CORE IDEA 3: ENERGY AND EQUILIBRIUM
TOPIC 3.2: CELLULAR RESPIRATION

Learning Outcomes:

(a) Identify components of mitochondria in drawings, photomicrographs and electronmicrographs.

(f) Outline the process of glycolysis, highlighting the location, raw materials used and products formed. (Knowledge of details of the intermediate compounds and isomerization is not required.)

(g) Outline the process of the link reaction and Krebs cycle, highlighting the location, raw materials used and products formed. (In terms of dehydrogenation and decarboxylation.)

(h) Outline the process of oxidative phosphorylation including the role of oxygen and the electron transport chain in aerobic respiration (names of complexes in the ETC are not required).

(i) Explain the production of a small yield of ATP from respiration in anaerobic conditions in yeast and in mammalian muscle tissue.

(j) Explain the significance of the formation of ethanol in yeast and lactate in mammals in the regeneration of NAD.

(k) Investigate the effect of factors such as substrate concentration, type of substrate and temperature on the rate of respiration.

(l) Outline chemiosmosis in respiration. (Names of complexes in the ETC are not required.)

References:

Campbell and Reece. *Biology.* (11th edition). Chapter 10: Cell Respiration, P. 236 – 256 (or corresponding chapter in other editions)

Note: This textbook is available in our library. You may wish to borrow it to supplement your reading when necessary.

1 INTRODUCTION

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

- Photosynthesis allows plants to convert energy from light into chemical energy stored in organic molecules, while cellular respiration occurs to break down the organic molecules to yield adenosine triphosphate (ATP), which drives many cellular processes.

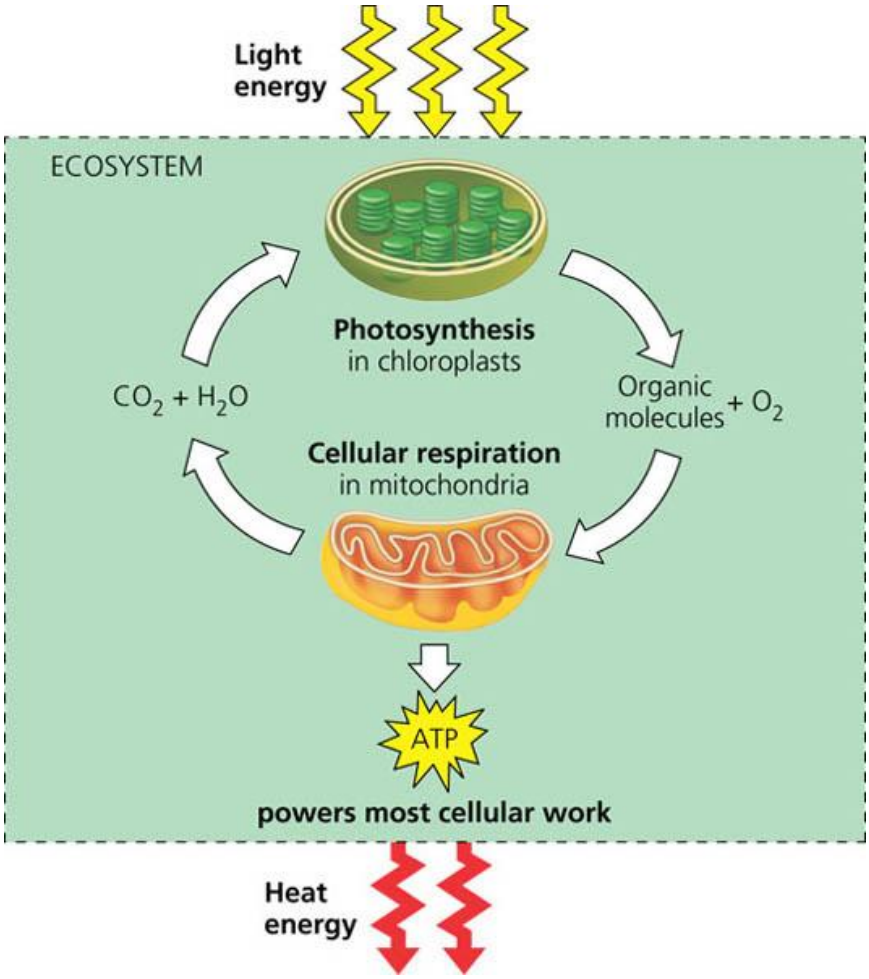


Fig 1.1: Energy flow and chemical recycling in ecosystems

1.1 ENERGY AND ATP

- Respiration involves the release of chemical energy, in the form of **adenosine triphosphate (ATP)**, from organic molecules through a series of oxidation-reduction (redox) reactions.

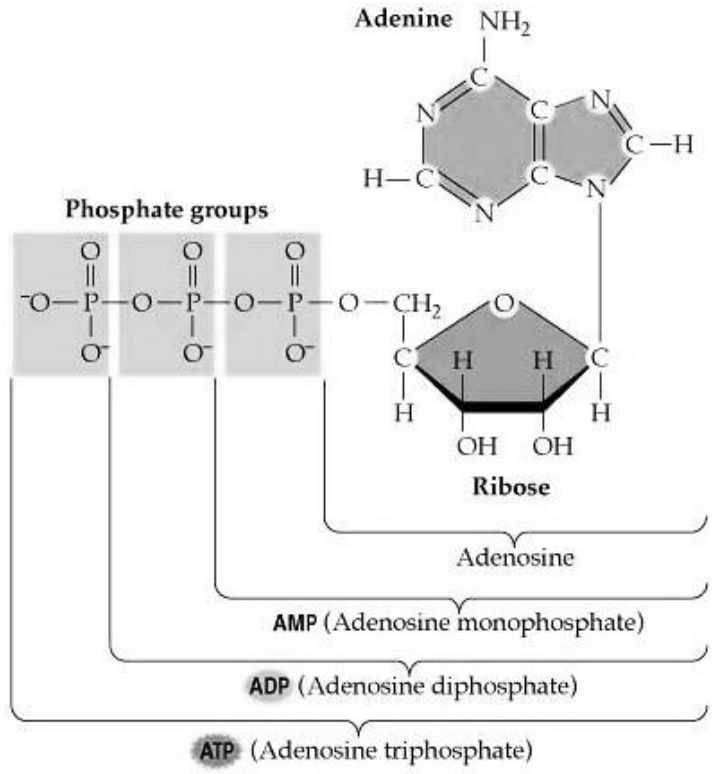


Fig. 1.1.1: Structure of ATP, ADP and AMP

The roles of ATP in cells include:

(a) Energy Currency

- ATP serves as the **energy currency** of the cell. Energy is **released** when ATP is hydrolysed to ADP (30.6 kJ per mole of ATP, 1 J = 0.239 cal). The energy released can be used in various cellular processes, such as enzyme reactions, protein synthesis, active transport, muscular contractions, etc.

- ATP can be resynthesised from ADP by the addition of a phosphate group, catalysed by the enzyme ATP synthase.

(b) Regulation of Metabolic Reactions

- ATP has an **allosteric effect** on the **regulation of metabolic reactions** (e.g. ATP acts as an allosteric inhibitor of phosphofructokinase in glycolysis). The concentration of ATP relative to ADP and AMP acts as an index of the energy status of the cell, determining the rates of reactions of regulatory enzymes involved in the metabolic pathway.

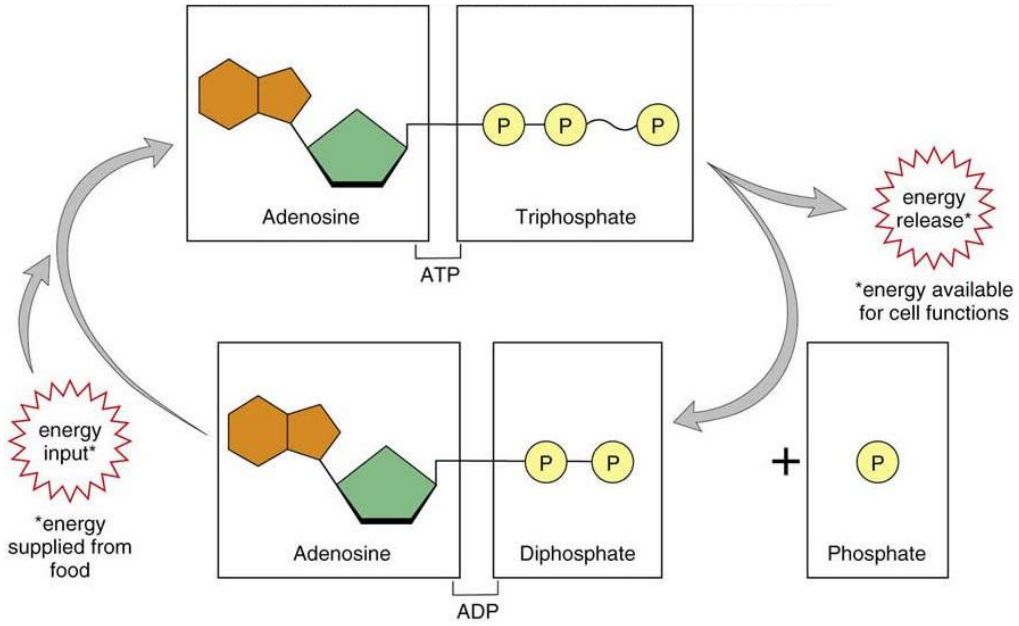


Fig. 1.1.2: Hydrolysis of ATP and condensation of ADP

1.2 THE MITOCHONDRION

Learning Outcome (a):
Identify components of mitochondria in drawings, photomicrograph and electron micrographs.

[Retrieval Practice] Refer to Topic 1.1 Organelles and Cellular Structures lecture notes.

- The mitochondrion is involved in **aerobic respiration**, which results in the formation of **ATP**.

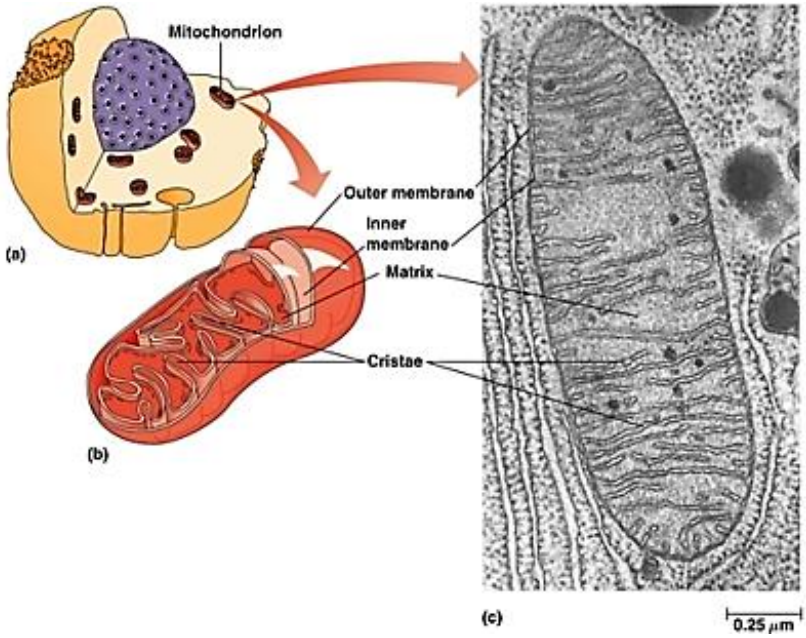


Fig. 1.2.1: (a) Mitochondrion in an animal cell; (b) Structure of a mitochondrion; (c) Electron micrograph of a mitochondrion

- It is an organelle bound by a **double membrane**. The outer membrane is smooth, while the inner membrane is extensively folded to form **cristae**. The enzyme **ATP synthase**, as well as the **electron transport chain (ETC)**, are embedded on the inner membrane.

- The **mitochondrial matrix** contains **enzymes, circular DNA, RNA,** and **70S ribosomes**.

1.2 CELLULAR RESPIRATION

- Respiration refers to a series of cellular processes by which organic compounds (glucose and other substrates) are broken down with the help of enzymes to release energy, in the form of ATP, for other cellular processes.

- Cells can undergo respiration in the presence of oxygen (**aerobic respiration**) or absence of oxygen (**anaerobic respiration**).

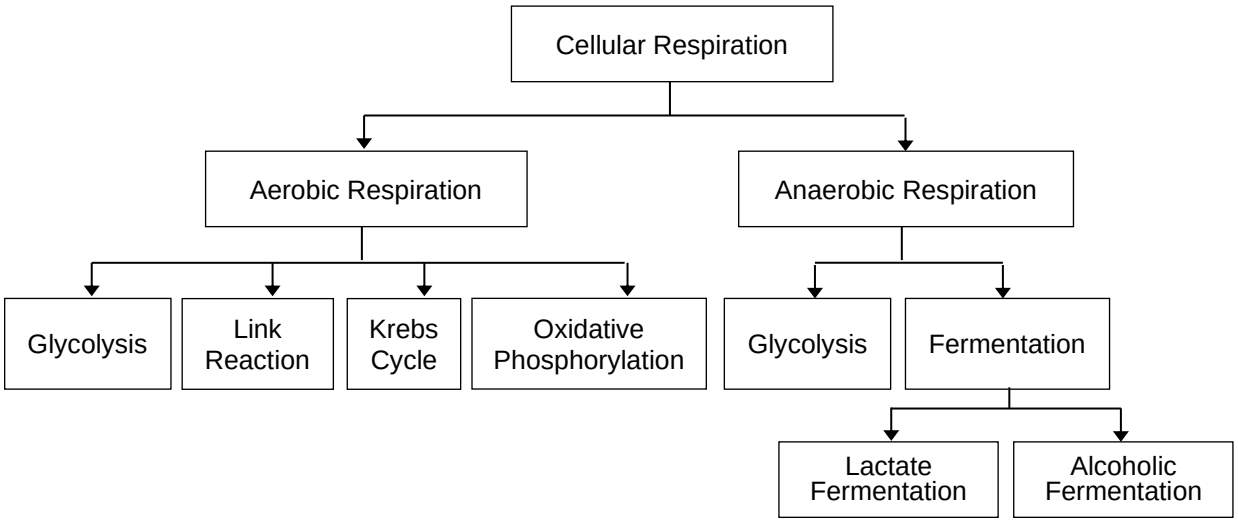


Fig. 1.3.1: Overview of cellular respiration

2 AEROBIC RESPIRATION

- In the presence of oxygen, glucose is broken down to yield carbon dioxide, water and energy. The oxidation of one molecule of glucose yields sufficient energy to produce **32 ATP** molecules.

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow\rightarrow 6\ CO_2 + 6\ H_2O + \text{Energy}$$

- Aerobic respiration occurs in four stages:

No.	Stage	Process	Location
1	Glycolysis	Breakdown of glucose (6C) to form 2 molecules of pyruvate (3C)	Cytosol
2	Link Reaction	Conversion of pyruvate (3C) to acetyl-CoA (2C)	Mitochondrial Matrix
3	Krebs Cycle	Oxidation of acetyl-CoA (2C) via a series of reactions	Mitochondrial Matrix
4	Oxidative Phosphorylation	Electron transfer down the electron transport chain resulting in the production of ATP	Inner Mitochondrial Membrane

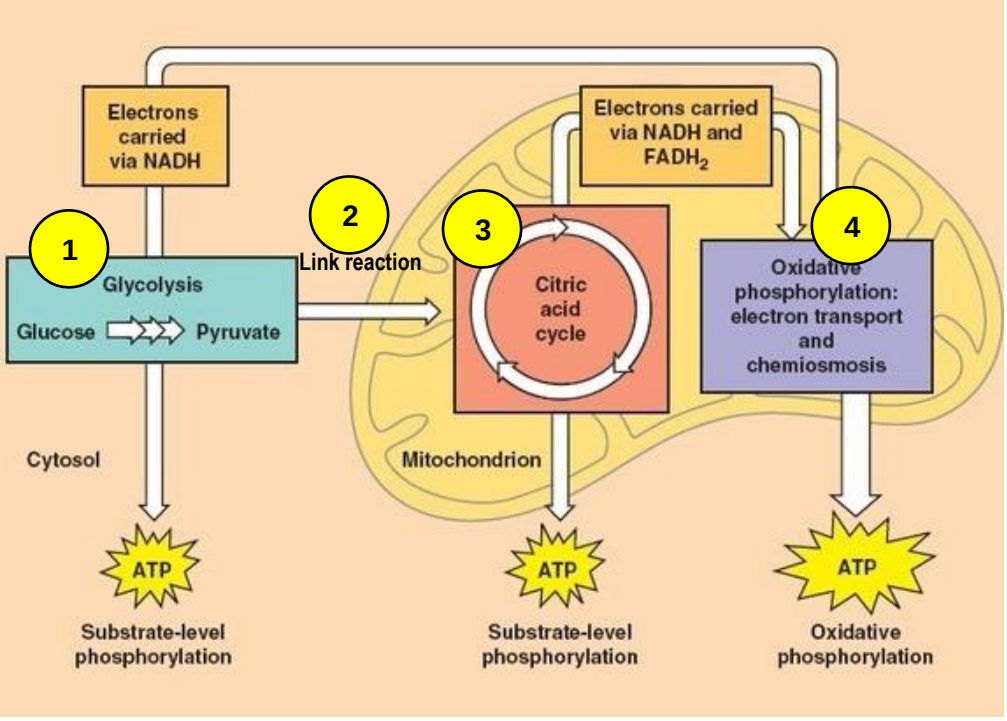


Fig. 2.1: Overview of aerobic respiration (Simplified)

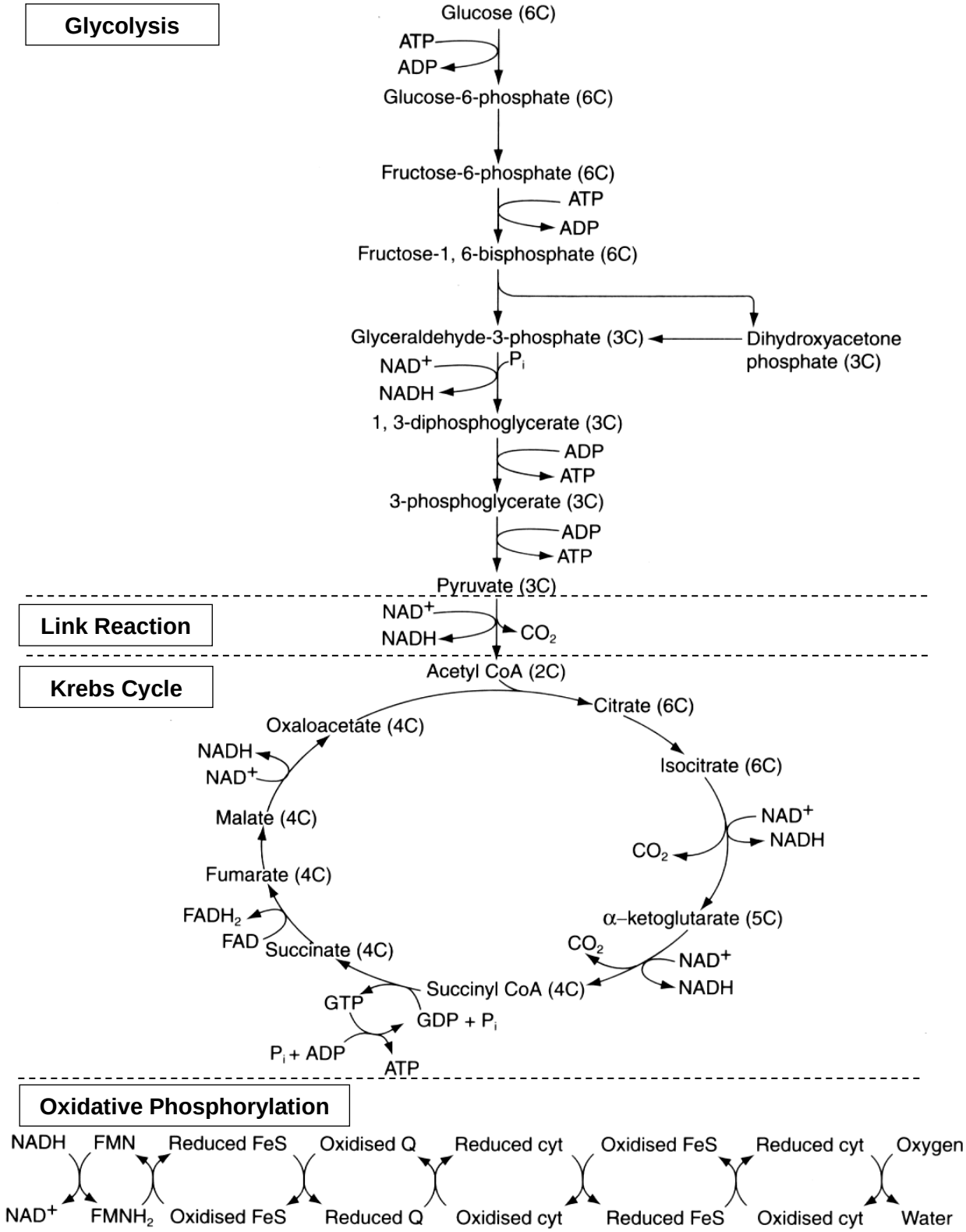


Fig. 2.2: Overview of aerobic respiration

2.1 GLYCOLYSIS

Learning Outcome (f):
Outline the process of glycolysis, highlighting the location, raw materials used and products formed.

- Glycolysis occurs under <u>both</u> **aerobic and anaerobic** conditions, and takes place in the **cytosol**.

- Glycolysis involves the breakdown of one molecule of **glucose (6C)** to two molecules of **pyruvate (3C)**.

- Glycolysis consists of an **energy investment phase** and an **energy payoff phase**.

  - During the energy investment phase, 2 molecules of ATP are used per glucose molecule.
  - During the energy payoff phase, 4 molecules of ATP are synthesised per glucose molecule.
  - Therefore, the **net yield** of glycolysis is **2 ATP per glucose molecule**.

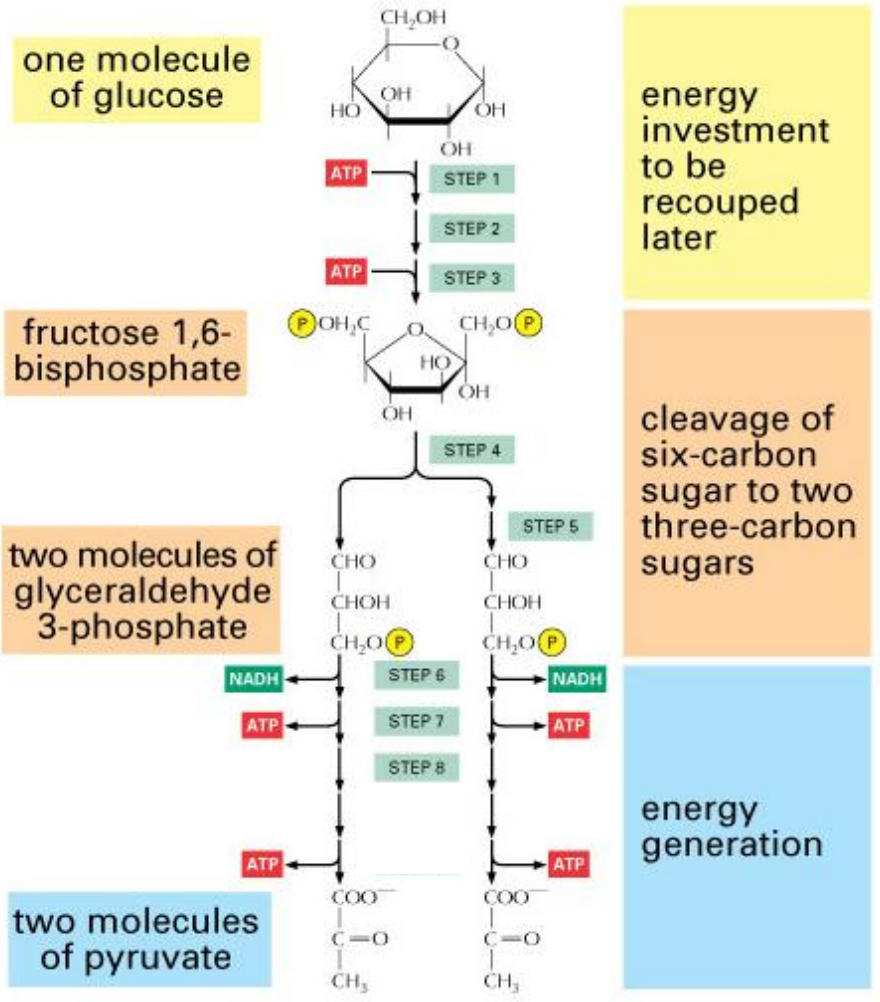


Fig. 2.1.1: Overview of glycolysis

Steps in Glycolysis

Step 1: Phosphorylation
Glucose (6C) is phosphorylated to **glucose-6-phosphate** (G6P) using a molecule of **ATP**. This step is the 1st priming reaction. It functions to: **1) Keep respiratory substrate in cell** Glucose is a polar molecule that can diffuse across the cell surface membrane via glucose transporters. Phosphorylation confers a negative charge on G6P, preventing it from moving out of the cell **2) Maintain concentration gradient of glucose** Rapid conversion of glucose to G6P keeps the intracellular concentration of glucose low, thus favouring the diffusion of glucose into the cell. Enzyme: hexokinase
Step 2: Isomerisation
Glucose-6-phosphate is isomerised to fructose-6-phosphate.
Step 3: Phosphorylation
Fructose-6-phosphate is phosphorylated to **fructose-1,6-bisphosphate** using a second molecule of **ATP**. This step is the 2nd priming reaction. Enzyme: phosphofructokinase
Step 4: Lysis
Fructose-1,6-bisphosphate is cleaved to form 2 molecules of **glyceraldehyde-3-phosphate / triose phosphate** (3C).
Step 5: Oxidation and Phosphorylation
Glyceraldehyde-3-phosphate is oxidised and phosphorylated to 1,3-bisphosphoglycerate. Inorganic phosphate is added to produce a high-energy compound, and NAD^+ is used as a hydrogen acceptor in the reaction, forming **NADH** (**reduced NAD**).
Step 6: Substrate Level Phosphorylation
One phosphate group of 1,3-bisphosphoglycerate is transferred to ADP, forming 3-phosphoglycerate / glycerate-3-phosphate and one molecule of **ATP**.
Step 7: Substrate Level Phosphorylation
One phosphate group of 3-phosphoglycerate / glycerate-3-phosphate is transferred to ADP, forming **pyruvate** and another molecule of **ATP**. Enzyme: pyruvate kinase

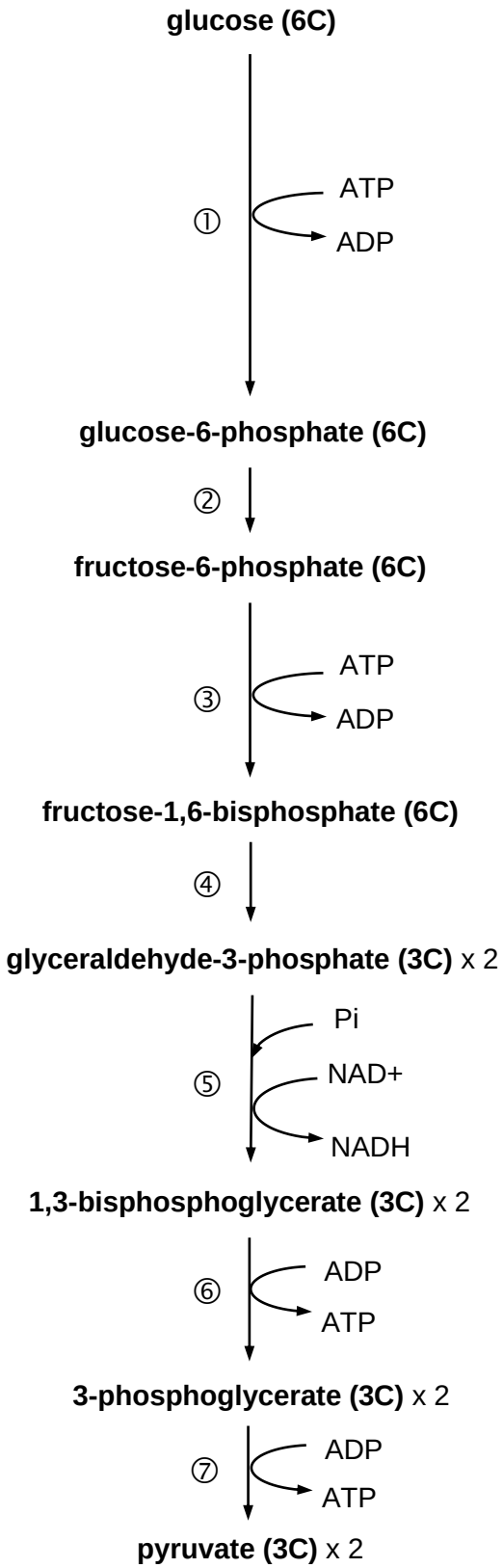


Fig 2.1.2: Steps in glycolysis

What are NAD^+ and FAD?

NAD^+ and FAD are **coenzymes** to dehydrogenase involved in cellular respiration. They can switch between the oxidised and reduced states easily, which allows them to function as **proton and electron carriers**. The reduced forms are more energetically valuable.

Oxidised Nicotinamide Adenine Dinucleotide (NAD^+)

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

$$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+$$

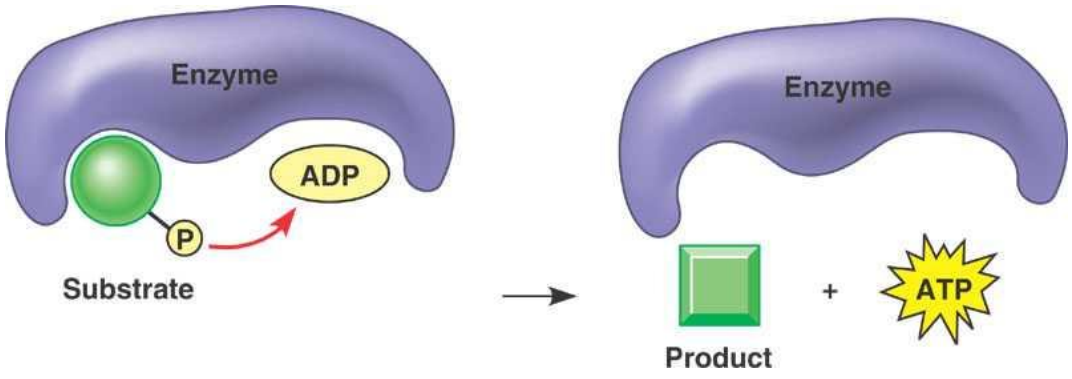
Flavin Adenine Dinucleotide (FAD)

- During aerobic respiration, glucose is oxidised by a series of dehydrogenation reactions.
- During Krebs cycle, protons and electrons are released and transferred to oxidised FAD (FAD) to form reduced FAD ($FADH_2$).

$$FAD + 2H^+ + 2e^- \rightarrow FADH_2$$

What is 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**).
- Only a small amount of ATP is generated by substrate level phosphorylation compared to oxidative phosphorylation (final step of aerobic respiration).



CHECKPOINT 1

Fill in the blanks to indicate the number of products formed from glycolysis (per glucose molecule).

- _____ **NADH** (**reduced NAD**) molecules – which will be used in oxidative phosphorylation (in aerobic respiration) or fermentation (in anaerobic respiration)
- _____ **ATP** molecules (net yield)
- _____ **pyruvate** (3C) molecules – which will be used in the link reaction (in aerobic respiration) or fermentation (in anaerobic respiration)

Regulation of Glycolysis

- The following enzymes are involved in the regulation of glycolysis:

(a) Hexokinase (HK)

- HK is regulated by direct **feedback inhibition** of its product **glucose-6-phosphate**.

(b) Phosphofructokinase (PFK)

- PFK is **allosterically inhibited** by **ATP**. When ATP concentration is sufficiently high, PFK activity falls and glycolysis is 'turned off'. When ATP concentration is low, PFK activity increases and glycolysis is 'turned on'. Thus, rate of glycolysis changes in response to the energy status of the cell.

(c) Pyruvate kinase (PK)

- PK is **activated** by **fructose-1,6-bisphosphate** and **inhibited** by high levels of **ATP**.

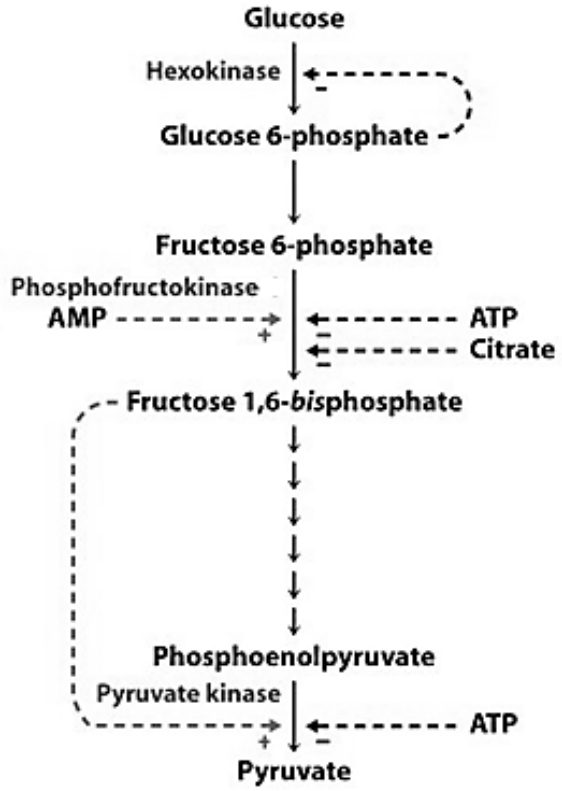


Fig. 2.1.3: Regulation of glycolysis

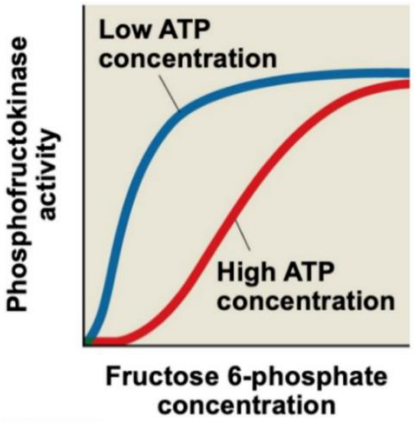


Fig. 2.1.4: Rate of reaction catalysed by phosphofructokinase (PFK) at high and low concentrations of ATP

2.2 LINK REACTION

Learning Outcome (g):
Outline the process of the link reaction and Krebs cycle, highlighting the location, raw materials used and products formed.

- Link reaction occurs only in **aerobic** conditions, and takes place in the **mitochondrial matrix**.

- **Pyruvate (3C)** enters the mitochondrial matrix from the cytosol via a transport protein. It is converted to **acetyl-CoA (2C)** through **oxidative decarboxylation**, which involves:

  (i) **Decarboxylation** of pyruvate (3C), releasing one molecule of carbon dioxide.

  (ii) The remaining two-carbon fragment undergoes an **oxidation** reaction to form acetate, where protons and electrons are removed and transferred to NAD^+ to form **NADH**.

  (iii) Acetate then reacts with **coenzyme A** (CoA) to form **acetyl CoA (2C)**.

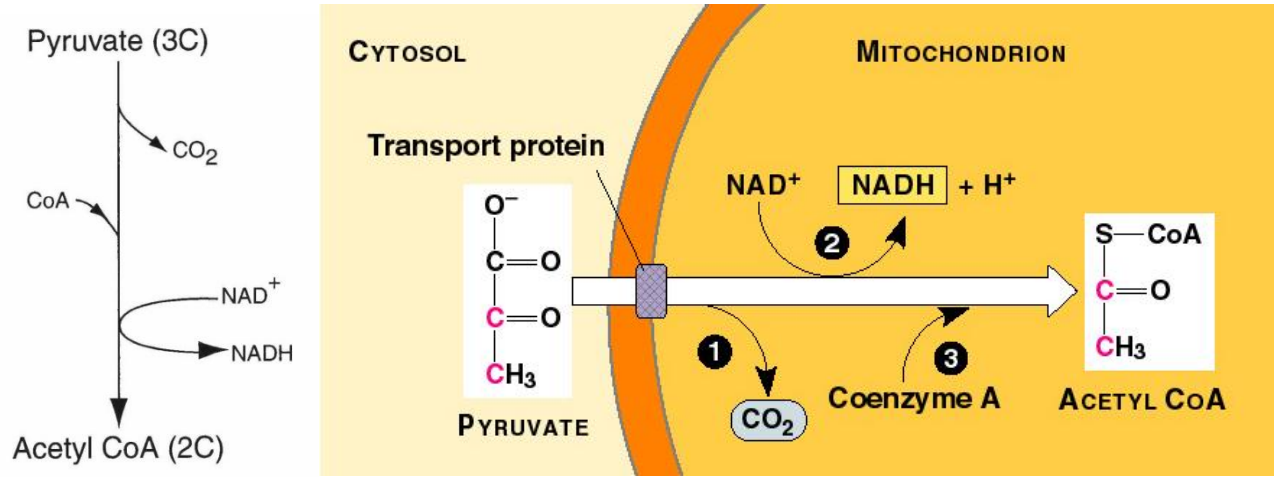


Fig. 2.2.1: Link reaction

CHECKPOINT 2

Fill in the blanks to indicate the number of products formed from link reaction (per glucose molecule).

- _____ **acetyl CoA** (2C) molecules – which will be used in Krebs cycle
- _____ **NADH** (**reduced NAD**) molecules – which will be used in oxidative phosphorylation
- _____ **CO_2** molecules – which are eventually exhaled from the lungs (in animals) or used for photosynthesis (in plants)

2.3 KREBS CYCLE (CITRIC ACID CYCLE / TRICARBOXYLIC ACID CYCLE)

- Krebs cycle, also known as the citric acid cycle or tricarboxylic acid (TCA) cycle, only occurs in **aerobic** conditions, and takes place in the **mitochondrial matrix**.

- Acetyl CoA (2C) synthesised from the link reaction enters the Krebs cycle. The main function of the Krebs cycle is to **oxidise acetyl CoA**, through a series of **decarboxylation** and **dehydrogenation** reactions, where each step is catalysed by a specific enzyme. In the process, reduced coenzymes NAD and $FADH_2$ are produced.

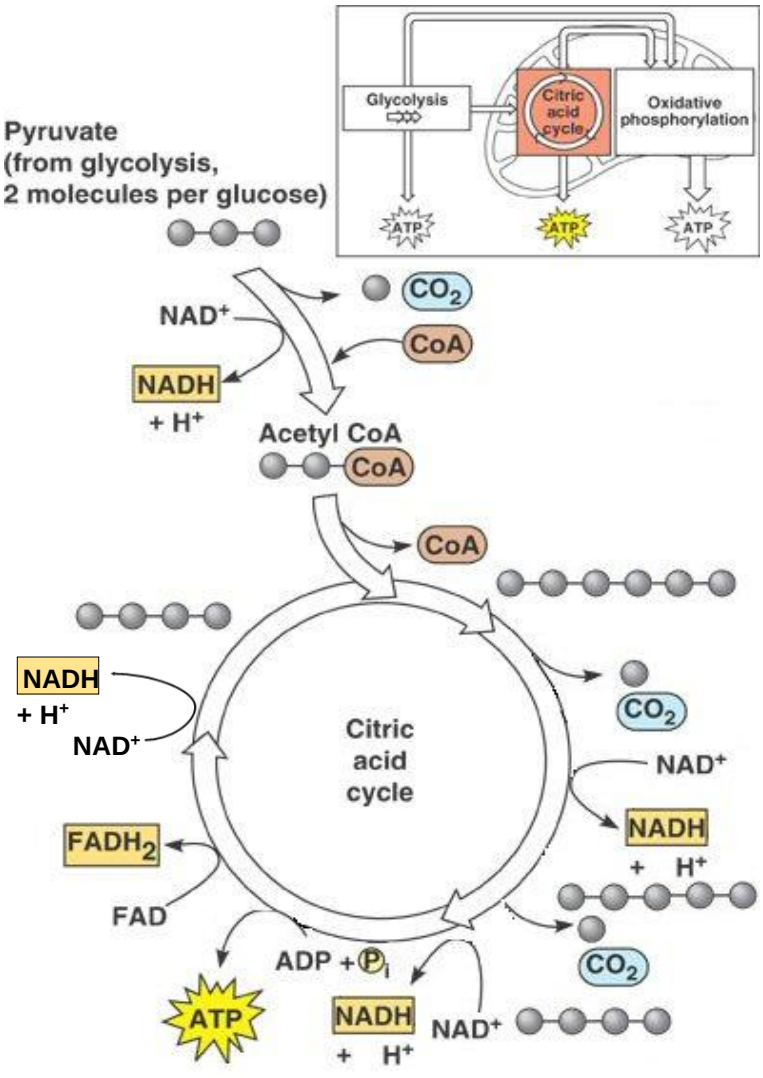


Fig. 2.3.1: Overview of the Krebs cycle

Steps in Krebs Cycle

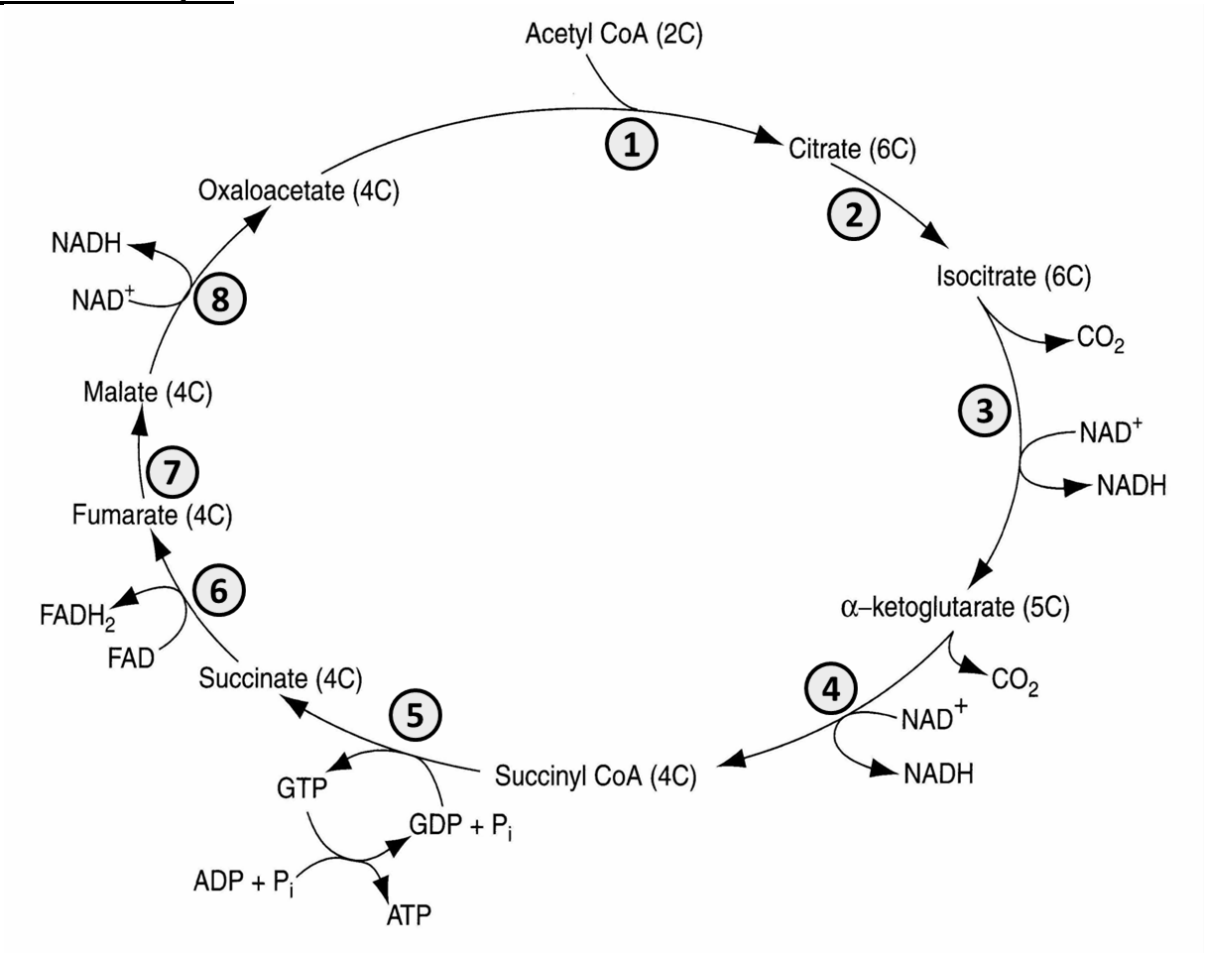


Fig. 2.3.2: Krebs cycle
(names of intermediates are not required)

Steps ① & ②: Formation of 6C Compound

- The two-carbon acetyl group of **acetyl CoA** (2C) is added to **oxaloacetate** (4C) to form **citrate** (6C). Citrate (6C) is converted to its isomer isocitrate (6C).

Steps ③ & ④: Oxidative Decarboxylation

- Isocitrate (6C) undergoes a series of oxidative decarboxylation reactions to form α-ketoglutarate (5C), and subsequently to succinyl CoA (4C). **2 carbon dioxide** molecules are lost, and NAD^+ acts as a proton and electron acceptor, forming **2 NADH**.

Step ⑤: Substrate level phosphorylation

- Succinyl CoA (4C) is converted to succinate (4C). 1 GTP is formed by substrate level phosphorylation, which may then be converted to **ATP**.

Step ⑥ - ⑧: Dehydrogenation

- Succinate (4C) is converted to fumarate (4C), then malate (4C) and subsequently to oxaloacetate (4C) via a series of dehydrogenation reactions. FAD and NAD^+ act as proton and electron acceptors, forming **$FADH_2$** and **NADH** respectively. Thus, **oxaloacetate is regenerated** and able to react with another molecule of acetyl-CoA.

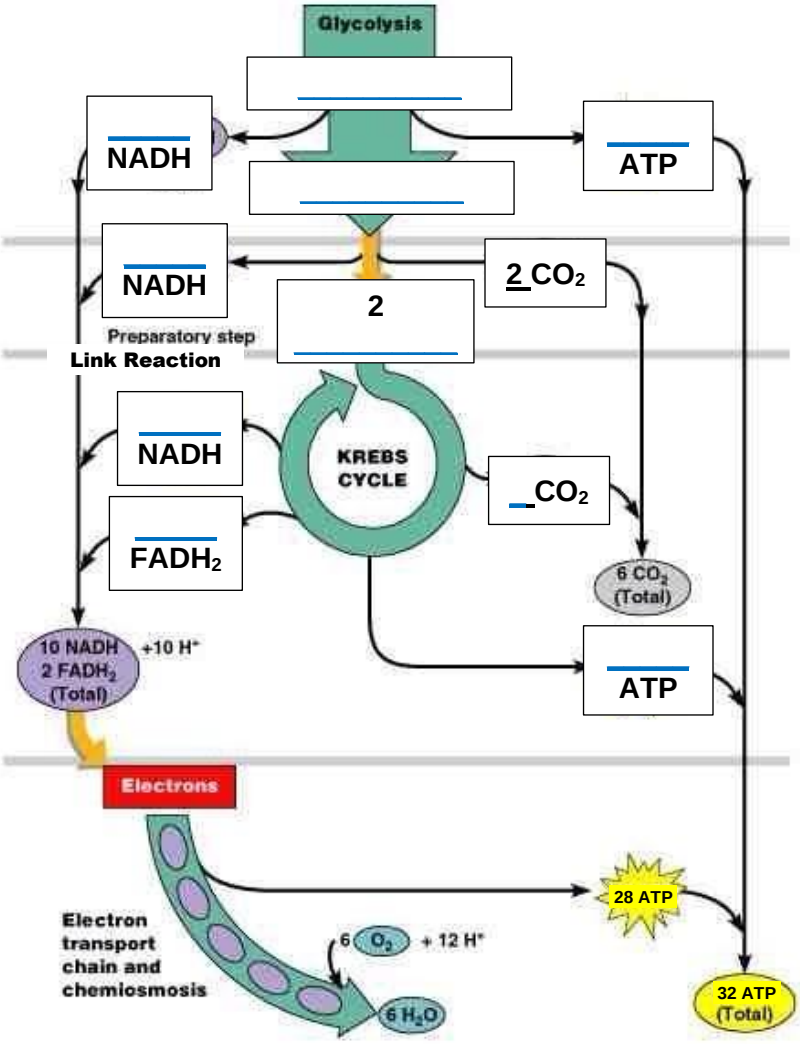
CHECKPOINT 3

Fill in the blanks to indicate the number of products formed from Krebs cycle (per glucose molecule).

- _____ **oxaloacetate** (4C) molecules regenerated – which reenters Krebs cycle
- _____ **NADH** molecules – which will be used in oxidative phosphorylation
- _____ **$FADH_2$** molecules – which will be used in oxidative phosphorylation
- _____ **ATP** molecules
- _____ **CO_2** molecules – which are eventually exhaled from the lungs (in animals) or used for photosynthesis (in plants)

CHECKPOINT 4

Fill in the blanks to indicate the starting materials and products of the first 3 stages of aerobic respiration.



So far, how many molecules of ATP, NADH and $FADH_2$ are produced per glucose molecule?

_____ ATP, _____ NADH and _____ $FADH_2$

2.4 OXIDATIVE PHOSPHORYLATION

Learning Outcome (h):
Outline the process of oxidative phosphorylation.
including the role of oxygen and the electron transport chain in aerobic respiration.

- Oxidative phosphorylation occurs <u>only</u> in **aerobic** conditions and takes place on the **inner mitochondrial membrane**.

- **Oxidative phosphorylation** is the process by which ATP is synthesised as **protons and electrons are transferred from reduced coenzymes (NADH and $FADH_2$)** (which are the products of glycolysis, link reaction and Krebs cycle) to **oxygen** via a series of **electron carriers** in the electron transport chain. Energy released from this electron transfer is used to build up a **proton gradient** across the **inner mitochondrial membrane**, where ATP is synthesised from the potential energy of this proton gradient.

- Oxidative phosphorylation serves to:
  (a) synthesise ATP, and
  (b) regenerate NAD^+ and FAD for use in glycolysis, link reaction and Krebs cycle

<u>Steps in Oxidative Phosphorylation</u>

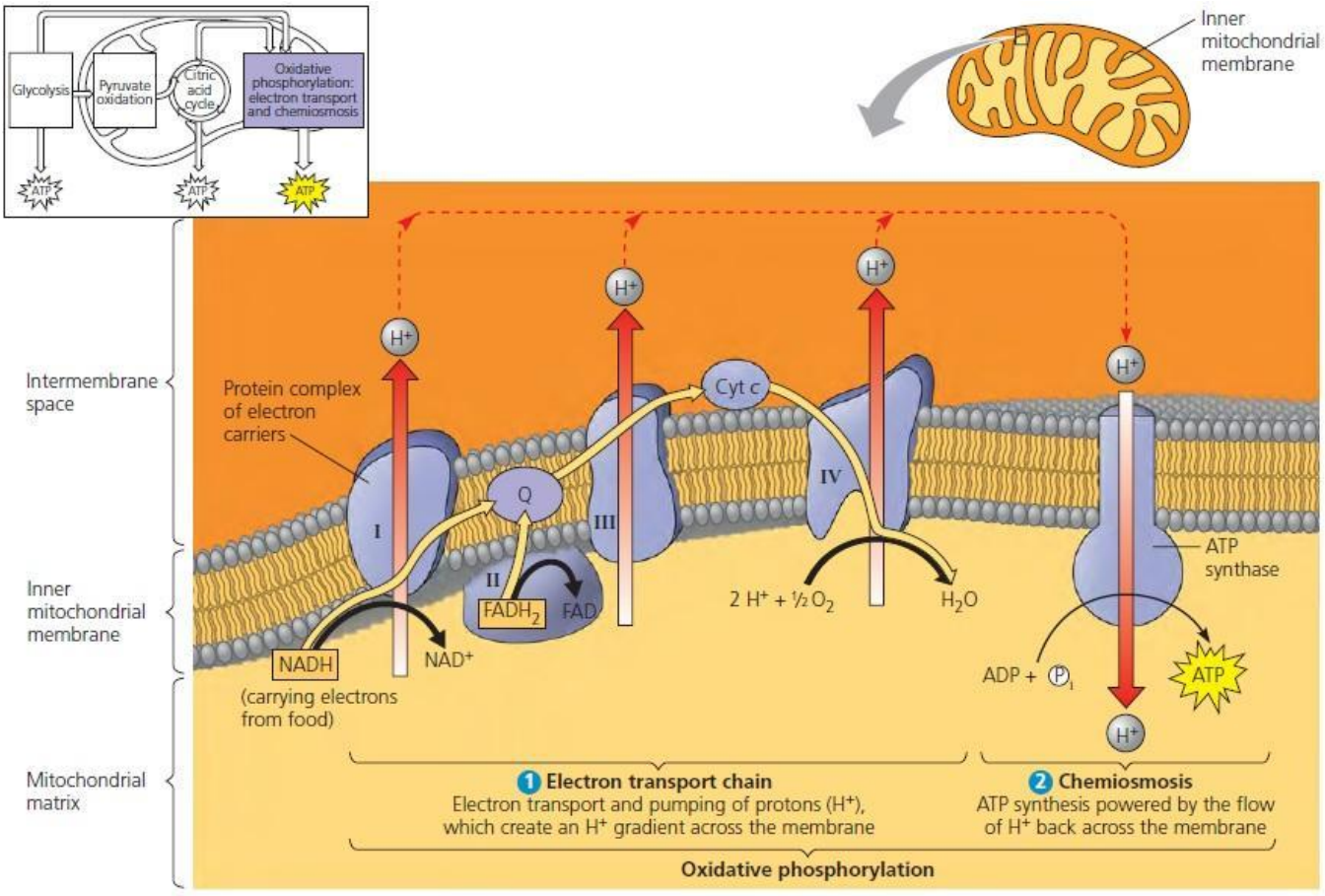


Fig. 2.4.1: Oxidative phosphorylation

Stage 1: Electron Transport Chain (ETC)

Step 1a:

- **NADH and $FADH_2$** (produced during glycolysis, link reaction and Krebs cycle) **donate electrons** to the electron carriers of the ETC. In the process, they are are **oxidised** to NAD^+ and FAD respectively.

Step 1b:

- Electrons are **passed down a series of electron carriers** via a series of redox reactions. Electrons are eventually passed to **oxygen**, the **final electron acceptor** of the ETC. *(names of electron carriers are not required)*

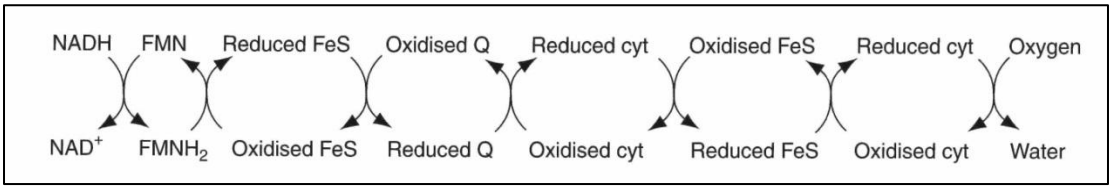


Fig. 2.4.2: Electron carriers of ETC undergo a series of redox reactions as electrons are being passed from NADH to oxygen

- The enzyme **cytochrome oxidase** combines oxygen with electrons and protons to form **water** (see equation in Fig. 2.4.1).

Step 1c:

- Since the electron carriers of the ETC are of **progressively lower energy levels**, **energy is released** during the electron transfer. This energy is used by proton pumps to **pump H^+ ions** from the **mitochondrial matrix**, across the **inner mitochondrial membrane**, into the **intermembrane space**.

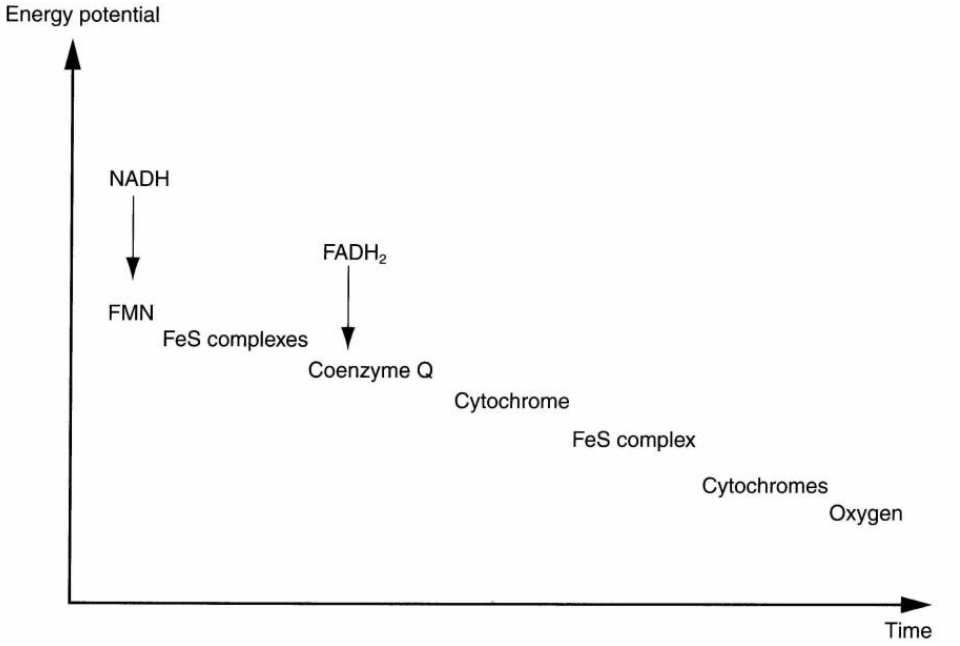


Fig. 2.4.3: Series of electron carriers of ETC have progressively lower energy levels.

- The **accumulation of H^+ ions** in the intermembrane space builds up a **proton gradient**, resulting in the generation of a **proton-motive force**.

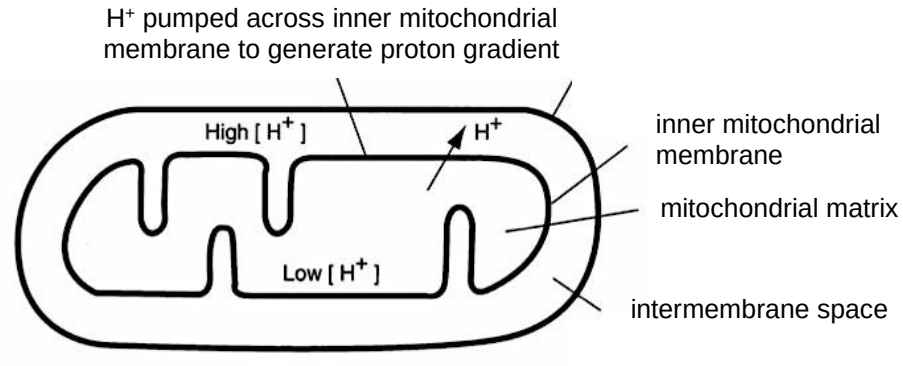


Fig. 2.4.4: Generation of proton gradient across inner mitochondrial membrane

Stage 2 (Chemiosmosis)

- As the inner mitochondrial membrane is impermeable to H^+ ions, **H^+ ions move from the intermembrane space back into the mitochondrial matrix** via **facilitaed diffusion**, through **ATP synthase** complex located in the inner mitochondrial membrane.

- As H^+ ions diffuse through ATP synthase complex, **ADP is phosphorylated to form ATP**.

- The process of using the kinetic energy of passing H^+ ions down their concentration gradient to generate ATP is called **chemiosmosis**.

- Thus, since the oxidation of reduced coenzymes (NADH & $FADH_2$) at the ETC is coupled to ADP phosphorylation, ATP is said to be produced via **oxidative phosphorylation**.

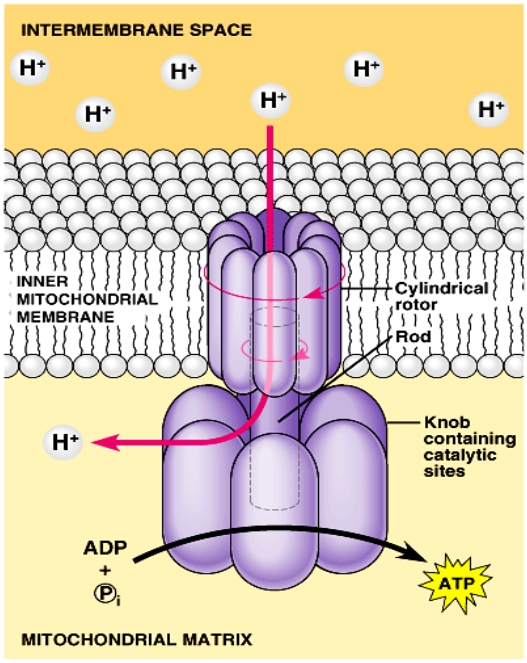


Fig. 2.4.5: Chemiosmosis and the generation of ATP via ATP synthase

- The oxidation of 1 molecule of **NADH** yields approximately **2.5** molecules of ATP, while the oxidation of 1 molecule of **$FADH_2$** yields approximately **1.5** molecules of ATP. This is because NADH donates its electrons to electron carriers of a higher energy level in the ETC as compared to $FADH_2$ (Fig. 2.4.3).

CHECKPOINT 5

Complete the following table to determine the ATP yield per glucose molecule at each stage of aerobic respiration.

Stage of aerobic respiration	ATP produced by substrate level phosphorylation	reduced NAD produced	reduced FAD produced	ATP produced from reduced NAD or FAD (oxidative phosphorylation)	Total ATP produced
Glycolysis					
Link reaction					
Krebs cycle					
Total					

(a) What is the number of ATP produced from substrate level phosphorylation? _____

(b) What is the number of ATP molecules produced from oxidative phosphorylation? _____

(c) What is the total number of ATP molecules produced as a result of the complete oxidation of one glucose molecule? _____

DID YOU KNOW?

Effects of 3 classes of respiratory poisons provide evidence for chemiosmosis and its dependence on the structural organisation of the mitochondrial membrane.

1) Poisons that block electron flow

Various poisons block the movement of electrons down the ETC. Chemicals such as carbon monoxide, cyanide and hydrogen sulphide block the transport of electrons from cytochrome a_3 to oxygen. In this case, ATP production is completely inhibited.

2) Poisons that inhibit ATP synthase

This class of poisons, which includes the antibiotic oligomycin, directly inhibit ATP synthase. Although the proton gradient becomes larger than normal, its potential energy cannot be tapped to make ATP.

3) Poisons that make the inner mitochondrial membrane leaky to protons

These poisons, such as dinitrophenol, are known as uncoupling agents. By allowing protons to leak back across the membrane, the proton gradient is dissipated, i.e. the ETC and proton gradient buildup are uncoupled. Since no proton gradient is formed, no ATP can be made by oxidative phosphorylation. Rather, the energy derived from electron transport is released as heat.

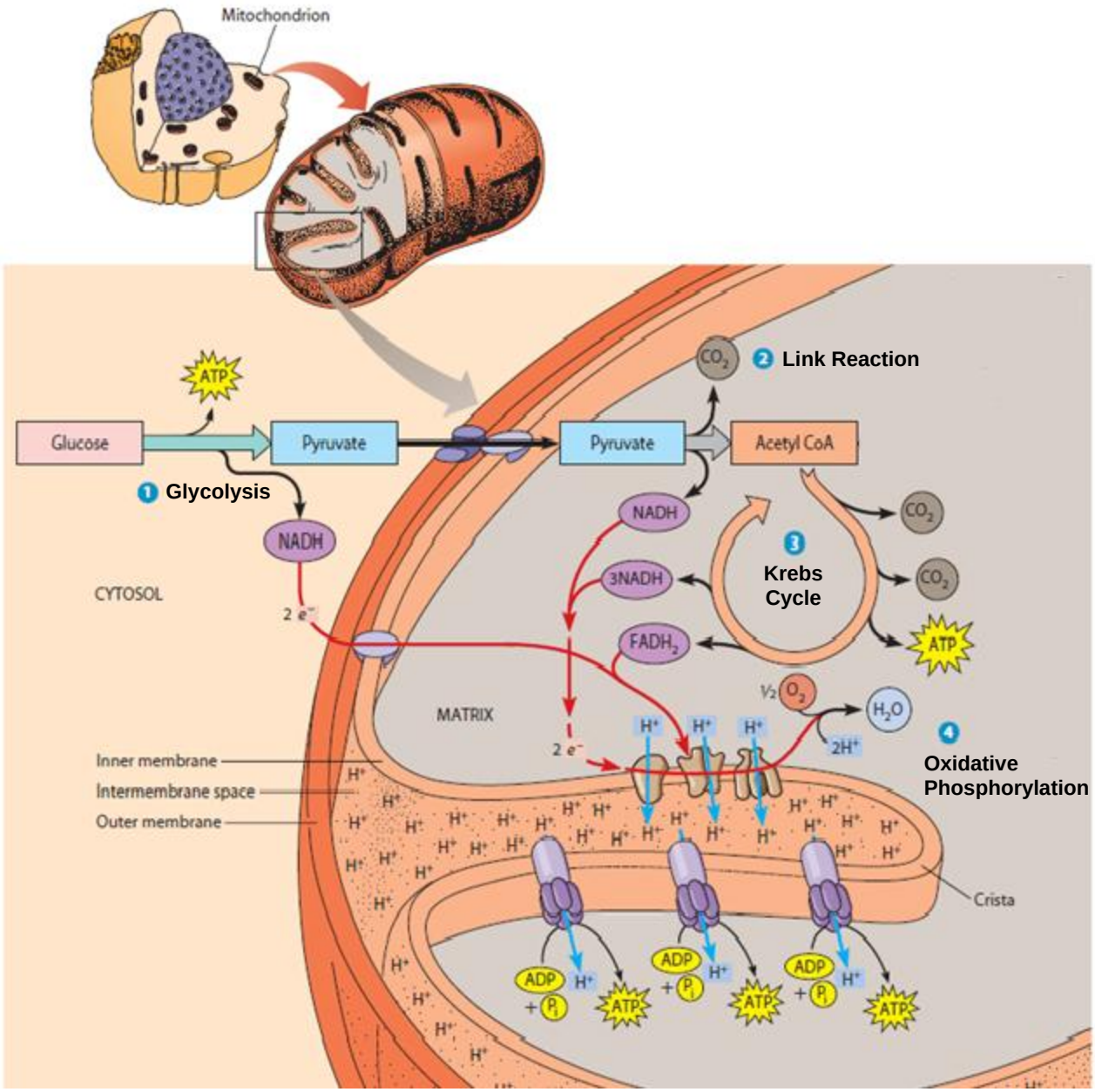


Fig. 2.4.6: Summary of aerobic respiration

CHECKPOINT 6

Distinguish between substrate level phosphorylation and oxidative phosphorylation.

Features	Substrate level phosphorylation	Oxidative phosphorylation
Definition		
Location		
Involvement of ETC		
Involvement of ATP Synthase		
Involvement of oxidation		
Number of ATP molecules formed per glucose molecule oxidized		

3 ANAEROBIC RESPIRATION

Learning Outcome (i):
Explain the production of a small yield of ATP from respiration in anaerobic conditions in yeast and in mammalian muscle tissue.

Learning Outcome (j):
Explain the significance of the formation of ethanol in yeast and lactate in mammals in the regeneration of NAD.

- In the absence of oxygen, **glycolysis** can still occur to generate ATP via **substrate level phosphorylation**, but the link reaction, Krebs cycle and oxidative phosphorylation are blocked.

- Oxidative phosphorylation is blocked as **oxygen,** the **final electron acceptor,** is **absent**. Therefore, electron carriers in the ETC will **remain reduced** after accepting electrons as they cannot pass electrons to the next carrier.

- This in turn prevents NADH and $FADH_2$ from being oxidised, depleting the supply of NAD^+ and FAD causing the link reaction and Krebs cycle to eventually cease.

- For glycolysis to continue under anaerobic conditions, a sufficient supply of NAD^+ must be available for accepting electrons during the oxidation step of glycolysis. Therefore, cells must undergo fermentation to **regenerate NAD^+**.

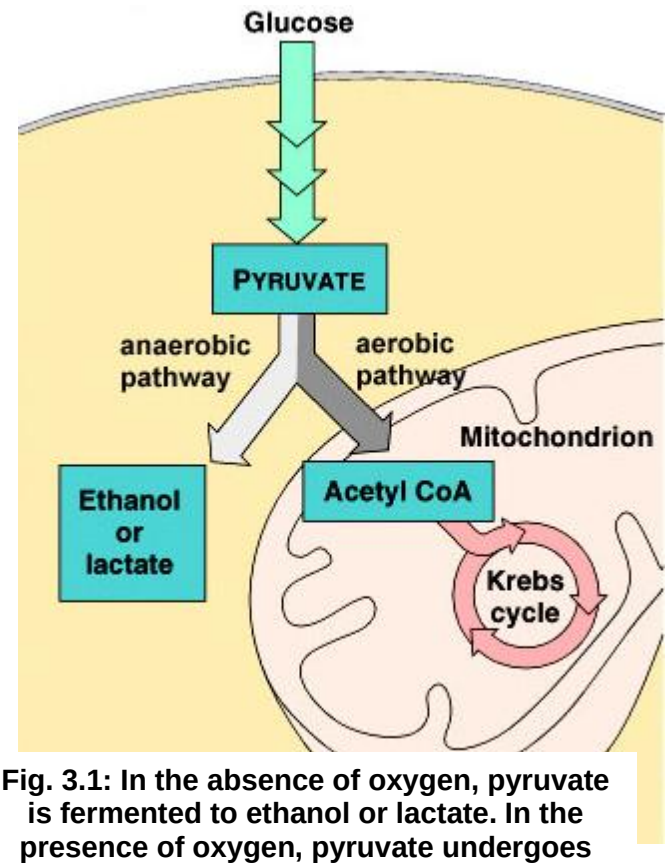


Fig. 3.1: In the absence of oxygen, pyruvate is fermented to ethanol or lactate. In the presence of oxygen, pyruvate undergoes the link reaction to form acetyl CoA.

- Two types of fermentation include: (i) **Alcoholic fermentation**, and (ii) **Lactate fermentation**. Both types of fermentation occur in the **cytosol / cytoplasm**.

Cytoplasm or Cytosol: What's the difference?
The **cytoplasm** encompasses everything within the cell membrane, excluding the nucleus, and includes the cytosol, organelles, and other structures. The **cytosol**, on the other hand, is the fluid portion of the cytoplasm, consisting of water, ions, proteins, and small molecules, excluding organelles. While the cytoplasm acts as the **site for cellular processes** and houses organelles, the cytosol serves as the **medium for metabolic reactions and molecular transport**.

- Both lactate fermentation and alcoholic fermentation **do not produce ATP**. They are mechanisms that **regenerate NAD^+** from NADH to **keep glycolysis going**.

- Note that **Anaerobic respiration = Glycolysis + Lactate fermentation OR Alcoholic fermentation**.

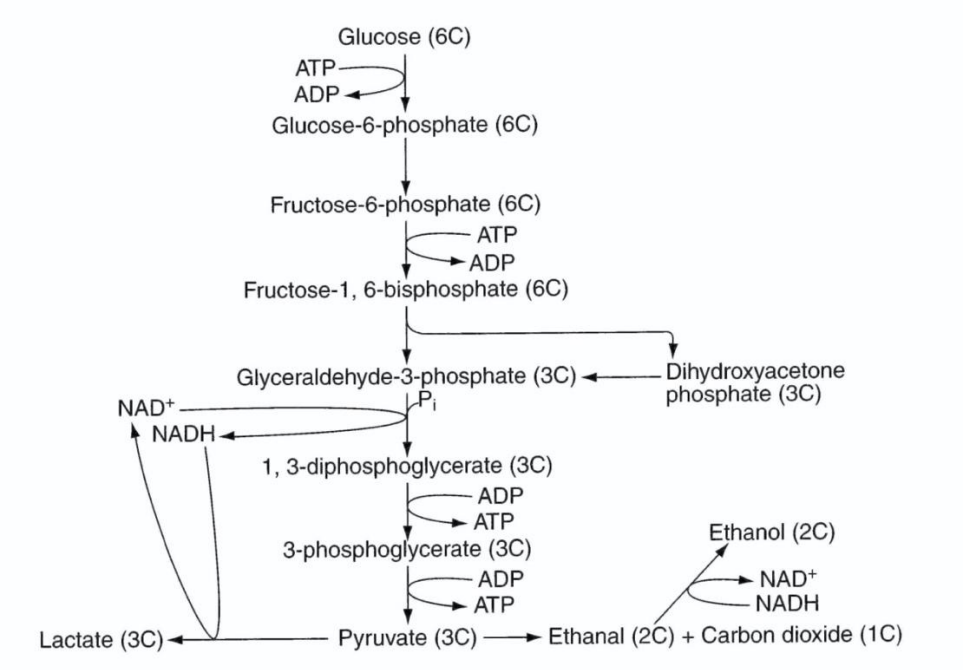


Fig. 3.2: Anaerobic respiration comprises Glycolysis coupled with lactate fermentation (left) or alcoholic fermentation (right)

3.1 ALCOHOLIC FERMENTATION

- Alcoholic fermentation occurs in yeasts and plants.

- Following glycolysis, the following steps occur:

  1) **Decarboxylation**
  Pyruvate (3C) is decarboxylated to **ethanal (acetaldehyde)** (2C), releasing carbon dioxide.

  2) **Reduction**
  Ethanal (acetaldehyde) (2C) is reduced to **ethanol** (2C), resulting in the **regeneration of NAD^+** from NADH.

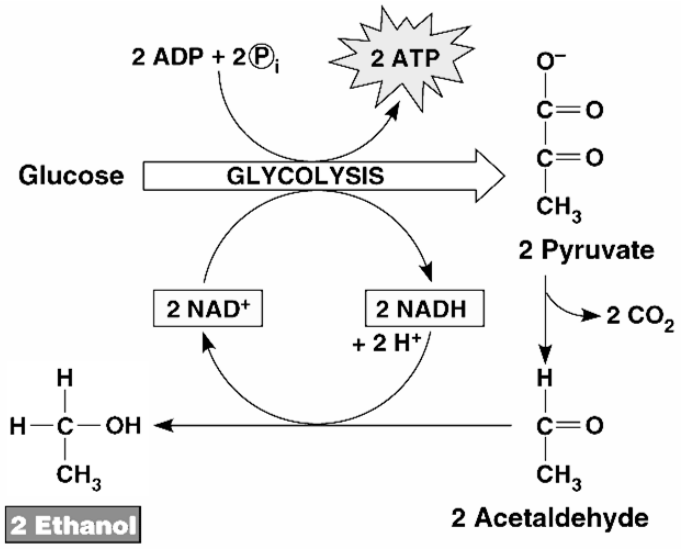


Fig. 3.1.1: Alcoholic fermentation

- When aerobic conditions resume, plants are unable to make use of ethanol as ethanol is volatile and evaporates easily to prevent toxicity to plants and yeast. Hence, a lot of energy remains locked in and lost through ethanol.

3.2 LACTATE FERMENTATION

- Lactate fermentation occurs in animals. It occurs in the muscles of animals during strenuous exercise in rapidly contracting skeletal muscles when oxygen is rapidly consumed, to generate ATP in the absence of oxygen.

- Following glycolysis, the following step occurs:

  1) **Reduction**
  Pyruvate (3C) is reduced to **lactate** (lactic acid) (3C), resulting in the **regeneration of NAD$^+$** from NADH.

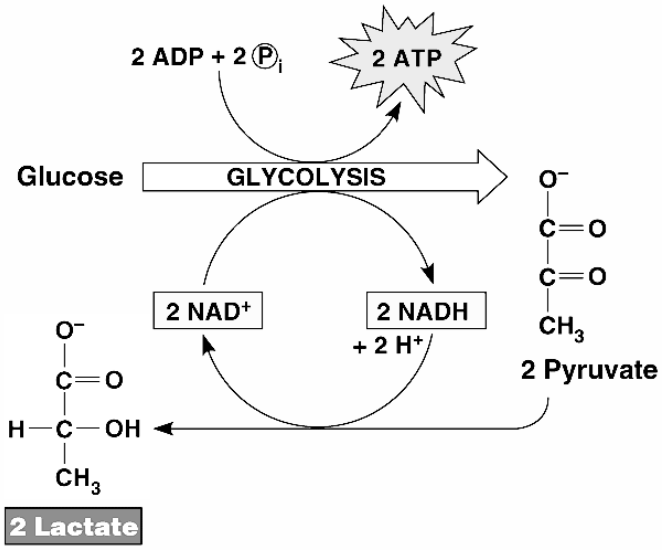


Fig. 3.2.1: Lactate fermentation

- Prolonged exercise results in lactate accumulation, lowering pH in the cellular environment, leading to muscle fatigue and cramps. When aerobic conditions resume, lactate is transported to the liver and reconverted to glucose. Glucose can then: (i) enter glycolysis, or (ii) be converted to glycogen and stored in the liver or muscle.

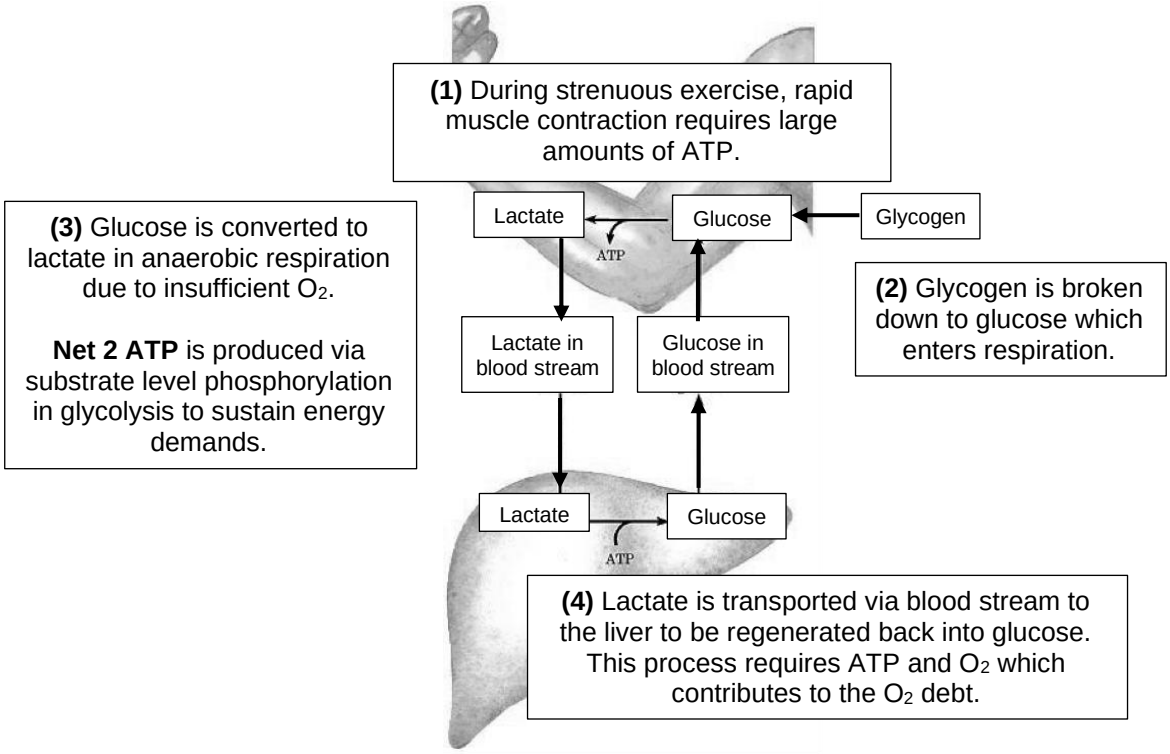


Fig. 3.2.2: Anaerobic respiration during strenuous exercise

CHECKPOINT 7

Complete the table to compare alcoholic fermentation and lactate fermentation.

Features	Alcoholic Fermentation	Lactate Fermentation
Reactions involved		
Products formed		
Final electron acceptor		
Occurs in		

4 RESPIRATORY QUOTIENT

Learning Outcome (k):
Investigate the effect of factors such as substrate concentration, type of substrate and temperature on the rate of respiration.

- The respiratory quotient (RQ) is the ratio of the amount of carbon dioxide produced to the amount of oxygen used, in a given time. **RQ is an indicator of the energy source** being used as different respiratory substrate results in different RQ.

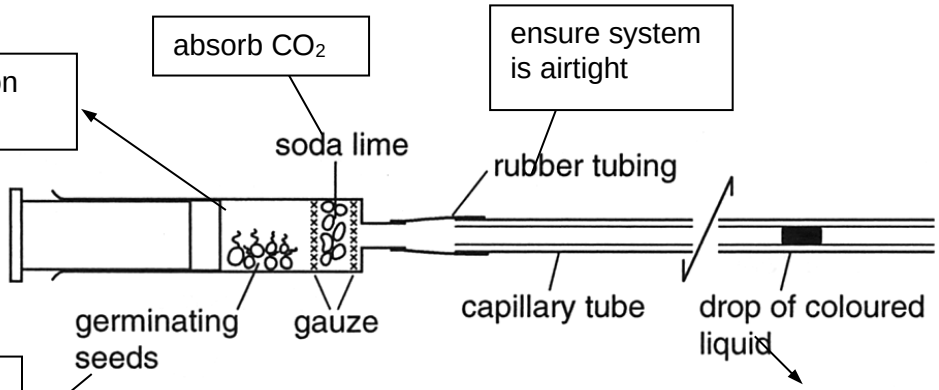
$$RQ = \frac{V_{CO_2}\ produced}{V_{O_2}\ consumed}$$

(Formula will be provided in assessment if calculation is required.)

Substrate	RQ
Carbohydrates	1.0
Oleic Acid (Fats)	0.7
Protein	0.9

- RQ may be measured using a **respirometer**, where two set-ups are used (one with soda lime and one without). A respirator may also incorporate a manometer.

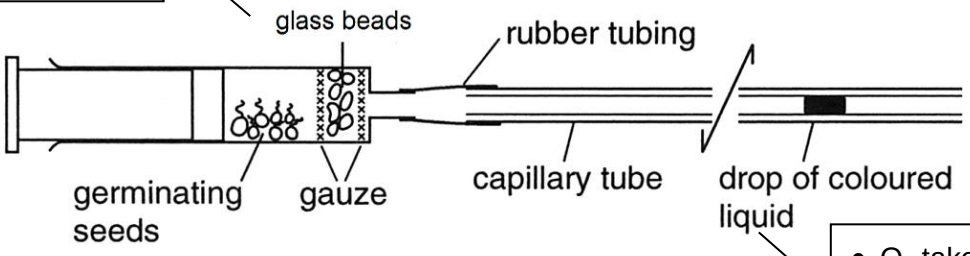
Respirometer (Set-Up with Soda Lime)



- O_2 taken in → pull droplet towards chamber.
- CO_2 given out → absorbed by soda lime.
- Droplet moves inwards.

Respirometer (Set-Up without Soda Lime)

To occupy same vol as soda lime, to keep air vol in chamber constant in both set ups



- O_2 taken in → pull droplet inwards
- CO_2 given out → push droplet outwards
- Net movement of droplet depends on vol of O_2 vs CO_2

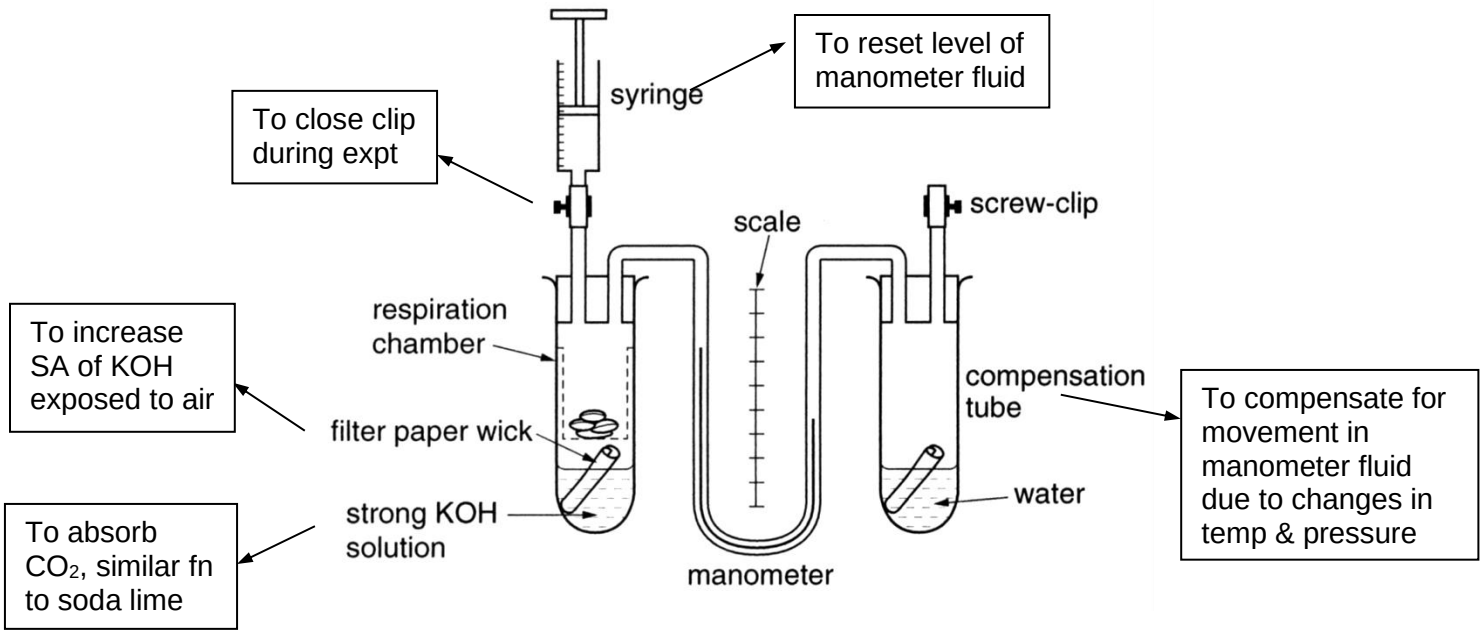
distance moved solely due to O_2

Condition	Distance travelled by droplet towards respiration chamber / mm	Volume / mm^3		RQ
		O_2	CO_2	
with soda lime	8	$8 \times \pi(0.2)^2 = 0.32\pi$	$5 \times \pi(0.2)^2 = 0.2\pi$	$\frac{0.2\pi}{0.32\pi} = 0.625$
without soda lime	3			

net distance moved due to:
(1) O_2 uptake pulling droplet in &
(2) CO_2 produced pushing droplet out

Note: Diameter of capillary tube is 0.4 mm.

Respirometer set-up using Manometer



The set up above will measure volume of oxygen taken in. A similar setup can be done by replacing strong KOH solution with equal volume of water to measure the volume of carbon dioxide given out.

5 FACTORS AFFECTING RATE OF RESPIRATION

- Each stage in aerobic respiration is catalysed by many enzymes, therefore **factors which influence rate of enzymatic reactions will also affect the rate of respiration**. Examples of key enzymes involved in respiration are provided in the table.

Stage of Aerobic Respiration	Key enzymes
Glycolysis	Hexokinase, Phosphofructokinase & Pyruvate kinase
Link reaction	Pyruvate dehydrogenase
Krebs Cycle	Various enzymes for each step
Oxidative Phosphorylation	Cytochrome oxidase & ATP synthase

5.1 TEMPERATURE

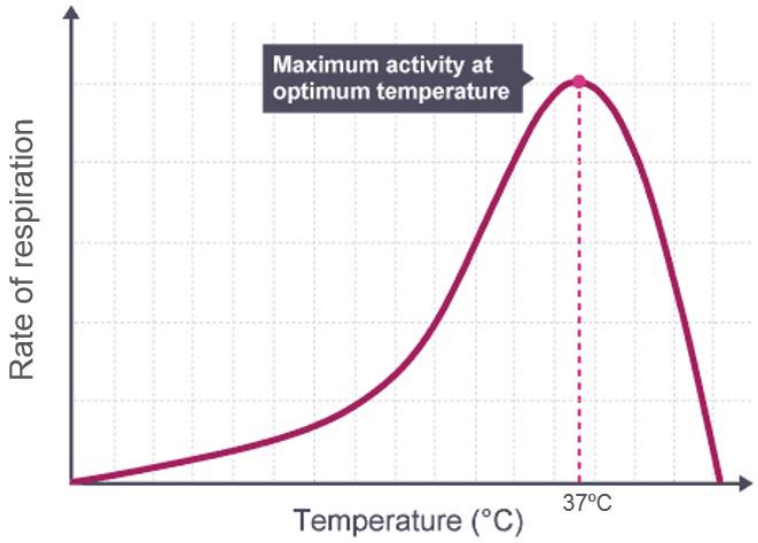


Fig. 5.1.1: Effect of temperature on rate of respiration

- At low temperatures, an increase in temperature results in an increase in rate of respiration.
  - An increase in temperature results in **increase in kinetic energy** of enzymes and substrates, resulting in an **increase in frequency of effective collisions** between enzyme and substrate molecules.
  - This results in an **increase in rate of ES complex formation**, and an **increase in rate of product formation**.

- At optimum temperature, there is maximum rate of respiration.
  - There is **maximum frequency of effective collisions** between enzyme and substrate molecules and **maximum rate of ES complex formation**. The **rate of product formation is at maximum**, resulting in maximum rate of respiration.

- At temperatures beyond optimum temperature, the rate of respiration decreases.
  - **Thermal agitation** of enzyme molecules **disrupts weaker hydrophobic interactions and hydrogen bonds**. This results in **loss of specific 3-dimensional conformation of enzyme**.
  - Enzymes are **denatured**, where the **active site of the enzyme is no longer complementary to substrate**. Hence the **rate of formation of ES complexes decreases**, resulting in a decrease in rate of respiration.

5.2 SUBSTRATE CONCENTRATION

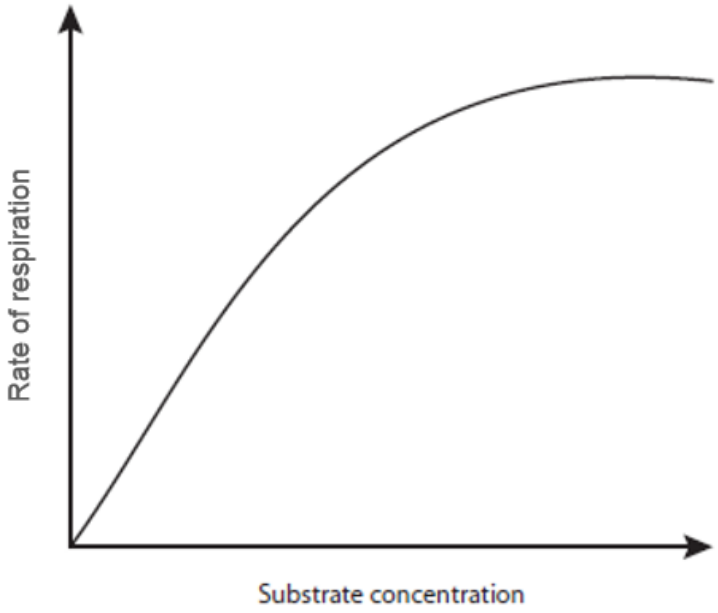


Fig. 5.2.1: Effect of substrate concentration on rate of respiration

- At low substrate concentration, an increase in substrate concentration results in an increase in rate of respiration.
  - An increase in substrate concentration **increases the frequency of effective collisions** between enzyme and substrate molecules, leading to an **increase in rate of ES complex formation**. This results in an **increase in the rate of formation of products**, hence increasing the rate of respiration.
  - At this point, substrate concentration acts as the limiting factor.

- At higher substrate concentration, a further increase in substrate concentration does not further increase the rate of reaction.
  - There is **maximum frequency of effective collisions** between enzyme and substrate molecules, resulting in **maximum rate of ES complex formation per unit time**. **Rate of formation of products is at its maximum**, resulting in maximum rate of respiration.
  - **Enzyme active sites are saturated**, where all enzyme active sites are being occupied by the substrate at any one time.
  - At this point, other factors (e.g. enzyme concentration) act as the limiting factor.

5.3 TYPE OF SUBSTRATE

- **Glucose** is the main respiratory substrate. The rate of respiration differs when other respiratory substrates are used, such as other types of carbohydrates, lipids and proteins.

- The rate of respiration when using other types of carbohydrates (e.g. sucrose, lactose) is lower as compared to glucose. The rate of respiration may differ due to a variety of factors including:

(a) Additional steps for conversion into glucose

- Other forms of carbohydrates will first need to be broken down or converted into glucose to enter glycolysis.

- For example, lactose is broken down by lactase to galactose and glucose, before glucose can enter glycolysis to be broken down.

(b) Uptake of carbohydrate

- The rate of carbohydrate uptake into the cells by the respective transporters will affect the rate at which the carbohydrate can be utilised.

- The rate of uptake is dependent on the number of transporters.

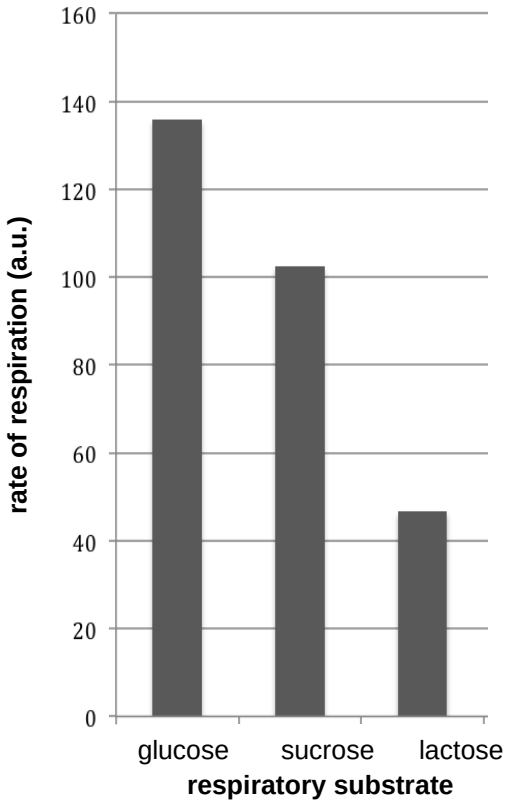


Fig. 5.3.1: Effect of respiratory substrate on rate of respiration

- The use of proteins or lipids as respiratory substrates will also result in a lower rate of respiration as compared to glucose. Proteins and lipids require several additional steps before they can be converted into suitable forms to enter various stages of respiration.

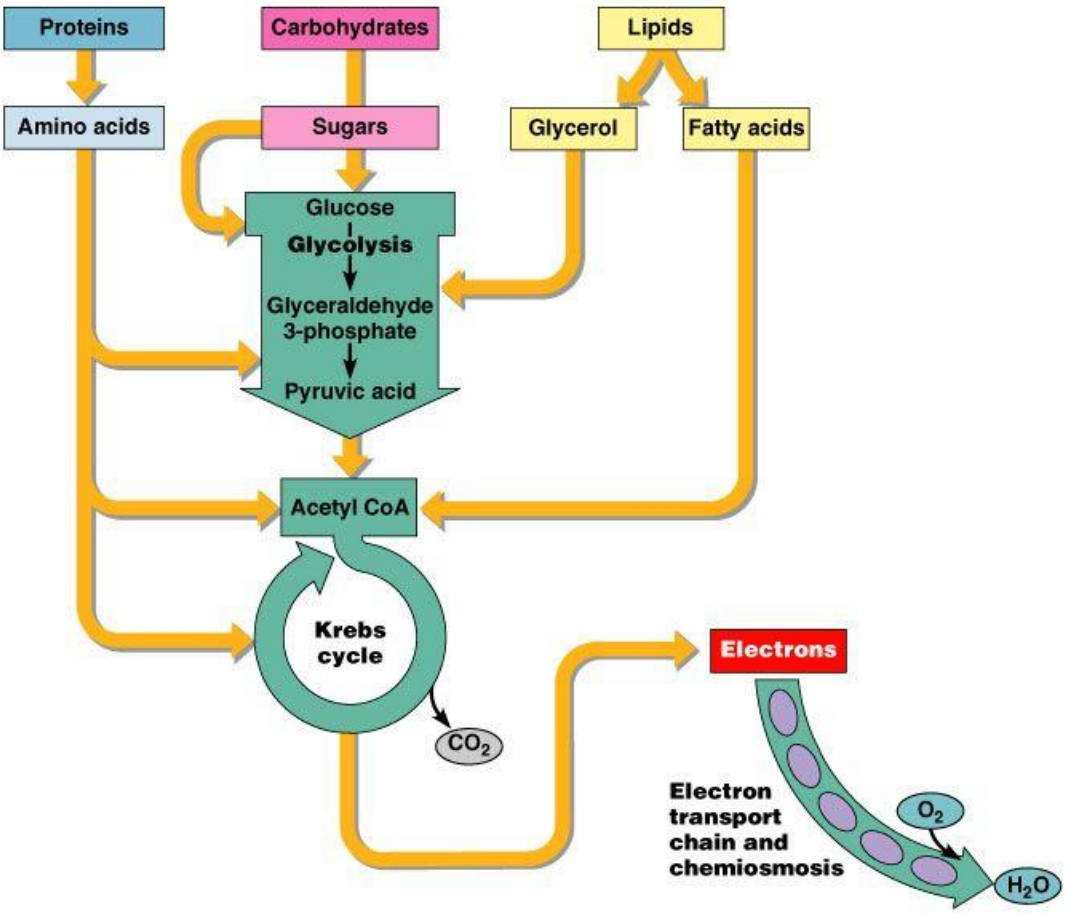


Fig. 5.3.2: Metabolic pathways of protein, carbohydrate and lipid catabolism