A study material for M.Sc. Biochemistry (Semester: II) Students

on the topic (CC-8; Unit II)

Carbon Fixation During Photosynthesis

The process of generation of food for all the living organisms on the Earth

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Carbon Fixation

Dark Reaction : The Phase of ATP and NADPH production is known as light phase or light reaction and the page in which ATP and NADPH is used to fix CO_2 into carbohydrates (Glucose, Fructose etc.) is known as Dark reaction. They are called so because the light reaction needs the direct involvement of light to excite electrons while the dark reaction is dependent on the light reaction product i.e., ATP and NADPH, but not directly on light. But it doesn't mean that the dark reaction occurs in dark, even if a small discontinuation of about 30 seconds of light can stop dark reaction because absence of light stops light reaction and after sometime when ATP and NADPH becomes short dark reaction also stops and hence, the word light reaction and dark reaction are only assumed and are misnomers.

Dark reaction is also called Biosynthetic Phase of photosynthesis. In this phase CO_2 combines with H_2O in presence of ATP and NADPH to produce $(CH_2O)_n$ or sugars.

Just after World War II, Melvin Calvin used Radioactive C^{14} in algal photosynthesis to study the steps of CO_2 fixation and also its first product. He found that the first product or compound of CO_2 fixation is a three carbon compound organic acid. It was the 3-phosphoglyceric acid. He also used paper chromatography to separate different intermediate compounds from Complex mixture and also autoradiography to identify on a chromatogram those compounds that are radioactive and therefore involved in the photosynthetic assimilation of $^{14}CO_2$. Later the complete biosynthesis pathway was named Calvin cycle after him.

The problem of sequencing different products was solved by an apparatus having a pot with algal suspension a CO_2 giving apparatus a pressure gauge. A narrow transparent tube dipping its end into methanol boiling. Radioactive $^{14}CO_2$ was introduced somewhere in the pipe. This set up was flow through the system.

Calvin and Benson obtained evidence that the five carbon compound Ribulose 1-5, Bis-Phosphate (RUBP) is the initial acceptor of CO_2 molecule. RUBP is carboxylate and then cleaved in geometrically to form two 3 PGA molecules. The enzyme required for this reaction is Ribulose 1-5, Bis- phosphate carboxylase Oxygenase (RuBisCO), probably one of the most abundant enzymes in photosynthetic tissues.

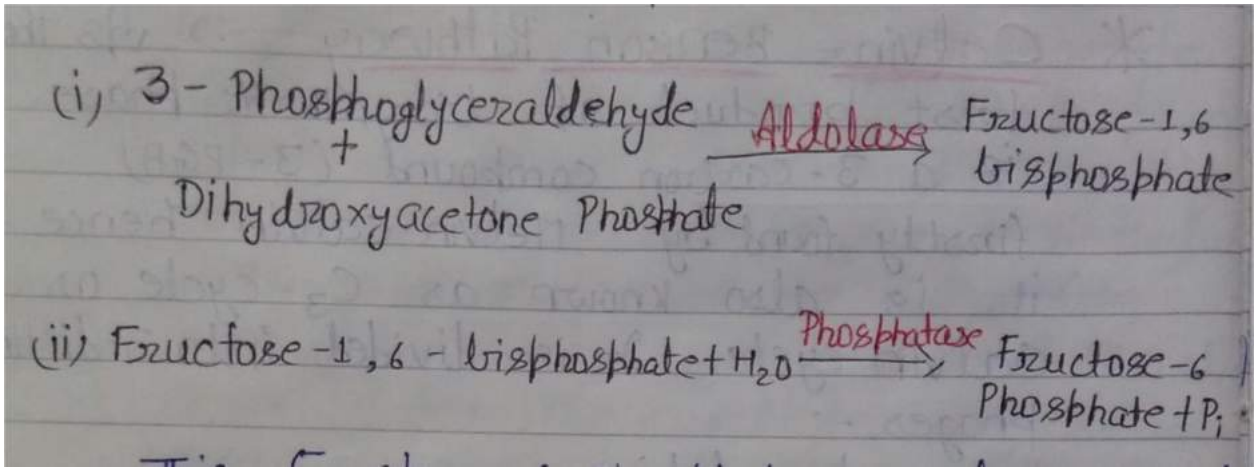
Calvin Benson pathway: As the first product of biosynthetic phase is a three carbon compound 3-PGA, firstly found by Melvin Calvin, hence it is also known as C_3 cycle or Calvin cycle. It is divided into three distinct phases

1. Carboxylation
2. Glycolytic reversal or reduction and
3. Regeneration of RUBP

1. **Carboxylation**: Formation of 3-PGA from CO_2 and Ribulose 1-5, Bis-phosphate (RUBP) . It is found that, if light is turned off there is a sharp increase in 3 PGA. It shows that the carboxylation process does not need ATP or NADPH (produced by light reaction).

2. **Glycolytic Reversal:** It is conversion of 3 – PGA (3 Phosphoglyceric acid) into 1-3 Bis-phosphoglycic Acid and then to 3- Phospho glyceraldehyde. It needs the involvement of ATP and NADPH, it can also be known by the sharp increase of 3-PGA when light is turned off.

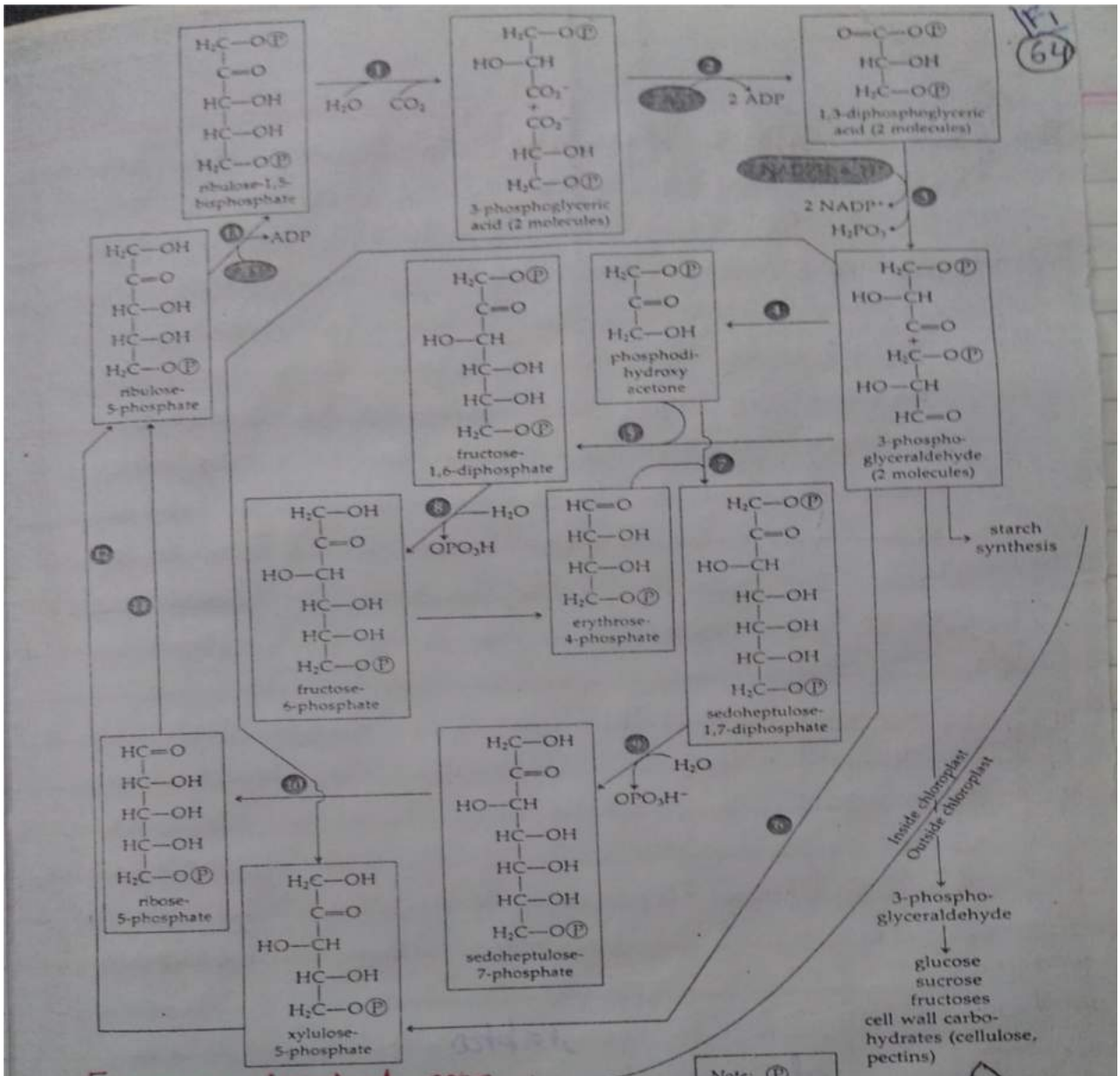
   Sugar Formation: Here produced 3 phosphoglyceraldehyde is converted into Fructose 1- 6, bisphosphate and later into Fructose 6 phosphate.



3. **Regeneration of RUBP:** Some molecules of 3 phosphoglyceraldehyde converts to Fructose 1-6 Bis-phosphate; phospho dihydroxy acetone and Xylose 5 phosphate molecules to finally recover the Ribulose 1-5, Bis-phosphate by different Pathways and mediated by different enzymes to complete the cycle.

In	**Out**	For every CO_2 entering Calvin Cycle, 3 molecules of ATP and 2 Molecules of NADPH are required
6 CO_2	Glucose ($C_6H_{12}O_6$)	
18 ATP	18 ADP	
12 $NADPH+H^+$	12 $NADP^+$	

Different enzymes used in this cycle and steps are as follows:

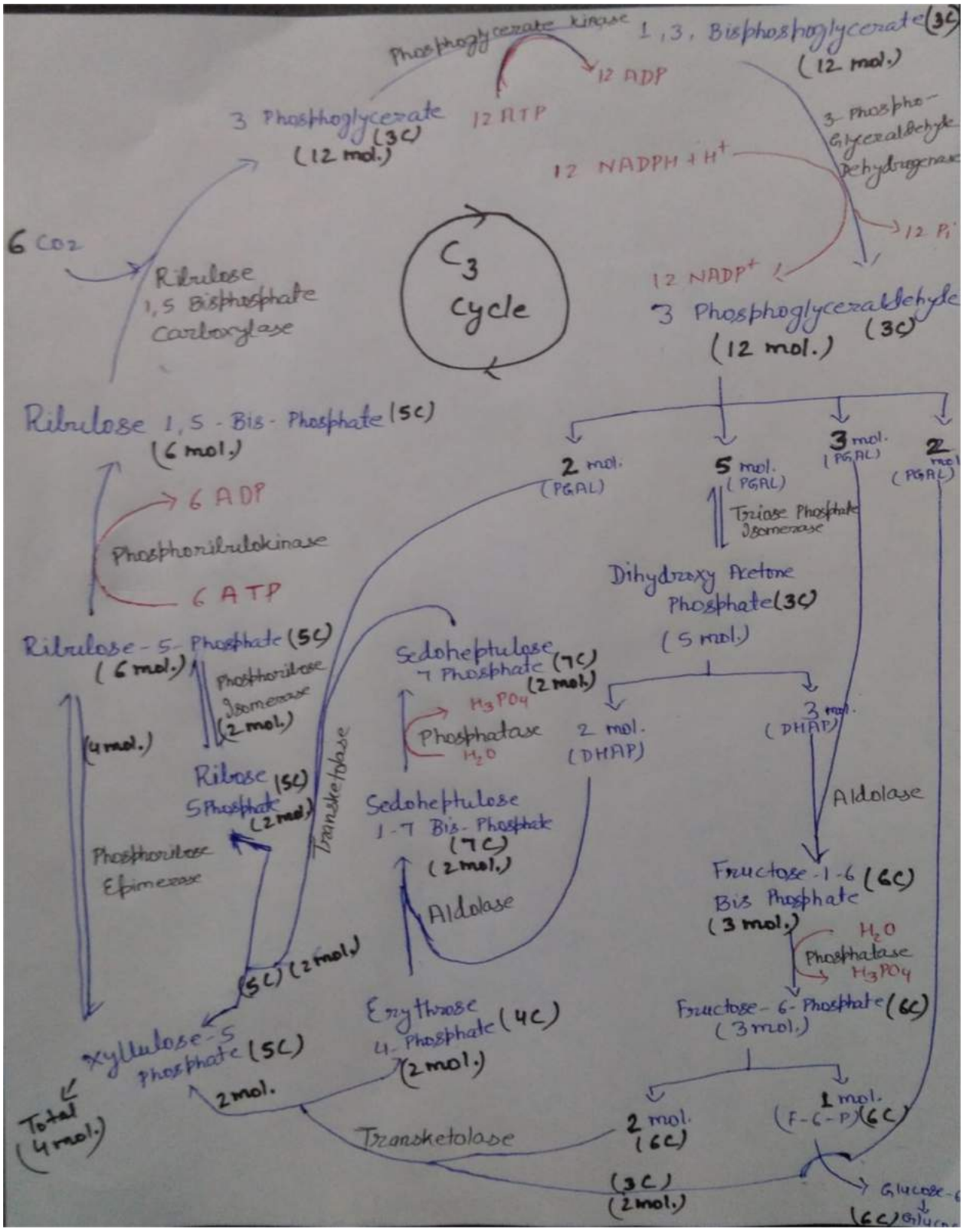


Calvin Benson cycle

Enzymes involved are –:

(1) Ribulose bisphosphate carboxylase
(2) 3- Phosphoglyceric acid kinase
(3) 3- Phosphoglyceraldehyde dehydrogenase
(4) Triose- Phosphate isomerase
(5) Aldolase (6) Transketolase (7) Aldolase
(8) Fructose-1,6 diphosphatase
(9) Sedoheptolose-1,7-diphosphatase
(10) Transketolase (11) Ribose phosphate isomerase
(12) Ribulose Phosphate epimerase
(13) Ribulose Phosphate kinase.

Simplified form of Calvin Benson Pathway may be given as:

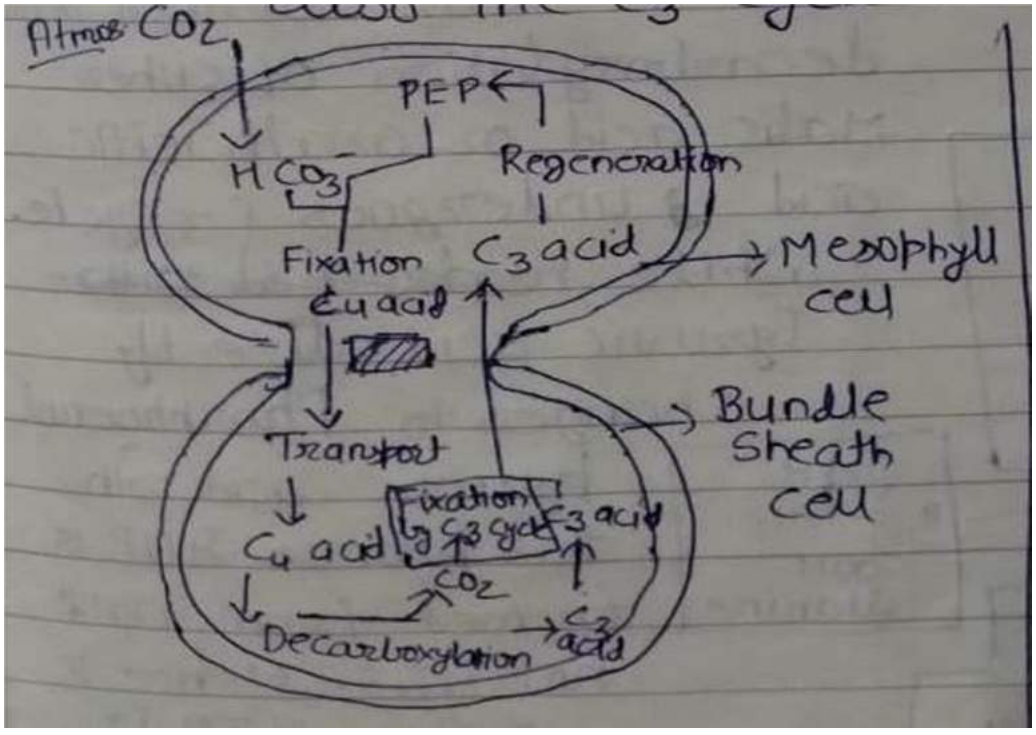


C4 plant and Hatch - Slack pathway: In 1957 Kortschak and coworkers reported Four Carbon organic acid as first stable product of photosynthesis which was different from that of C_3 pathway that is Calvin cycle. Later two scientists Hatch and Slack throughly investigated the complete pathway which was further named as Hatch and Slack pathway. It is also called C_4 cycle (due to first stable product is 4- Carbon compound) and the plants exhibit this cycle are known as C_4 plants.

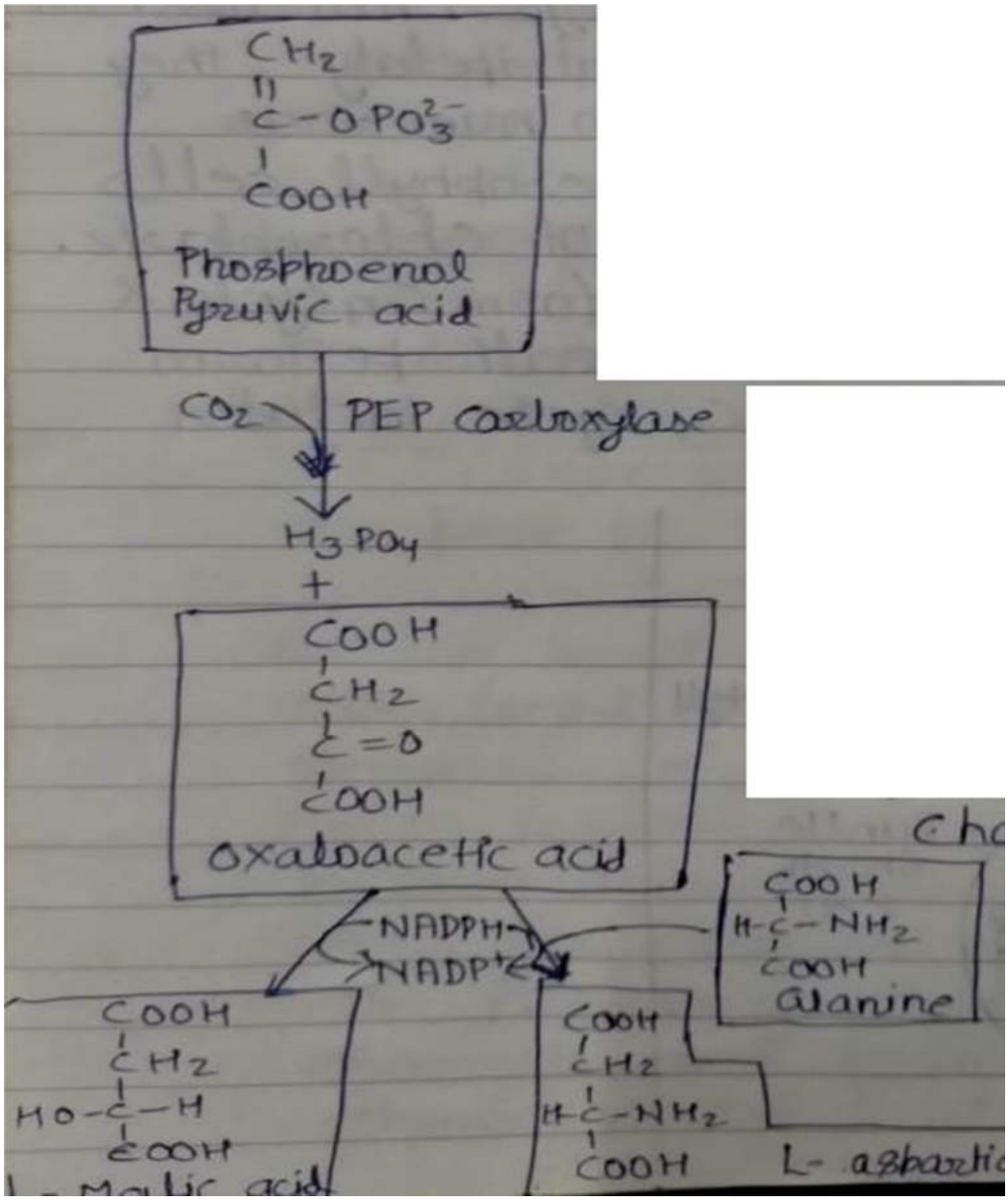
C_4 Plants: Hatch and Slack were are able to detect the relatively unstable C^{14} labelled Oxaloacetic acid as the first carboxylation product of PEP (Phosphoenolpyruvic acid) which acts as the first acceptor of CO_2 in this system.

There are more than 900 species in which C_4 cycle occurs. They are found in tropical (both in dry and hot) and subtropical regions environment, example: Sugarcane, Maize, Sorghum*, Cyperus rutendus*, *Digitaria brownii,* Amaranthus etc. About 300 are Dicots and others are monocot. The leaves of C_4 plants are characterized by a sheath of parenchyma cells that are radially arranged around each vascular bundle in turn is enclosed by loosely packed spongy mesophyll cells. This closed vascular bundle sheath arrangement is known as **Kranz anatomy** (German word that means Halo or Wreath).

The leaf cells of C_4 plants have dimorphic chloroplast i.e., too morphologically distinct types. Within the bundle sheath cells are large chloroplast that are arranged centripetally, they lack grana and contain numerous starch grains. The mesophyll cells contain normal type of chloroplast. The mesophyll cells perform C_4 cycle and the cells of bundle sheath perform C_3. Hence C4 cycle includes the C3 cycle too.



C_4 Cycle: In C_4 cycle firstly CO_2 is fixed as a 4 carbon compound of Oxalo-acetic acid (OAA) with the help of first Phosphoenol Pyruvic acid (PEP) and the enzyme Phosphoenolpyruvate carboxylase in mesophyll cells. Mesophyll cell has very less quantity of RUBP carboxylase which is higher in bundle sheath cells. Then Oxaloacetic acid converts to either Malic acid or Aspartic acid then Malic acid or Aspartic acid transports to bundle sheath cells, where malic acid gets decarboxylated and and change into pyruvic acid while aspartic acid firstly changes to Oxalo-acetic acid and after decarboxylation then pyruvic acid. The CO_2 obtained from decarboxylation of either Malic acid or Oxaloacetic acid undergoes C_3 cycle. While Pyruvic acid directly changes to Phosphoenolpyruvic acid. In this process AMP is formed from ATP not ADP. Hence two extra ATP is required to regenerate ATP from AMP and hence 12 additional ATP are needed in C_4 cycle.



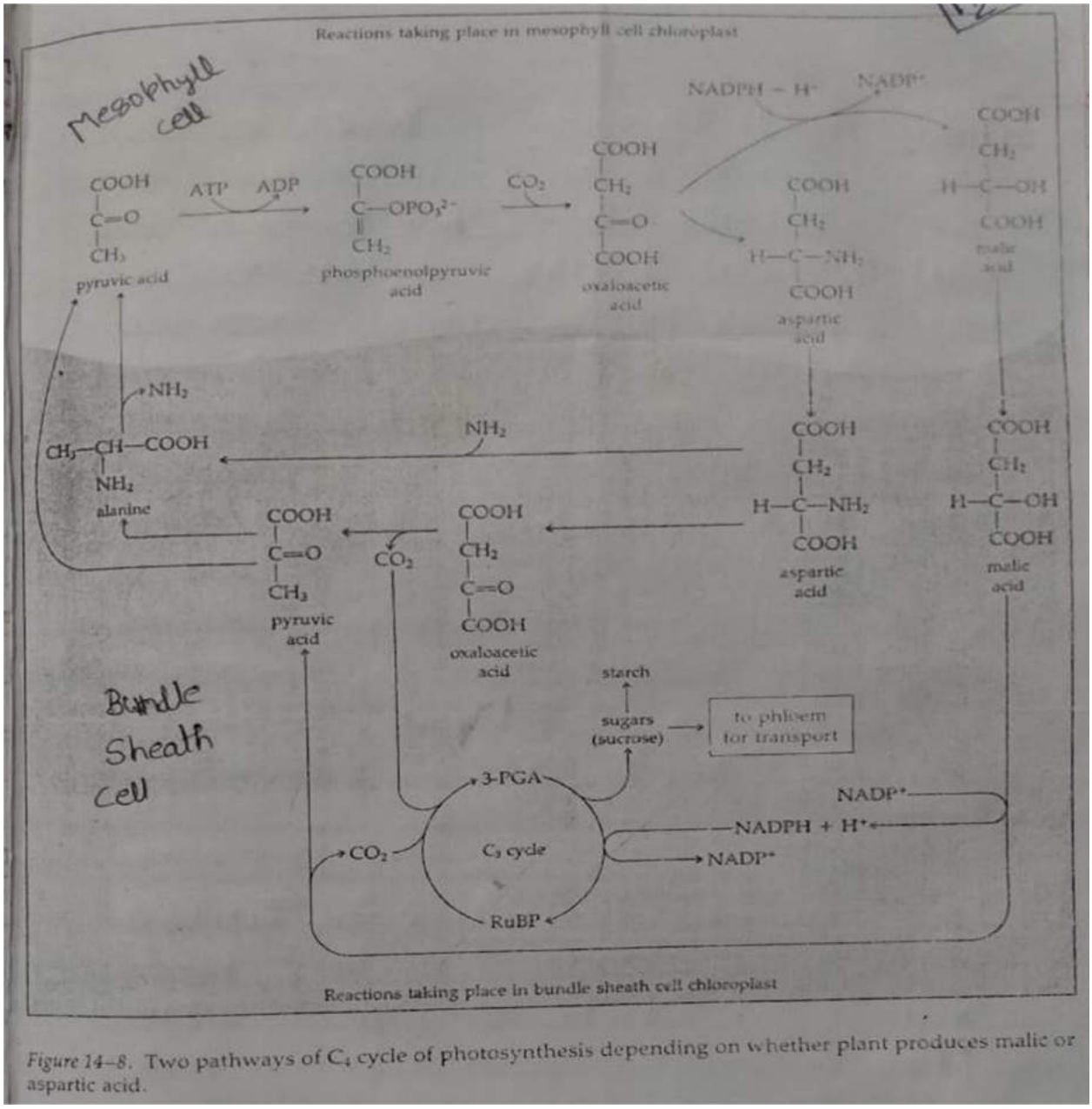


Figure 14–8. Two pathways of C_4 cycle of photosynthesis depending on whether plant produces malic or aspartic acid.

In	**Out**	For every CO_2 entering Calvin Cycle, 5 molecules of ATP and 2 Molecules of NADPH are required
6 CO_2	1 Glucose ($C_6H_{12}O_6$)	
30 ATP	30 ADP	
12 NADPH+H^+	12 $NADP^+$	

Significance of C_4 Cycle: The C_4 plants can absorb CO_2 even from a much low CO_2 concentration when the C_3 plants fail to avail it. Hence it can perform high rate of photosynthesis even when the stomata are closed (e.g., in water shortage) they are better adapted to tropical and desert areas and can tolerate halophytic conditions.

- C_4 plants require more light energy to fix CO_2 to as compared to C_3 plants
- C_4 cycle is more energy expensive (30ATP /Glucose molecule) with respect to C_3 cycle (18 ATP per glucose molecule).

Photorespiration: In 1920 Otto Warburg observed that presence of excess O_2 in the atmosphere inhibits photosynthesis in green algae and later observed in other higher plants. This type of photosynthetic inhibition due to Excess O2 was named as Warburg effect.

Later Decker and Tio in 1959 found this process in tobacco and it was also observed that O2 consumption becomes double in presence of light. This light respiration is similar to true aerobic respiration which is found in many plants and animals and which is also characterized by O2 uptake and CO_2 liberation, but that in light respiration, no energy liberation occurs (No ATP production occurs from phosphorylated sugars). This form of respiration is called photo respiration because of its similarity to true respiration with regard to the gaseous exchange process.

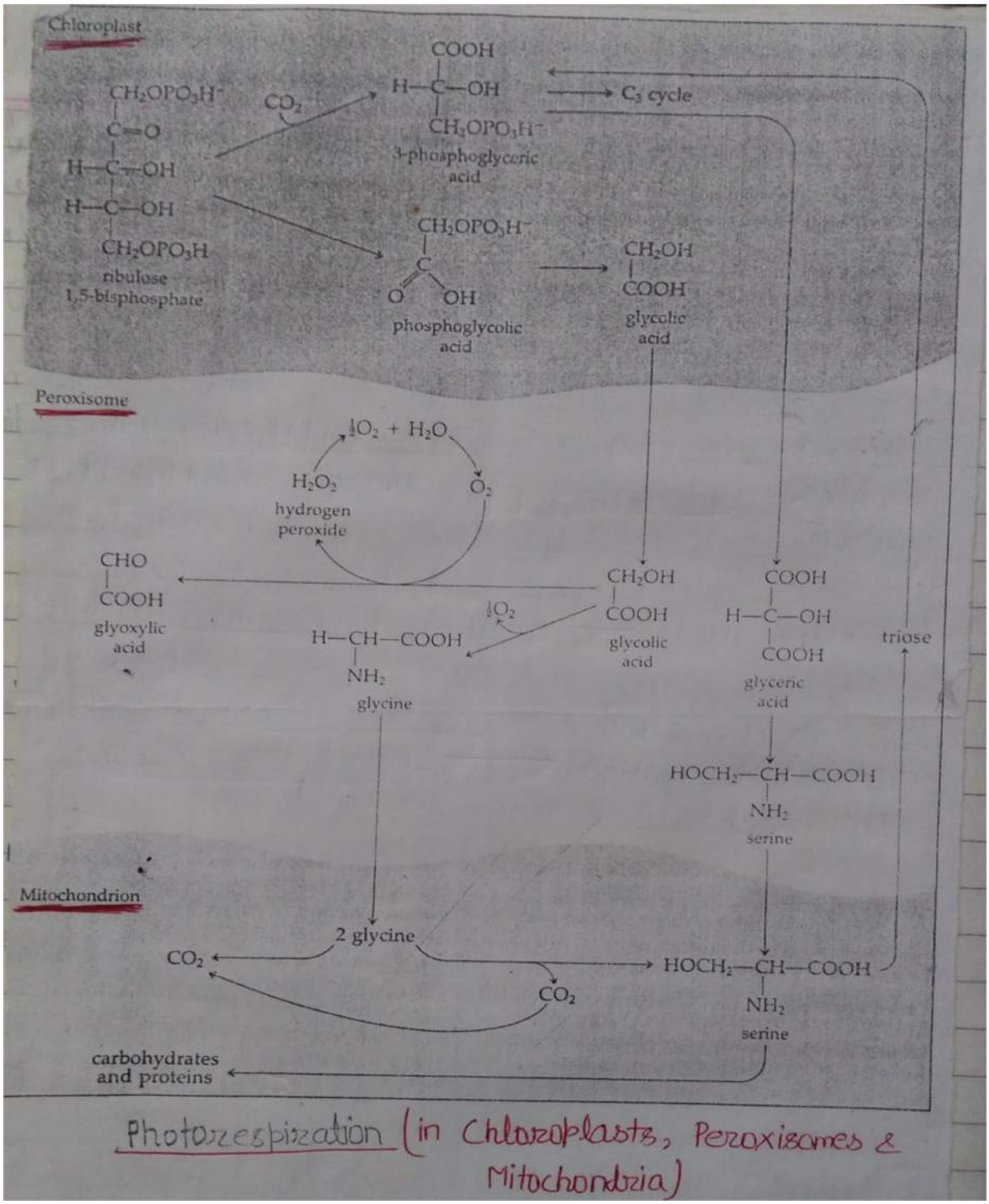
The process photorespiration interferes with successful functioning of Calvin cycle because the carbon is used to regenerate RUBP with no gain in carbohydrate synthesis for respiration or storage. Also the ATP and NADPH are required to regenerate the RUBP for photophosphorylation as well as CO_2 fixation.

Mechanism: It takes place in chloroplast, peroxisomes and mitochondria. The site of action of O_2 is Ribulose Bis-phosphate Carboxylase and substrate is Ribulose 1-5, Bis-phosphate. In the presence of oxygen the enzyme operate as an oxygenage catalyzing the oxidation (addition of oxygen). of RUBP to Phosphoglycolic acid. O_2 therefore competes for the RUBP with CO_2 , thus causing the net effect of a reduced rate of CO_2 fixation and diminution of phosphorylated sugar synthesis.

As we know that Ribulose 1-5 Bis-phosphate carboxylase oxygenase (RuBisCO) is the most abundant enzyme on the earth, but it has two active sites one for CO_2 and another for oxygen. Plant cells have both mitochondria and chloroplast. They synthesize food in chloroplast with the help of ATP (photophosphorylation by producing Oxygen) and catabolize glucose to produce ATP in mitochondria with the help of oxygen. We can say that one side they need high CO_2 concentration for the fixation of glucose and another side they require high oxygen concentration for the catabolism of glucose in mitochondria. If the concentration of CO_2 increases it will cause the mitochondria to stop respiration but if the concentration of oxygen increases it will compete RuBisCO in chloroplast. We can say that plants have to make a balance

between the concentration of CO_2 and oxygen in their self and it is a tough task there to perform both photosynthesis and respiration.

The whole process can be given as follows:



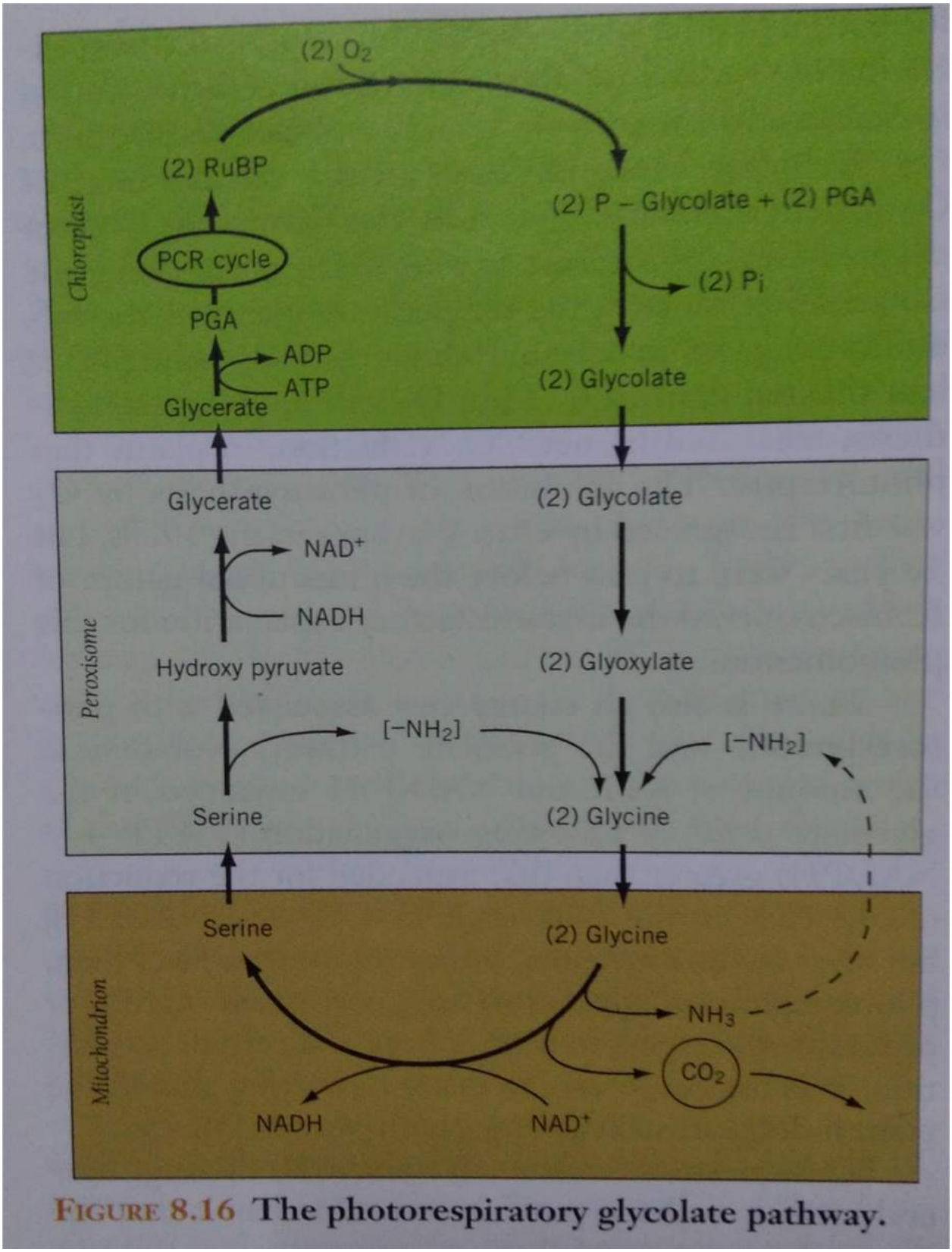
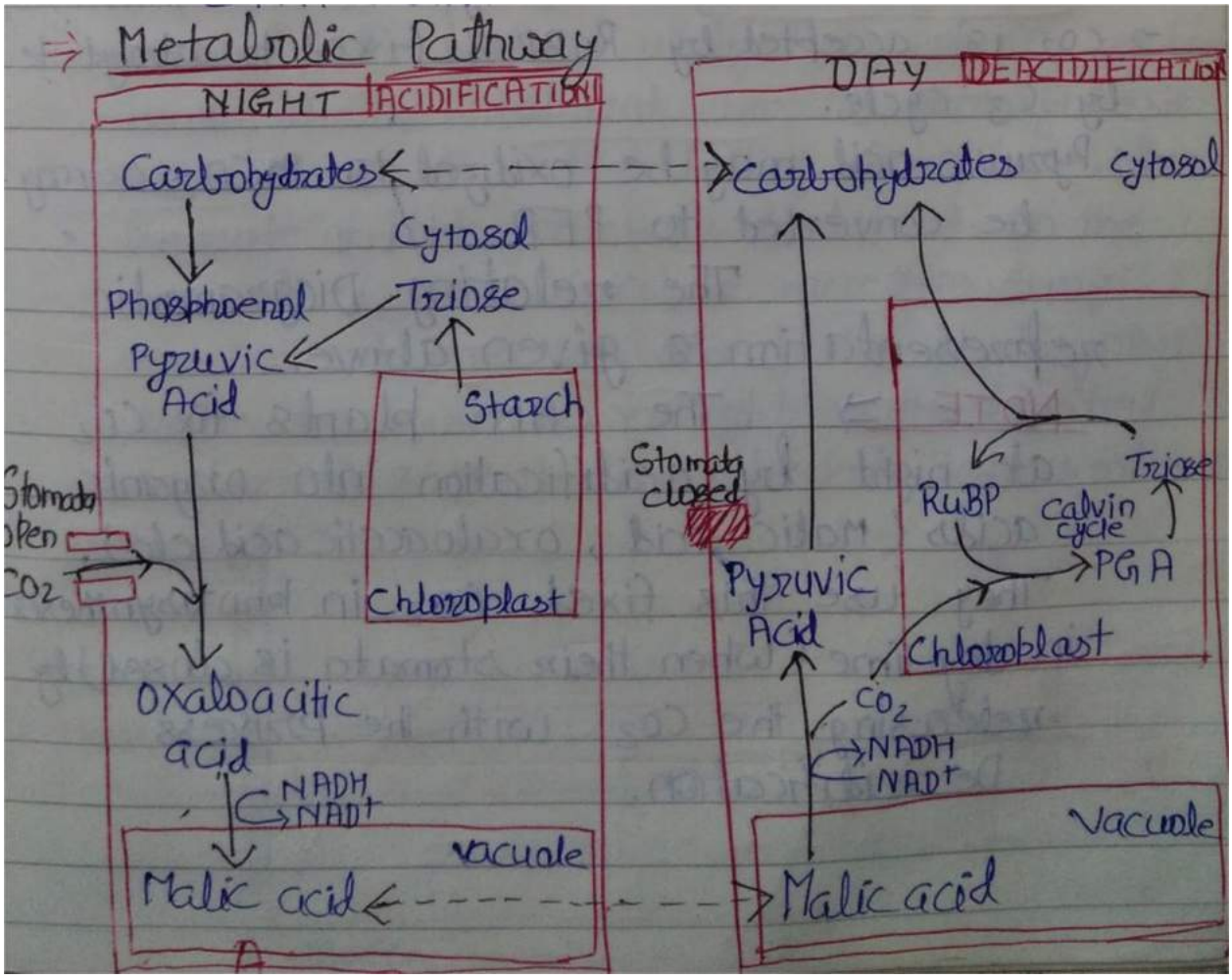


FIGURE 8.16 The photorespiratory glycolate pathway.

(Image Source: Google)

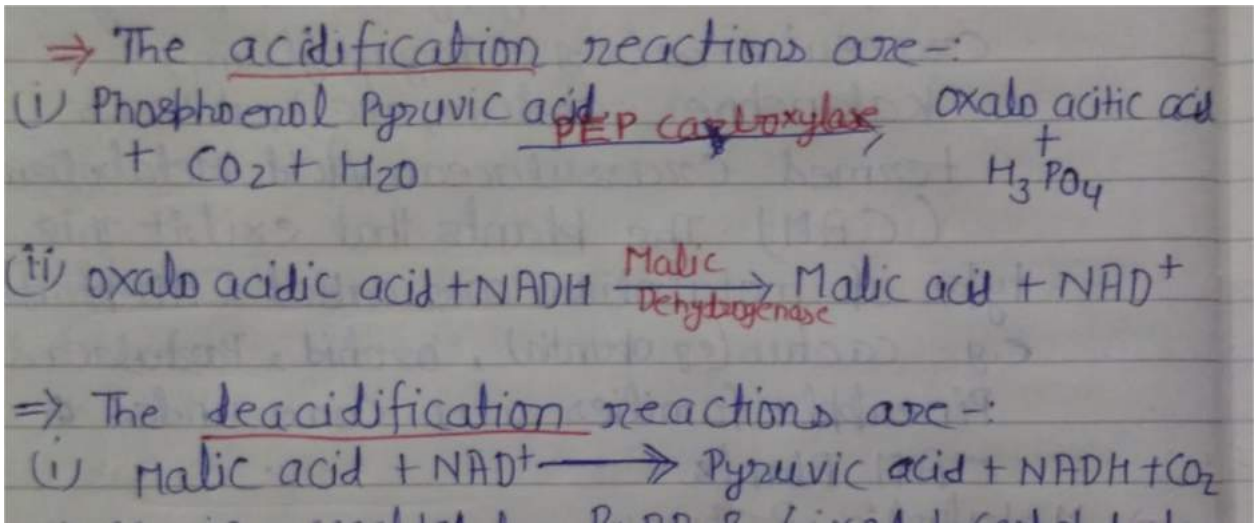
Crassulacean Acid Metabolism: Some plants especially succulent which grow under extremely xeric condition, fix atmospheric CO_2 in dark. Since the process was first observed in the plants belonging to the family Crassulaceae (e.g., Bryophyllum, Kalanchoe, Sedum etc.). It was termed Crassulaceaen Acid Metabolism (CAM). The plants that exhibit this type of metabolism are called CAM plants e.g., Cactus (Opuntia), Orchid, Partulaca and Pineapple families also come under CAM plants.

The metabolic pathway of CAM involves acidification in night and deacidification in day.



As we know that the plant which are already in deficiency of water, i.e., xeric plants have a tough situation to open their stomata during day time because the day time opening of stomata. It will cause them excess loss of water due to transpiration. So they open their stomata in night. The opening of stomata is essential for the exchange of gases, but there is a problem the product of light reaction that is ATP and NADPH are not available in night and hence the photosynthesis cannot occur in night and in daytime they cannot open their stomata so they convert CO_2 in different organic acid at the time of night and store the organic acids in there vacuole. At the daytime when the sunlight is available and the product of light reaction is available they convert the organic acids back into CO_2 and fix this to carbohydrates. In this way they can manage the CO_2 without opening their stomata at day time.

In night organic content increases, cell sap, pH decreases and storage carbohydrate decreases while in daytime the organic content decreases, cell sap, pH increases and stored carbohydrate increases in the CAM plants.



- CO_2 is accepted by RUBP and fixed to carbohydrate by C_3 cycle.
- Pyruvic acid may be oxidised to CO_2 or may be converted to phosphoenolpyruvic acid.

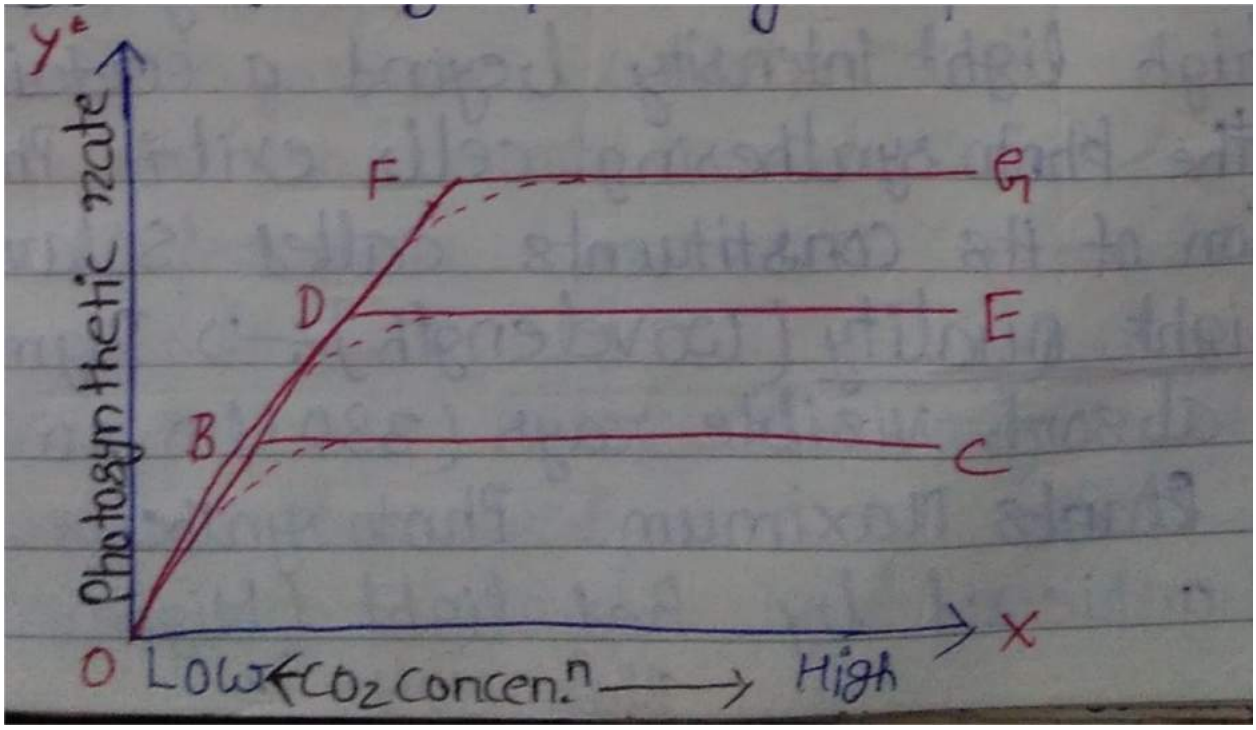
Note: The CAM plants fix CO_2 at night by acidification into organic acids (malic acid or oxaloacetic acid etc.). They use this fixed CO_2 in photosynthesis in day time (when their stomata is closed) by releasing that CO_2 with the process of de-acidification.

Factors Affecting Photosynthesis

Blackman's Law of Limiting Factors

In 1843 Liebig proposed the law of minimum, which states that "the rate of process is limited by the base of the slowest factor". Later F.F. Blackman extended it in his own words as "when a process is conditioned as to its rapidity by a number of separate factors the rate of the process is limited by the pace of slowest factor".

Suppose the light intensity on a leaf is as such the leaf can utilize 5 mg of CO_2 per hour. If CO_2 is absent then no photosynthesis occur. Here CO_2 is the limiting factor. Now the rate of photosynthesis will depend on the amount of CO_2. If CO_2 ismore than 5 mg then CO_2 will not affect the photosynthetic rate, because now light is the limiting factor. Its graph may be plotted as follows:



Criticism:

1. The rate of decline of photosynthetic rate will be shown as curvature because all chloroplast may or may not be affected by the limiting factors simultaneously.

2. Simultaneously two or more factors may be limiting

Factor Affecting Photosynthesis

A. External Factors

1. **Light:** Light varies in intensity, quality, wavelength and duration

a. Light Intensity: Some plants require low light intensity for optimum photosynthesis (Sciophytes) while some other require high light intensity (Heliophytes).

Under low light intensity usually the rate of photosynthesis is low, increase in light intensity causes increase in the rate of photosynthesis until some other factors becomes limiting. At very high light intensity beyond a certain point the photosynthetic cells exhibit photo-oxidation of its constituents called solarization.

b. **Light Quality (Wavelength):** Pigments absorb visible rays (380 μm to 760 μm). Maximum photosynthesis is achieved by red light (higher plants), green light in case of red algae and blue light in case of brown algae.

c. **Duration of Light:** Longer light duration (10-12 hours) favors photosynthesis. Plants can actively exhibit photosynthesis under continuous light without being damaged. Light can also affect photosynthesis by the opening and closing of stomata, by which it can affect the CO_2concentration and water availability (transpiration).

2. **Carbon Dioxide**: The rate of photosynthesis increases with the increase in CO_2 concentration (only upto 1%). If light and temperature are not limiting factor very high concentration of CO_2 becomes toxic to plants and inhibits photosynthesis

3. **Temperature**: Normally the rate of photosynthesis increases with the increase in temperature upto 40ºC, after that the enzyme becomes deactivated and the photosynthesis stops, except some conifers (even at minus 35ºC) and some BGA (Blue green algae) and bacteria (even at 70ºC of hot springs etc.).

4. **Water:** Plant use only 1% of the water absorbed in the photosynthesis, hence it rarely act as limiting factor, while the decrease in water supply can decrease the rate of photosynthesis and can cause wilting effect

5. **Oxygen**: Oxygen can inhibit photosynthesis in C_3 plants and can cause photo respiration due to

a. It oxidizes chlorophyll and destroys its excited state.

b. It competes with CO_2.

c. It favors rapid respiration which utilizes common intermediates.

6. **Minerals:** Some minerals are essential for photosynthesis and their deficiency may decrease the rate of photosynthesis, e.g., Magnesium, Manganese, Iron, Copper, Chlorine and Phosphorus

7. **Other Factor**s: Some liquids Chemicals, solid metallic and gaseous pollutants decreases photosynthesis rate, e.g., Ozone, SO_2, HF, HCN and H_2S, Chloroform, Ether extra

B. Internal Factors

1. **Chlorophyll Content:** The loss of chlorophyll in a plant is called chlorosis. Chlorophyll are the main pigments of photosynthesis, hence the decrease in chlorophyll content may decrease the rate of photosynthesis.

2. **Protoplasmic Factor: S**ome factors in protoplast are useful in photosynthesis. Their absence or excess presence can affect the photosynthesis. It includes some enzymes also.

3. **Accumulation of Carbohydrates**: The amount of carbohydrate present in the chloroplast can affect the rate of photosynthesis, because it reduces the effective surface in the chloroplast and hence the rate. Its accumulation can be obtained by not trans locating the produced carbohydrates.

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