Respiration

1. [Tutorial 9286/08/2/7a] Outline the main stages of glycolysis. [8]

Glycolysis occurs in the cytoplasm and does not require the presence of oxygen. It involves the oxidation / breakdown of glucose to yield two molecules of pyruvate;
Glycolysis can be divided into two phases: energy investment phase and energy pay-off phase;
During the energy investment phase, energy in the form of 2 ATP is used per glucose molecule;
Activation of glucose occurs to make it more chemically reactive with the phosphorylation of glucose using ATP to produce glucose-6-phosphate. This reaction is catalysed by hexokinase;
Glucose-6-phosphate is then isomerised to fructose-6-phosphate by isomerase;
Phosphorylation of fructose-6-phosphate using ATP produces fructose-1,6-bisphosphate. This reaction is catalysed by phosphofructokinase;
Cleavage / Splitting of fructose-1,6-bisphosphate produces 2 molecules of glyceraldehyde-3-phosphate (GALP);
During the energy payoff phase, energy in the form of 4 ATP is produced via substrate level phosphorylation;
Each GALP is subsequently converted to pyruvate via multiple steps where 2 ATP are generated via substrate level phosphorylation and protons and electrons released via dehydrogenation are transferred to 1 oxidised NAD to form 1 reduced NAD;
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;

Or Breakdown down 1 molecule of glucose to 2 pyruvate, 2 reduced NAD, with net gain of 2 ATP;

[AJC 2013] Explain the significance of the steps in glycolysis. [5]

- Glycolysis is a common step in both anaerobic and aerobic respiration;
- Phosphorylation of glucose (by 2 ATP) is to activate it;
- Phosphorylation of glucose (by 2 ATP) / glucose-6-phosphate results in glucose being trapped in the cytosol/ unable to leave the cell through the same glucose carrier protein/ committed to the end of glycolysis;
- PFK which catalyse the phosphorylation of fructose phosphate also control rate of glycolysis/ high rate of ATP act as allosteric inhibitor to PFK;
- ATP synthesis by substrate level phosphorylation;
- Forms two glyceraldehyde-3-phosphate/ triose phosphate from one glucose;
- Forms two reduced NAD (NADH) (by dehydrogenation);
- Which later give 6 ATP by oxidative phosphorylation;
- Pyruvate can enter into link reaction/ mitochondria or be converted to lactate (in mammals) or ethanol and carbon dioxide (in yeast);
- Pyruvate is small enough to enter mitochondrion for aerobic respiration;

[ACJC 2010 H1 P2] Describe the role of glycolysis in respiration. [7]

1. **glycolysis - oxidation of glucose to pyruvate;**
2. **phosphorylation of glucose;**
3. **add (2) phosphate groups to glucose;**
4. **from (2) molecules of) ATP;**

5. **to produce (1 molecule of) fructose-1,6-bisphosphate;**
6. **to raise the energy level of glucose;**
7. **so that useful energy can be harvested in later steps in the pathway;**
8. **oxidation / conversion of (2 molecules of) glyceraldehydes;**
9. **into (2 molecules of) pyruvate;**
10. **removal of H from glyceraldehyde;**
11. **to hydrogen carriers NAD^+;**
12. **produce (2 molecules of) reduced NAD / NADH + H^+;**
13. **form (4 molecules of) ATP;**
14. **by substrate-level phosphorylation;**
15. **using organic phosphates;**
16. **reduced NAD allows for ATP production in the mitochondria;**
17. **by oxidative phosphorylation;**
18. **pyruvate to enter mitochondria for further oxidation;**
19. **or converted to lactate / ethanol in anaerobic respiration;**
20. **to regenerate NAD^+ to allow glycolysis to continue;**

;@1/2m, max 7

2. Outline the link reaction. [2]

Takes place in mitochondrial matrix;

1) Decarboxylation

- Carboxyl group of **pyruvate** (3C) is removed and **carbon dioxide (CO_2) is releas**

2) **Oxidation** via dehydrogenation

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

3) Addition of Coenzyme A (CoA)

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

Substrate to product (1M)

By-products & processes(1M)

3. [Tutorial 02/7a] Outline the main features of the Krebs cycle. [8]
[BT 2016] Outline the main stages of the Krebs cycle. [8]

Krebs cycle occurs in mitochondrial matrix;

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

At 2 stages in the Krebs cycle, carbon is removed from the intermediate compounds via oxidative decarboxylation, forming intermediate 5C and 4C compounds respectively. 2 molecules of carbon dioxide are produced per cycle and carbon dioxide diffuses out of the mitochondrion, and out of the cell;

1 molecule of ATP is produced per cycle via substrate level phosphorylation where the phosphate group was derived from Guanosine triphosphate (GTP);

Intermediate compounds undergo oxidation via dehydrogenation whereby protons and electrons are transferred to oxidised NAD (nicotinamide adenine dinucleotide) and oxidised FAD (flavin adenine dinucleotide) and reduced to reduced NAD and reduced FAD respectively;

These reduced coenzymes subsequently transfer these <u>high energy protons and electrons</u> to the <u>electron transport chain</u> for the <u>synthesis of ATP</u>;

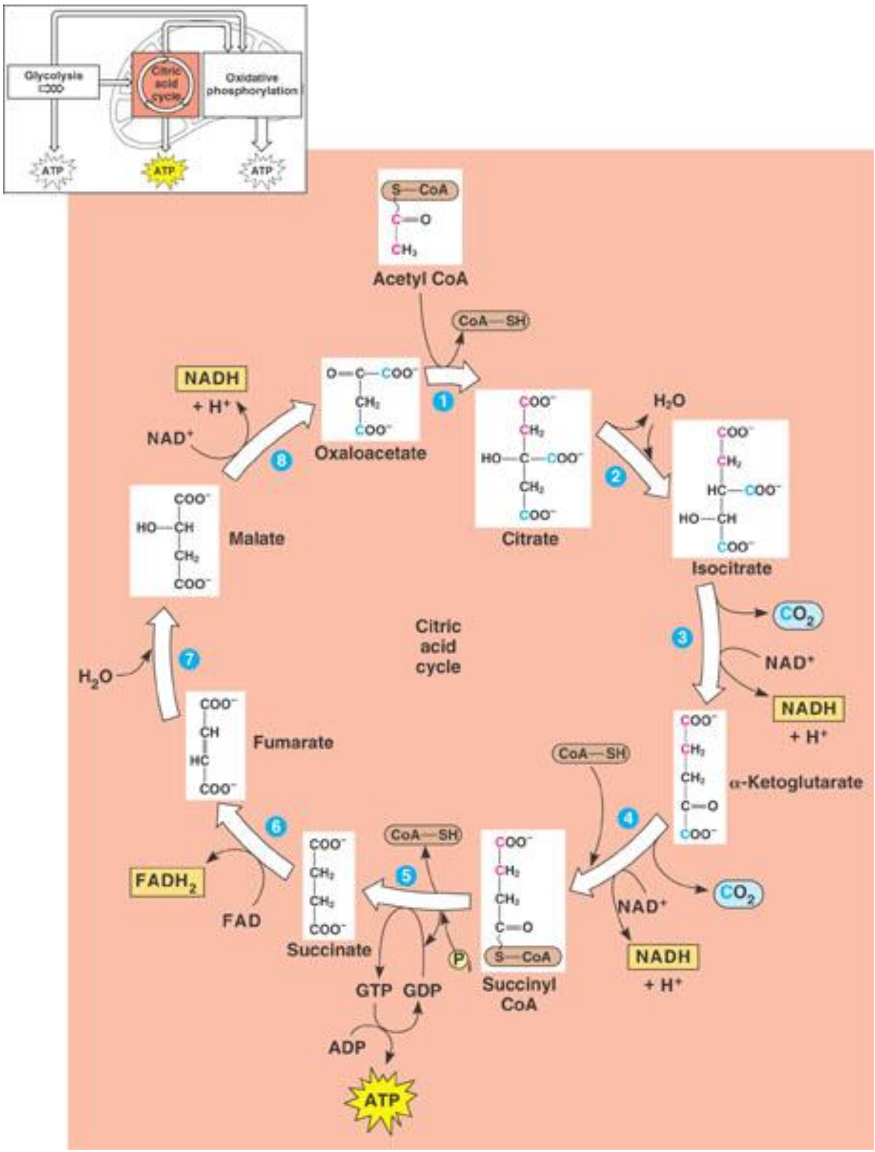
Since 2 molecules of acetyl CoA are formed per glucose molecule during the link reaction, the <u>Krebs cycle runs twice</u> to completely utilise them;

Therefore for each glucose molecule, the products of Krebs cycle are <u>4 molecules of CO_2, 6 molecules of reduced NAD, 2 molecules of reduced FAD and 2 molecules of ATP</u>;

Examiner's comments:

The term 'inorganic phosphate' is only applicable for Oxidative phosphorylation.

Substrate- level phosphorylation involves an enzyme transfers a phosphate group from a substrate molecule to ADP, rather than adding an inorganic phosphate (free lying ions) to ADP as in oxidative phosphorylation.



4. [BT 2016] Describe the role of NAD and FAD in cellular respiration. [6]

NAD and FAD are <u>co-enzymes</u> which function as <u>protons</u> and <u>electrons</u> carrier;

® acceptor

....

Oxidized NAD reduced to reduced NAD formed during glycolysis (cytosol), link reaction and Krebs cycle (both in mitochondria matrix) while oxidized FAD reduced to reduced FAD during Krebs cycle only;

During the oxidation of respiratory substrate via dehydrogenation reactions, protons and electrons are released and transferred to oxidized FAD to form reduced FAD.; (Learning point)

Reduced NAD and FAD transfer high energy protons and electrons to electron carriers on electron transport chain embedded in the inner mitochondrial membrane, where oxidative phosphorylation occurs;

Leading to the regeneration of oxidised NAD and FAD to allow glycolysis, link reaction and Krebs cycle to continue;

As the donated electrons travel down a series of electron carriers that are progressively electronegative, the energy released is coupled to the pumping to these H+ ions from the mitochondrial matrix into the intermembrane space;

Leading to a build-up of a proton-motive force/ electrochemical proton gradient/ H+ ion gradient across the inner mitochondrial membrane which is essential for the formation of ATP from ADP + Pi via chemiosmosis using ATP synthase;

[TPJC 2010] Outline the role of NAD in aerobic respiration. [6]

1. NAD acts as co-enzymes of the NADH dehyrogenase
2. removes H atoms from their active sites
3. NAD acts as electron acceptors at glycolysis, link reaction and Krebs cycle, receiving H+ and electrons from the organic intermediates via dehydrogenases
4. they act as carriers of H+ and electrons to the ETC
5. NADH is then oxidized at the ETC when it transfers H+ and electrons to the electron carriers of the ETC to produce ATP at the inner membrane of the mitochondria
6. helps to generate proton gradient for the production of ATP
7. NAD is regenerated for continual reactions to take place

[RI 2010] Outline the role of NAD in aerobic and anaerobic respiration. [6]

Aerobic Respiration

1. NAD^+ is a coenzyme involved in redox reactions and functions as a mobile electron (and proton) carrier to carry high energy electrons and H^+ from organic molecules to the electron transport chain on the cristae of mitochondria;
2. Organic molecules are oxidized during glycolysis, link reaction and Krebs cycle and the electrons and H^+ from the oxidation process are transferred to NAD^+ to form NADH;
3. The electrons in NADH are used to reduce the electron acceptors of the electron transport chain, while NADH itself gets re-oxidised;
4. As electrons pass down the chain, the release of energy in a series of redox reactions is coupled to the phosphorylation of ADP to form ATP;
5. The protons liberated in the oxidation of NADH is used to establish the proton motive force necessary for ATP synthesis;
6. 1 NADH yields 3 ATP;
7. Reoxidation of NADH allows the regeneration of the coenzyme NAD^+, allowing it to pick up more protons and electrons from the Krebs cycle, link reaction and glycolysis so that these reactions can continue; max 5

Anaerobic Respiration
8. NAD regenerated when electrons from NADH transferred to pyruvate/ ethanal;
9.NAD reused in glycolysis to generate 2 ATP; max 1

[TPJC 2010 H1 P2] Outline the main features of glycolysis and Krebs cycle which lead to the production of NAD. [10]

1.phosphate groups added to glucose with the **use of 2ATP**
2. subsequent steps results the conversion (of PGAL) to **pyruvate**
3. with the **release of 4ATP/ net of 2 ATP**
4. **2 NADH produced for every glucose that enters glycolysis**
5. Dehydrogenase removes H atoms from organic intermediates (PGAL) → forms NADH
6. acetyl co-A enters Krebs cycle and combines with oxaloacetate to form **citrate**
7. citrate/isocitrate is dehydrogenated/oxidised to form **alpha-ketoglutarate** → **NAD is reduced**
8. alpha-ketoglutarate is dehydrogenated/oxidised to form succinyl co-A → **NAD is reduced**
9. succinyl co-A is subsequently **dehydrogenated and decarboxylated to form malate**
10. **Malate is dehydrogenated/oxidized to oxaloacetate → NAD is reduced**
11. (given**) 3 NADH produced per cycle** of Krebs cycle (if points 7,8 and 10 not given);
12. requires dehydrogenase to remove H atoms from intermediates of Krebs cycle;

5. 9286/08/2/7b Describe oxidative phosphorylation. [6] (refer to next qns ans)

[Tutorial] Describe oxidative phosphorylation and distinguish it from oxidative decarboxylation [6]

Describe oxidative phosphorylation

- Oxidative phosphorylation is the mechanism of ATP synthesis which occurs only in the presence of oxygen and takes place in the inner mitochondrial membrane;
- Reduced NAD and reduced FAD transfer high energy electrons and protons to the electron transport chain (ETC) for the 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;
- Energy released from the flow of electrons is used to actively pump protons from matrix of the mitochondrion into the intermembrane space, therefore producing a high concentration of H^+ in the intermembrane space, setting up a steep electrochemical proton gradient and generating the proton motive force;
- H^+ diffuse through stalked particles containing ATP synthases embedded in the inner mitochondrial membrane, 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).
- Oxygen functions as the final proton and electron acceptor to form water, catalysed by cytochrome oxidase;
- Oxidised NAD and oxidised FAD are regenerated at the end of oxidative phosphorylation;

not needed in ans

Amount of ATP synthesized per glucose molecule:

Stage	No. of reduced NAD / reduced FAD	No. of ATP by O.P	No. of ATP by S.L.P	Total no. of ATP
Glycolysis	2 reduced NAD	2 x 2.5 = 5	Net 2	**7**
Link reaction	2 reduced NAD	2 x 2.5 = 5	0	**5**
Krebs cycle	6 reduced NAD & 2 reduced FAD	6 x 2.5 = 15 2 x 1.5 = 3	2	**22**
Total no. of ATP		**28**	**4**	**32**

Note: In reality, about 2.5 ATP is synthesized for every reduced NAD and 1.5 ATP is synthesized for every reduced FAD.

Distinguish from oxidative decarboxylation

Features	Oxidative Decarboxylation	Oxidative Phosphorylation
Location	In matrix of mitochondrion (Link reaction and Krebs cycle)	Carried out by electron transport chain and stalked particles found embedded in the inner mitochondrial membrane
Reactions	Involves single reactions e.g. from pyruvate to acetyl CoA, where the carboxyl group is removed as a molecules of CO_2 and pyruvate is oxidized.	Involves redox reactions where the electron carriers alternate between reduced and oxidised states as they accept and donate electrons OR Involves redox reactions, along a series of electron carriers, each with an energy level lower than the one preceding it.
Enzymes	Catalysed by decarboxylases and dehydrogenases, e.g. pyruvate dehydrogenase removes CO_2 from pyruvate to form acetyl CoA Point for teaching: To be more specific, steps involving oxidative decarboxylation are catalyzed by dehydrogenases but reactions with decarboxylation alone are carried out by decarboxylases	Catalysed by ATP synthase and cytochrome oxidase
Involvement of electron transport chain	No	Yes. Involves redox reactions, along a series of electron carriers, each with an energy level lower than the one preceding it, until the electron is finally accepted by oxygen, resulting in the formation of water.

Involvement of decarboxylation	Yes. Removal of carbon from substrates and carbon dioxide is released e.g. Pyruvate forming acetyl coenzyme A (acetyl CoA) during link reaction. CO_2 is exhaled from the lungs eventually.	No
Involvement of oxidation	Substrate undergoes oxidation via dehydrogenation when protons and electrons are transferred to oxidised NAD and oxidised FAD	Reduced NAD and reduced FAD undergoes oxidation when they transfer protons and electrons to the electron transport chain for synthesis of ATP.
Production of ATP	No	Yes

6. List the differences between substrate-level phosphorylation and oxidative phosphorylation.

Substrate-level phosphorylation VS oxidative phosphorylation

Features	Substrate-level Phosphorylation	Oxidative Phosphorylation
Definition	Synthesis of ATP involves an enzyme transferring a high-energy phosphate group from a phosphorylated substrate to ADP.	ATP synthesis occurs as electrons are transferred from reduced NAD or reduced FAD to O_2 by an electron transport chain. The energy coupling occurs through a electrochemical H^+ gradient, whereby the potential energy of the electrochemical proton gradient is used for the synthesis of ATP from ADP and Pi, catalysed by ATP synthase.
Location	In the cytoplasm (Glycolysis) and matrix of mitochondrion (Krebs cycle).	On the inner membrane of mitochondrion, where stalked particles are found.
Reactions	Phosphate group from a substrate molecule is transferred to ADP to form ATP and this process is enzyme-catalysed. For example in glycolysis, from phosphoenolpyruvate to pyruvate. For example in Krebs cycle, from Succinyl CoA to Succinate.	ATP synthase found in stalk particle catalyzes formation of ATP from ADP & Pi, using energy from flow of H^+.
Involvement of electron transport chain	No.	Yes. Involves redox reactions, along a series of electron carriers, each with an energy level lower than the one preceding it, until the electron is finally accepted by oxygen, resulting in the formation of water.
Involvement of oxidation	No.	Yes, Reduced NAD & reduced FAD are oxidized to NAD^+ & FAD respectively by transferring electrons to electron transport chain.
No. of ATP formed per glucose	4 (Net 2)	28

7. [BT 2016] Explain how ATP is produced in anaerobic respiration. [6]

Glycolysis can still occur in the absence of oxygen in cytoplasm;;

Oxidation/ breakdown of glucose (6C) to form two molecules of pyruvate (3C), reduced nicotinamide adenine dinucleotide (reduced NAD) / NADH + H^+ and ATP;

Glycolysis can be divided into 2 phases – energy investment phase (2 ATP used per glucose mol to convert glucose into two mol of GALP) and energy pay-off phase (4 ATP produced). During energy pay-off phase, 2ATP is produced in subsequent conversion of each GALP to pyruvate;

Only net of 2 molecules of ATP is synthesised from one molecule of glucose via substrate level phosphorylation in glycolysis;

Alcoholic or lactate fermentation occurs to regenerate oxidised NAD for glycolysis to continue;

Elaboration of either type of fermentation in detail (type of reaction + enzymes) [max 1]

Oxidative phosphorylation cannot take place in the absence of oxygen, therefore oxygen cannot act as the final electron and hydrogen acceptor at the electron transport chain. There is no regeneration of oxidized NAD and FAD at the electron transport chain (since there is no flow of electrons along ETC);

Examiner's comments:

When accounting for the number of products formed during the reaction, please state clearly with reference to the number of starting glucose molecule.

Do not use short forms like ETC, OP. These are not proper/official short forms.

Anaerobic respiration is NOT fermentation. Fermentation DO NOT result in production of ATP. (Then why need fermentation?) Please revise this again.

8. [Tutorial] Explain the small yield of ATP from anaerobic respiration in both yeast and mammals [8]

1m

Anaerobic respiration comprises glycolysis and fermentation. Glycolysis can still occur in the absence of oxygen in cytoplasm.

Only 2 molecules of ATP is synthesised from one molecule of glucose via substrate level phosphorylation in glycolysis;

No oxygen to act as the final proton and electron acceptor at the end of the electron transport chain, therefore oxidative phosphorylation cannot occur;

No ATP synthesised through oxidative phosphorylation;

No flow of electrons along ETC resulting in no regeneration of oxidised NAD and oxidised FAD, therefore link reaction and Krebs cycle cannot take place;

Alcoholic or lactate fermentation occurs to regenerate oxidised NAD for glycolysis to continue;

During alcoholic fermentation which occurs in plants and yeast, pyruvate is first converted into ethanal / acetaldehyde which is then reduced to ethanol and carbon dioxide;
OR

During alcoholic fermentation which occurs in plants and yeast, pyruvate is first converted to ethanal with the release of CO_2 (catalyzed by decarboxylase).

Ethanal is then converted to ethanol, whereby reduced NAD is oxidised back to oxidised NAD^+ (catalyzed by alcoholic dehydrogenase);

During lactate fermentation in mammals, pyruvate is reduced directly into lactate; whereby reduced NAD is oxidised back to oxidised NAD^+ (catalyzed by lactate dehydrogenase);

Glucose is incompletely oxidised / broken down, therefore a lot of energy is still trapped in lactate and ethanol.

Lactate is then reconverted to pyruvate in the liver when the oxygen supply is restored;

9286/08/2/7c Explain what happens in an animal cell when there is insufficient oxygen for aerobic respiration. [6]

[PJC 2010 H1 P2] Explain the continual production of a small yield of ATP from anaerobic respiration in yeast. [6]

1. **In anaerobic respiration, there is no oxygen to act as the final electron acceptor in ETC, thus ETC is blocked;**

2. **only glycolysis can take place to break down one glucose molecule into 2 pyruvate molecules;**

3. **in cytosol;**
4. **glycolysis process requires 2NAD⁺ as electron and proton acceptor → NADH**
5. **With production of a total of 4 ATPs by substrate level phosphorylation;**
6. **But 2 ATPs are used up to phosphorylate glucose in the initial stages of glycolysis;**
7. **So there is only a net gain of <u>2 ATPs per glucose molecule</u> during glycolysis;**
8. **fermentation then takes place where pyruvate is first decarboxylated (removal of CO_2) to acetaldehyde / ethanal;**
9. **process is catalysed by enzyme <u>pyruvate decarboxylase</u>;**
10. **NADH then donates its H atoms and reduces acetaldehyde to ethanol;**
11. **process is catalysed by the enzyme <u>alcohol dehydrogenase</u>;**
12. **NAD^+ is regenerated;**
13. **Which is needed for the <u>continuation of glycolysis</u>;**
14. **<u>No ATP</u> from fermentation process;**
15. **a lot of energy is still locked in the ethanol;**
16. **only 2 ATP per glucose molecule**

Photosynthesis

9. [12/2/9a] Outline the light dependent reactions of photosynthesis. [7]

Tutorial 11/2/8c Outline the main features of photo-phosphorylation. [8]

Light-dependent reactions / photophosphorylation occurs in the grana of the chloroplast. The objective of these reactions is to provide ATP and reduced nicotinamide adenine dinucleotide phosphate (reduced NADP / NADPH + H+) for the light-independent reactions (Calvin cycle).
As the energy for the synthesis of ATP comes from light, it is called photophosphorylation. There are 2 types of photophosphorylation – cyclic and non-cyclic.
Photosystems, which are embedded in thylakoid membranes, comprise of accessory pigments (chlorophyll a and b, and carotenoids) in light harvesting complex and primary pigments (special chlorophyll a) and primary electron acceptors in the reaction centres.
In non-cyclic photophosphorylation, light of particular wavelengths strikes an accessory pigment molecule in a light harvesting complex of PSII and PSI and this energy is relayed to neighbouring accessory pigment molecules, until it accumulates and reaches one of the two P680 chlorophyll a molecules in the reaction centres of PSII. The same occurs for the P700 chlorophyll a molecules in the reaction centre of PSI.
This excites one of the P680 or P700 electrons to a higher energy state, which subsequently gets emitted and captured by the primary electron acceptor within each PS, leaving behind a positive 'hole' in the chlorophyll a molecules.
Photolysis of water occurs when an enzyme catalyses the splitting of a water molecule into protons, electrons and molecular oxygen. The electrons are used to fill up the positive 'holes' in the reaction centre of PSII to return $P680^+$ to ground state.

The photoexcited electron passes from primary electron acceptor of PSII to P700$^+$ in PSI to fill the positive 'hole' in P700$^+$. This occurs via an electron transport chain made of electron carriers, each with an energy level lower than the one preceding it.

Energy from the electron transfer down the chain of electron carriers is used to actively pump protons from the stroma into the thylakoid space, generating an electrochemical proton gradient for the synthesis of ATP, as the protons diffuse through stalked particles containing ATP synthase, back into the stroma.

Electrons and protons are passed down a second electron transport chain from the primary electron acceptor of PSI to the protein ferredoxin (the last electron carrier).

The enzyme NADP reductase catalyses the transfer of electrons from ferredoxin to oxidised NADP (the final electron and proton acceptor) to form reduced NADP.

In cyclic photophosphorylation, PSI is now both a donor and acceptor of electrons. The excited electrons in the primary electron acceptor of PSI pass to ferredoxin and back to the cytochrome complex in the electron transport chain. The electrons eventually return to the PSI reaction centre.

The energy released during the cycle of electrons down the chain of electron carriers allows protons to be actively pumped from the stroma into the thylakoid space, generating an electrochemical proton gradient across the thylakoid membrane, just like in non-cyclic photophosphorylation.

10. Compare non-cyclic and cyclic photophosphorylation. [6]

Features	Non-cyclic	Cyclic
~~Role of Process~~	(Both processes happen simultaneously. So not a valid point of comparison)	
Pathway of e-	Linear	Cyclic
PS involved	PSI and PSII	Only PSI
1st e^- donor	Water	PSI
Final e- acceptor	$NADP^+$	PSI
Establishing of H^+ gradient for ATP synthesis	1. Photolysis of water in the thylakoid space to produce electrons and protons. 2. Active transport of H^+ ions from the stroma, across the thylakoid membrane, into the thylakoid space (energy from movement of electrons down the ETC)	Only via active transport of H^+ ions from the stroma, across the thylakoid membrane, into the thylakoid space (energy from movement of electrons down the ETC)
Products	ATP, reduced NADP, O_2	Only ATP
Conditions under which process occurs	when plants require ATP & NADPH	When plants require ATP only

Photophosphorylation VS oxidative phosphorylation

11. Compare photophosphorylation with oxidative phosphorylation. [6]

Similarities

- Both involve the transport of high energy electron along the electron transport chain from one electron carrier to another, each with an energy level lower than the one preceding it.
- Energy released from electron transport is used to create electrochemical proton gradient, by actively pumping protons from stroma into thylakoid space;
- Potential energy of electrochemical proton gradient used for synthesis of ATP from ADP & P_i, Protons diffuse down the gradient through the stalked particle containing ATP synthase, which catalyses the phosphorylation of ADP to ATP.

Features	Photophosphorylation	Oxidative Phosphorylation
Energy conversion	Light energy to chemical energy (reduced NADP and ATP)	Chemical energy (food) to chemical energy (ATP)
Location	Thylakoid membrane of chloroplast	Inner membrane of mitochondria
Involvement of light energy	Light energy required for photolysis of water and photoactivation of primary pigment molecule in the reaction center of photosystem, where electron of the P680/P700 chlorophyll a molecule gets excited to a higher energy level and gets emitted and then captured by primary electron acceptor.	Light energy is not required
Source of energy for synthesis of ATP	Energy for synthesis of ATP comes ultimately from light	Energy for synthesis of ATP comes from the oxidation of glucose

Electron donors	Non- cyclic: Water, PSI and PSII Cyclic: PSI	Reduced NAD and reduced FAD
Electron and proton (H^+) acceptors	Non-cyclic: $NADP^+$ is the final electron and proton acceptor in the non-cyclic pathway. Cyclic: PSI	Oxygen is the final electron and proton acceptor & is reduced to water, catalyzed by cytochrome oxidase
Establishing proton gradient for the synthesis of ATP	Protons are pumped inwards/ active transport/pumping of H^+ ions, from stroma, across the thylakoid membrane, into the thylakoid space	Protons are pumped outwards, from mitochondrial matrix, across the inner membrane, into the intermembrane space

[2014 RI ANS]

	Features	Photophosphorylation	Oxidative Phosphorylation
1	**Location**	***Thylakoid membrane*** * of chloroplast	***Inner membrane of mitochondrion/Cristae****
2	**Source of energy for synthesis of ATP**	Energy for synthesis of ATP comes ultimately from ***light****.	Energy for the synthesis of ATP comes from the oxidation of glucose which stores chemical energy.
3	**Electron donors**	**Water** is the electron donor in the non-cyclic pathway while; ***Photosystem I**** is the electron donor in the cyclic pathway.	***Reduced NAD*** and reduced ***FAD****
4	**Electron acceptors**	***Oxidised NADP**** is the final electron and proton acceptor in the **non-cyclic pathway** ; while ***Photosystem I*** *is the final electron acceptor in the **cyclic pathway.**	***Oxygen**** is the final electron and proton acceptor (and it combines with H^+) and is reduced to water.
5	**Establishing proton gradient for ATP synthesis**	Protons are actively pumped from ***stroma****, across the thylakoid membrane, into the **thylakoid space*.**	Protons are pumped, from the ***matrix****, across the inner membrane, into the ***intermembrane space****.
6	**By-product**	***Oxygen****	**Water**
7	**Coenzyme**	Oxidised NADP	Oxidised NAD and oxidised FAD

Similarities:

- As the donated electrons travel down a series of electron carriers that are progressively electronegative, the energy released is coupled to the **pumping to H^+ ions** across membrane;
- Leading to a build-up of a proton-motive force/ electrochemical gradient/ H^+ ion gradient across the inner mitochondrial membrane which is essential for the formation of ATP via *chemiosmosis* using **ATP synthase**;
- Both processes involved the use of **coenzymes** to carry protons and electrons;

RVHS Prelim 2010 H1 P2

(b) Describe how photophosphorylation differs from oxidative phosphorylation. [8]

	Feature	photophosphorylation	oxidative phosphorylation
B1	Organelle	Chloroplast	Mitochondria
B2	Location	Thylakoid membrane	Inner Mitochondrial membrane
B3	Proton reservoirs	Thylakoid space	Intermembrane space
B4	Energy conversion	Light to chemical	Chemical to chemical
B5	Role of coenzymes	NADP is reduced in the light rxn as final electron acceptor	NAD is reduced in glycolysis, link rxn and Kreb's cycle
B6	Sources of electrons	Water	NADH and $FADH_2$
B7	Roles of O_2	By-product of photolysis	Final electron acceptor

B8	Roles of water	Photolysis: To produce H^+ which will then accumulate in the thylakoid space and electrons to replace the electron lost at the primary reaction centre	By-product of O_2 as the final electron acceptor

12. [12/2/9b] Explain the role of membranes in the chloroplast. [7]

1. Chloroplast is bound by double membrane. The outer membrane is a smooth and continuous boundary, an inner membrane and also a membrane system inside the chloroplast that gives rises to thylakoids and lamellae. Thylakoids are stacked to form grana.
2. * Extensive folding of thylakoid membrane increases surface area for proteins such as photosystems, electron carriers and stalked particles containing ATP synthase to be embedded in the thylakoid membrane.

Photosystems

3. Photosystems, which are embedded in thylakoid membranes, comprise of accessory pigments (chlorophyll a and b, and carotenoids) in light harvesting complex, and primary pigments (special chlorophyll a) and primary electron acceptors in the reaction centres;
4. During * photophosphorylation, one of the P680 or P700 electrons get excited to a higher energy state, which subsequently gets emitted and captured by the primary electron acceptor, leaving behind a positive 'hole';

Electron transport chain

5. * Photoexcited electrons from PSII are passed down the electron transport chain made of electron carriers, found embedded on the thylakoid membrane, each with an energy level lower than the one preceding it, until it fills the positive 'hole' in PSI.
6. Energy released from the electron transfer down the chain of electron carriers is used to actively pump protons from stroma into the thylakoid space, generating electrochemical proton gradient for the synthesis of ATP
7. * Thylakoid membranes are also impermeable to protons which allows the steep electrochemical proton gradient to be set up, with high concentrations of H^+ in the thylakoid space.

Stalked particles containing ATP synthase

8. * As protons diffuse through the stalked particles containing ATP synthase embedded on thylakoid membrane, this catalyses the synthesis of ATP from ADP and inorganic phosphate.
9. The electron carriers, stalked particles containing ATP synthase, photosystems embedded in the thylakoid membrane together with the impermeability of thylakoid membrane to protons enables the non-cyclic (and cyclic) photophosphorylation to take place, producing ATP, reduced NADP and oxygen.

Compartmentalisation to form stroma, thylakoid space

10. * Thylakoid membranes also compartmentalize enzymes and other substances involved in photosynthesis;
11. E.g. such that enzymes like Rubisco, that is required for carbon fixation is concentrated within the stroma; (other e.g. include concentration of H^+ in the thylakoid space)

[NJC 2010 H1 P2] Describe the roles of membranes in photosynthesis and in respiration. [8]

Describe the roles of membranes in respiration. [8]

1. Mitochondrion is bound by a double membrane, whereby the outer membrane is a smooth and continuous boundary whereas the inner membrane is extensively folded to form cristae.
2. * Extensively folded cristae increases surface area for proteins such as electron carriers and stalked particles containing ATP synthase to be embedded in the inner mitochondria membrane
3. * Oxidative phosphorylation is the mechanism of ATP synthesis when high energy electrons (released from oxidation of intermediates in glycolysis, links reaction and Krebs cycle) are transferred from reduced coenzymes, e.g. reduced NAD and reduced FAD to the electron transport chain at the inner mitochondria membrane.

   Electron transport chain
4. * When high energy electrons are passed down the electron transport chain made of electron carriers, each with an energy level lower than the one preceding it, energy released from the electron transfer down the chain of electron carriers is used to actively pump protons from matrix into the intermembrane space, generating electrochemical proton gradient for the synthesis of ATP
5. * Inner membrane is also impermeable to protons which allows the steep electrochemical proton gradient to be set up, with high concentrations of H^+ in the intermembrane space.

   Stalked particles containing ATP synthase
6. * As protons diffuse through the stalked particles containing ATP synthase embedded on inner mitochondria membrane which catalyses the synthesis of ATP from ADP and inorganic phosphate.
7. The electron carriers, stalked particles containing ATP synthase together with the impermeability of inner mitochondria membrane to protons enables oxidative photophosphorylation to take place, producing ATP, oxidised NAD and FAD and water.

   Compartmentalisation to form mitochondrial matrix, intermembrane space
8. The inner and outer membranes are separated by an intermembrane space, where high concentration of H^+ is present to generate steep electrochemical proton gradient across the inner membrane
9. The semi-fluid mitochondrial matrix contains enzymes of the Link reaction and Krebs cycle, allowing oxidation of pyruvate (3C) to form acetyl-CoA / further oxidation of acetyl-CoA occurs via a series of dehydrogenation reactions respectively

[2016 IJC] Describe the roles of membranes in photosynthesis and in respiration. [8]

1. contains electron carriers of the ETC;
2. during e^- transfer, energy is released to pump H^+ from mitochondrial matrix and stroma into mitochondrial intermembrane space and thylakoid space;
3. impermeability of membrane to ions;
4. allows formation of proton gradient across inner mito memb and thylakoid memb;
5. contains ATP synthase to harness proton motive force / which allows H^+ to diffuse through;
6. to phosphorylate ADP to form ATP;
7. thylakoid membrane contains photosynthetic pigments that absorb light energy during photosynthesis;
8. membranes of mitochondrion contains transport proteins to allow transport of metabolites e.g. glucose / triose phosphate / pyruvate;
9. the envelopes of mito and chloroplast allows for compartmentalisation of the cell as mito. and chloroplast enz require different / specific conditions from the rest of the cell to function;

[AJC 2013] With reference to photosynthesis and cellular respiration, explain the importance of compartmentalization within a plant cell. [10]

- The inner membrane of the chloroplast encloses the stroma where enzymes for Calvin cycle are
- Thylakoid membrane houses light harvesting complexes / photosystems for harnessing light energy to excite electrons in the reaction centre down the ETC
- with NADP reductase as final electron carrier which is reduced to NADPH for Calvin cycle
- Flow of electrons down ETC allows pumping of H^+ from the stroma to the thylakoid space, setting up a proton gradient
- Diffusion of the H^+ from the thylakoid space to the stroma via ATP synthase / stalked particles in thylakoid membrane allows phosphorylation of ADP to form ATP required in Calvin cycle
- The inner mitochondrial membrane encloses the matrix where enzymes of the Krebs cycle are localised
- Enzymes localised in the cytoplasm allow glycolysis to occur
- The inner mitochondrial membrane is the site of oxidative phosphorylation
- and is highly folded forming cristae to increase surface area for more ETCs / stalked particles
- Flow of electrons down ETC allows pumping of H^+ from the matrix to the intermembrane space, setting up a proton gradient since the inner mitochondrial membrane is impermeable to H^+
- Diffusion of the H^+ from the inner mitochondrial space to the matrix via ATP synthase / stalked particles in the inner mitochondrial membrane allows phosphorylation of ADP to form ATP

13. [12/2/9c] Describe the effect of <u>increasing</u> light intensity on the <u>rate</u> of photosynthesis. [6]

1. Light intensity is an important <u>limiting factor</u> in the <u>light dependent stage</u> as light is required to excite chlorophyll molecules for photophosphorylation.
2. As light intensity increases, the rate of light-dependent reactions <u>increases</u>, therefore rate of photosynthesis increases

3. When light strikes an <u>accessory pigment molecule</u> in a light harvesting complex of PSII and PSI, this energy is relayed to neighbouring accessory pigment molecules, until it accumulates and reaches one of the two <u>P680 or P700 chlorophyll a</u> molecules in the reaction centres of PSII or PSI respectively.
4. This excites one of the P680 or P700 electrons to a <u>higher energy state</u>, which is then emitted and captured by the primary electron acceptor, leaving behind a positive hole.
5. The higher the light intensity, the <u>more chlorophyll molecules in the photosystems will be excited</u>, therefore <u>more electrons are excited to higher energy state</u> and gets emitted.
6. resulting in <u>larger number of excited electrons</u> passed down the <u>electron transport chain</u>, made of electron carriers, <u>each with an energy level lower than the one preceding it</u>, until it fills the positive 'hole' in PSI.
7. Energy released from the transfer of electrons down the chain of electron carriers is used to <u>actively pump more protons</u> from the stroma into the thylakoid space, generating electrochemical proton gradient for the synthesis of ATP, as the protons diffuse through stalked particles containing <u>ATP synthase</u>.
8. More <u>oxidised NADP</u>, which acts as final electron and proton acceptor, are reduced
9. resulting in more products, <u>ATP and reduced NADP</u> being formed

10. ATP and reduced NADPH are required for the reduction of glycerate 3 phosphate to triose phosphates in the light-independent stage / Calvin cycle;
11. ATP is required for the regeneration of RuBP;

12. However at higher light intensities, the photosystems are saturated with light and photosynthesis is occurring at maximum rate, therefore there is maximum rate of production of ATP and reduced NADP.
13. The rate of carbon dioxide assimilation / carbon fixation also plateaus/level off as it is limited by amount of ATP and reduced NADP produced from light-dependent reactions.
14. Light intensity is no longer the limiting factor of rate of photosynthesis at higher light intensities, but other factors such as temperature.

Examiner's comments:

Candidates were able to state the broad relationship between light intensity and the rate of photosynthesis, but descriptions often lacked further details, such as consideration of how the relationship changes at higher light intensities. References to the results of increased light intensity on the light dependent and light independent stages of photosynthesis would also have been relevant.

14. [Tutorial 04/2/10] Explain how ATP is synthesized using light energy in photosynthesis. [6]

Photosystems, which are embedded in thylakoid membranes, comprise of accessory pigments (chlorophyll a and b, and carotenoids) in light harvesting complex and primary pigments (special chlorophyll a) and primary electron acceptors in the reaction centres.
In non-cyclic photophosphorylation, light of particular wavelengths strikes an accessory pigment molecule in a light harvesting complex of PSII and PSI and this energy is relayed to neighbouring accessory pigment molecules, until it accumulates and reaches one of the two P680 chlorophyll a molecules in the reaction centres of PSII. The same occurs for the P700 chlorophyll a molecules in the reaction centre of PSI.
This excites one of the P680 or P700 electrons to a higher energy state, which subsequently gets emitted and captured by the primary electron acceptor within each PS, leaving behind a positive 'hole' in the chlorophyll a molecules.
Photolysis of water occurs when an enzyme catalyses the splitting of a water molecule into protons, electrons and molecular oxygen. The electrons are used to fill up the positive 'holes' in the reaction centre of PSII to return $P680^+$ to ground state.
The photoexcited electron passes from primary electron acceptor of PSII to $P700^+$ in PSI to fill the positive 'hole' in $P700^+$. This occurs via an electron transport chain made of electron carriers, each with an energy level lower than the one preceding it.
Energy from the electron transfer down the chain of electron carriers is used to actively pump protons from the stroma into the thylakoid space, generating an electrochemical proton gradient for the synthesis of ATP, as the protons diffuse through stalked particles containing ATP synthase, back into the stroma.

[RI 2010 H1 P2] Describe the process of chemiosmosis in the chloroplast, highlighting the processes that generate and maintain the proton gradient. [6]

1. Chemiosmosis: energy-coupling mechanism using proton-motive force to drive ATP synthesis
2. As electrons travel down a series of electron carriers that are progressively more electronegative, the free energy released is used
3. by certain electron transport chain proteins in the thylakoid membrane pump rotons/H^+ ions across the membrane, from stroma into the thylakoid space;
4. which contributes towards a high H^+ concentration in the thylakoid space relative to the stroma.
5. As H^+ ions diffuse down the concentration gradient from the thylakoid space into the stroma via the ATP synthase (or ATP synthetase);
6. ADP is phosphorylated to ATP in the process;(awarded if point 1 not mentioned)
(For points 1-6, max 4 marks)

7. splitting of H_2O into H_2O, H^+ and electrons in the thylakoid space contributes to the accumulation of H^+ in the thylakoid space;
8. $NADP^+$ is reduced to NADPH by $NADP^+$ reductase;
takes up H^+ ions from the stroma (thus reducing the relative H^+ concentration in the stroma);

15. [08/2/8c] & [TJC 2010 H1 P2] State the similarities between ATP production in mitochondria and chloroplasts and suggest why these similarities exist. [5]

Maximum 4 marks from points 1 - 6

1. Electron transport chain is found in thylakoid membrane in chloroplasts and inner membrane in mitochondria;

2. Electrons are passed along the electron transport chain from one electron carrier to the next, each with an energy level lower than the one preceding it;

3. Energy released is used to pump protons from matrix of the mitochondrion into the intermembrane space during oxidative phosphorylation of respiration and from stroma of chloroplast into thylakoid space during photophosphorylation of Calvin cycle;

4. This produces a high concentration of H^+ due to impermeable nature of membranes to protons, generating a steep electrochemical proton gradient;

5. Stalked particles containing ATP synthases ® ATPase are embedded on inner mitochondria membrane / thylakoid membrane;

6. Protons diffuse through them, synthesizing ATP by the phosphorylation of ADP with inorganic phosphate (Pi);

7. The endosymbiotic origin of mitochondria and chloroplasts states that they were once prokaryotes, thus need to synthesize their own ATP / have the structures to allow ATP synthesis;

Examiner's comments:

Again this question was well done by the vast majority of candidates. Marks were commonly gained for references to an electron transport chain, the generation of a proton gradient, the relevant membranes involved and the role of ATP synthetase. Some candidates incorrectly referred to ATPase in this context. The question asks why there are similarities in ATP production in mitochondria and chloroplasts and therefore references to the fact they were once prokaryotes and their endosymbiotic origin were required.

16. [Tutorial 04/2/10] Describe the main stages of the Calvin cycle. [8]

Involves a series of enzyme-catalyzed reactions which occurs in the stroma of chloroplast;

RuBP carboxylase-oxygenase (Rubisco) is present in large amounts in the stroma of the chloroplast.

It catalyses the fixation of CO_2 by a 5C sugar known as ribulose bisphosphate (RuBP), which gives an unstable 6C intermediate

that immediately breaks down to 2 molecules of 3C compound known as glycerate-3-phosphate (GP).

GP can be converted to pyruvate which is used to synthesize fatty acids and amino acids.

The reducing power of reduced NADP and energy from the hydrolysis of ATP are used to convert GP to GALP. GALP is the first carbohydrate made in photosynthesis.

About 1/6 of the total amount of GALP is used to synthesize other carbohydrates (e.g. sucrose and starch) and glycerol

About 5/6 of the total amount of GALP has to be used to regenerate the RuBP consumed in the first reaction. This process requires energy from the hydrolysis of ATP.

[2016 YJC] Outline the three phases of the Calvin cycle [6]

Phase 1 (CO_2 fixation) /carboxylation

1. **Carbon dioxide acceptor is** ribulose bisphosphate **(RuBP), a five-carbon sugar.**

2. **This is process is known as** carboxylation **/ Carboxylation is catalysed by the enzyme** RuBP carboxylase (RUBISCO)**.**

3. **The intermediate six-carbon product is** unstable**/This intermediate six-carbon product will be broken down to two molecules of** glycerate 3-phosphate **(GP) / 3-phosphoglycerate, a three-carbon organic acid.**

Phase 2 (Reduction)

4. **Glycerate-3-phosphate is** phosphorylated **by** ATP **to form** 1, 3-bisphosphoglycerate**.**

5. 1, 3-bisphosphoglycerate is reduced by NADPH to form glyceraldehyde 3-phosphate (G3P) / triose phosphate (TP).

Phase 3 (Regeneration of CO_2 acceptor RuBP)

6. Glyceraldehyde 3-phosphate **(G3P) / triose phosphate has to be used to regenerate the RuBP.**

7. ATPs **are used to form glucose. Therefore, more ATPs are used in total compared to NADPH in Calvin cycle.**

8. **To form a** glucose molecule **(6-carbon),** two G3P **(3-carbon) are combined together.**

17. [08/2/8a & 11/2/8a] Compare Calvin cycle and Krebs cycle. [7]

Feature	Krebs Cycle	Calvin Cycle
Site	Occurs in matrix of mitochondria	Occurs in stroma of chloroplasts;
Type of reaction	Respiration + (Catabolic reaction) involving breakdown of acetyl coA / glucose	Photosynthesis + (Anabolic reaction) involving formation of carbohydrates;
Role of ATP	Synthesis of ATP by substrate level phosphorylation	Energy from hydrolysis of ATP is used in the reduction of GP to GALP/triose phosphate & regeneration of RuBP.
Coenzymes involved	NAD^+ and FAD function as electron and proton acceptors	NADPH + H^+ function as electron and proton donors; ® carriers
Role of Carbon dioxide	(2) molecules of CO_2 is released by oxidative decarboxylation	(1) molecule of CO_2 is accepted by RuBP catalyzed by RuBP carboxylase-oxygenase;
Type of redox reaction	Reactions are mainly oxidative as many dehydrogenation reactions occur / many reduced NAD formed	Reactions are mainly reductive as reduced NADP is used up;
Type of cells that undergo these processes	Occurs in all aerobically respiring cells	Occurs in plants cells / algae / blue-green bacteria;
Input of reactants	Acetyl-CoA combines with oxaloacetate to form citrate	CO_2 combines with RuBP to form an unstable 6C molecule which break down into two 3C compounds / GP;
Conversion of Products	All citrate are converted back to oxaloacetate	1/6 of GALP is used to convert to carbohydrates and 5/6 of GALP is used for regeneration of RuBP;
Fate of Water	Water is required in the conversion of fumarate to malate	Water is used in carbon fixation step, combining with RuBP and CO_2 to form 2 GP;

Formation of glycerate-3-phosphate after 6C intermediate breaks down

reduced NAD becomes oxidised to form oxidised NAD

Regenerating of starting material

Examiner's comments:

The differences between the Calvin cycle and the Krebs Cycle were very well known. Candidates often presented their answer in tabular form which was probably a useful strategy enabling them to quickly check their points of comparison. Points which gained credit were in relation to the site of action, the anabolic/catabolic nature of the process, the roles of carbon dioxide, coenzymes and ATP.

accept:

Products formed	CO_2 enters the cycle whereby RuBP accepts CO_2 to form an unstable 6C compound, catalysed by RuBP carboxylase-oxygenase. ATP is hydrolysed to form ADP and P_i during the reduction phase and regeneration of RuBP. Reducing power from reduced NADP is used to convert glycerate-3-phosphate (GP) to glyceraldehyde-3-phosphate (GALP), therefore $NADP^+$ + P_i is formed. For every 3 molecules of CO_2 that enter the cycle, there is a net output of 1 molecule of Glyceraldehyde-3-phosphate (GALP) while the other 5 molecules of GALP are used to regenerate 3 molecules of RuBP.	Carbon is removed from the intermediate compounds via oxidative decarboxylation, in the form of CO_2. GTP is readily used for the synthesis of one molecule of ATP via substrate-level phosphorylation. NAD^+ and FAD are then reduced to reduced NAD and reduced FAD respectively during dehydrogenation/ oxidation of substrates. For each glucose molecule, the products of Krebs cycle per glucose molecule include 4 molecules of CO_2, 6 molecules of reduced NAD, 2 molecules of reduced FAD and 2 molecules of ATP.

18. [Tutorial 04/2/10] Outline the role of NADP in photosynthesis. [6]

(Answer must address BOTH oxidised and reduced NADP). Location: $NADP^+$ is located in the stroma of the chloroplast;

$NADP^+$ is a coenzyme;

which can be reduced as it can carry high energy electrons and protons;

$NADP^+$ is reduced in the light-dependent reaction as the final proton and electron acceptor during non-cyclic photophosphorylation;

Reduced NADP provides the reducing power to convert glycerate-3-phosphate to glyceraldehyde phosphate in the Calvin cycle;

$NADP^+$ is regenerated;

19. [Tutorial 06/2/7] Explain the effect of carbon dioxide and light as limiting factors on the rate of photosynthesis. [6]

A limiting factor is one which at its minimum value, will limit the rate of the overall reaction;

Carbon dioxide is a major limiting factor as its concentration in the atmosphere is low (0.03 – 0.04%);

It is the raw material for the Calvin cycle and its increased concentration will increase the rate of photosynthesis;

Light intensity is an important limiting factor in the light-dependent stage to excite chlorophyll molecules for photophosphorylation to occur;

However, it is seldom the limiting factor during daylight hours (except in the case of shaded plants);

At low light intensities, rate of photosynthesis increases (linearly) with increasing light intensity.

The rate at which ATP and NADPH are produced is too slow to allow the dark reactions to proceed at maximum rate.

Hence, light is a limiting factor in photosynthesis at low light intensities.

Wavelength of light is also a limiting factor as demonstrated by comparing the action and absorption spectra for photosynthesis. Rate of photosynthesis is highest at the red and blue-violet regions of the spectrum and lowest at the green region;

Guard cells are the only cells found on lower epidermis of leaf that can photosynthesize and the synthesis of sugars increase the solute potential within these cells, causing water to enter and subsequently causing stomata to open, allowing gaseous exchange;

Please teach the highlighted point.

Examiners' Comments

(c) This part was well answered by most candidates. That carbon dioxide was the raw material for the Calvin cycle and that its increased concentration increased the rate of photosynthesis were well known. Its low level (0.04%) in the atmosphere and consequent major role in limiting photosynthesis were not seen so often. There were many references to light intensity and its importance in the light dependent stage to excite electrons but fewer references were made to wavelength of light, compensation point, the fact that it is rarely limiting during daylight hours and its role in opening stomata.

Discuss limiting factors in photosynthesis and carry out investigations on the effects of limiting factors, such as light intensity, CO_2 concentration and temperature, on the rate of photosynthesis. Please read up on investigations.

Ans same as above for limiting factors of light intensity, CO_2 concentration.

1. Temperature is an important limiting factor as it affects the rate of enzyme reactions;
2. Such as during light-dependent reactions (e.g. NADP reductase) and light-independent reactions (e.g. Rubisco);

2010 H1 A Level P2 FRQ 6(c)

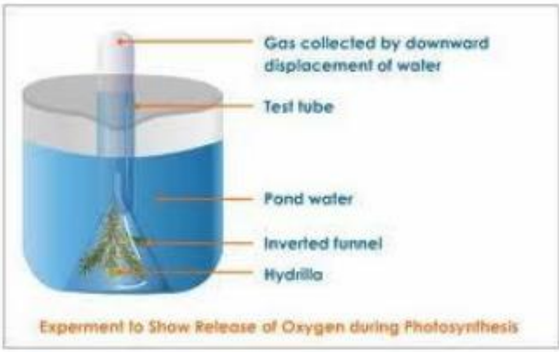
6(c) Describe how would you carry out an investigation to determine the effect of temperature on the rate of photosynthesis. [10 marks]

1. Dependent variable (method to measure rate of photosynthesis)
    - Measure volume of oxygen released (count number of bubbles of oxygen formed per minute / use gas syringe to measure volume of oxygen;
    - Oxygen is released through photolysis of water;
    - Higher the rate of photosynthesis, more oxygen will be released;
2. Independent variable;
    - Use water bath kept at constant temperature by using hot water /
      Use light to shine at the plant at different distance. The nearer the lamps to a beaker containing water plant, the higher the temperature
3. Standardised variables:
    - Age of the water plant / hydrilla;
    - Equilibration time to allow plant to equilibrate to the desired temperature;
4. At least 5 different temperatures;
5. Carry out 2 repeats and calculate average;
6. Table of results;

Temperature / °C Or Distance from the lamp / cm	Number of bubbles counted in 3 minutes				Number of bubbles per minute
	Expt 1	Expt 2	Expt 3	Average	

7. Plot the graph of number of bubbles per minute against temperature;
8. Draw diagram;

E.g.



(c) In order to gain credit, responses needed to describe how the investigation could be carried out, rather than the biochemistry of photosynthesis. Most candidates used a water plant, with standardised variables and counted the number of bubbles of oxygen in a set time. It must be emphasised to candidates that in investigations such as this, it is necessary to ensure that the plant samples are as similar as possible and to allow for the plant to become equilibrated at each temperature before counting the number of bubbles. In addition, some method of preventing the lights from warming up the water would reduce the error in an investigation like this. The most common error made in answering this question was to try to use the iodine test to show that starch has been formed in destarched leaves. This would not give data suitable to determine a rate.

Can use LED lamps that produce less heat.

School / Year : SAJC / 2011

Topic : Photosynthesis

Worksheet Title : To investigate the effect of light intensity on rate of photosynthesis

Aim: In this experiment you will be assessed on your ability to:

- Define the question/problem using appropriate knowledge and understanding
- Give a clear logical account of the experimental procedure to be followed
- Describe how the data should be used to reach a conclusion
- Assess the risks of the experiment and describe precautions that should be taken to keep risks to minimum

Description: Fig. 4.1 shows an aquatic plant called *Elodea canadensis* (Canadian pondweed) which lives entirely underwater and is commonly used in aquariums.

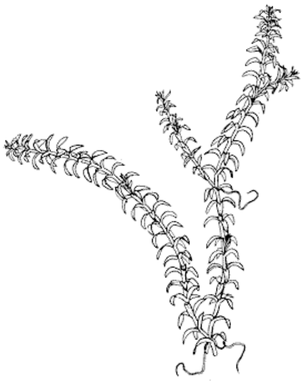


Figure 4.1

Design an experiment using *Elodea* to test the hypothesis that **the rate of photosynthesis is dependent on the light intensity**.

Your plan should:

- a description of the method used including the scientific reasoning behind the method
- an explanation of the dependent and independent variables involved
- relevant, clearly labelled diagrams
- how you will record your results and ensure they are as accurate and reliable as possible
- proposed layout of results tables and graphs with clear headings and labels
- the correct use of technical and scientific terms
- relevant risks and precautions taken

Materials/ Apparatus: Your planning must be based on the assumption that you have been provided with the following equipment and materials which you must use:

- *Elodea* (Canadian pondweed)
- sodium hydrogen carbonate
- distilled water
- table lamp
- a room which can be made dark
- sharp knife
- ruler
- plastacine
- burette
- filter funnel
- thermometer
- stopwatch
- beaker
- Beakers

[Total:12]

Theory

Energy from the light dependent reaction comes from light, which is harvested by photosynthetic pigments embedded in the thylakoid membranes of the chloroplasts. This excites one of the P680 or P700 electrons to a higher energy state, which subsequently gets emitted and captured by the primary electron acceptor, leaving behind a positive 'hole'. Photolysis of water results in the production of protons, electrons and molecular oxygen, and the electrons are used to fill up the positive 'holes' in the reaction centre of PSII to return $P680^+$ to ground state. The photoexcited electron passes from primary electron acceptor of PSII to PSI via an electron transport chain made of electron carriers, each with an energy level lower than the one preceding it, until it fills the positive 'hole' in PSI. This results in the production of ATP, which is used as a source of energy for the production of triose phosphate from carbon dioxide in Calvin cycle.

Dependent variable

Volume of oxygen evolved per minute

Independent variable

Light intensity (measured by the distance between Elodea and lamp)

Constant variables

Amount of sodium hydrogen carbonate, carbon dioxide concentration in water, temperature, same piece of Elodea should be used each time in order to make sure that each experiment is being carried out with the same leaf surface area

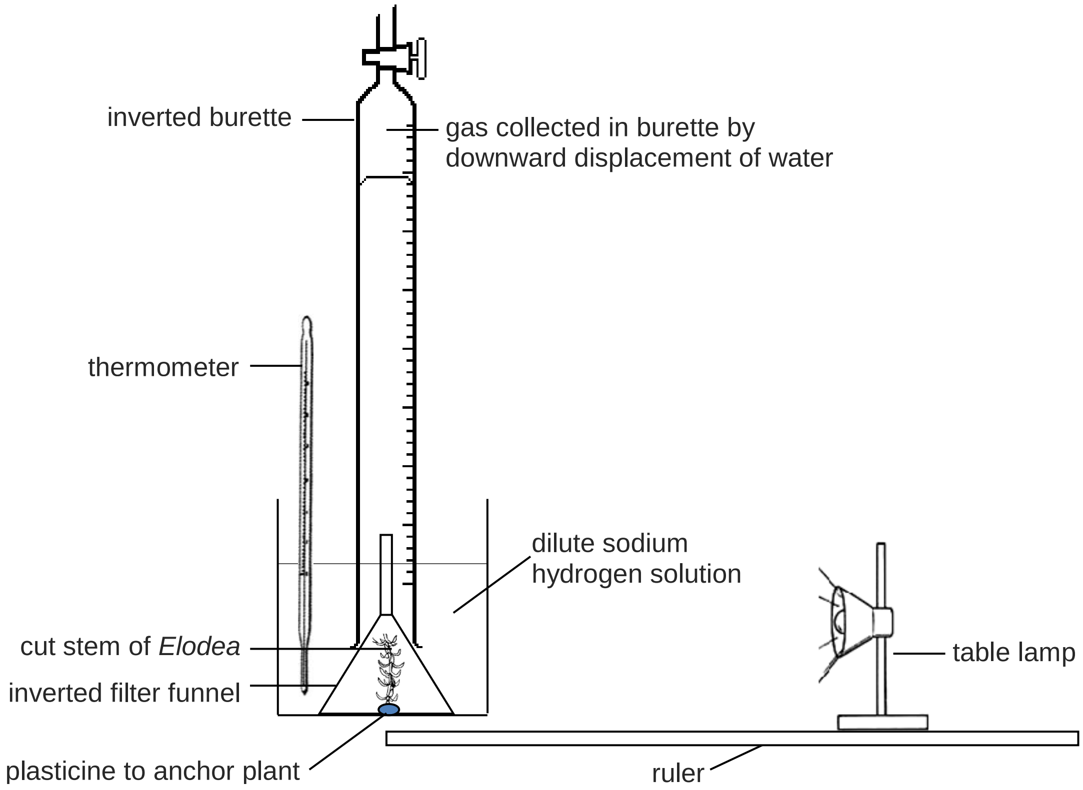
Hypothesis

As light intensity increases, the volume of oxygen evolved per minute increases, therefore the rate of photosynthesis increases.

Procedures – A SCAR

1. Add 2 g of sodium hydrogen carbonate to 600 cm^3 of distilled water in a beaker.
2. Cut the stem of piece of *Elodea* to 5 cm length with a sharp knife.
3. Use plasticine to attach the *Elodea* to the base of the beaker of prepared solution with the cut surface upwards.
4. Use a thermometer to monitor the temperature of the water bath at intervals throughout the experiment.
5. Set up the apparatus as below:

Annotated diagram



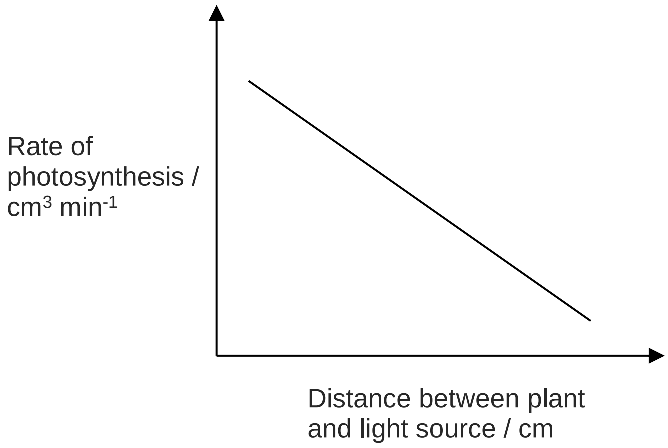
6. Darken the room. Place the table lamp 10 cm from the *Elodea*.
7. Allow the plant to adjust to the light intensity (equilibrate) for 2 minute. Ensure that the rate of bubbling is constant.
8. Collect a suitable volume of gas in 5 min. Measure the volume of gas collected in the burette by downward displacement of water.
9. **R**epeat steps 1 to 8 using the same cut *Elodea* stem, with increasing distances between the table lamp and *Elodea,* such as 20, 30, 40, 50 and 60 cm. In each case, allow time for the plant to equilibrate. The intensity of light falling on a given object is inversely proportional to the square of the distance from the source. *Light intensity* $\propto 1/d^2$ *where d* is the distance between object and light source.
10. Repeat steps 7 to 8 twice, replenishing the set-up with fresh hydrogen carbonate indicator solution between each replicate. This is to obtain a total of three readings for each distance.
11. Set up a **C**ontrol by placing the same setup in the dark. The control is being subjected to same environmental factors as that for the experiment. The rubber tubing/aquarium plastic plant (inanimate object) is used to replace Elodea.

    ® Take away Elodea. The variable that is removed must be substituted to ensure same setup.
12. The amount of CO_2 evolved during respiration is then measured for the control setup so as to determine the actual amount of O_2 evolved during photosynthesis. It is also to ensure that only Elodea plant is giving out CO_2 during experiment.

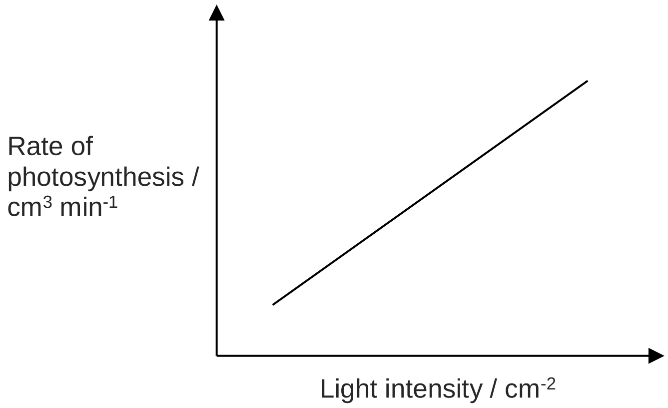
Data Presentation

Distance between plant and light source / cm	**Light intensity /cm^{-2}**	**Volume of oxygen gas evolved in 5 min / cm^{-3}**				**Rate of photosynthesis (Volume of oxygen gas evolved per min) / cm^3 min^{-1}**
		Replicate 1	Replicate 2	Replicate 3	Mean	
10						
20						
30						
40						
50						
60						

Plot a graph with rate of photosynthesis on the y-axis (volume of oxygen gas evolved per min) and distance between plant and light souce (cm) / rate of photosynthesis on the y-axis (volume of oxygen gas evolved per min) and light intensity on the x-axis (as $1/d^2$)



OR



Conclusion: The rate of respiration is affected by light intensity. When light intensity increases, the number of photons striking chlorophyll molecules also increases. This would increase the rate at which the electrons are being excited from the primary reaction centres/ primary pigment molecule of the photosystems. The rate at which photolysis of water increases, therefore there is an increased production of molecular oxygen.

Risk assessment

Care with sharp knife to avoid cuts when trimming stems
Care when handling the lamp to prevent scalding with bulb
Dry hands when operating the lamp to prevent electric shock

20. [2016 DHS] Discuss the effects of varying carbon dioxide and oxygen levels on photosynthesis. [6]

1. Under normal field conditions, carbon dioxide is the major limiting factor in photosynthesis, since its concentration in the atmosphere is about 0.03%.
2. Increasing carbon dioxide concentration leads to a linear increase until limited by other factors.
3. Ribulose bisphosphate carboxylase oxygenase (Rubisco), the enzyme that captures carbon dioxide in the light-independent reactions, has a binding affinity for both carbon dioxide and oxygen.
4. When the concentration of carbon dioxide is high, Rubisco will fix carbon dioxide in Calvin Cycle which increases the rate of photosynthesis.
5. If the carbon dioxide concentration is low and oxygen concentration is high, oxygen will out-compete carbon dioxide for the active site of the enzyme Rubisco during the dark stage of the reaction.
6. Therefore, a high concentration of oxygen lowers the rate of photosynthesis.