# Edexcel International A Level (IAL) Biology

Your notes

## Respiration

### Contents

Your notes

# Overview of Respiration

- **Glucose** is the main respiratory substrate used by cells
- Aerobic respiration is the process of breaking down a respiratory substrate in order to **produce ATP using oxygen**
- The equation for aerobic respiration:

**glucose + oxygen → carbon dioxide + water + energy**

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 2870kJ$$

- The energy that is released during the process is used to phosphorylate (add a phosphate) ADP to form ATP
- ATP provides energy for other biological processes in cells
- The process of aerobic respiration using glucose can be split into four stages which each occurs at a particular location in a eukaryotic cell:
  - **Glycolysis** takes place in the cell cytoplasm
  - The **Link reaction** takes place in the matrix of the mitochondria
  - The **Krebs cycle** takes place in the matrix of the mitochondria
  - **Oxidative phosphorylation** occurs at the inner membrane of the mitochondria
- These chemical reactions are controlled by **intracellular enzymes** that catalyses reactions within the cell
  - Ensuring that the energy trapped within the chemical bonds of the glucose molecule is **released gradually** and not all at once
- A sudden release of such a large amount of energy would result in an increase in body temperature to levels that would **denature enzymes**
- The enzyme that catalyses these reactions the **slowest** will determine the **overall rate of aerobic respiration**
- Several **coenzymes** are required during respiration to **transfer** various molecules involved in the process
  - **NAD** and **FAD** are the coenzymes responsible for **transferring hydrogen** between molecules
  - Depending on whether they give or take hydrogen, they are able to **reduce** or **oxidise** a molecule
  - **Coenzyme A** is responsible for the **transfer of acetate** (also known as acetic acid) from one molecule to another

- Although glucose is the main fuel for respiration, organisms can also break down other molecules (such as fatty acids or amino acids) to be respired

**Four Stages of Respiration Table**

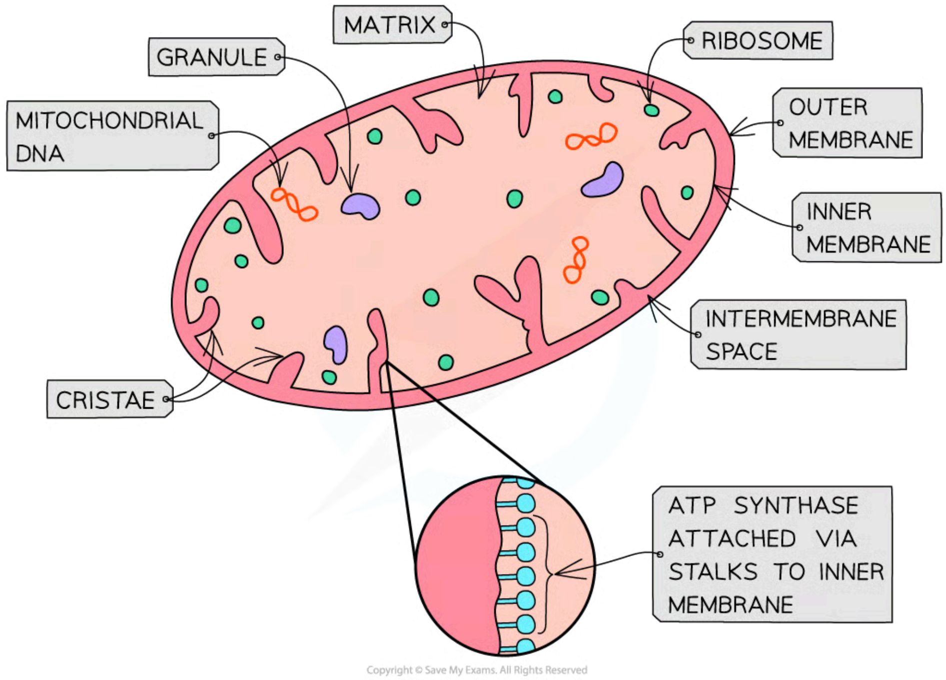
| Stage | Description | Location |
|---|---|---|
| **1. Glycolysis** | Phosphorylation and splitting of glucose | Cell cytoplasm |
| **2. Link reaction** | Decarboxylation and dehydrogenation of pyruvate | Matrix of mitochondria |
| **3. Krebs cycle** | Cyclical pathway with enzyme–controlled reactions | Matrix of mitochondria |
| **4. Oxidative phosphorylation** | Production of ATP through oxidation of hydrogen atoms | Inner membrane of mitochondria |



## Structure of mitochondria

- Mitochondria have two phospholipid membranes
- The **outer membrane** is:
  - Smooth
  - Permeable to several small molecules
- The **inner membrane** is:
  - Folded (cristae)
  - **Less permeable**
  - The site of the **electron transport chain** (used in oxidative phosphorylation)
  - Location of **ATP synthase enzymes** (used in oxidative phosphorylation)
- The **intermembrane space**:
  - Has a low pH due to the **high concentration of protons**
  - The concentration gradient across the inner membrane is formed during oxidative phosphorylation and is **essential for ATP synthesis**
- The **matrix**:
  - Is an aqueous solution within the inner membranes of the mitochondrion

- Contains ribosomes, enzymes and circular mitochondrial DNA necessary for mitochondria to function




*The structure of a mitochondrion*

### Examiner Tips and Tricks

It's important to know the exact locations of each stage. It is not enough to say the Krebs cycle takes place in the mitochondria, you need to say it takes place in the **matrix** of the mitochondria.

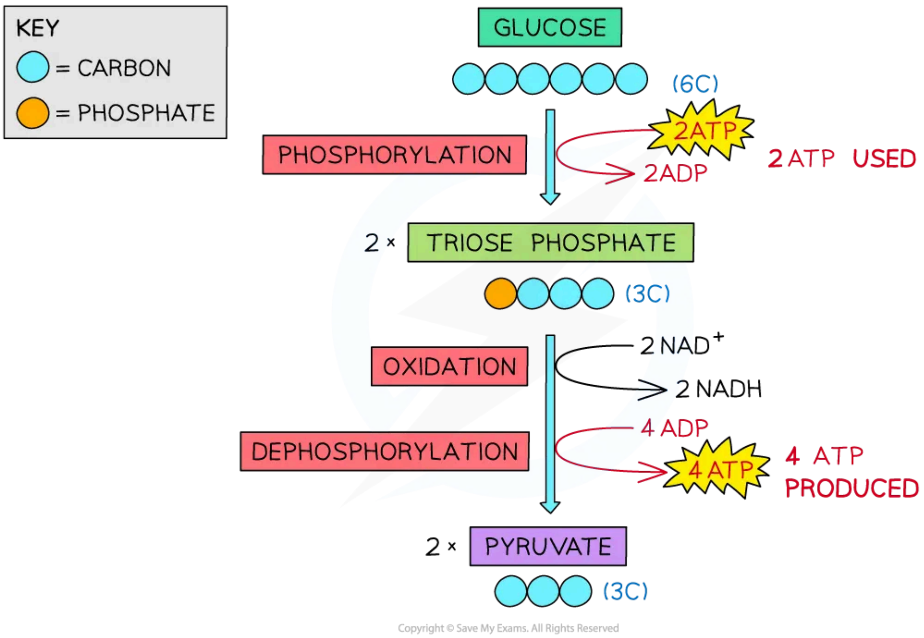
Your notes

# Glycolysis

- Glycolysis is the first stage of respiration
- It does not require oxygen to take place and is therefore the first step for both aerobic and anaerobic respiration
- Glucose is only **partially oxidised** during glycolysis
- It takes place in the cytoplasm of the cell and involves:
  - **Trapping glucose** in the cell by **phosphorylating** the molecule
  - **Oxidising** triose phosphate (by losing hydrogen)
- It results in the production of
  - **2 Pyruvate** (3C) molecules which moves into the matrix of mitochondria to be used during the link reaction
  - Net gain **2 ATP**
  - **2 reduced NAD**, which will be used during a later stage called oxidative phosphorylation
- Under **anaerobic** conditions, glycolysis produces **lactic acid or lactate** instead of pyruvate

## Steps of glycolysis

- **Phosphorylation** of glucose (a hexose sugar)
  - Two molecules of ATP are required to provide the **two phosphates** needed for the phosphorylation of glucose
  - This produces
    - **Two molecules of triose phosphate**
    - **Two molecules of ADP**
- **Oxidation** of triose phosphate
  - After triose phosphate **loses hydrogen**, it forms **two molecules of pyruvate**
  - The **hydrogen ions are collected by NAD** which reduces the coenzyme
  - This forms **two reduced NAD or NADH**
  - Even though a total of four ATP molecules were produced during glycolysis, two of them were used to phosphorylate glucose
  - There was therefore a **net gain of two ATP molecules**




*The process of glycolysis*

### Examiner Tips and Tricks

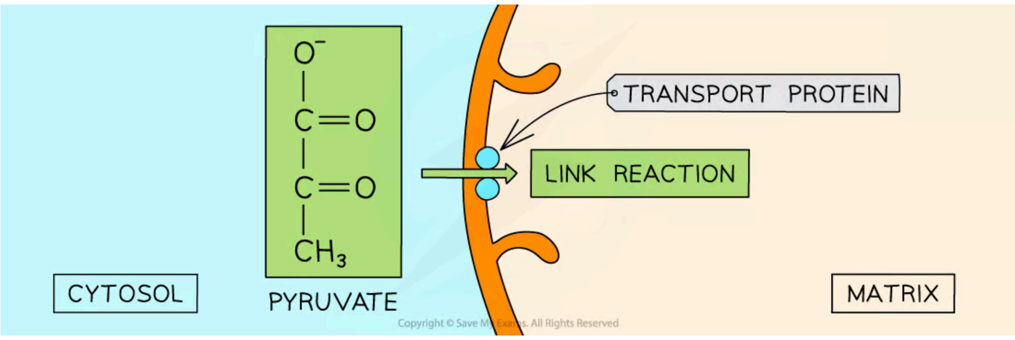
It may seem strange that ATP is used and also produced during glycolysis. At the start ATP is used to **make glucose more reactive** (it is usually very stable) and to lower the activation energy of the reaction. Since 2 ATP are used and 4 are produced during the process, there is a **net gain of 2 ATP per glucose molecule.**

Your notes

# Link Reaction & Krebs Cycle

## The Link reaction

- The end product of glycolysis is **pyruvate**
- Pyruvate contains a substantial amount of chemical energy that can be further utilised in respiration to produce more ATP
- The enzymes and coenzymes that are required for the link reaction are found in the mitochondrial matrix
- When **oxygen is available** pyruvate will **enter the mitochondrial matrix** and **aerobic** respiration will continue
- Pyruvate moves across the double membrane of the mitochondria via **active transport**
  - It requires a transport protein and a small amount of ATP
- Once in the mitochondrial matrix **pyruvate** takes part in the **link reaction**



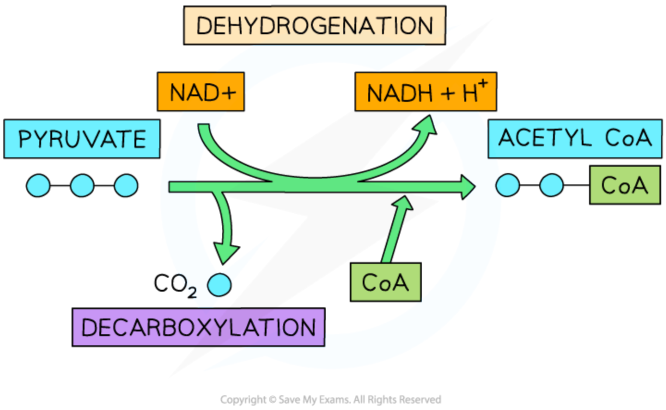

***Pyruvate enters the mitochondrial matrix from the cytosol (cytoplasm) by active transport***

- The link reaction takes place in the matrix of the mitochondria
- It is referred to as the link reaction because it **links glycolysis** to the **Krebs cycle**
- The steps are:
  - **Pyruvate is oxidised** (hydrogen is removed) by enzymes to produce **acetate, $CH_3CO(O)$** (also known as acetic acid)
  - Pyruvate is also **decarboxylated** (carbon is removed) in the form of **carbon dioxide**
  - **Reduction of NAD** to NADH or reduce NAD by collecting hydrogen from pyruvate
  - **Acetate combines with coenzyme A** to form **acetyl coenzyme A (acetyl CoA)**
- **No ATP is produced** during the link reaction

Your notes

- It produces:
  - Acetyl coA
  - Carbon dioxide ($CO_2$)
  - Reduced NAD (NADH)

**pyruvate + NAD + CoA → acetyl CoA + carbon dioxide + reduced NAD**



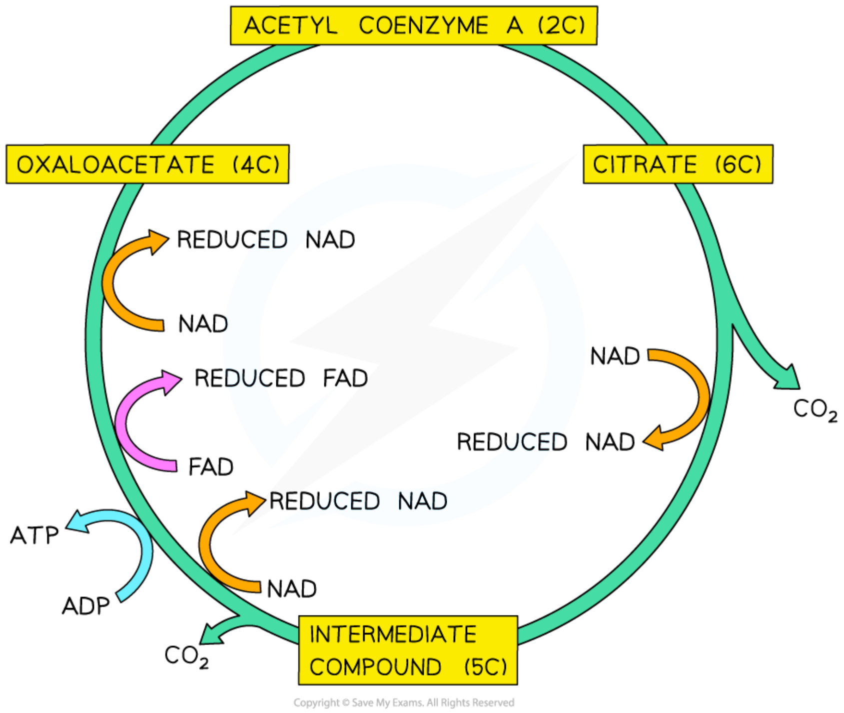

***The link reaction occurs in the mitochondrial matrix. It dehydrogenates and decarboxylates the three-carbon pyruvate to produce the two-carbon acetyl CoA that can enter the Krebs Cycle***

- Every **molecule of glucose** produces **two pyruvate molecules**
- The link reaction and the Krebs cycle will therefore **occur twice** for every molecule of glucose
- Thus, each molecule of glucose will produce:
  - **Two** molecules of **acetyl CoA**
  - **Two** molecules of **$CO_2$**
  - **Two** molecules of **reduced NAD**

## The Krebs Cycle

- The Krebs cycle (sometimes called the citric acid cycle) consists of a **series of enzyme-controlled reactions**
- **2 carbon (2C) Acetyl CoA** enters the circular pathway from the **link reaction** in glucose metabolism
  - Acetyl CoA formed from **fatty acids** (after the breakdown of lipids) and **amino acids** enters directly into the Krebs Cycle from other metabolic pathways
- 4 carbon (**4C**) **oxaloacetate** accepts the **2C acetyl** fragment from acetyl CoA to form the 6 carbon (**6C) citrate**
  - Coenzyme A is released in this reaction to be reused in the next link reaction

- **Citrate is then converted back to oxaloacetate** through a series of oxidation-reduction (redox) reactions




*The Krebs Cycle uses acetyl CoA from the link reaction and the regeneration of oxaloacetate to produce reduced NAD, reduced FAD and ATP*

## Regeneration of oxaloacetate

- Oxaloacetate is regenerated in the Krebs cycle through a series of **redox reactions**
- **Decarboxylation** of citrate
  - Releasing **2 $CO_2$** as waste gas
- **Oxidation** (dehydrogenation) of citrate
  - Releasing H atoms that **reduce coenzymes NAD and FAD**
  - These will be used during oxidative phosphorylation
  - 3 NAD and 1 FAD → **3NADH + $H^+$ and 1 $FADH_2$**
- **Substrate linked phosphorylation**
  - A phosphate is transferred from one of the intermediates to ADP, forming **1 ATP** to supply energy
- Because **two acetyl-CoA molecules** are produced from each glucose molecule, **two cycles are required per glucose** molecule
- Therefore, at the end of two cycles, the **products are:**
  - **Two** ATP

Your notes

- **Six** NADH (reduced NAD)
- **Two** $FADH_2$ (reduced FAD)
- **Four** $CO_2$

### Examiner Tips and Tricks

The Krebs cycle is often referred to as cyclical or circular. This is because the acceptor molecule oxaloacetate is regenerated throughout the reaction so that it can start all over again by adding another acetyl CoA.

You may be asked to name the important molecules in the Krebs cycle like oxaloacetate and citrate.

It is also worth noting how the number of carbon atoms in the substrate molecule changes as the cycle progresses.

Your notes

# The Electron Transport Chain

- **Oxidative phosphorylation** is the last stage of aerobic respiration
    - It takes place at the **inner mitochondrial membrane**
    - It results in the **production of many molecules of ATP** and the **production of water from oxygen**
- The current model for oxidative phosphorylation is the **chemiosmotic theory**
    - The model states that **energy from electrons** is passed through a chain of proteins in the membrane, known as the **electron transport chain**
    - This energy is used to **pump protons (hydrogen ions) against their concentration gradient** into the intermembrane space
    - The protons are then allowed to **flow by facilitated diffusion** through a channel enzyme called **ATP synthase** into the matrix
    - The energy of the protons flowing down their concentration gradient is harnessed (a bit like water flowing through a hydroelectric damn) resulting in the **phosphorylation of ADP into ATP** by ATP synthase

## Outline of oxidative phosphorylation

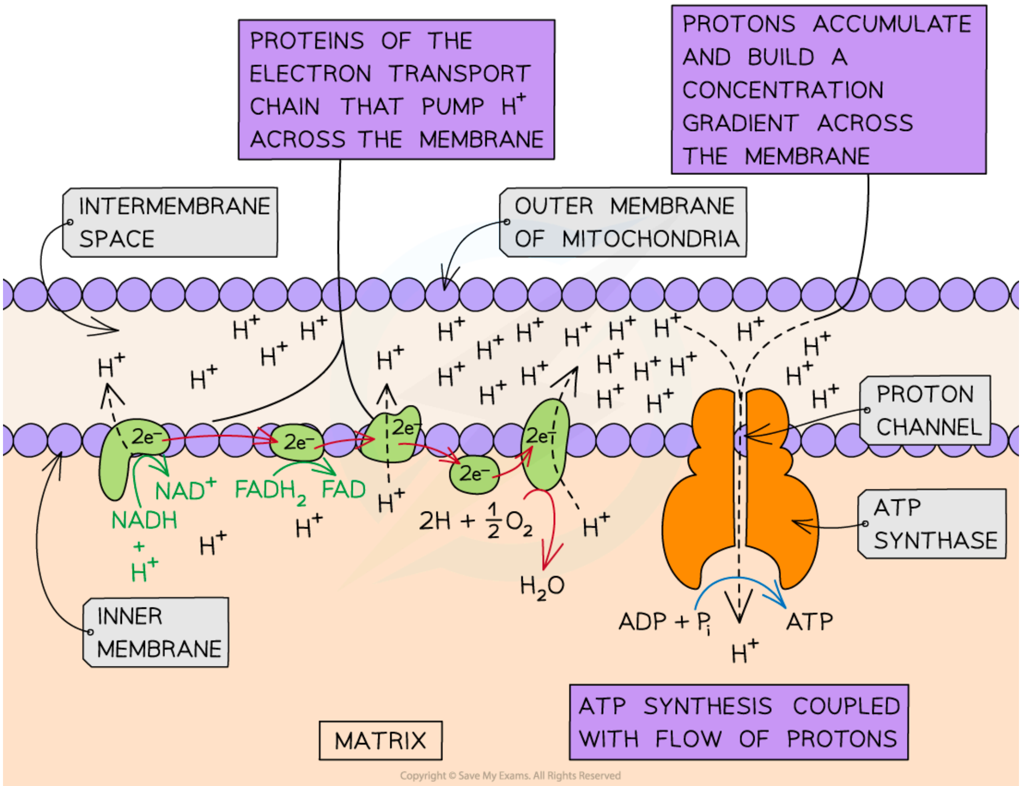
- Hydrogen atoms are donated by reduced NAD (NADH) and reduced FAD ($FADH_2$) from the Krebs Cycle
- Hydrogen atoms **split into protons ($H^+$ ions) and electrons**
- The high energy electrons enter the **electron transport chain** and **release energy** as they move through the electron transport chain
- The energy released is used to **transport protons** across the inner mitochondrial membrane **from the matrix into the intermembrane space**
- A **concentration gradient** of protons is established between the intermembrane space and the matrix
- The protons return to the matrix via **facilitated diffusion** through the **channel enzyme ATP synthase**
- The movement of protons down their concentration gradient provides energy for **ATP synthesis**
- **Oxygen** acts as the '**final electron acceptor**' and combines with protons and electrons at the end of the electron transport chain to **form water**

## The electron transport chain

- The electron transport chain is made up of a **series of membrane proteins**/ electron carriers

- They are positioned close together which allows the electrons to pass from carrier to carrier
- The inner membrane of the mitochondria is impermeable to hydrogen ions so these electron carriers are required to **pump the protons across the membrane** to establish the concentration gradient




***Oxidative phosphorylation via the chemiosmotic theory occurs on the inner mitochondrial membrane and requires NADH and FADH$_2$ from the Krebs Cycle. It produces water and many molecules of ATP***

- Oxidative phosphorylation **uses energy** from reduced NAD and FAD to **produce ATP**
  - **3 ATP** molecules for every reduced **NAD** molecule
  - **2 ATP** molecules for every reduced **FAD** molecule
- For every molecule of glucose a total of **38 ATP molecules** can be produced during aerobic respiration

**Number of ATP Molecules Produced During Aerobic Respiration per Glucose Molecule Table**

Your notes

| Stage of aerobic respiration | Molecules produced | Number of ATP molecules produced |
|---|---|---|
| Glycolysis | 2 ATP | 2 |
| Glycolysis | 2 reduced NAD | 6 |
| Link reaction (x2) | 2 reduced NAD | 6 |
| Krebs cycle (x2) | 2 ATP | 2 |
| Krebs cycle (x2) | 6 reduced NAD | 18 |
| Krebs cycle (x2) | 2 reduced FAD | 4 |
| | Total ATP molecules | 38 |



### Examiner Tips and Tricks

Examiners often ask why oxygen is so important for aerobic respiration. Oxygen acts as the **final electron acceptor.** Without oxygen the electron transport chain cannot continue as the electrons have nowhere to go. Without oxygen accepting the electrons (and hydrogen ions) the reduced coenzymes NADH and $FADH_2$ cannot be oxidised to regenerate NAD and FAD, so they can't be used in further hydrogen transport.

It is important to use the correct terminology when describing hydrogen; ensure you understand when to use "hydrogen" and when to use "hydrogen ions/protons".

Your notes

# Anaerobic Respiration

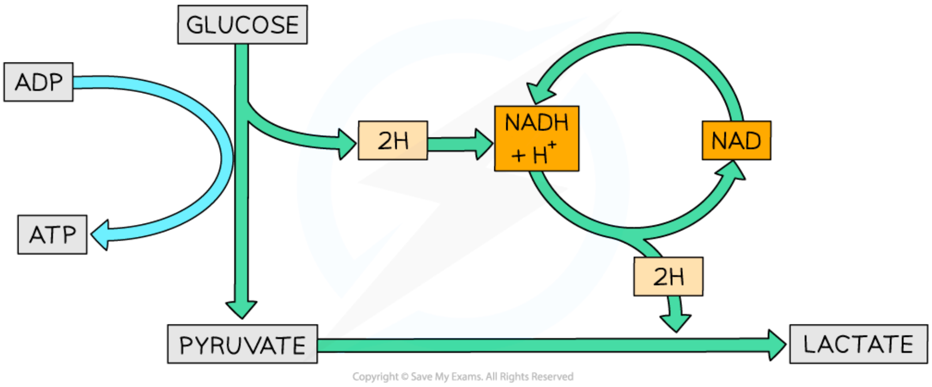
- Sometimes cells experience conditions with **little or no oxygen**
- There are several consequences when there is not enough oxygen available for respiration:
  - There is **no final acceptor** (oxygen) **of electrons** from the electron transport chain
  - The **electron transport chain stops** functioning
  - **No more ATP** is produced **via oxidative phosphorylation**
  - Reduced NAD and FAD **aren't oxidised** by an electron carrier
  - No oxidised NAD and FAD are available for **dehydrogenation** in the Krebs cycle
  - The **Krebs cycle stops**
  - The **link reaction also stops**
- However, there is still a way for cells to produce some ATP in low oxygen conditions through **anaerobic respiration**

## Anaerobic pathways

- Some cells are able to **oxidise the reduced NAD** produced during glycolysis so it can be used for further hydrogen transport
- This means that **glycolysis can continue** and **small amounts of ATP** are still produced
- Different cells use different pathways to achieve this
  - Yeast and microorganisms use **ethanol fermentation**
  - Other microorganisms and mammalian muscle cells use **lactate fermentation**

## Lactate fermentation

- In this pathway reduced NAD transfers hydrogen to pyruvate to form lactate
- **NAD** can now be **reused in glycolysis**
- **Pyruvate is reduced** to lactate by enzyme lactate dehydrogenase
- Pyruvate is the hydrogen acceptor
- The final product lactate can be further metabolised
- A small amount of ATP is produced




*The pathway of lactate fermentation*

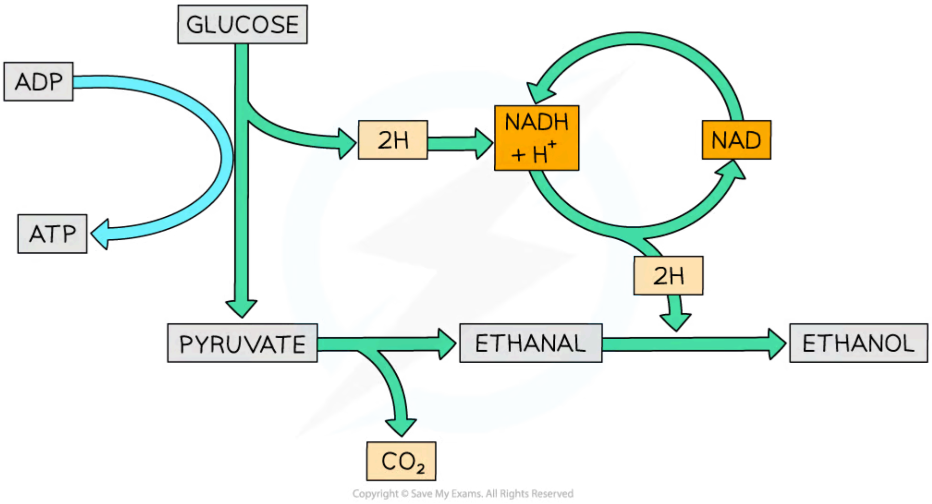
Your notes

## Processing Lactate

- Lactate (lactic acid) can build up in the cells after a period of time
- After lactate is produced two things can happen:
  - It can be **oxidised back to pyruvate** which is then channelled into the Krebs cycle for ATP production
  - It can be **converted into glucose** by the liver cells for use during respiration or for storage (in the form of glycogen)
- The oxidation of lactate back to pyruvate needs extra oxygen
  - This extra oxygen is referred to as an **oxygen debt**
  - It explains why animals **breathe deeper and faster after exercise**

## Ethanol fermentation

- In this pathway reduced NAD transfers its hydrogens to ethanal to form ethanol
- In the first step of the pathway **pyruvate is decarboxylated** to ethanal
  - Producing **$CO_2$**
- Then **ethanal is reduced** to ethanol by the enzyme alcohol dehydrogenase
- Ethanal is the hydrogen acceptor
- Ethanol cannot be further metabolised; it is a waste product
- Ethanol fermentation occurs in yeast and plant cells




*The pathway of ethanol fermentation*

## Examiner Tips and Tricks

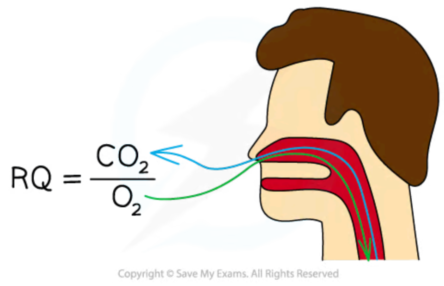
Note that ethanol fermentation is a two-step process (lactate fermentation is a one-step process). Carbon dioxide is also produced alongside the waste ethanol. This waste ethanol is what makes yeast vital in producing alcoholic drinks like beer!

Your notes

# Respiratory Quotient

- The **respiratory quotient** (**RQ**) is the ratio of carbon dioxide molecules produced to oxygen molecules taken in during respiration

**RQ = $CO_2 \div O_2$**




*The formula for the Respiratory Quotient*

## RQ values of different respiratory substrates

- **Carbohydrates**, **lipids** and **proteins** have different typical RQ values
- This is because of the **number of carbon-hydrogen bonds** differs in each type of biological molecule
    - More carbon-hydrogen bonds means that more hydrogen atoms can be used to create a proton gradient
    - More hydrogens means that more ATP molecules can be produced
    - More oxygen is therefore required to breakdown the molecule (in the last step of oxidative phosphorylation to form water)
- When **glucose** is **aerobically respired** equal amounts of carbon dioxide are produced to oxygen taken in, meaning it has an RQ value of 1

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$



***During the breakdown of glucose, six molecules of oxygen is used and six molecules of carbon dioxide is produced. This results in an RQ value of 1 ($6CO_2 \div 6O_2$)***

**RQ Values For Different Respiratory Substrates Table**

Your notes

| Respiratory substrate | RQ |
|---|---|
| Carbohydrate | 1.0 |
| Lipid | 0.7 |
| Protein | 0.9 |



- Some questions may ask you to suggest what substrate is being respired during an experiment based on the RQ value – so make yourself familiar with the values in the table

## Calculating RQ values

- The respiratory quotient is calculated from respiration equations
- It involves comparing the ratios of carbon dioxide given out to oxygen taken in
- The formula for this is:

$$RQ = \frac{\text{MOLES OR MOLECULES OF CARBON DIOXIDE GIVEN OUT}}{\text{MOLES OR MOLECULES OF OXYGEN TAKEN IN}}$$



***Equation to calculate the RQ value of a respiratory substrate***

- For **aerobic respiration** the RQ will typically be less than 1 since oxygen is being used to break down the substrate
- Should the RQ of an organism be greater than 1, it may indicate that **anaerobic respiration** is occurring, since very little to no oxygen is used
- If you know the **molecular formula** of the substrate being aerobically respired then you can create a balanced equation to calculate the RQ value
- In a **balanced** equation the **number before the chemical formula** can be taken as the **number of molecules/moles** of that compound
  - This is because the same number of molecules of any gas take up the same volume e.g. 12 molecules of carbon dioxide take up the same volume as 12 molecules of oxygen
- **Glucose has a simple 1:1 ratio** and RQ value of 1 but other substrates have more complex ratios leading to different RQ values

### Worked Example

Linoleic acid (a fatty acid found in nuts) has the molecular formula $C_{18}H_{32}O_2$. Calculate the RQ value of this lipid.

Answer:

**Step 1: Write down the respiration equation**

$$C_{18}H_{32}O_2 + O_2 \rightarrow CO_2 + H_2O$$

**Step 2: Balance the equation**

$$C_{18}H_{32}O_2 + 25O_2 \rightarrow 18CO_2 + 16H_2O$$

**Step 3: Use the RQ formula**

$$RQ = CO_2 \div O_2$$

$$RQ = 18 \div 25$$

$$RQ = 0.72$$

## Worked Example

During ethanol fermentation in lettuce roots, glucose is partially broken down. This process is represented by the following equation:

**glucose → ethanol + carbon dioxide**

Calculate the RQ value.

**Answer:**

**Step 1: Write down the respiration equation**

$$C_6H_{12}O_6 \rightarrow C_2H_5OH + CO_2$$

**Step 2: Balance the equation**

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

**Step 3: Use the RQ formula**

$$RQ = CO_2 \div O_2$$

$$RQ = 2 \div 0$$

$$RQ = \infty \text{ (infinity)}$$

## Examiner Tips and Tricks

Your notes

Make sure the respiration equation you are working with is **fully balanced** before you start doing any calculations to find out the RQ value.

Your notes

# Investigation of Respiration in Yeast

- A **redox indicator** is a substance that changes colour when it is reduced or oxidised
- **DCPIP** and **methylene** blue are redox indicators
  - They are used to investigate the effects of **temperature and substrate concentration** on the **rate of anaerobic respiration** in yeast
- These dyes can be added to a suspension of living **yeast cells** as they don't damage cells
- Yeast can respire both aerobically and anaerobically, in this experiment it is their rate of anaerobic respiration that is being investigated

## Mechanism

- **Dehydrogenation** happens regularly throughout the different stages of aerobic respiration
- The hydrogens that are removed from substrate molecules are transferred to the final stage of aerobic respiration, oxidative phosphorylation, via the hydrogen carriers NAD and FAD
- The enzyme **dehydrogenase** catalyses the production of **reduced NAD** in **glycolysis**
- When DCPIP or methylene blue are present they take up hydrogens from the organic compounds and get reduced instead of NAD
- Both redox indicators undergo the same colour change when they are reduced
  - **Blue → colourless**
- The faster the rate of respiration, the faster the rate of hydrogen release and the faster the dyes get reduced and change colour
  - This means that the **rate of colour change** can correspond to the rate **dehydrogenase** would be working at and therefore, **the rate of respiration** in yeast
- The rate of respiration is inversely proportional to the time taken

$$\textbf{Rate of respiration (sec}^{-1}\textbf{) = 1} \div \textbf{time (sec)}$$

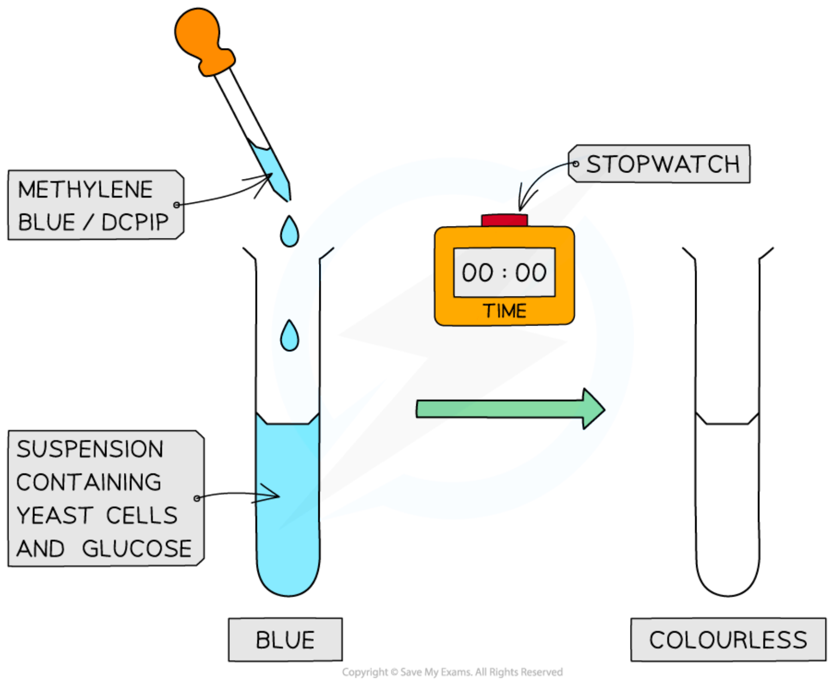
## Apparatus

- Yeast
- Glucose solution
- Test tubes
- Stopwatch

- DCPIP

## Method – Temperature

1. Add a set volume of yeast suspension to test tubes containing a certain concentration of glucose
2. Put the test tube in a **temperature-controlled water bath** and leave for 5 minutes to ensure the water temperature is correct and not continuing to increase or decrease
3. Add a set volume of DCPIP to the test tube and start the stopwatch immediately
4. Stop the stopwatch when the solution becomes colourless or lose all blue colour
    - This is subjective and therefore the same person should be assigned this task for all repeat experiments
5. Record the time taken for a colour change to occur once the dye is added
    - Repeat across a range of temperatures. For example, 30°C, 35°C, 40°C, 45°C
6. The effect of substrate concentration can be investigated by adding **different concentrations of a substrate** to the suspension of yeast cells and recording the time taken for a colour change to occur once the dye is added
    - For example, 0.1% glucose, 0.5% glucose, 1.0% glucose




*Methylene blue or DCPIP is added to a solution of anaerobically respiring yeast cells in a glucose solution. The rate at which the solution turns from blue to colourless gives the rate of dehydrogenase activity*

## Controlling other variables

Your notes

- It is important when investigating one variable to ensure that the other variables in the experiment are being controlled
  - **Volume of dye added**: if there is more dye molecules present then the time taken for the colour change to occur will be longer
  - **Volume of yeast suspension**: when more yeast cells are present the rate of respiration will be inflated
  - **Type of substrate**: yeast cells will respire different substrates at different rates
  - **Concentration of substrate**: if there is limited substrate in one tube then the respiration of those yeast cells will be limited
  - **Temperature**: an increase or decrease in temperature can affect the rate of respiration due to energy demands and kinetic energy changes. The temperature of the dye being added also needs to be considered
  - **pH**: a buffer solution can be used to control the pH level to ensure that no enzymes are denatured

## Interpretation of results

- A **graph** should be plotted of temperature against time
- As the **temperature increases**, the rate of respiration also increases so the time taken for the solution to go colourless reduces
  - This means hydrogens are released by the reactions more quickly, hence the DCPIP accepts electrons/hydrogens more quickly until all molecules of DCPIP are reduced. This means that it will take less time to turn from blue to colourless
- At extreme **high** temperatures, the enzyme may **denature** and meaning the colour change may not occur

### Examiner Tips and Tricks

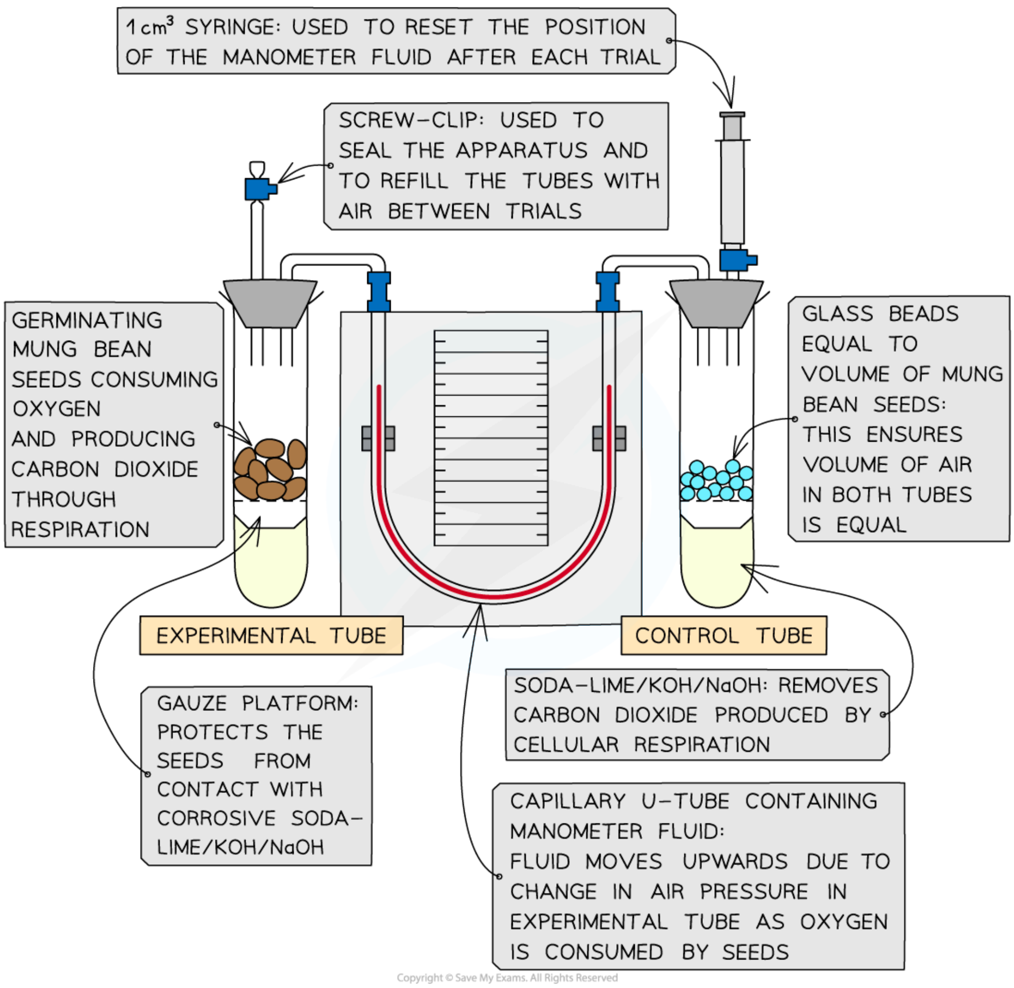
Although the DCPIP and methylene blue undergo a colour change from blue to colourless it is important to remember that the **yeast suspension in the test tube may have a slight colour** (usually yellow). That means when the dye changes to colourless there may still be an overall yellow colour in the test tube. If this is the case it can be useful to have a control tube containing the same yeast suspension but with no dye added, then you can tell when the dye has completely changed colour.

# Using a Respirometer to Calculate RQ

- Respirometers are used to measure and investigate the **rate of oxygen consumption** during respiration in organisms
- They can also be used to calculate respiratory quotients
- The experiments usually involve organisms such as seeds or invertebrates

## Apparatus

- Respirometer
- Glass beads
- Germinating seeds
  - These will be actively respiring and consuming oxygen
- Test tubes
- Soda-lime pellets (or potassium hydroxide)
  - To absorb the carbon dioxide produced
- Stopwatch




Your notes

*A respirometer set up to measure the rate of respiration*

## Method

1. **Measure oxygen consumption**: set up the respirometer and run the experiment with both tubes for a set amount of time (e.g. 30 minutes)
2. As the seeds consume oxygen, the **volume of air** in the test tube **will decrease** ($CO_2$ produced during respiration is absorbed by soda lime or KOH)
3. This **reduces the pressure** in the capillary tube and **manometer fluid will move towards** the test tube containing the seeds
4. Measure the distance moved by the liquid in a given time
5. Use this measurement to calculate the change in gas volume within a given time, $x$ cm$^3$ min$^{-1}$
6. **Reset the apparatus**: allow air to re-enter the tubes via the screw cap and reset the manometer fluid using the syringe
7. Run the experiment again: **remove the soda-lime** from both tubes and use the manometer reading to calculate the change in gas volume in a given time, $y$ cm$^3$ min$^{-1}$

## Equation for calculating change in gas volume

- The volume of oxygen consumed ($cm^3\ min^{-1}$) can be worked out using the diameter of the capillary tube *r* (cm) and the distance moved by the manometer fluid *h* (cm) in a minute using the formula:

$$\pi r^2 h$$

## Calculations

- *x* tells us the volume of oxygen consumed by respiration within a given time
- *y* tells us the volume of oxygen consumed by respiration within a given time minus the volume of carbon dioxide produced within a given time
  - ***y* may be a positive or negative** value depending on the direction that the manometer fluid moves (up = positive value, down = negative value)
- The two measurements *x* and *y* can be used to calculate the RQ

$$RQ = \frac{CO_2}{O_2}$$

$$\boxed{RQ = \frac{x + y}{x}}$$



*Equation to calculate RQ values using a respirometer*

### Worked Example

During a respirometer experiment using blow fly larvae, the volume of oxygen consumed was 2.9 $cm^3min^{-1}$. The soda lime was removed from both test tubes and the experiment was repeated. The change in gas volume was -0.8 $cm^3min^{-1}$. Calculate the RQ value for the blow fly larvae.

**Answer:**

*x* = 2.9 $cm^3min^{-1}$

*y* = -0.8 $cm^3min^{-1}$

**Step 1: Write down equation**

$$RQ = \frac{x + y}{x}$$

**Step 2: Substitute values**

$$RQ = \frac{2.9 - 0.8}{2.9}$$

Your notes

**Step 3: Calculate RQ**

$$RQ = 0.72$$

Your notes

## Interpretation of results

- Respirometers can be used in experiments to investigate how different factors affect the RQ of organisms over time
  - E.g. temperature – using a series of water baths
- When an **RQ value changes** it means **the substrate being respired has changed**
- Some cells may also be **using a mixture of substrates** in respiration e.g. An RQ value of 0.85 suggests **both** carbohydrates and lipids are being used
  - This is because the RQ of glucose is 1 and the RQ of lipids is 0.7
- Under normal cell conditions the order substrates are used in respiration: carbohydrates, lipids then proteins
- The RQ can also give an indication of under or overfeeding:
  - An RQ value of more than 1 suggests excessive carbohydrate/calorie intake
  - An RQ value of less than 0.7 suggests underfeeding

### Examiner Tips and Tricks

There are several ways you can manage variables and increase the reliability of results in respirometer experiments:

- Use a controlled water bath to keep the **temperature** constant
- Have a control tube with an equal volume of inert material to the volume of the organisms to compensate for changes in atmospheric **pressure**
- Repeat the experiment multiple times and use an **average**