



RESPIRATION

Learning Outcome

Core Topic 6 – Cellular Physiology and Biochemistry

Candidates should be able to:

- (d) List and give an overview of the 4 stages of aerobic respiration and indicate where each stage takes place in an eukaryotic cell and mitochondria, and add up the energy captured (as ATP, reduced NAD and FAD) in each stage.
- (e) Explain the production of a small yield of ATP from anaerobic respiration and the formation of ethanol in yeast and lactate in mammals.

Content Outline

1. Introduction
 - (a) The Need for Energy in Living Organisms
 - (b) Overview of Cellular Respiration
 - (c) Role of Adenosine Triphosphate (ATP)
 - (d) Role of Nicotinamide Adenine Dinucleotide (NAD) and Flavin Adenine Dinucleotide (FAD)
2. Aerobic Respiration
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3. Anaerobic Respiration
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4. Regulation of Respiration
5. Respiratory Quotient
6. Respirometer
7. Comparison Tables (for Photosynthesis and Respiration)

References

1. Campbell, N.A. & Reece, J.B. (2010) Biology. Chapter 9: Cellular Respiration and Fermentation. 9th Edition. Pearson Education Inc.



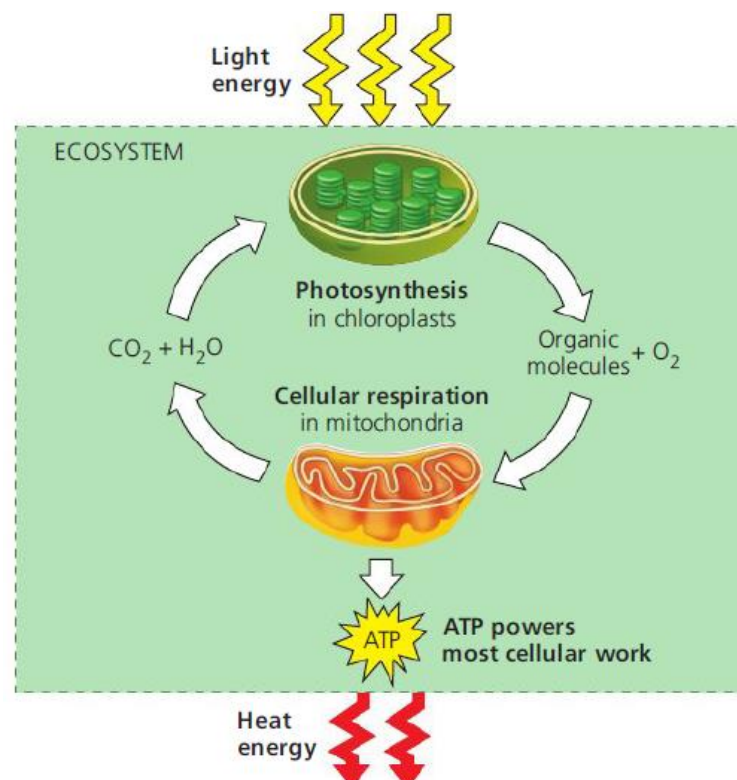
1. Introduction

(a) The Need For Energy in Living Organisms

The life processes of every cell are driven by energy. Energy flows into the ecosystem as sunlight and leaves as heat while the chemical elements essential to life are recycled.

Photosynthesis allows plants to convert energy from sunlight into chemical potential energy stored in organic molecules of food. Animals then obtain this energy by consuming plants and other animals. Cellular respiration is then carried out to break down these energy fuels into **adenosine triphosphate (ATP)** which drives most cellular work.

Living cells require ATP for energy-consuming activities such as assembling polymers, pumping substances across membranes, moving and reproducing.



Energy flow and chemical recycling in ecosystems

(b) Overview of Cellular Respiration

Cellular respiration is the process by which chemical energy in organic molecules (e.g. carbohydrates, fats and proteins) is released by **oxidation**. The energy released is then used to generate **ATP**.

Cellular respiration includes two processes: **aerobic respiration** (requires oxygen) and **anaerobic respiration** (does not require oxygen).

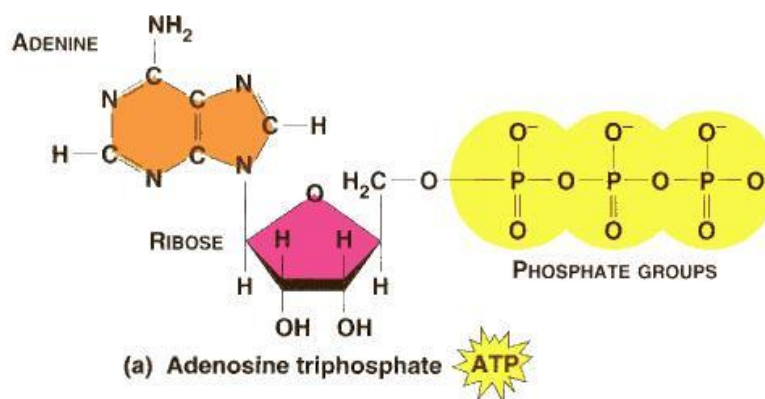
The presence of oxygen, therefore, determines the type of respiration that will take place in the living cell.

**(c) Role of Adenosine Triphosphate (ATP)**

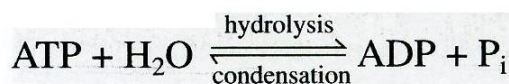
Adenosine Triphosphate (ATP) is an instant source of energy found in all living cells and is therefore known as the **universal energy carrier** or energy currency in living organisms. Energy released during respiration, is thus stored in the form of ATP.

(i) Structure of ATP

- ATP consists of a **ribose sugar**, an **adenine** base and **3 phosphate groups**.

**(ii) Properties of ATP**

- ATP is **soluble** and can transport chemical energy to energy-consuming processes anywhere within the cell.
- Hydrolysis** of ATP requires the addition of water and releases energy.
 - ATP is converted to **ADP** and **inorganic phosphate (Pi)**.
- Phosphorylation** of ADP can form ATP. This reaction releases water and is known as **condensation**. The enzyme that catalyses the reaction is called **ATP synthase**.
 - ADP** is phosphorylated with **inorganic phosphate (Pi)** to form ATP.



$$\Delta H_c = -30.6 \text{ kJ mol}^{-1}$$



(d) **Role of Nicotinamide Adenine Dinucleotide (NAD) and Flavin Adenine Dinucleotide (FAD)**

NAD and FAD are **coenzymes** to dehydrogenases involved in cellular respiration. Their ability to exist in the oxidised or reduced state allows them to function as proton and electron carriers. The reduced form is more energetically valuable.

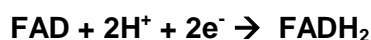
(i) **Nicotinamide Adenine Dinucleotide (NAD)**

- During aerobic respiration, glucose is oxidised by a series of dehydrogenation reactions.
- At each of these reactions (in glycolysis, link reaction and Krebs cycle), protons (H^+) and electrons (e^-) are released and transferred to **oxidised NAD** (NAD^+) to form **reduced NAD** ($NADH + H^+$).



(ii) **Flavin Adenine Dinucleotide (FAD)**

- During aerobic respiration, glucose is oxidised by a series of dehydrogenation reactions.
- At each of these reactions (in Krebs Cycle), protons (H^+) and electrons (e^-) are released and transferred to **oxidised FAD** (FAD) to form **reduced FAD** ($FADH_2$).



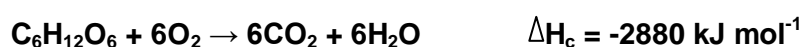


2. Aerobic Respiration

(a) Overview

Although carbohydrates, proteins and fats are all fuels that can act as respiratory substrates for the production of ATP, it is important to learn about the stages of aerobic respiration particularly by tracking the oxidation or breaking down of glucose molecules as it is the most common fuel utilised by living cells.

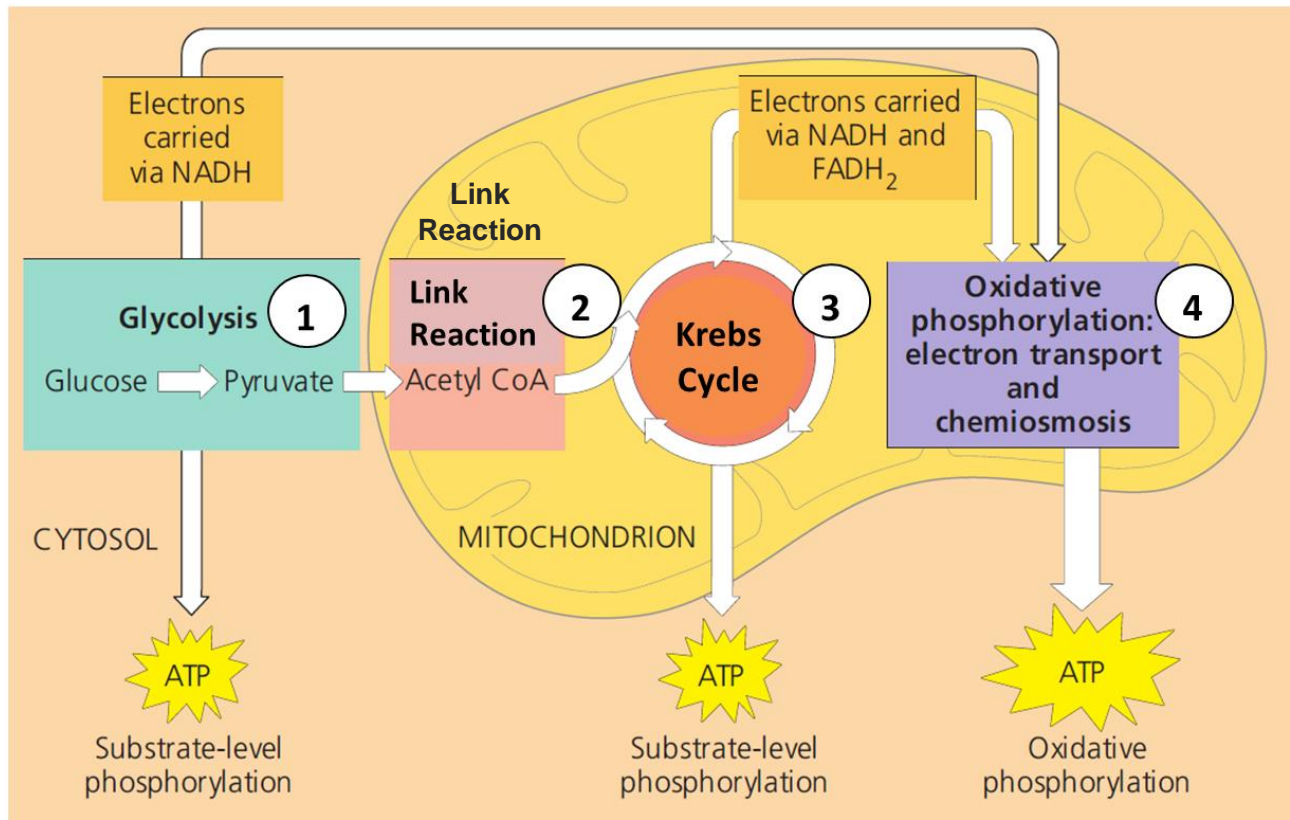
The general equation below shows the oxidation of glucose in aerobic respiration. This is a simplified equation as aerobic respiration does not take place in a single reaction but the cumulative result of four major sequential stages. Each stage is comprised of a series of reactions. Each reaction is catalysed by a specific enzyme.



Aerobic respiration takes place through **four** main stages.

Stage	Requirements for oxygen	Location	Processes
1) <u>Glycolysis</u>	Does not require the presence of oxygen	<u>Cytoplasm</u>	Oxidation of glucose (6C) to form two molecules of <u>pyruvate</u> (3C).
2) <u>Link Reaction</u>	Occurs only in the presence of oxygen (Note: Oxygen is not directly involved in this stage)	<u>Mitochondrial matrix of eukaryotic cells</u> or cytosol of prokaryotes	Oxidation of pyruvate (3C) to form <u>acetyl-CoA</u>
3) <u>Krebs Cycle</u> (also known as Citric Acid / Tricarboxylic Acid (TCA) Cycle)	Occurs only in the presence of oxygen (Note: Oxygen is not directly involved in this stage)	<u>Mitochondrial matrix of eukaryotic cells</u> or cytosol of prokaryotes	Further oxidation of <u>acetyl-CoA</u> occurs via a series of reactions.
4) <u>Oxidative Phosphorylation</u>	Occurs only in the presence of oxygen (Note: Oxygen is directly involved in this stage)	<u>Inner mitochondrial membrane of eukaryotes</u> or cell surface membrane of prokaryotes	Electrons released via oxidation in the first three stages are passed along the <u>electron transport chain</u> , resulting in production of large amounts of ATP.

Specific processes occurring in each of these stages will be elaborated in the later sections.



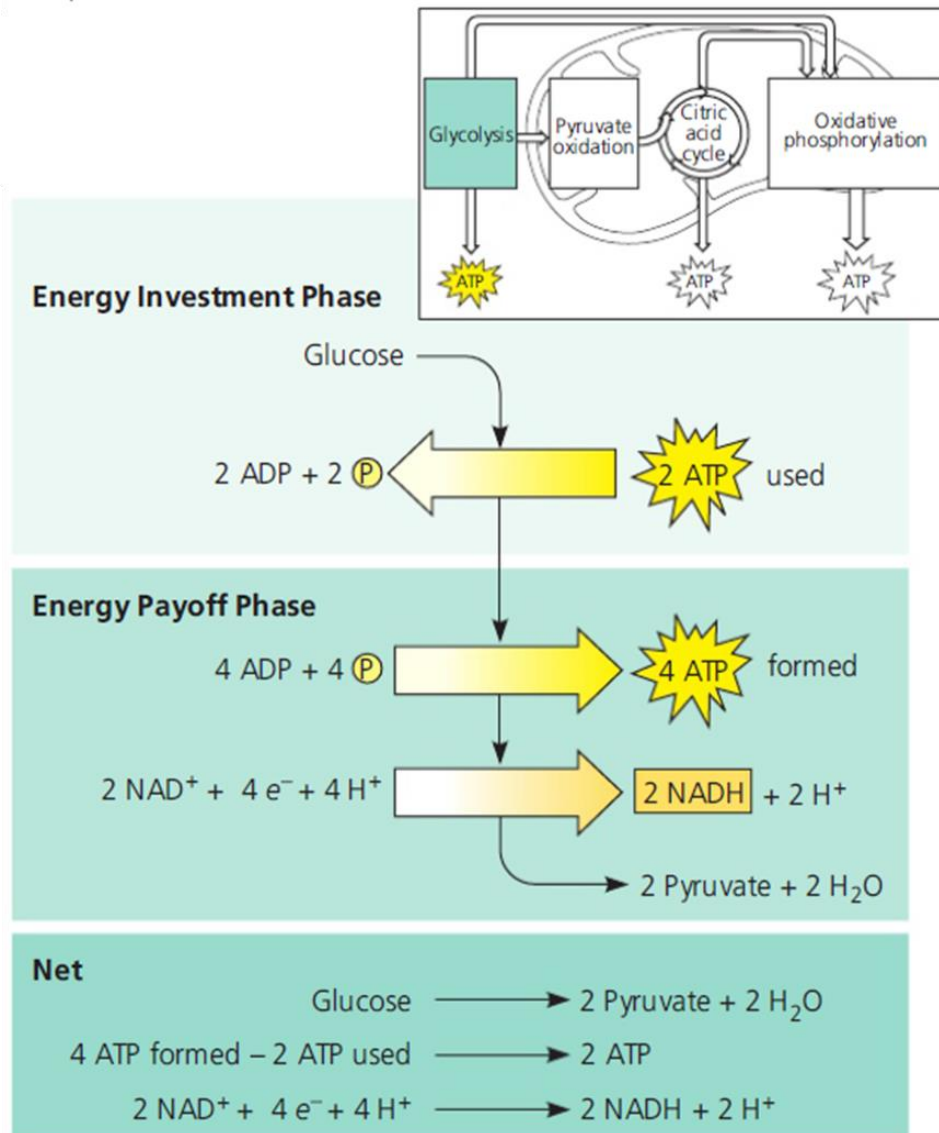
Overview of the four stages of aerobic respiration

**(b) Glycolysis**

Glycolysis occurs in the **cytoplasm** (independent of oxygen) and it involves the breakdown of **glucose** (6C) to yield **pyruvate** (3C), **reduced nicotinamide adenine dinucleotide (reduced NAD)** / $\text{NADH} + \text{H}^+$ and **ATP**. No CO_2 is released in glycolysis.

Glycolysis can be divided into two phases:

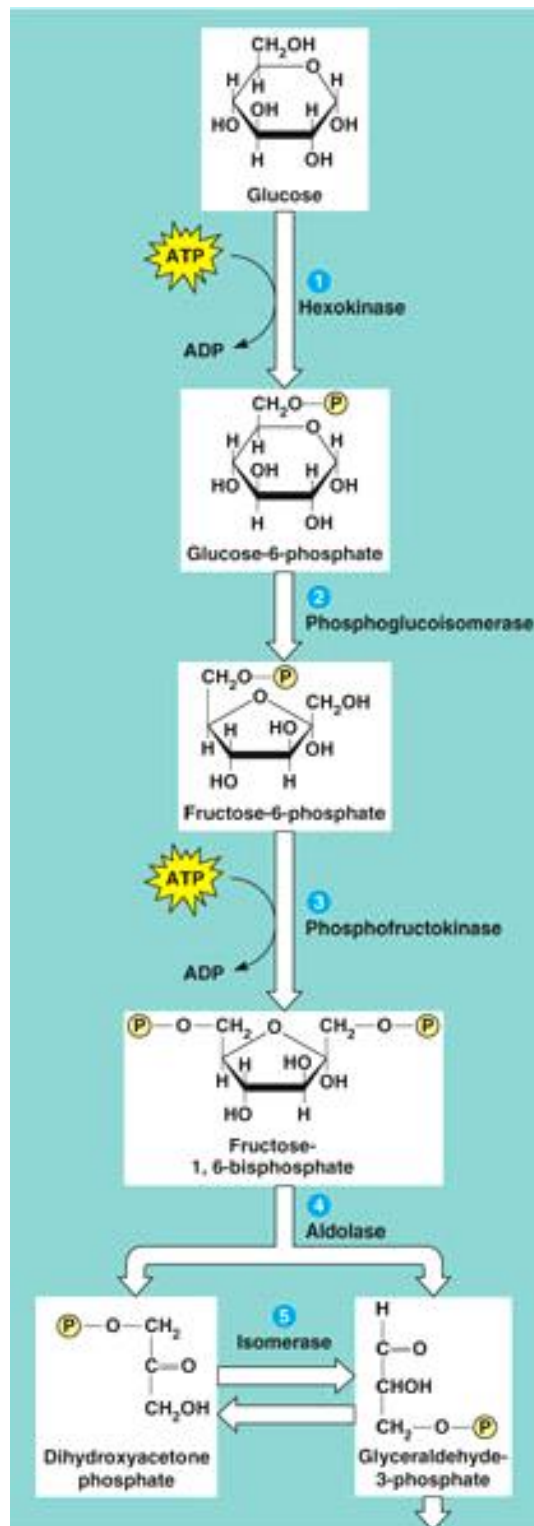
- (i) **Energy Investment Phase**
- (ii) **Energy Pay-Off Phase**



Energy input and output of glycolysis

(i) **Energy Investment Phase** (also known as the Preparatory Phase)

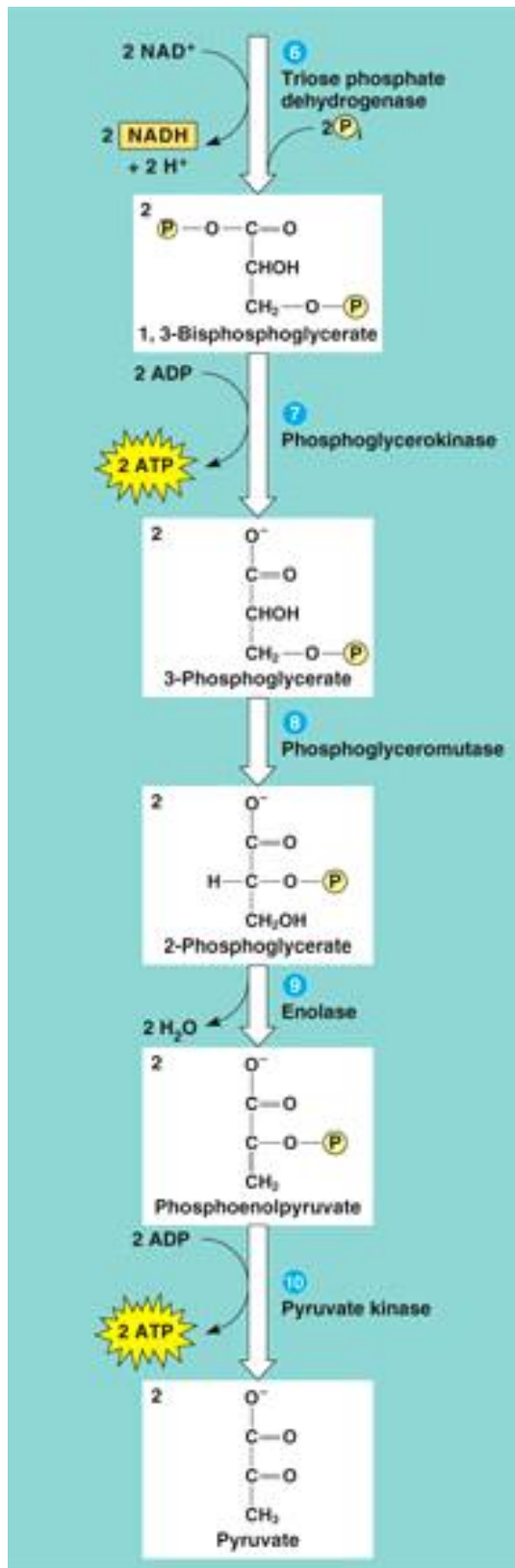
Energy in the form of ATP is used in this phase of glycolysis (2 ATP per glucose molecule).



- 1) **Activation** of glucose occurs to make it more chemically reactive
 - **Phosphorylation** of glucose using **ATP**
 - Glucose → **Glucose-6-phosphate**
 - Catalysed by **hexokinase**
- 2) Isomerisation of glucose-6-phosphate to **fructose-6-phosphate**
- 3) **Phosphorylation** of fructose-6-phosphate using **ATP**
 - Fructose-6-phosphate → **Fructose-1,6-bisphosphate**
 - Catalysed by **phosphofructokinase**
- 4) Cleavage of fructose-1,6-bisphosphate (6C) into **2 triose phosphates** (3C), which are **dihydroxyacetone phosphate** and **glyceraldehyde-3-phosphate** (GALP / GP / G3P)
- 5) Isomerisation of dihydroxyacetone phosphate into glyceraldehyde-3-phosphate
 - At the end of step 5, **2 molecules of glyceraldehyde-3-phosphate** (GALP) have been formed from a single glucose molecule.

(ii) **Energy Payoff Phase**

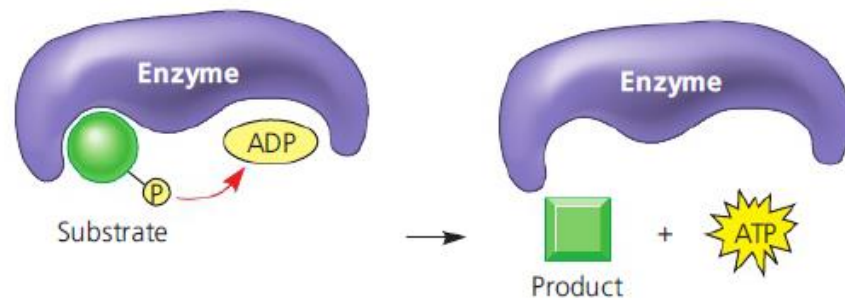
Energy in the form of **ATP** is produced via **substrate level phosphorylation** (4 ATP per glucose molecule) in this phase.

**Steps 6-10:**

- Subsequent conversion of **each** GALP to **pyruvate** via multiple steps:
 - Generates **2 ATP** via **substrate-level phosphorylation**
 - Releases **protons (H⁺)** and **electrons (e⁻)** via **dehydrogenation** which are transferred to **1 oxidised NAD (NAD⁺)** to form **1 reduced NAD (NADH + H⁺)**
- Since 2 molecules of GALP is formed from 1 glucose molecule, therefore, **2 pyruvate**, **4 ATP** and **2 reduced NAD** are produced per glucose molecule.

**Substrate level phosphorylation**

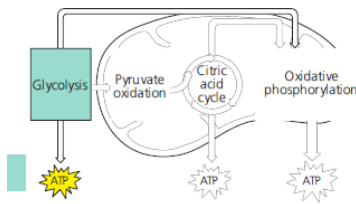
- Mode of ATP synthesis whereby an enzyme **transfers a phosphate group from a substrate molecule to ADP.**
- Occurs in cytoplasm (during **glycolysis**) and in the mitochondrial matrix (during **Krebs cycle** in which ATP is generated).
- Only a small amount of ATP is generated by substrate level phosphorylation compared to oxidative phosphorylation (final step of aerobic respiration).



▲ **Figure 9.7 Substrate-level phosphorylation.** Some ATP is made by direct transfer of a phosphate group from an organic substrate to ADP by an enzyme. (For examples in glycolysis, see Figure 9.9, steps 7 and 10.)

SUMMARY OF GLYCOLYSIS:

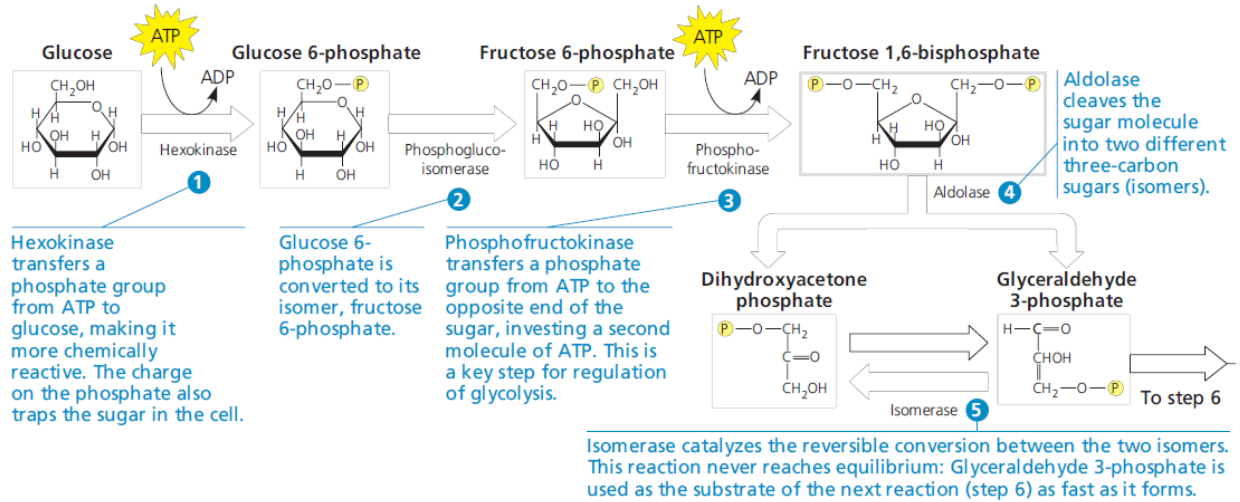
Products per glucose molecule		
Pyruvate (3C)	ATP (net gain)	Reduced NAD (NADH + H ⁺)
2	2	2



▼ **Figure 9.9 A closer look at glycolysis.** The orientation diagram on the left relates glycolysis to the entire process of respiration. Note that glycolysis is a source of ATP and NADH.

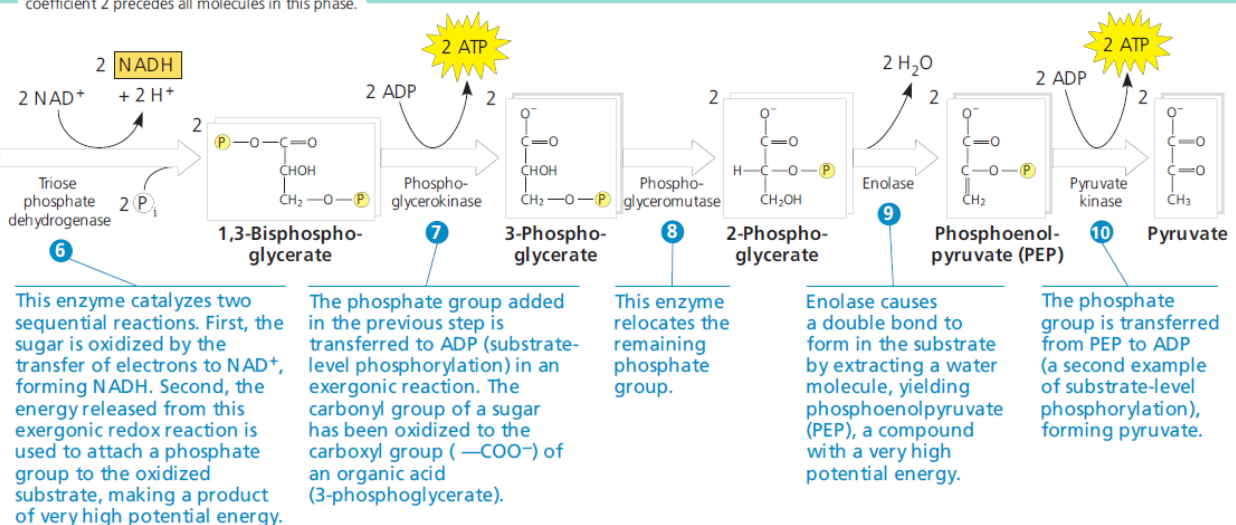
WHAT IF? What would happen if you removed the dihydroxyacetone phosphate generated in step 4 as fast as it was produced?

Glycolysis: Energy Investment Phase



The energy payoff phase occurs after glucose is split into two three-carbon sugars. Thus, the coefficient 2 precedes all molecules in this phase.

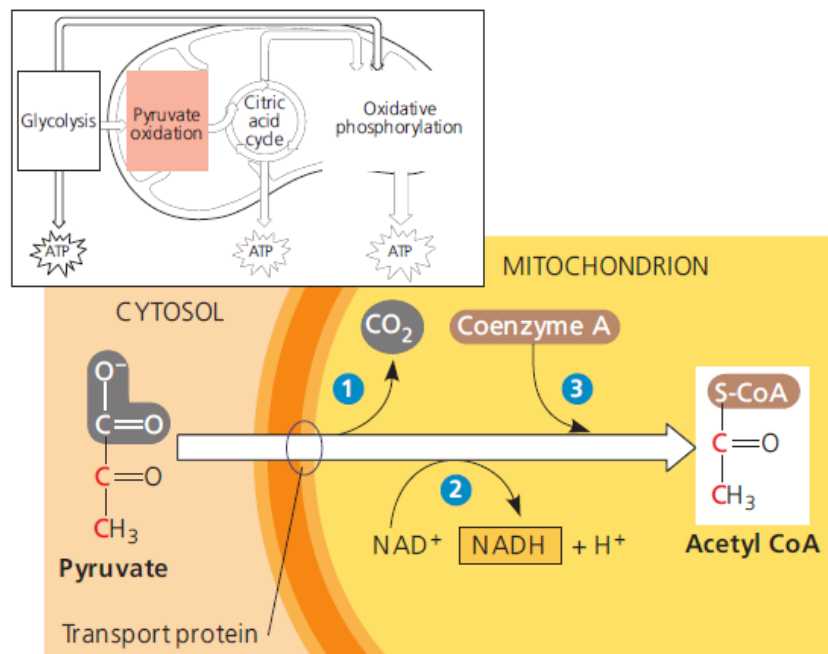
Glycolysis: Energy Payoff Phase



Detailed steps of glycolysis

(c) Link Reaction

If oxygen is available, pyruvate passes into the mitochondrion through the outer and inner mitochondrial membranes via active transport. In the **mitochondrial matrix**, **pyruvate is converted to acetyl Coenzyme A (acetyl-CoA)** by **oxidative decarboxylation**.

**Link Reaction: Oxidation of pyruvate to acetyl-CoA**

This step, linking glycolysis and Krebs cycle, is carried out by a multi-enzyme complex which catalyses **three** reactions:

1) Decarboxylation

- Carboxyl group of pyruvate (3C) is removed and **carbon dioxide (CO₂) is released**
- First carbon dioxide molecule produced from respiration

2) Oxidation (Dehydrogenation)

- Remaining 2C molecule undergoes oxidation via **dehydrogenation** by transferring **protons and electrons** to oxidised NAD, therefore converting it to **reduced NAD**
- Acetate (CH₃COO⁻) is produced

**Oxidative
Decarboxylation**3) Addition of Coenzyme A (CoA)

- Coenzyme A** is attached to acetate to form **acetyl-CoA** (2C)

SUMMARY OF LINK REACTION:

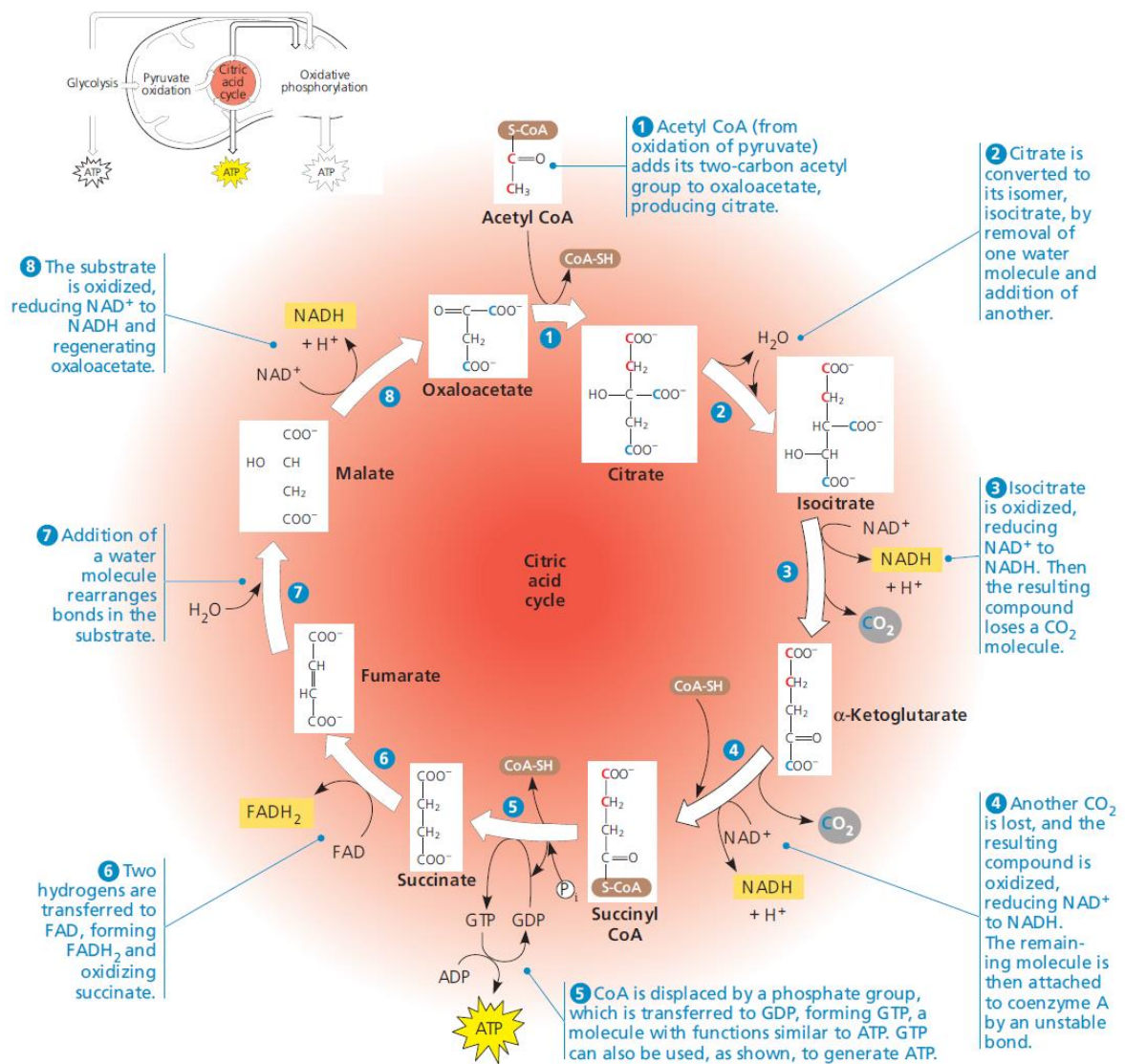
Products per glucose molecule		
Acetyl-CoA	CO ₂	Reduced NAD (NADH + H ⁺)
2	2	2

**(d) Krebs Cycle**

Following link reaction, **Krebs cycle**, (also known as Citric Acid / Tricarboxylic Acid (TCA) Cycle), occurs in the **mitochondrial matrix**. The cycle consists of a series of reactions which results in acetyl- CoA being completely oxidised to CO_2 and H_2O .

Since each glucose (6C) is converted into 2 pyruvate (3C) via glycolysis, 2 acetyl-CoA (2C) are formed after link reaction. Therefore, for each glucose molecule, the Krebs cycle runs twice to completely utilise the acetyl-CoA.

During Krebs cycle, acetyl-CoA (2C) is attached to a 4C compound called **oxaloacetate**. The resulting 6C compound, **citrate** is then gradually **re-converted to oxaloacetate**, making it a cycle.

**Krebs Cycle**



There are 8 steps in the Krebs cycle, each catalysed by a specific enzyme.

Essential steps to take note of (details of each step on diagram in previous page):

- (i) At 2 steps in the Krebs cycle (Steps 3 and 4), **carbon** is removed from the intermediate compounds via **decarboxylation**.
 - 2 molecules of carbon dioxide per cycle are produced
 - Carbon dioxide diffuses out of the mitochondrion and out of the cell
- (ii) 1 ATP is produced per Krebs cycle (Step 5) via **substrate level phosphorylation**.
- (iii) Intermediate compounds undergo oxidation via **dehydrogenation** (Steps 3, 4, 6 and 8)
 - Protons and electrons released are transferred to **oxidised NAD** and **oxidised FAD (flavin adenine dinucleotide)**
 - Oxidised NAD (NAD^+) is reduced to **reduced NAD** ($\text{NADH} + \text{H}^+$), oxidised FAD reduced to **reduced FAD** (FADH_2)
- (iv) These coenzymes subsequently transfer these electrons to the electron transport chain (ETC) for the production of ATP.

SUMMARY OF KREBS CYCLE:

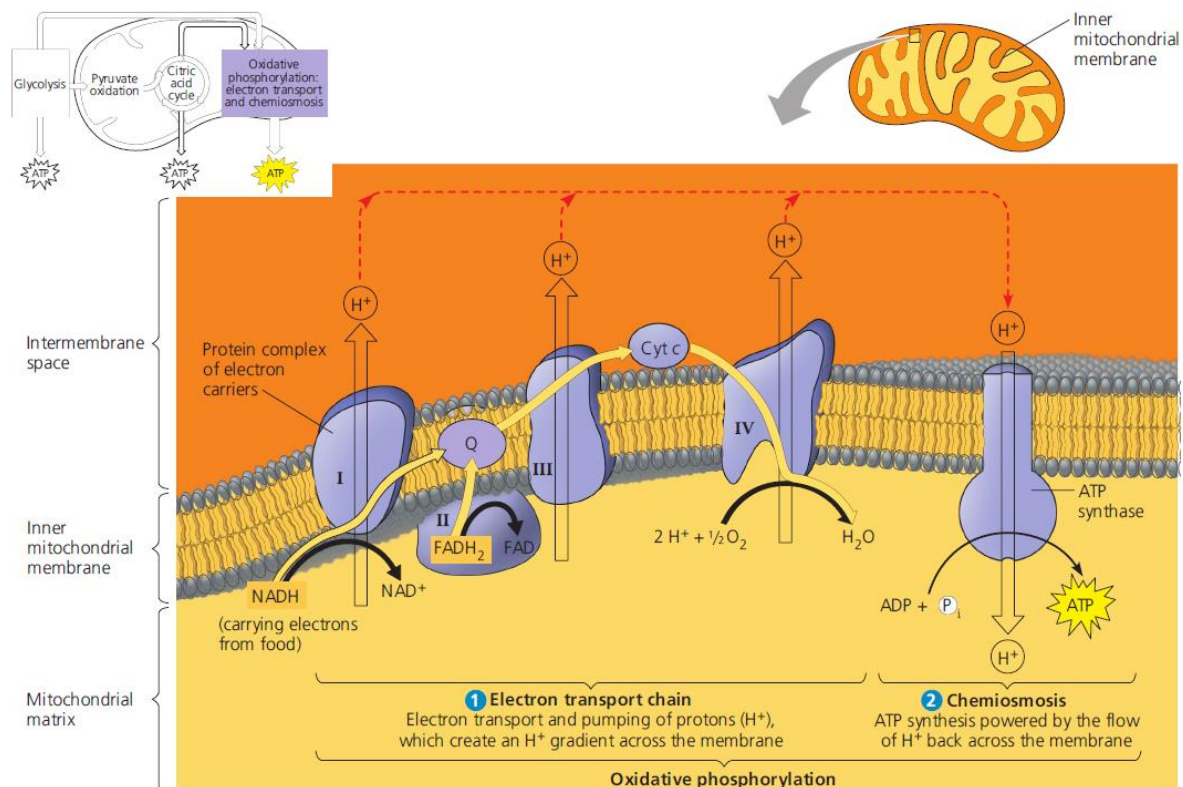
Products per cycle				
Oxaloacetate	CO_2	Reduced NAD ($\text{NADH} + \text{H}^+$)	Reduced FAD (FADH_2)	ATP
1	2	3	1	1

Products per glucose molecule				
Oxaloacetate	CO_2	Reduced NAD ($\text{NADH} + \text{H}^+$)	Reduced FAD (FADH_2)	ATP
2	4	6	2	2

**(e) Oxidative Phosphorylation**

Oxidative phosphorylation is the mechanism of **ATP synthesis** where electrons (released from oxidation in the first three stages) are transferred from coenzymes, reduced NAD and reduced FAD, to the electron transport chain (ETC).

It occurs only in the **presence of oxygen** and takes place in the **inner mitochondrial membrane** where the ETC and stalked particles containing ATP synthase are embedded.



▲ **Figure 9.15 Chemiosmosis couples the electron transport chain to ATP synthesis.**

① NADH and FADH₂ shuttle high-energy electrons extracted from food during glycolysis and the citric acid cycle into an electron transport chain built into the inner mitochondrial membrane. The gold arrows trace the transport of electrons, which finally pass to oxygen at the “downhill” end of the chain, forming water. As Figure 9.13 showed, most of the electron carriers of the chain are grouped into four complexes. Two mobile carriers, ubiquinone (Q)

and cytochrome c (Cyt c), move rapidly, ferrying electrons between the large complexes. As complexes I, III, and IV accept and then donate electrons, they pump protons from the mitochondrial matrix into the intermembrane space. (In prokaryotes, protons are pumped outside the plasma membrane.) Note that FADH₂ deposits its electrons via complex II and so results in fewer protons being pumped into the intermembrane space than occurs with NADH. Chemical energy originally harvested from food is transformed into a proton-motive

force, a gradient of H⁺ across the membrane.

② During chemiosmosis, the protons flow back down their gradient via ATP synthase, which is built into the membrane nearby. The ATP synthase harnesses the proton-motive force to phosphorylate ADP, forming ATP. Together, electron transport and chemiosmosis make up oxidative phosphorylation.

WHAT IF? If complex IV were nonfunctional, could chemiosmosis produce any ATP, and if so, how would the rate of synthesis differ?

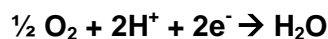
Steps in oxidative phosphorylation

1) **Electron Transport Chain**• **Organisation of ETC:**

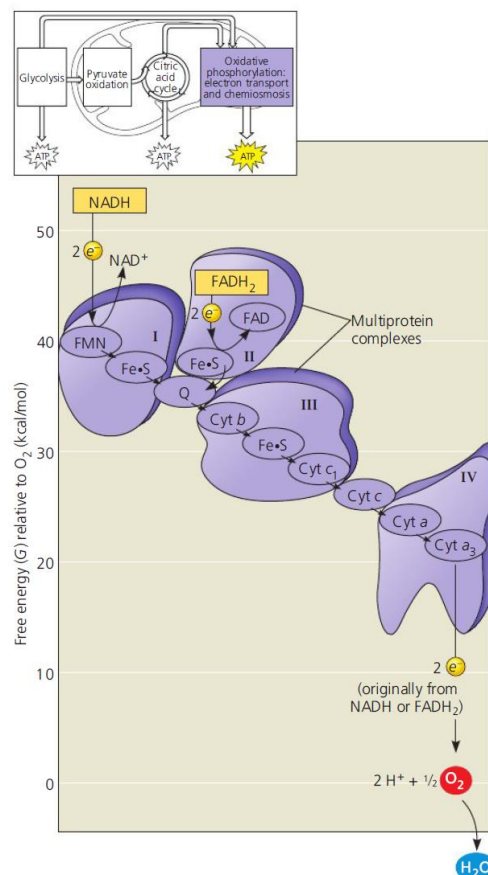
- Composed of a collection of **electron carriers** embedded in the **inner mitochondrial membrane**.
- Extensively folded inner mitochondrial membrane (cristae) **increases surface area** to provide space for thousands of copies of the chain in each mitochondrion.
- The electron carriers are numbered I through IV in **decreasing levels of free energy**.

• **Processes:**

- **Reduced NAD** and **reduced FAD** transfer **high energy protons and electrons** to the ETC for synthesis of ATP.
- Electrons are passed along the **electron transport chain (ETC)** from **one electron carrier to the next, each with an energy level lower than the one preceding it**.
- Electron carriers alternate between reduced and oxidised states as they accept and donate electrons.
- The last electron carrier passes the electrons to **oxygen**, which functions as the **final proton and electron acceptor** to form **water**, catalyzed by **cytochrome oxidase**:



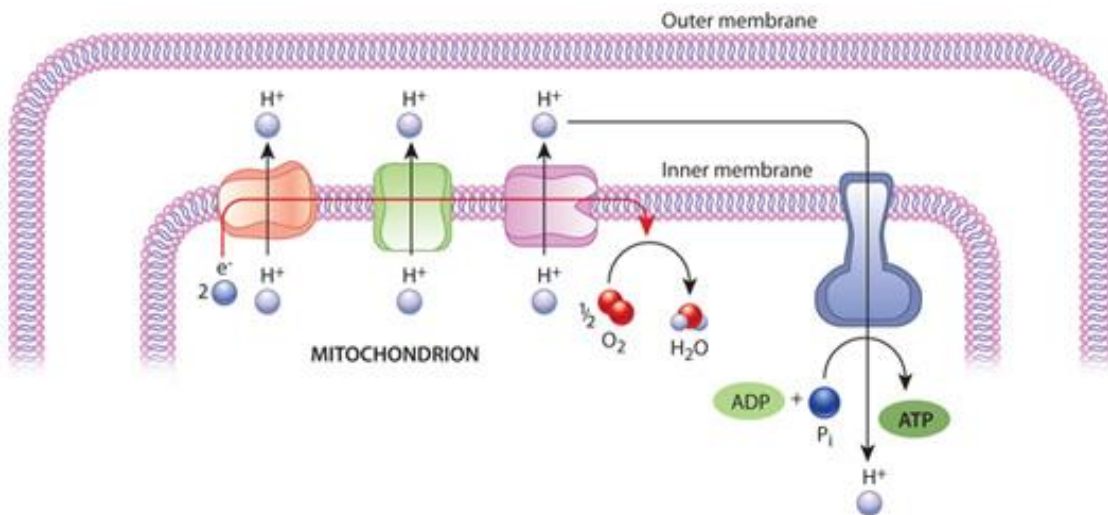
- **Oxidised NAD** and **oxidised FAD** are **regenerated** in the process.

**Electron transport down the ETC**



2) Chemiosmosis

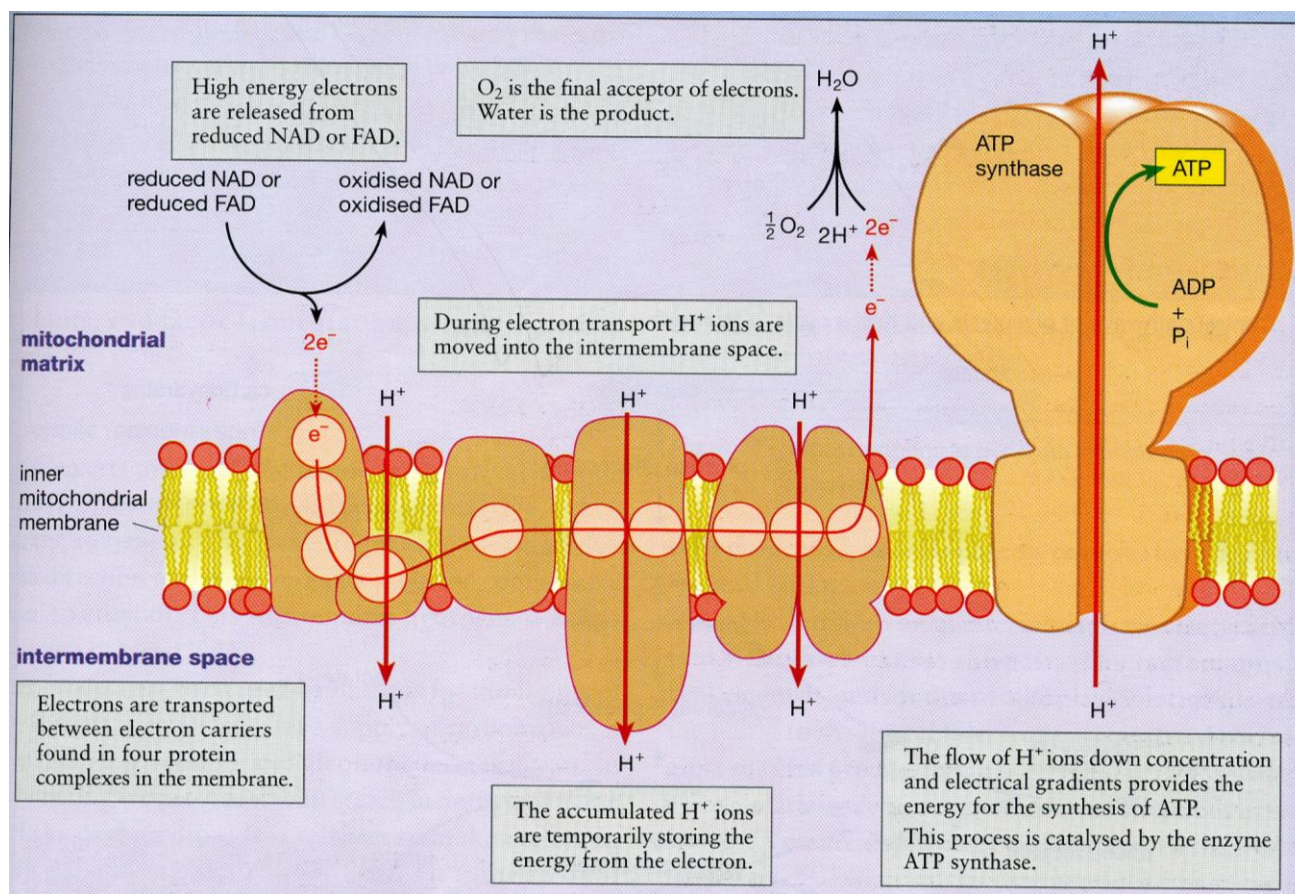
- Coupling of ETC to ATP synthesis
 - As the electrons are passed from one electron carrier to the next, energy released is used to pump protons from the matrix of the mitochondrion into the intermembrane space.
 - High concentration of H^+ in the intermembrane space → steep electrochemical proton gradient → proton motive force
 - This impermeable nature of inner mitochondrial membrane to H^+ allows this gradient to be established.
 - Stalked particles each containing ATP synthase are embedded on inner mitochondrial membrane. H^+ diffuse through them, down the electrochemical proton gradient, back into the matrix.
 - This provides enough energy to synthesise ATP by the phosphorylation of ADP with inorganic phosphate (P_i).



Chemiosmosis couples the ETC to ATP synthesis



SUMMARY OF OXIDATIVE PHOSPHORYLATION:

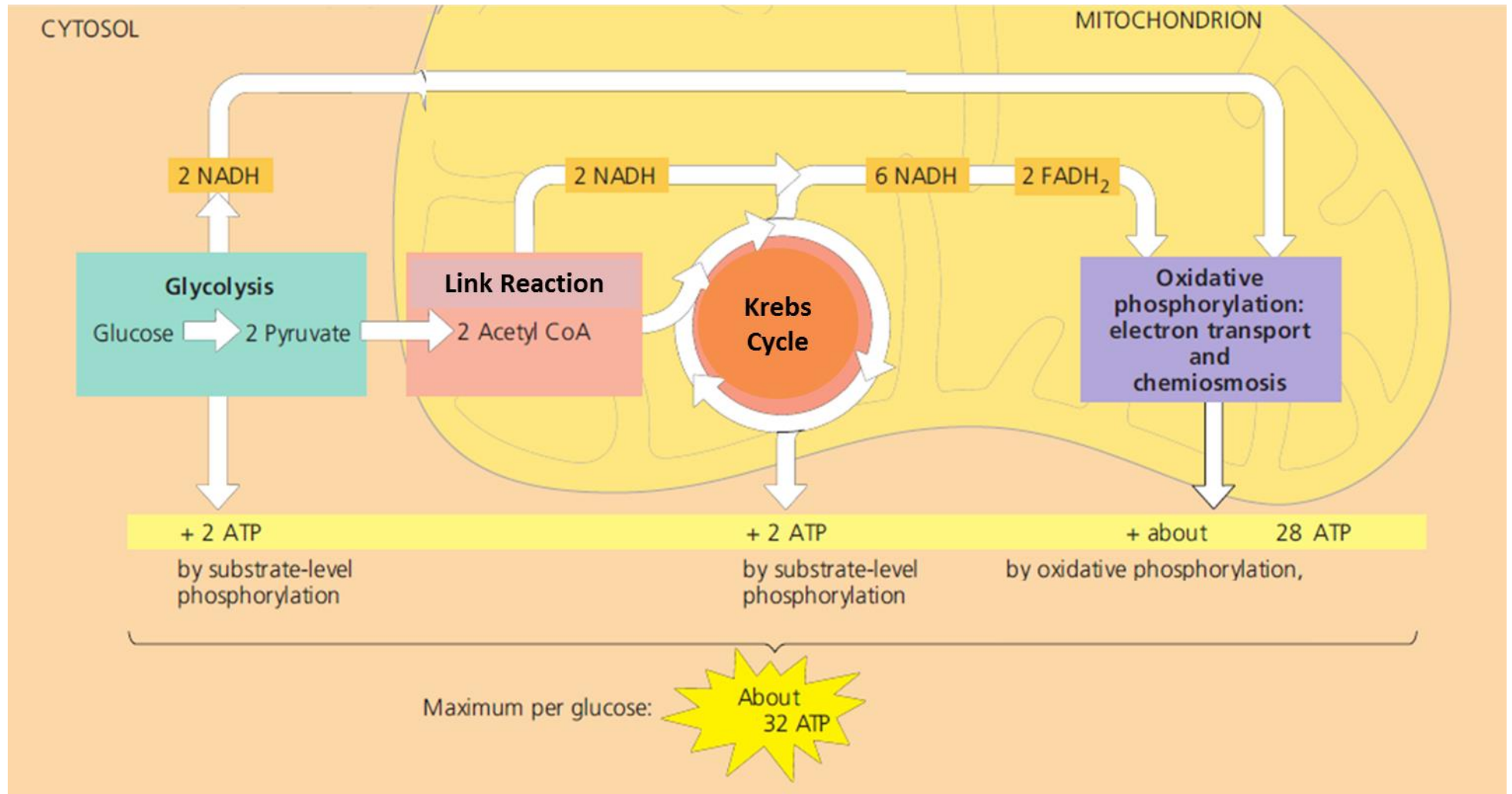


- Electrons from each reduced NAD ($NADH + H^+$) enter the ETC chain at the beginning, yielding **2.5 ATP**.
- Electrons from each reduced FAD ($FADH_2$) enter later in the chain at a lower energy level, synthesising only **1.5 ATP**.

Through aerobic respiration, the maximum amount of ATP synthesized per glucose molecule:

Stage	No. of reduced NAD and reduced FAD	No. of ATP by S.L.P	No. of ATP by O.P	Total no. of ATP
Glycolysis				
Link reaction				
Krebs cycle				
Total no. of ATP				

Key: O.P. = oxidative phosphorylation; S.L.P. = substrate level phosphorylation



ATP yield per molecule of glucose at each stage of aerobic respiration



3. Anaerobic Respiration

Anaerobic respiration occurs in the **absence of oxygen**. During anaerobic respiration, **glycolysis occurs**, producing **pyruvate** and a **small yield of ATP**. This is followed by **fermentation** which **regenerates oxidised NAD** by transferring protons and electrons from reduced NAD to pyruvate (lactate fermentation) or to derivatives of pyruvate (alcohol fermentation).

Note:

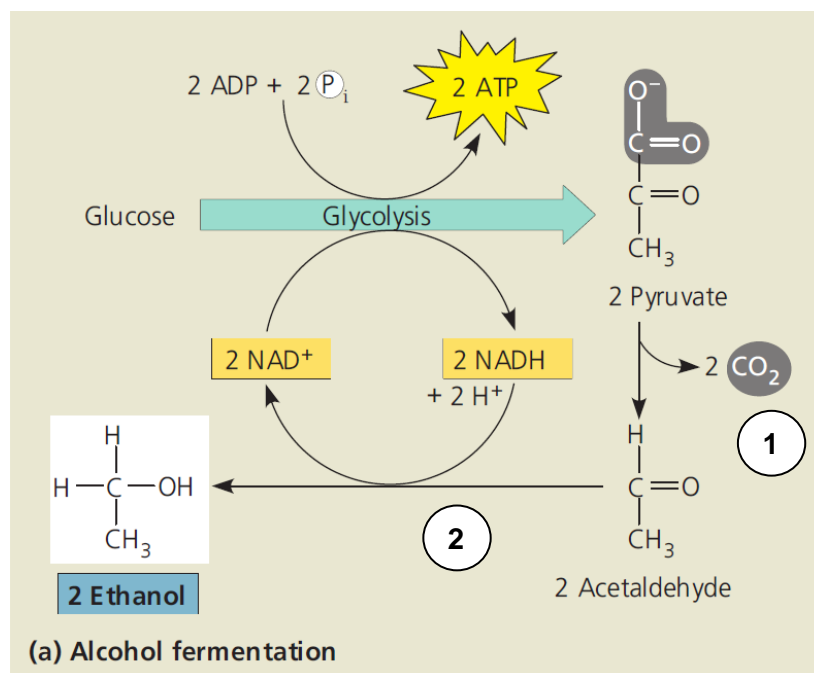
Link reaction and Krebs cycle cannot take place in the absence of oxygen because oxidative phosphorylation cannot occur to regenerate oxidised NAD and oxidised FAD without oxygen being present to act as the final proton and electron acceptor.

Glucose is thus incompletely oxidised in anaerobic respiration. A lot of energy is trapped in lactic acid or ethanol, and therefore anaerobic respiration is less efficient than aerobic respiration.

(a) Alcoholic Fermentation

During alcoholic fermentation in **plants** and **yeast**, **pyruvate** (product of glycolysis) is converted to **ethanol** in two steps:

- 1) **Carbon dioxide is released** from pyruvate (3C) to produce **acetaldehyde / ethanal** (2C). This step is catalysed by a **decarboxylase**.
- 2) **Acetaldehyde / Ethanal** is reduced to **ethanol** by **reduced NAD** catalysed by **alcohol dehydrogenase**. Therefore **oxidation of reduced NAD** takes place and **regenerates oxidised NAD** which allows glycolysis to continue.

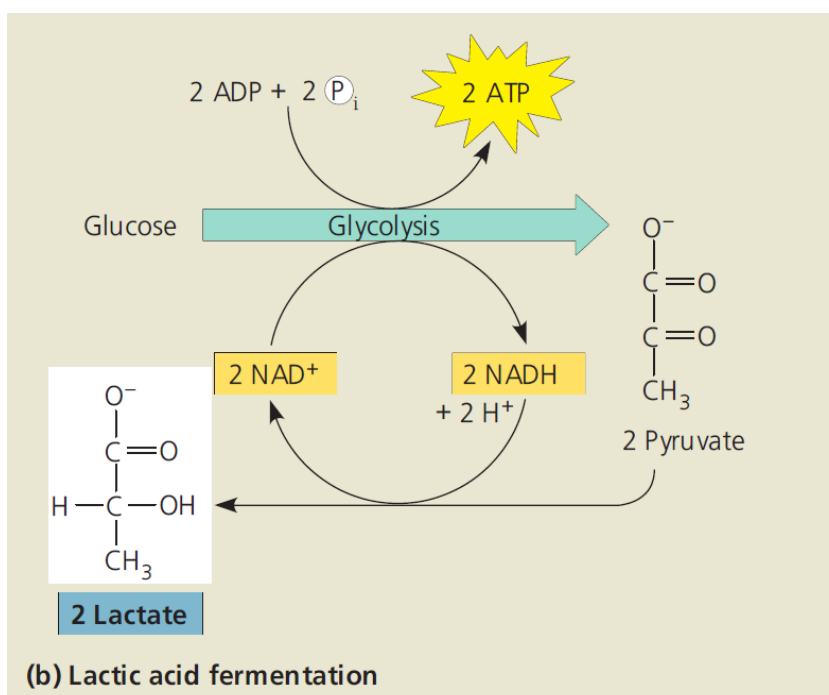




(b) Lactate Fermentation

Pyruvate is reduced directly to lactate (ionised form of lactic acid) by reduced NAD. This occurs in a single step catalysed by lactate dehydrogenase.

- (i) Lactate has to be removed from muscle cells by the blood to prevent muscle fatigue. An accumulation of lactate can be hazardous to the tissue as it is acidic.
- (ii) As lactate is a waste product which still contains a lot of energy, it is reconverted to pyruvate in the liver when oxygen supply is restored. Pyruvate then enters Krebs cycle and is fully oxidised to carbon dioxide and water, releasing more ATP.



SUMMARY OF ANAEROBIC RESPIRATION:

Overall, anaerobic respiration yields only 2 molecules of ATP per glucose molecule (via substrate level phosphorylation in glycolysis).

Products per glucose molecule
ATP
2



4. Regulation Of Respiration

Metabolism is highly regulated to **prevent wastage of energy and resources**. This is to ensure that the cell does not make more than what it needs. The most common regulation mechanism is via **negative feedback** such as the **end-product inhibition** of phosphofructokinase.

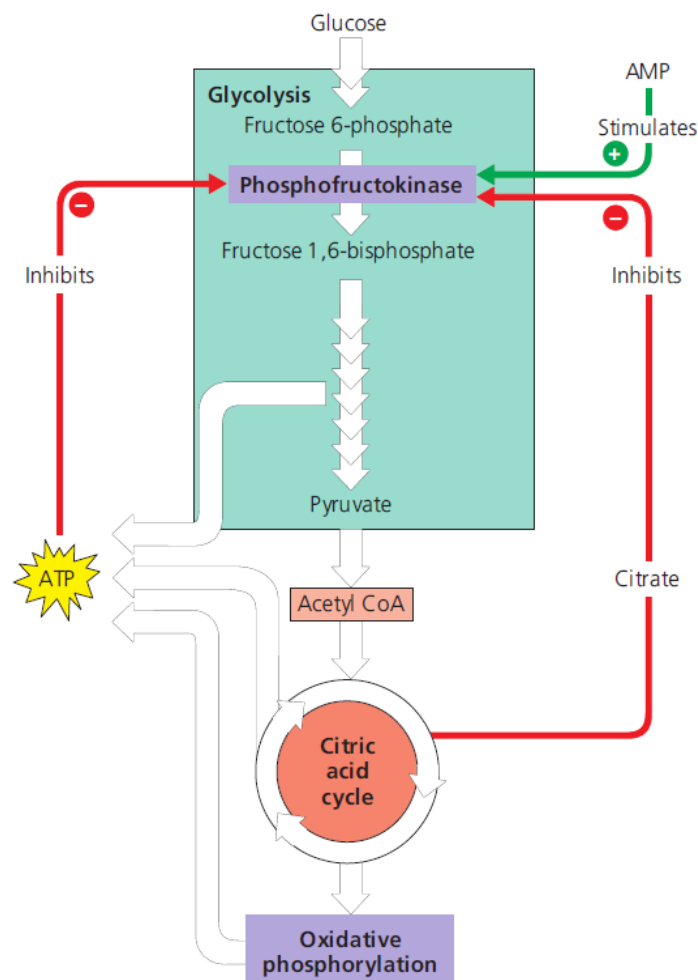
If the cell is undergoing high metabolic activity and its ATP concentration begins to drop, respiration speeds up. When there is plenty of ATP to meet demand, respiration slows down, sparing valuable organic molecules for other functions.

▪ Phosphofructokinase

This allosteric enzyme (with receptor sites for specific inhibitors and activators) catalyses one of the steps in glycolysis.

- Stimulated by **adenosine monophosphate (AMP)** (derived from ADP)
- Inhibited by the **accumulation of citrate** and **high ATP: ADP ratio**

This feedback regulation adjusts the rate of respiration as the cell's catabolic and anabolic demands change.



Control of cellular respiration



5. Respiratory Quotient

When different respiratory substrates are used, the ratio of carbon dioxide given out to oxygen taken in may vary. This ratio is called the **respiratory quotient (RQ)**.

$$\text{RQ} = \frac{\text{Volume of CO}_2 \text{ evolved}}{\text{Volume of O}_2 \text{ absorbed}} \text{ per unit time}$$

RQ can identify the type of respiratory substrate that is being oxidised. (The lower the RQ, the more oxygen is required for complete oxidation of the substrate, hence the greater the potential yield of ATP.)

- When glucose is the respiratory substrate, exactly the same number of molecules (and hence volume) of carbon dioxide and oxygen are produced and used respectively. This means that the RQ is **exactly 1**.
- However, if other substances are used as the main respiratory substrate, then the RQ is **less than 1**:

Respiratory substrate	RQ
Carbohydrate, eg glucose	1.0
Fat	0.7
Protein	0.9



6. Respirometer

A respirometer is a device used to measure the rate of respiration of a living organism by measuring its rate of exchange of oxygen and carbon dioxide.

The rate of respiration can be estimated by measuring the rate of gas exchange (change in volume of a gas). If aerobic respiration is occurring and the substrate oxidised is glucose, then the volume of oxygen taken up equals to the volume of carbon dioxide released. Therefore the total volume of gases should remain constant.

Principle behind a simple respirometer:

- If a compound that absorbs carbon dioxide, e.g. soda lime / potassium hydroxide, is placed inside the closed vessel, the pressure in the vessel decreases as the seeds/organism take up oxygen during respiration.
- The rate at which the pressure decreases is a measure of the rate at which the respiring tissue is taking up oxygen.

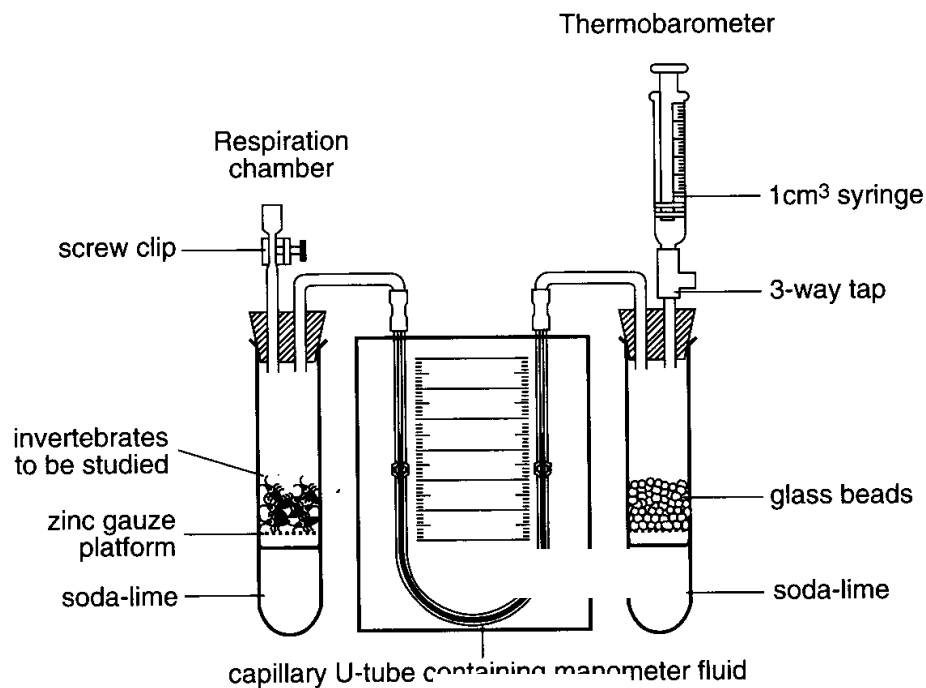


Figure 10.13 A respirometer.

**7. Comparison Tables (for Photosynthesis and Respiration)**Non-cyclic VS cyclic photophosphorylation

Features	Non-cyclic	Cyclic
Role of process		
Pathway of electrons		
PS involved		
First electron donor		
Final electron acceptor		
Establishing of H ⁺ gradient for ATP synthesis		
Products		

Photophosphorylation VS oxidative phosphorylation*Similarities*

-
-
-

Differences

Features	Photophosphorylation	Oxidative Phosphorylation
Energy conversion		
Location		
Involvement of light energy		
Source of energy for synthesis of ATP		
First electron donor		
Final electron and proton acceptor		
Establishing proton gradient for the synthesis of ATP		

Substrate-level phosphorylation VS oxidative phosphorylation

Features	Substrate-level Phosphorylation	Oxidative Phosphorylation
Definition		
Location		
Reactions		
Involvement of electron transport chain		
Involvement of oxidation		
No. of ATP formed per glucose		

Oxidative decarboxylation VS oxidative phosphorylation

Features	Oxidative Decarboxylation	Oxidative Phosphorylation
Location		
Reactions		
Enzymes		
Involvement of electron transport chain		
Involvement of decarboxylation		
Involvement of oxidation		
Production of ATP		

Calvin cycle VS Krebs Cycle

Features	Calvin Cycle	Krebs Cycle
Site		
Coenzymes Involved		
Role / Fate of carbon dioxide		
Role / Fate of ATP		
Products formed		
Regeneration of starting material		