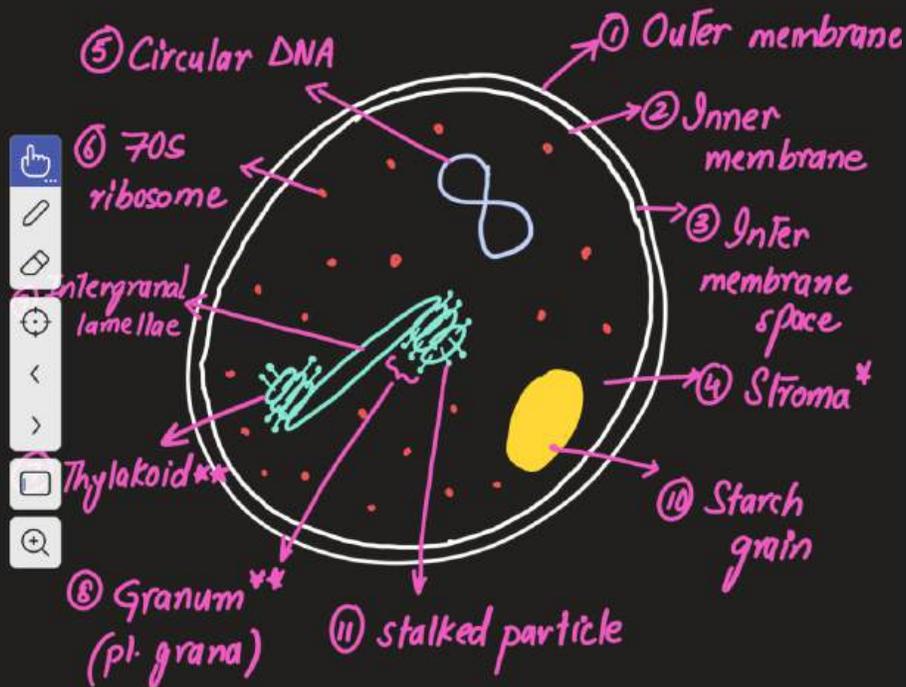


Photosynthesis

CHLOROPLASTS

STRUCTURE :



** light dependent reactions
* light independent reactions
(Calvin cycle)

With
Mohammad Hussham Arshad, MD

ADVANCED LEVEL BIOLOGY 9700 UNIT 13: Photosynthesis

Learning Objectives:

- **Introduction To Photosynthesis**
- **Palisade mesophyll and Chloroplasts**
- **Photosystems and pigments 1**

Video Lecture 1 Slides
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Biology Department

Photosynthesis

Photosynthetic organism → Plant

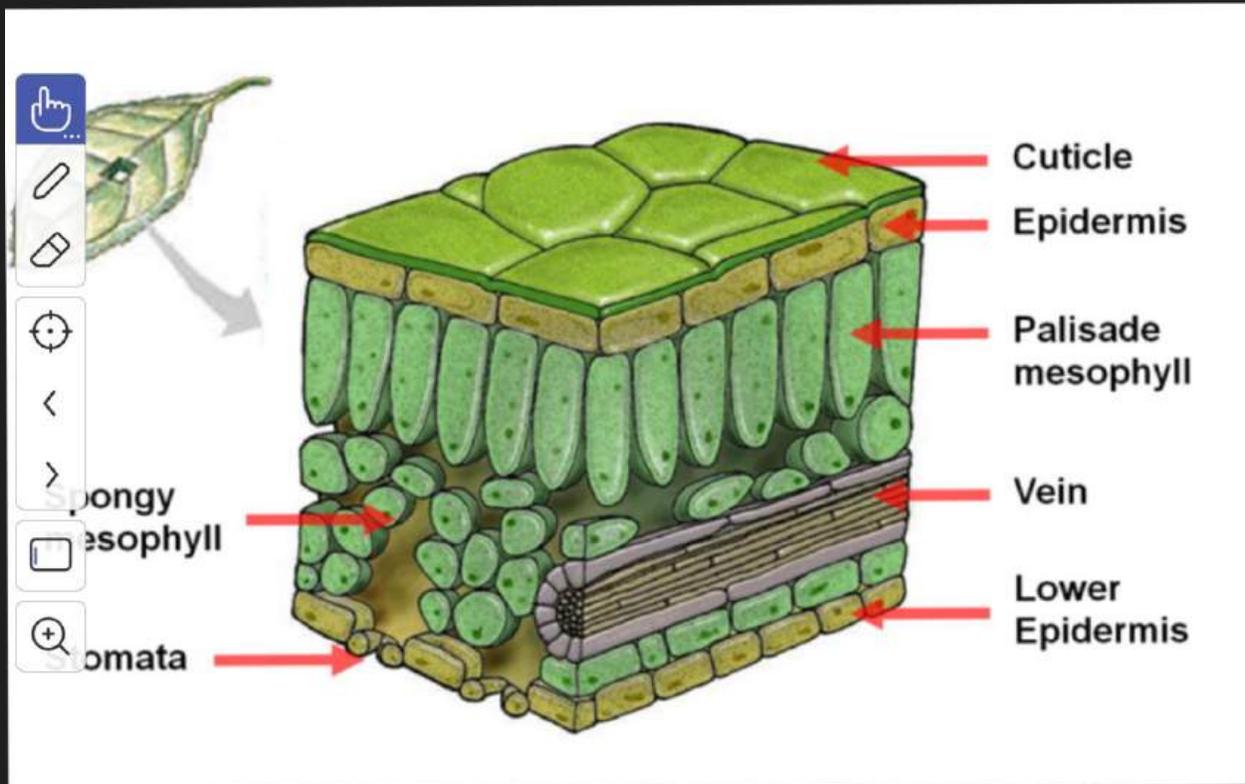
Photosynthetic organ → Leaf

Photosynthetic tissue → Palisade mesophyll

Photosynthetic cells → Palisade mesophyll cells

Photosynthetic organelles → Chloroplast

T.S. of a dicotyledonous leaf

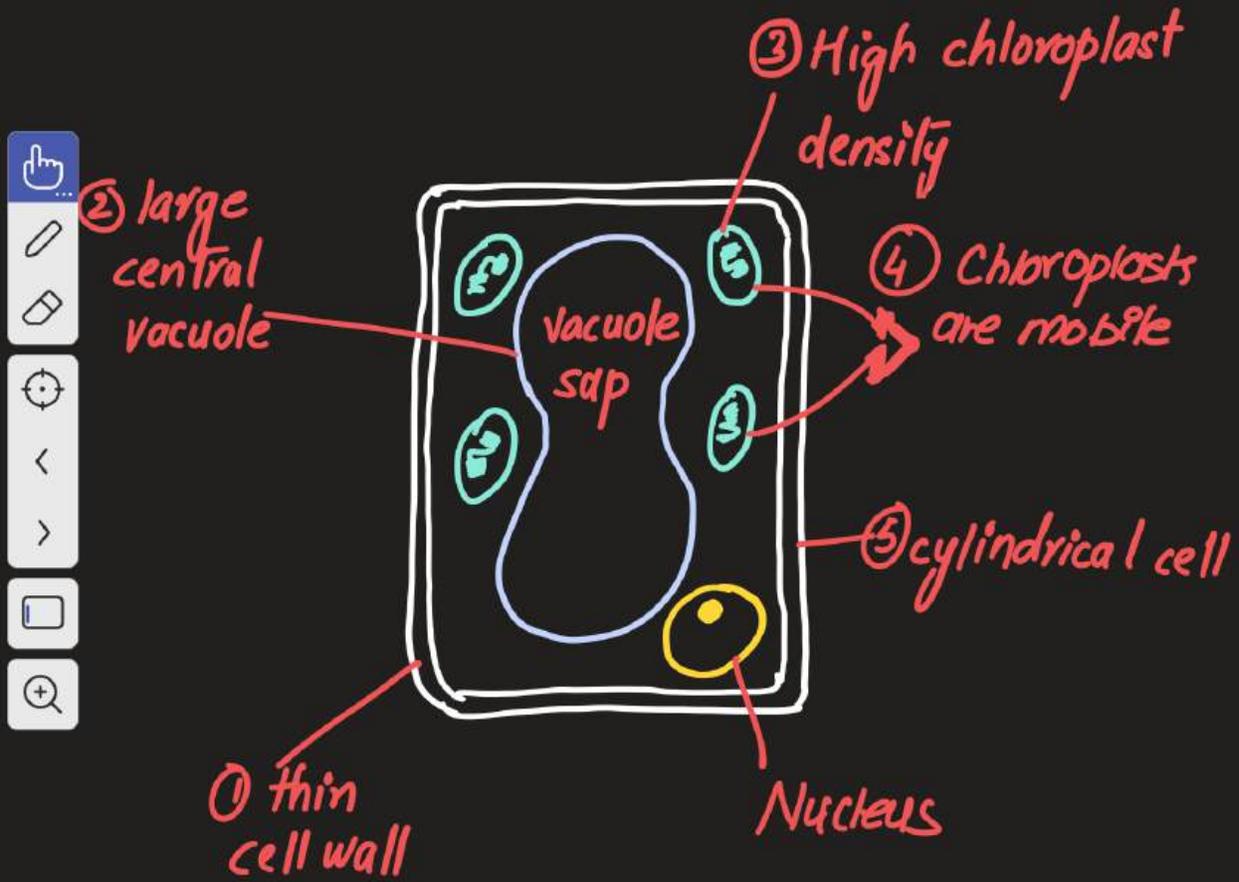


* T.S (plan diagram) of a dicot leaf:



Palisade mesophyll cell

"Main photosynthetic cells"



Structural features of palisade mesophyll & their functional adaptations

① Thin cell wall → minimises diffusion distance for exchange of O_2 , CO_2 , H_2O , etc.

② Large central vacuole → pushes chloroplasts towards the edges of a cell. This increases the efficiency of light absorption

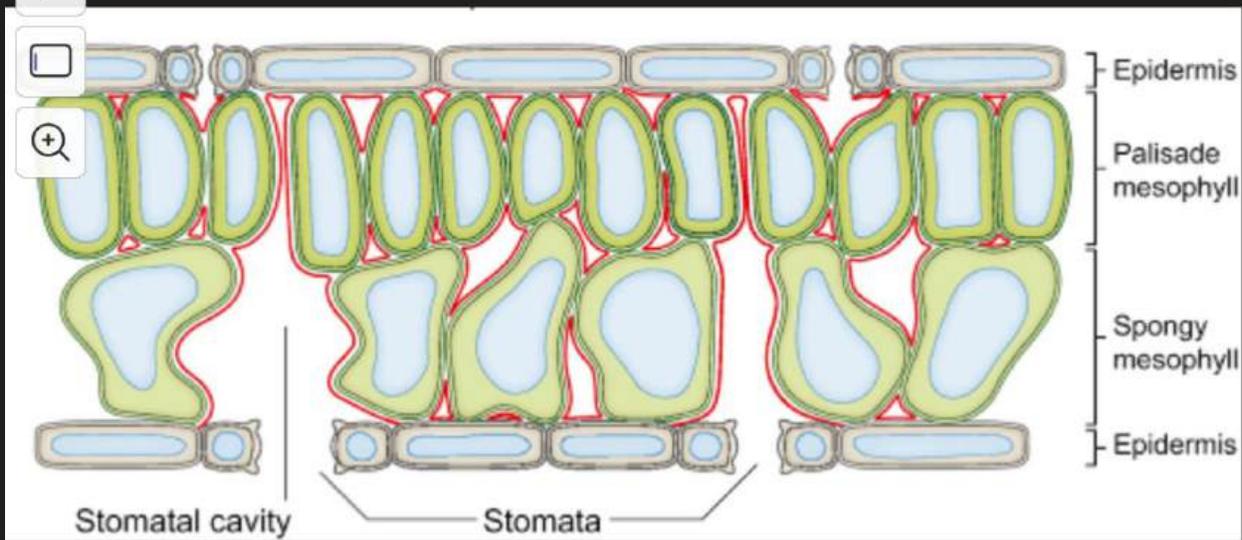
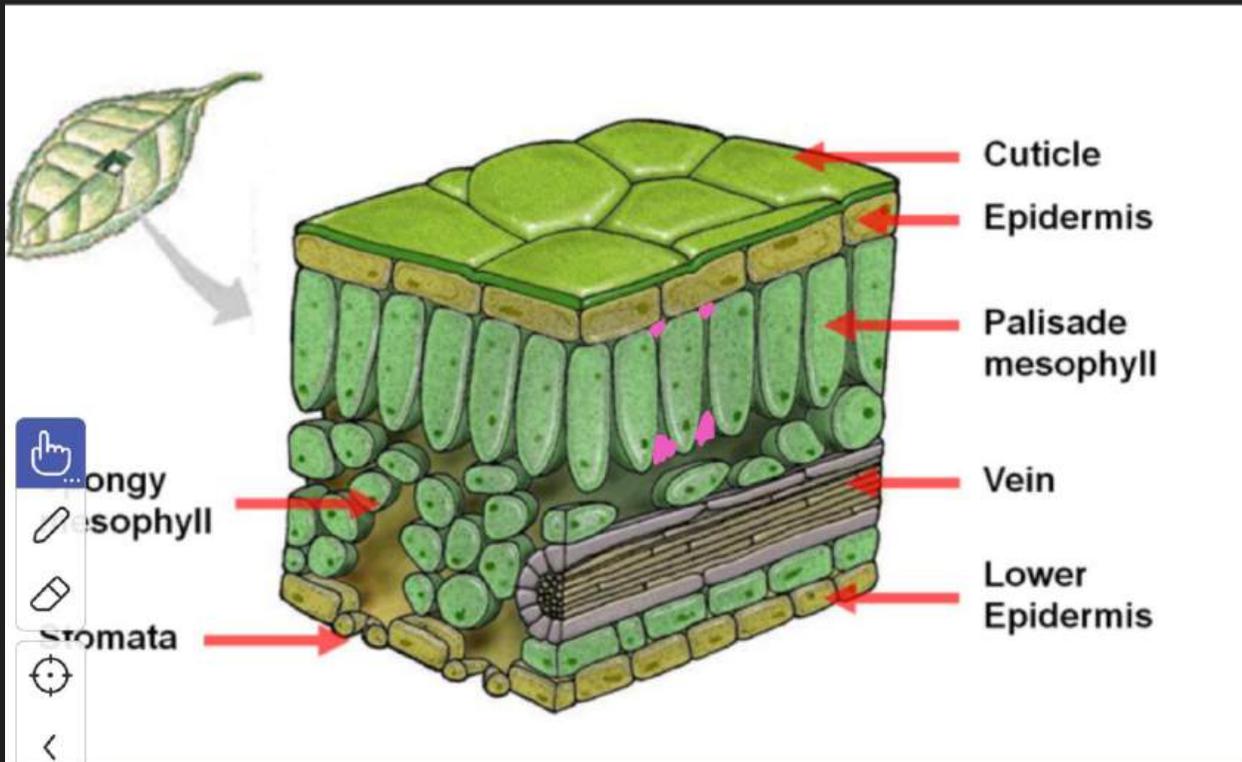
③ High chloroplast density → to maximise light absorption

④ Chloroplasts are mobile →

- chloroplasts can move TOWARDS light to absorb maximum light

- chloroplast can move AWAY from high light intensities to avoid damage to pigments

⑤ Cylindrical cells → produces small air spaces between cells which serve as a reservoir of CO_2

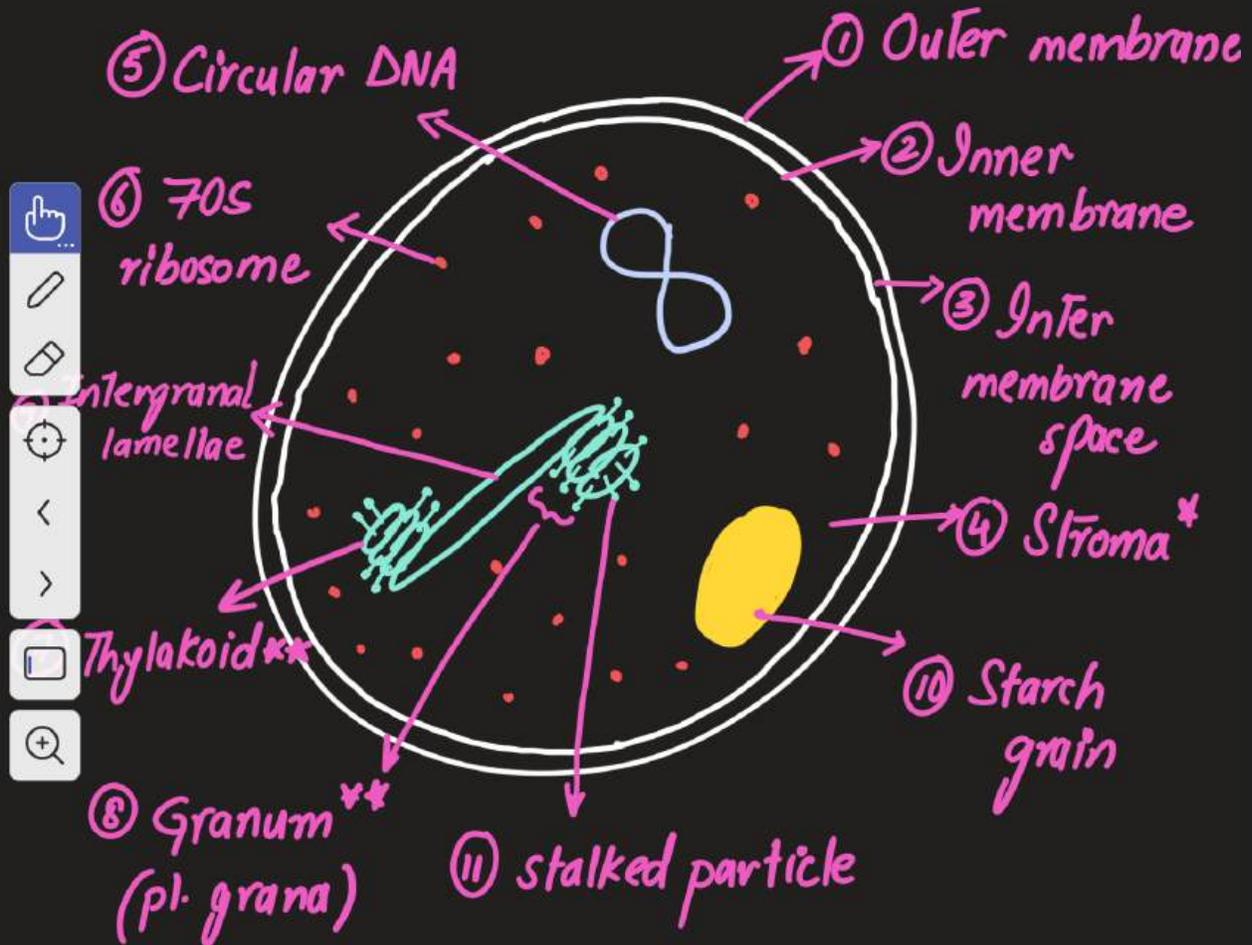


Structural features of palisade mesophyll & their functional adaptations ...

- 1) Closely packed cells → to absorb more light
- 2) Cells near the upper surface of the leaf → to maximise light interception
- 3) Cells arranged at right angles to the leaf surface → to reduce the number of light absorbing walls

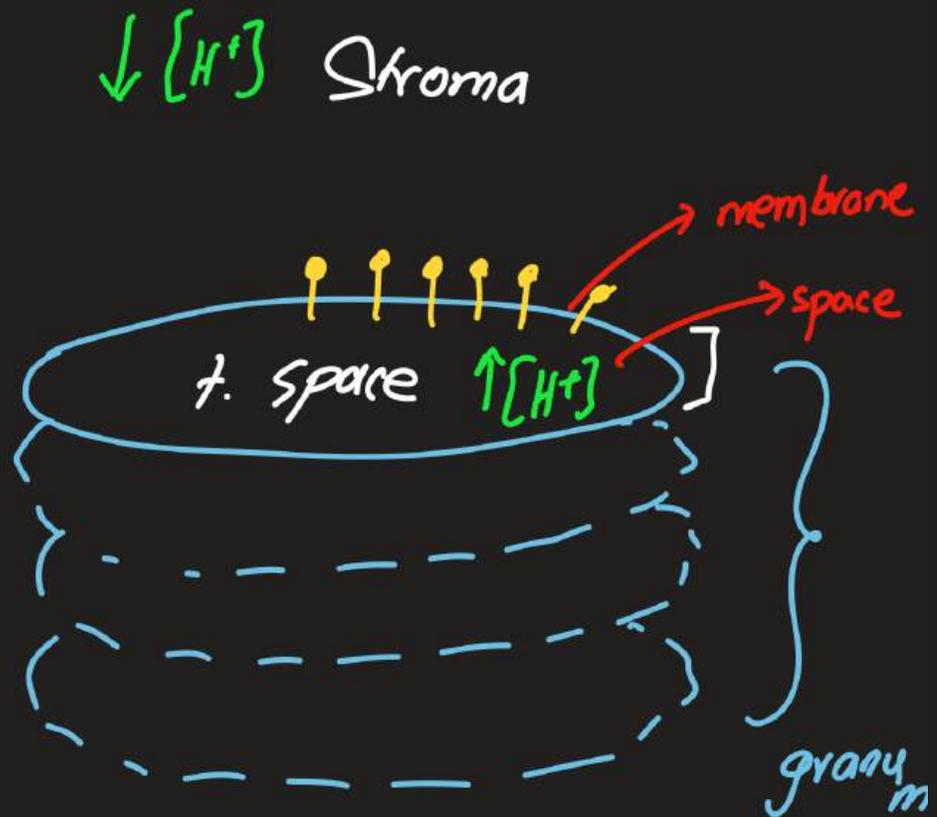
CHLOROPLASTS

STRUCTURE :



** light dependent reactions
* light independent reactions
(Calvin cycle)

Thylakoid and granum



Chloroplast → Structural details

- * Chloroplasts are biconvex disc-like
- * 3-10 μm in diameter

→ organelle surrounded by a double membrane

→ only found in photosynthetic cells.

→ Within the inner membrane lies the stroma.

→ Stroma contains;

- 70s ribosomes

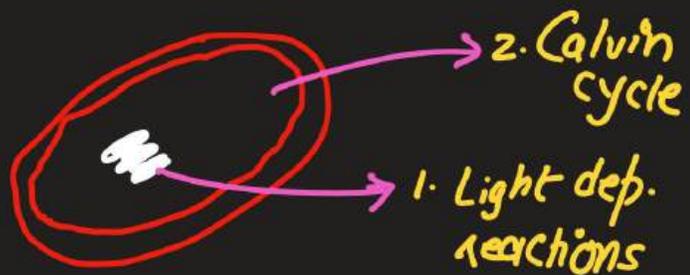
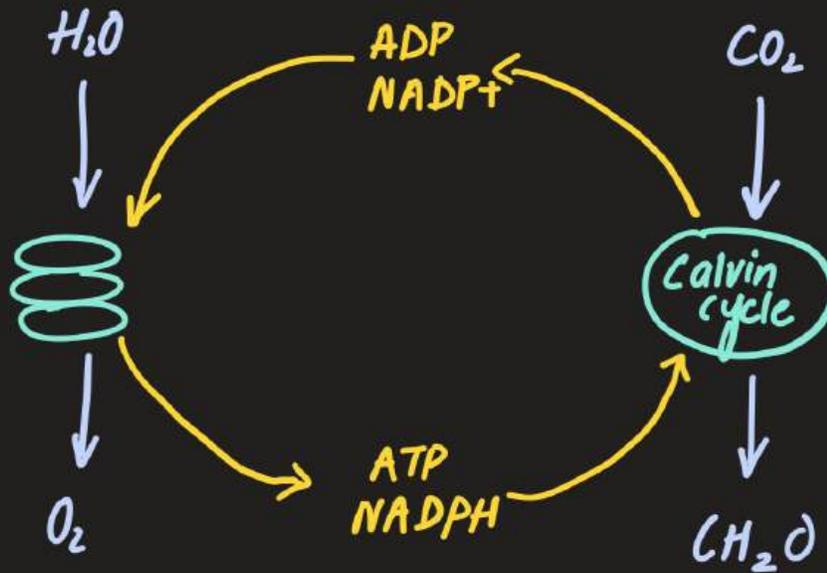
- circular DNA

- starch grains

- Grana

- * A granum is a stack of thylakoids.
- * Thylakoids provide a large surface area for enzyme reactions.
- * Stalked particles contain the enzyme ATP synthase for synthesis of ATP.

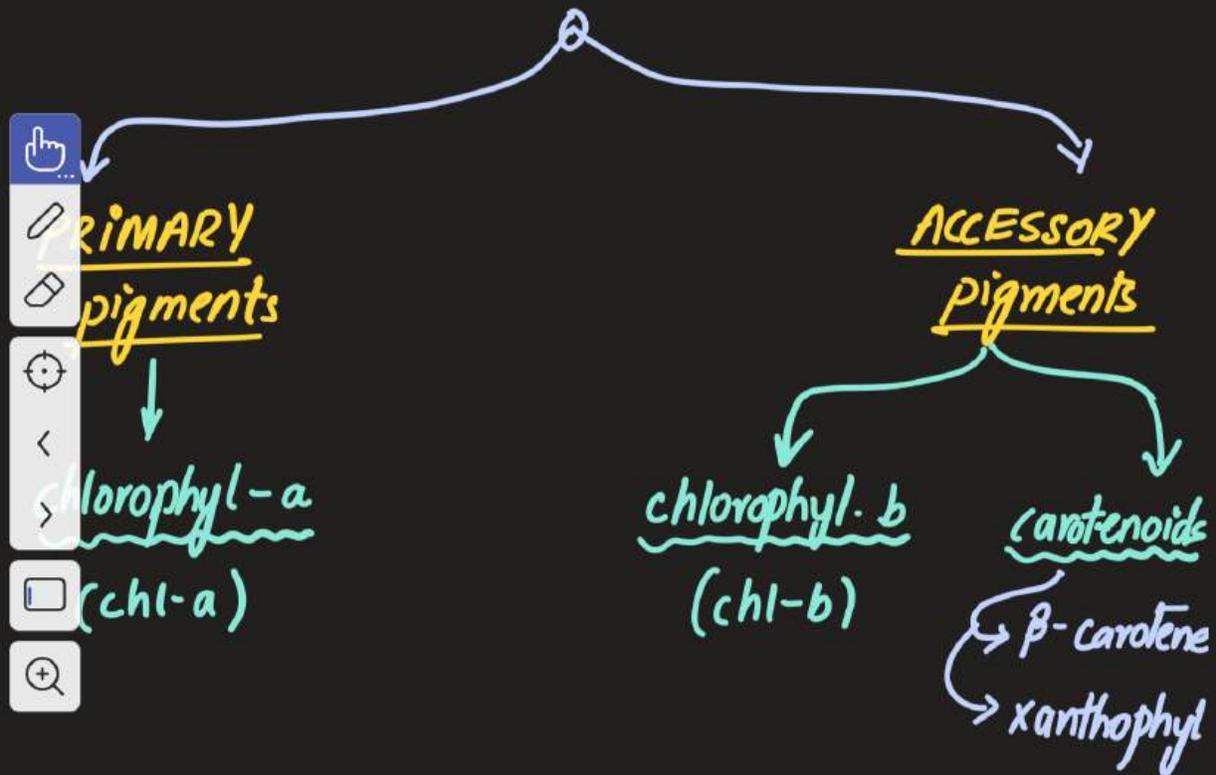
PHOTOSYNTHESIS - Summary





PIGMENTS
&
PHOTOSYSTEMS

Pigments used in photosynthesis



Q: Where are these pigment molecules located within chloroplasts?

Ans: They are located on thylakoid membranes

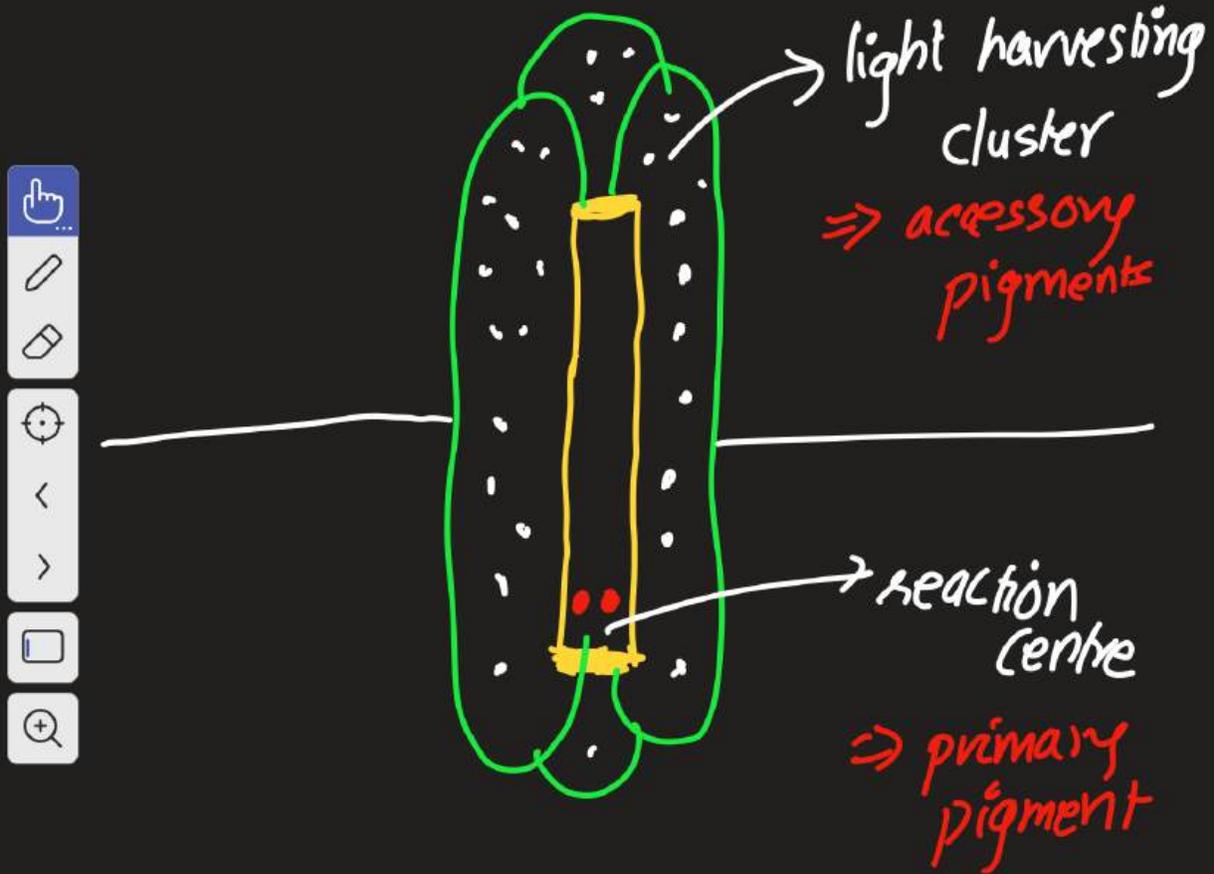
within structures known as PHOTOSYSTEMS.

Thylakoid membranes provide a large surface area to accommodate these photosystems.

Q: What is a photosystem?

Ans: A photosystem is a complex combination of proteins and pigment molecules.

Structure of a photosystem

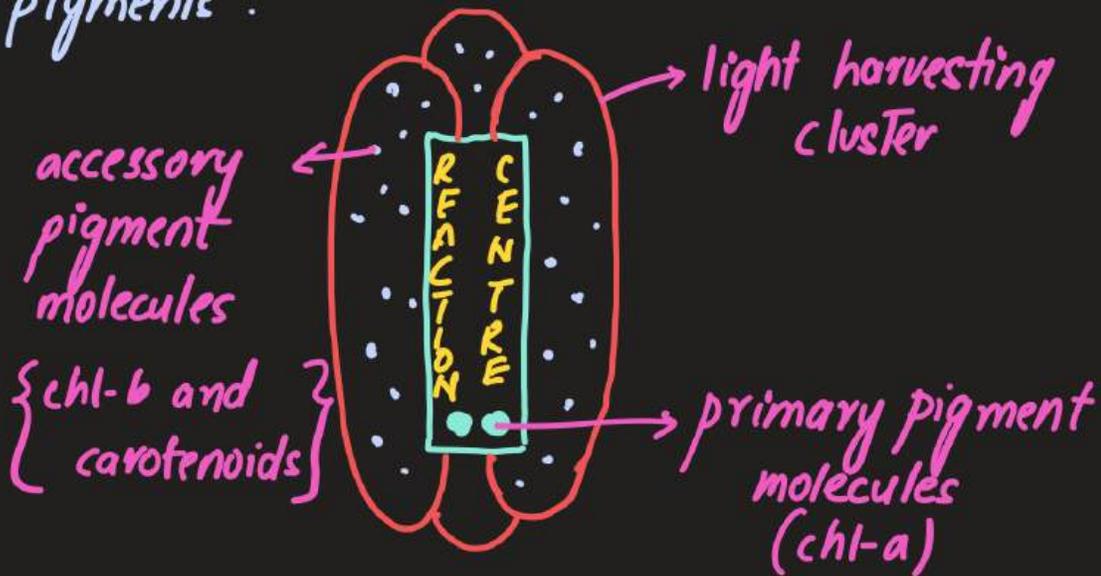


Structure of a photosystem

* A photosystem consists of a **reaction centre** which contains primary pigment molecules.



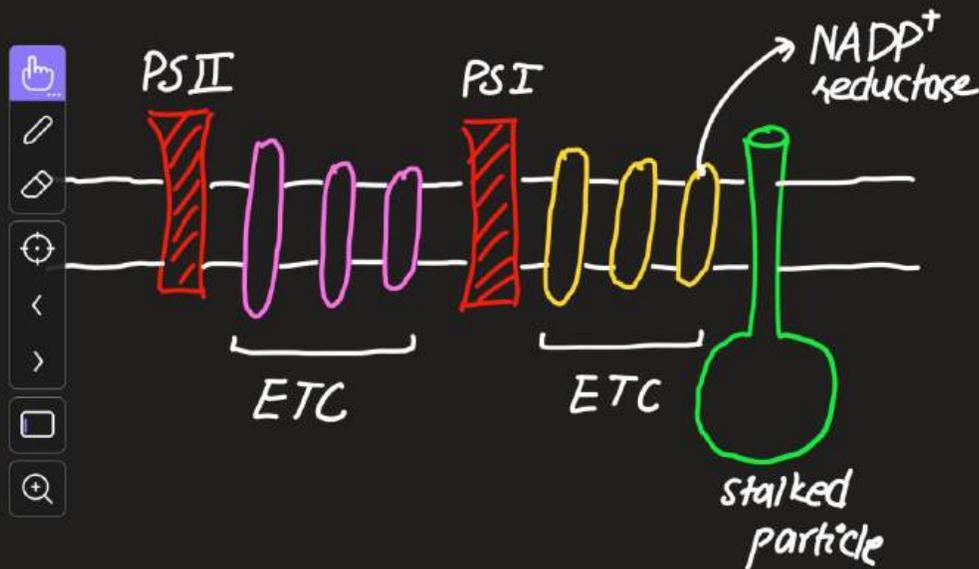
* Surrounding the reaction centre are **light harvesting clusters** which contain accessory pigments.



Photosynthesis

Again

Site B = thylakoid space
 $\uparrow [H^+]$



site A = stroma
 $\downarrow [H^+]$

With
Mohammad Hussham Arshad, MD

ADVANCED LEVEL BIOLOGY 9700 UNIT 13: Photosynthesis

Learning Objectives:

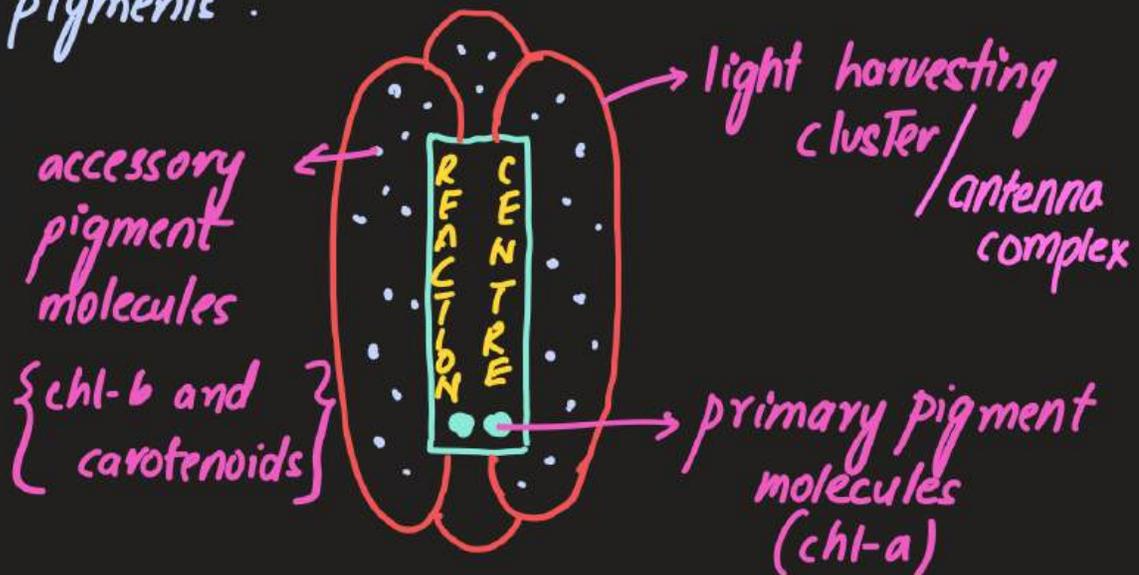
- Photosystems and Pigments 2
- Coenzyme NADP⁺
- Role of Thylakoid and Light

Video Lecture 2 Slides
Mohammad Hussham Arshad, MD
Biology Department

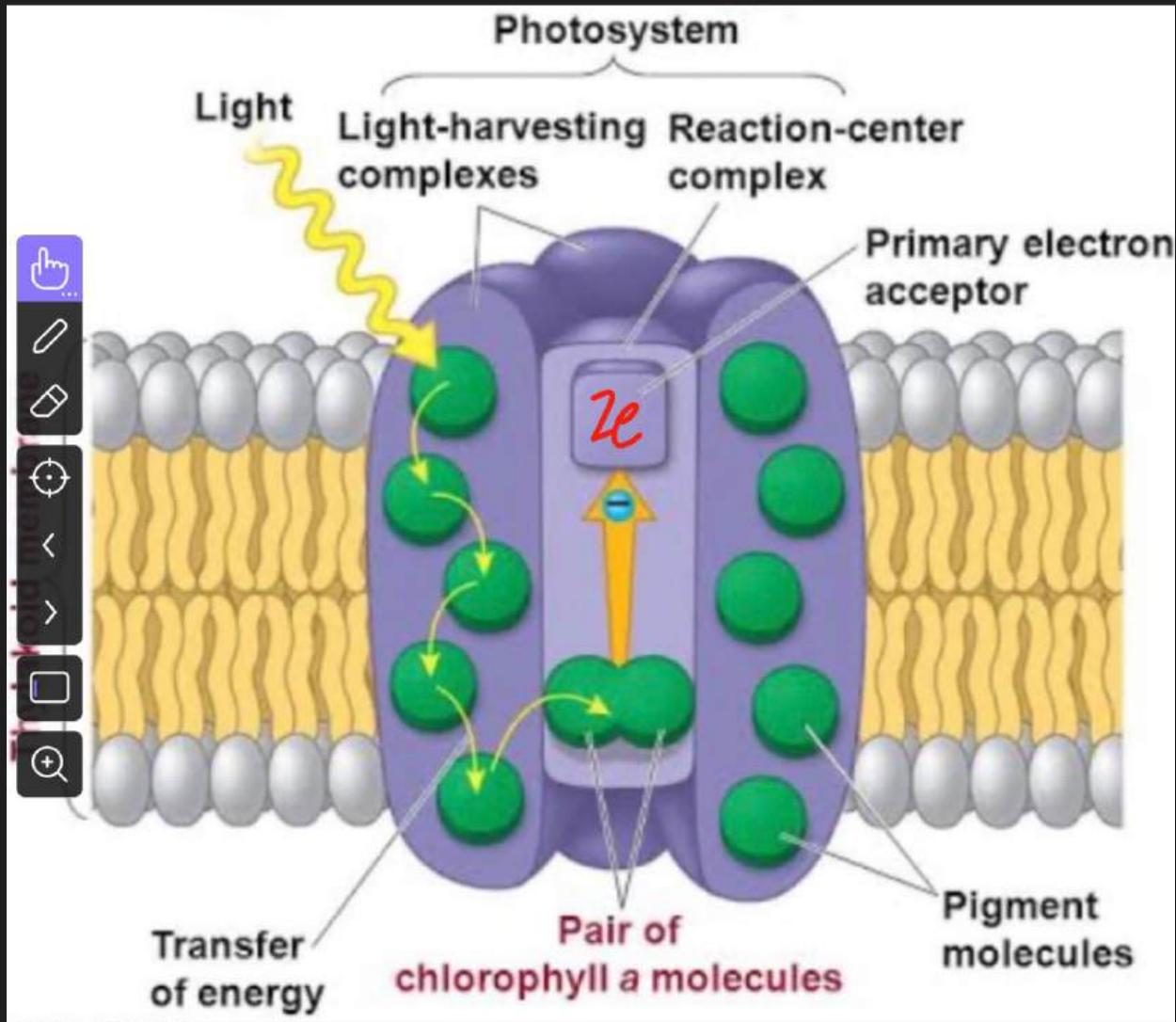
Structure of a photosystem

* A photosystem consists of a **reaction centre** which contains primary pigment molecules.

* Surrounding the reaction centre are **light harvesting clusters** which contain accessory pigments.



Role of accessory pigments



400 nm \longrightarrow 700 nm
blue red

Role of accessory pigments

* The accessory pigment absorb wavelengths of light not absorbed by the primary pigments.



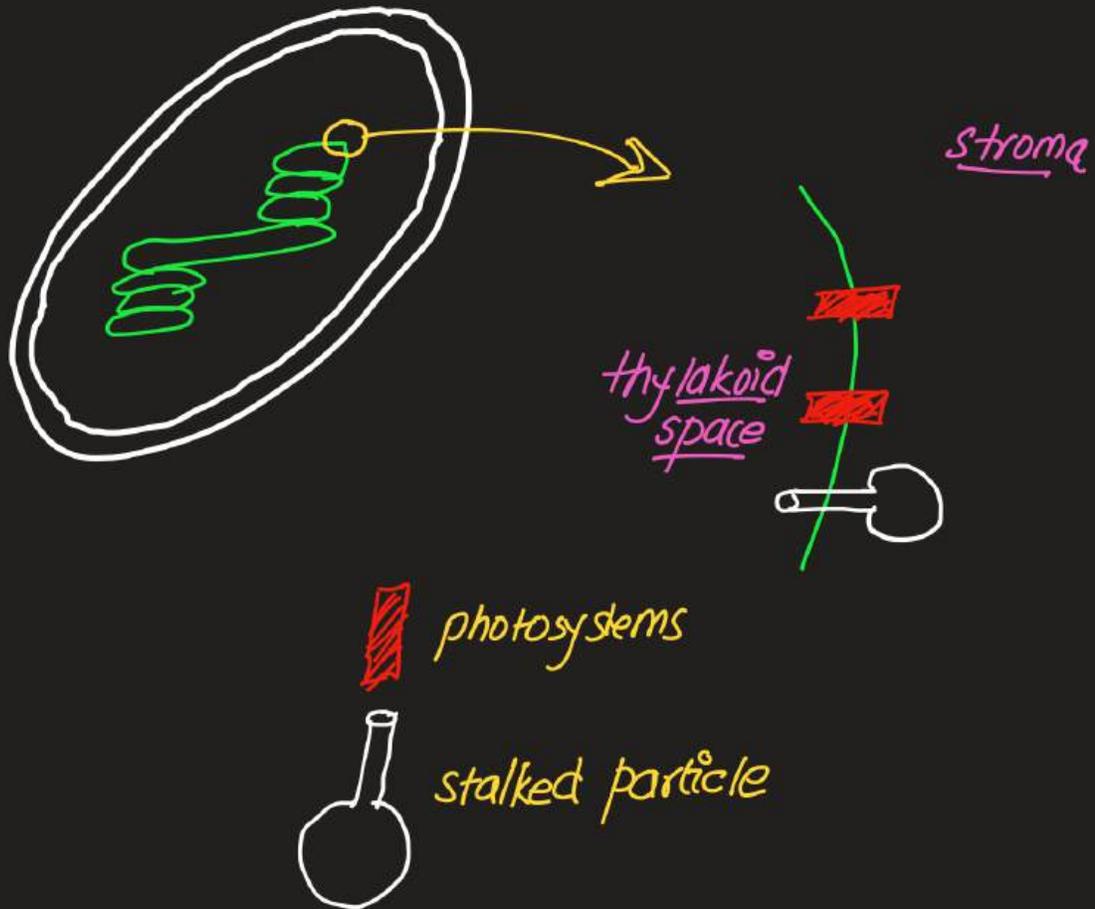
The accessory pigments absorb light (photons) energy and transfer it to primary pigment molecules within the reaction centre.

→ Accessory pigments also protect the primary pigments from excessive light (photo protection)

* Before we continue to elaborate further on photosystems, take note that.....

the thylakoid membranes provide a large surface area to accommodate

- * stalked particles and
- * the photosystems



* Continuing with the photosystems



.... let's have a look at the
different types of photosystems

* There are Two types of photosystems:

① Photosystem I (PS I)

- ① The primary pigments in PSI absorb at an optimum wavelength of 700 nm.
- ② PSI is therefore also termed as P700.
- ③ PSI does NOT contain the **water splitting centre**.

④ Photosystem II (PS II)



① The primary pigments in PS II absorb at an optimal wavelength of 680 nm.

② PS II is therefore also termed as P680.

③ PS II contains the **water splitting centre**

(an enzyme complex responsible for splitting water)

PS I → 700 nm

PS II → 680 nm
⇒ 8-6=2

Q. Differentiate between photosystem I and photosystem II.



Visible light & colour of the pigment

NOTE: ① visible light has a range of wavelengths from 400-700 nm.

② for simplicity, we can divide visible light into three regions:



chlorophylls → red
 → blue reflect green

carotenoids → blue + green
 reflect red

* chlorophylls generally absorb in the blue and red regions of the spectrum.

⇒ thus appear green in colour.

* carotenoids absorb in the blue and green regions of the spectrum.

⇒ thus appear brown, orange or red in colour.

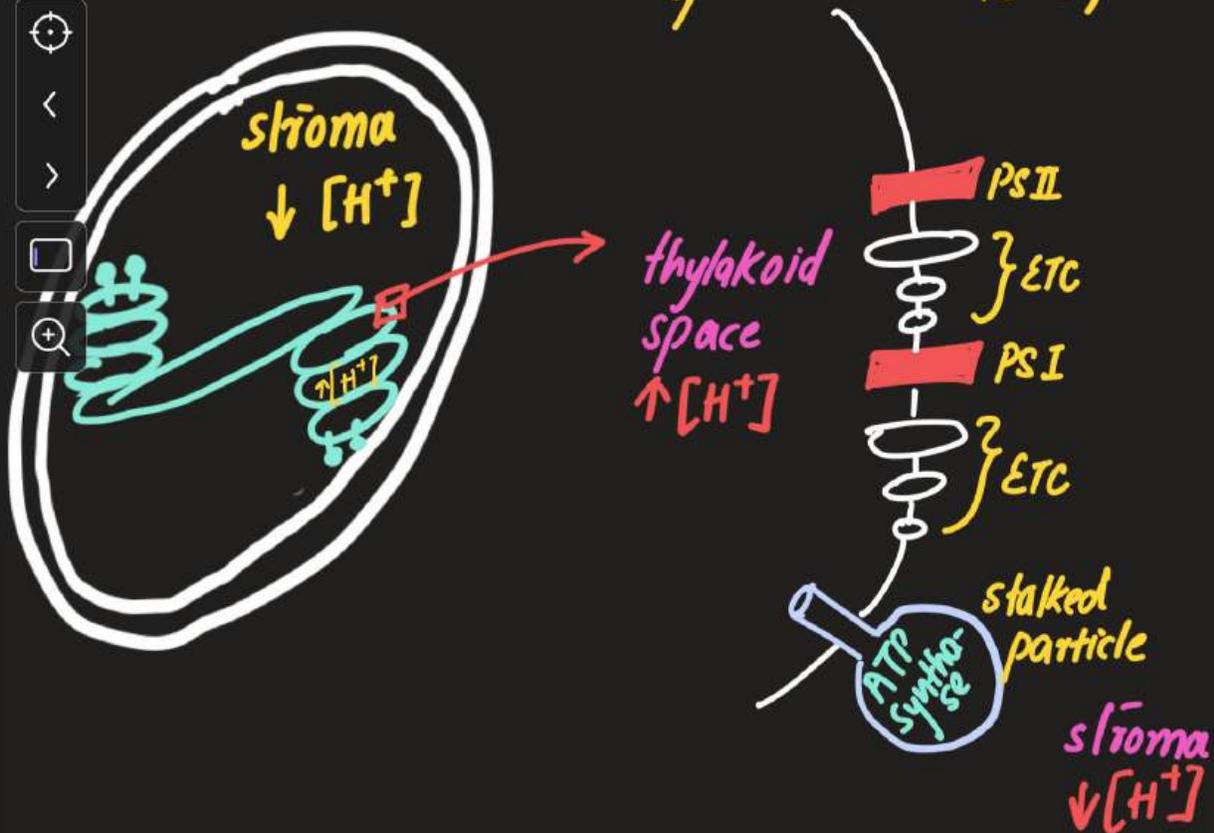


Role of thylakoid membranes
and
light in photosynthesis

Role of thylakoid membranes

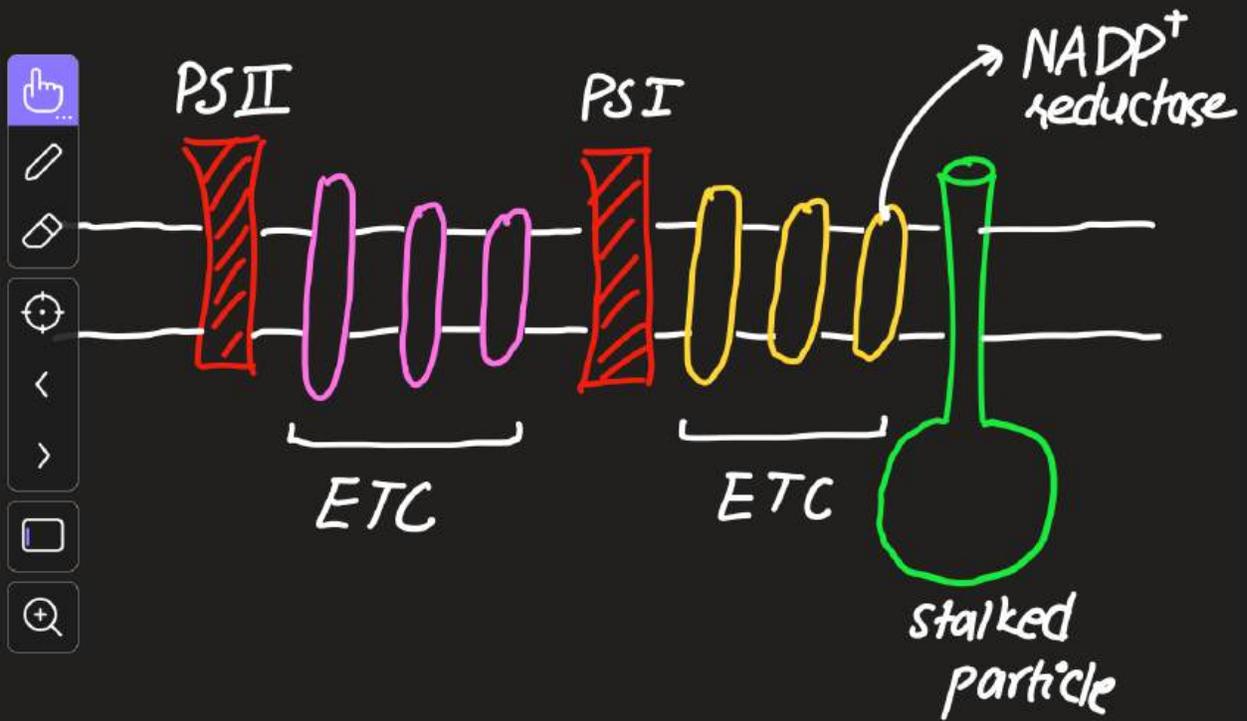
* Thylakoid membranes provide a large surface area to accommodate;

- ① Photosystems
- ② Stalked particles
- ③ Electron Transport chain (ETC)



Again

site B = thylakoid space
 $\uparrow [H^+]$



site A = stroma
 $\downarrow [H^+]$

Role of light in photosynthesis

PHOTOACTIVATION OF PIGMENT MOLECULES

-  * Accessory pigments absorb light energy and transfer it to primary pigment molecules.
- 
- 
- 
- 
- 
- 
- 
- * Primary pigment molecules undergo excitation and lose electrons (oxidation)
- * This is termed as photoactivation of pigment molecules

PHOTOLYSIS OF WATER

- * occurs in the presence of the water splitting centre within PSII using energy in the form of photons.
- * occurs in two steps

Photoactivation of chlorophyll a molecules

Accessory pigments absorb light energy (photons) of different wavelengths

↓
light energy transferred to primary pigments in the reaction centre

↓
primary pigments absorb energy which causes excitation of electrons

↓
electrons lost from primary pigments



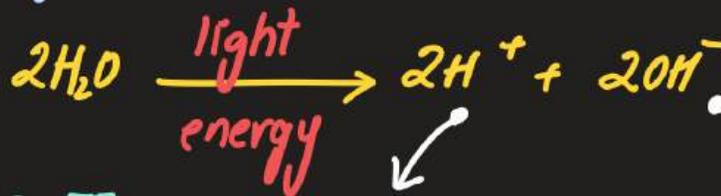
PHOTOLYSIS OF H₂O

Two Steps of photolysis of water:

STEP I

* water is broken down to form protons

and hydroxide ions;



used to reduce
NADP⁺

further
broken down

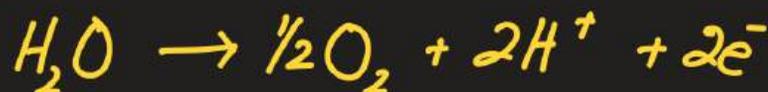
STEP II

* OH⁻ are further broken down to release

electrons, O₂ (g) and some water;



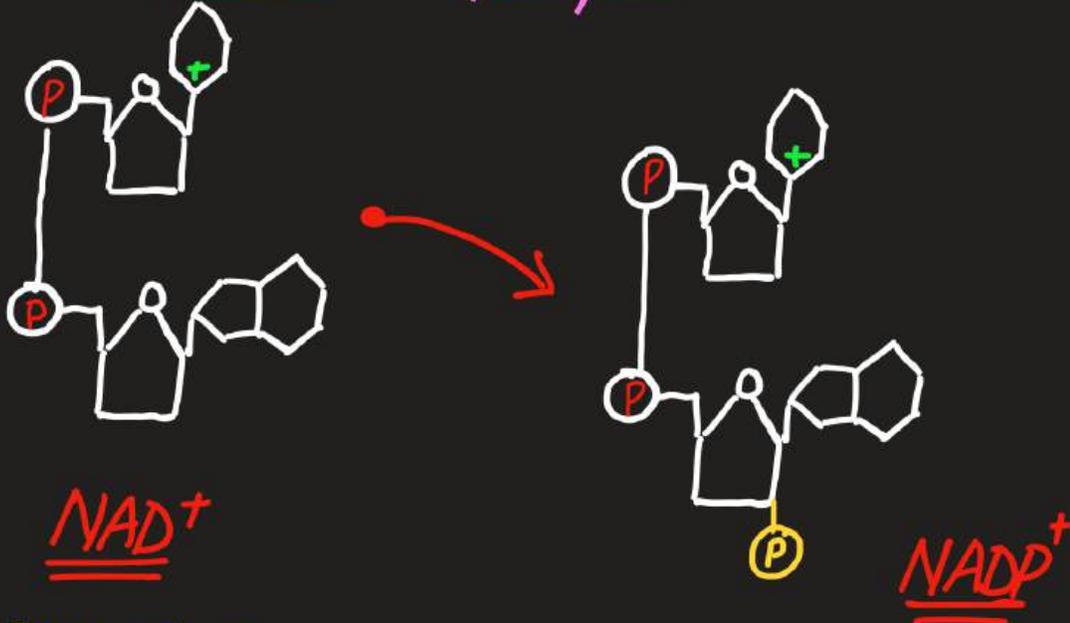
OVERALL:





Coenzyme used in photosynthesis

NADP⁺ (nicotinamide adenine dinucleotide phosphate)



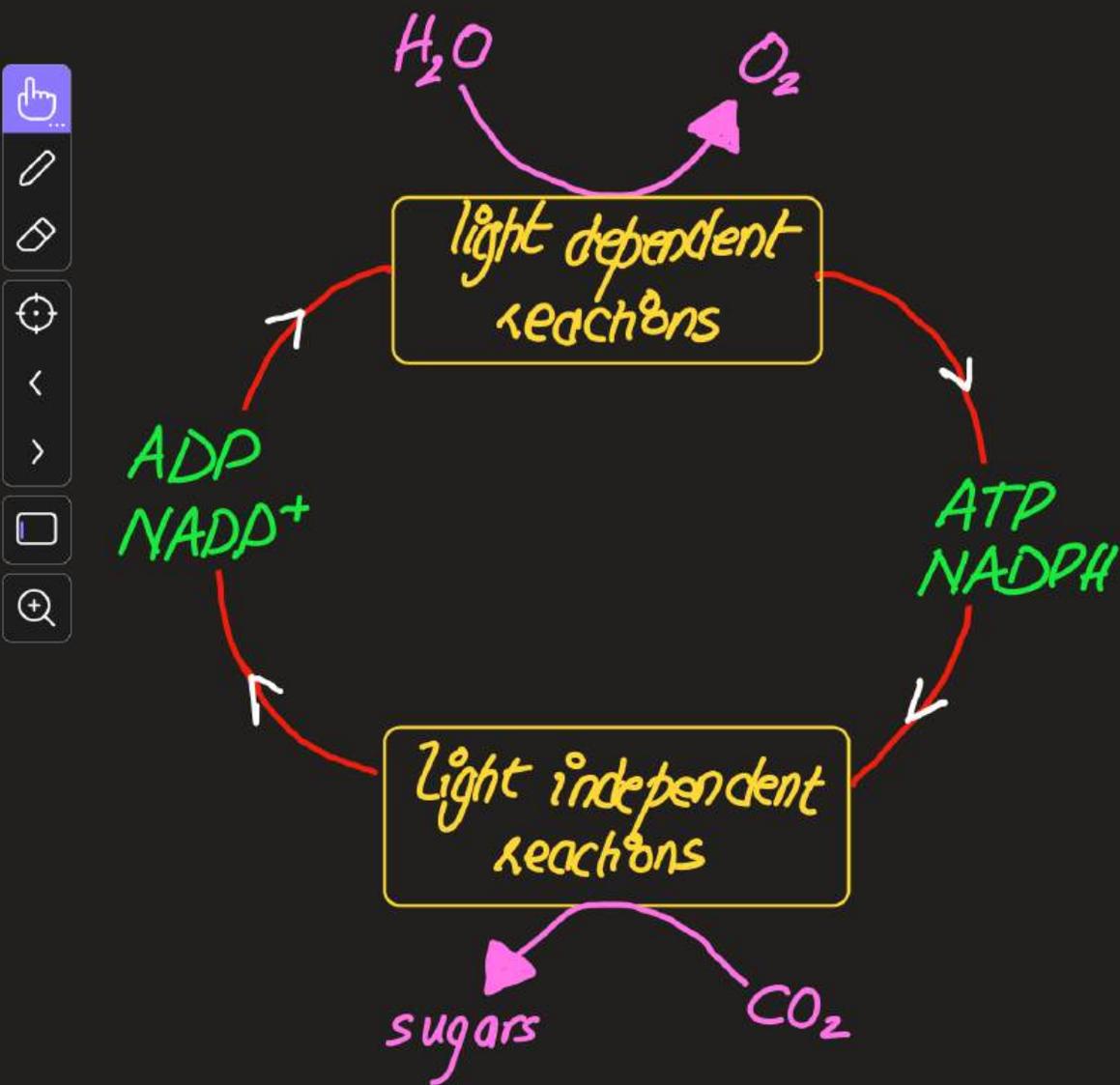
- * NADP⁺ is a coenzyme
- * It accepts protons and electrons in the presence of NADP reductase to form NADPH (reduced NADP).



- * NADPH is used to reduce CO₂ in the Calvin cycle to form sugars.

Coming up next.....

Summary of Photosynthesis





Questions

Question 1

1 Fig. 1.1 is a diagram of a palisade cell from a dicotyledonous leaf.

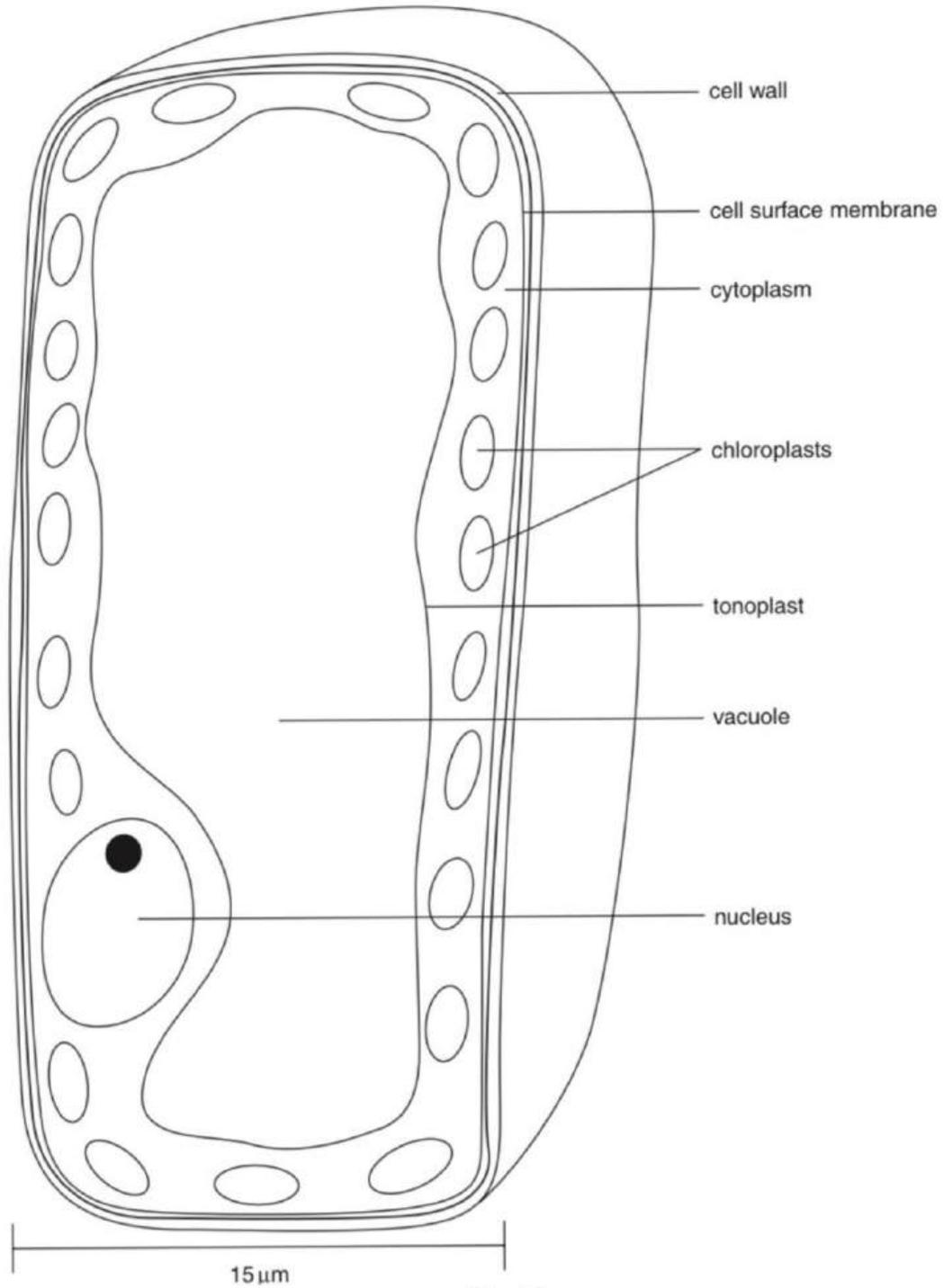


Fig. 1.1



(a) Describe how these cells are arranged in the leaf.

* upper half of the leaf beneath the upper epidermis

* closely packed * right angles to leaf surface [2]

(b) With reference to Fig. 1.1, explain how the structure of this cell is related to its function in photosynthesis.

* thin cell wall →

* large numbers of chloroplast →

* large central vacuole →

[3]

Question 2

- 1 Fig. 1.1 shows the arrangement of photosystems, protein complexes containing chlorophyll molecules, on the thylakoid membrane of a plant chloroplast.

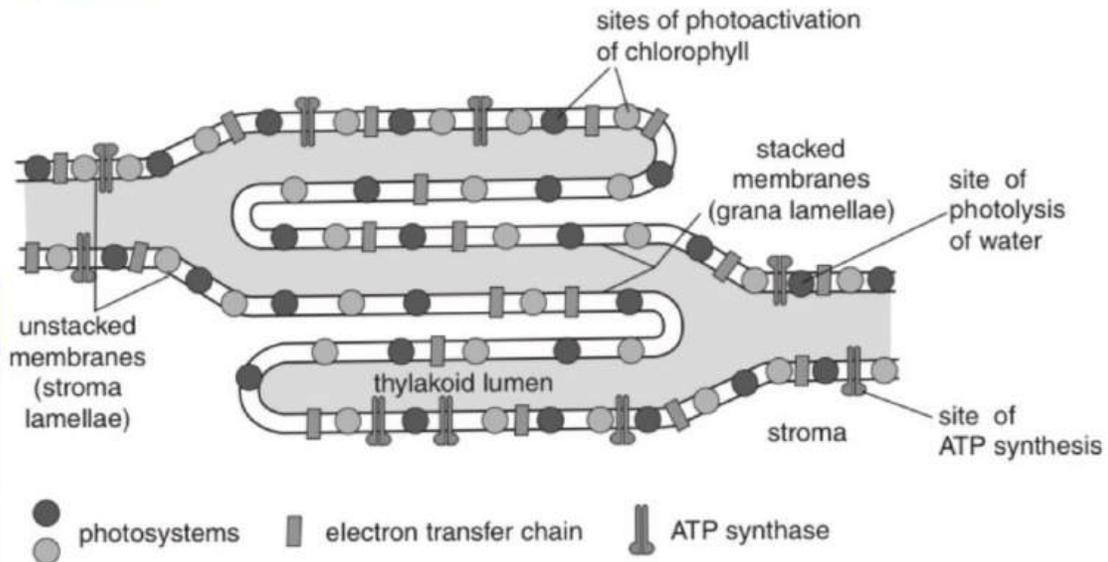


Fig. 1.1

- (a) Describe the photoactivation of chlorophyll.

* chlorophylls absorb mainly blue and red light
* light absorbed by antenna complexes
* energy transferred to primary pigments in reaction centre
* electron lost from chlorophyll

[3]

- (b) Explain how the photolysis of water occurs.

* water is split into H^+ and OH^-
* H^+ used to form reduced NADP
* OH^- split further to form O_2 and H_2O and electrons

[3]

Question 3

(d) A palisade mesophyll cell is adapted to carry out photosynthesis. The table below lists some of the adaptations of a palisade mesophyll cell.

Complete the table to show how these adaptations help the cell to carry out photosynthesis.

adaptation	how the adaptation helps photosynthesis
thin cell wall	* short diffusion distance
cylindrical shape	* air spaces to serve as reservoirs of CO_2
large vacuole	* chloroplasts pushed towards the edges of the cell
chloroplasts can be moved within the cell	* absorb maximum light * avoid excessive light

[4]

Question 4

(d) Fig. 8.3 shows the relationship between the light-dependent and light-independent reactions in a chloroplast.

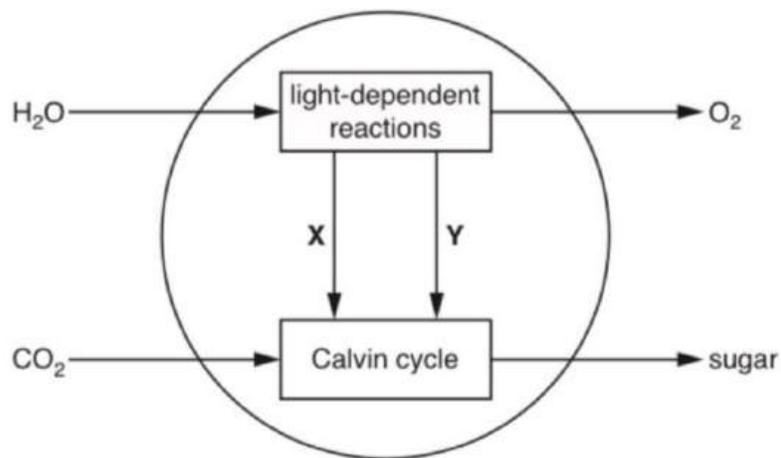


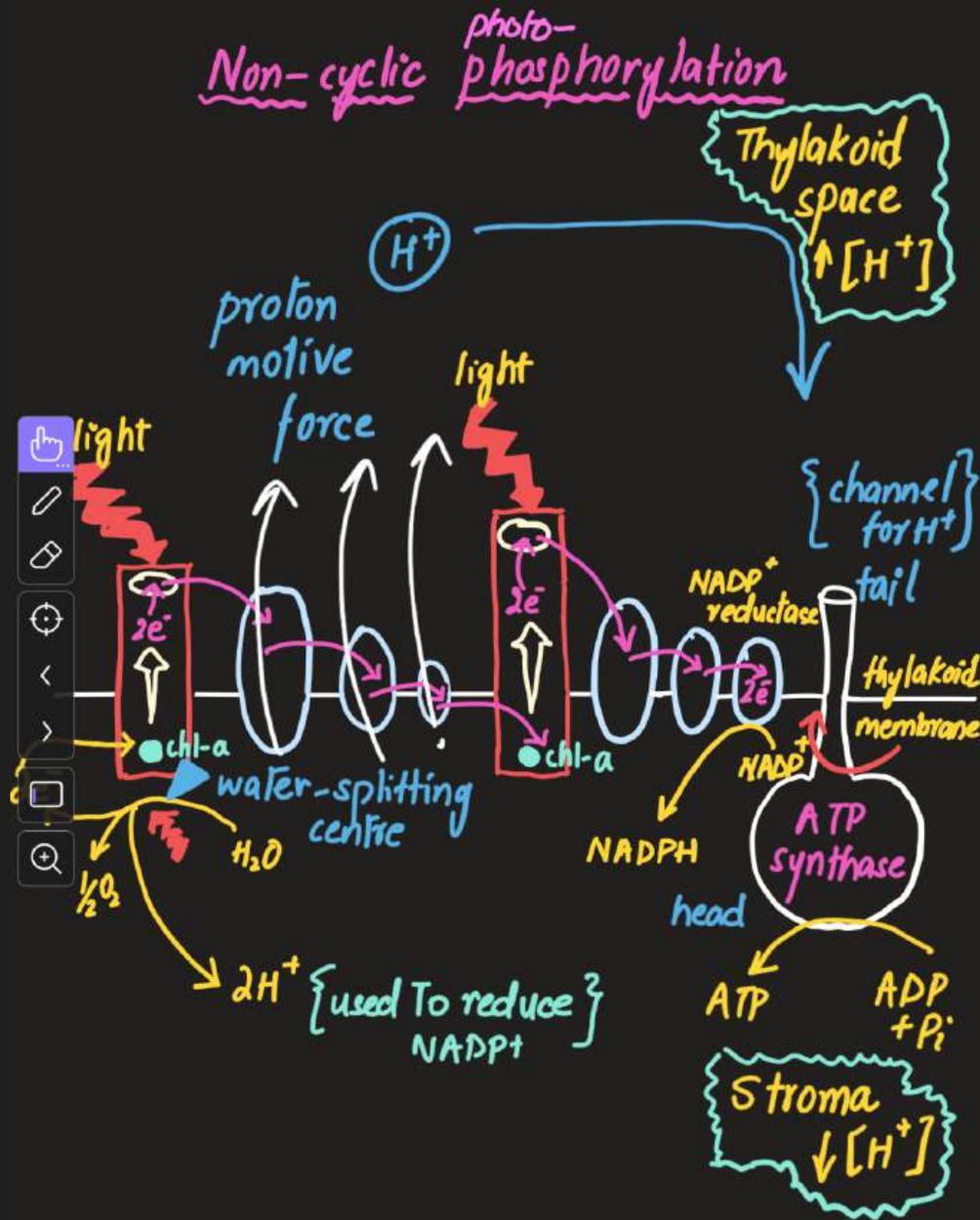
Fig. 8.3

Name the substances X and Y in Fig. 8.3.

X ATP

Y NADPH [2]

Photosynthesis



With
Mohammad Hussham Arshad, MD

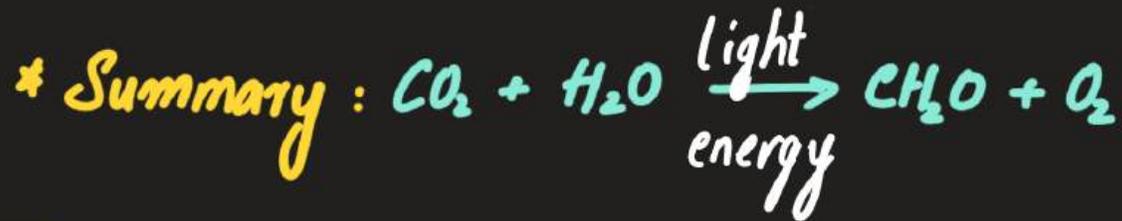
ADVANCED LEVEL BIOLOGY 9700 UNIT 13: Photosynthesis

Learning Objectives:

- Light Dependent Reactions

Video Lecture 3 Slides
Mohammad Hussham Arshad, MD
Biology Department

Photosynthesis



Photosynthesis occurs in two steps:

① Light dependent reactions

② Light independent reactions

Light Dependent Reactions

* occurs on the thylakoid membrane.

* involves the production of **ATP** and **NADPH**

using light energy. ATP and NADPH are required for the Calvin cycle.

* **Oxygen** (O_2) is produced as the by-product of the reaction.

* Light dependent reactions involve;

- (a) Non-cyclic photophosphorylation
- (b) Cyclic photophosphorylation

Q: What does ^{photo-} phosphorylation mean?

Ans: Photophosphorylation refers to the

production of ATP in the presence of proton motive force using energy derived from light.

light energy \longrightarrow chemical energy
(ATP)

* Given below are the differences between non-cyclic and cyclic phosphorylation.

NON-CYCLIC

CYCLIC

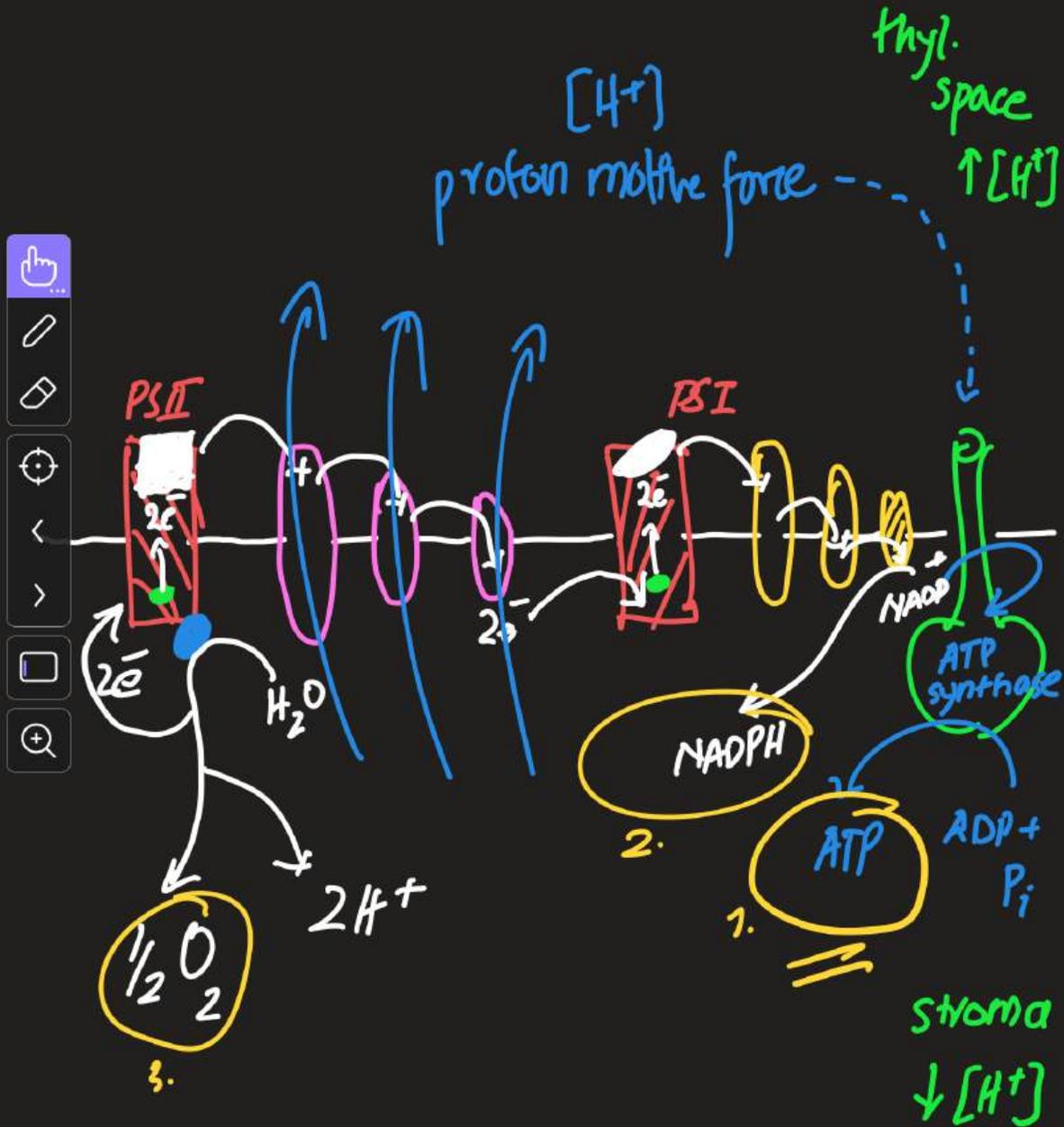
PHOTOPHOSPHORYLATION

PHOTOPHOSPHORYLATION

- ① Non-cyclic flow of electrons.
- ② first electron donor is water.
- ③ Last electron acceptor is $NADP^+$
- ④ Uses both PSI and $PSII$.
- ⑤ Involves photolysis of water.
- ⑥ Products : O_2 , ATP & NADPH

- ① Cyclic flow of electrons.
- ② first electron donor is PSI .
- ③ last electron acceptor is PSI (thus cyclic)
- ④ Uses only PSI .
- ⑤ Does not involve photolysis of water.
- ⑥ Products : ATP only

Non-cyclic photophosphorylation
($\text{NADP}^+ + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{NADPH} + \text{H}^+$)



Non-cyclic photophosphorylation

- 1) Accessory pigments in light harvesting complexes in PSI and PSII absorb light energy
- 2) This light energy is passed to primary pigment molecules in the reaction centre

Chl-a molecules are the primary pigments

Electrons in chl-a molecules excited to a higher energy level.

These electrons are picked up by electron acceptors and pass down the ETC to produce;

* ATP via chemiosmosis

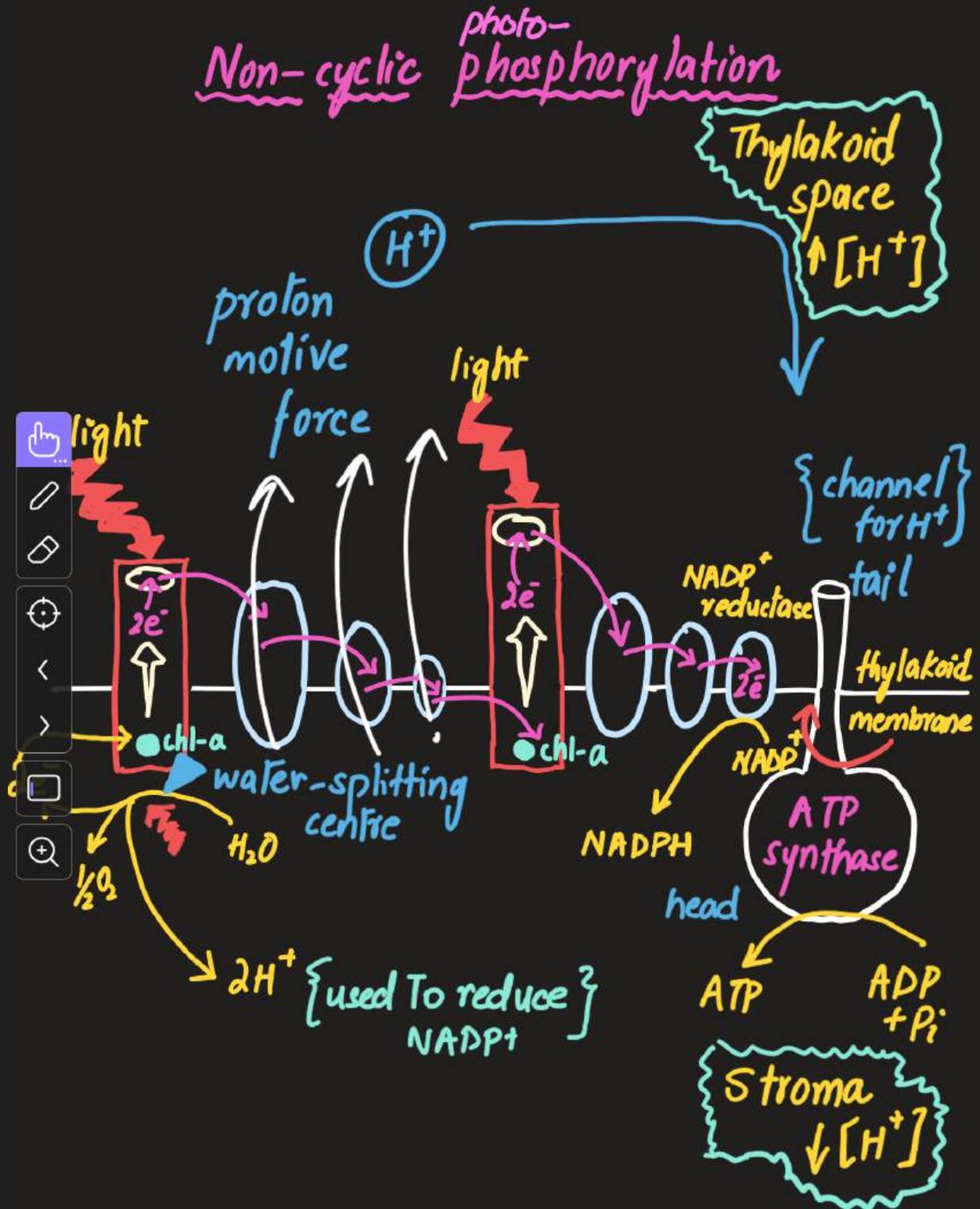
* NADPH by combining NADP^+ + protons + electrons

Electrons lost by PSI are replaced by electrons from PSII.

7) Electrons lost by PSII are replaced by electrons produced via photolysis of H_2O .

8) Photolysis occurs in PSII which contains the water splitting centre. Photolysis of H_2O produces oxygen, protons and electrons.







Products of photolysis of H_2O



* used to reduce $NADP^+$



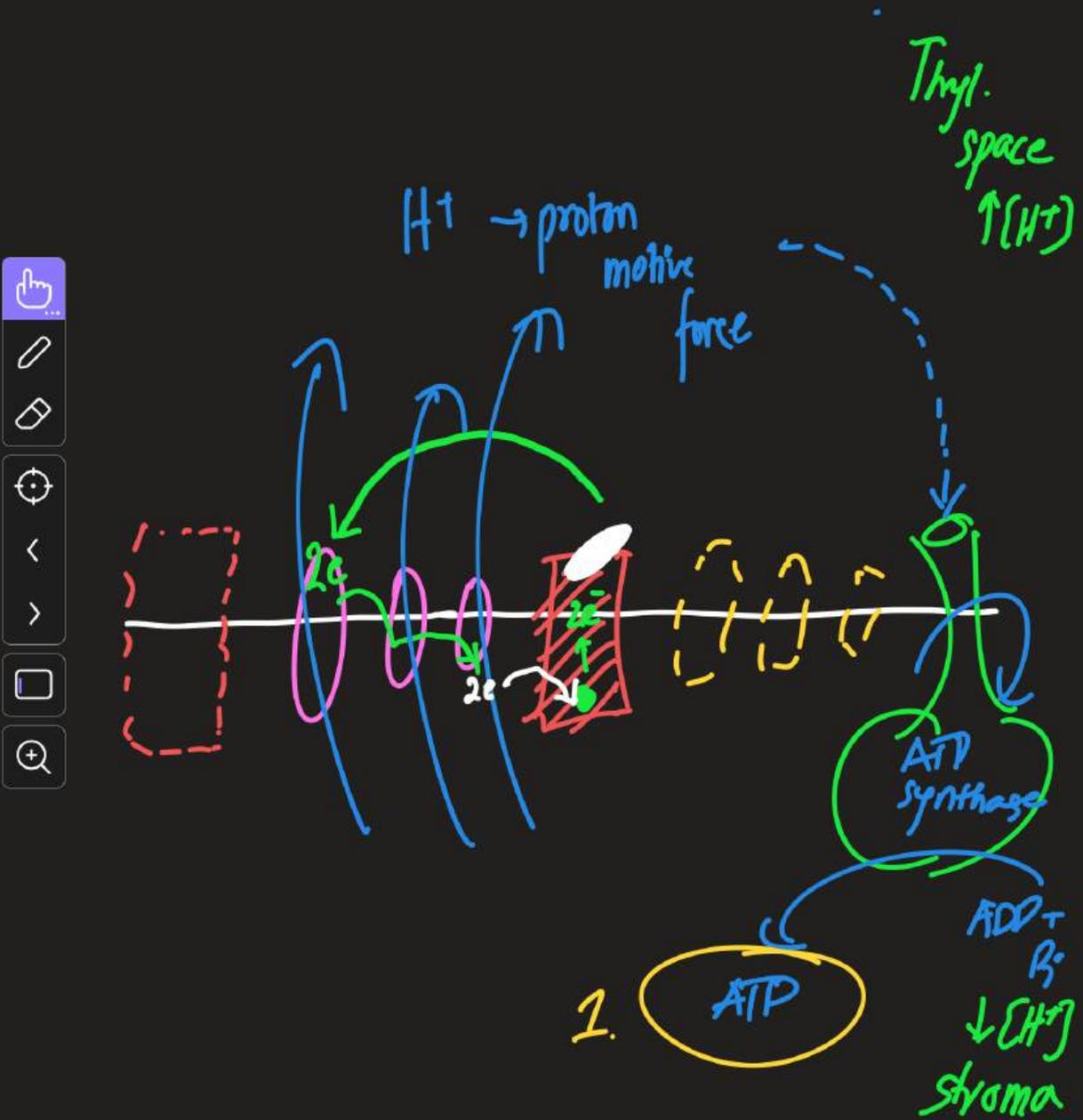
* replace the electrons lost by PSII in non-cyclic photophosphorylation



* diffuses out of the chloroplast and can.....

- 1) either be used for aerobic respiration, or
- 2) diffuse out via the stomata

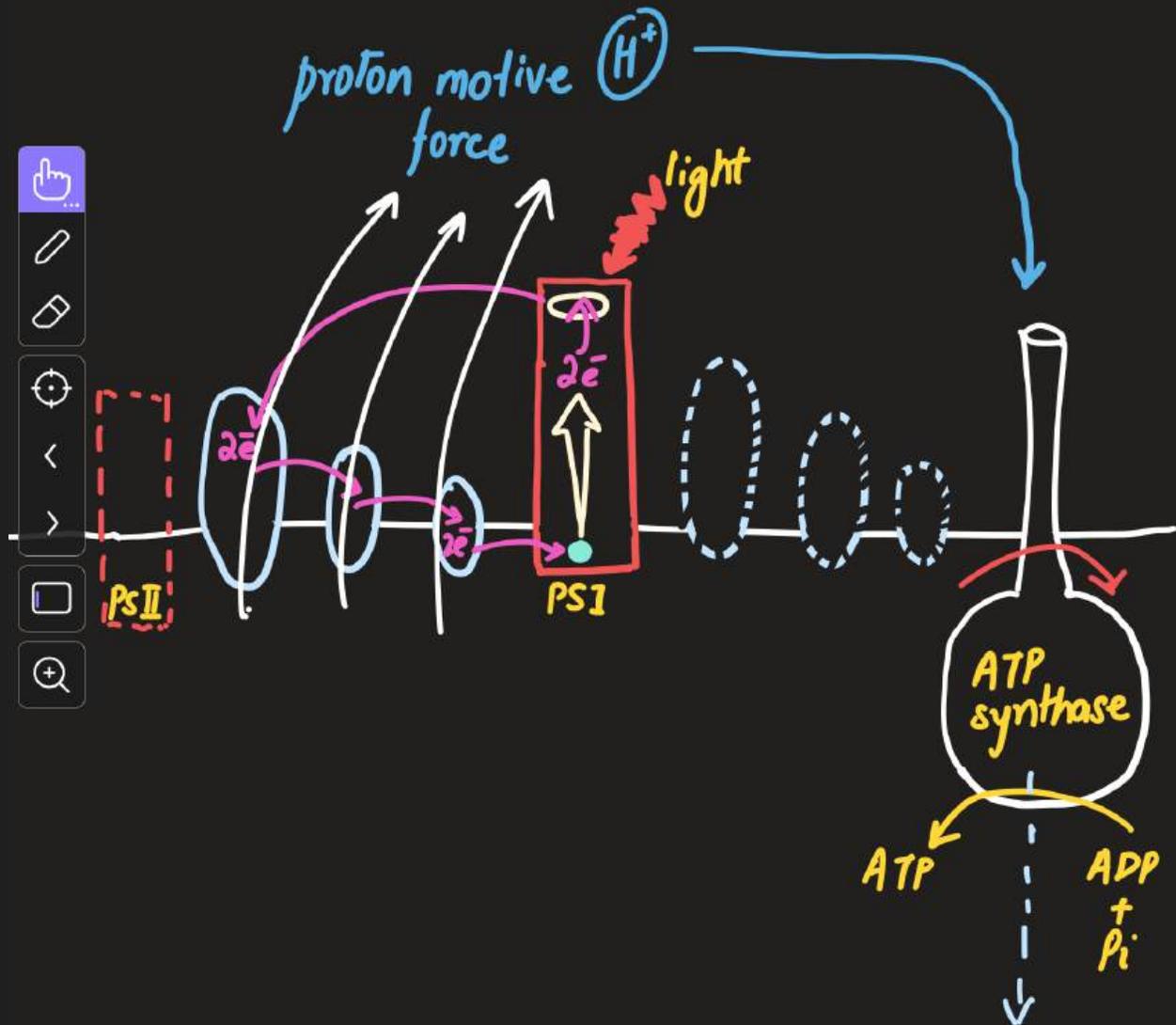
Cyclic photophosphorylation

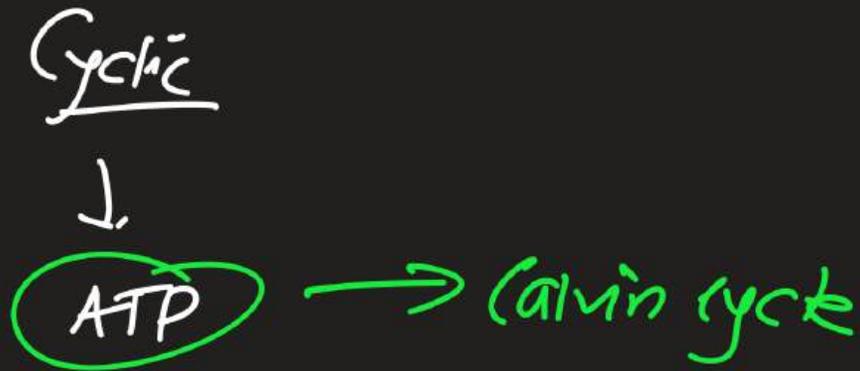
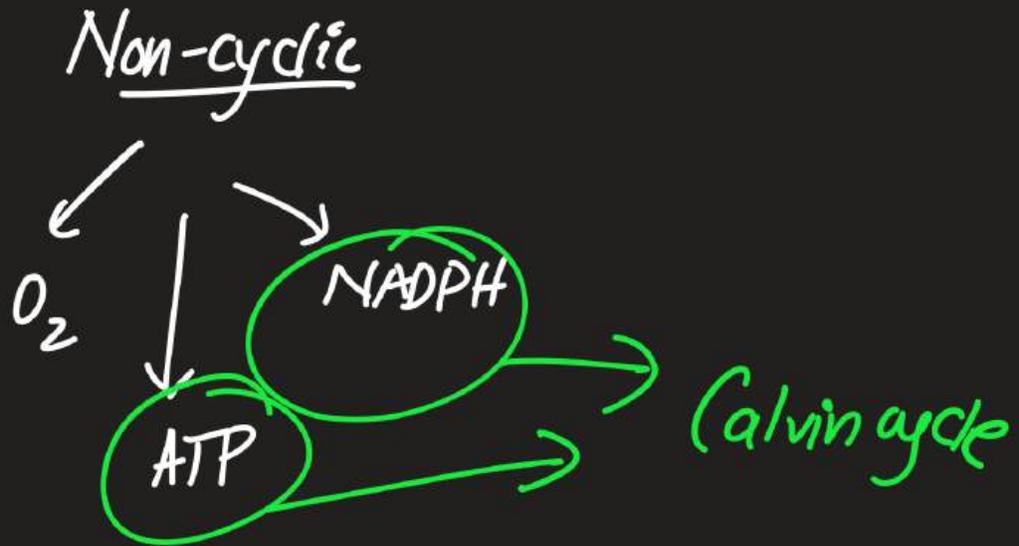


Cyclic photophosphorylation

- 1) Accessory pigments in light harvesting clusters of PSI absorb light energy and pass it to primary pigments within the reaction centre.
- 2) Chl-a molecules are the primary pigment molecules.
- 3) The electrons in chl-a are excited to a higher energy level and emitted.
- 4) The electrons are picked up by a primary electron acceptor (ferredoxin) and passed to a chain of electron carriers.
- 5) ATP is synthesized via chemiosmosis.
- 6) electrons return to PSI.

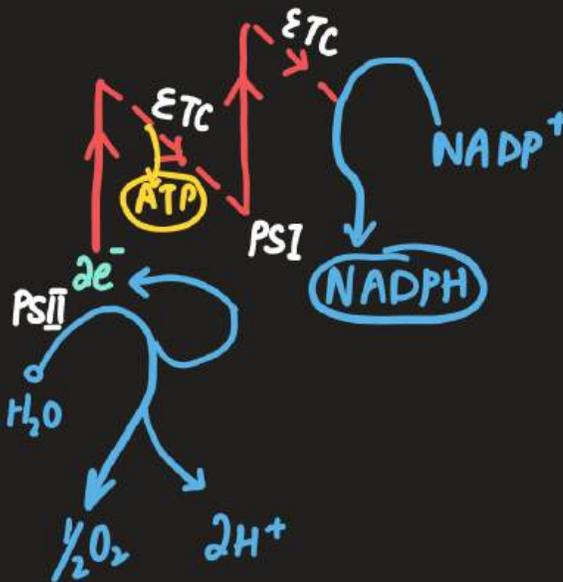
Photo- Cyclic Phosphorylation





Summary of the flow of electrons in photophosphorylation

NON-CYCLIC



CYCLIC



* Given below are the differences between non-cyclic and cyclic phosphorylation.

NON-CYCLIC

CYCLIC

PHOTOPHOSPHORYLATION

PHOTOPHOSPHORYLATION

- ① Non-cyclic flow of electrons.
- ② first electron donor is water.
- ③ Last electron acceptor is NADP^+
- ④ Uses both PSI and PSII .
- ⑤ Involves photolysis of water.
- ⑥ Products : O_2 , ATP & NADPH

- ① Cyclic flow of electrons.
- ② first electron donor is PSI .
- ③ last electron acceptor is PSI (thus cyclic)
- ④ Uses only PSI .
- ⑤ Does not involve photolysis of water.
- ⑥ Products : ATP only

Q1: Outline the structure of the photosystems? [8]

Q2: Briefly describe photolysis of water? [6]

Q3: Describe how non-cyclic phosphorylation leads to production of ATP and NADPH? [10]

Q4: Outline the process of cyclic photophosphorylation? [6]

Q5: Differentiate between cyclic and non-cyclic photophosphorylation? [6]



PAST PAPER QUESTIONS

Question 1:

(i) Cyclic and non-cyclic photophosphorylation take place in the light-dependent stage of photosynthesis.

(i) Describe the role of accessory pigments in photophosphorylation.

- absorb light energy
- pass light energy to primary pigments

[2]

(ii) Write a balanced equation that summarises photolysis.



[1]

(iii) State **precisely** the location of photosynthetic pigments within a chloroplast.

thylakoid membrane

[1]

Question 2:

Q35 (N13/43/q7)

The light-dependent stage of photosynthesis takes place on the thylakoids of the chloroplast.

Fig. 7.1 shows some of the components involved in the light-dependent stage.

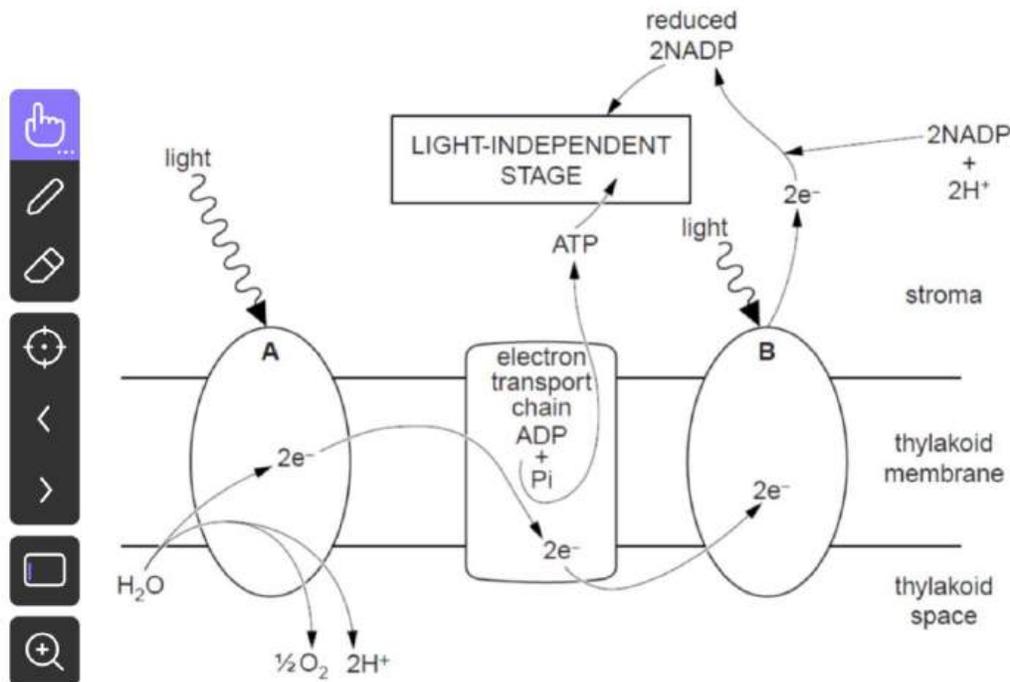


Fig. 7.1

(a) With reference to Fig. 7.1, identify structures A and B.

- A PS II
- B PS I [2]

Question 3 :



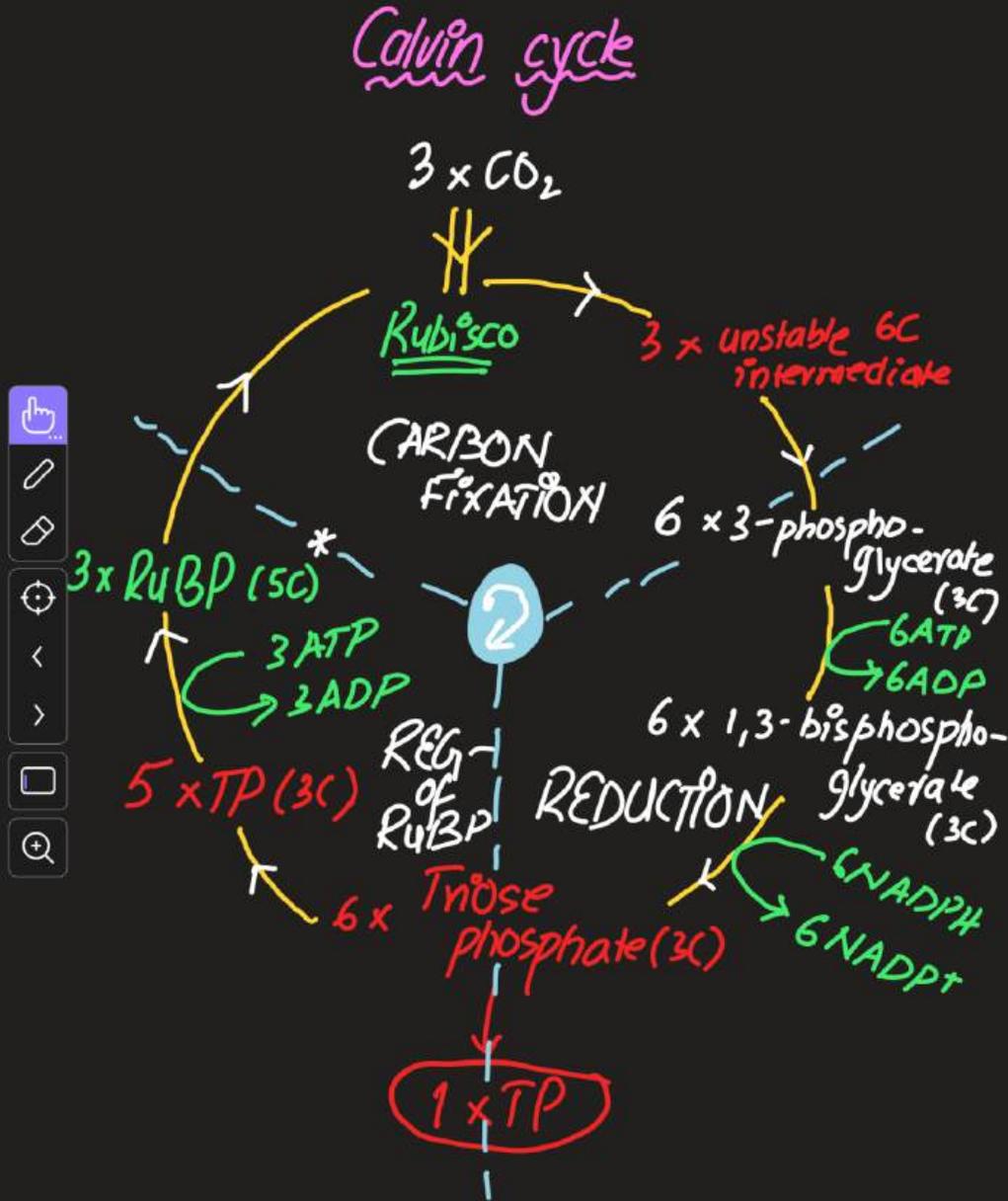
Complete the following paragraph by using the most suitable words to fill in the gaps.

In a photosystem, several hundred accessory pigment molecules surround a primary pigment molecule, called chlorophyll - a, in the thylakoid membrane.

The position of the primary pigment is also called the reaction centre.

Light energy is absorbed by the accessory pigments and passed on to the primary pigment. Electrons are excited to a higher energy level. They are emitted from the primary pigment and are captured by electron acceptors and eventually pass along the electron transport chain, producing ATP. [4]

Photosynthesis



With
Mohammad Hussham Arshad, MD

ADVANCED LEVEL BIOLOGY 9700 UNIT 13: Photosynthesis

Learning Objectives:

- Calvin Cycle (light independent reaction)

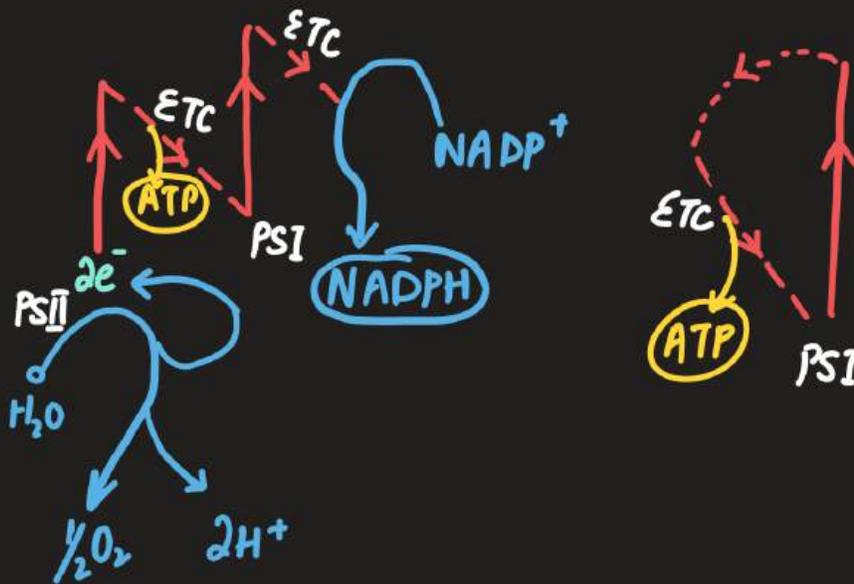
Video Lecture 4 Slides
Mohammad Hussham Arshad, MD
Biology Department

Previously.....

Summary of the flow of electrons in photophosphorylation

NON-CYCLIC

CYCLIC



* Given below are the differences between non-cyclic and cyclic phosphorylation.

NON-CYCLIC

PHOTOPHOSPHORYLATION

- ① Non-cyclic flow of electrons.
- ② first electron donor is water.
- ③ Last electron acceptor is $NADP^+$
- ④ Uses both PSI and $PSII$.
- ⑤ Involves photolysis of water.
- ⑥ Products : O_2 , ATP & NADPH

CYCLIC

PHOTOPHOSPHORYLATION

- ① Cyclic flow of electrons.
- ② first electron donor is PSI .
- ③ last electron acceptor is PSI (thus cyclic)
- ④ Uses only PSI .
- ⑤ Does not involve photolysis of water.
- ⑥ Products : ATP only

Q1 : Outline the structure of photosystems?

Ans : Photosystem is the complex combination of proteins and pigment molecules . Photosynthetic pigment are subdivided into primary and accessory pigments. Primary pigment (chlorophyll a) are present within the reaction centre and the accessory pigments are located in the light harvesting clusters of a photosystem. The accessory pigment absorbs light energy and pass it onto the primary pigment molecules which thereafter undergo photo activation.

There are two types of photosystems . Photosystem I (PSI) and Photosystem II (PS II) . The primary pigments in PSI absorb light at an optimal wavelength of 700nm. The primary pigment within PSII absorb light at an optimal wavelength of 680 nm. PSII also contain an enzyme known as the water splitting centre.



Q2: Outline how the non cyclic photophosphorylation leads to production of ATP and NADPH ?

Ans : Non cyclic photo phosphorylation involves non cyclic flow of electrons through a chain of electron carriers to produce ATP and NADPH . Both PSI and PSII are involved in the process. The accessory pigments in light harvesting clusters absorb light energy and transfer it to primary pigments in the reaction centre. Chlorophyll-a molecules are the primary pigment molecules. The electrons in these pigment molecules are excited to a higher energy level and emitted. These electrons are picked up by electron acceptors and passed to a chain of electron carriers. ATP is produced via chemiosmosis. Photolysis of water occurs in PSII in the presence of water splitting centre to produce oxygen, protons and electrons. These electrons replace the electrons lost by PSII. The electrons lost by PSI are replaced by electrons of PSII. Reduced NADP is produced by accepting protons and electrons in the presence of NADP reductase. The products of non cyclic photophosphorylation are ATP, NADPH and O₂.

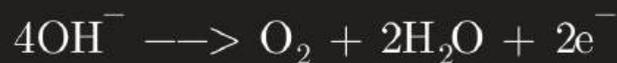


Q3: Outline the process of photolysis of water ?

Ans : Photolysis of water refers to the breakdown of water molecules using energy in the form of light . The process occurs within PSII in the presence of the enzyme the water splitting centre . Photolysis of water is a two step process . The first step involves the breakdown of water molecules to form protons and hydroxide ions;



In the second step hydroxide ions are further broken down to form molecular oxygen, water and electrons;



The overall yield of photolysis of water is therefore ;



The electrons replace the electron lost by Photosystem II. The proton are used to reduce NADP, molecular oxygen can either escape via stomata or can be used for aerobic respiration.



Q4: Outline the process of cyclic photo phosphorylation?

Ans : Cyclic photophosphorylation leads to production of ATP as the electrons pass through a chain of electron carriers . It only involve PSI which serve as the first electron donor and the last electron acceptor .



Accessory pigments (chl-b & carotenoids) absorb light energy and pass it to primary pigments in the reaction centre. Photo activation of primary pigment (chlorophyll a) in Photosystem I leads to emission of high energy electrons which are picked up by a primary electron acceptor (Ferredoxin) . The electrons lose energy as they travel through the electron transport chain. This energy is used to transport protons from the stroma into the thylakoid space creating the proton motive force . The protons re enter the stroma via stalked particles which rotate combining ADP and inorganic phosphate to form ATP in the presence of the enzyme ATP synthase.

Q5: Differentiate between cyclic and non cyclic photophosphorylation ?

Non-cyclic photo phosphorylation



- 1) Non cyclic flow of electrons.
- 2) First electron donor is water.
- 3) Last electron acceptor is PSI.
- 4) Uses both PSI and PSII.
- 5) Involves photolysis of water.
- 6) Products : O_2 , ATP and NADPH

Cyclic photo phosphorylation

- 1) Cyclic flow of electrons.
- 2) First electron donor is PSI
- 3) Last electron acceptor is PSI. (thus cyclic)
- 4) Uses only PSI.
- 5) Does not involve photolysis of water.
- 6) Products : ATP only



More past paper questions.....

Question 4:

Q49 (J18/41/q7)

7 (a) Fig. 7.1 outlines the process of non-cyclic photophosphorylation in the chloroplast of a leaf mesophyll cell.

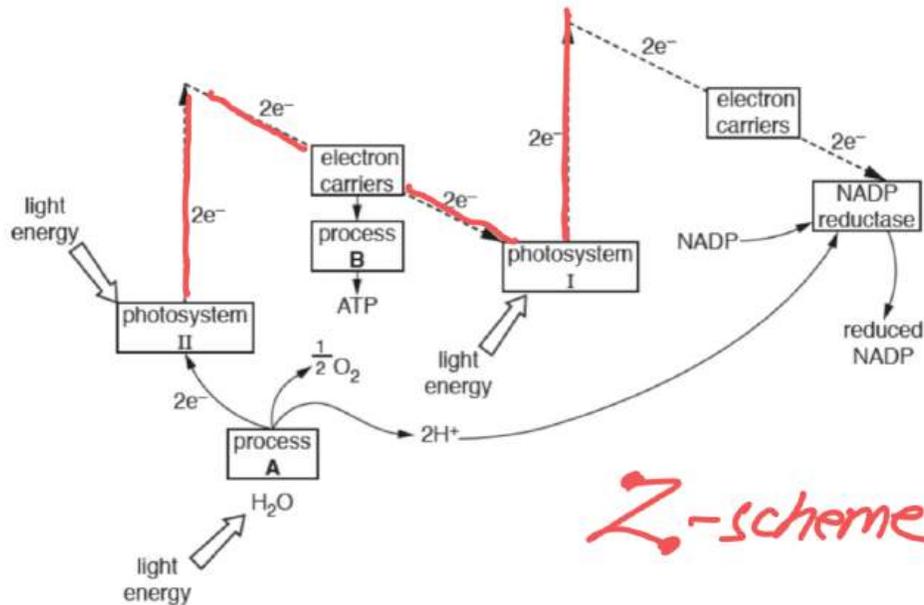


Fig. 7.1

- (i) State precisely where non-cyclic photophosphorylation occurs in the chloroplast.
thylakoid membrane [1]
- (ii) With reference to Fig. 7.1, name processes A and B.
 A *photolysis of H_2O*
 B *chemiosmosis / photophosphorylation* [2]
- (iii) State what happens to the oxygen produced by process A.
1) used in aerobic resp.
2) diffuses out of the stomata [1]
- (iv) Name the primary pigment in photosystem I and photosystem II.
chlorophyll-a [1]
- (v) Name **two** compounds shown in Fig. 7.1 that are used in the conversion of glycerate-3-phosphate (GP) to triose phosphate (TP) in the Calvin cycle.
~~ATP~~
~~reduced NADP~~
 [1]

Question 5:

Q56 (N19/43/q6)

Mining may result in the release of heavy metal ions, causing pollution of lakes and rivers.

High concentrations of these heavy metal ions, such as cadmium (Cd^{2+}) and copper (Cu^{2+}), decrease the rate of photosynthesis in plants.

a) Cadmium ions disrupt the function of photosystem II in chloroplasts.

(i) Name the part of the chloroplast where photosystem II is located.

thylakoid membrane

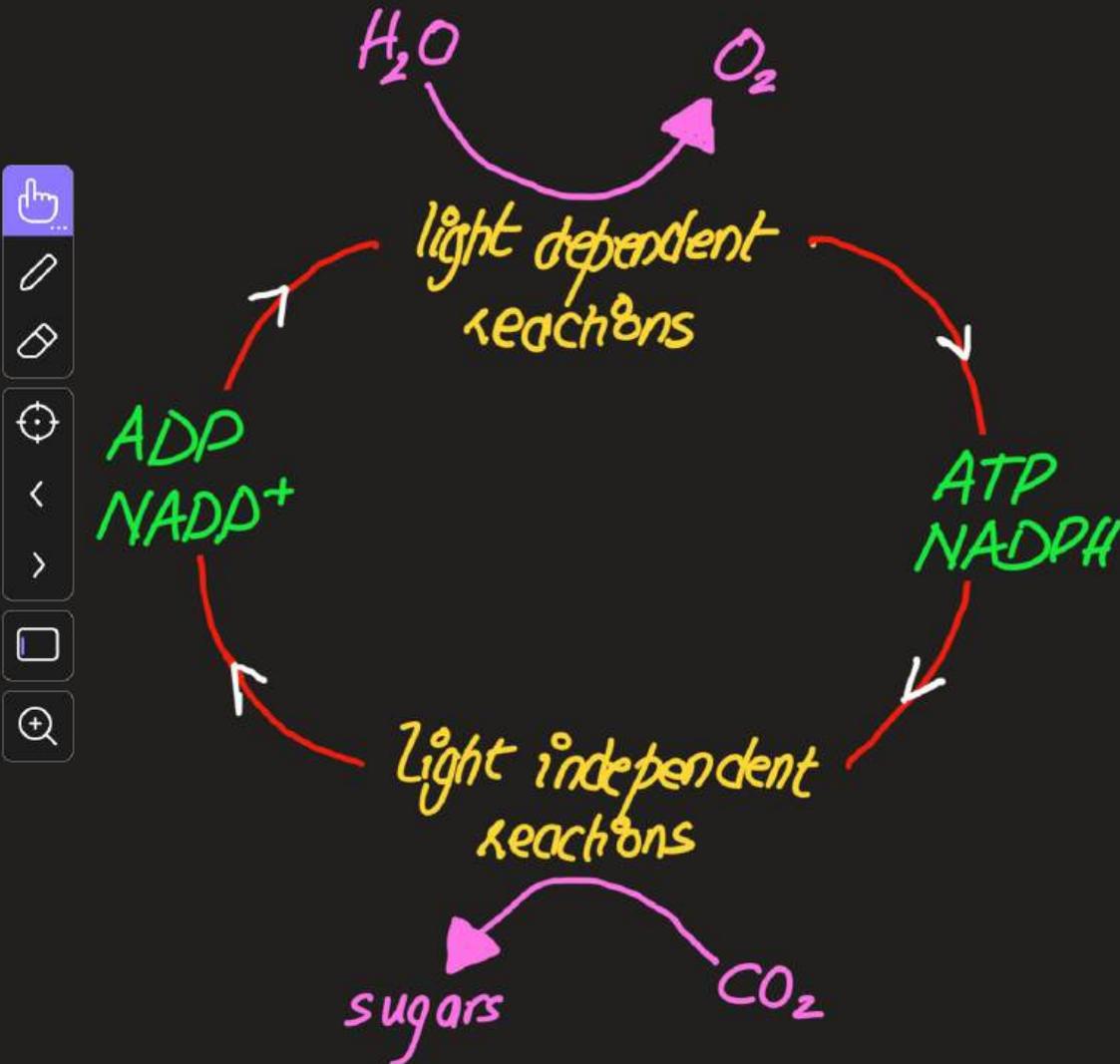
[1]

(ii) Describe the role of photosystem II in the absorption of light.

* Accessory pigments in light harv. clusters absorb light energy and pass it to primary pigments in the reaction centre
* chl-a → primary pigment molecules

[3]

Summary of Photosynthesis





CALVIN CYCLE
~~~~~ *~~~~~*
(light - independent reactions)

Light Independent Reactions (Calvin cycle)

* cyclic enzyme controlled process.

* occurs within the stroma of the chloroplast.

* stroma contains an important enzyme

Rubisco and a 5C pentose sugar RuBP.

* Rubisco = ribulose bisphosphate carboxylase
oxygenase

RuBP = ribulose bisphosphate

* Rubisco is perhaps the most abundant protein on this planet!

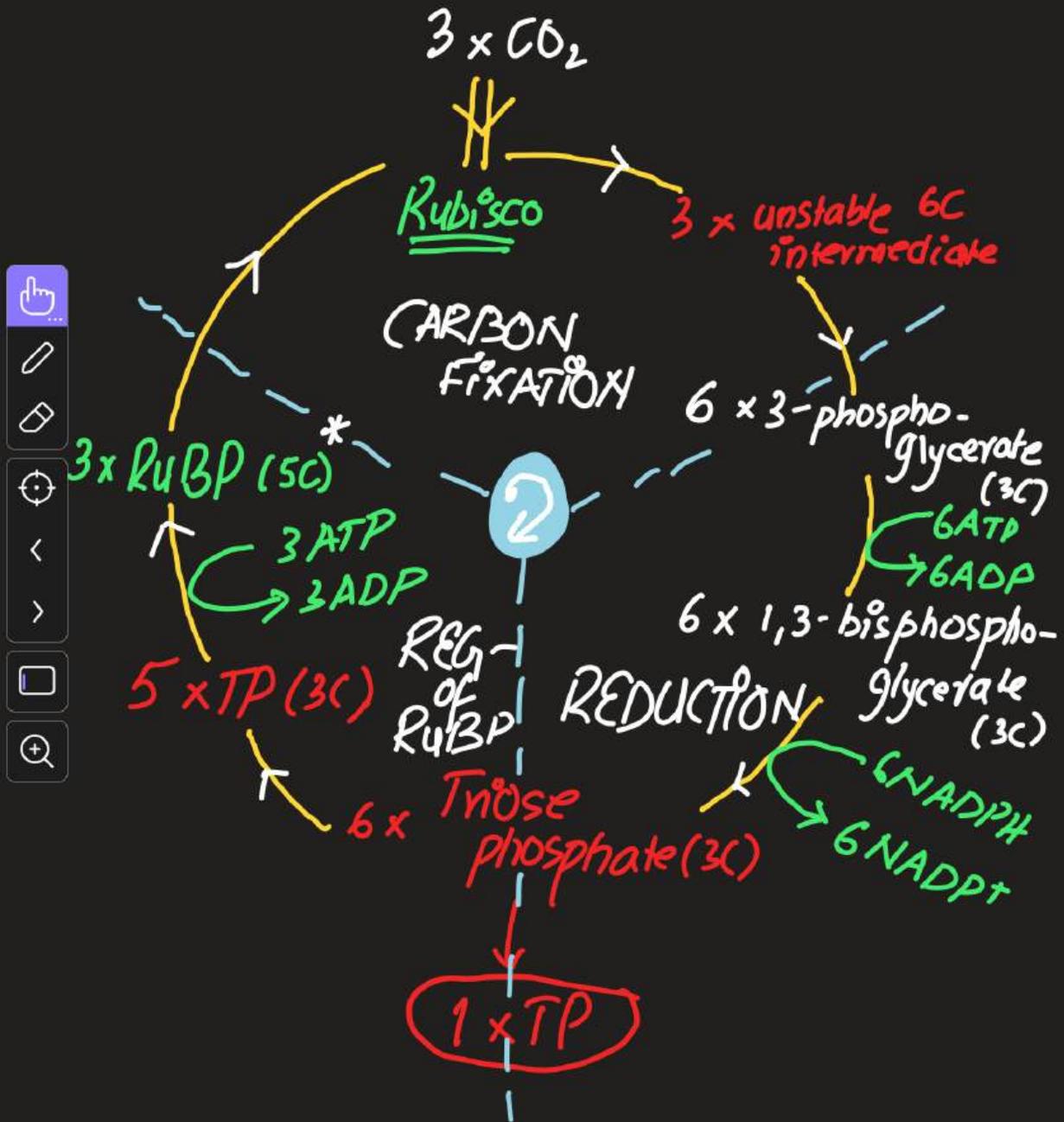
* Calvin cycle makes use of **ATP** and **NADPH**
(from light dependent reactions) AND **CO₂**
to synthesise sugars (triose phosphates).
=TP



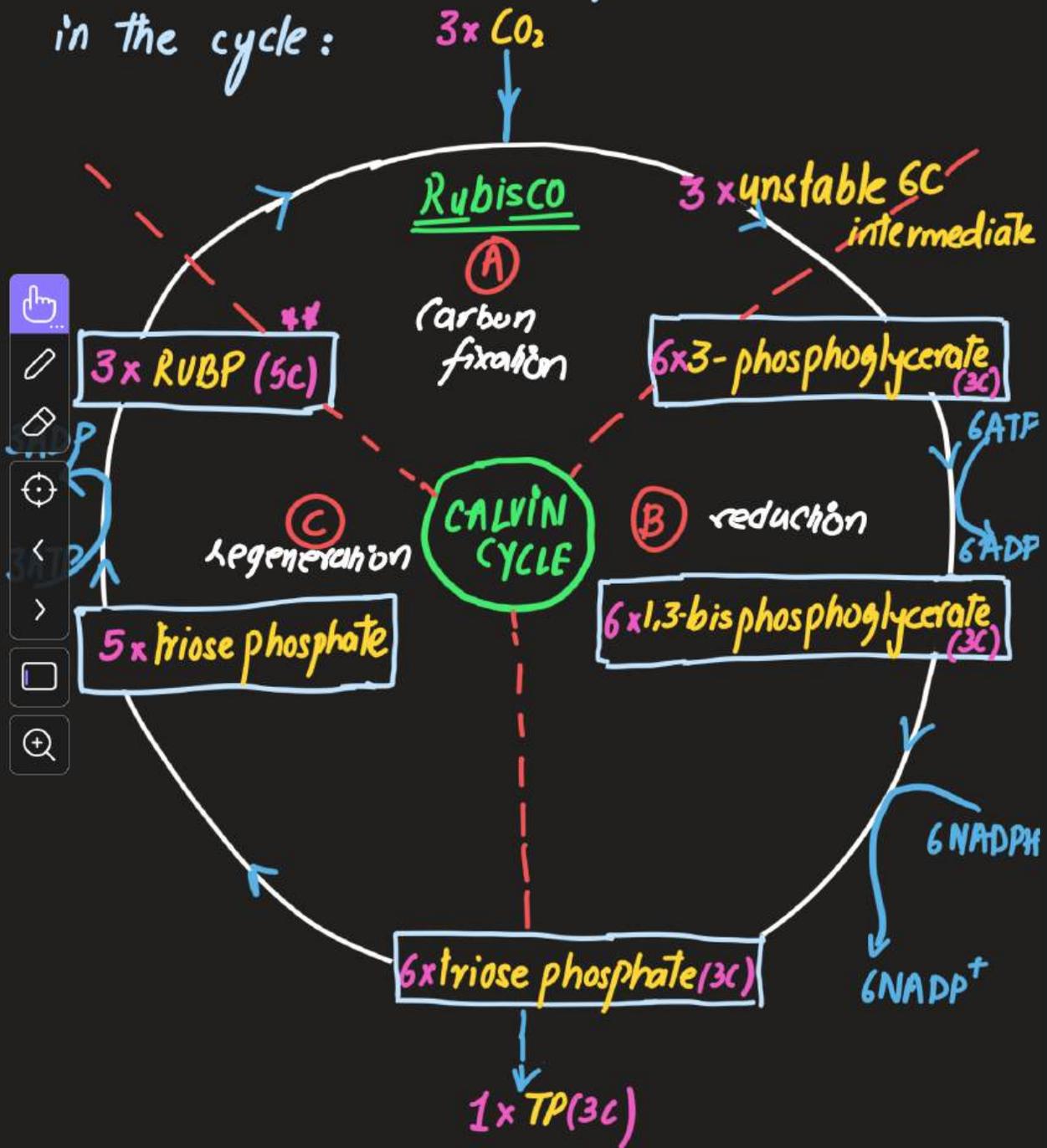
* Calvin cycle can be simplified into **Three**
phases: **(a) Carbon fixation**
(b) Reduction
(c) Regeneration of RuBP.

* One complete turn of the cycle consumes
9 ATP molecules and 6 NADPH molecules
to form one molecule of triose phosphate
(TP).

Calvin cycle



* Given below are the sequences of events in the cycle:



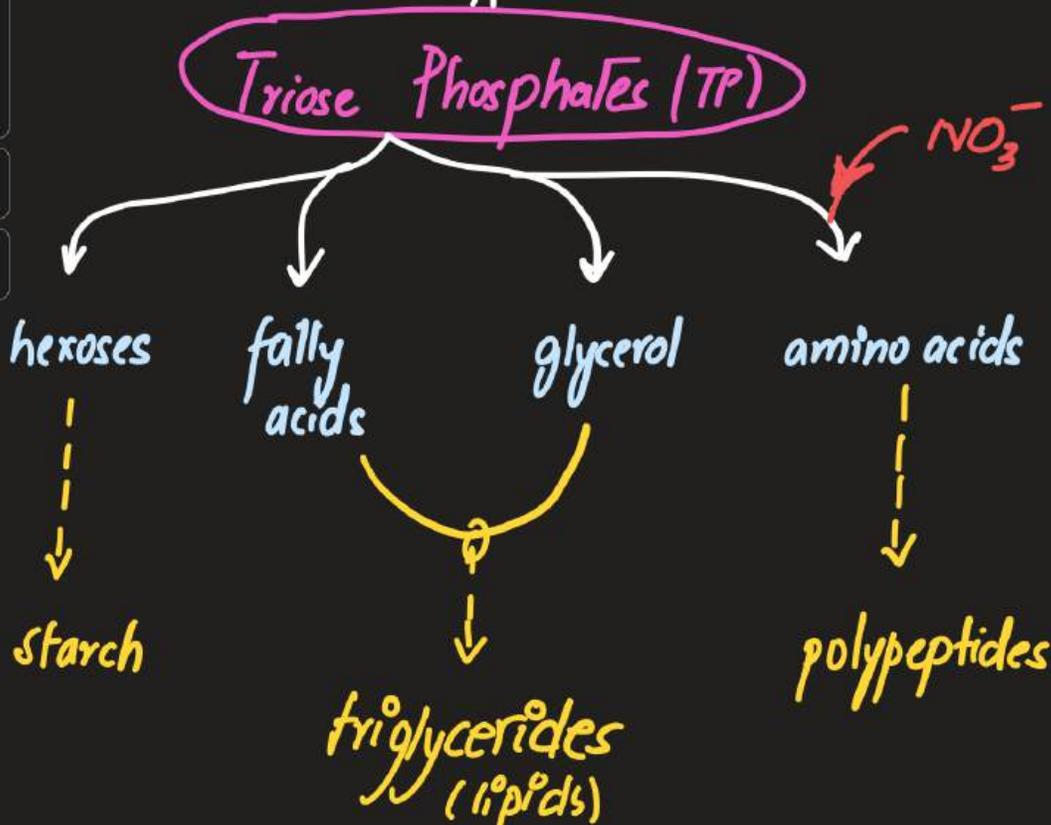
NOTE:
2

3-phosphoglycerate } GP
1,3-bisphosphoglycerate }
triose phosphate } TP

FATE OF TRIOSE PHOSPHATE MOLECULES



Most TP is used to regenerate RuBP to allow Calvin cycle to continue



Q₁: Outline Calvin cycle? [8]

Q₂: Briefly describe The role of the following

in Calvin cycle:

(a) NADPH → reducing agent
→ reduces GP → TP
→ regenerates oxidised NADP

(b) ATP → energy
→ phosphate donor
→ regenerate RuBP

(c) Rubisco → RuBP + CO₂ → carbon fixation
→ to form unstable 6C interm.

→ RuBP accepts CO₂ in
→ carbon fixation
→ to form unstable 6C interm.



Q3: Differentiate between Kreb's cycle & Calvin cycle?

- | | |
|---|---|
|  ① Catabolic process | ① Anabolic process |
|  ② Exothermic process
since it involves
release of energy in
the form of ATP. | ② Endothermic process
because it uses ATP
as a donor of energy. |
|  ③ Involves removal of
CO ₂ in decarboxylation
reactions. | ③ Involves consumption
of CO ₂ in carbon
fixation. |

④ Involves oxidation in the form of dehydrogenation reactions. NAD^+ serve as the oxidising agent.

⑤ Oxaloacetate (4C) is regenerated at the end of the cycle

⑥ Krebs' cycle stops in the absence of oxygen.

⑦ Krebs' cycle occurs in the matrix of the mitochondrion

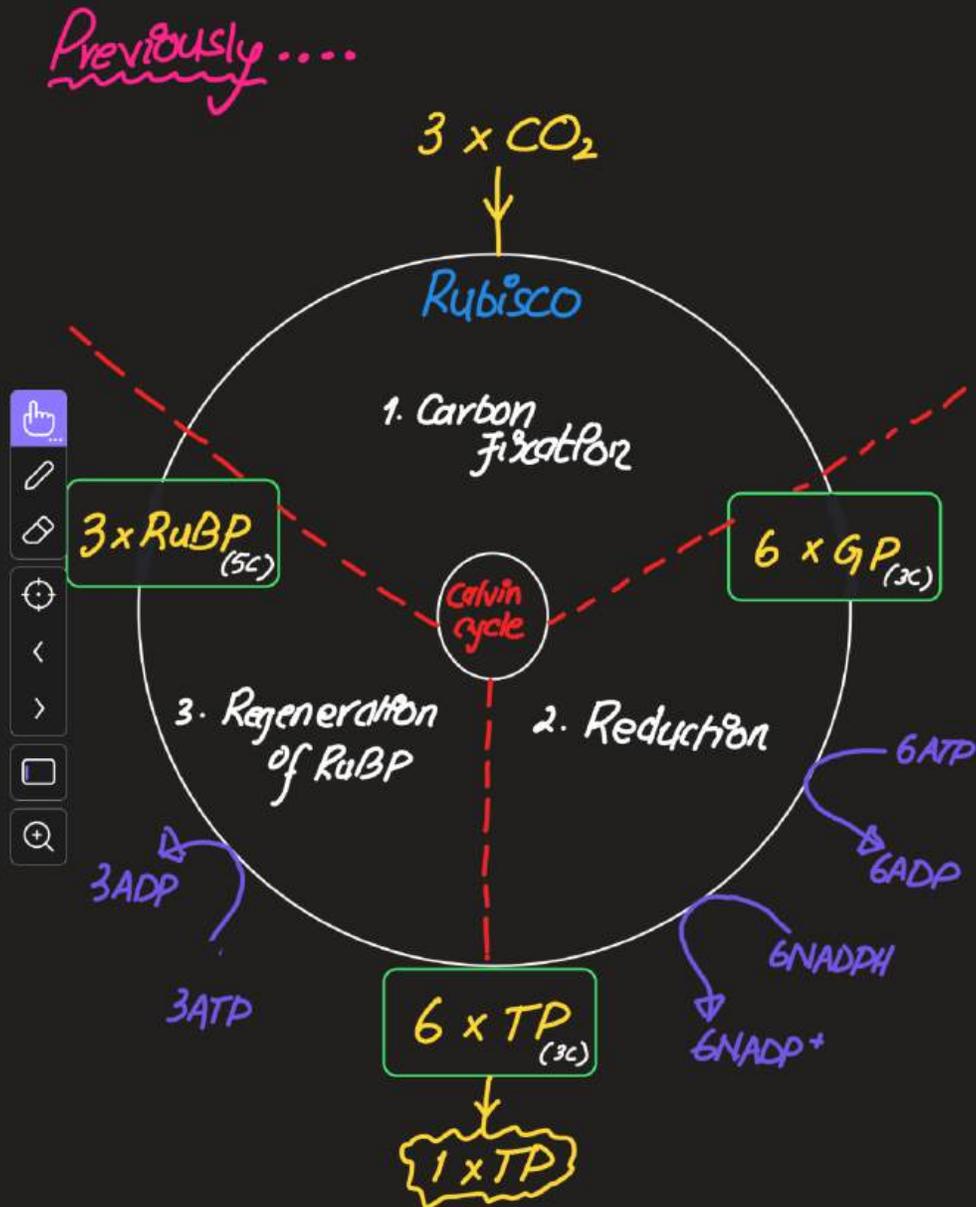
④ Involves reduction to convert GP into TP. NADPH serves as the reducing agent.

⑤ RuBP (5C) is regenerated at the end of the cycle.

⑥ Calvin cycle is an oxygen-independent process.

⑦ Calvin cycle occurs in the stroma of the chloroplast

Photosynthesis



With
Mohammad Hussham Arshad, MD

ADVANCED LEVEL BIOLOGY 9700 UNIT 13: Photosynthesis

Learning Objectives:

- Light dependent & light independent stages
- Revision & past paper Questions

Video Lecture 5 Slides
Mohammad Hussham Arshad, MD
Biology Department

Previously



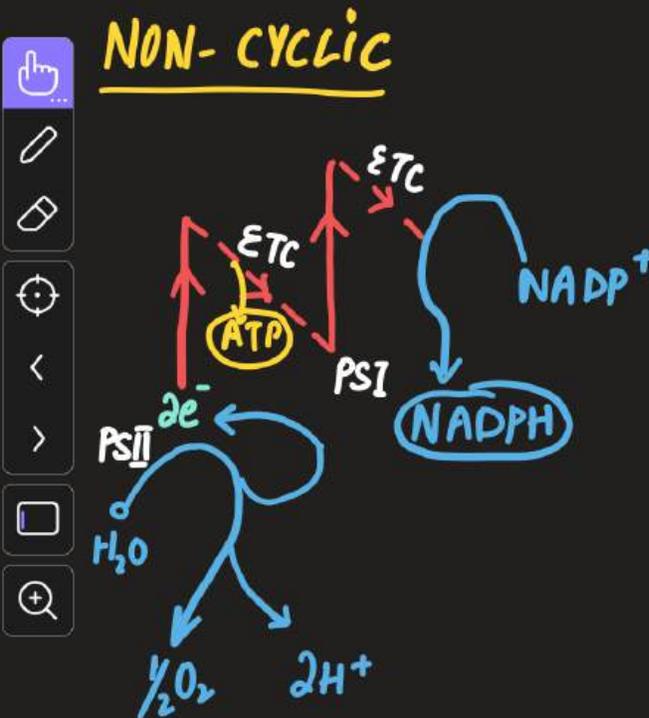
1) We discussed about the thylakoid membranes and the photosystems, ETC, the stalked particles.

2) We discussed the details of ;

- * light dependent reactions
- * Calvin cycle

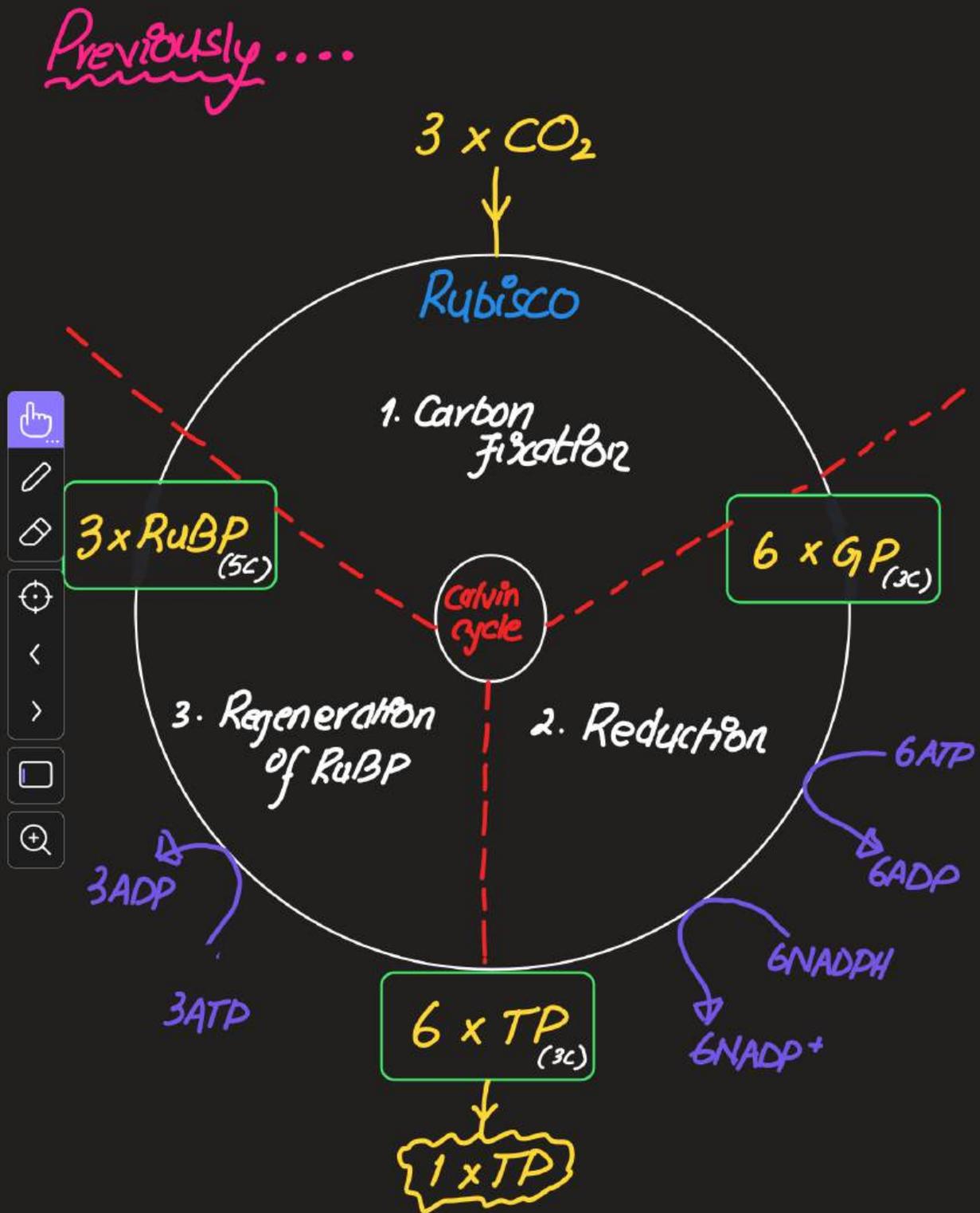
Previously....

Summary of the flow of electrons in
photophosphorylation



- * non cyclic flow of e^-
- * produces ATP , $NADPH$ and O_2
- * involves PSI and $PSII$
- * involves photolysis

- * cyclic flow of e^-
- * produces ATP only
- * involves PSI only
- * NO photolysis



Previously....

Differences between Calvin cycle and
Kreb's cycle.....



1. Anabolic vs catabolic

2. Endothermic vs Exothermic

3. Carbon fixation vs decarboxylation

4. Reduction vs oxidation

5. Coenzyme; $NADP^+$ vs NAD^+

6. Regeneration of RuBP vs oxaloacetate

7. Site ; stroma of the chloroplast vs matrix of the mitochondrion

⇒ Similarities between kreb's cycle and Calvin cycle?



① Enzyme controlled temperature dependent processes.

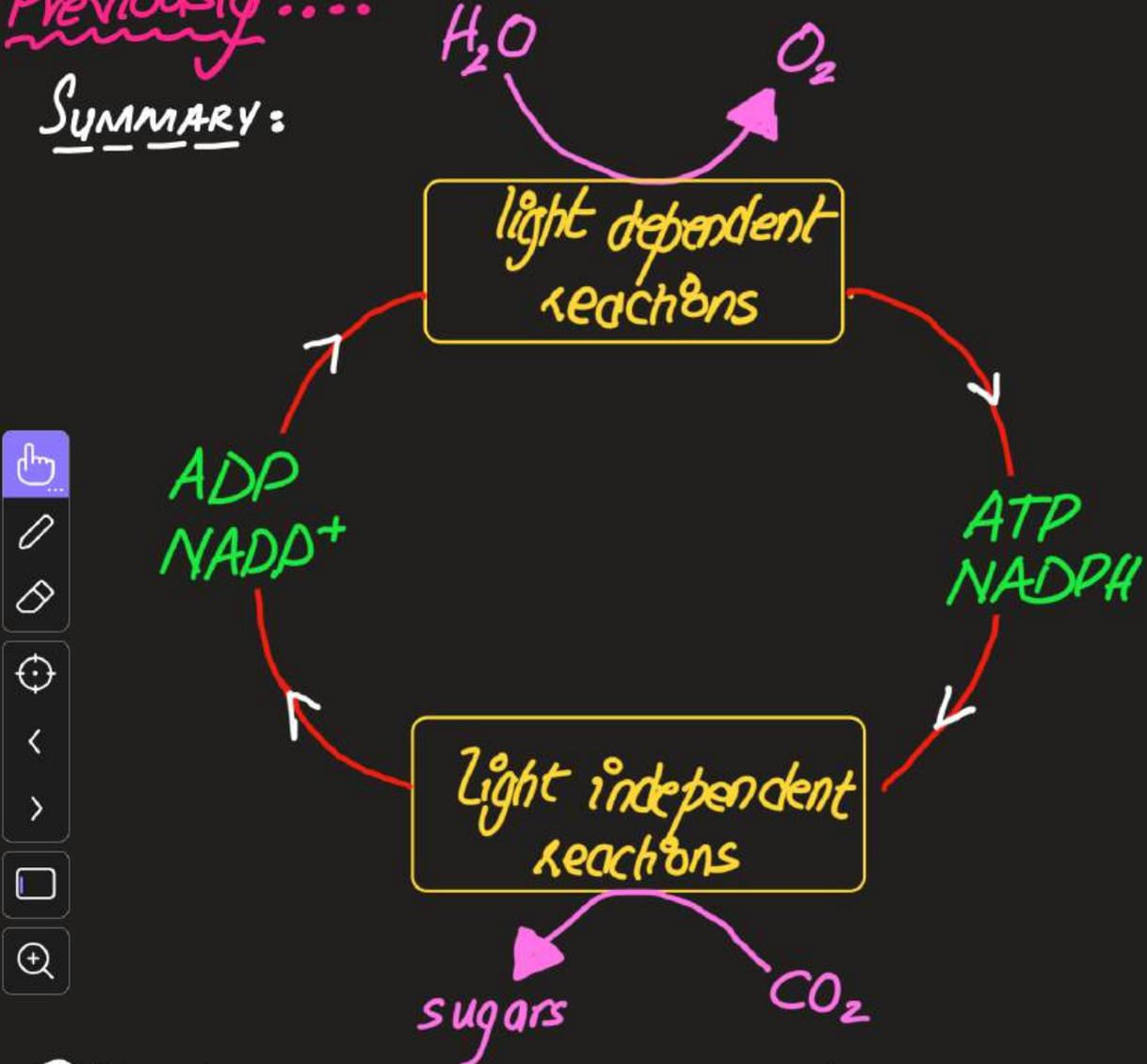


② Cyclic processes.



Previously....

SUMMARY:



Q. Based on the figure above, predict with a YES or NO if;

- light independent reactions will occur in the absence of light?
 - light dependent reactions will occur if Calvin cycle stops?
- } No



Past paper questions

Question 1:

(b) Describe the roles of the following substances in the light-independent stage of photosynthesis:

(i) RuBP

- CO_2 fixation
- production of GP

[2]

(ii) reduced NADP

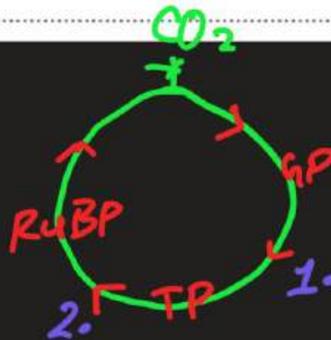
- reduction of GP
- to TP

[2]

(iii) ATP.

- Supplies energy and phosphate groups
- ✓ • to convert GP to TP
- ✓ • to regenerate RuBP

[2]



Question 2:

Q36 (N14/P41/Q8)

(a) Fig. 8.1 shows some of the reactions that take place inside a palisade mesophyll cell.

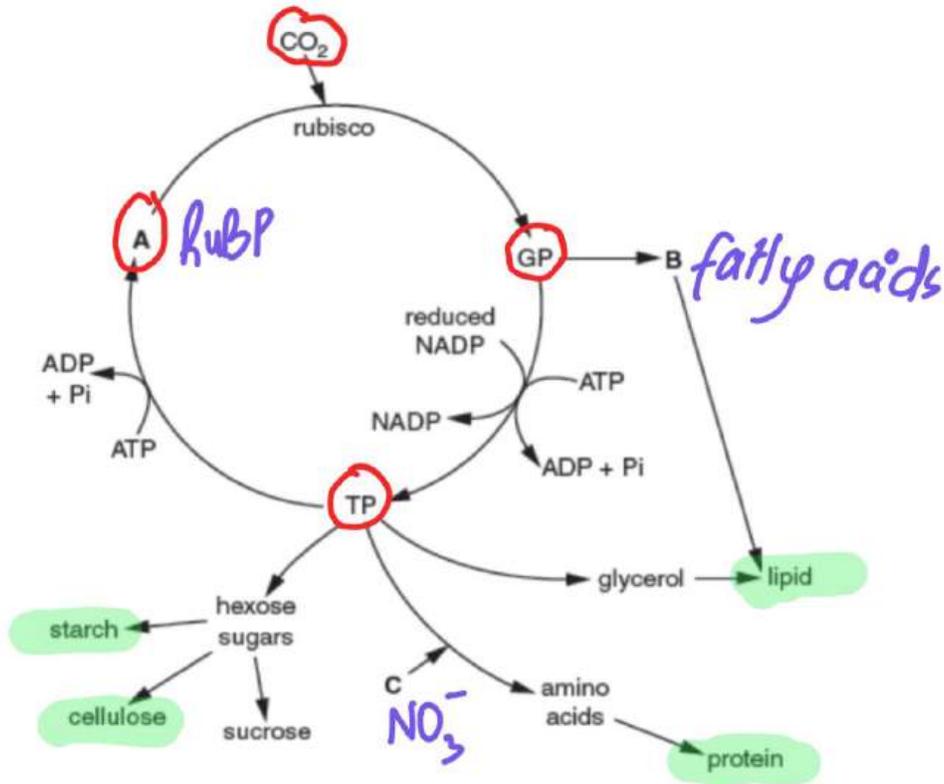


Fig. 8.1

(i) Identify substances A, B and C.

A *RUBP*
B *fatty acids*
C *NO₃⁻*

[3]

(ii) Name precisely the process that produces reduced NADP.

..... *non cyclic photophosphorylation* [1]

Question 3:

c) Complete the following paragraph by naming the most suitable compounds to fill in the gaps.

Rubisco is involved in the fixation of CO₂ by RuBP (ribulose biphosphate) in the Calvin cycle. The resulting six carbon compound immediately splits to give two molecules of glycerate-3-phosphate (GP). GP is converted to triose phosphate (TP) using ATP and reduced NADP produced in the light-dependent stage. Some of the TP produced is used to regenerate ribulose biphosphate so that the Calvin cycle can continue. The remaining TP may be used to synthesise other compounds including acetyl CoA which can == directly enter the Krebs cycle. [4]

Question 4:

 Describe the role of rubisco in the Calvin cycle.









** catalyses the reaction between RuBP and CO₂*
** to form an unstable 6C intermediate*
** which splits to form two G3P molecules*

.....[3]

Question 6:

(a) Fig. 7.1 is a transmission electron micrograph of a chloroplast.

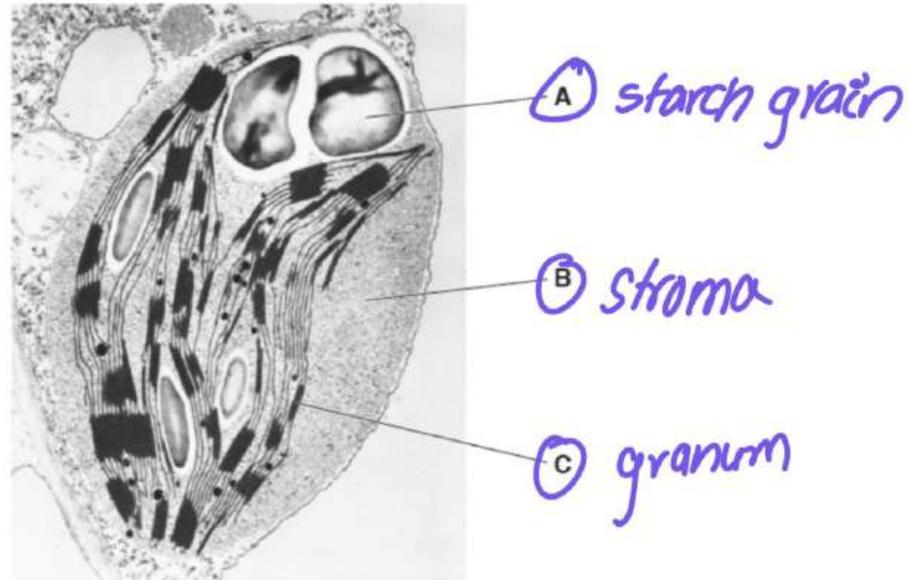


Fig. 7.1

Many compounds and structures involved in photosynthesis are located in a chloroplast.

Using the labels **A**, **B** or **C**, complete Table 7.1 to show the location of four of these compounds or structures.

You may use each of the letters **A**, **B** and **C** once, more than once, or not at all.

Table 7.1

compound or structure	location
ATP synthase C
rubisco B
starch grain A
phospholipid bilayer C

[3]

Question 7

a) Fig. 5.1 shows the seaweed *Laminaria hyperborea*. This is a photosynthetic protist found in the coastal waters around Norway.

The seaweed is grown commercially to obtain the glucose polysaccharide called alginate. This is used in certain food products.

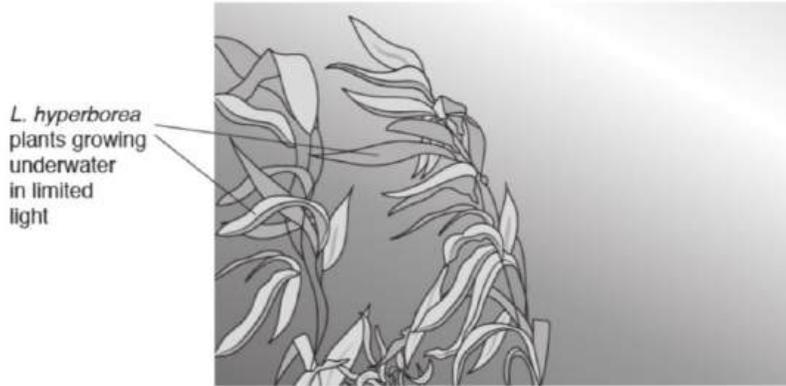


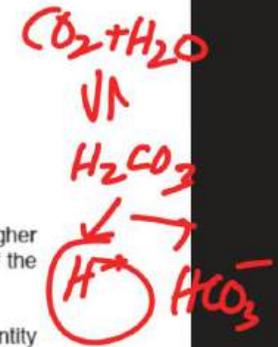
Fig. 5.1

An increase in carbon dioxide concentration in the atmosphere has resulted in higher concentrations of carbon dioxide in the ocean. This has caused a decrease in the pH of the ocean and has resulted in ocean acidification.

Scientists are studying seaweeds such as *L. hyperborea* because they absorb a large quantity of carbon dioxide during photosynthesis. This may help to increase the pH of the ocean and reverse ocean acidification.

(i) State where light absorption occurs in the chloroplasts of *L. hyperborea*.

thylakoid



[1]

(ii) Name **one** product of the light dependent stage of photosynthesis.

reduced NADP

[1]

iii) Outline the reactions occurring in the stroma that lead to the production of a polysaccharide, such as alginate.

The first sentence has been completed for you.

Carbon dioxide binds to RuBP.

- * RUBISCO catalyses this reaction
- * unstable 6C intermediate compound
- * splits into 2 GP molecules
- * GP reduced to TP
- * using NADPH & ATP
- * TP used to form glucose
- * glucose forms alginate

Question 8:

Fig. 7.1 outlines some of the events in the process of photosynthesis in a chloroplast.

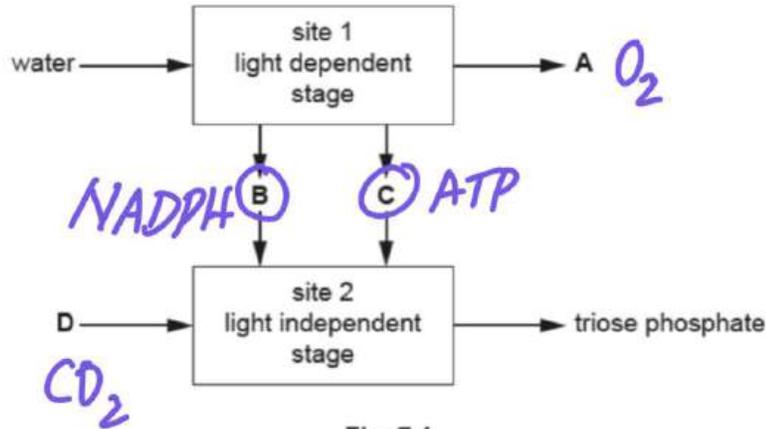


Fig. 7.1

(a) Name the parts of a chloroplast labelled site 1 and site 2 in Fig. 7.1.

site 1 *thylakoid membrane*
site 2 *stroma*

[2]

Name the substances labelled A, B, C and D in Fig. 7.1.

The substances labelled B and C may be named in either order.

A *O₂*
B *NADPH*
C *ATP*
D *CO₂*

[3]

Question 9:

(c) Most of the triose phosphate produced in the light independent stage is used to regenerate RuBP so that the Calvin cycle can continue. Some of the triose phosphate is used to make other organic compounds with a range of functions.

State **three** different functions of these other organic compounds in a plant cell and give **one** example of an organic compound for each function.

function produce energy (ATP) via cellular respiration

compound glucose, sucrose

function to synthesize the cell wall

compound cellulose

function to serve as an energy store

compound starch, lipids

[3]

Question 10:

- 9 In the majority of photosynthetic organisms, fixation of carbon dioxide occurs in the Calvin cycle.

Fig. 9.1 is an outline of this cycle.

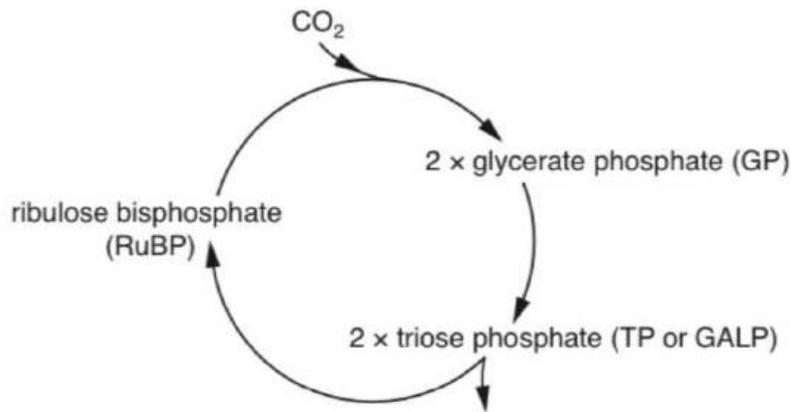


Fig. 9.1

(a) State,

- (i) the name of the five carbon sugar in the cycle

ribulose biphosphate (RuBP) [1]

- (ii) the name of the enzyme that fixes carbon dioxide

rubisco [1]

- (iii) where in the chloroplast the Calvin cycle occurs

stroma [1]

- (iv) the name of another compound that is produced in the light-dependent stage of photosynthesis that is used in the Calvin cycle.

ATP / NADPH [1]

(b) Fig. 9.2 shows the changes in the relative concentrations of RuBP and GP produced in the Calvin cycle before and after a light source is switched off. All other conditions are constant.

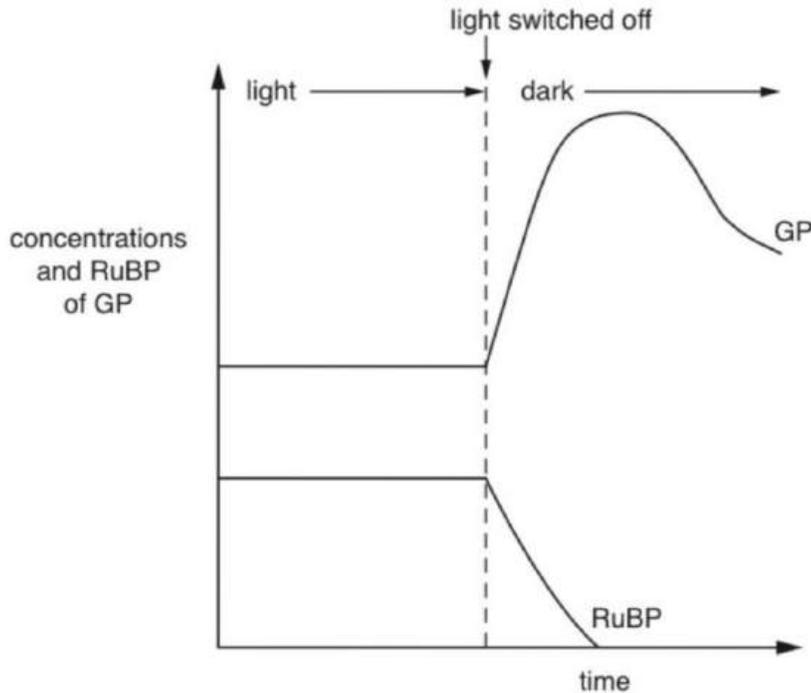


Fig. 9.2

Explain the changes in the relative concentrations of RuBP and GP **after** the light source is switched off.

- * light independent reactions continue
- * RuBP converted to GP until it's used up
- * GP rises initially and then drops as it's converted to TP
- * No ATP / NADPH produced by light depend. reactions
- * RuBP not regenerated

[4]

Q11: Outline Calvin cycle ?

Ans: Calvin cycle is a cyclic enzyme controlled process which involves reduction of carbon dioxide in the presence of ATP and NADPH to form sugars. The process occurs in the stroma of the chloroplast .

A molecule of RuBP accepts CO_2 in the presence of the enzyme Rubisco to form an unstable 6 carbon intermediate which splits to form 2 molecules of phosphoglycerate (GP) . The combination of RuBP with CO_2 is known as **carbon fixation** . Each phosphoglycerate molecule undergoes **reduction** using ATP and NADPH to form triose-phosphate . Most triose phosphate molecules use energy in the form of ATP to **regenerate RuBP** . Other triose phosphate molecules are used in making hexose sugars, fatty acids, glycerol and amino acids. For every triose phosphate molecule produced in the Calvin cycle, 6NADPH and 9ATPs are required.



Q12: Briefly outline the role of a) ATP , b) NADPH, c) CO₂ , d) RubisCO , e) RuBP during Calvin cycle ?

Ans:

A) ATP serves as a donor of energy and phosphate groups. It provides energy to convert GP to TP and to regenerate RuBP from TP.



B) NADPH is responsible for reducing GP to TP, regenerating oxidised NADP in the process.



C) CO₂ serves as a substrate for the enzyme RubisCO . It combines with RuBP in the presence of the enzyme RubisCO to form an unstable 6 carbon compound. CO₂ eventually undergoes reduction to form sugars.



D) Rubisco catalyses the reaction between RuBP and CO₂ to form an unstable intermediate. This is known as carbon fixation. The intermediate splits to form two molecules of GP.



E) RuBP accepts CO₂ in Calvin cycle to form an unstable intermediate. This is known as carbon fixation. The reaction is catalysed by Rubisco. The intermediate splits to form two molecules of GP.



Factors affecting the rate
of photosynthesis

Factors affecting the rate of photosynthesis

External factors

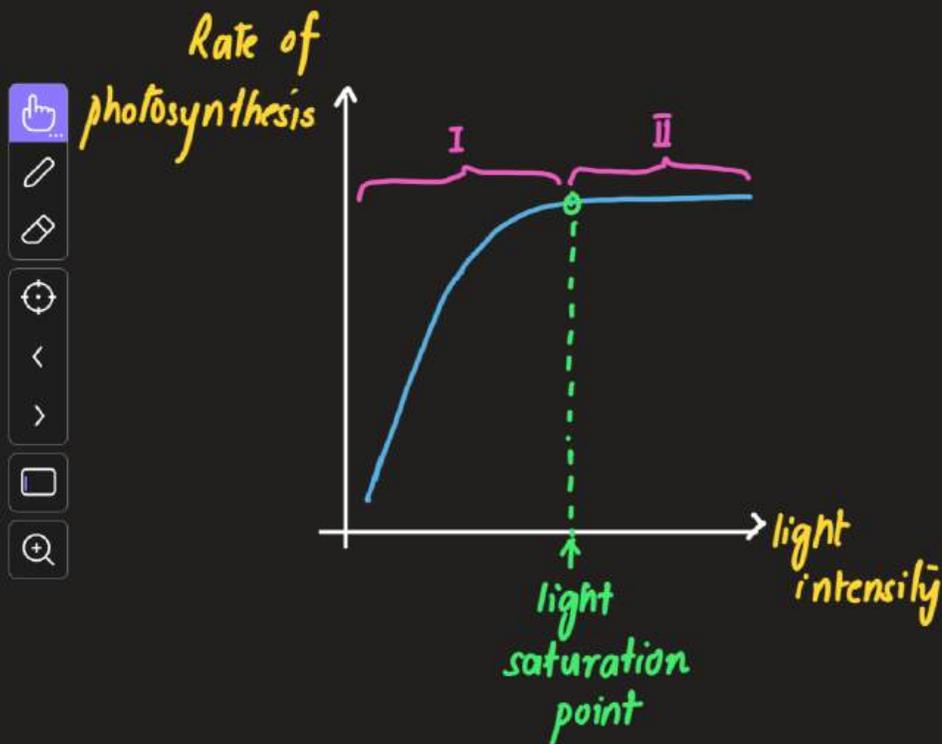
- ① Temperature
- ② CO_2 concentration [CO_2]
- ③ Light intensity
- ④ Wavelength of light

Internal factors

- ① Surface area of leaves
- ② Chloroplast density
- ③ Stomatal density

Photosynthesis

© LIGHT INTENSITY



With
Mohammad Hussham Arshad, MD

ADVANCED LEVEL BIOLOGY 9700 UNIT 13: Photosynthesis

Learning Objectives:

- Factors affecting photosynthesis
- Photorespiration
- Absorption & action spectrum

Video Lecture 6 Slides
Mohammad Hussham Arshad, MD
Biology Department



*Factors affecting the rate
of photosynthesis*

Factors affecting the rate of photosynthesis



External factors

- ① Temperature
- ② CO_2 concentration [CO_2]
- ③ Light intensity
- ④ Wavelength of light

Internal factors

- ① Surface area of leaves
- ② Chloroplast density
- ③ Stomatal density

* But before that, let's see how we determine the rate of photosynthesis....

• Rate of photosynthesis can be measured by determining;

a) the rate of O_2 produced

b) the net CO_2 uptake

$$= \overset{\text{rate of}}{CO_2 \text{ uptake in photosynthesis}} - \text{rate of } CO_2 \text{ produced in respiration}$$



+

rate of PS
>>>
rate of resp

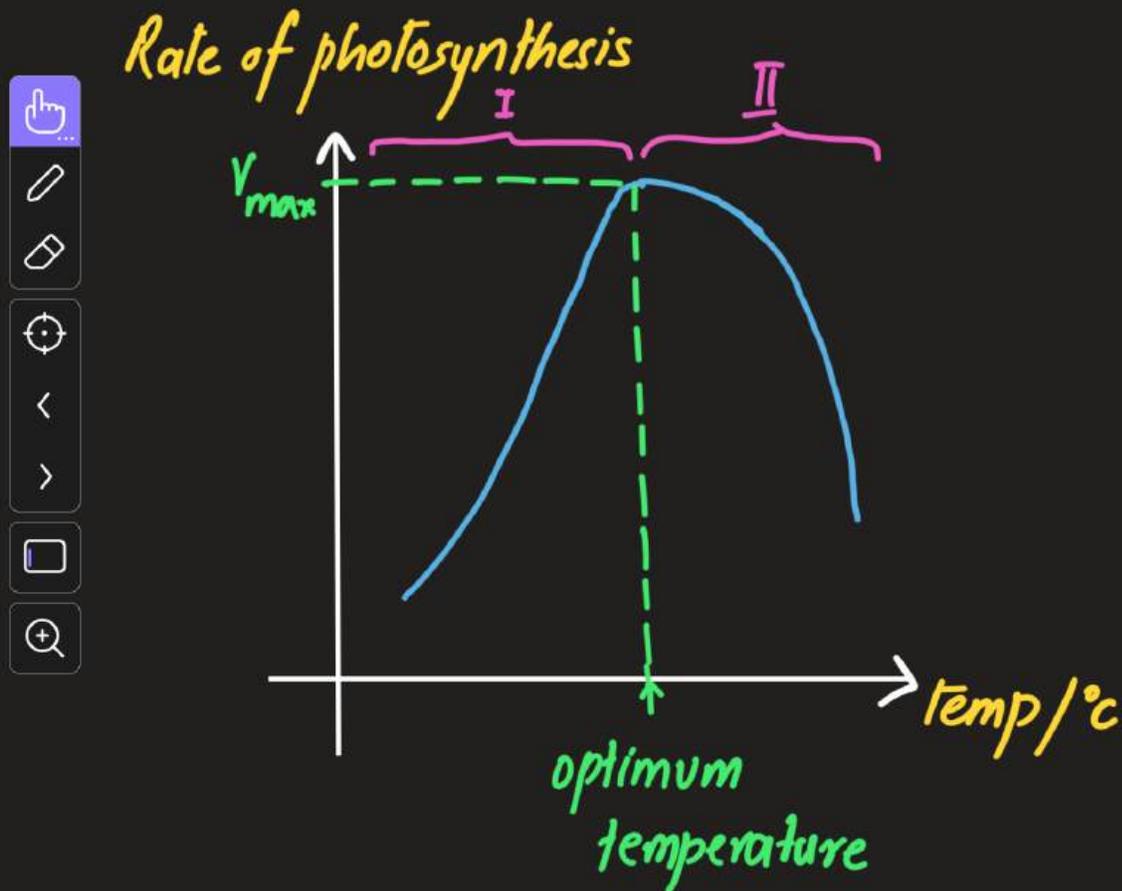
0

rate of PS
≡
rate of resp

-

rate of PS
<<<
rate of resp

(A) TEMPERATURE



Description :

* Increase in the temp. increases the rate

of photosynthesis until it reaches V_{max} at

the optimum temperature (I).

* The rate of photosynthesis decreases

beyond the optimum temperature (II).

Explanation:

1:

* Photosynthesis is an enzyme controlled process.

* Light dependent reactions use The water splitting centre and The NADP^+ reductase.

* Calvin cycle uses Rubisco besides other enzymes.

* Increase in temp increases the kinetic energy of the enzyme and substrate molecules \rightarrow increase in frequency of successful collisions \rightarrow increase in the rate of formation of E-S complexes \rightarrow increase in the rate of photosynthesis.



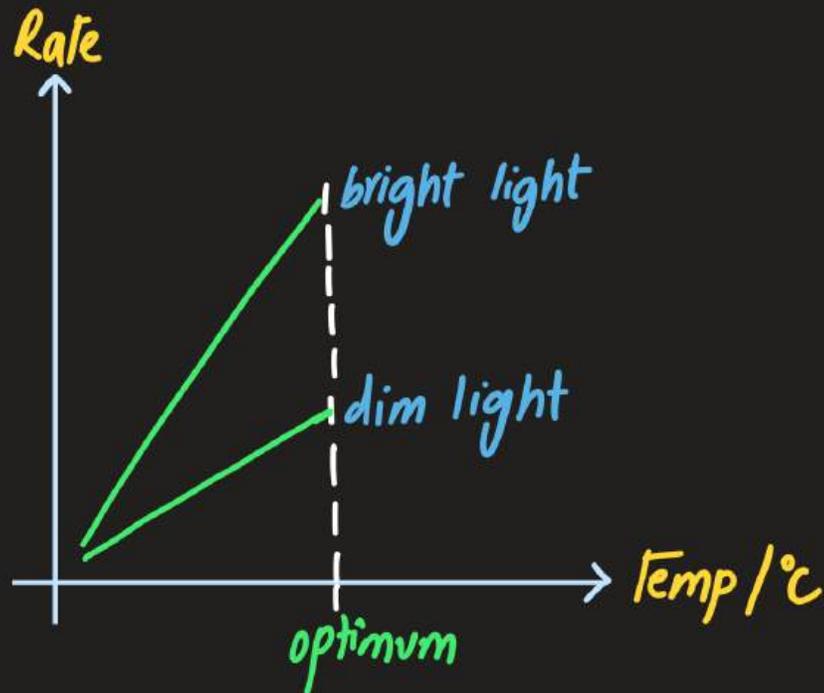
* Beyond The optimum temperature, The enzymes lose their tertiary structure

due to breakage of hydrogen bonds,

hydrophobic interactions and ionic bonds.

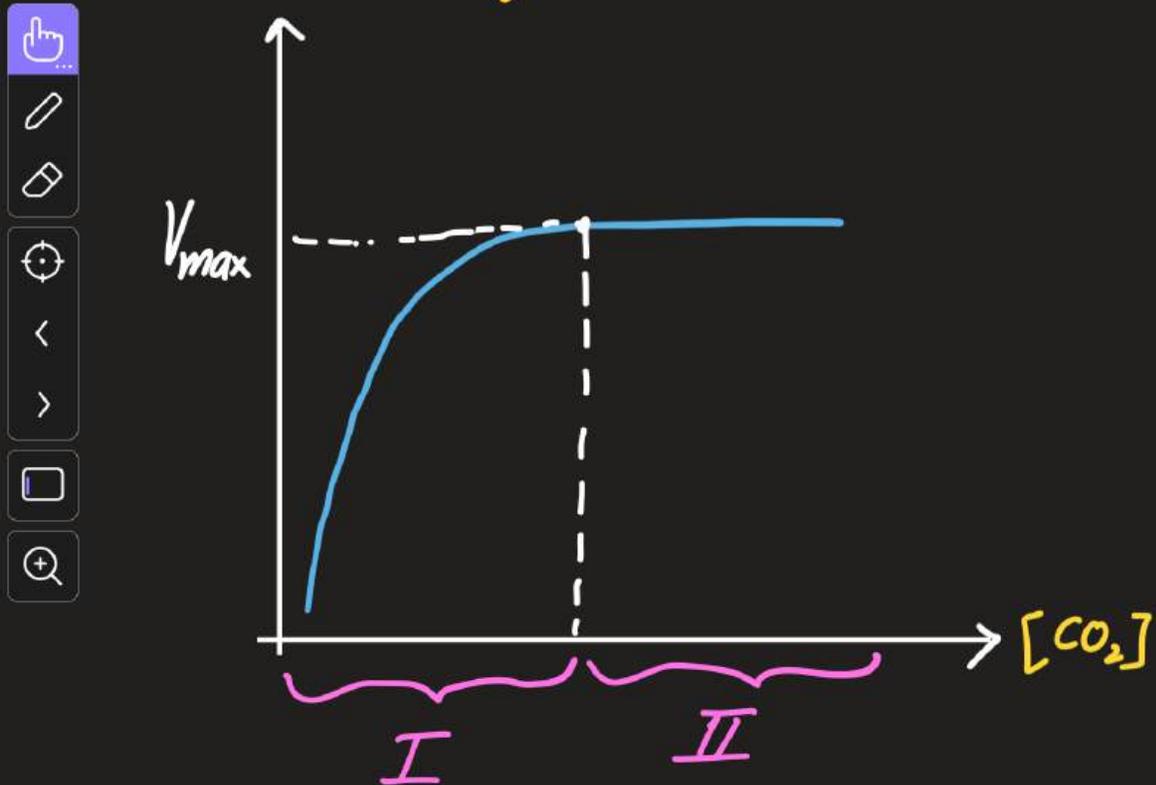
* Enzymes lose the shape of the active site \rightarrow decreases the rate of formation of E-S complexes \rightarrow decreases the rate of photosynthesis.

NOTE: Increase in temp does NOT significantly increase the rate of photosynthesis if light intensity is low:



(B) CO₂ CONCENTRATION

Rate of photosynthesis



Description :

* Rate of photosynthesis increases with increase in $[CO_2]$ until the rate becomes constant.

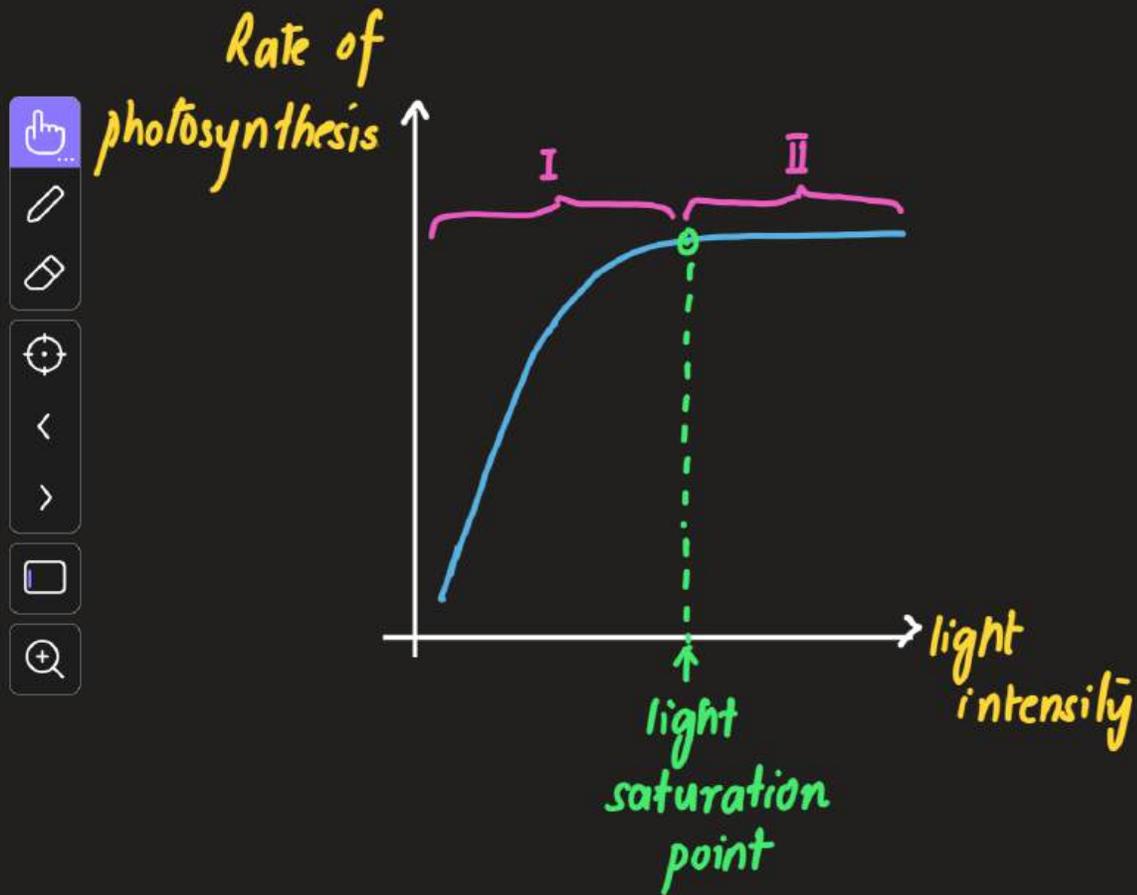
Explanation:

- * The amount of CO_2 in the atmosphere is 0.04%.
- * At low $[CO_2]$, CO_2 is the limiting factor.
- * Increasing $[CO_2]$ increases the substrate available for carbon fixation \rightarrow increase in the rate of Calvin cycle \rightarrow increase in the rate of photosynthesis.

* At high $[CO_2]$, some other factors such as the light intensity may be the limiting factor, or

* The enzymes are saturated and working at their optimum rate.
maximum

© LIGHT INTENSITY



Description :



* Increase in light intensity increases the rate of photosynthesis till the light saturation point is reached (I).

* Beyond the light saturation point, the rate becomes constant (II).

Explanation:

I * At low light intensities, light is the limiting factor.

 * Increase in light intensity provides more energy in the form of photons.




<
>

 * There is a consequent increase in the rate of light dependent reactions → increasing the rate of production of ATP and NADPH.

* ATP and NADPH increase the rate of Calvin cycle ⇒ thereafter increasing the overall rate of photosynthesis.

* Increasing light intensity also raises the temperature slightly which contributes to the rising rate of photosynthesis.

II



* Beyond the light saturation point (high light intensity is no longer the limiting factor.

* Other factors such as $[CO_2]$ might be limiting the process.

* The rate of photosynthesis may also be limited by **photorespiration** at high light intensities due to high $[O_2]$ in the mesophyll cells.

What is photorespiration?

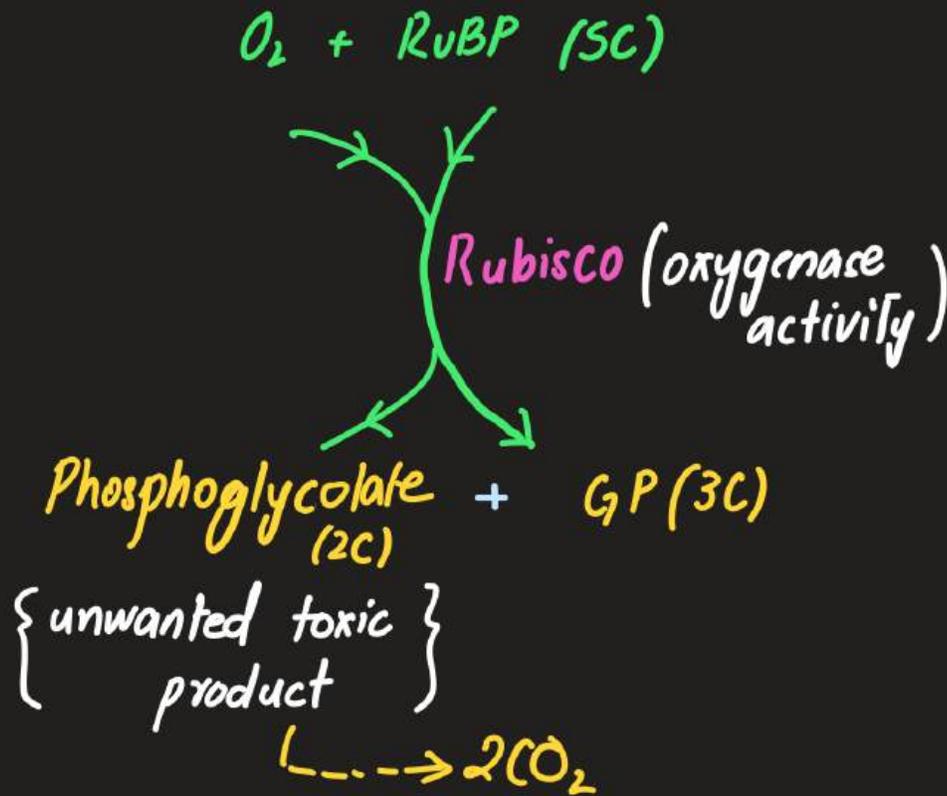
* Photorespiration occurs when plants take up O_2 and give out CO_2 in the presence of light (contrary to photosynthesis).

It occurs in plant cells when the $[O_2]:[CO_2]$ is relatively higher than normal. \Rightarrow such as at high light intensities and high temperatures.

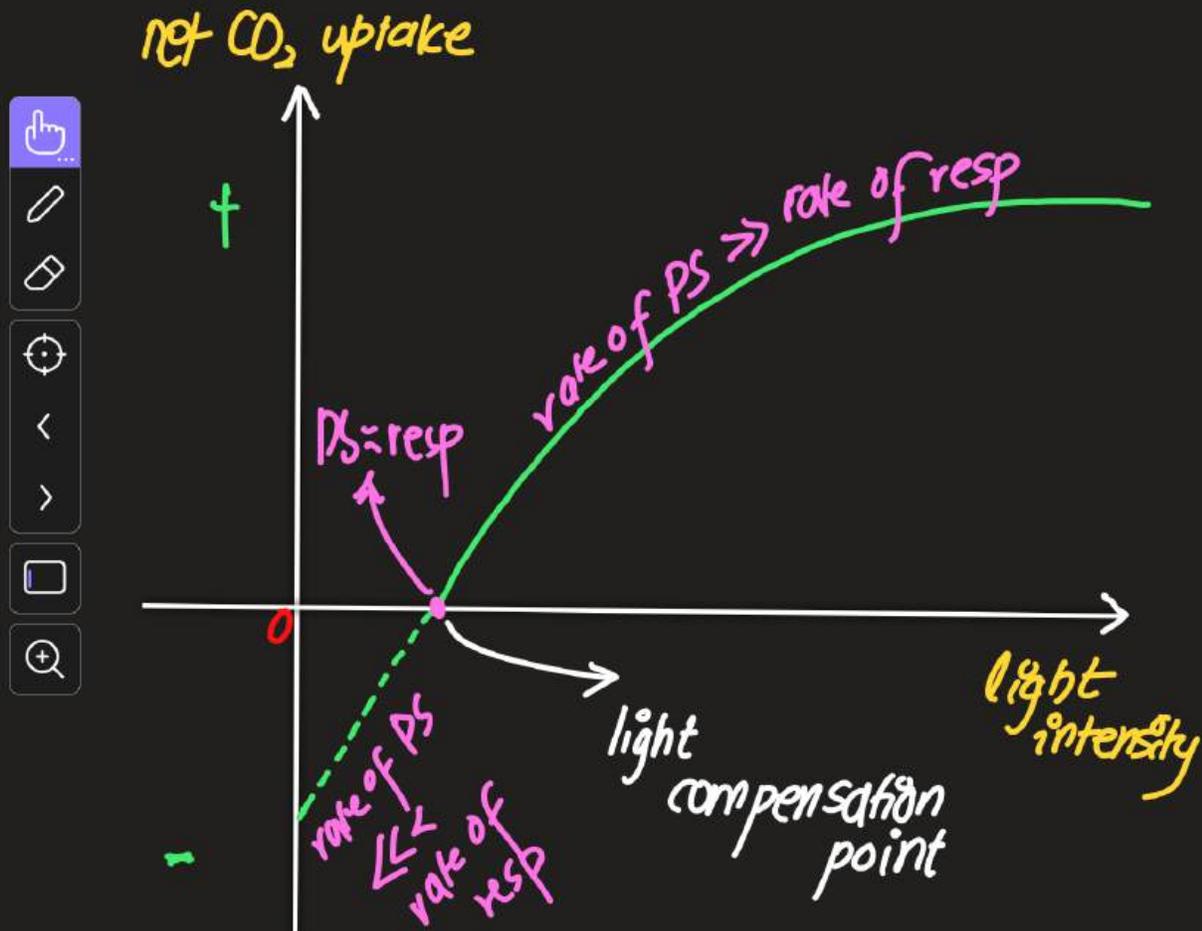
* In this process, the enzyme Rubisco combines O_2 with RuBP rather than CO_2 to form a 2C unwanted product



known as phosphoglycolate:



NOTE: The graph of net CO_2 uptake against light intensity is an important graph for you to remember;

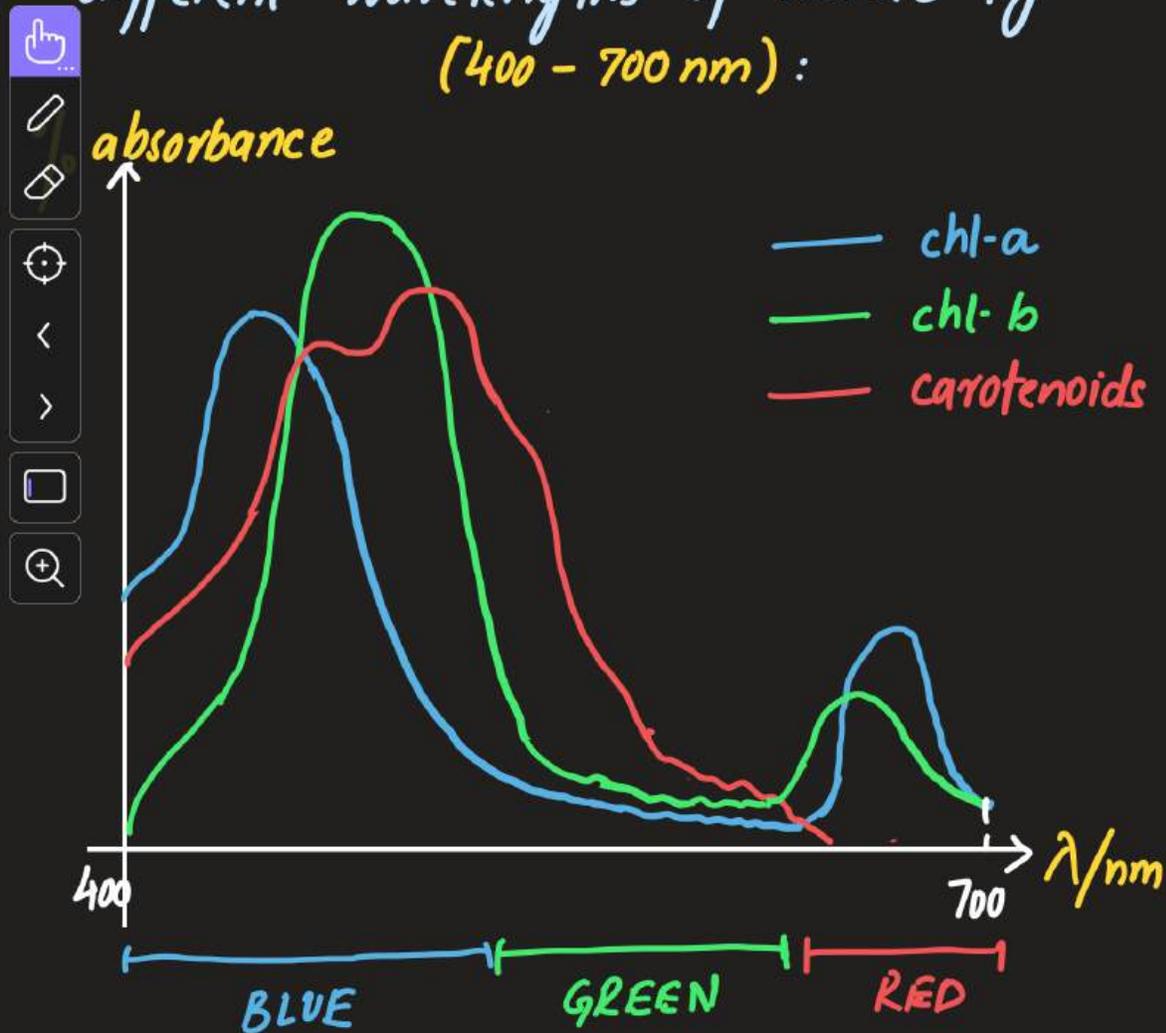


Before we move to the last factor (wavelength), let's understand what we mean by the term "limiting factor"....

a limiting factor is the factor in the shortest supply amongst several factors that influence the rate

④ WAVELENGTH

* The graph below shows the % absorbance of different photosynthetic pigments at different wavelengths of visible light (400 - 700 nm):



* Chlorophylls primarily absorb light in the blue and the red regions of the spectrum.



• chl-a has a higher peak than chl-b in the red region.

• chl-b has a higher peak than chl-a in the blue region.

* Carotenoids absorb light in the blue and the green regions of the spectrum.

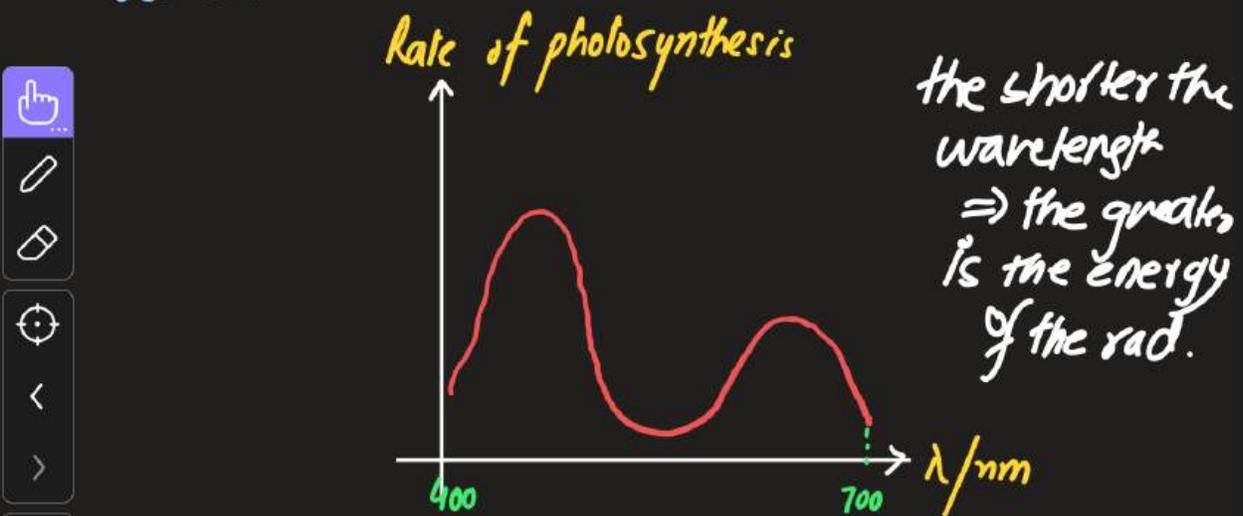
* The graph above showing the relationship between the % absorbance of different pigments against the wavelength of

light is known as the **absorption spectrum**.

* The absorption spectrum correlates with the **action spectrum** which shows

the rate of photosynthesis at different wavelengths of light.

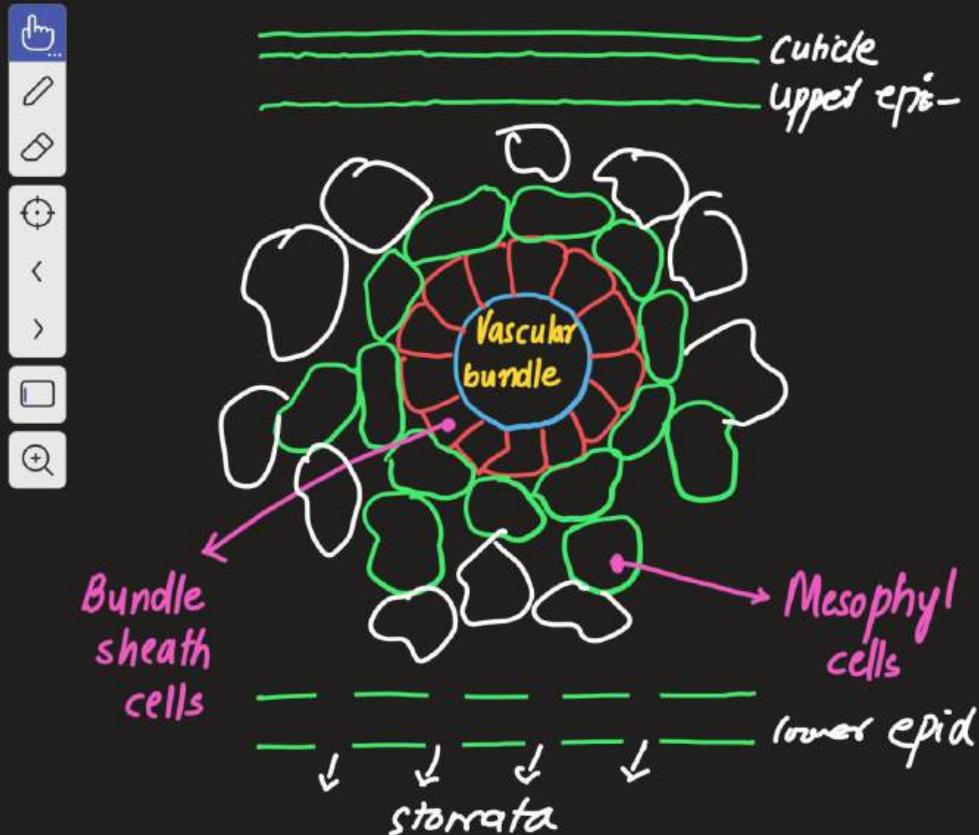
* For the absorption spectrum above, The action spectrum will look like The one below:



The graph above shows higher rate of photosynthesis in the blue and red regions of the spectrum and relatively lower rates in the green region of the spectrum.

Photosynthesis

* Given below is a section of a leaf of a C_4 plant to illustrate the main features of Kranz anatomy:



With

Mohammad Hussham Arshad, MD

ADVANCED LEVEL BIOLOGY 9700 UNIT 13: Photosynthesis

Learning Objectives:

- C4 Plants -1

Video Lecture 7 Slides
Mohammad Hussham Arshad, MD
Biology Department

Previously.....

Factors affecting the rate of photosynthesis



External factors

- ① Temperature
- ② CO_2 concentration [CO_2]
- ③ Light intensity
- ④ Wavelength of light

Internal factors

- ① Surface area of leaves
- ② Chloroplast density
- ③ Stomatal density



Past paper questions

Question 1

(c) Fig. 8.3 shows the absorption spectra of the photosynthetic pigments of a flowering plant.

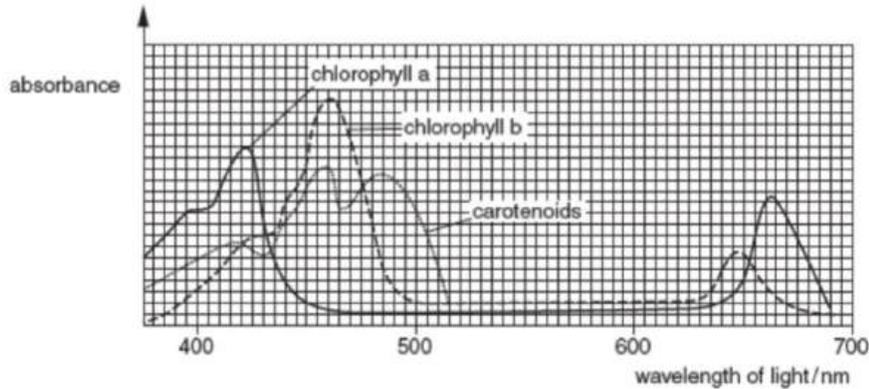


Fig. 8.3

(i) Name the accessory pigment(s) shown in Fig. 8.3.

chl-b and carotenoids

[1]

(ii) Outline the role of the accessory pigments in photosynthesis.

- absorb light energy at wavelengths not readily absorbed by primary pigments*
- then pass energy to primary pigment in reaction centre.*

[3]

(iii) Very little light of wavelength 550 nm is absorbed by the photosynthetic pigments.

State what happens to most of this light.

reflected

[1]

(iv) A graph can also be drawn to show the relationship between the wavelength of light and the rate of photosynthesis.

State the name of this type of graph.

action spectrum

[1]



Question 2

Q39 (N15/41/q7)

- (a) One way to estimate the rate of photosynthesis is to measure the rate of uptake of carbon dioxide.

Fig. 7.1 shows the relationship between light intensity and relative carbon dioxide uptake and production in a dicotyledonous plant.

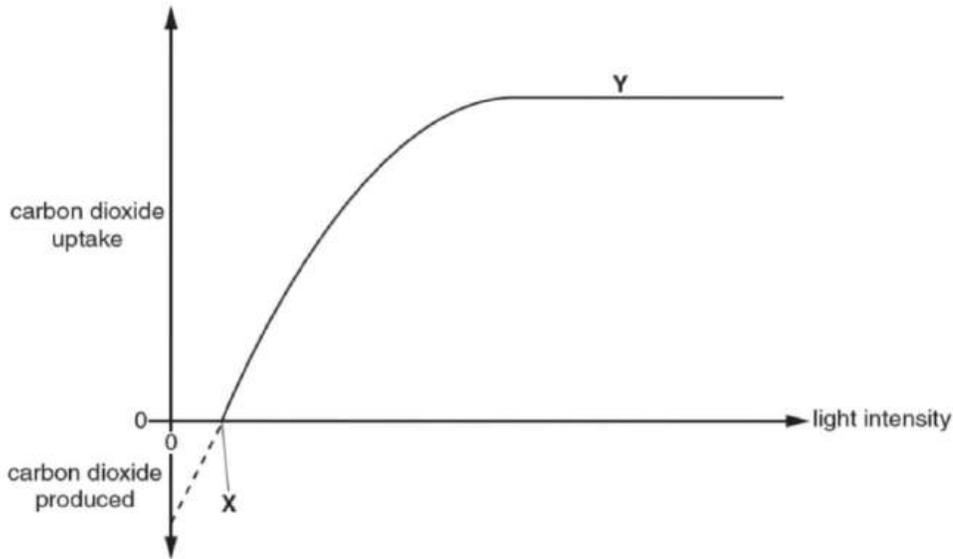


Fig. 7.1

- (i) State one physical factor that may limit the rate of photosynthesis at Y.
..... *CO₂ concentration / temperature* [1]
- (ii) State two features of a dicotyledonous leaf that can affect the rate of photosynthesis.
..... ** surface area of the leaf*
..... ** chloroplast density* [2]
- (iii) Explain the shape of the curve as light intensity increases from 0 to X.
..... *• respiration rate greater than photosynthesis*
..... *• overall net production of CO₂*
..... *• X = compensation point, so photosynthesis rate = respiration rate*
..... [2]

Question 3

Q40 (N15/43/q7)

7 (a) Fig. 7.1 shows the absorption spectra of chlorophyll a and chlorophyll b and a corresponding action spectrum.

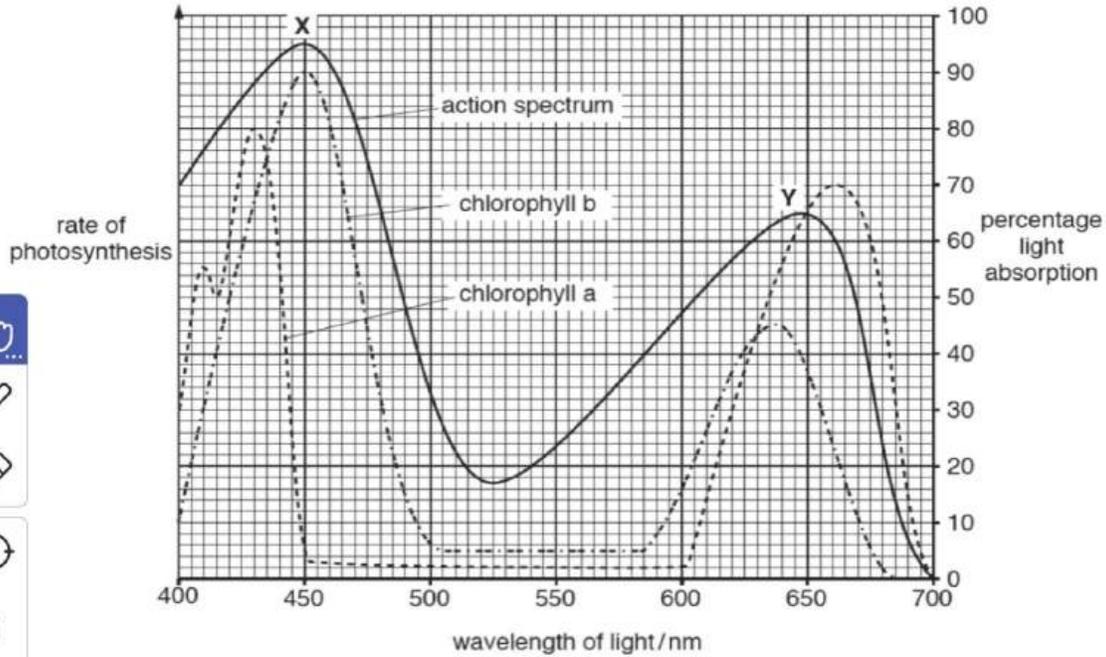


Fig. 7.1

(i) Explain why peak X of the action spectrum is higher than peak Y.

- more light absorbed by chlorophyll
 - short wavelengths have more energy
- so greater rate of photosynthesis

[2]

(ii) Explain why most plants appear green.

- contain chlorophyll
- reflects green light

[2]



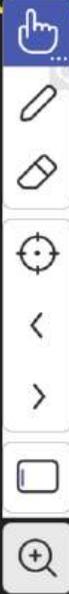
(iii) Chlorophyll b is an accessory pigment.

Outline the role played by accessory pigments in the light-dependent stage of photosynthesis.

- absorb light in wavelengths not absorbed by primary pigments
- pass light energy to primary pigments

[2]

Question 4



41 (J16/41/q2)

The concentration of carbon dioxide in the atmosphere and the light intensity often limit the rate of photosynthesis.

(a) Explain what is meant by a *limiting factor* in relation to photosynthesis.

- in shortest supply
- one of the many factors that affect rate.

[2]

Question 5

Q42 (J16/42/q2)

- (a) Fig. 2.1 shows the effect of temperature on the rate of photosynthesis of a plant at low light intensity and at high light intensity.

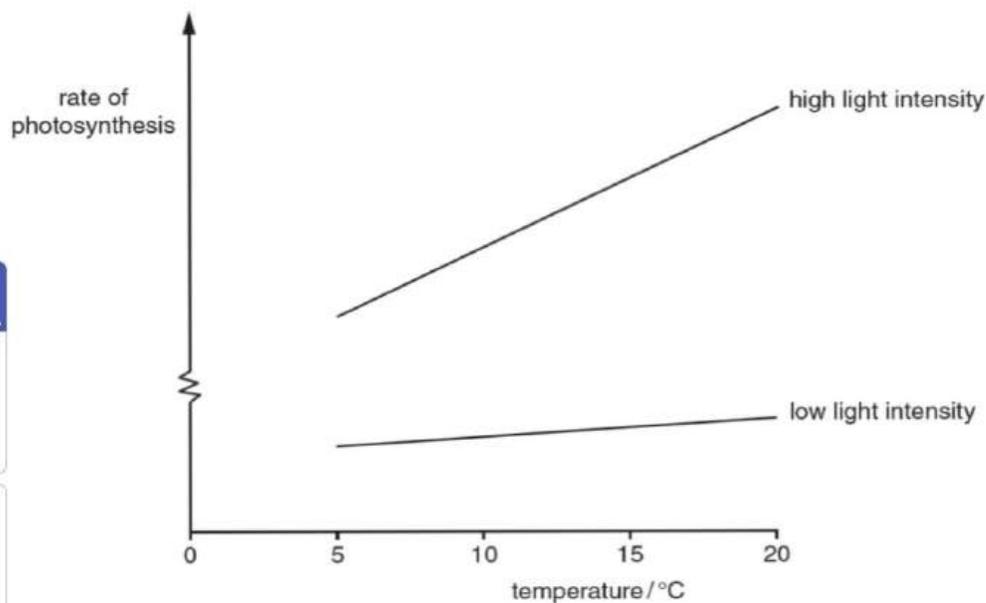


Fig. 2.1

With reference to Fig. 2.1, describe and explain the effect of temperature on the rate of photosynthesis.

- increased temp increases the rate at high light intensities
- increased temp has little effect on rate at low light intensities.
- increased kinetic energy → increased no. of collisions
- increased rate

[4]

(b) Fig. 2.2 shows an absorption spectrum for chloroplast pigments and a photosynthetic action spectrum for the same plant.

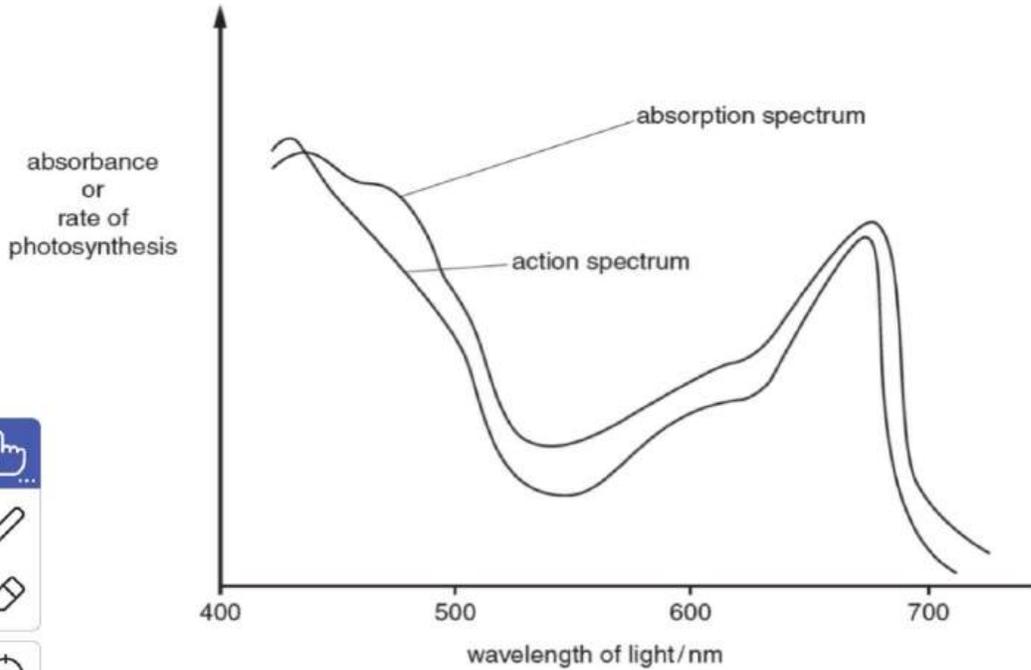


Fig. 2.2

(i) Distinguish between an absorption spectrum and an action spectrum.

absorption spectrum The graph that shows absorbance of photosynthetic pigment at each λ of light.

action spectrum The graph rate of photosynthesis against the λ of light [2]

(ii) Explain why the two curves shown in Fig. 2.2 have similar shapes.

- light energy absorbed is used in photosynthesis
- greater rate of photosynthesis at wavelengths absorbed most

[2]

Question 6

(b) The active sites of rubisco accept ribulose biphosphate (RuBP) and **either** carbon dioxide or oxygen and can catalyse the two reactions shown below.

either



or



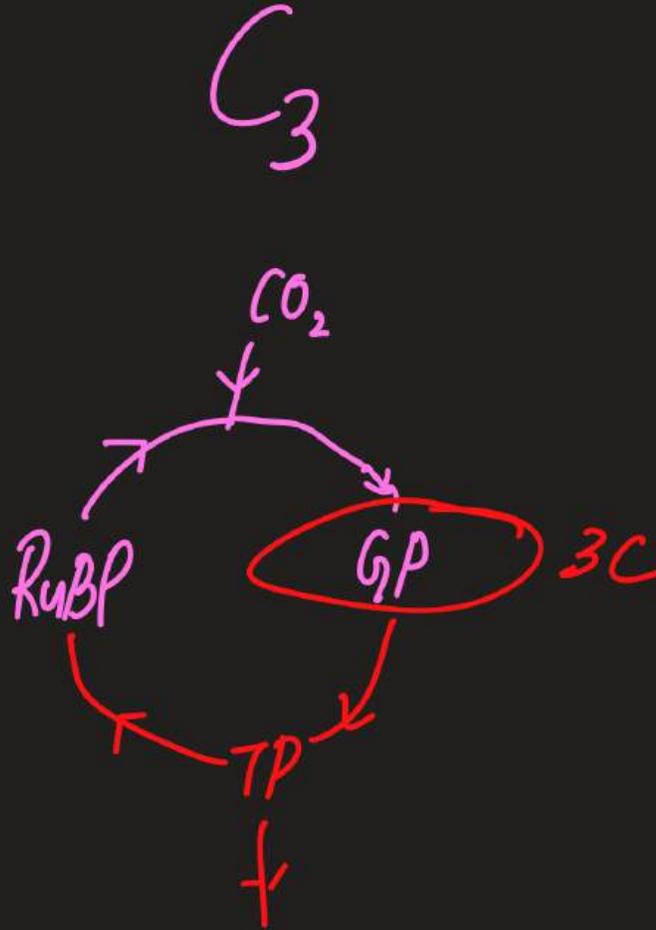
Explain the consequences to the plant of the reaction involving oxygen.

- no CO₂ fixed
- photosynthesis decreased

[2]



C_4 plants



C₄ plants

* Tropical and sub-tropical plants are exposed to :

a) high light intensity

b) high temperatures



* Under these conditions, the rate of photorespiration is high due to high concentration of O₂ in the air spaces.

* O₂ binds to the active site of rubisco (thus competes with CO₂) to form unwanted products.

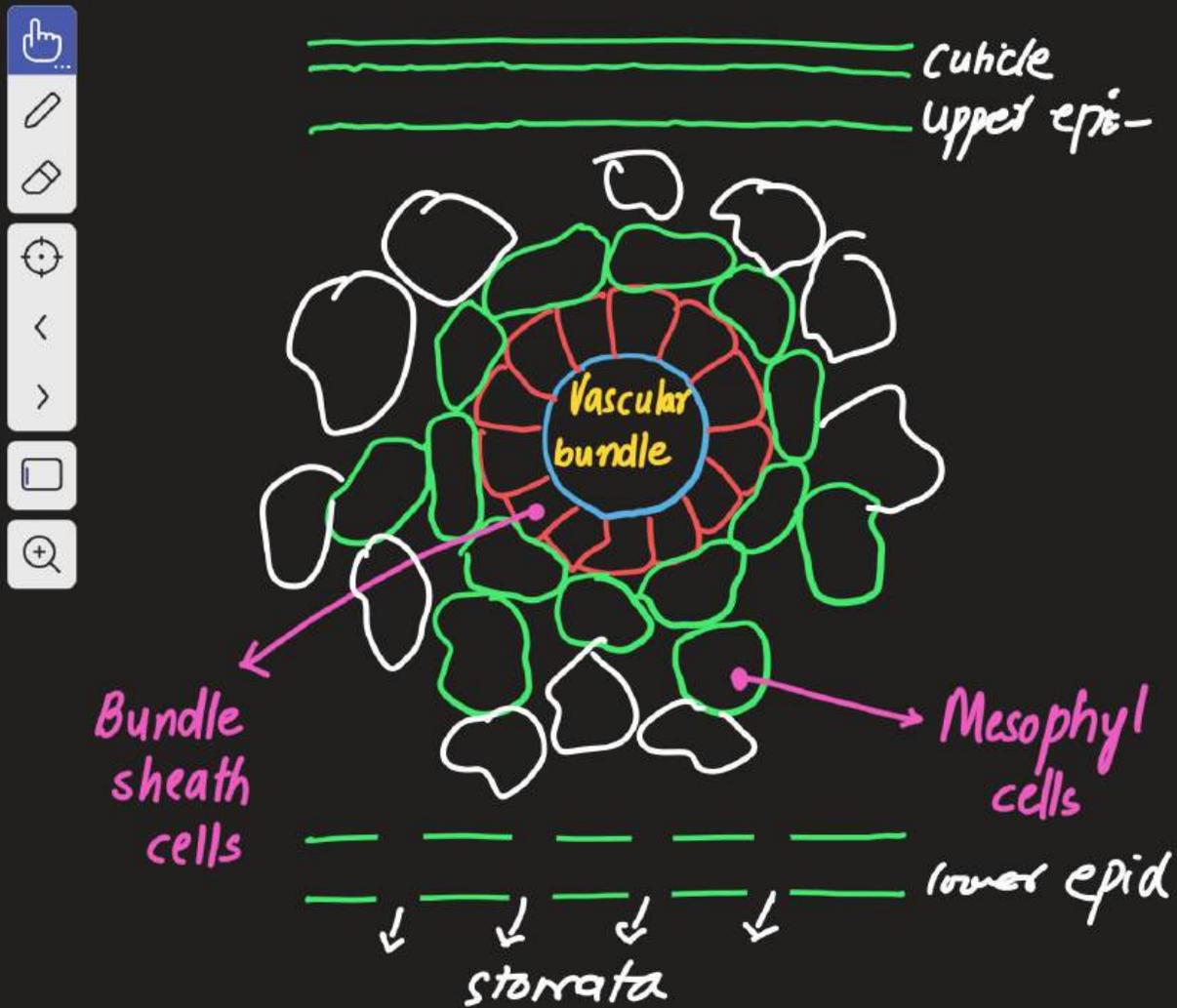
* This reduces the amount of RuBP available for carbon fixation.

* Certain tropical and sub-tropical

plants such as maize, sugarcane and sorghum therefore have a specialised anatomy to reduce the rates of photorespiration. The specialised anatomy is known as the **Kranz anatomy**

* Plants with Kranz anatomy are known as C_4 plants and can carry out C_4 pathway (discussed later)

* Given below is a section of a leaf of a C_4 plant to illustrate the main features of Kranz anatomy:



BUNDLE SHEATH CELLS

① form an air tight ring of cells around the vascular bundle => NOT directly exposed to air spaces

② main photosynthetic cells

- light dependent reactions
- Calvin cycle

③ Contain the enzyme rubisco and it's substrate RuBP.

MESOPHYL CELLS

① surround the bundle sheath cells and exposed to air spaces.

② site of C_4 pathway.

③ contain the enzyme PEP carboxylase

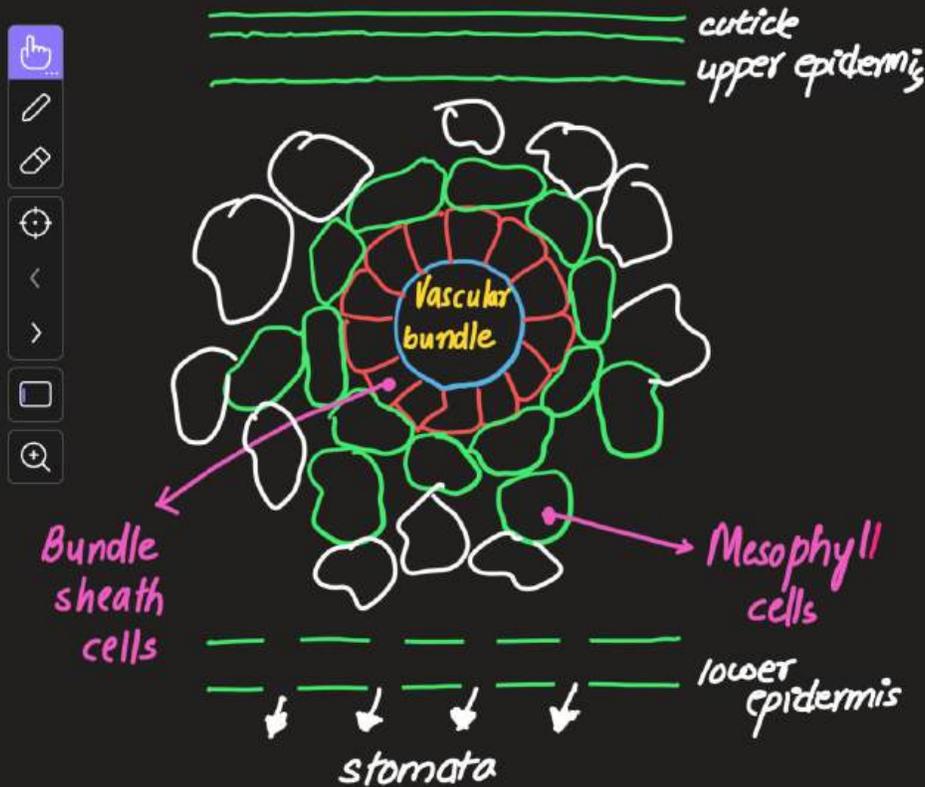
and its substrate PEP*

PEP = phosphoenol pyruvate (3C)

Photosynthesis

Previously.....

* Kranz anatomy of C₄ plants to avoid photorespiration;



With
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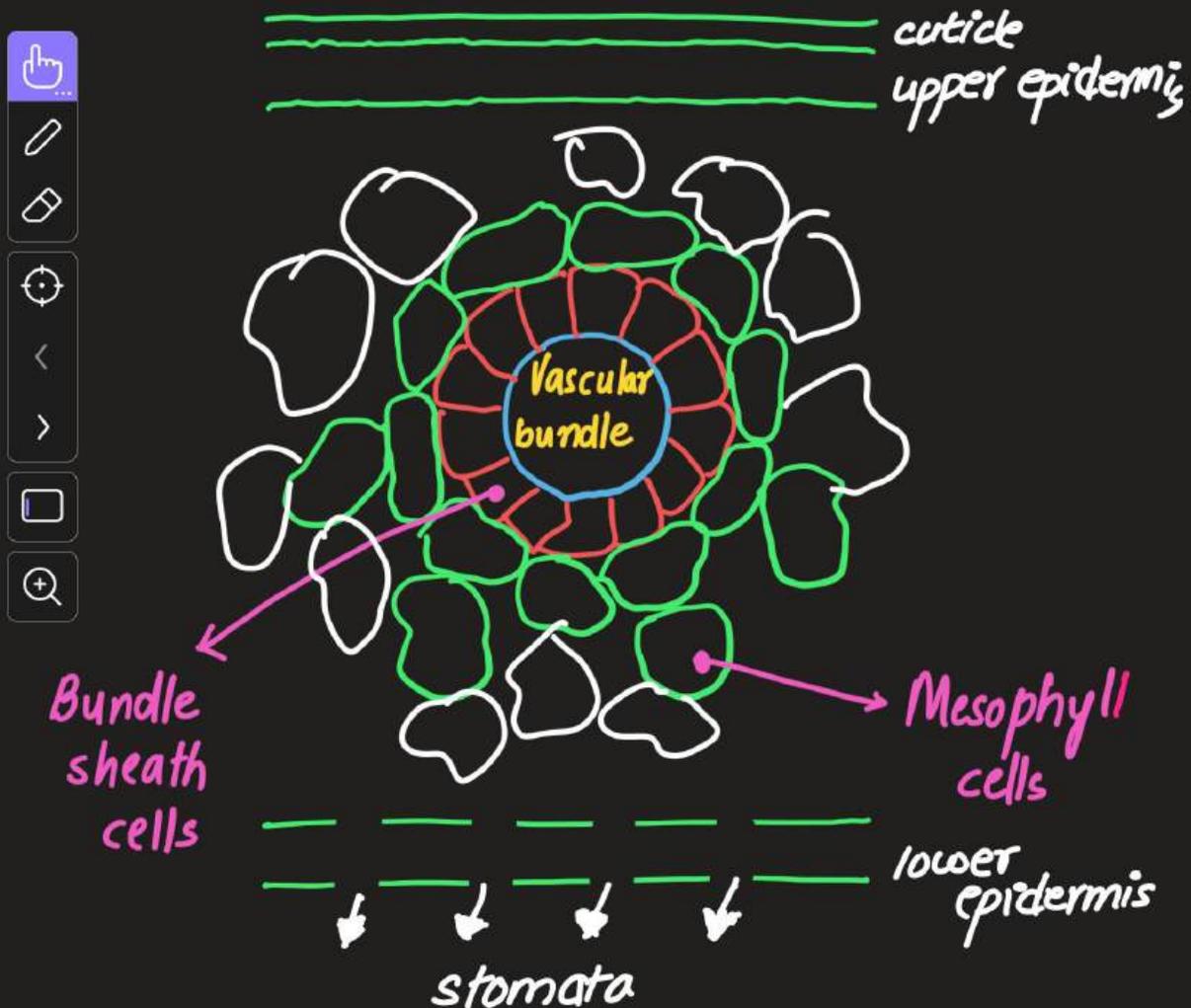
Learning Objectives:

- C4 Plants -2

Video Lecture 8 Slides
Mohammad Hussham Arshad, MD
Biology Department

Previously.....

* Kranz anatomy of C₄ plants to avoid photorespiration;



Previously....

Q. What is photorespiration?

Ans. Photorespiration is a metabolic process in plants where the enzyme Rubisco combines O_2 with RuBP in the presence of light to form unwanted products. The process releases CO_2 .

Photorespiration is also termed as the oxidative photosynthetic cycle or C_2 photosynthesis.



Previously....

Q. Suggest why the rate of photorespiration \uparrow increases at high light intensities?

Ans. \uparrow high light intensities

↓
increase in the rate of light dependent reactions and photolysis

↓
thus increasing the rate of production of oxygen

↓
this leads to an increase in $[O_2] : [CO_2]$

↓
thereby favouring the oxygenase activity of rubisco combining RuBP with O_2



Previously....

Q. State and explain what happens to the rate of photosynthesis as a result of photorespiration.

Ans.

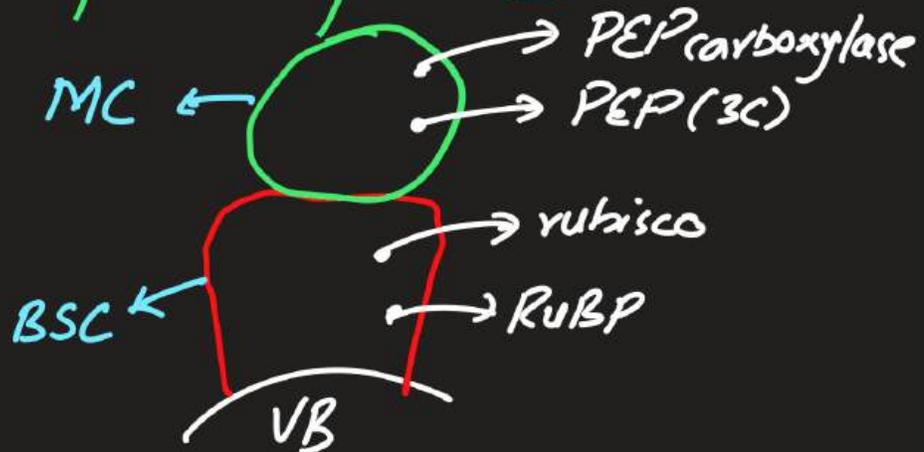
- * The rate of photosynthesis decreases
- * less RuBP available for carbon fixation
- * less GP and less TP produced
- * reduction in the rate of Calvin cycle and hence the overall rate of photosynthesis.

Previously . . .

Q. Describe and explain how the anatomy of C4 plants helps them minimise photorespiration.

Ans.

- ① Bundle sheath cells surrounded by an airtight ring of mesophyll cells
- ② Mesophyll cells stop air/oxygen from getting into bundle sheath cells
- ③ RuBP and rubisco inside the bundle sheath cells
- ④ O_2 therefore does not combine with RuBP in the presence of rubisco



Previously....

Q. Describe what is meant by a C₃ and a C₄ plant.

Ans. A plant is said to be a C₃ plant if its first photosynthetic product is a three carbon compound (GP).

Most plants are C₃ plants.

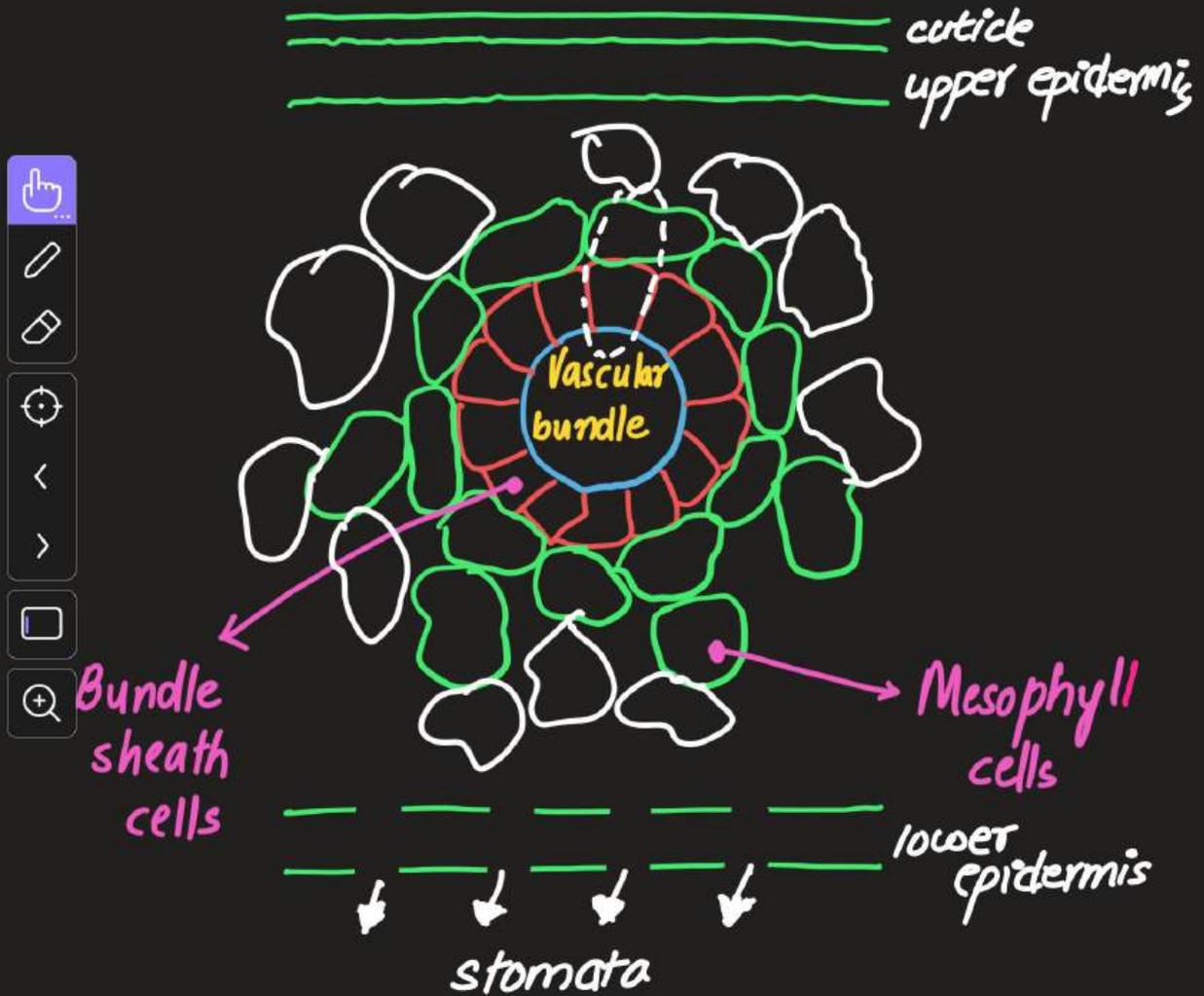
A plant is said to be a C₄ plant if the first photosynthetic product is a four carbon compound (oxaloacetate).

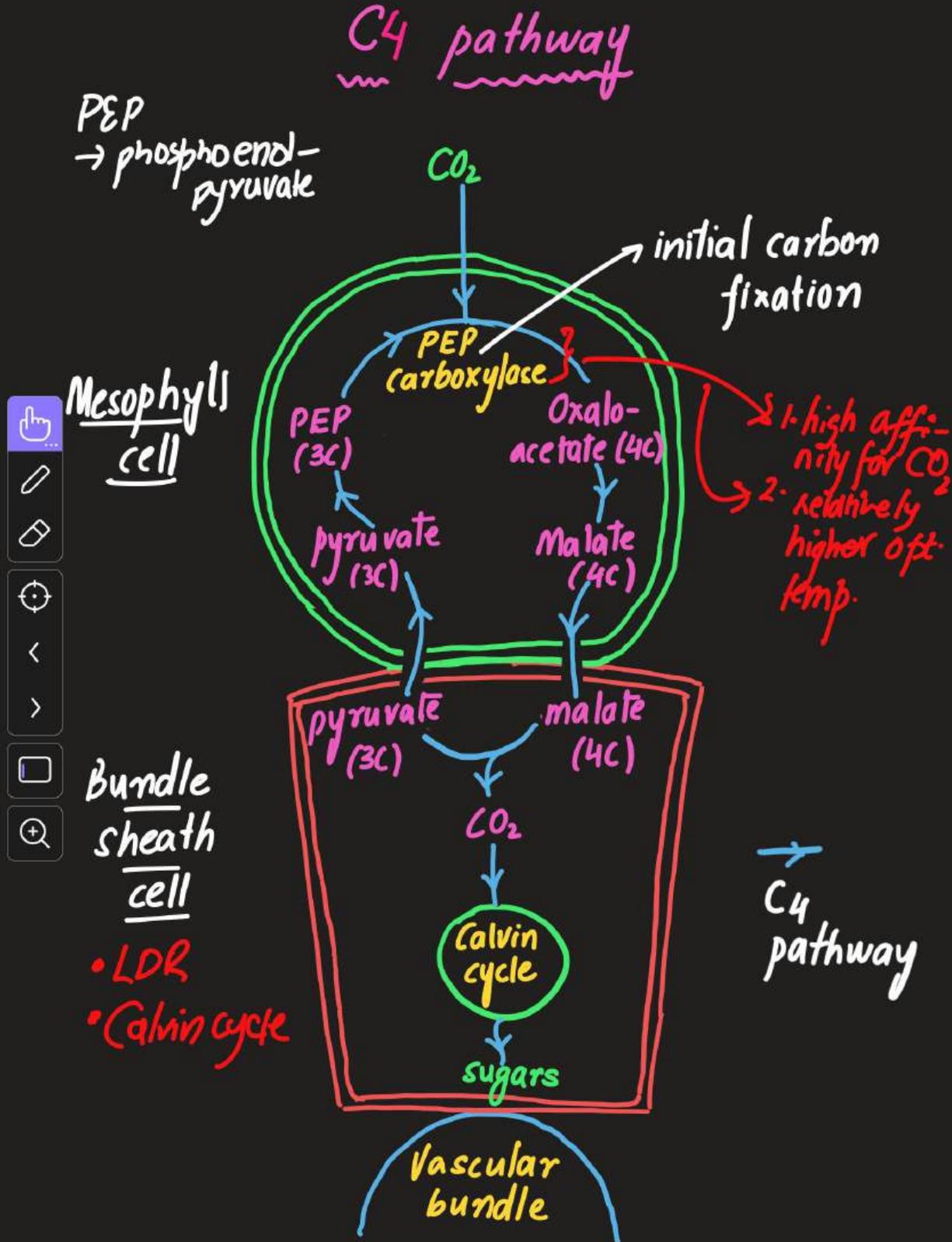




Let's have a closer look at the
C₄ pathway now

Cells involved in C₄ pathway....





* PEP carboxylase carries out initial carbon fixation in the mesophyll cells

to form a 4C stable product → oxaloacetate.

* Thus the name C₄ pathway.

* PEP carboxylase has two important properties;

- a) a higher optimum temperature
- b) a very high affinity for CO₂

* Oxaloacetate is converted into malate before being transported to bundle sheath cells.

* Malate undergoes decarboxylation releasing CO_2 and forms a 3C product pyruvate.

* CO_2 is then used in the Calvin cycle along with ATP and NADPH from light dependent reactions (in the bundle sheath).

* Light dependent reactions are said to be spatially separated from initial carbon fixation (occur in different cells)

C₃ vs C₄

Structural differences /
anatomical
differences

Biochemical
differences



Biochemical differences

C3 plant



1) The primary acceptor of CO_2 is RuBP.



2) The first stable product in C3 process is a 3 carbon compound



phosphoglycerate.



3) C3 photosynthesis occurs only in the mesophyll cells of the leaves.



4) In C3 pathway the light dependent reactions and the carbon fixation occur in the same cell.



C4 plant

1) The primary acceptor of CO_2 is phosphoenol pyruvate

2) The first stable product in C4 process is a 4 carbon compound oxaloacetate.

3) C4 photosynthesis occurs in the mesophyll cells and bundle sheath cells of the leaves.

4) In C4 pathway the initial carbon fixation is spatially separated from the light dependent reactions.



Past paper questions

Question 1:

Grass crops such as maize, sorghum and sugarcane are C4 plants. They are common grass crops of tropical regions.

Oats and wheat, commonly grown in temperate regions, are C3 plants. Most plants are C3 plants. They are termed 'C3' because the first product of photosynthesis is a three carbon compound.

(a) Outline how the biochemistry of C4 plants differs from that of C3 plants.

- first product of photosynthesis is 4 carbon compound
 - oxaloacetate
 - first CO₂ acceptor → PEP
- [2]

The C4 pathway for fixing carbon dioxide was worked out in 1966 by Hatch and Slack. During their investigation they measured the rates of fixation of carbon dioxide at high light intensities in leaves removed from both temperate and tropical grasses.

They also measured the rates of activity of two carboxylase enzymes in the leaves, ribulose biphosphate carboxylase (rubisco) and PEP carboxylase.

All rates were measured at 30 °C.

Some of their results are shown in Table 2.1.

Table 2.1

grass crop	rate of fixation of carbon dioxide / arbitrary units	rate of activity of rubisco / arbitrary units	rate of activity of PEP carboxylase / arbitrary units
maize	3.5	0.62	17.50
sorghum	3.1	0.35	15.80
sugarcane	2.9	0.30	18.50
oats	1.6	4.50	0.33
wheat	1.7	4.70	0.29

(i) With reference to Table 2.1, compare the rates of fixation of carbon dioxide in C₃ and C₄ grasses.

- rate in C₄ higher than C₃
- mean rate in C₄ = 3.17 a.u ,
mean rate in C₃ = 1.65 a.u
- more variation in C₄ plants

[2]

(ii) With reference to Table 2.1, suggest reasons for the differences in activity of the two carboxylase enzymes in C₃ and C₄ grasses.

- PEP carboxylase has higher rate of activity in C₄
- C₄ plants can live in high temps
- rubisco has higher rate of activity in C₃
- due to high concentration of CO₂

[4]

Question 2:

Fig. 7.1 shows part of a section through the leaf of a C₄ plant such as maize. The letters **A**, **B** and **C** show three types of cell found in the leaf.

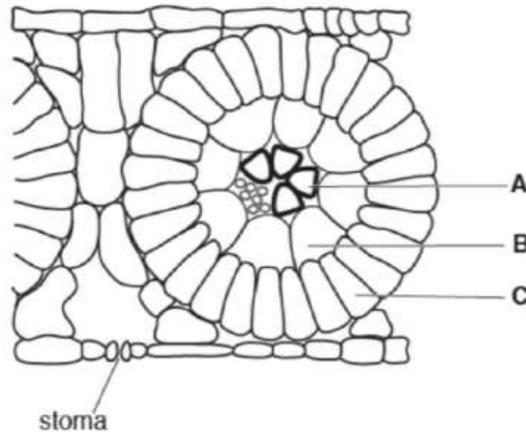


Fig. 7.1

- (a) Complete Table 7.1 by using the letters **A**, **B** or **C** from Fig. 7.1 to show the location of several compounds associated with photosynthesis in C₄ plants.

You may use **A**, **B** and **C** once, more than once, or not at all.

Table 7.1

compound	location
PEP carboxylase	C
RuBP	B
rubisco	B

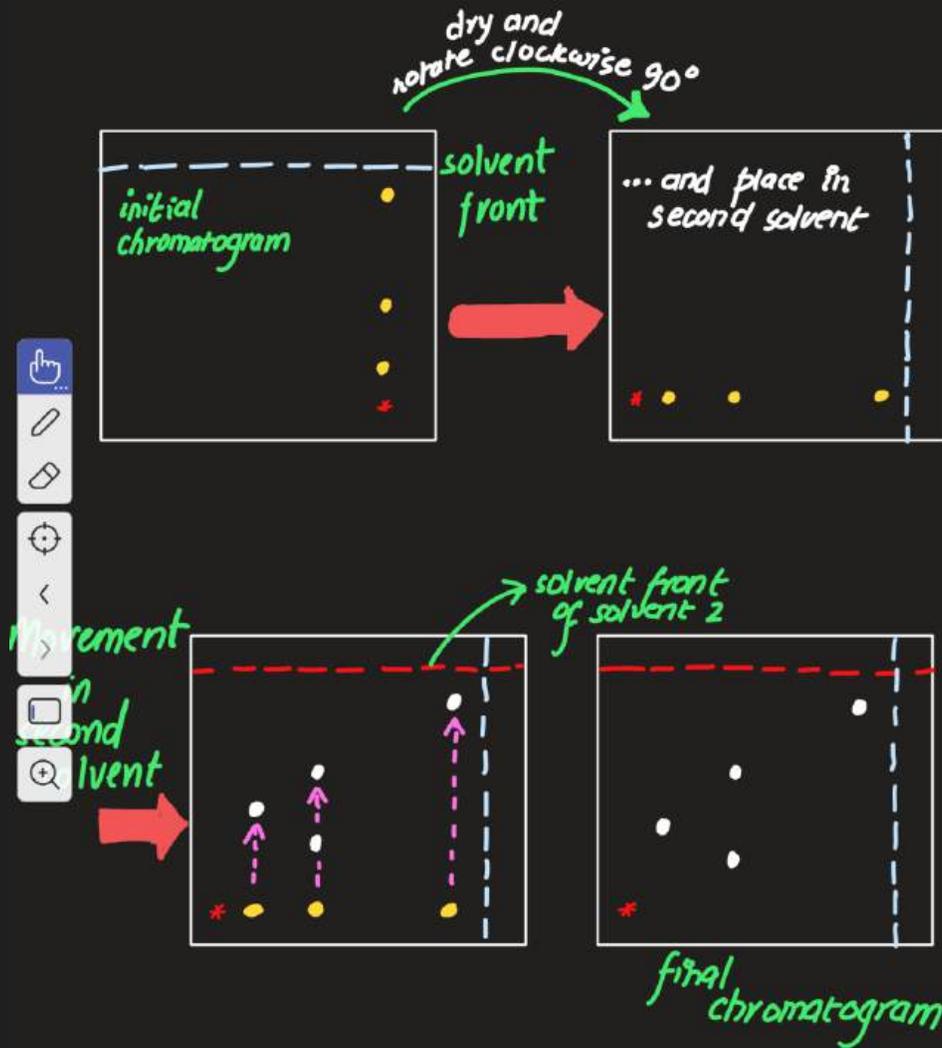
[3]

- (b) Explain why the cells in **C** form a tight ring around the cells in **B**.

- to stop oxygen from getting to cells **B**
- so rubisco doesn't catalyse reaction of RuBP with oxygen
- hence reduce photorespiration.

[2]

Photosynthesis



With
Mohammad Hussham Arshad, MD

ADVANCED LEVEL BIOLOGY 9700 UNIT 13: Photosynthesis

Learning Objectives:

- C4 plants questions
- Chloroplast isolation
- Separation of pigments using chromatography

Video Lecture 9 Slides
Mohammad Hussham Arshad, MD
Biology Department

Previously

We discussed about,

- 1) the presence of C₄ plants in tropics where high light intensities / high temperatures promote photorespiration
- 2) the Kranz anatomy in C₄ plants to minimize photorespiration
- 3) the C₄ pathway in mesophyll cells and bundle sheath cells
- 4) the features of PEP carboxylase
- 5) the spatial separation of light dependent reactions and the initial carbon fixation in C₄ plants



More past paper questions.....

Question 3:

- 4 Ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco) is an important enzyme involved in the light independent stage (Calvin cycle) of photosynthesis. It fixes carbon by combining carbon dioxide with RuBP.

In certain situations, the active site of rubisco becomes occupied by a sugar phosphate, making the enzyme inactive. Rubisco can become active again in the presence of another enzyme, rubisco activase.

- (a) Name all the bonds that are likely to hold a molecule of rubisco in shape.

* peptide bonds
* hydrogen bonds * hydrophobic bonds
* ionic bonds
* disulfide bonds [2]

- (b) Suggest how rubisco activase can activate rubisco.

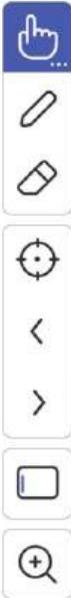
* via removal of the sugar phosphate from the active site [1]

- (c) C4 plants such as maize have adaptations that allow them to have high rates of carbon fixation at high temperatures. Without these adaptations, some plants (C3 plants) are affected at high temperatures by a process known as photorespiration.

In photorespiration, rubisco combines oxygen with RuBP. This leads to a decrease in the rate of photosynthesis.

- (i) Describe **and** explain how the anatomy of the leaves of C4 plants such as maize allows them to have high rates of carbon fixation at high temperatures.

* RuBP and rubisco are in the bundle sheath cells
* mesophyll cells form an air tight ring around the bundle sheath cells
* thereby stopping entry of oxygen into the bundle sheath cells [3]



- (ii) In C3 plants, the rate of photorespiration increases at high light intensities as well as at high temperatures.

Suggest why the rate of photorespiration increases at high light intensities in C3 plants.

* high L:I \rightarrow high rate of photolysis \rightarrow increase the rate of formation of oxygen \rightarrow \uparrow the ratio of $[O_2] : [CO_2]$ \Rightarrow favour the oxygenase activity of rubisco [2]

- (iii) Explain why the rate of photosynthesis decreases as a result of photorespiration in C3 plants.

* less RuBP available to combine with CO_2
* less GP produced \rightarrow less TP
* reduction in the rate of Calvin cycle
..... [2]

[Total: 10]



Question 4:

- 2 Most plants are C₃ plants and are so-called because their first photosynthetic product is a three carbon compound.

The enzyme ribulose biphosphate carboxylase/oxygenase (rubisco) catalyses the fixation of carbon dioxide in the Calvin cycle and is used by both C₃ and C₄ plants.

Each molecule is made up of eight large polypeptides and eight small polypeptides. Fig. 2.1 shows a side view of the molecule.

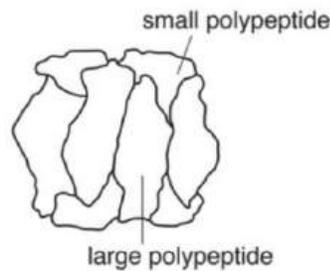
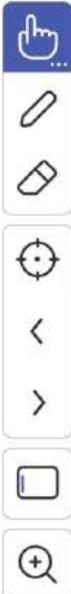


Fig. 2.1



- (a) (i) Outline how the biochemistry of C₄ plants differs from that of C₃ plants.

* PEP combines with CO₂ to form oxaloacetate
* this reaction is catalysed by PEP carboxylase

[2]

- (ii) State why rubisco is said to have quaternary structure.

* it has more than one polypeptide

[1]

- (iii) Explain what makes a molecule such as rubisco soluble.

* hydrophilic R-groups point outwards
* hydrophobic R-groups point inwards
* making the protein globular

[2]

(b) The active sites of rubisco accept ribulose biphosphate (RuBP) and **either** carbon dioxide or oxygen and can catalyse the two reactions shown below.

either



or



Explain the consequences to the plant of the reaction involving oxygen.

- * less RuBP available for carbon fixation
- * less GP and less TP produced
- * which reduces the rate of photosynthesis

[2]

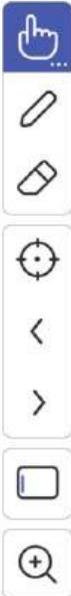
(c) In the absence of light, rubisco changes shape from an active form to an inactive form.

Explain why rubisco does **not** need to be in an active form in the absence of light.

- * no light \rightarrow no light dependent reactions
- * no ATP or NADPH produced
- * no Calvin cycle

[3]

[Total: 10]



Question 5:

(b) Explain how the leaves of maize or sorghum are able to maximise carbon dioxide fixation at high temperatures. [9]

Markscheme:

- RuBP in bundle sheath cells
- away from oxygen
- to prevent photorespiration
- CO_2 combines with PEP
- by PEP carboxylase
- in mesophyll cells
- to form oxaloacetate
- oxaloacetate converted to malate
- malate passes to bundle sheath cells
- malate releases CO_2
- RuBP combines with CO_2



Chloroplast isolation and
its uses

Isolation of chloroplasts from plant cells

Select large green leaves

↓
Wash and devein

↓
Cut into small pieces

↓
Place them in a blender with GIB solution (ice cold)

↓
Homogenise the leaf tissue

↓
filter through 8 layers of cheese cloth

↓
← centrifuge the filtrate to obtain the chloroplast pellet
dilute
↓
wrap it in Al foil → storage



Isolation of chloroplasts from plant cells

The following steps may be followed to extract chloroplasts from plant cells:



1. Select large green leaves such as the spinach or lettuce leaves.

2. Wash the leaves and remove large veins.

3. Cut the leaves into small pieces.

4. Place them in a blender which contains ice-cold phosphate buffer solution. This solution is also termed as the chloroplast isolation buffer.

The ice-cold solution slows the enzyme activity. The phosphate buffer provides an optimum pH and osmolarity for the isolation of chloroplast.



5. Blending achieves homogenisation; a process which involves breakage of the cell so that the cellular contents pour into the external solution. The resulting mixture after homogenisation is termed as 'homogenate'.

6. The homogenate is filtered through 8 layers of cheese cloth to remove debris.

7. The filtrate undergoes centrifugation

To obtain the chloroplast pellet.

8. The chloroplast pellet contains intact and damaged chloroplast.

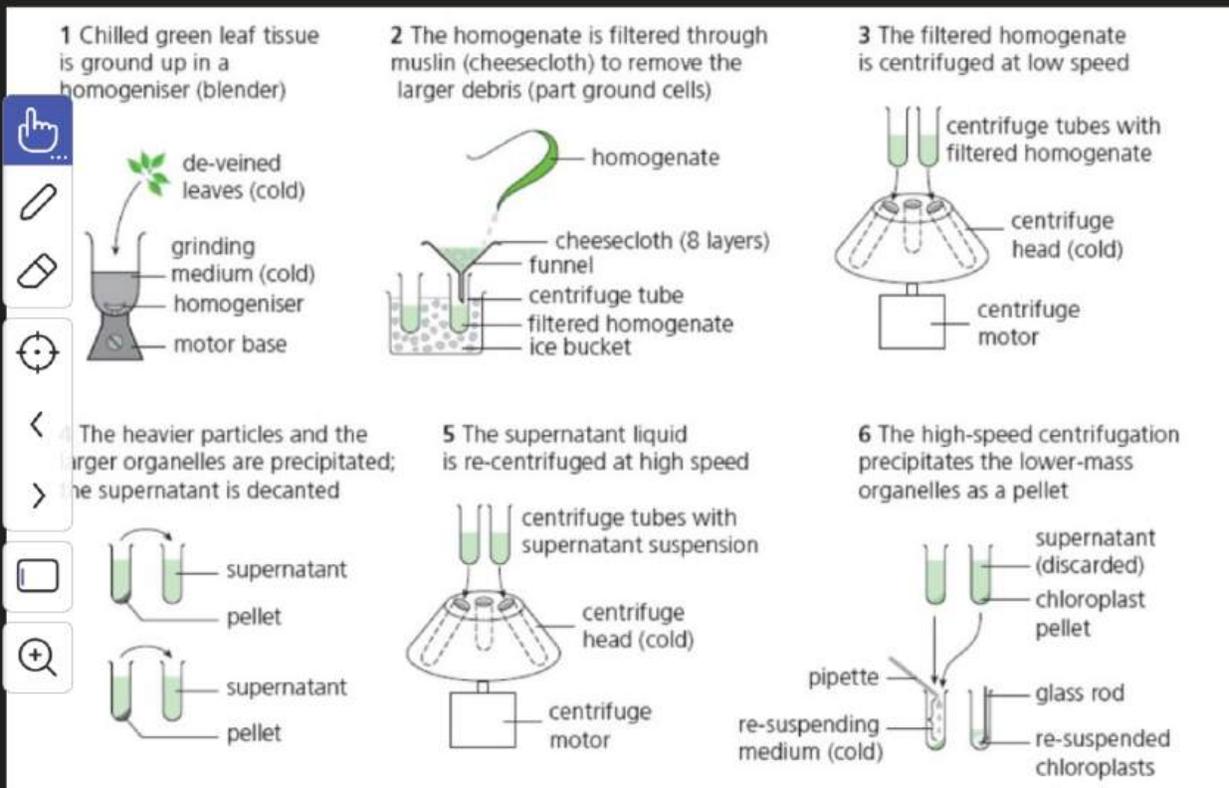
9. The chloroplast extract is diluted using acetone and wrapped in aluminium foil for storage. The aluminium foil offers photoprotection.

10. The chloroplast extract may be used
for :

a) isolation of photosynthetic pigments

b) Hill reaction





Isolation of photosynthetic pigments using chromatography

1. The chloroplast extract is concentrated via evaporation.



2. Prepare a chromatography paper by drawing the start line using a pencil.

3. Place a drop of the chloroplast extract on the start line using a pin head.

4. Place the chromatography paper in a petri dish containing suitable organic solvent such that the start line is above the level of the solvent.

5. Cover The entire apparatus with a lid to prevent evaporation of the organic solvent.



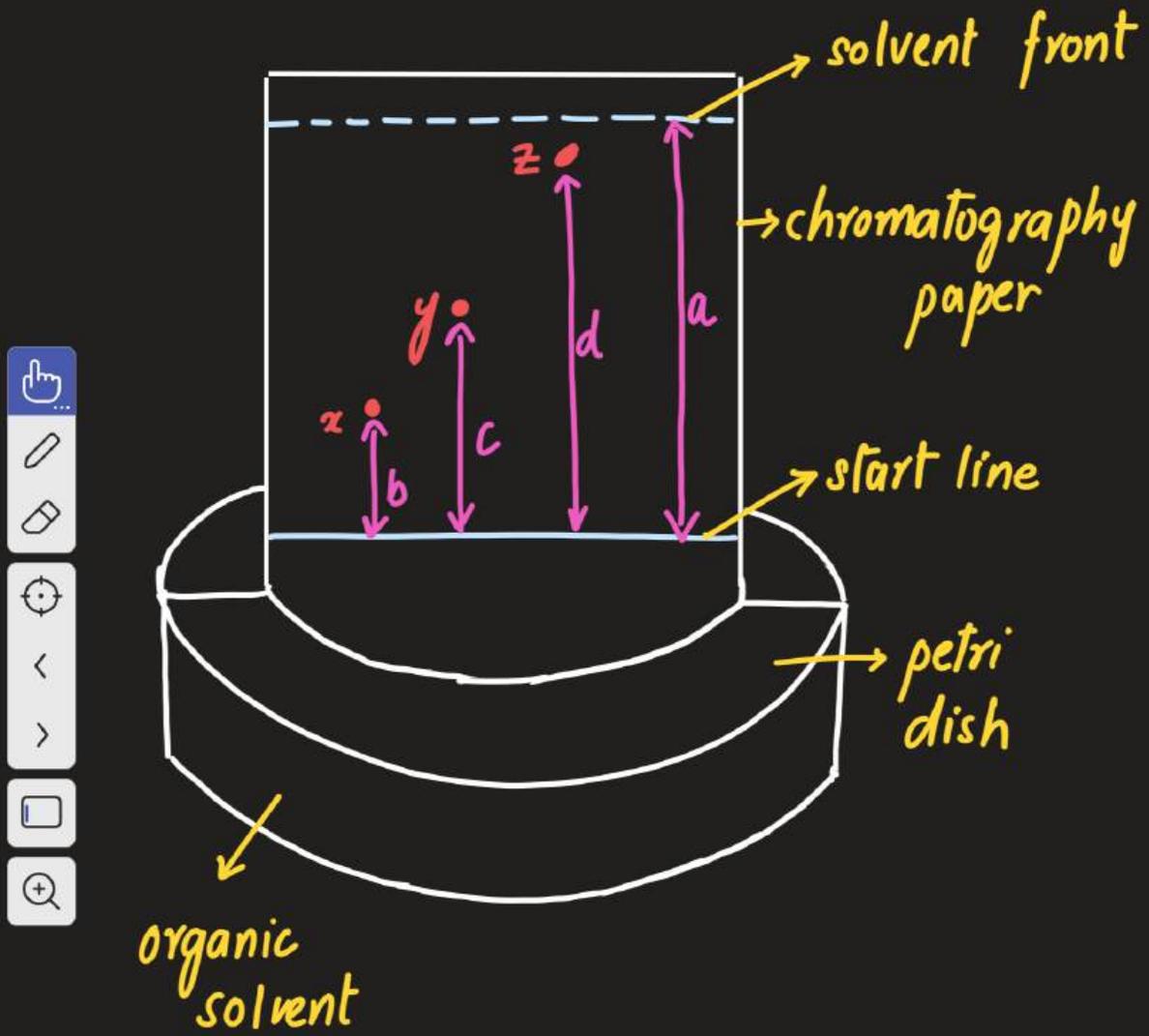
6. Allow The organic solvent to ascend the chromatography paper until The solvent front is reached.

7. The spots that appear on the chromatogram can be identified using the R_f values (Retention factor values)

8. The R_f value is calculated using The expression:

$$R_f = \frac{\text{distance moved by the pigment}}{\text{distance b/w the start line and solvent front}}$$

R_f values ≤ 1.0
↳ NO UNIT



9. At times a pigment may not be separated using this form of chromatography. This may occur due to:



a) The pigment being insoluble in the organic solvent used.



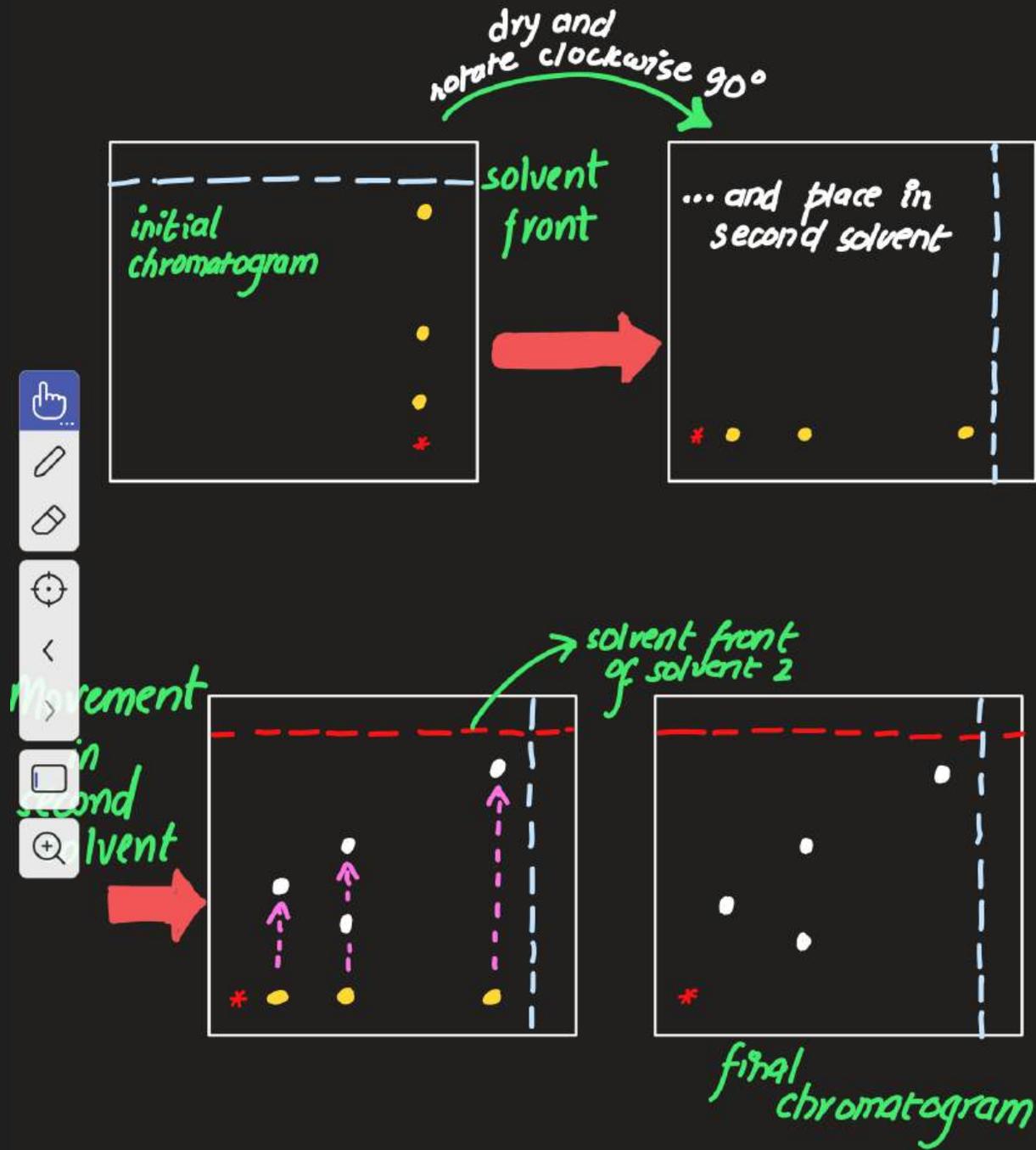
b) The pigment may be masked by another pigment.



10. In such cases two way paper chromatography is used.

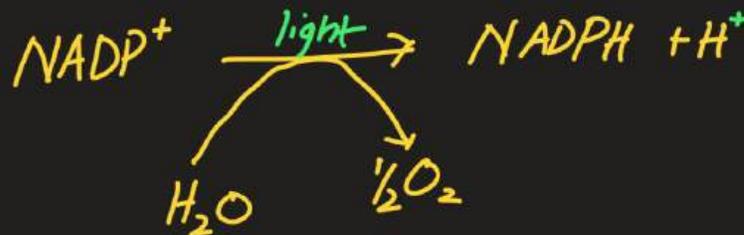
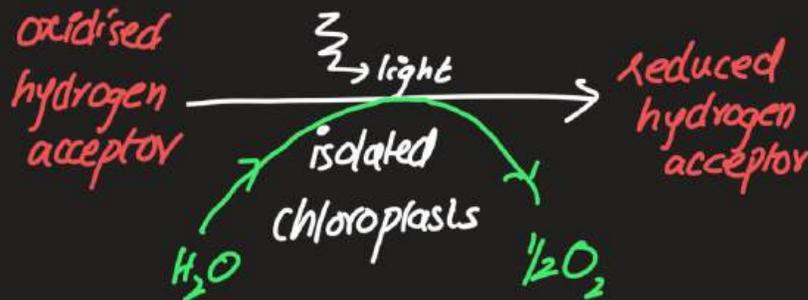
11. In two way paper chromatography the initial chromatogram is dried, rotated by 90° and placed in another organic solvent. The new R_f values obtained may be used to identify the unknown pigment.





Photosynthesis

Hill reaction



With
Mohammad Hussham Arshad, MD

ADVANCED LEVEL BIOLOGY 9700 UNIT 13: Photosynthesis

Learning Objectives:

- Hill Reaction

Video Lecture 10 Slides
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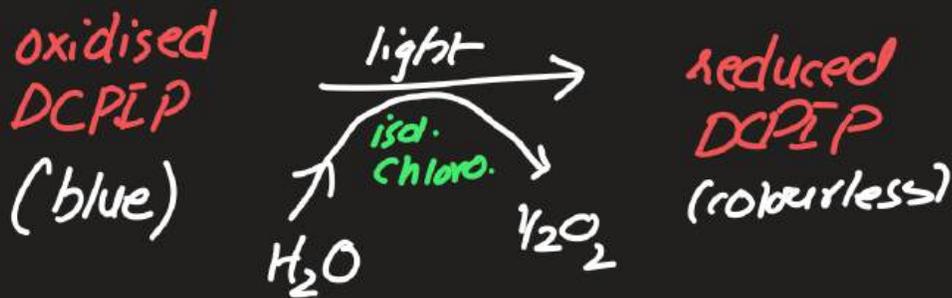
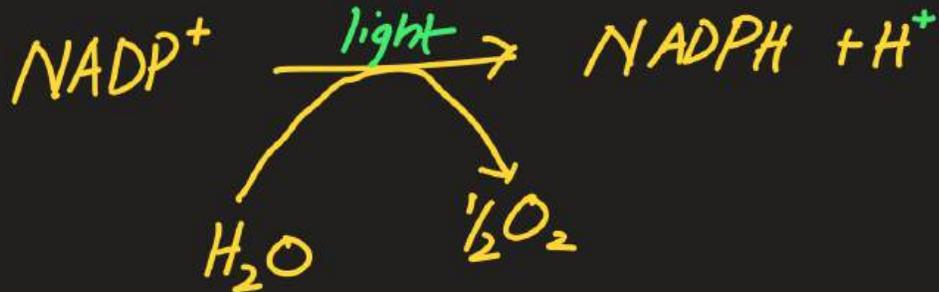
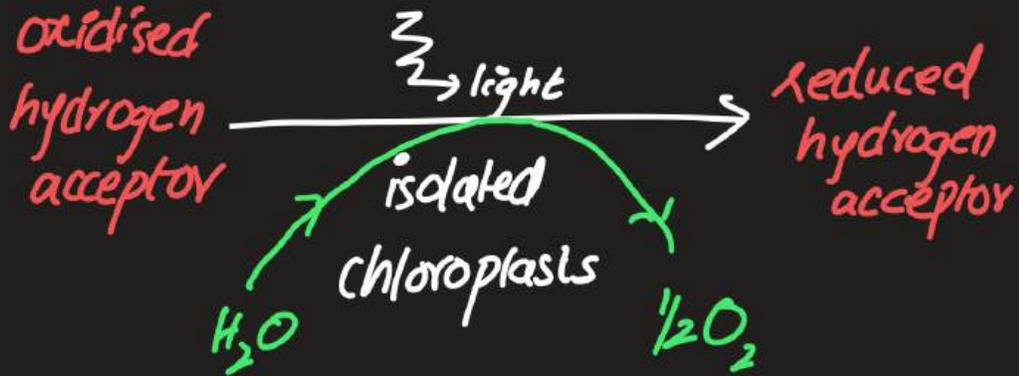
Previously,

We discussed,

- * Isolation of chloroplasts
- * Separation of pigments using chromatography



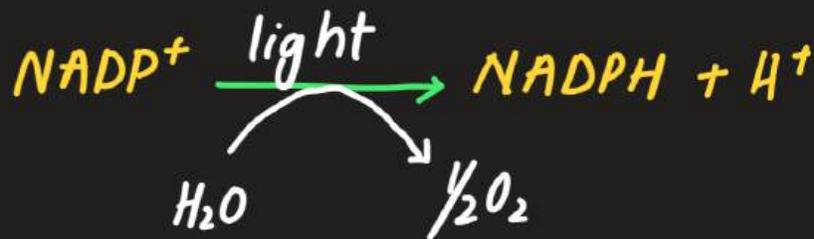
Hill reaction



Hill reaction

1. Hill reaction states that isolated chloroplasts can photoreduce a hydrogen acceptor (oxidising agent) using protons and electrons from H_2O evolving O_2 in the process.
2. Hill reaction is named after its discoverer Robert Hill.
3. Hill reaction is the measure of light dependent reaction.

4. In plant cell, $NADP^+$ serve as the electron and proton acceptor {oxidising agent}:

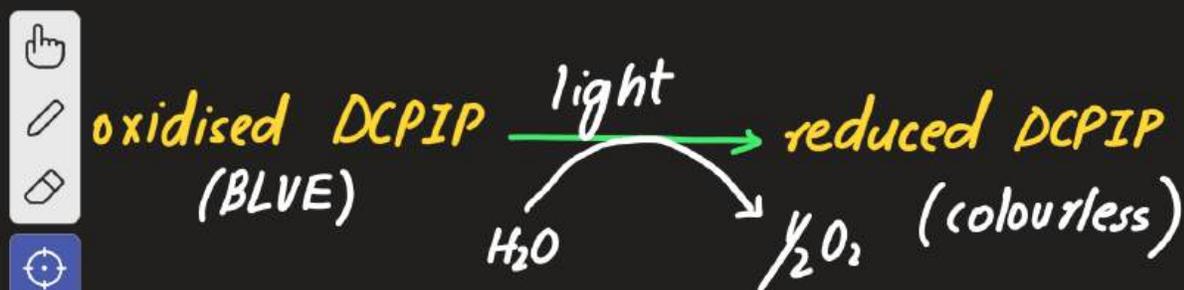


5. We can use an artificial oxidising agent in the lab though instead of $NADP^+$.

6. The oxidising agent used is **DCPIP**.



7. DCPIP is bright blue when oxidised and colourless when reduced.



8. The rate of decolourisation of DCPIP can be taken as a measure of the rate of Hill reaction = rate of light dependent reaction = rate of photosynthesis

Effect of light intensity on rate of Hill reaction

* Independent variable: light intensity

Dependent variable: Rate of decolourisation of DCPIP

* Controls: pH, temperature, volume of chloroplast extract, volume of DCPIP

* How is The light intensity varied?

→ By using different shades of grey filters and Al foil (shown below)



* How is the rate of decolourisation calculated?

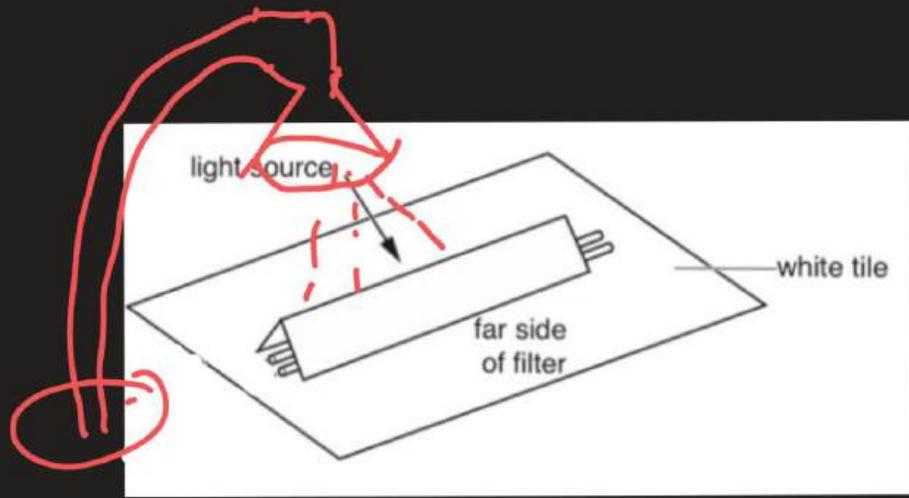
→ Use of stopwatch to note The time taken (s) for decolourisation.

→ $\frac{1}{\text{time}}$ is a measure of the rate in s^{-1} .

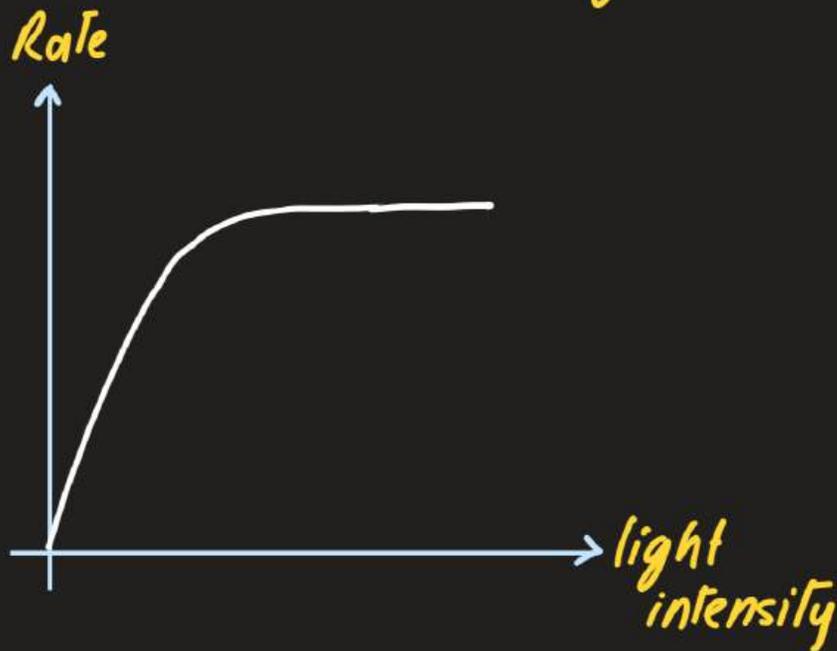
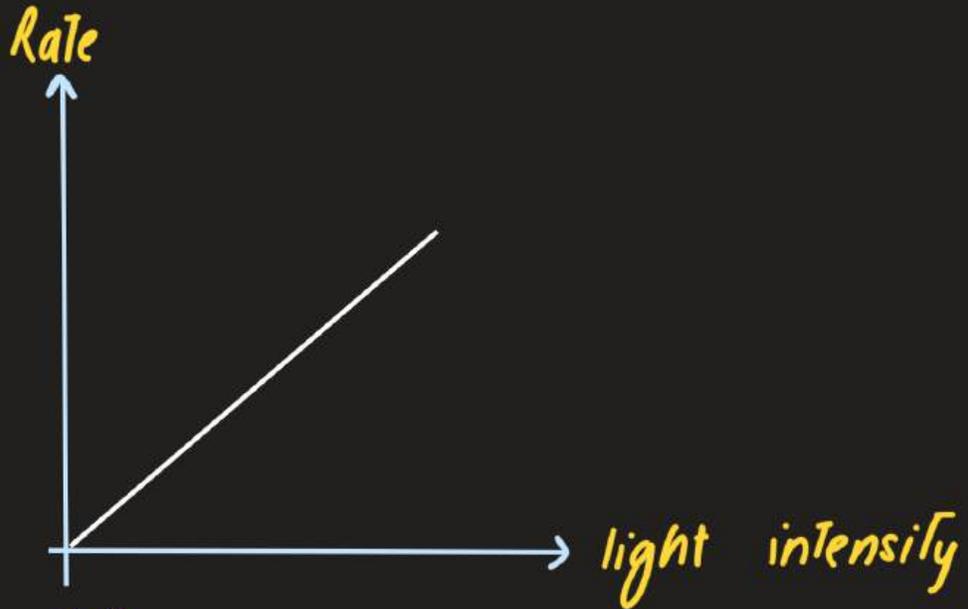


Capillary tube	Content	filter	% light Transmitted	time/s	Rate/s ⁻¹
A	Extract	No filter	100		
B	Extract + DCPIP	No filter	100		
C	"	pale grey	75		
D	"	medium grey	50		
E	"	dark grey	25		
F	"	Al foil	0		





Analysis of results



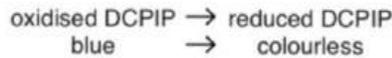


Past paper questions

Question 1

- 2 The light dependent stage of photosynthesis in a suspension of isolated chloroplasts can be investigated using the Hill reaction.

Dichlorophenolindophenol (DCPIP) can be used to follow the process. DCPIP is a blue dye which turns colourless when it is reduced by accepting hydrogen and electrons.



- (a) (i) DCPIP is an artificial hydrogen acceptor that can be used in the Hill reaction.

Name the natural hydrogen acceptor found in chloroplasts that is replaced by DCPIP in the Hill reaction.

..... *NADP⁺ / oxidised NADP* [1]

- (ii) Outline the way in which hydrogen is made available to reduce the hydrogen acceptor in the light dependent stage of photosynthesis.

..... ** photolysis of water*

..... ** which produces O₂, protons and*

..... *electrons in the presence of the*

..... *water splitting centre*

..... [2]

- (b) A suspension of isolated chloroplasts for measuring the rate of the Hill reaction can be prepared by carrying out the following steps:

- prepare buffer solution with the same water potential as the stroma of chloroplasts
- liquidise (homogenise) spinach leaves in ice cold buffer solution
- filter the liquid and obtain the filtrate
- centrifuge the filtrate to obtain a pellet of chloroplasts
- add the chloroplast pellet to fresh buffer solution in a beaker and mix to obtain a suspension.

Explain the reason for:

- (i) keeping the temperature very low

..... ** to slow down the enzyme activity*

..... ** thereby preventing chloroplast*

..... *damage*

..... [2]



(ii) using a buffer solution

* to provide an optimum pH
* which ensures that the enzymes
are NOT denatured

[2]

(iii) using a solution of the same water potential as the stroma of chloroplasts.

* this prevents osmosis
* thereby preventing damage to
the chloroplasts

[2]

(c) An experiment was carried out to measure the time taken for decolourisation of DCPIP mixed with a suspension of chloroplasts.

The results are shown in Table 2.1.

Table 2.1

replicate	time taken for DCPIP to decolourise/s	rate/s ⁻¹
1	38	
2	43	
3	48	
mean	43	23.3

Complete Table 2.1 by calculating:

(i) for the three replicates, the mean time taken for the DCPIP to decolourise [1]

(ii) the mean rate using the formula:

$$\text{rate} = \frac{1000}{t} \quad \text{where } t = \text{time in seconds.} \quad [1]$$



(iii) The time taken to decolourise DCPIP was measured at a range of light intensities.

State **and** explain the expected relationship between light intensity and time taken to decolourise DCPIP.

expected relationship ... *as light intensity increase,
the time taken for decolourisation
decrease*

explanation ... *more light intensity*

*↓
more energy in the form of
photons*

*↓
↑ rate of photolysis*

*↓
↑ rate of formation
of protons and
electrons*

*↓
and hence increasing
the rate of reduction
of DCPIP*

[4]

[Total: 15]

Photosynthesis

LOLLIPOP EXPERIMENT

- Radioactive carbon-14 is added to a 'lollipop' apparatus containing green algae (Chlorella).
- Light is shone on the apparatus to induce photosynthesis (which will incorporate the carbon-14 into organic compounds)

With

Mohammad Hussham Arshad, MD

ADVANCED LEVEL BIOLOGY 9700 UNIT 13: Photosynthesis

Learning Objectives:

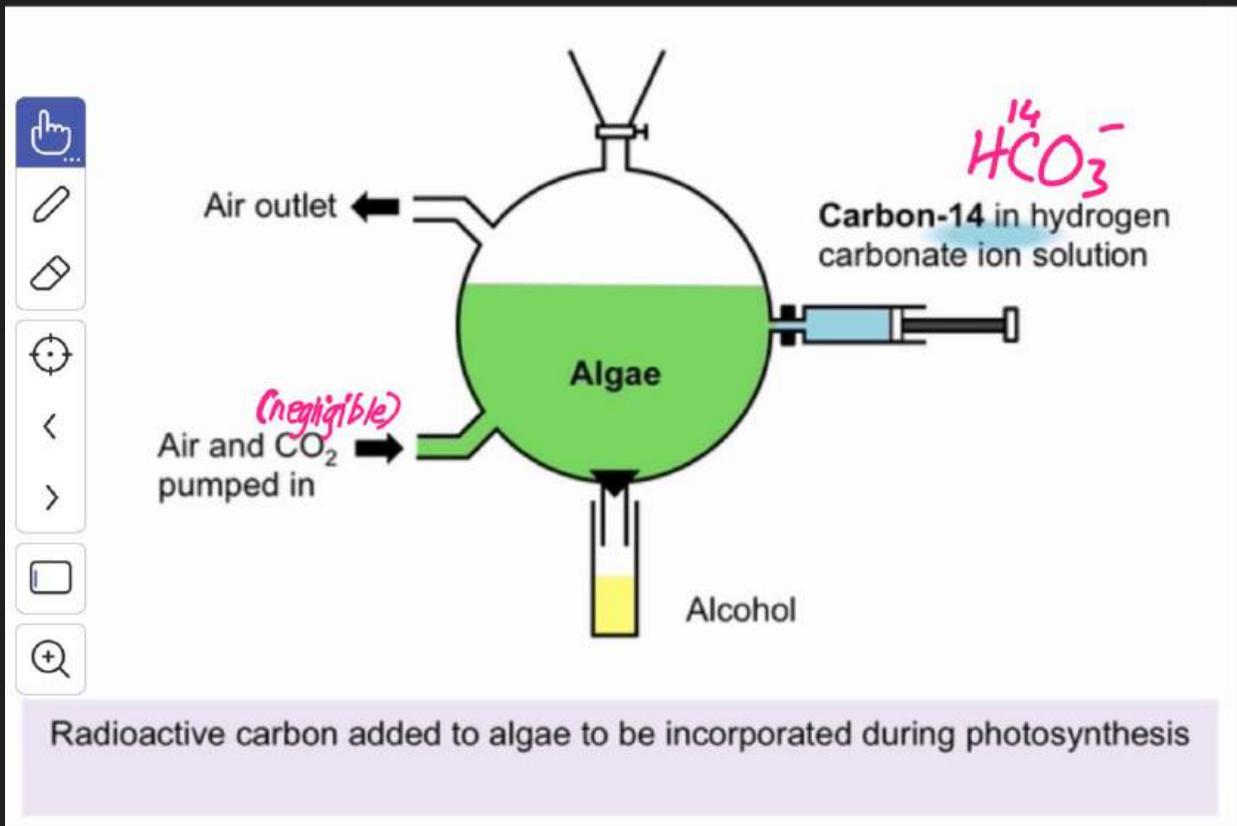
- Lollipop experiment
- Sun & Shade leaves

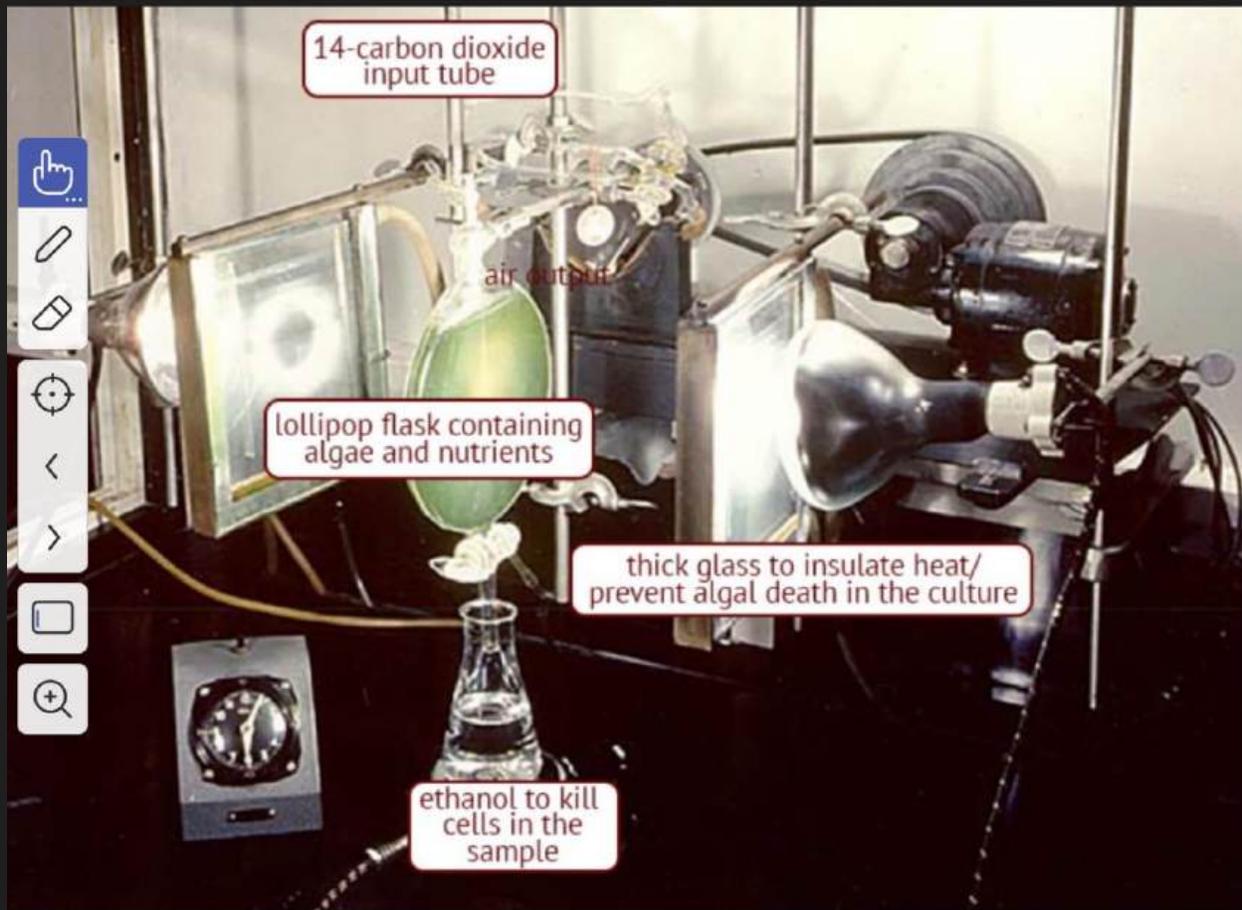
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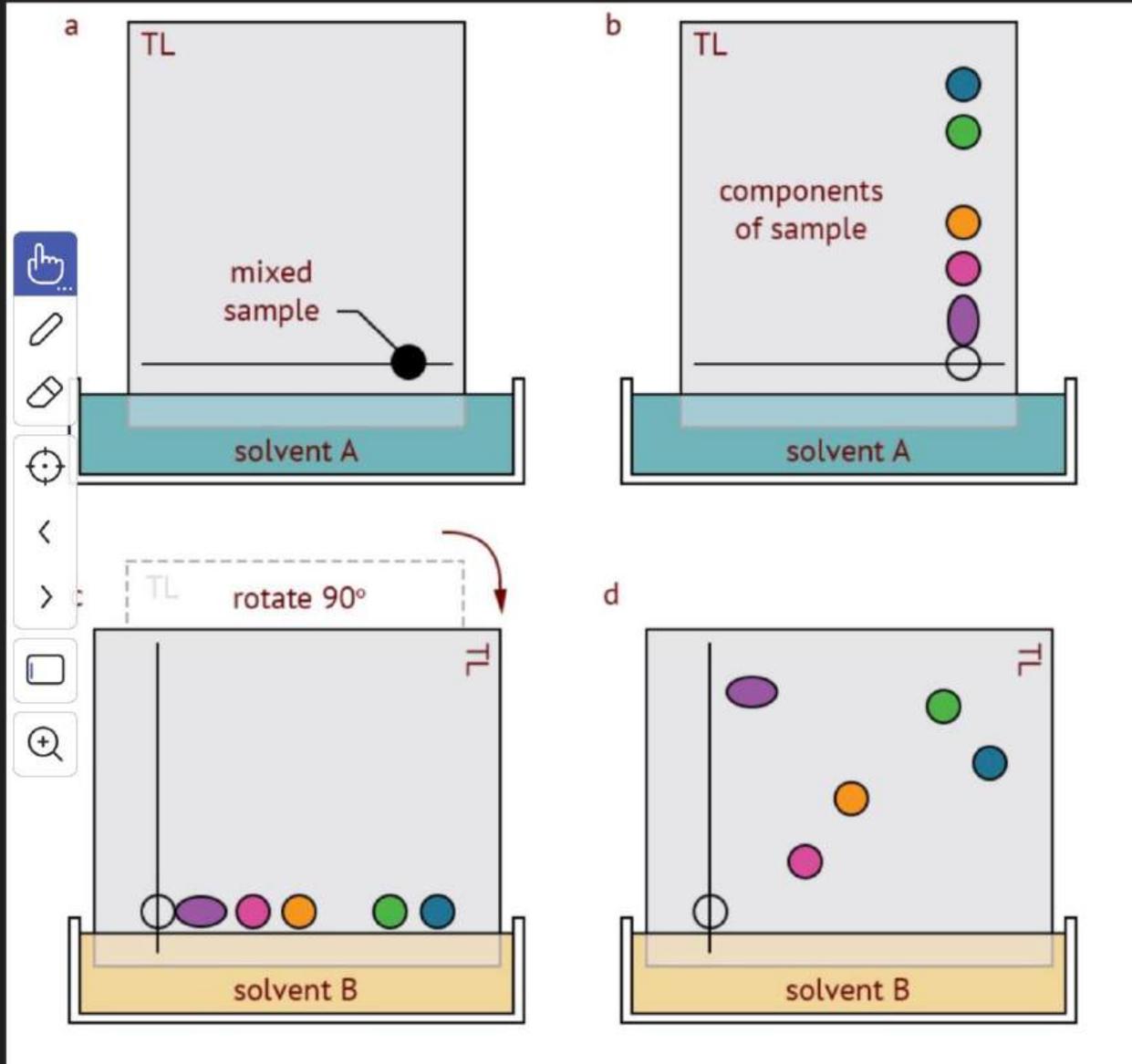


Lollipop experiment
(Melvin (alvin))

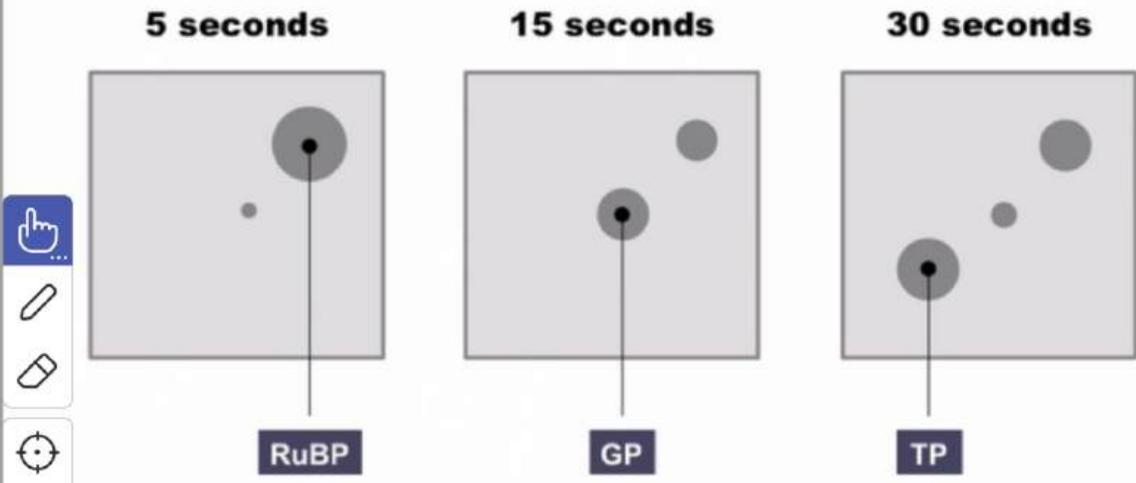
doliplop vessel → set up
Algae: Chlorella







Autoradiograph Time Course:



Lollipop experiment conducted for a number of different time periods
Order in which compounds form is used to determine progression of cycle



LOLLIPOP EXPERIMENT

• Radioactive carbon-14 is added to a

 'lollipop' apparatus containing green algae



(Chlorella).

• Light is shone on the apparatus to

induce photosynthesis (which will

incorporate the carbon-14 into organic

compounds)

- After different periods of time, the algae is killed by running it into a solution of heated alcohol.
- Dead algal samples are analysed using 2-way chromatography, which separates out the different carbon compounds.

- Any radioactive carbon compounds on the chromatogram were then identified using autoradiography. (X-ray film exposure)

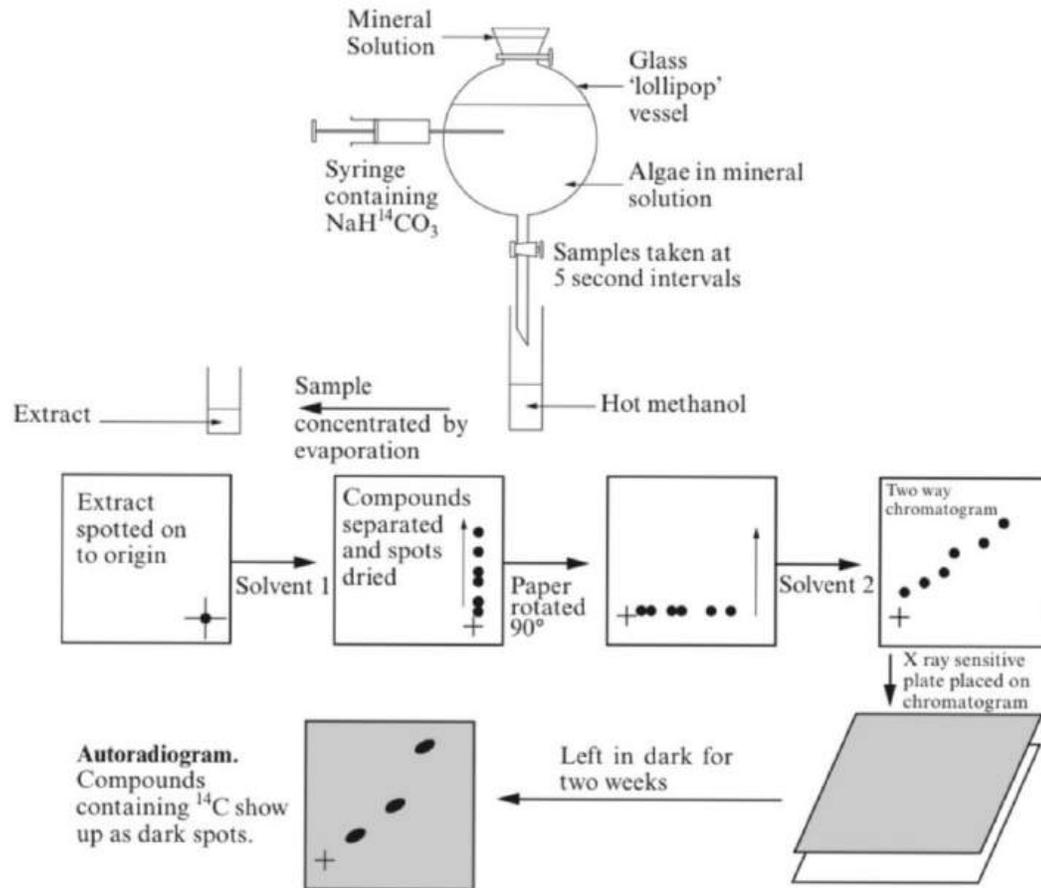


- By comparing different periods of light exposure, the order by which carbon compounds are generated was determined.

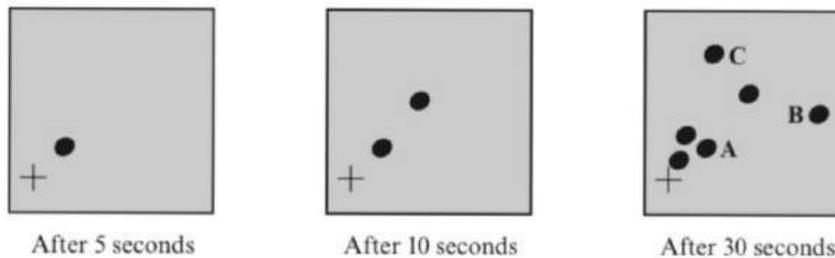
- Calvin used this information to propose a sequence of events known as The Calvin cycle (light independent reactions)

Question 1:

2. Calvin did experiments on a series of reactions which is now called the light independent stage of photosynthesis. The diagram shows one such experiment. The apparatus was set up as shown and brightly illuminated. The clock was started on the introduction of radioactive hydrogen carbonate ions.



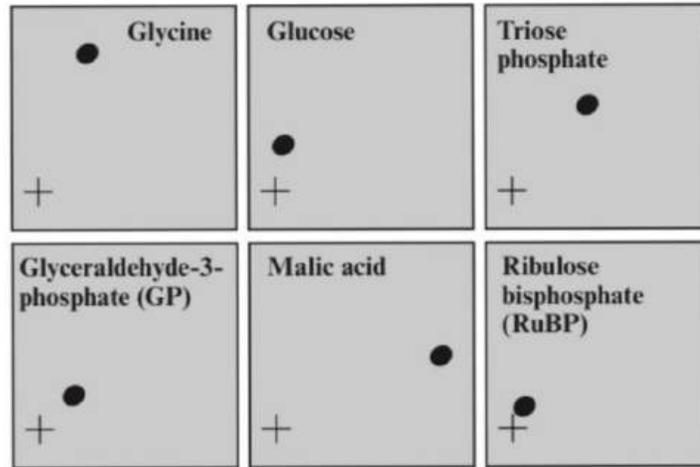
Autoradiograms from one such experiment are shown below:



(a) What is the main difference between these three autoradiograms? [1]

increased number of spots on the autoradiogram

(b) To identify the substances represented by the dark spots, Calvin made autoradiograms of known substances. He then compared their positions with those of the dark spots. The results of some of these are shown below.



Use these autoradiograms and the ones shown in part (a) to identify compounds represented by spots A-C. [1]

Spot	Name of compound
A	<i>GP</i>
B	<i>malic acid</i>
C	<i>glycine</i>

(c) Use the autoradiograms to determine which were the first and second substances formed. [2]

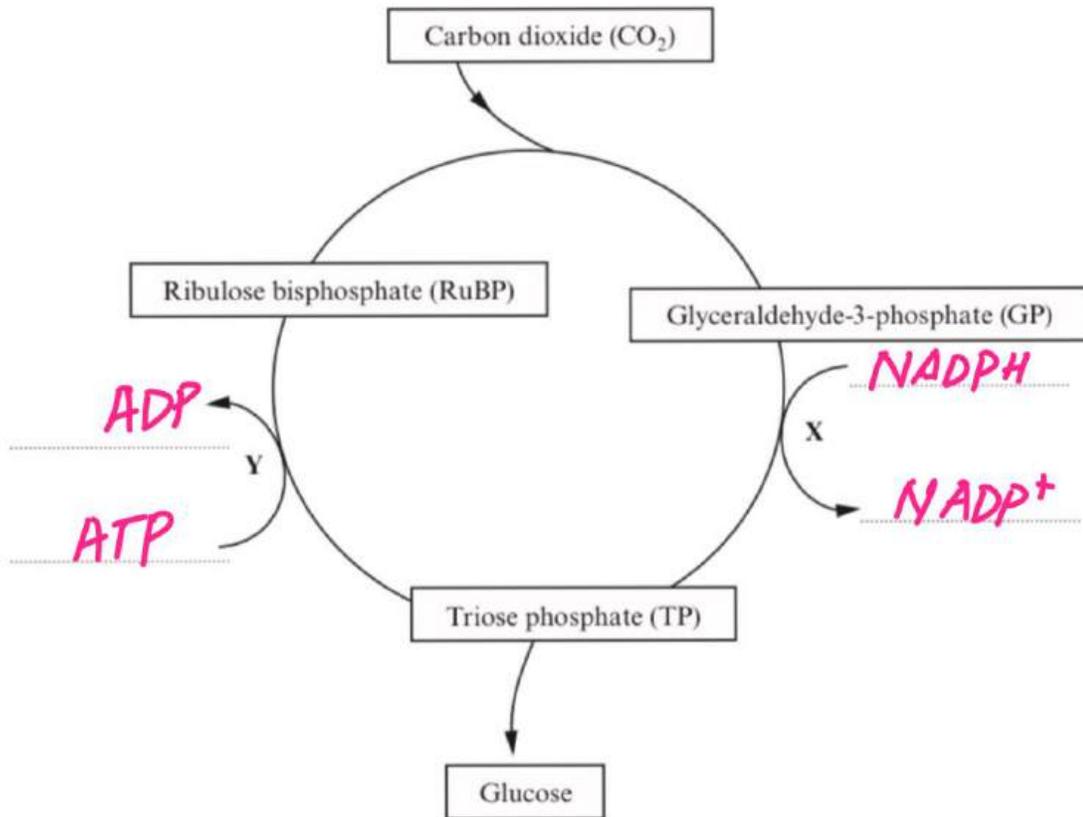
First *GP*
 Second *TP (triose phosphate)*

(d) Glycine is an amino acid. Which chemical element would have been needed in the mineral solution in order for the algae to have made this compound? [1]

nitrogen



(e) Calvin worked out that the ribulose bisphosphate is regenerated so that the reactions are in the form of a cycle, which is summarised below:



Compound X is a hydrogen carrier and compound Y is the universal energy currency in cells.

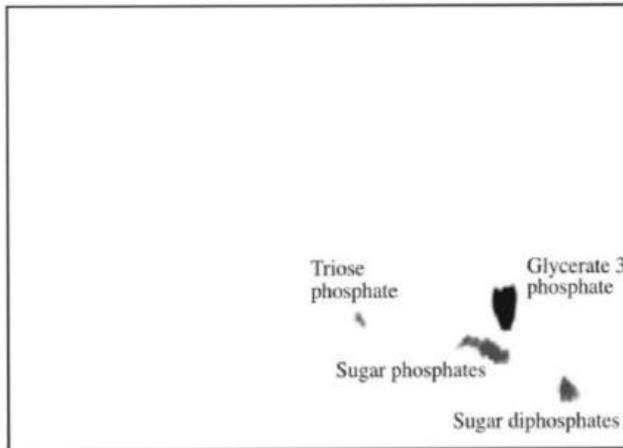
- (i) Complete the diagram to show how compounds X and Y change during the cycle. [2]
- (ii) Which series of reactions provides the compounds X and Y in chloroplasts? [1]
light dependent reactions
- (iii) State precisely where the production of X occurs in chloroplasts. [1]
thylakoid membranes
- (f) How many molecules of triose phosphate would be needed to synthesise three molecules of glucose? [1]
6

(Total 10 marks)

Question 2:

7. To investigate the sequence of events in the Calvin cycle, the following experiment was carried out.
1. The alga *Chlorella* was exposed to $^{14}\text{CO}_2$.
 2. After time intervals of 5 seconds and 30 seconds a sample of algae was added to hot ethanol to stop enzyme reactions in the cells.
 3. The radioactive compounds were separated by paper chromatography. Autoradiographs of the results are shown below.

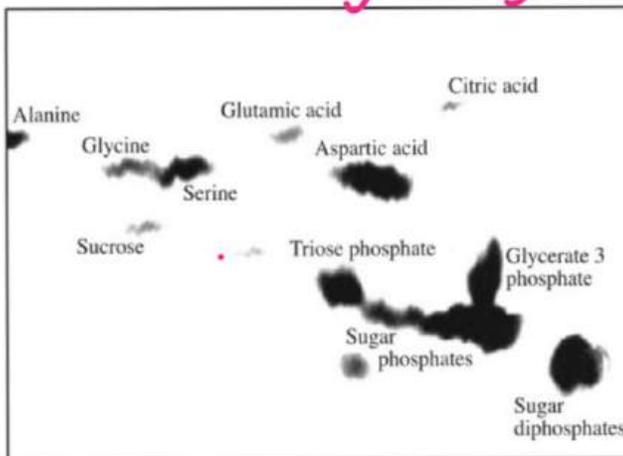
The darker, more intense the spots, the more of that compound is present.



Chromatography paper showing radioactively labelled compounds extracted from *Chlorella* after 5 seconds photosynthesis with radioactive CO_2 .

- (a) Using your knowledge of the Calvin cycle explain the quantitative results of the autoradiograph after 5 seconds. [2]

** dark spot of glycerate phosphate indicating a lot has been synthesized * first product of after $^{14}\text{CO}_2$ is GP.*



Chromatography paper showing radioactively labelled compounds extracted from *Chlorella* after 30 seconds photosynthesis with radioactive CO_2 .

From Basham TA (1965) "Photosynthesis: The path of carbon." In *Plant Biochemistry*, 2nd ed., J Bonner & E Varner, eds. Academic Press NY pp. 875-902

(b) What further information can be determined from the autoradiograph after 30 seconds? [3]

- * more sugar phosphates produced
- * more triose phosphates produced
- * sucrose present
- * presence of amino acids

(c) What further compounds would you expect to see after 300 seconds? [2]

- * Starch
- * Lipids
- * Proteins
- * cellulose

(d) The Calvin cycle depends on products of the light dependent stage of photosynthesis. Name these two products. [1]

NADPH
ATP

(e) The light dependent stage involves both cyclic and non-cyclic photophosphorylation. Explain what is meant by

(i) Cyclic photophosphorylation [2]

- * cyclic flow of electrons → electron released by and return to PSI
- * ATP produced using proton motive force

(ii) Non-cyclic photophosphorylation [4]

- * Light absorbed by both PS
- * electrons travel through ETC
- * Photolysis of water replaces electrons lost by PSII
- * PSII replaces electrons lost by PSI
- * ATP and NADPH produced.

(Total 14 marks)



Photosynthesis

<u>SUN LEAVES</u>	<u>SHADE LEAVES</u>
ANATOMICAL DIFFERENCES	
THICKNESS Thicker with more tightly packed cells	Thinner with less densely packed cells
SIZE Smaller in size with less surface area	Greater surface area exposed.
CHLOROPLASTS Fewer number of chloroplasts	Greater number of chloroplasts
COLOUR Lighter in colour when compared with shade leaves	Darker green in colour.
STOMATA Stomatal pores are smaller but more dense	Stomatal pores are larger but less dense.

With

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ADVANCED LEVEL BIOLOGY 9700 UNIT 13: Photosynthesis

Learning Objectives:

- Lollipop experiment
- Sun & Shade leaves

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SUN AND SHADE PLANTS

- Leaves are usually adapted to grow in direct sunlight or shaded conditions.

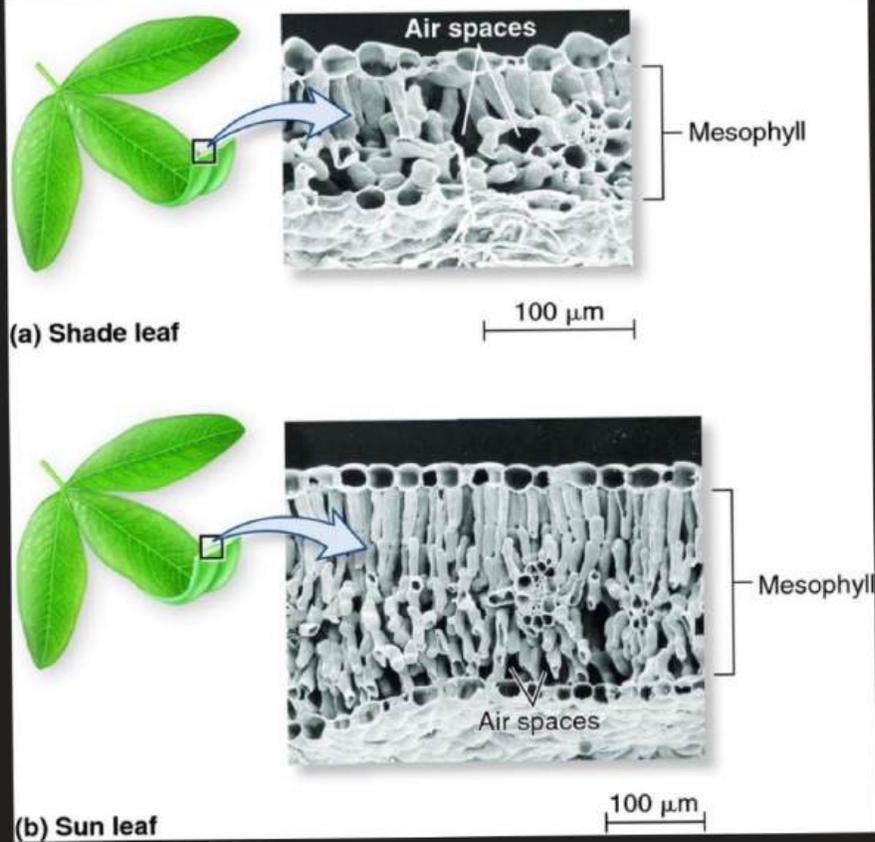
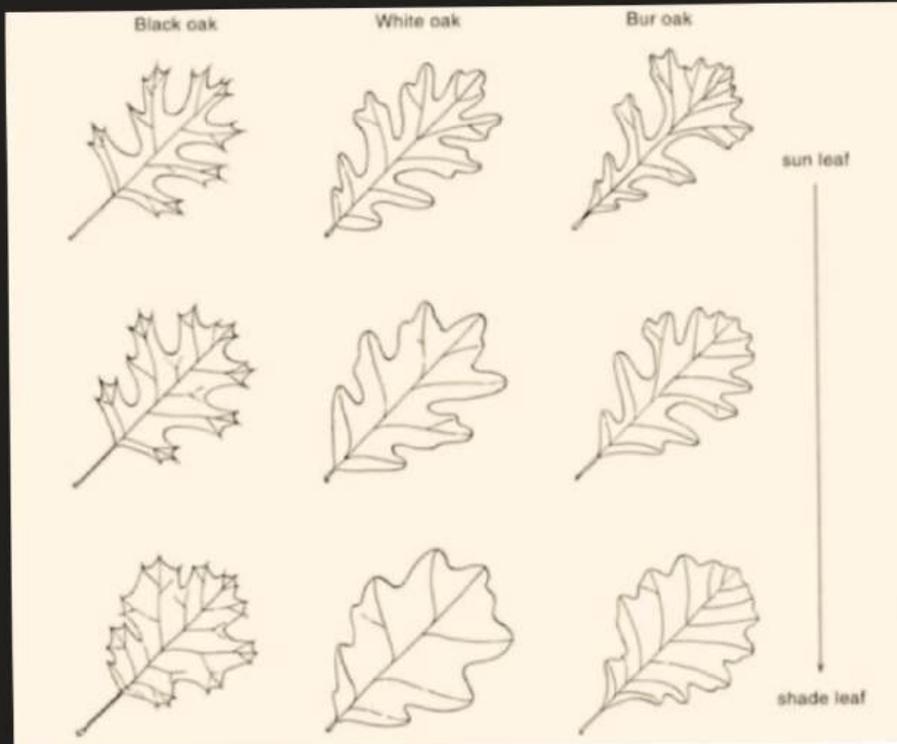


- The leaves which develop under the shade of other leaves are anatomically and metabolically different from those that grow on exposed canopy surfaces.

- Shade-type leaves typically are thinner, have more surface area, and contain more chlorophyll than those of sun leaves.



- As a result, shade leaves are more efficient in harvesting sunlight at low light levels.



SUN LEAVES

SHADE LEAVES

ANATOMICAL DIFFERENCES

THICKNESS Thicker with more tightly packed cells

Thinner with less densely packed cells

SIZE Smaller in size with less surface area

Greater surface area exposed.

CHLOROPLASTS Fewer number of chloroplasts

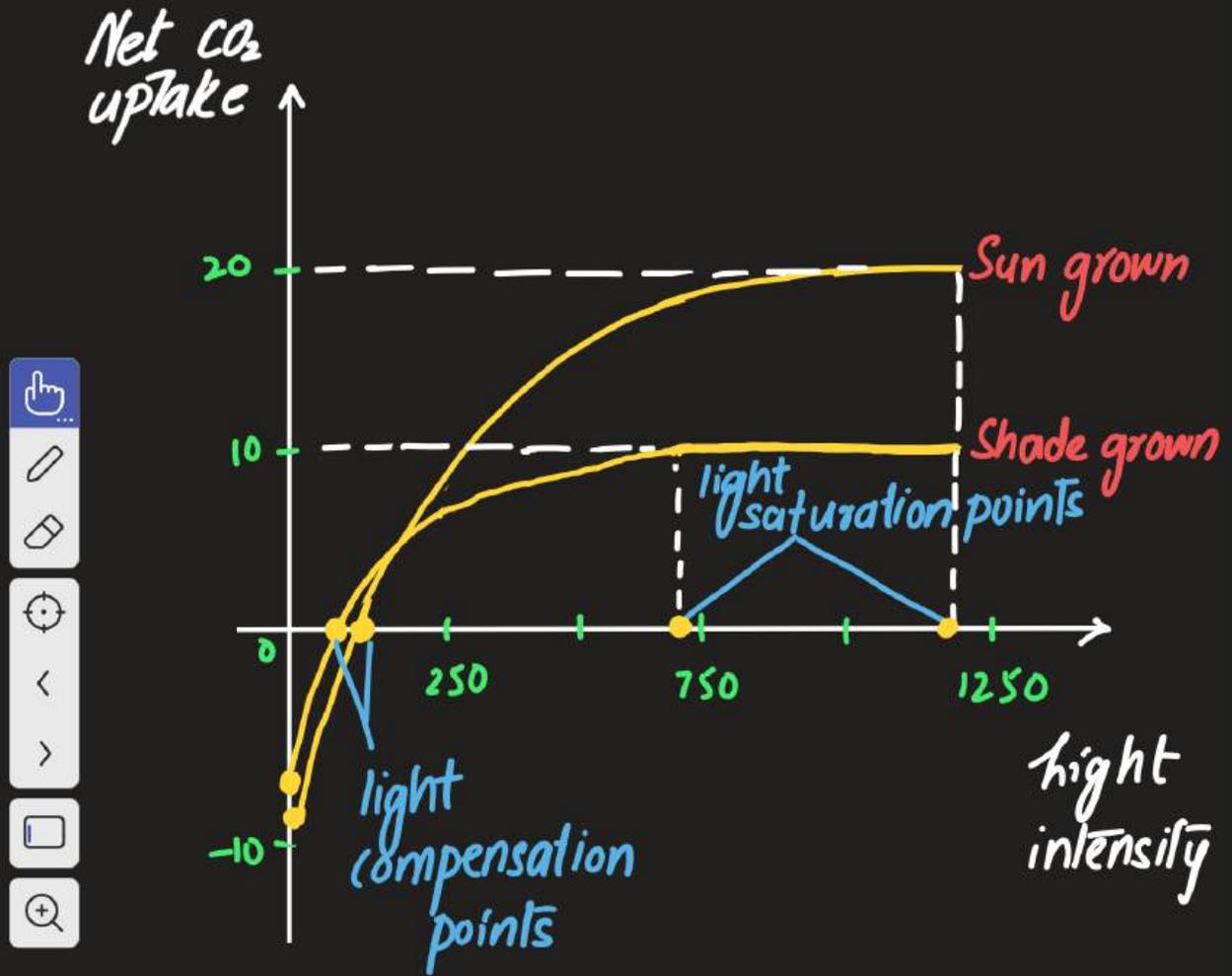
Greater number of chloroplasts

COLOUR Lighter in colour when compared with shade leaves

Darker green in colour.

STOMATA Stomatal pores are smaller but more dense

Stomatal pores are larger but less dense.



PHOTOSYNTHETIC EFFICIENCY (figure next)

SUN LEAVES

- Higher rates of photosynthesis (CO_2 uptake) at higher light intensities
- Reaches compensation point at higher light intensity
- More rapid increase in rate of photosynthesis
- Reaches light saturation point at higher light intensities

SHADE LEAVES

- Higher rates of photosynthesis (CO_2 uptake) at lower light intensities.
- Reaches compensation point at lower light intensity.
- Increase in rate of photosynthesis less
- Reaches light saturation point at lower light intensities



- Positive net CO_2 uptake :

rate of photosynthesis \gg rate of respiration



- Negative net CO_2 uptake :

rate of photosynthesis \ll rate of respiration

- Zero net CO_2 uptake :

rate of photosynthesis = rate of respiration

Question 1:

- 1 Large trees produce sun leaves on the outside of the canopy and shade leaves inside the canopy. Fig. 1.1 shows the rate of carbon dioxide uptake or production of a sun leaf and a shade leaf when exposed to increasing light intensity.

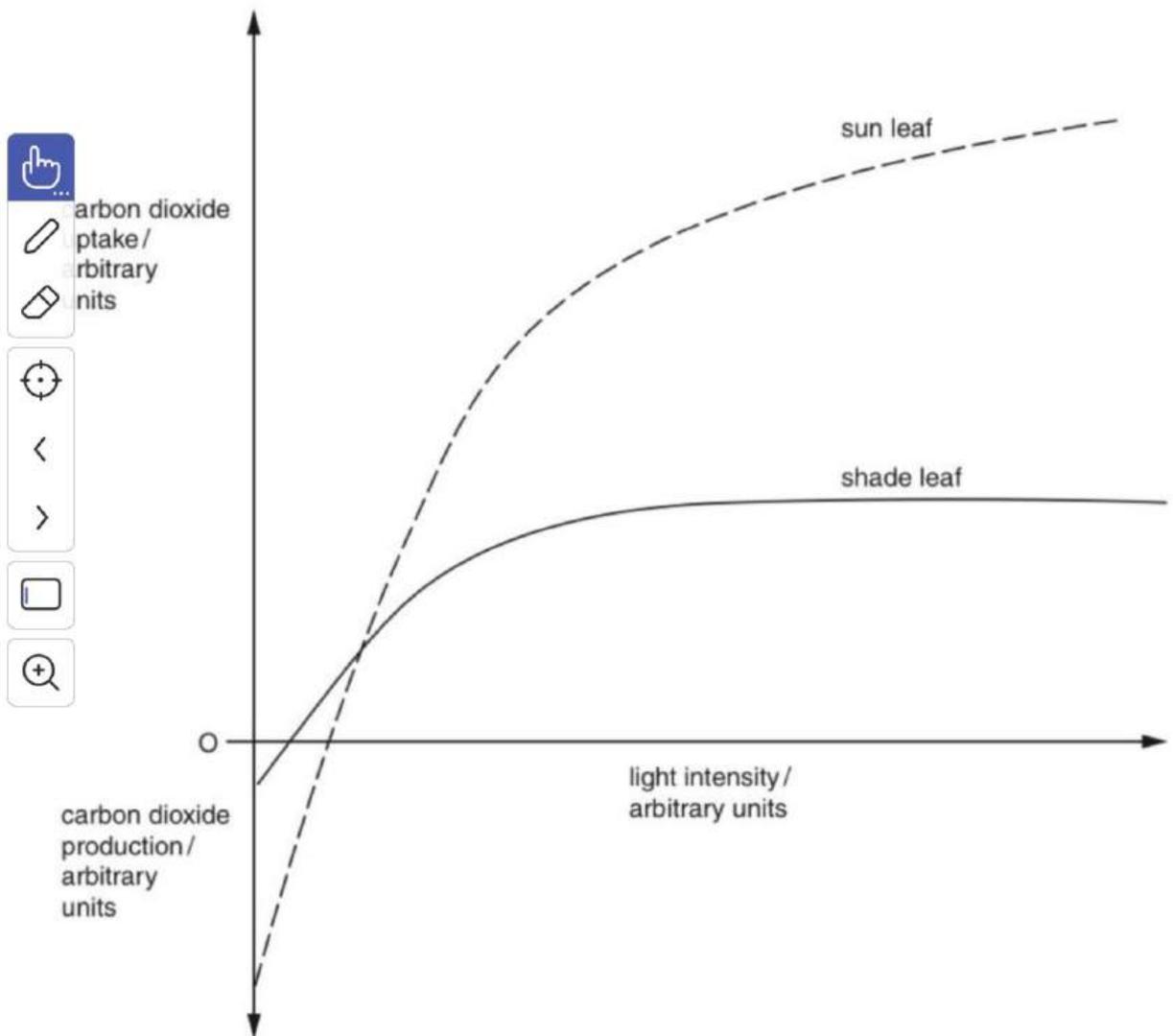


Fig. 1.1

(a) With reference to Fig. 1.1, describe three ways in which the sun and shade leaf differ in their response to increasing light intensity.

- 1 sun leaves reach compensation point at higher light intensity
- 2 rate of photosynthesis increases more rapidly in sun leaves
- 3 higher rate of photosynthesis in sun leaves at higher light intensity [3]

(b) Explain why the carbon dioxide uptake levels off in the shade leaf as the light intensity increases.

- * light no longer limiting
- * some other factor limiting such as CO_2 conc, temperature [3]

The results shown in Fig. 1.1 were taken at a temperature of 20°C .

Describe briefly how increasing the temperature to 25°C would affect the results in the sun leaf.

- * at little light intensity, no effect
- * at high light intensity, temp increases the rate of photosynthesis
- * temperature increases the rate of enzyme catalysed reactions [3]

[Total : 9]