Limiting Factors of Photosynthesis

The rate of photosynthesis is determined by many interacting factors: light, temperature, carbon dioxide concentration, nitrate concentration etc. This is because photosynthesis is made up of many small reactions. Temperature affects the enzyme action in the Calvin cycle, carbon dioxide concentration affects the enzyme action of RUBISCO and slows the Calvin cycle and light intensity affects the light dependent reaction. As so many factors are involved, the rate of photosynthesis fluctuates in different environments.

The rate of photosynthesis is determined by the limiting factor – the factor in the shortest supply and therefore the factor with the least favourable value. All physiological processes are limited by the factor with the least favourable value. For example, at low light intensities, the amount of light that hits the plant is the limiting factor of photosynthesis. This is the factor in the shortest supply, so it doesn't matter if the temperature, carbon dioxide concentration and the water concentration are low. When the light intensity increases, the rate of photosynthesis increases, but after a certain point, the rate will stop increasing no matter how much the light intensity is increased by. This is because, past a certain light intensity, it is another factor that is in the shortest supply and therefore limits the rate.

The rate of photosynthesis is measured in two main ways: volume of carbon dioxide taken up and volume of oxygen produced.

When light is the limiting factor, the rate of photosynthesis is proportional to the increase in light intensity. This means that as the light intensity increases, the rate will also increase (shown by the increase in oxygen production and carbon dioxide uptake). At a certain point, the carbon dioxide taken up and the oxygen produced in photosynthesis will balance with the carbon dioxide produced and the oxygen taken up in respiration. At this point, no net gas exchange takes place, and it is known as the light compensation point. After this point, the rate will continue to increase with the light intensity until another limiting factor prevents a further increase in the rate.

Carbon dioxide is usually the limiting factor of photosynthesis, as it makes up such a tiny proportion of air. Carbon dioxide is combined with ribulose biphoshate to start the Calvin cycle, so a low carbon dioxide concentration will cause a reduced rate of reaction in the Calvin cycle and reduced enzyme activity of RUBISCO. In commercial greenhouses, farmers enrich the air with carbon dioxide in order to increase the rate of photosynthesis and hence the growth rate of the plants.

Temperature is also an important factor as it affects the enzyme activity of all enzymes involved in the Calvin cycle. The rate of photosynthesis is directly proportional to temperature – between 0^{O} C and 25^{O} C, the rate of photosynthesis will double for every 10^{O} C temperature increase. 25^{O} C is the optimum temperature of photosynthesis so past this temperature the increase levels off and then declines at higher temperatures. Temperature doesn't affect the photochemical light dependent reaction, so the fact that photosynthesis is affected by temperature suggested to scientists that the reaction had a chemical component as well.

Measuring photosynthesis:

Food Chains and Food Webs

Food chains and food webs visually show the transfer of energy between different organisms in an ecosystem. They are made up of producers, consumers and decomposers, and are split into trophic levels.

Producers are autotrophs – they synthesise their own chemical energy from a simple, inorganic source of carbon. Photoautotrophs are autotrophs that use light to synthesise chemical energy by photosynthesis. They include plants, algae and some bacteria. Chemoautotrophs use a source of carbon that is different to the source used by the photoautotrophs. They also do not use photosynthesis. Autotrophs are called producers because they capture chemical energy and are the entry point of the energy into an ecosystem.

Consumers are heterotrophs – they cannot synthesis their own chemical energy and must consume an external, organic source. Primary consumers are those that feed on producers, secondary consumers feed on primary consumers etc.

Decomposers break down dead plant and animal matter. They absorb the soluble products of this breakdown to supply their own nutritional and metabolic needs. This breakdown also releases important minerals and elements back into the ecosystem, which are then recycled through the ecosystem.

Food chains can be constructed which show the flow of energy through an ecosystem marked by the different consumers. Each stage of the food chain is a trophic level. There are considerable energy losses between each trophic level. In practice, food chains are inaccurate as organisms in an ecosystem have a varied diet and feed on a number of different organisms. To reflect this, food chains are combined to form food webs, which show the interaction between different species. However, food webs can also be inaccurate, as the feeding patterns of different species changes seasonally and due to other abiotic and biotic factors.

However, the disadvantages to biological controls are that they take much longer to have an effect than pesticides, by which time the crops may have already become irreversibly damaged. There is also a possibility that the control organism itself becomes a pest.

Integrates pest control systems combine chemical, biological and physical controls to maximise the efficiency of pest control while doing as little damage possible to the environment.

- Livestock/plant varieties are chosen that are as resistant to the pest as possible
- The agricultural ecosystem they are grown in is designed to provide habitats and food sources to predators of the pests
- The pest levels are monitored regularly
- Pests are removed mechanically and physically (by hand/machine)
- Biological controls are introduced
- Pesticides are used as a last resort

Environmental consequences of fertiliser use

Nitrogen increases productivity by increasing growth. As the plant grows larger, the leaves grow bigger. This results in an increase leaf surface area, which leads to greater light and CO_2 absorption that increases the rate of photosynthesis. However, nitrogen fertilisers can have some detrimental effects on the surrounding environment.

- Nitrogen fertilisers reduce species diversity in ecosystems. This is because nitrate concentration is usually a limiting factor of photosynthesis. Soils with high nitrate concentration favour fast growing plant species that out-compete slower growing species. For increased species diversity, the nitrate concentration must be low in order to allow the slower species to compete with the faster species. The result is weeds and grasses out-competing other species such as wild flowers and other grasses, leading to low biodiversity.
- Leaching takes place. Leaching is the process by which nutrients leave the soil. Soluble nutrients such as nitrate are carried deep into the soil by rain water. They then enter watercourses. High nitrate concentrations in sources of drinking water can have health effects of humans. High nitrate concentration can reduce the oxygen carrying capacity of haemoglobin in babies and can increase the risk of stomach cancer. Leaching also results in eutrophication.
- Eutrophication is a natural process that takes place when the nitrate concentration of a natural aquatic ecosystem increases exponentially and ceases to be a limiting factor of photosynthesis.

1. In natural aquatic ecosystems, the nitrate concentration of the environment is low and is therefore the limiting factor of photosynthesis for plants and algae
2. When nitrates leach into the ecosystem, the concentration increases and it ceases to be the limiting factor.
3. Algae and plant species grow exponentially as the photosynthesis rate increases. Algae grow at the surface of the water, in algal bloom.
4. Algal bloom prevents light from reaching aquatic plants that live deeper in the water. They are starved of light and die as their rate of photosynthesis was too low to support them.
5. Saprobiotic algae and saprophytes break down the dead plant matter, releasing nitrates and further increasing the concentration in the watercourse.
6. The saprophytes are aerobic organisms and so require oxygen. As their numbers increase, the oxygen in the ecosystem is gradually depleted as the oxygen concentration of the water is not high enough to support the saprophytes and the other organisms in the ecosystem. Oxygen is also prevented from entering the ecosystem by the algal bloom.
7. Organisms such as fish die due to the lack of oxygen, and are broken down by saprobiotic algae and saprophytes, releasing more nitrates into the ecosystem.
8. Due to the lack of oxygen, aerobic organisms die off and anaerobic organisms increase in numbers as there is less competition. Anaerobic organisms further decompose dead organic matter, releasing nitrates and toxic compounds which make the water putrid.

X^hY = male that suffers from haemophilia. He possesses the haemophilia allele on his X chromosome and lack the homologous portion on his Y chromosome that contains the dominant clotting factor allele.

Receptors – Cone and Rod Cells

Receptors only respond to specific stimuli, so the body has many different receptors that respond to their different respective stimuli. Also, as some stimuli such as temperature and light are continuous, the body must have different receptors that respond to different intensities of stimuli. Rod cells and cone cells are secondary receptors as they detect stimuli and create a generator potential which is transferred to a bipolar cell before the sensory neurone.

Rod and cone cells are two photoreceptors that respond to different intensities of light. Rod cells respond to lower light intensities and are therefore more sensitive to light. In the dark, rod and cone cells have their sodium ion channels open, as their pigment is inactivated. This depolarization causes them to constantly release the neurotransmitter glutamine. The glutamine creates an action potential which causes some bipolar cells to be hyperpolarized and some to be depolarized. In light, the pigment is activated and the sodium ion channels close, causing the release of glutamate to cease. This causes hyperpolarized bipolar cells to depolarize and depolarized bipolar cells to hyperpolarize. This change in light intensity causes action potentials to be sent to the brain, which processes the information to form an image.

Rod cells cannot distinguish between different wavelengths of light, so images from rod cells are always black and white. Rod cells contain the pigment rhodopsin. This pigment is broken down at low light intensities, which causes sodium ion channels to close. Rod cells show retinal convergence, which further helps them to respond to low light intensities. To create a generator potential, a threshold value of energy must be achieved before it is trascduced into electrical energy (a nerve impulse). At very low light intensities, it is unlikely that this threshold value will be achieved by one cell, as a single rod cell is unlikely to absorb all the necessary light energy to create the generator potential. To get around this, many rod cells attach to one bipolar cell and one neurone. Collectively, at low light intensities, they can overcome the threshold value and create a generator potential. This is how we can see at night. However, retinal convergence results in low visual acuity, as many rod cells are attached to one neurone, so only one nerve impulse is created, no matter how many different sources of light there are. As a result, the brain cannot distinguish between many different sources of light, as two different sources may be represented by one nerve impulse.

There are three different types of cone cell, each of which responds to a different wavelength of light. This means that we can see in colour when cone cells are activated. Cone cells only respond to high light intensities, which is why we cannot see bright colours in the dark, as only rod cells are stimulated. Cone cells don't show retinal convergence, so one cone cell is connected to one bipolar cell and one sensory neurone. This means that a higher threshold value is required to create a generator potential from a cone cell. Cone cells have the pigment iodopsin, which breaks down at higher light intensities than rhodopsin, and so higher light intensities are required to create the generator potential. An advantage of the lack of retinal convergence is the higher visual acuity of cone cells. As each cone cell is attached to one bipolar cell and sensory neurone, a single nerve impulse is generated from each cone cell, allowing the brain to distinguish between different sources of light.

The distribution of rod and cone cells throughout the retina changes. At the fovea, there is the highest concentration of cone cells, as this is the region that experiences the highest light intensity.

At the peripheries of the retina, the concentration of the rod cells increases and the concentration of the cone cells decreases.

The Nerve Impulse

The nerve impulse is an electrical impulse that is transmitted along the action potential. It is not an electrical current, but a reversal in the electrical potential difference in the axon membrane. The reversal is between two states: resting potential and action potential.

The reversal of electrical potential is caused by the movement of Na+ and K+ ions across the membrane. Their movement is controlled by the axon membrane.

- The phospholipid bilayer is not water soluble, so prevents Na+ and K+ ions diffusing across it.
- The Na+ and K+ ions must therefore diffuse through intrinsic protein channels in the cell membrane. Some protein channels are permanently open, allowing the free diffusion of ions. Some channels are gated – they open and close according to different cellular conditions. Stretch gated channels open when the membrane changes shape and is stretched, causing the channels themselves to physically widen (e.g. the channels in the Pacinian corpuscle). Ligand gated channels open when certain ligands (e.g. neurotransmitters) bind to the protein channel and cause a conformational change in the protein, leading to the opening of the channels. Voltage gated channels open when there is a change in the electrical potential of the membrane.
- The sodium-potassium pump is a protein complex that actively transports Na+ ions out of the cell and K+ ions into the cell. For every three Na+ ion pumped out, there are two K+ pumped in.

The resting potential:

1. Na+ ions are actively transported out of the axon by the sodium-potassium pump, and K+ ions are actively transported into the axon.
2. As three Na+ ions are transported out for every two K+ that are transported in, there are more Na+ ions on the outside of the axon than inside the axon, and more K+ inside the axon than outside. There is an overall loss of positive ions from the axon interior.
3. Na+ ions start to diffuse back into the axon and K+ ions diffuse out of the axon through the open protein channels.
4. However, at resting potential, most of the gated sodium ion channels are closed, whereas most of the gated potassium ion channels are open.
5. This means that Na+ ions are prevented from moving back into the axon, and potassium are allowed to move out of the axon, leading to a loss of positive ions from the axon interior. This polarises the axon membrane, giving it an overall negative charge. The exterior of the axon is very positive as there is a high concentration of Na+ and K+ ions.
6. At a certain point, the diffusion of K+ out of the axon slows as an electrical gradient slows the rate of diffusion. The outside of the membrane is very positive and K+ ions, being positive, are repelled. They therefore stay within the axon, which has an overall negative charge.
7. The movement of ions balances and equilibrium is established where there is no net movement of ions. The overall charge of the axon interior is -65mV.

Hormones and the Regulation of Blood Glucose

Blood glucose is regulated by the interacting hormones that are released from the pancreas and other glands, such as the adrenal glands. The pancreas releases the main hormones that regulate the blood glucose: insulin and glucagon.

Hormones are released by glands and organs into the blood stream when these organs are stimulated. The hormones travel in the blood stream to the target cells. Target cells are cells which have receptors on their membranes that are complementary to the hormones. The hormones then bring about changes within the cell when they are attached.

Hormones have two different modes of action. One of these is the second messenger model. The second messenger model works as follows:

1. The hormone acts as the first messenger. It is released by glands and organs into the blood stream. It then binds to target cells.
2. The hormone binds to complementary receptors on the cell membrane to form a hormone receptor complex. This binding activates an enzyme within the cell which produces a new molecule. This product molecule is the second messenger.
3. The second messenger molecule then activates other enzymes, which catalyse reactions and bring about the desired changes within the cell.

Glucagon, adrenaline and thyroxin all work in the same way following the second messenger model. The process of adrenaline action is the same for glucagon and thyroxin:

1. Adrenaline is released in the adrenal glands and travels in the blood stream.
2. Adrenaline reached target cells and binds to the complementary receptors on the membranes of these cells, forming an adrenaline-receptor complex.
3. This activates the enzyme adenylate cyclase, which synthesises cyclic AMP from ATP.
4. cAMP acts as the second messenger, and activates the enzyme glycogen phosphorylase, which carries out glycogenolysis and deactivates enzymes that carry out glycogenesis.

In the case of glucagon, the hormone also activates enzymes that carry out gluconeogenesis but doesn't deactivate glycogenesis enzymes.

Blood glucose is regulated in order to keep the blood glucose level high enough to supply all body cells with sufficient glucose for respiration. It is also prevented from rising too high, which would lower the water potential of the blood and cause osmotic problems with cells, which could have a damaging effect on the organism. The blood glucose changes throughout the day as a result of glucose influx via food and glucose expenditure, as work is done by cells (exercise or mental work).

Insulin and glucagon are produced in the pancreas. The pancreas is made up of mostly exocrine cells, which produce digestive enzymes in a fluid called pancreatic juice. Pancreatic juice is injected into the duodenum in order to digest food. Throughout the pancreas, there are groups

Diabetes

Diabetes is a disease where the body is unable to metabolise glucose, leading to a rise in the blood glucose concentration. This can affect the water potential of the cellular environment and can be fatal. There are two main types of diabetes: type 1 and type 2

Type 1 diabetes is where sufferers cannot produce insulin. This may be due to the immune system attacking the beta cells in the pancreas, leaving the body unable to detect rises in blood glucose concentration or produce insulin to lower glucose concentration. Type 1 usually develops in childhood over a few weeks. The symptoms are usually obvious: high blood glucose level, increased thirst and hunger, glucose in urine, need to urinate excessively, tiredness, genital itching, weight loss and blurred vision.

Type 2 diabetes is where the sufferer has a reduced ability to respond to insulin or produces inadequate quantities of insulin, or a combination of both. A reduced ability to respond to insulin arises from a change in tertiary structure of the glycoprotein insulin receptors on body cells. As a result of this change, the insulin can no longer bind to body cells and can therefore no longer increase the glucose uptake of cells. The blood glucose concentration stays high. Type 2 usually develops slowly in people over 40, but poor diet and obesity are increasing the numbers of people with type 2, including those under 40. The symptoms are less severe than type 1, so they develop slowly are more likely to go unnoticed. There is a stronger link between lifestyle and risk of developing type 2 than lifestyle and type 1, which is more due to genetics.

Type 1 diabetes is controlled by regular injections of insulin. As insulin is a protein, it cannot be taken orally as it would be denatured and digested as it passed through the digestive system. Insulin is taken with meals, mimicking the increase in insulin production that normally occurs after a meal due to an increase in blood glucose. The amount of insulin must be matched to the amount of carbohydrate consumed. If the amount of insulin injected is too high, the blood glucose concentration will decrease too much, which leads to low blood glucose and unconsciousness as the cells have insufficient glucose for respiration.

Type 2 diabetes is controlled by regulating the amount of carbohydrate eaten and taking regular exercise, as this naturally helps to lower blood glucose levels. This can be complemented by taking small insulin injections or drugs that stimulate insulin production. Also, drugs can be taken which slow the absorption of glucose from the small intestine to ensure that the blood glucose levels don't rise too high too fast, and complex carbohydrates can be eaten, which are naturally digested slowly.

Feedback mechanisms

In almost all homeostatic control in the body, a feedback loop is established that helps to maintain the set point. When an effector has produced a response, the response is detected by receptors which send new information on the state of the set point to the control centre in the CNS. There are two types of feedback mechanisms: positive and negative.

Negative feedback is where the corrective measures carried out by the effectors are turned off by a return to the set point. This is by far the most common form of feedback. It is carried out in order to prevent effectors from being overstimulated, which would cause a deviation from the set point in the reverse direction, which could damage the organism. Two examples are thermoregulation and the regulation of blood glucose.

In thermoregulation, thermoreceptors in the skin and hypothalamus detect a change in body temperature (e.g. a rise). This information is sent to the heat loss centre of the hypothalamus. The heat loss centre then stimulates effectors in order to lose excess heat, to prevent damage to the organism. This involves vasodilation and increased sweating, as well as a decrease in metabolic rate and a relaxation of body hairs. The body temperature falls as a result and returns to normal. This change is detected by thermoreceptors and sent back to the hypothalamus. The hypothalamus then inhibits the effectors so as not to lose too much heat.

In the regulation of blood glucose, a change in blood glucose (e.g. a rise) is detected by the beta cells in the islets of Langerhans. They then release insulin into the blood stream, which binds to target cells, causing changes in the structure and metabolic reactions of the cell in order to increase its glucose uptake. As a result, the blood glucose concentration falls back to normal. This fall is detected again by the beta cells in the pancreas, which stop producing insulin as a result.

In positive feedback, the feedback causes the corrective measures carried out to remain turned on, which causes an even larger deviation from the set point. Positive feedback is rare, as a large deviation from the set point can be detrimental to the organism. One example of positive feedback is in the transmission of the action potential. When sodium ions diffuse across the axon membrane, the reversal in the electrical potential of the membrane causes sodium voltage gated channels further down the membrane to open. This increases the permeability of the axon membrane and ensures that the action potential is transmitted rapidly down the axon. Positive feedback also occurs when regulatory systems break down. In typhoid fever, thermoregulatory systems in the body break down, causing an uncontrollable rise in temperature which can be fatal.

RNA and the Genetic Code

DNA codes for proteins. It is a sequence of nucleotide bases, three of which are known as a codon. Each codon corresponds to a different amino acid. The sequence that the codons are arranged in dictate the primary structure of the protein being synthesised – the amino acid sequence.

As DNA is too big to leave the nucleus, and leaving the nucleus would risk damaging the DNA, it is transcribed into a single stranded molecule known as messenger RNA (mRNA). mRNA carries the genetic code – the sequence of nucleotide bases that correspond to amino acids, which are joined together to form a protein. The mRNA base sequence is complementary to that of the DNA, so it is not exactly the same. After transcription, the mRNA leaves the nucleus via the nuclear pore and enters the cytoplasm. Here, it associates with the ribosomes and tRNA is used to synthesise the protein that it codes for.

The main features of the genetic code:

- Each amino acid is coded for by a codon (triplet base sequence)
- The code is degenerate, so a single amino acid is coded for by more than one codon. Some amino acids are coded for only be a single codon, but most have more than one.
- There are three codons which don't code for any amino acid. Instead they are placed at the end of the base sequence for a particular protein and signal the ribosomes to stop transcribing as the synthesis is finished. They are the stop codons.
- The code is non-overlapping, as each codon is read as distinct, three base sections.
- The code is universal – the same codon codes for the same amino acid in almost all organisms.

mRNA and tRNA are both made up of ribonucleic acid, instead of deoxyribonucleic acid.

RNA is a polymer made up of many nucleotide monomers. The nucleotides are molecules of ribonucleic acid. This is comprised of the pentose sugar ribose, a nitrogenous base and a phosphate group. The nitrogenous bases in RNA are the same as those in DNA except for thymine, which is replaced in RNA with uracil. Adenine binds to uracil and thymine.

mRNA and tRNA differ in their structure and function, but are both made up of RNA.

mRNA is a long, single stranded molecule of RNA. It is complementary the DNA section from which it was transcribed. It is small and soluble, allowing it to leave the nucleus via the nuclear pore and associate with ribosomes. The fact that it is single stranded means that it's nitrogenous bases are exposed, which allows tRNA molecules with complementary anticodons to bind to the exposed bases. The tRNA molecules carry amino acids, which are then joined together by the enzyme peptidyl transferase. The mRNA is easily broken down by enzymes in the cytoplasm, so proteins are not continually transcribed when they are not needed.

tRNA is a small, clover shaped molecule of RNA, made up of around 80 nucleotides. Hydrogen bonds form between complementary bases within the molecule, which forms the clover shape. Each different type of tRNA carries a different amino acid. Each different tRNA molecule also has a different anticodon. The anticodon is a codon which is complementary to the codon on the mRNA. As the mRNA codes for a specific tRNA molecule, via the anticodon, the codon on the mRNA therefore codes for the amino acid carried by this tRNA molecule. In this way, the tRNA and the mRNA both contribute to determining the amino acid sequence of the protein.

Totipotency and Cell Specialisation

All cells in the body have the same genes and can therefore, theoretically, produce the same proteins. E.g. a muscle cell has the gene which codes for insulin, so can in theory produce insulin, while an epithelial cell has the genes which code for myosin and actin. However, cells are very different and produce different proteins in order to perform their particular function. They don't produce certain proteins that other cell types produce. This is because, although all cells contain the same genes, different cells have different genes that are switched on. A gene can only be transcribed if it is switched on. If it is switched off, the gene is not translated and the protein that it codes for is not produced. The switching on and off of different genes is differentiation.

Some genes are permanently switched on and expressed in all cells, such as the genes that code for respiratory enzymes. Different genes are permanently switched off depending on the cell. For instance, the gene that codes for insulin is permanently switched off in muscle cells but permanently switched on in beta cells in the pancreas. Some genes are switched on and off depending on the need for certain proteins at different stages in the cell cycle. For example, proteins that make up to spindle fibres are only needed during cell division, so the genes that code for them are only switched on when the cell enters cell division.

Cells differentiate due to the switching on and off of genes, which give them different abilities and functions. An organism beings from a zygote (fertilised egg). A zygote must be able to differentiate into all cell types in the body, including placenta and umbilical cells, for an organism to develop from the zygote. The zygote is therefore totipotent – it can differentiate into all cell types including placenta and umbilical cells. The zygote produces embryonic stem cells which are pluripotent – these cells can develop into any body cell, but cannot mature into placenta or umbilical cells. Totipotent and pluripotent cells split and develop into every type of body cell, such as muscle cells and epithelial cells, which are specialised to carry out a particular function. This is because the genes of the totipotent and pluripotent cells are switched on and off by methylation (the addition or removal of a methyl group). This is differentiation. The other genes that are not switched on are prevented from being transcribed and translated by preventing transcription and preventing mRNA from being translated.

In normal body cells, differentiation is an irreversible process: they will not be reversed back into pluripotent cells. However, in the adult body, there are adult stem cells in the bone marrow, skin, and stomach lining that can differentiate into more than one type of cell. These cells are multipotent – they can differentiate into a few different types of cell, usually a single tissue. For example, multipotent stem cells in the bone marrow can mature into platelets, erythrocytes and leucocytes, but cannot differentiate into other cell types. Multipotent adult stem cells and pluripotent embryonic stem cells are being used to treat a variety of conditions.

In plants, many cells are pluripotent. Under suitable conditions, a plant cell can be taken and an entirely new plant can be grown, as the cell can differentiate into any type of plant cell. The resultant plant would be a clone, as all the cells that develop from the original cell would have the same genetic information as the original cell, although the genes expressed would be different in each cell type.

this gene is. siRNA is also used in medicine to treat diseases causes by genes, as the siRNA can prevent the expression of these genes. siRNA can be used to treat viral and bacterial diseases in this way, as it can prevent the production of toxic proteins produced by bacteria and viruses.

Producing DNA Fragments

Many diseases are caused by the lack of the ability of an organism to produce certain chemicals needed in metabolic reactions or homeostasis. For example, some people lack the ability to produce insulin, resulting in diabetes, or certain clotting factors, resulting in haemophilia. Many of these substances are proteins, or are made up of proteins and other non-protein groups, and enzymes which produce these chemicals. The lack of the ability to produce these substances is therefore due to a defect in the genes that code for them – an allele that has a different base sequence to the normal allele, and therefore produces a non functional protein.

In order to treat these patients, the substances that they lacked were extracted from other organisms and introduced into the patient. This method carried the risk of rejection of the substance by the immune system and infection, as well as being very expensive. An advantage of DNA technology is the ability to produce pure substances that will not be rejected by the immune system that can be given to the patient, using bacteria or viruses to produce them. This involves extracting the genes that produce these substances and introducing them into the bacteria, which then produce the substance. DNA of two different organisms that is combined like this is called recombinant DNA, and the organism itself is genetically modified.

There are 5 main stages to genetically modifying an organism in this way:

1. Isolation of DNA fragments that contain the desired gene.
2. Insertion of the DNA fragments into a vector.
3. Transformation (also called transfection) of a host organism – the transfer of the DNA into the cells of the host organism
4. Identification of the host cells which have taken up the gene using DNA markers
5. Cloning of host cells to produce the desired substance

There are two main ways of isolating and extracting the DNA contains the gene for a particular substance: using reverse transcriptase and restriction endonuclease.

Reverse transcriptase is an enzyme used by retroviruses to convert mRNA into DNA, reversing the transcription process.

1. A cell that readily produces the desired substance is identified (e.g. a beta cell in the pancreas for insulin)
2. This cell will have lots of mRNA that code for the desired substance in its cytoplasm.
3. The mRNA is extracted and reverse transcriptase is used to convert the mRNA into a single strand of DNA. The DNA is known as complementary DNA (cDNA) as its base sequence is complementary to the sequence of the mRNA.
4. DNA polymerase is then used to produce the second strand of DNA, which forms a double stranded DNA molecule which contains the desired gene.

Restriction endonucleases are enzymes that bacteria use to defend against viruses. When viruses enter a bacterial cell, they produce DNA that enters the nucleus of the bacteria. To combat this, bacteria produce restriction endonucleases, which cut the DNA into pieces. There are many different types of restriction endonuclease. Each type cuts DNA at a different sequence of bases. The sequence at which a restriction endonuclease cuts is its recognition sequence. Sometimes the

Locating and Sequencing Genes

In order to carry out gene therapy and genetic screening, genes must be sequenced and located. This means that the base sequence of the desired genes must be determined and their location on the DNA molecule must be found.

DNA sequencing is used to determine the base sequence of a desired gene. For small genes with short base sequences, the Sanger method is used. For larger sequences, restriction mapping is used.

The Sanger method uses terminator nucleotides. These are nucleotides which cannot attach to the next base in the sequence – they do not allow another nucleotide to be attached to them once they are added into the chain and therefore are the last nucleotides in the sequence. The terminator nucleotides can carry one of the four DNA bases. To sequence DNA using the Sanger method:

1. DNA is treated to cause it to separate. This releases single stranded molecules of DNA into the solution, each of which can act as a template strand for the synthesis of another, complementary strand. This DNA is then separated into four test tubes.
2. Nucleotides are added to each test tube for DNA synthesis.
3. One of the four terminator nucleotides are added to each test tube. E.g. adenine terminator is added to test tube 1, thymine terminator is added to test tube 2 etc.
4. DNA primers are added to each test tube. These are radioactively or fluorescently labelled.
5. DNA polymerase is added to each test tube.
6. DNA synthesis then takes place. As the addition of nucleotides onto a new strand of DNA is a random process, the addition of a terminator nucleotide is as likely as the addition of a normal nucleotide. As a result, the strands produced in each tube will be of different lengths, as the addition of the terminator nucleotide will have occurred at different points in the DNA synthesis. In each test tube, every strand will end with the same nucleotide, as the terminator nucleotide will be the last nucleotide added into the new strand. These DNA strands can be identified using the labelled primers.

Once the stands have been identified, the DNA is then separated out according to length by gel electrophoresis. The DNA fragments are placed on agar gel and a voltage is applied across it. The resistance of the gel means that the larger the fragments, the more slowly they move and the shorter the distance that they move. In this way, DNA of different lengths is separated out. If radioactive primers were used, then photographic film is then placed over the gel for several hours. The radiation emitted develops the film and shows the positions of the fragments.

The shortest fragment will only have one nucleotide. This will be the first nucleotide in the base sequence of the gene. The next fragment will have two nucleotides, which will be the first two nucleotides in the base sequence. The fragments are read in this way, from smallest to largest, and the base sequence of the gene is determined. This is only useful for genes with base sequences of less than 500 nucleotides. Larger genes and genomes are sequenced by restriction mapping.

Restriction mapping involves cutting up a large section of DNA into smaller sections using restriction endonucleases. These sections are then put through gel electrophoresis and separated out according to length. The base sequence is then determined by reading off the fragments, from smallest to largest. Different restriction endonucleases are used on the same peice of DNA to get fragments of

Genetic Fingerprinting

Genetic fingerprinting is used to test to see how closely related two individuals or populations are, as well as being used to determine if a DNA sample matches the DNA of an individual.

DNA fingerprints are a result of the introns in a person's DNA. Introns are non-coding sections of DNA which contain repetitive sequences of bases called core sequences. The core sequences in each individual are unique, so the core sequences of two individuals must be different (apart from identical twins). The more closely two individuals are to each other, the more similar the core sequences will be. This means that the core sequences can be used to identify an individual from a DNA sample, as the specific pattern of the core sequence of the DNA sample can only correspond to a single individual.

Making a genetic fingerprint consists of five stages: extraction, digestion, separation, hybridisation and development

1. Extraction: the DNA is extracted from the tissue sample of the individual. As the DNA quantity is usually small, it is amplified using PCR.
2. Digestion: restriction endonucleases are used to cut the DNA into fragments. The restriction endonucleases used are those that cut around but not into core sequences.
3. Separation: the DNA is then separated according to size using gel electrophoresis. The DNA fragments are then treated with alkali in order to separate the double strands into single strands. The DNA strands are transferred to a nylon membrane using a technique called Southern blotting. Southern blotting involves placing a nylon membrane over the gel, and then covering the membrane with absorbent paper. The liquid containing the DNA is drawn up into the nylon membrane by capillary action. This transfers the DNA fragments into the nylon membrane in the same positions as they were in the gel. They are then fixed to the membrane by UV light.
4. Hybridisation: marked DNA probes are then added to the nylon membrane. The DNA probes are each complementary to different core sequences and therefore bind to them.
5. Development: the membrane is covered with a photographic plate which is exposed by the radioactively labelled DNA probes (if they were fluorescently labelled they are identified visually). The pattern of bands shows the specific combination of core sequences possessed by the individual; hence the pattern is unique to every individual.

DNA fingerprints are then interpreted by checking to see if there is a match between two DNA samples. If there is a match, the samples are passed through an automated scanning machine, which calculates the length of the DNA in each band and the probability of someone else having an identical fingerprint.

DNA fingerprinting is used extensively in forensics, where it is used to determine if DNA taken at the scene of a crime matches that of a suspect. This is then used to investigate and convict a suspect. However, the DNA may not be incriminating as the DNA could have been left there innocently, the DNA could have been left there before the crime took place, and the DNA could have been left when the person touched the victim. Also, the calculation done by the automated scanning machine assumes that the DNA is distributed randomly throughout the community, which may not be the case.