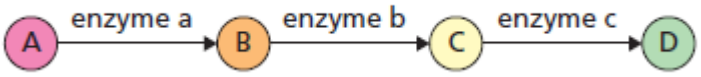
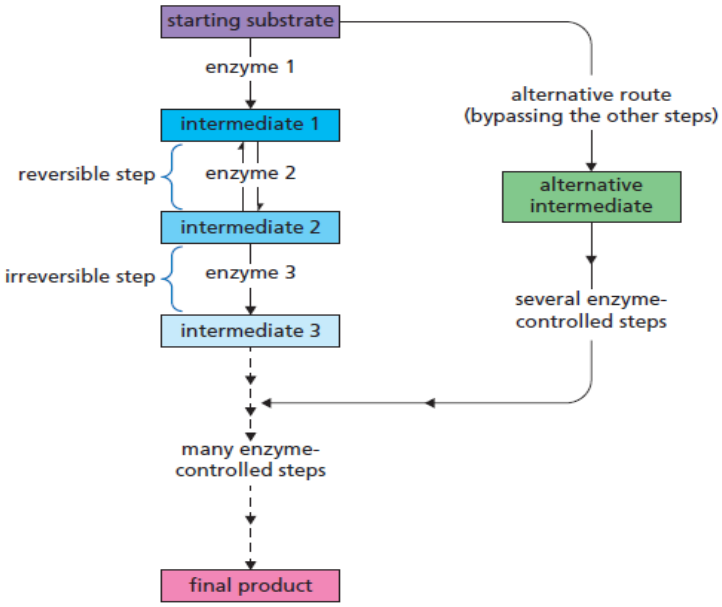
Unit 2 Metabolism & Survival

Key Area 1: Metabolic Pathways

Metabolic Pathways are integrated and controlled pathways of **enzyme-catalysed** reactions within a cell.



Metabolic Pathways can have **reversible steps**, **irreversible steps** and **alternative routes**.

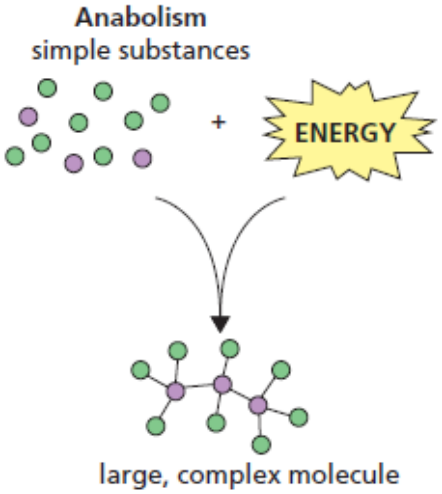


Catabolic and Anabolic Reactions

Reactions within metabolic pathways can be anabolic or catabolic.

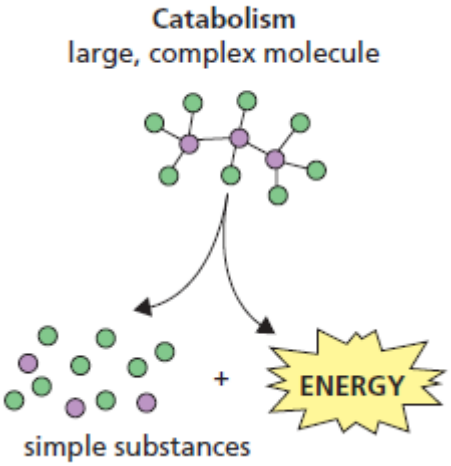
Anabolic Reactions

Anabolism **build up large molecules from small molecules and require energy**.



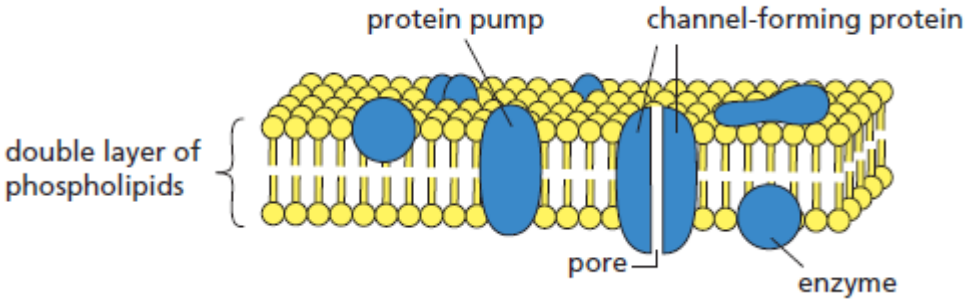
Catabolic Reactions

Catabolic reactions **break down large molecules into smaller molecules and release energy**.



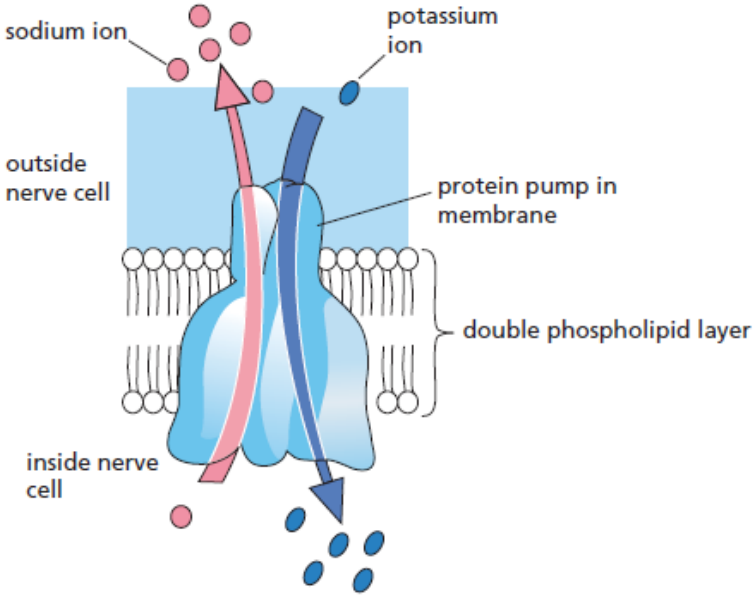
Protein pores, pumps and enzymes are embedded in membranes.

Membranes consist of proteins and phospholipids. The phospholipids create a bilayer and are constantly moving. This gives the membrane flexibility.



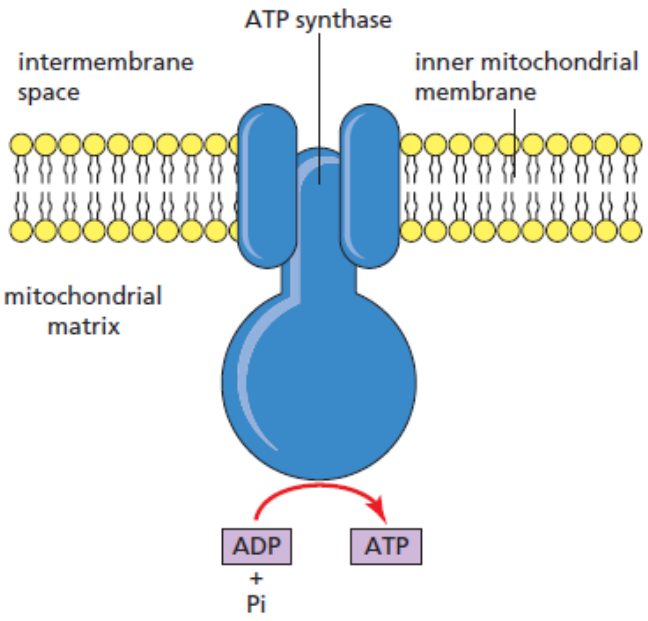
Channel-forming proteins create **pores** which control the **diffusion** of small molecules across the cell. Diffusion= movement of molecules from an area of high concentration to an area of low concentration.

Active transport = movement of molecules from an area of low concentration to an area of high concentration and requires energy in the form of ATP. This ATP is generated during aerobic respiration.



The Sodium-Potassium pump is an example of a carrier protein involved in active transport. Details of this is not required.

Some of the proteins embedded in the membranes of mitochondria and chloroplasts act as **enzymes**. ATP Synthase is an example of an membrane embedded enzyme which produces ATP from ADP + Pi.



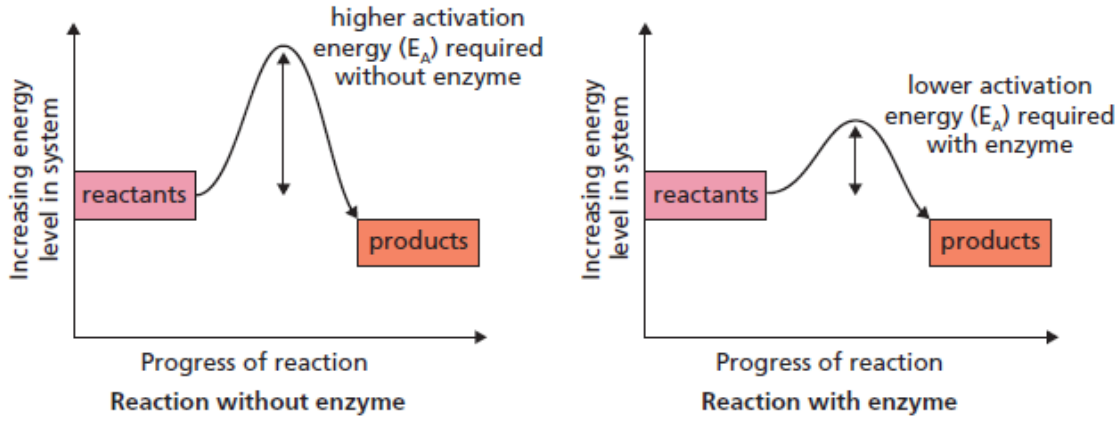
Role of Enzymes in Metabolic Pathways

Metabolic pathways are controlled by the presence or absence of particular enzymes and the regulation of the rate of reaction of key enzymes.

Activation Energy

The energy required to initiate a chemical reaction is known as the activation energy.

Enzymes **lower the activation energy** required for a reaction to take place.



Affinity

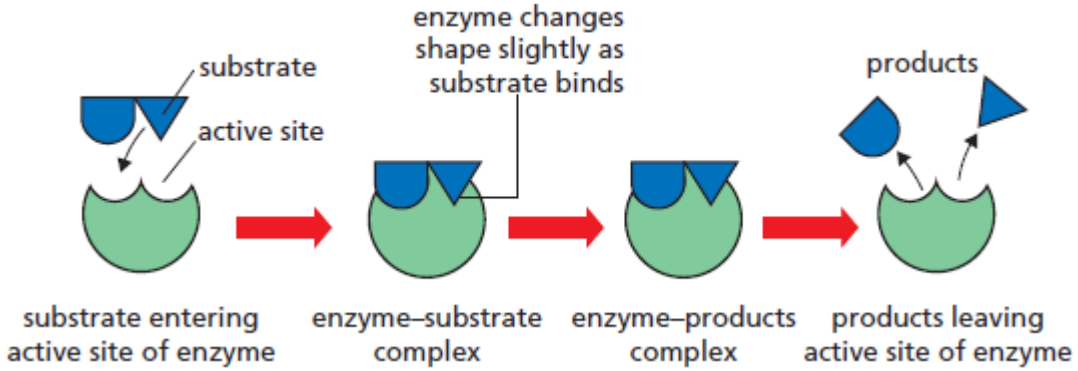
The activity of enzymes depends on their flexible and dynamic shape.

Substrate molecules have **high affinity** for the **active site** of an enzyme (bind readily).

Products of enzyme reactions have a **low affinity** for the **active site** of an enzyme, allowing them to leave the active.

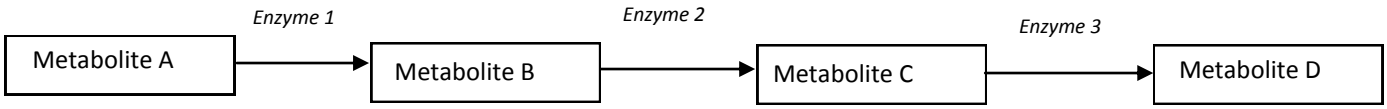
Induced Fit

The enzyme is flexible and the **substrate can induce the active site to change shape** to **better fit the substrate** after the substrate binds. This is known as **INDUCED FIT**.



Direction of enzyme-controlled reactions

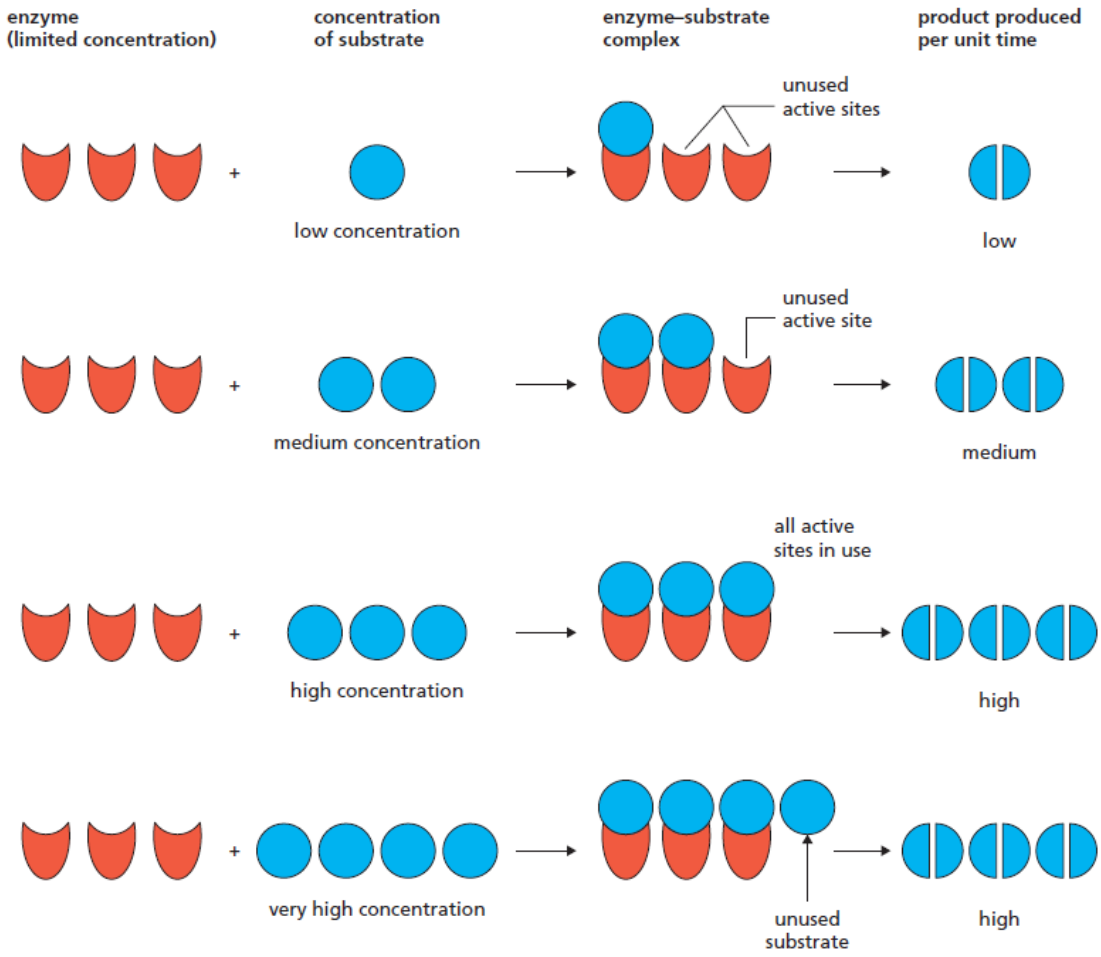
Some metabolic reactions are **reversible** and the presence of a substrate or the removal of a product will drive a sequence of reactions in a particular direction.



In the above example, the presence of Metabolite A activates Enzyme 1 and metabolite A is then converted to metabolite B. An increase in metabolite B then activates enzyme 2 and metabolite B is converted to metabolite C etc. Most enzymes can also work in reverse. If, for example, Metabolite C levels were to increase to an unusually high level whilst Metabolite B levels were low, enzyme 2 would work in reverse to convert Metabolite C back into Metabolite B.

Effect od Substrate and Enzyme Concentration

As the concentration of Substrate or Enzyme increases, the rate of an enzyme-controlled reation increases since more active sites will be occupied by substrate and therefore more enzyme-substrate complexes will be formed. If however**, either enzyme or substrate concentration is limited, the rate reaction will only increase up to a point**. Beyond this point, the rate of reaction remains **Constant** because either active sites will remain unoccupied (if substrate concentration is limited) or substrate molecules will have no active sites to occupy (if enzyme concentration is limited).



Enzyme Inhibition

An Inhibitor is a substance which reduces the rate of an enzyme controlled reaction.

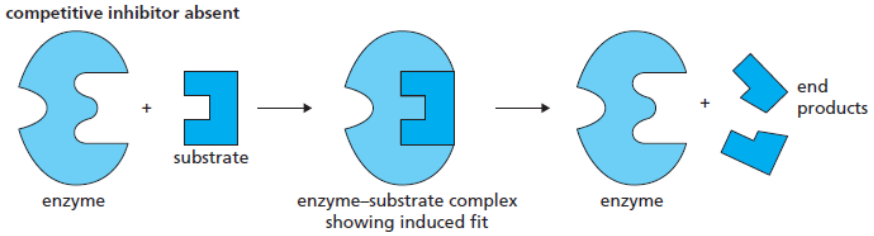
There are 2 main types of inhibitor :

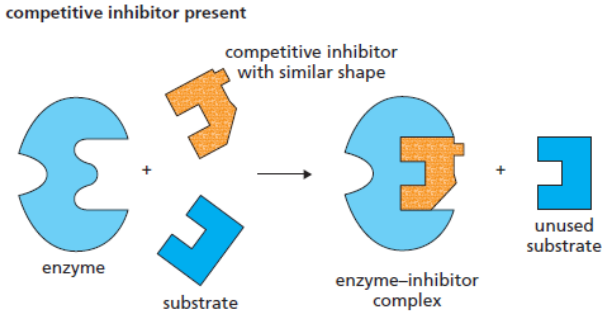
- Competitive
- Non-competitive

Competitive Inhibitors

Competitive inhibitors **bind at the active site** of the enzyme, **preventing the substrate from binding**.

Competitive inhibition **can be reversed** by **increasing substrate concentration**.

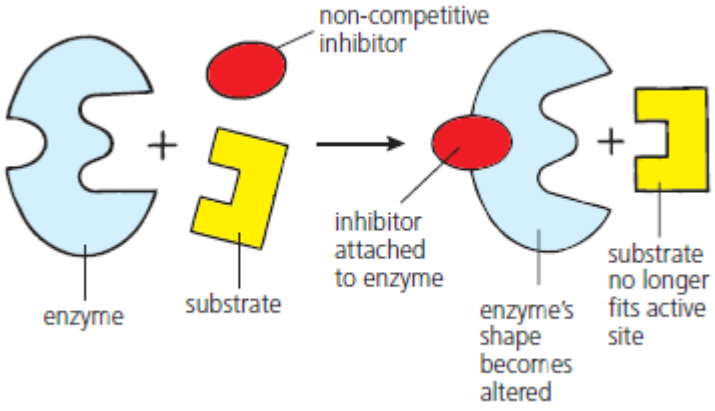




Non-competitive Inhibitors

Non-competitive inhibitors **bind away from the active site** (at an **allosteric site**) but **change the shape of the active site**, **preventing the substrate from binding**.

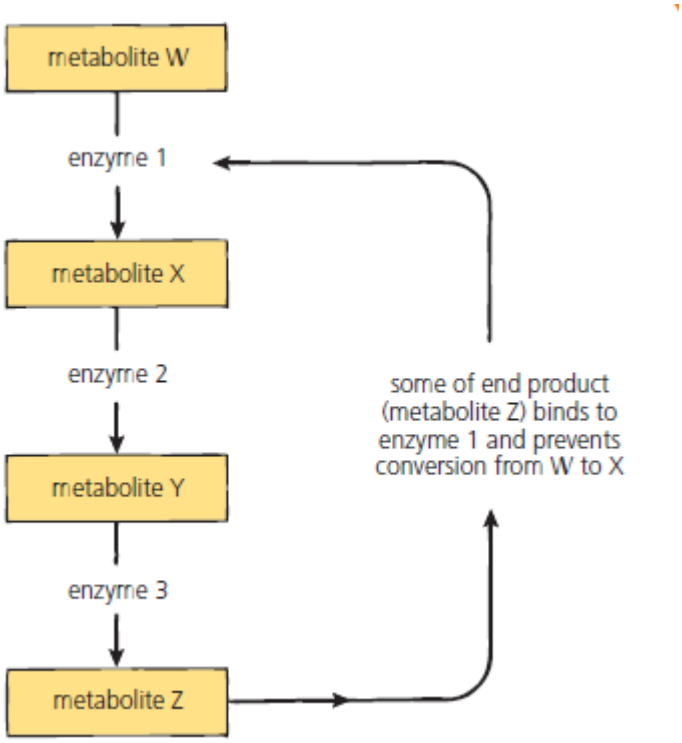
Non-competitive inhibition **cannot be reversed** by increasing substrate concentration.



Feedback Inhibition

Feed back inhibition occurs when the **end-product** in the metabolic pathway reaches a **critical concentration**.

The end-product then **inhibits an earlier enzyme**, blocking the pathway and so **prevents further synthesis of the end-product**.



Unit 2 Metabolism & Survival

Key Area 2: Cellular Respiration

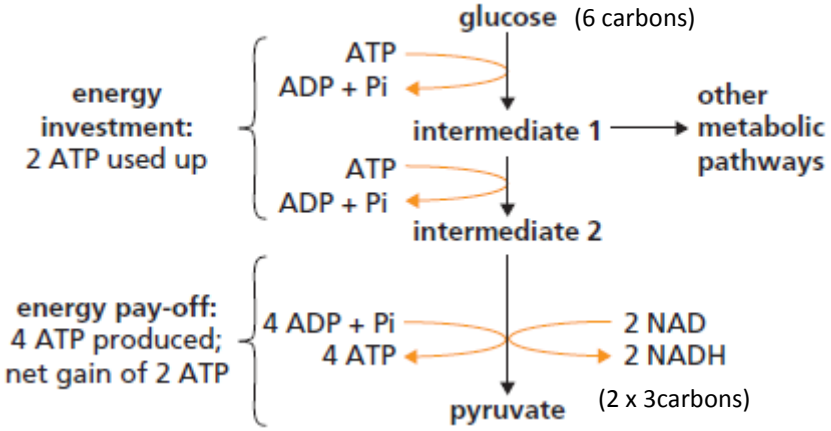
Cellular respiration is an enzyme-controlled series of reactions in which a respiration substrate such as Glucose is broken down to generate energy in the form of ATP.

Glycolysis

The 1st step is known as Glycolysis and involves the **breakdown of Glucose to Pyruvate**.

Glycolysis occurs in the **cytoplasm** of the cell.

ATP is required for the **phosphorylation of glucose and intermediates** during the **energy investment phase** of glycolysis. This leads to the **generation of more ATP** during the energy **pay-off stage** and results in a **net gain of ATP**.

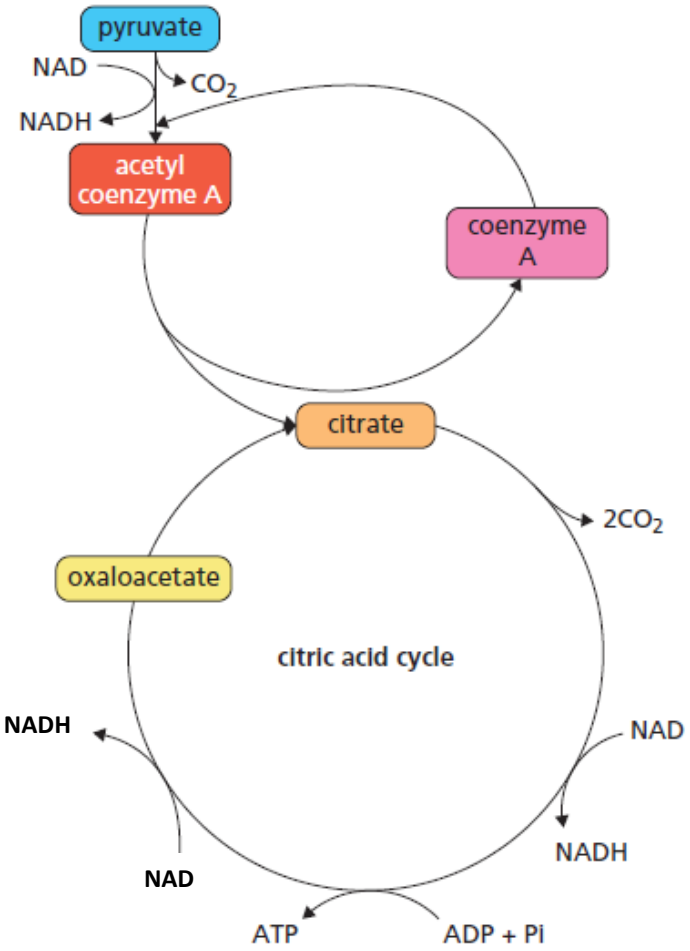


Citric Acid Cycle

The Aerobic phase of respiration takes place in the **Matrix of Mitochondria**.

In the presence of oxygen, **Pyruvate** is then **broken down** to an **Acetyl Group** that **combines with Coenzyme A** forming **Acetyl Coenzyme A**.

In the Citric Acid Cycle, the **Acetyl group** from Acetyl Coenzyme A **combines with Oxaloacetate to form Citrate**.



During a series of enzyme controlled steps, **Citrate** is gradually **converted back** into **Oxaloacetate** which results in the **generation of ATP** and **release of Carbon Dioxide**.

Dehydrogenase enzymes remove hydrogen ions and electrons and pass them to the Coenzyme **NAD**, forming **NADH**. This occurs in both Glycolysis and the Citric Acid Cycle.

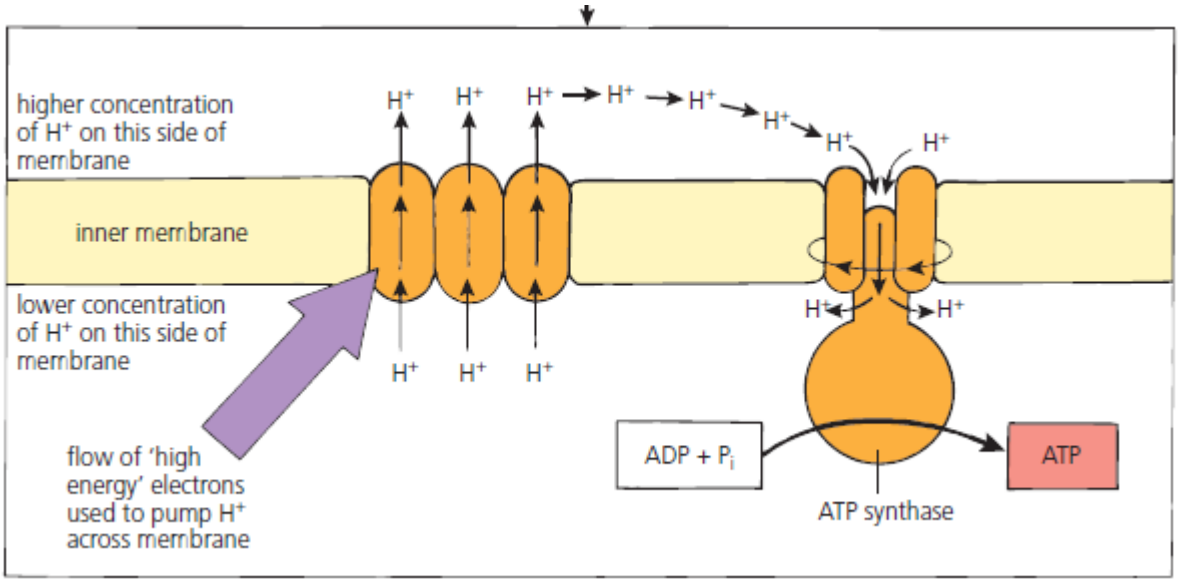
The **hydrogen ions and electrons from NADH** are passed to the **Electron Transport Chain** on the **inner mitochondrial membrane**.

ATP Synthesis

The **electron transport chain** is a series of carrier proteins attached to the inner mitochondrial membrane.

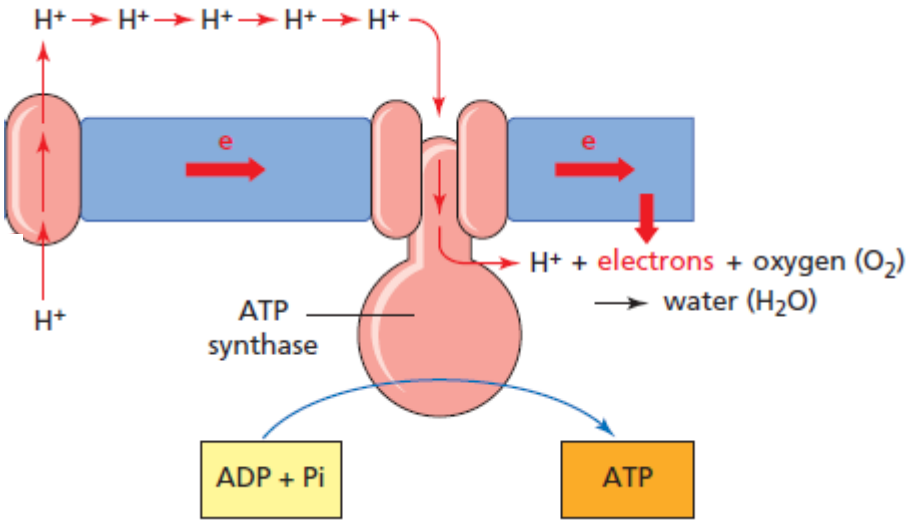
Electrons are passed along the **Electron Transport Chain releasing energy**. This energy allows **Hydrogen ions to be pumped across the inner mitochondrial membrane**.

The **flow of these ions** back through the membrane protein **ATP Synthase**, results in the **production of ATP**.



Finally, **hydrogen ions and electrons combine with oxygen to form water.**

Oxygen is required during the aerobic phase of respiration since it is the **final hydrogen acceptor,** combining with hydrogen ions & electrons to form water.



Fermentation

In the **absence of oxygen**, **fermentation** takes place in the **cytoplasm**.

Glycolysis takes place as normal, however the Pyruvate formed cannot enter the Citric Acid cycle. Instead, the Pyruvate is then fermented as follows:

Animal Cells

In animal cells, the **Pyruvate is converted to Lactate** in a reversible reaction.

Pyruvate $\rightleftharpoons$ Lactate

Plant & Yeast Cells

In plant & yeast cells, the Pyruvate is converted to Ethanol and CO_2 in a reversible reaction.

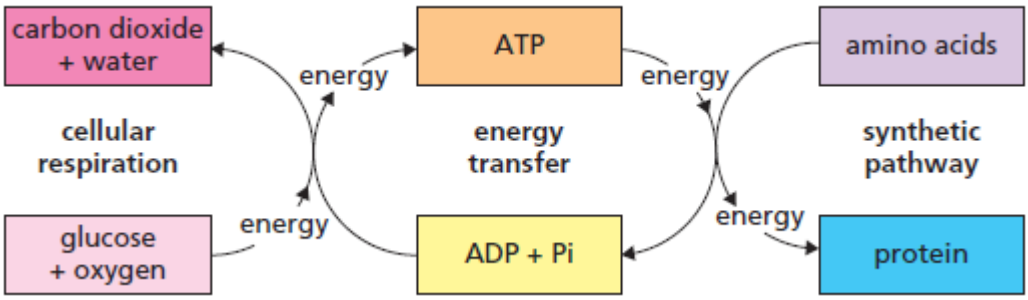
Pyruvate $\rightleftharpoons$ Ethanol + Carbon Dioxide

Fermentation results in much less ATP being produced than in aerobic respiration.

Role of ATP

ATP is used to transfer energy to cellular processes which require energy.

E.g the ATP released during aerobic respiration provides the energy required for Protein Synthesis.



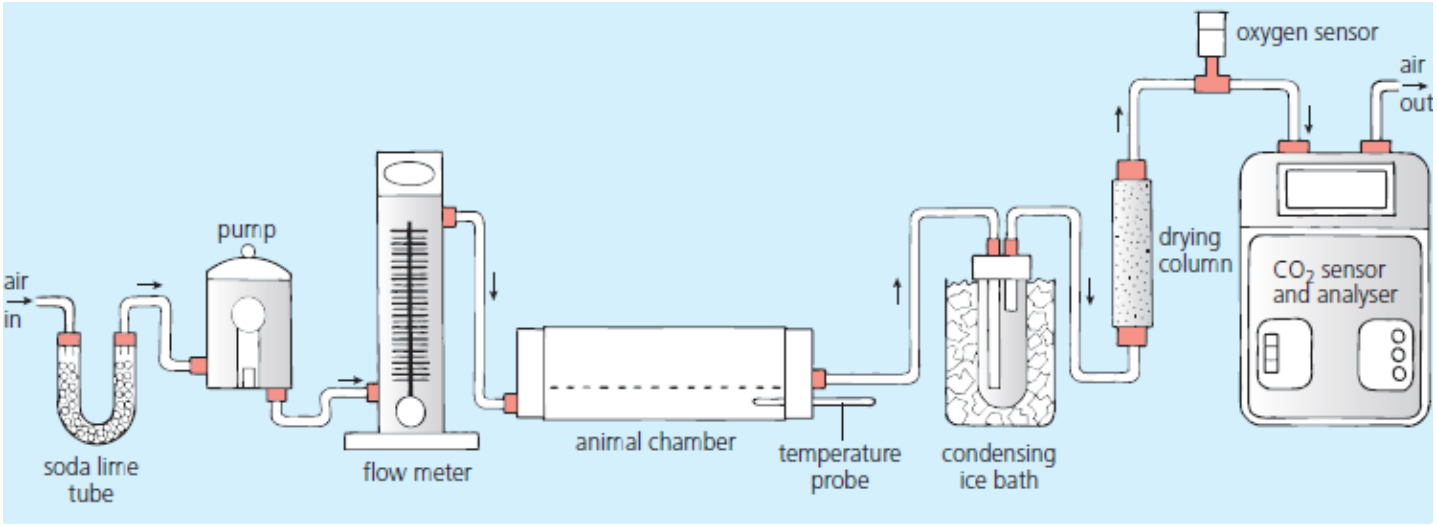
Unit 2 Metabolism & Survival

Key Area 3 : Metabolic Rate

Metabolic rate can be measured either by measuring the rate of:

- **Oxygen Consumption**
- **Carbon Dioxide Production**
- **Heat Production**

This involves the use of **respirometers, oxygen probes, carbon dioxide probes & calorimeters**.



Organisms with High Metabolic Rates

Organisms with high metabolic rates require more efficient delivery of oxygen to cells.

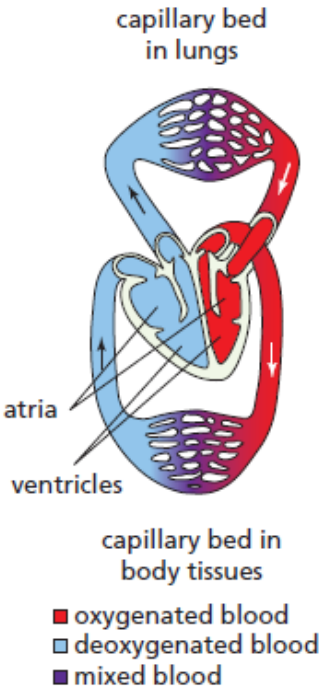
Birds and Mammals have **higher metabolic rates** than **Reptiles and Amphibians**, which in turn have higher metabolic rates than **Fish**.

(Birds & Mammals > Reptiles & Amphibians > Fish)

Circulatory Systems

Birds & Mammals

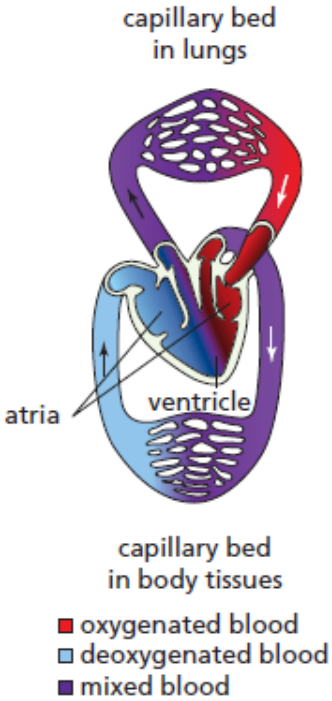
Birds & Mammals have a **Complete Double** circulatory system consisting of **2 Atria & 2 Ventricles.**



Complete Double circulatory systems enable **higher metabolic rates** to be maintained. There is **no mixing of oxygenated & deoxygenated** blood and the oxygenated blood can be pumped out at a **higher pressure**. This enables more **efficient Oxygen delivery to cells**.

Amphibians & Reptiles

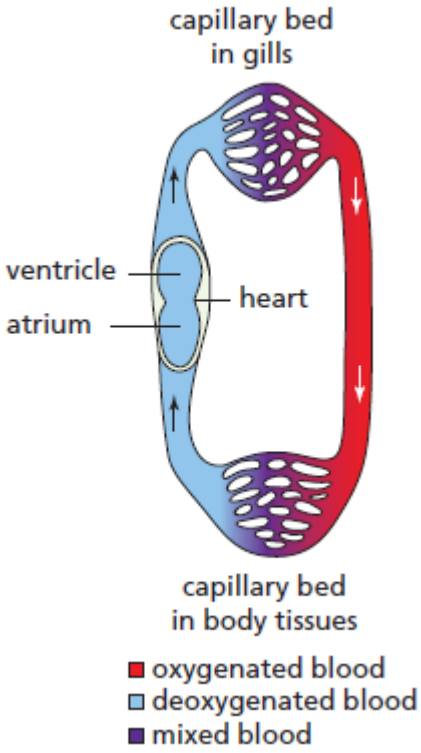
Amphibians & most reptiles have an **Incomplete Double** circulatory system consisting of **2 Atria & 1 Ventricle**.



Incomplete Double circulatory systems are less efficient in the delivery of oxygen to cells since there is **mixing of oxygenated and deoxygenated blood** in the single ventricle present.

Fish

Fish have a **single circulatory system** consisting of **1 Atrium & 1 Ventricle**. It is called a single circulatory system because the blood only passes through the heart ONCE in each complete circuit around the body.



As blood passes through a capillary bed (e.g. at the gills), there is drop in blood pressure. This means that blood is delivered to the capillary bed in the body tissues at **LOW PRESSURE**.

Unit 2 Metabolism & Survival

Key Area 4: Metabolism in Conformers and Regulators

The ability of an organism to maintain it's metabolic rate is affected by **external abiotic factors**.

Abiotic factors which affect an organisms ability to maintain metabolic rate include:

- **Temperature**
- **Salinity**
- **pH**

Conformers

A Conformers **internal environment is dependant upon it's external environment**.

Conformers use **Behavioural responses** to **maintain optimum metabolic rate**. E.g. Lizards can maintain their body temperature by basking in the sunshine.

Behavioural responses by conformers allow them to **tolerate variation** in their external environment to maintain optimum metabolic rate.

Conformers have **LOW METABOLIC COSTS** and a **NARROW RANGE OF ECOLOGICAL NICHES**.

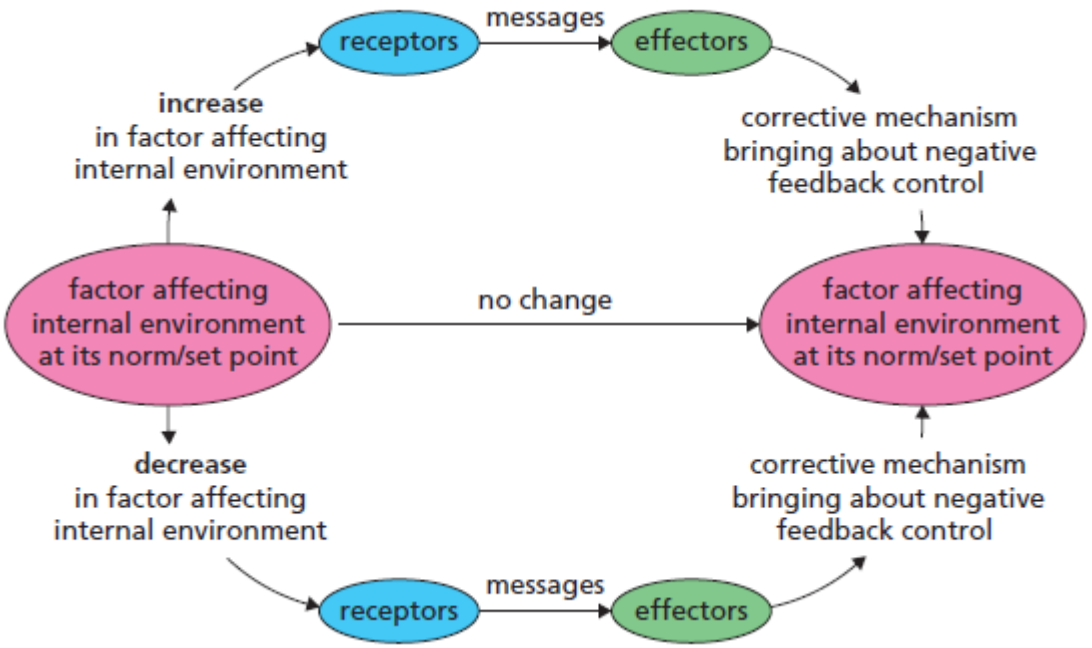
Regulators

A Regulator **can maintain it's internal environment regardless of it's external environment**.

Regulators use metabolism to control their internal environment, which **INCREASES THE RANGE OF POSSIBLE ECOLOGICAL NICHES**.

This regulation **requires ENERGY** to achieve **HOMEOSTASIS**. This **INCREASES THEIR METABOLIC COSTS**.

The control mechanism by which Regulators maintain Homeostasis is called **NEGATIVE FEEDBACK CONTROL**.



THERMOREGULATION BY NEGATIVE FEEDBACK CONTROL

The **Hypothalamus** is the temperature monitoring centre in the brain.

Information is communicated by electrical impulses through nerves to the effectors, which bring about corrective responses to return temperature to normal.

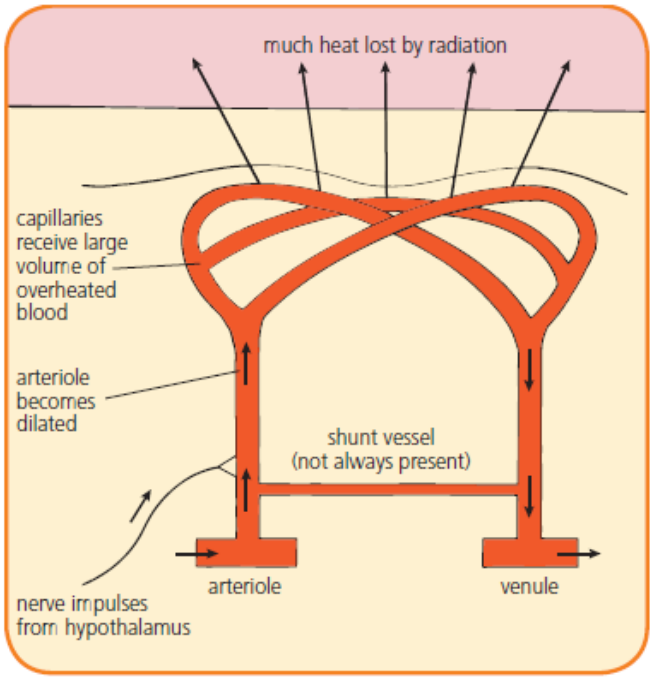
Response to an Increase in Body Temperature

When the Hypothalamus detects an **increase in body temperature**, **nerve impulses** are sent to **effectors** to make response measures which return temperature to normal. These include:

- Sweating
- Vasodilation of Blood Vessels
- Decreased metabolic rate

Sweating : body heat is used to evaporate water in the sweat, cooling the skin.

Vasodilation of Blood Vessels: dilation of blood vessels , **increases blood flow to the skin** (reason why we look red when hot) and allows **heat to be lost by radiation** from the skin surface.



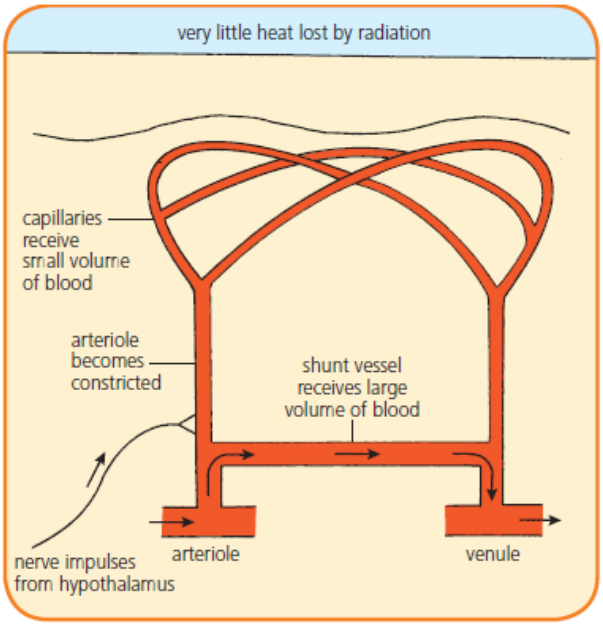
Response to a Decrease in Body Temperature

When the Hypothalamus detects a **decrease in body temperature**, **nerve impulses** are sent to **effectors** to make response measures which return temperature to normal. These include:

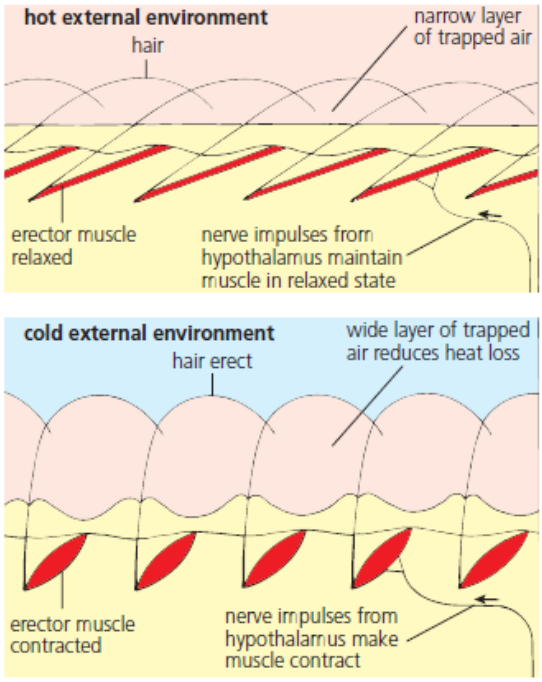
- Shivering
- Vasoconstriction of Blood vessels
- Hair erector muscles contracting
- Increased metabolic rate

Shivering: Muscle contraction generates heat to return body temperature to normal.

Vasoconstriction of Blood vessels: Decreased blood flow to the skin (reason why we look pale when cold) decreases heat loss from the skin surface.



Hair Erector muscles contract: this traps a layer of Insulating air.



Increased metabolic rate: more heat is produced to return body temperature to normal.

Importance of Regulating Temperature

Thermoregulation (control of internal body temperature to within tolerable limits) is essential for **optimal enzyme activity** and **high diffusion rates** to **maintain metabolism**.

Unit 2 Metabolism & Survival

Key Area 5: Metabolism and Adverse conditions

Many environments vary beyond the tolerable limits for normal metabolic activity for any particular organism. Some animals have adapted to survive these adverse conditions while others avoid them.

Surviving Adverse conditions by Dormancy

Dormancy is part of some organism' lifecycle to allow survival during a period when the costs of continued normal metabolic activity would be too high.

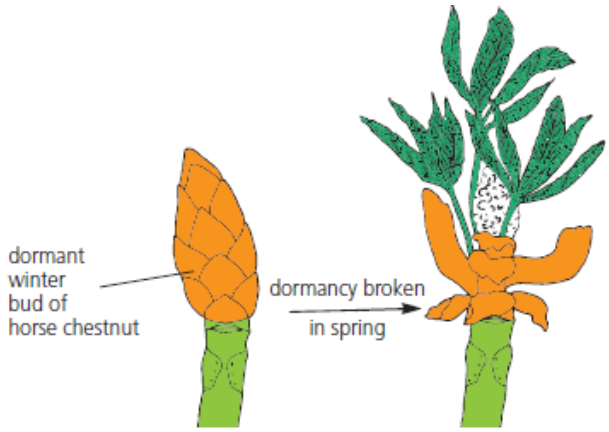
The **metabolic rate** can be **reduced** during **dormancy** to **save energy.**

During **dormancy** there is a **decrease in metabolic rate, heart rate, breathing rate and body temperature.**

Dormancy can be **predictive or consequential**.

Predictive Dormancy : **occurs before the onset of adverse conditions**. This is common in environments which have predictable seasons where the temperature and photoperiod (number of hours of daylight) can be used as triggers.

E.g. Many trees shed their leaves in Autumn and winter buds remain dormant until the Spring.



Consequential Dormancy: **occurs after the onset of adverse conditions**. This is common among organisms living in unpredictable environments. The advantage of this type of dormancy is that the organism may remain active for longer and continue to make use of the available resources. Unfortunately they run the risk of being killed off in the event of a sudden and severe environmental change.

Type of Dormancy

The main types of Dormancy include:

- **Hibernation** - Some mammals survive during **Winter/Low temperatures** by hibernating.

  E.g Hedgehogs slow their heart rate, breathing rate, body temperature and levels of activity so that the minimum amount of energy is expended to maintain vital cell activity.

- **Aestivation** - This allows survival in periods of **High temperature** or **Drought**.

  E.g. Lungfish buries itself with a cocoon of dried mucus while ex changing gases through a breathing tube. It can remain dormant for many months until the arrival of the next rainy season.

- **Daily Torpor** - This is a period of reduced activity in some animals with **high metabolic rates** (e.g. small birds & mammals).

<u>Avoiding Adverse Conditions by Migration</u>

Migration **avoids metabolic adversity** by **expending energy** to **relocate to a more suitable environment**.

Migratory behaviour can be **innate** (inherited) and **learned** (gained by experience).

<u>Tracking Migration</u>

Specialised techniques are used to study long-distance migration.

Examples include:

- Satellite tracking
- Leg rings

Unit 2 Metabolism & Survival

Key Area 6 : Environmental Control of Metabolism

Micro-organisms include :

- **Archaea**
- **Bacteria**
- **Some species of Eukaryotes (e.g. yeast & protozoans)**

Micro-organisms use a **wide-variety of substrates** for metabolism and **produce a range of products** from their metabolic pathways.

Micro-organisms are used because of their **adaptability**, **ease of cultivation (growing)** and **speed of growth**.

<u>Variations in Growth media and control of Environmental factors</u>

When culturing micro-organisms, their **growth media requires raw materials for biosynthesis** as well as an **energy source**.

Many micro-organisms produce all the complex molecules required for biosynthesis

e.g. **Amino acids**

Vitamins

Fatty acids

Other micro-organisms require these to be supplied in the growth media.

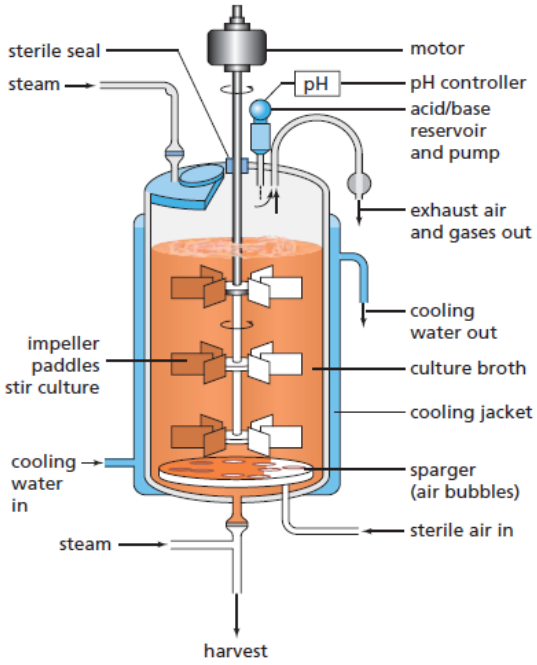
Growth media may contain simple substances suitable for specific micro-organisms or complex ingredients such as **beef extract**.

An **energy source** is derived either from **chemical substrates such as carbohydrates** or from **light in the case of photosynthetic micro-organisms**.

Culture Conditions

Culture conditions include: (Clue : STOP)

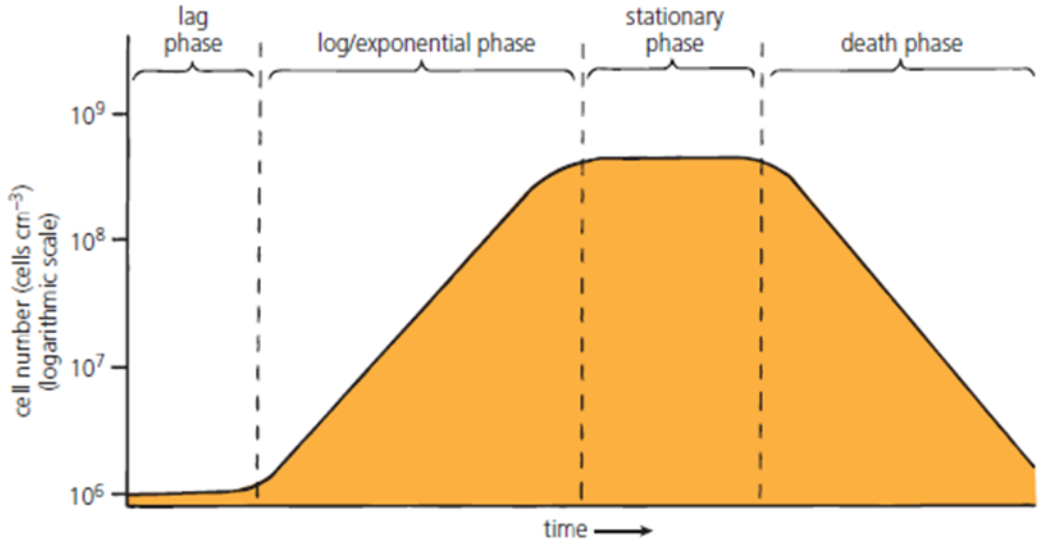
- **Sterility** - sterile conditions in fermenters reduce competition with desired micro-organisms for nutrients and reduce the risk of spoilage of the product.
- **Temperature** must be controlled
- **Oxygen** - Oxygen levels may be controlled by aeration (air pump).
- **pH** - pH can be regulated by adding pH Buffers or the addition of acid or alkali.



An industrial fermenter can be used to culture micro-organisms on a large scale. Sensors are used to monitor temperature, pH and oxygen levels.

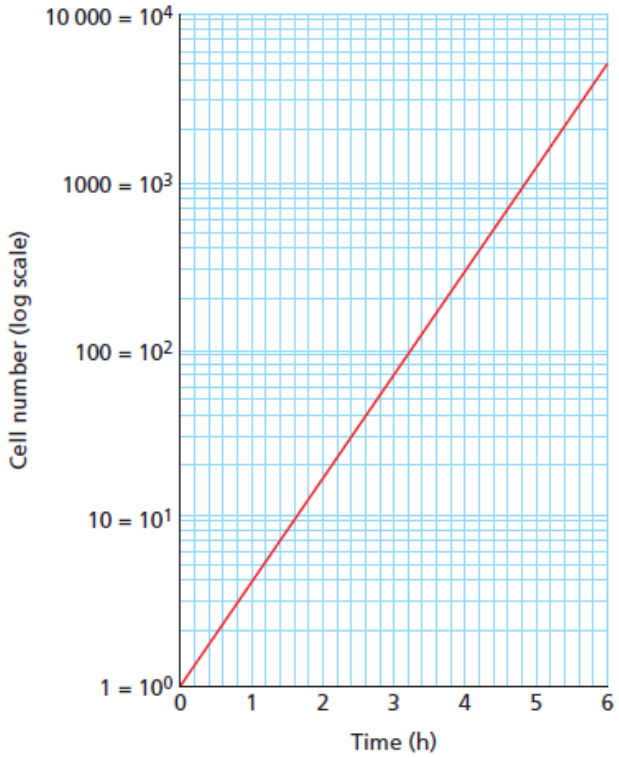
Phases of Growth and changes in culture conditions

Micro-organisms have 4 phases of Growth:



- **Lag** - During the Lag phases **ENZYMES ARE INDUCED** to metabolise substrates.
- **Log/Exponential** - This phase contains the **most rapid growth** of micro-organisms due to **plentiful supply of nutrients**.
- **Stationary** - This phase occurs due to the **nutrients in the culture media becoming depleted** and the **production of toxic metabolites**. **Secondary metabolites**, such as antibiotics, are **also produced**. In the wild these metabolites confer an ecological advantage by allowing the micro-organisms which produce them to outcompete other micro-organisms.
- **Death** - This phase occurs due to the **toxic accumulation of metabolites** or the **lack of nutrients** in the culture.

Semi-logarithmic scales can be used to show the exponential growth phase of micro-organisms. Use of semi-logarithmic graphs to plot the exponential growth phase, produces a straight line. On the cell number scale, the division between 1 and 10 is the same size as that between 10 and 100.



Viable and Total Cell Count

VIABLE cell counts involve counting **only the LIVING micro-organisms**.

TOTAL cell counts involve counting **viable and dead cells**.

Only viable cell counts show a death phase where cell numbers are decreasing.

Unit 2 Metabolism & Survival

Key Area 7: Genetic control of metabolism

Wild strains of micro-organisms can be improved by Mutagenesis or Recombinant DNA technology.

Mutagenesis

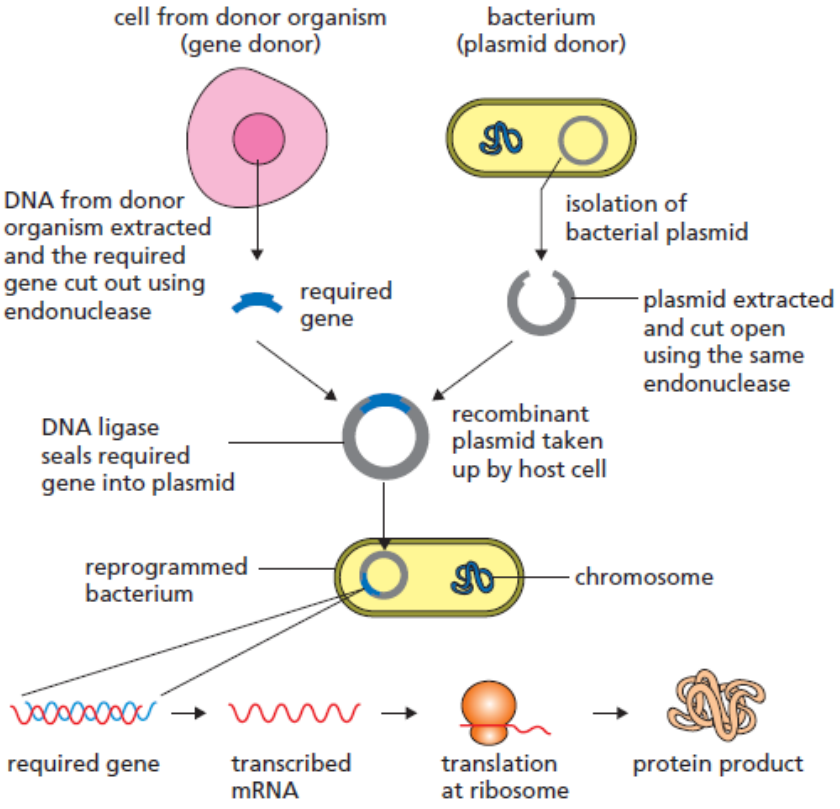
Mutagenesis is the process of inducing mutations.

Exposure to **UV light** and other forms of **Radiation** (e.g. X-rays or Gamma rays) or **Chemicals** (e.g Mustard gas) results in mutations. **Some of these mutations produce an improved strain of micro-organism.**

Recombinant DNA Technology

Recombinant DNA technology involves the use of **recombinant plasmids** and **artificial chromosomes** as **vectors**.

A vector is a DNA molecule used to carry foreign genetic information (DNA) from a donor organism into a host cell.

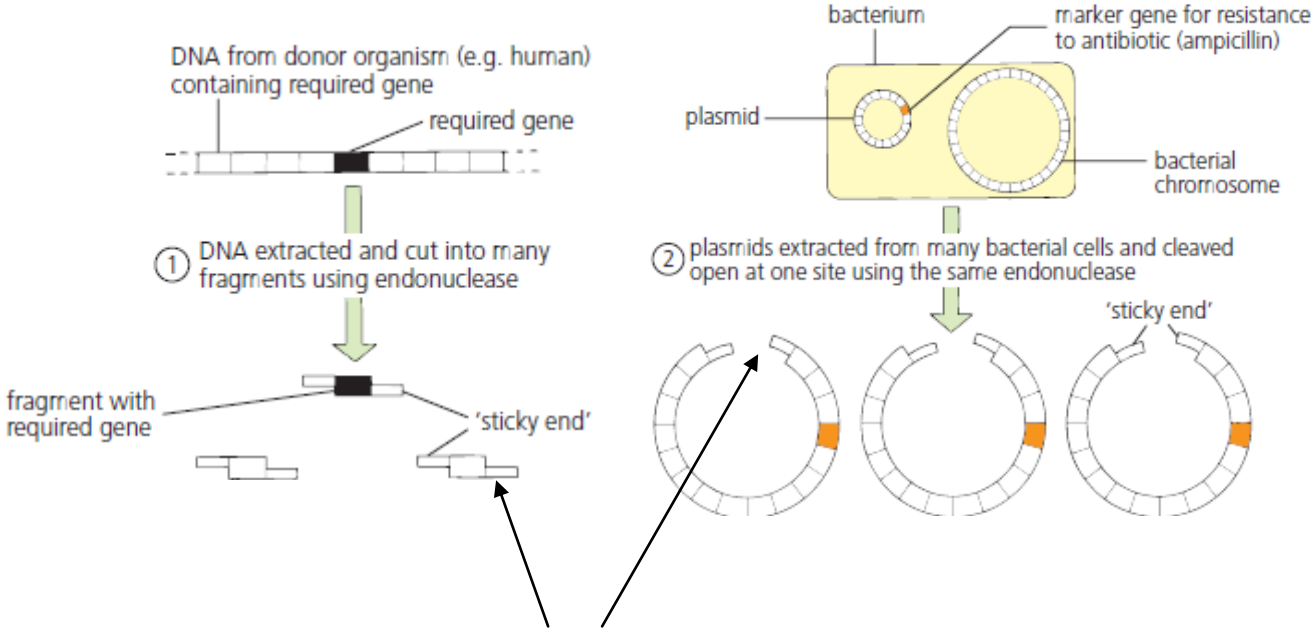


Artificial chromosomes are preferable to plasmids as vectors when **larger fragments** of foreign DNA are required to be inserted.

Restriction Endonuclease Enzymes

Restriction endonucleases **cut open plasmids** and **specific genes out of chromosomes** leaving **sticky ends**.

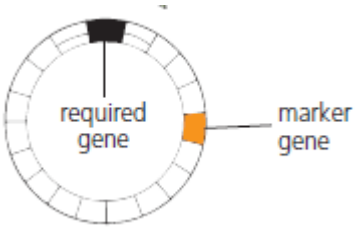
Complimentary sticky ends are produced when the **same restriction endonuclease** is used to **cut open the plasmid** and the **gene from the chromosome**.



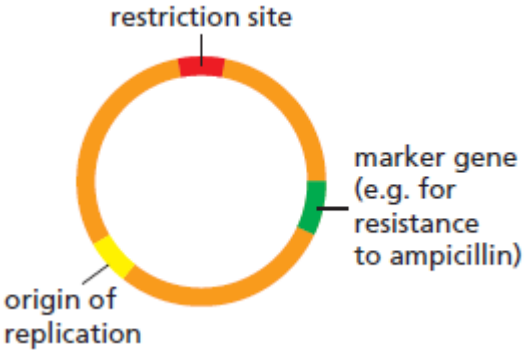
Complimentary sticky ends

Ligase

Ligase is the enzyme used to **seal the gene into the plasmid**.

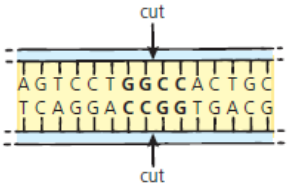
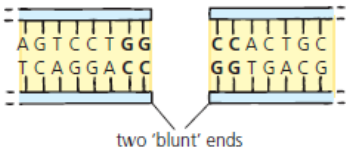
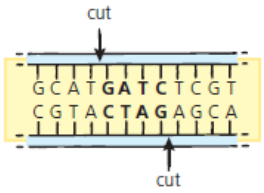
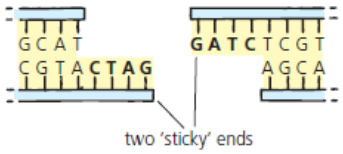
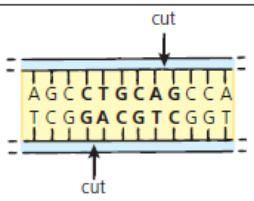
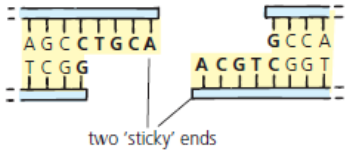


Recombinant plasmids and artificial chromosomes contain **restriction sites**, **regulatory sequences, an origin of replication** and **selectable markers**.



Restriction sites contain **target sequences of DNA where specific restriction endonucleases cut**.

E.g.

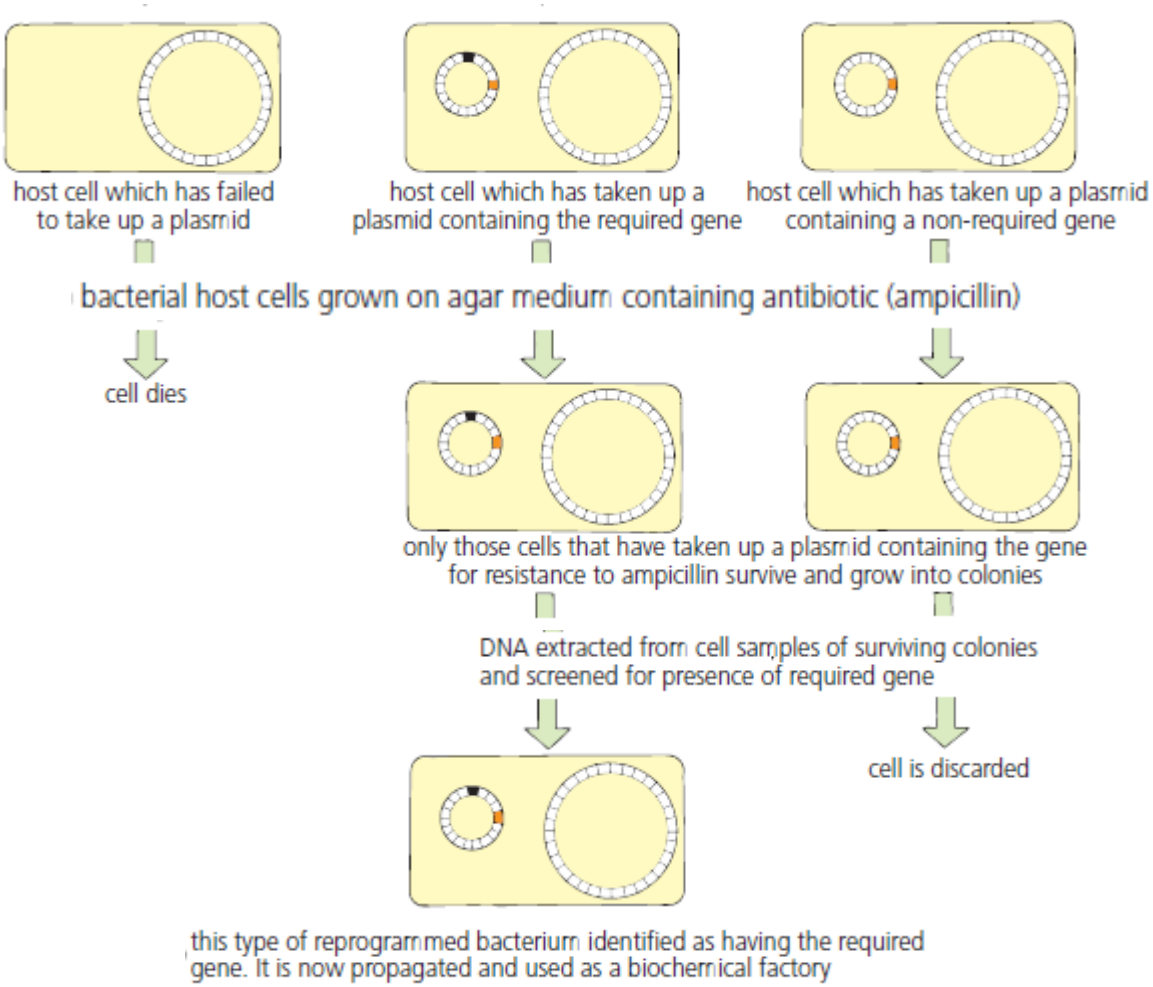
Enzyme	Recognition sequence on DNA	Position on DNA cut by enzyme	Result
1	GGCC CCGG		
2	GATC CTAG		
3	CTGCAG GACGTC		

Regulatory sequences control gene expression.

Origin of Replication allows **self-replication of the plasmid/artificial chromosome**.

Selectable Marker Genes

Selectable markers such as **antibiotic resistance genes** protect the micro-organism from a selective agent (antibiotic) that would normally kill it or prevent it growing.



Selectable marker genes present in the vector ensure that only micro-organisms that have taken up the vector grow in the presence of the selective agent (antibiotic).

i.e

To identify bacterial cells which have taken up the recombinant plasmid, the cells are grown in a nutrient medium containing an antibiotic. **Only bacterial cells which contain the recombinant plasmid survive** (due to the presence of the marker gene/antibiotic resistance gene). **Bacterial cells which have not taken up the recombinant plasmid do not have resistance to the antibiotic and so are killed**.

Safety precautions

As a safety mechanism, **genes are often introduced** that **prevent the survival of the micro-organism in an external environment**.

Limitations of using Prokaryotes in Recombinant DNA technology

Plant or animal (eukaryotic) DNA expressed in bacteria (prokaryotes) may result in **polypeptides being incorrectly folded** (since they are not capable of post-translational modification).

Recombinant Yeast Cells (which are eukaryotic) may be used instead to produce active forms of the protein which are inactive in bacteria.