

Transpiration rate is affected by light intensity, temperature, humidity and wind, as these all affect the rate of evaporation from the leaves.

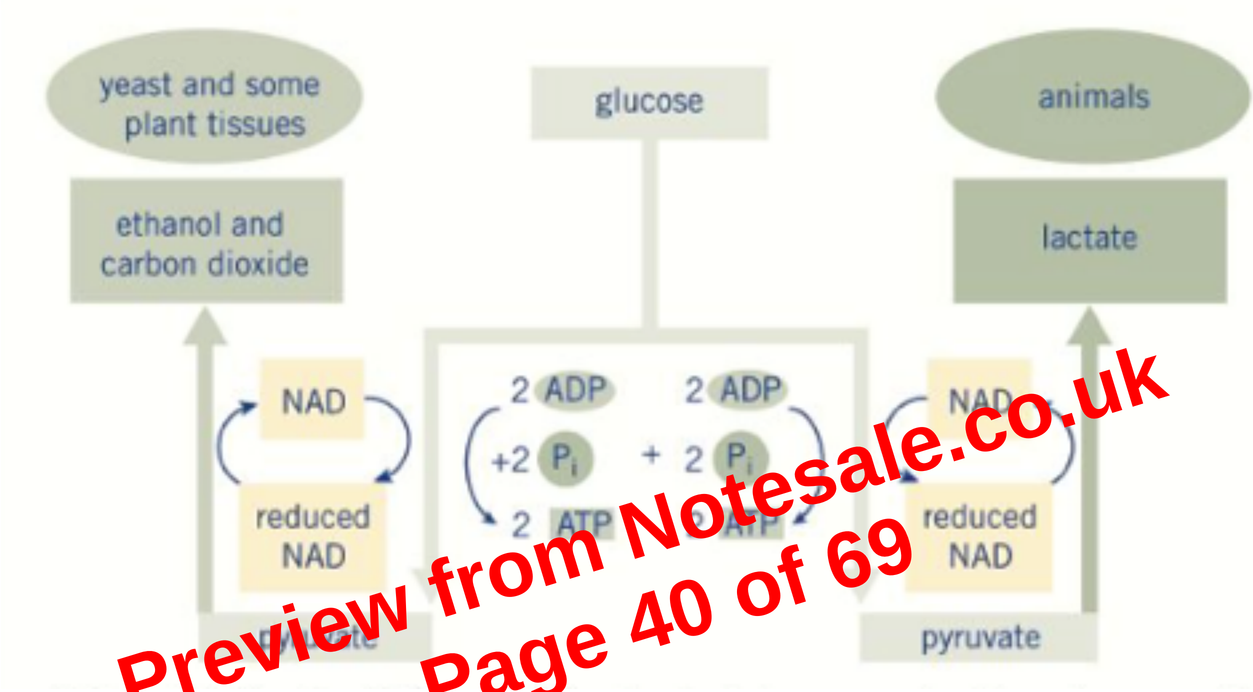
The movement of organic solutes and some mineral ions occurs in the phloem of a plant by a process called translocation. Phloem is made up of live sieve tube elements (that have no nucleus and few organelles) arranged end to end, with perforated end walls to form sieve plates. Each sieve tube has an associated companion cell.
The source is the site of production of organic molecules, where as a sink is the place where the organic molecule will be used. The solutes are sometimes referred to as assimilates.
Mass flow theory explains the mechanism of translocation:
1) Sucrose is produced in photosynthesising tissue and diffuses down the concentration gradient by facilitated diffusion into companion cells
2) Hydrogen ions are actively transported from companion cells into the spaces within cell walls using ATP, causing a concentration gradient. Hydrogen ions then diffuse through carrier proteins into the sieve tube elements, cotransporting sucrose molecules
3) Sieve tubes have a lower water potential than the xylem, so water moves from the xylem into the sieve tubes by osmosis, increasing the hydrostatic pressure. This leads to the mass transport of sucrose through the phloem
4) At respiring cells (the sink) sucrose is actively transported out of the sieve tubes. This lowers their water potential, so water moves from the sieve tubes and into the respiring cells by osmosis. This in turn lowers the hydrostatic of the sieve tubes again. Enzymes also maintain a concentration gradient from the source to the sink by changing the solutes at the sink

Evidence for the mass flow hypothesis:
- Sap is released when sieve tubes are cut, so they are under pressure
- Sucrose concentrations are higher in leaves (sources) than roots (sinks)
- Downward flow in phloem ceases when leaves are shaded/at night
- Increased sucrose concentrations in the leaves are later followed by increased sucrose concentrations in the phloem
- Metabolic poisons/anaerobic conditions inhibit translocation of sucrose in phloem
- Companion cells possess many mitochondria for ATP production

Evidence against the mass flow hypothesis:
- The function of sieve plates is unclear, as they should hinder mass flow
- Not all solutes move at the same speed, which would occur under mass flow
- Sucrose is delivered at the same rate to all regions, rather than reaching those with the lowest concentration first

atoms when they are broken down, there are more protons available for oxidative phosphorylation, which is why lipids release double the energy as the same mass of carbohydrates and lipids. Proteins can hydrolysed into their constituent amino acids. Following deamination they can be converted into 3-, 4- and 5-carbon molecules.

<u>Anaerobic respiration -</u>
Without oxygen, aerobic respiration cannot occur so there is a diminished ATP yield. The pyruvate is reduced to form either lactate or ethanol, as well as oxidised NAD. Lactate can later be oxidised into pyruvate again, for later use in the link reaction.
One glucose molecule makes 2 ATP during anaerobic respiration, where as it produces 32 through aerobic respiration.



Temporal summation occurs when one presynaptic neurone releases neurotransmitter several time over a given time period in order to make it accumulate to the threshold value.

Inhibitory synapses make it less likely that an action potential will be generated.
1) The presynaptic neurone releases a neurotransmitter that binds to chloride ion protein channels, causing them to open and allow chloride ions to move in via facilitated diffusion
2) The binding of the neurotransmitter also causes potassium ion protein channels to open, allowing potassium ions to diffuse out of the neurone
3) The postsynaptic membrane becomes more negative than the outside, causing hyper polarisation and therefore increasing the number of sodium ions needed to reach the threshold value and generate an action potential

The transmission of nerve impulses in the cholinergic synapse:
1) The arrival of an action potential in the end of the presynaptic neurone causes calcium ion protein channels to open and diffuse into the synaptic knob
2) The calcium ions cause the synaptic vesicles to fuse with the presynaptic membrane, releasing acetylcholine into the synaptic cleft
3) Acetylcholine binds to receptor sites on the sodium ion protein channels in the membrane of the postsynaptic membrane, causing them to open and allow sodium ions to diffuse into the postsynaptic neurone
4) This generate a new action potential in the neurone
5) Acetylcholinesterase hydrolyses acetylcholine into choline and ethanoic acid, which diffuse back into the presynaptic neurone. This is known as recycling and leads to the discrete transfer of information across synapses. Sodium ion protein channels close in the absence of acetylcholine
6) ATP released by mitochondria is used to recombine choline and ethanoic acid into acetylcholine, which is stored in the synaptic vesicles

<u>Muscles -</u>
Cardiac muscle - The muscle found in the heart
Smooth muscle - The muscle found in the walls of blood vessels
Skeletal muscles - Muscle attached to bone, which acts under voluntary control
Muscle fibres - Aggregations of muscle cells that share nuclei and sarcoplasm, which contains a large concentration of mitochondria and RER
Slow-twitch muscle fibres - Contract more slowly and less powerfully but work over a longer period, so are adapted to aerobic respiration by containing a large store of myoglobin, a rich supply of blood vessels and numerous mitochondria
Fast-twitch muscle fibres - Contract more rapidly and produce powerful contractions for a short period of time. They are thicker and have more myosin filaments, contain a high concentration of glycogen and enzymes associated with anaerobic respiration. They also contain a store of phosphocreatine to quickly regenerate ATP from ADP
Myofibrils - Groups of muscle fibres that make up individual muscles

Myofibrils are made up of two types of protein filament; actin and myosin.
Actin is thinner than myosin and consists of two twisted strands. Myosin consists of rod-shaped tails with bulbous heads that intertwine.
Thin filaments are made up of actin molecules, whereas thick filaments are made up of myosin molecules.
Tropomyosin is another protein found in muscles and forms a fibrous strand around actin filaments.
Sarcomeres are arranged in a long line so that when one contracts a little, there is an amplified response along the whole line. They also run parallel to each other so the force is generated in one direction.

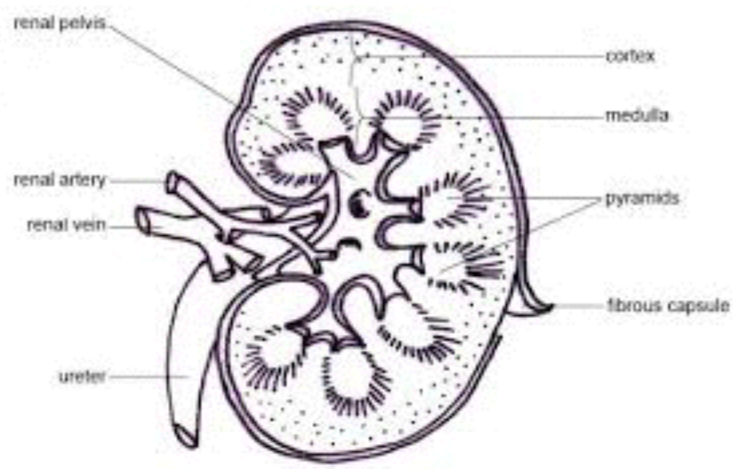
Within myofibrils there are different regions and bands.
I bands - Isotropic bands where thick and thin filaments do not overlap
A bands - Anisotropic bands where thick and thin filaments do overlap

Controlling blood water potential -

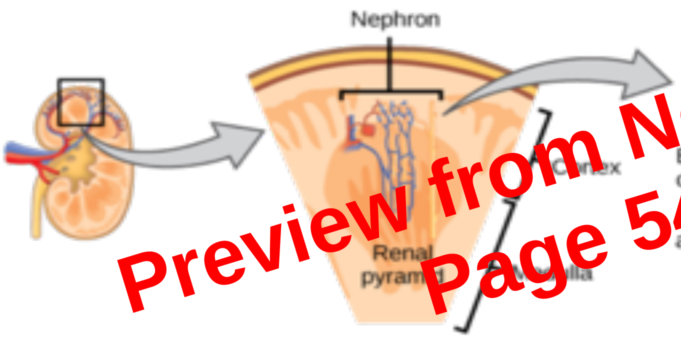
The homeostatic regulation of blood water potential is called osmoregulation and is necessary to maintain an optimum concentration of water and salts within the blood plasma. The overall gain in water and ions should balance the overall loss.

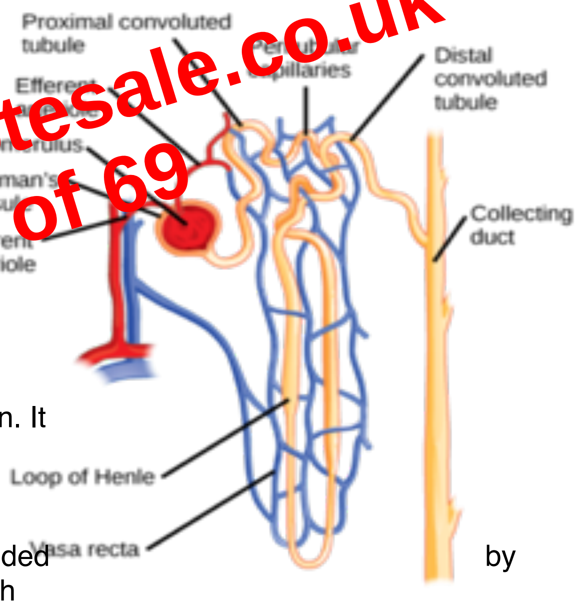
A typical person consumes 2.3 litres per day of water and produces around 0.2 litres as a by-product of metabolic processes. To counteract this, around 1.5 litres is passed as urine, 0.4 litres is expired, 0.6 litres is lost through evaporation from skin, faeces and sweat.

It is the kidneys that carry out osmoregulation in mammals. The mammalian kidneys are found at the abdominal cavity - one on each side of the spinal cord.



Fibrous capsule - the outer membrane that protects the kidney
Cortex - the outer section of the kidney, which contains renal Bowman's capsules, convoluted tubules and blood vessels
Medulla - the inner region of the kidney made up of loops of Henle, collecting ducts and blood vessels
Renal pelvis - funnel-shaped cavity that collects urine into the ureter
Ureter - carry urine from the kidney to bladder





- *Renal Bowman's capsule* - this the start of the nephron. It is cup-shaped and surrounded by a mass of blood capillaries known as the glomerulus. The inner layer is made up of podocytes.
- *Proximal convoluted tubule* - a series of loops surrounded by blood vessels. Its walls are made of epithelial cells with microvilli
- *Loop of Henle* - a loop that extends from the cortex of the kidney into the medulla
- *Distal convoluted tubule* - similar to the proximal convoluted tubule, but is surrounded by fewer capillaries
- *Collecting duct* - where the distal convoluted tubules converge to. It gets increasingly wider towards the pelvis of the kidney

The afferent arteriole arises from the renal artery. It supplies the nephron with blood and forms a glomerulus. The glomerulus is a branched knot of capillaries within the Bowman's capsule and is the site of ultrafiltration. The glomerulus recombine to form the efferent arteriole, which leaves the renal capsule. It has a smaller diameter than the afferent arteriole, and later branches to form the blood capillaries that surround the components of the nephron. They reabsorb mineral salts, glucose and water. These merge into venules and then the renal vein.

Osmoregulation

only inherited from the mother, who may be a carrier (heterozygous). Pedigree charts can be used to understand the genotypes of families based on who has/had a sex-linked disease.

Section 21: Recombinant DNA

Recombinant DNA -
Recombinant DNA is when the DNA of two different organisms is combined to form a transgenic/ genetically modified organism. Because the genetic code is universal, genes can be isolated and copied into the DNA of different species, without being rejected.

DNA fragments can be produced using:
- Reverse transcriptase to make complementary DNA from an mRNA template
- Restriction endonuclease enzymes, which recognise specific palindromic sequences (recognition sequences) and cut DNA at these places
- A gene machine to create oligonucleotides

Steps of gene transfer and gene cloning -
1) Isolation of the DNA fragments with the gene that codes for a specific protein
   This can be done using reverse transcriptase to produce cDNA from mRNA, using restriction endonuclease and creating the gene in a gene machine.
   Reverse transcriptase is an enzyme that catalyses the production of DNA from RNA. mRNA coding for the desired protein acts as a template for a single-stranded complementary copy of DNA (cDNA) to be formed using reverse transcriptase. This is then isolated by the hydrolysis of mRNA using an enzyme and the cDNA acts as a template for double stranded DNA to be formed using DNA polymerase.
   Restriction endonucleases are enzymes that cut DNA at recognition sequences. These sequences are palindromic. If the cuts occur at opposite base pairs the straight edge is known as a blunt end. If the DNA is cut in a staggered fashion, it is known as a sticky end, as DNA has exposed, unpaired bases. It is these unpaired bases that can be used to combine DNA from two different organisms, using the enzyme DNA ligase.
   The gene machine uses the desired sequence of nucleotide bases, which are fed into a computer and oligonucleotides (small, overlapping single strands of nucleotides) are assembled into the gene. The original sequences must first be checked for biosafety and biosecurity. The gene is replicated using the polymerase chain reaction, which also constructors the complementary strand of nucleotides to make the required double stranded gene. Using sticky ends, the gene can be inserted into bacterial plasmids, which act as a vector for the gene. The gene doesn't contain any introns. These artificial genes can be produced in as little as 10 days with great accuracy.

2) Insertion of the DNA fragment into a vector
   In vivo (within organisms) gene cloning involves transferring DNA fragments to a host cell using a vector.
   DNA is prepared for insertion the fragment has promoter and terminator regions attached. These regions indicates the appropriate points for RNA polymerase to act between.
   Once the DNA fragment has been isolated and the promoter and terminator regions have been added, the fragment can be inserted into a vector. This involves using the same restriction endonuclease to open up plasmids, so that the sticky ends are complementary to those of the DNA fragment. DNA ligase then binds the plasmids and DNA fragments when they have been incorporated.

3) Transformation of DNA into the host cells
   Plasmids containing the genes and bacteria are mixed together in a medium containing calcium ions. These calcium ions alongside changes in temperature affect the permeability of the bacterial cell surface membrane, allowing the plasmids to enter the bacterias cytoplasm. About 1% of bacteria cells will take up the plasmids, and some plasmids don't actually incorporate the new gene.

4) Identification of successful host cells using gene markers
   Marker genes are second, separate genes on the plasmid and they may be resistant to