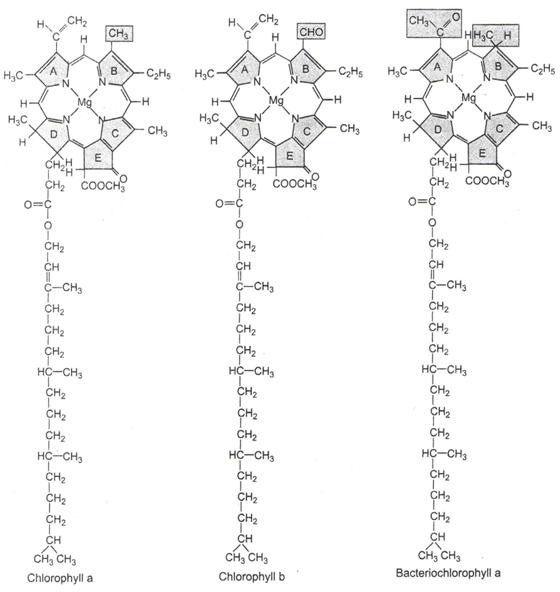
The Photosynthesis

The Photosynthetic products are energy rich organic compounds. The potential chemical energy of these compounds comes from the light energy.

The light energy to be effective in photosynthesis must be absorbed by a suitable pigment. This vital role is performed by the green pigment, chlorophylls, in plants.

#### Chlorophyll

There are at least seven types of chlorophylls known: **chlorophylls a, b, c, d** and **e**, **bacteriochlorophyll** and **bacterioviridin.**.



**Fig.5.1 Structures of Chlorophyll a, chlorophyll b and bacteriochlorophyll**

The molecular formulae of the chlorophylls are given below:

Chlorophyll a : C55H 72O5N4Mg Chlorophyll b : C55H70O6N4Mg

Both the chlorophylls a and b have hydrophilic Mg – Porphyrin head and a lipophilic phytol tail.

The chlorophylls are primarily located within the grana thylakoids. The chlorophyll molecules form a monolayer between the protein and lipid layers of the membranes of the thylakoids. The hydrophilic heads of the chlorophyll molecules are embedded within the protein layer while the lipophilic tails are located within the lipid layer.

### CONCEPT OF TWO PHOTOSYSTEMS

There are two reactions involved in photosynthesis. The first reaction requires light and is called the light or Hill reaction. The second reaction does not require light and is called the dark or Blackman reaction.

1. The light reaction is a photochemical reaction, while the dark reaction is a thermochemical reaction. The unit of photosynthesis is believed to consist of two types of centres,

photosystem I and photosystem II. These are excited at different wavelengths of light. The two systems are linked by redox catalysts. The light reaction involves two processes, photophosphorylation and photolysis of water. In photophosphorylation there is conversion of light energy into chemical energy. Photophosphorylation is of two types, cyclic photophosphorylation and noncyclic photophosphorylation.

1. The dark reaction takes place through a series of steps known as the Calvin-Benson cycle. The details of different stages of photosynthesis will now be taken up.

In higher plants and algae two pigment systems are involved in photophosphorylation. These are called photosystems I and II. The pigments of the two systems are known as pigment system I (PS I) and pigment system II (PS II), respectively. PS I and PS II are structurally distinct. PS I and PS II both contain chlorophyll a, chlorophyll b and carotenoids. The distribution of the two pigments however, varies in the two systems. PS I contain more carotenes than PS II. Xanthophyll predominates in PS II. The primary photosynthetic pigment of both systems is chlorophyll a. In blue-green and red algae phycobiliproteins (formerly phycoerythrin and phycocyanin) are present as accessory pigments.

#### .

### PHOTOPHOSPHORYLATION

There are two types of phtophosphorylations in the chloroplasts of plants:

1. Non cyclic photophosphorylation
2. Cyclic photophosphorylation.

###### Non-Cyclic Photophosphorylation

The famous Z scheme of non-cyclic phtophosphorylation was developed as a result of work of several research workers. According to Hill and Bendall (1960), Rabinowithch and Govindjee (1965) and others the photochemical reactions occur in series, the product of one being used up by the other. It has undergone modifications from time due to discovery of new electron carriers.

When the chlorophyll is photoexcited the electron gets dislodged from the chlorophyll molecule leaving a ‗hole‘ in it. The ‗hole‘ left by displaced electron is soon filled up by the return of the same electron or another one (cyclic and non-cyclic electron transport). In pigment system I there are 200 chlorophyll a molecules and just one P700 molecule. Any one or all of the chlorophyll a molecules can absorb red or blue photons of light and can pass on the excitation energy to P700 molecule which alone can lose the electron. Due to very tight arrangement of the chlorophyll a 683 molecules within PS I the singlet excitation energy resulting from the absorption of a light quantum by Chl a 683 migrates from one molecule to another by resonance transfer until it reaches a chlorophyll a 683 molecule which is adjacent to the long wave absorbing pigment (P700). The energy is then passed as an excitation to the P700 molecule which is the reaction centre. P700 attains the first excited singlet state and loses an electron to an electron acceptor. The PSI function has been compared to an antenna which causes the

‗funneling‘ of photons into an ‗energy trap‘ or ‗sink‘ (P700) or to a lens which concentrates light into a focal point (P700).

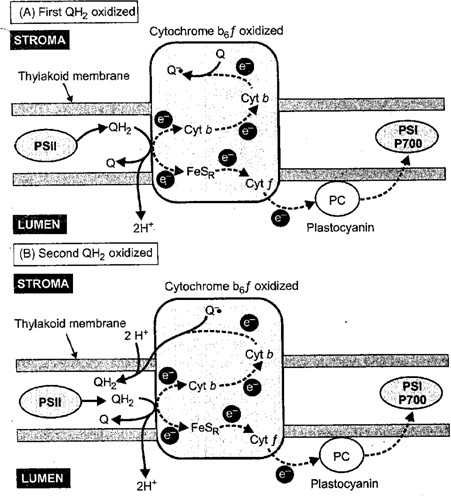
The sequential steps in the electron transport chain are discussed under the following headings:

1. **PS II AND Photolysis of water:** One of the two primary photochemical reactions in photosynthesis is the absorption of red light by PS II. According to Barber et al. (1999) PS II is a multi subunit protein super complex which has two unique chlorophyll a P680 reaction centers (dimer) and some antenna complexes. After receiving light the P680 reaction center gets photoexcited as a result of which an electron is ejected. The excited form of P680 known as P680\* loses its electron to pheophytin, which is the primary electron acceptor. Pheophytin is a type of chlorophyll a in which magnesium is substituted by two hydrogens. According to Okamura et al. (2000) the electron is transferred from pheophytin to plastoquinones QA and then to QB. The two are bound to the reaction center. The second plastoquinone QB after receiving two electrons is reduced to QB-2 which reacts with two protons of the stroma to form QH2 (plastohydroquinone). QH2 which is a small nonpolar molecule was earlier bound to PS II is freed from it. It moves into the non-polar part of the phospholipids and transfers the electrons to cytochrome b6f complex. (Fig 5.6)
2. **The Cytochrome Complex :** The cytochrome b6f complex is also a large multi subunit protein. It has many prosthetic groups. There are two b type hemes. a c type heme (cytochrome f), and a Rieske iron sulphur protein (FeSr) present in the complex.

In the non-cyclic or linear type of electron transfer QH2 transfers one electron to Rieske FeS protein which is present on the side of the lumen and transfers another electron to one or the cytochrome b. The Rieske FeS protein transfers its electron to cytochrome f, which is also present on the lumelal side of the thylakoid membrane. In the process two H+ ions are released into the lumen.

In another mechanism for the transfer of protons and electrons in the cytochrome b6f complex, called cyclic process, the other QH2 electron received by cytochrome b is transferred which after

passing through the two b type cytoquinone reduces the semiquinone to plastohydroquinone. In the process two protons are taken up from the stroma so that a total of 4 protons are released in the lumen. ( Fig 5.6).



**Fig.5.6 Diagram to show the non-cyclic type of electron transport in the cytochrome b6f complex.**

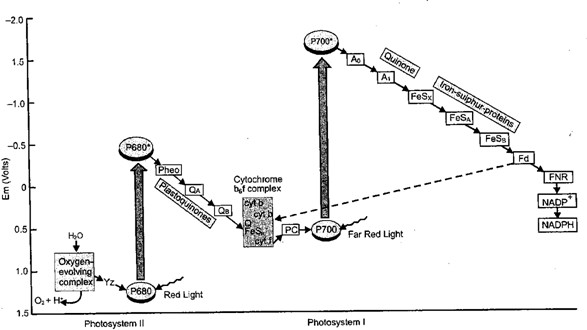
**A. Linear or cyclic transport; B. Cyclic transport (Redrawn from Taiz and Zeiger, 2002)**

1. **Plastocyanin, the Electron Donor to PS I:** The plastocyanin accepts electrons from cytochrome b6f complex and donates them to PS I. It is the only copper containing electron carrier in the electron transport chain of photosynthesis. It is a small water soluble protein and is present on the side of the lumen. Plastocyanin does not appear to be indispensable since in some algae non-cylic electron transport chain occurs even in its absence.
2. **The PS I:** According to Jordan *et al.,* the PS I is also a large multi subunit complex. It contains not only the reaction center P700 but also a number of components which participate in the transfer of electrons. They are all present around two big sized proteins called PsA and PsB and some small proteins. The P700 is positioned in such a manner in the membrane that the electron is easily ejected. The primary electron acceptor is A0 which is a type of chlorophyll. The next electron acceptor is A1 which is a quinone. The electron is then transferred to soluble ferredoxin (Fd) through a series of iron sulphur proteins namely Fe Sx, Fe SA and Fe SB.

The electrons far transferred from ferredoxin to ferredoxin-NADP+ reductase (FNR) which donates them to NADP+ to produce NADPH (Vishniac and Ochoa, 1951).

1. **The ATP Synthase:** It is a large complex enzyme and is known by several names such as ATPase, the coupling factor, and CF0-CF1\* This enzyme is situated only at the edges of granal thylakoids and in the stroma lamella and therefore, the protons coming from photolysis of H2O and from cytochrome b6f complex have to move great distances laterally to reach it. One of the two components of the enzyme called CF0 is hydrophobic and is bound to the membrane. The other component termed CF1 projects into the stroma.

According to Peter Mitchell‘s chemiosmotic theory the energy required for the synthesis of ATP is provided by proton motive force. The proton motive force is created by the proton chemical potential and the transmembrane electrical potential. The H+ ions released during photolysis of water accumulate in the lumen of the thylaoids.. The accumulated H+ ions in the lumen try to leak back into the stroma through the ATP synthase. The protons enter the channel formed by CF0 and when they cross through the catalytic sites of β-polypeptide of CF1, the proton motive force breaks, releases energy as a result of which ATP is generated . The latest detailed Z scheme is given in Fig. 5.7.



**Fig. 5.7 Current concept of the Z-scheme of light phase of photosynthesis. (After Blankenship and Prince, 1985). The cyclic electron transport is indicated by dotted line.**

1. **Cyclic Photophosphorylation**

Another type of photophosphorylation can also take place under certain conditions e.g. when the amount of available NADP+ is low or PS II is absent or monochromatic light beyond 680 nm is

given to the plant in the laboratory. This process involves only pigment system I and, therefore, photolysis of water and the consequent evolution of oxygen does not take place . Non cyclic electron transfer does not take place and NADPH is not formed. The CO2 assimilation is retarded (red drop). The photosynthetic enhancement can, however, take place if PS II wavelength of light is also given to bring into action the noncyclic phtophosphorylation.

In cyclic electron transfer the electron flows from photoexcited P700 to X, A and B, FRS and then to ferredoxin, which unable to pass electrons to NADP+ transfers them to cytochrome b6 (E0 = - 0.06 V). The electron is ultimately cycled back to P700 via PQ, FeS, cytochrome f and plastocyanin. ATP molecule is formed either between ferredoxin and cytochrome b6 or between cytochrome b6 and cytochrome f or at both steps.

It is however, doubtful whether cyclic process occurs in normal photosynthesis (VAN Niel, 1962), According to Ramirex et al. (1968) it may serve as the source of ATP for biosynthetic processes occurring in chloroplasts that are not on the main photosynthetic path of carbohydrate synthesis but branch off from this path for the synthesis of protein, DNA, RNA, starch, lipids, pigments etc. Part of the ATP requirement of the dark phase of photosynthesis is also met with by this process.

The cyclic electron transport chain was studied by Arnon. It is not inhibited by 3(3), 4- dichlorophenyl 1, 1-dimethylurea: DCMU). This inhibitor, however, inhibits non-cyclic transport of electrons. Cytocrome b6 is an electron carrier which participates exclusively in cyclic electron transport chain.

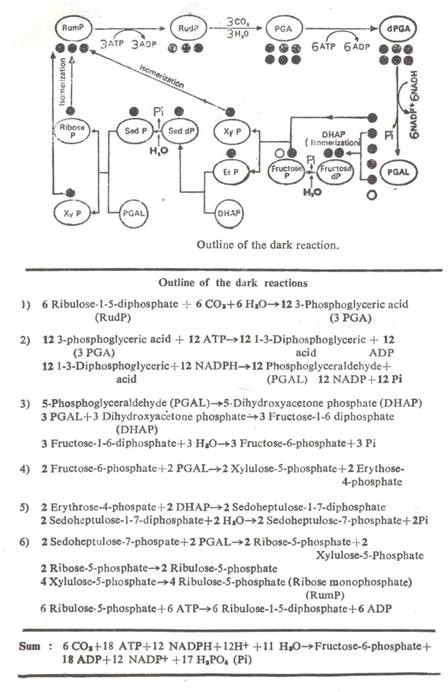
**Table 4: Difference between cyclic and non cyclic photophosphorylation**

|  |  |  |
| --- | --- | --- |
| **S.No.** | **Cyclic Photophosphorylation** | **Noncyclic Photophosphorylation** |
| 1 | Only photosystem I functions in cyclic photophosphorylation. | Both photosystem I and II function in noncyclic photophosphorylation. |
| 2 | Cyclic photophosphorylation functions in a closed loop. Electrons released from chlorophyll to acceptor return to chlorophyll. | An independent electron donor is necessary. Water is the ultimate source of electrons. NADP+ is the final electron acceptor. |
| 3 | There is no net production of reduced compounds. NADPH2 is not formed and assimilation of CO2 is retarded. | NADPH2 is the reduced compound formed. It is utilized in carbon assimilation. |
| 4 | Oxygen is not evolved. | ATP formation is coupled to evolution of oxygen. |
| 5 | In bacteria only cyclic | In green plants noncyclic |

|  |  |  |
| --- | --- | --- |
|  | photophosphorylation takes place. | photophosphorylation also occurs. |
| 6 | Cyclic photophosphorylation is not sensitive to either antimycin or dichlorophenyl-dimethly-urea (DCMU). | DCMU inhibits flow of electrons from water to NADP+ and thus stops noncyclic photophosphorylation. |
| 7 | The flow of electrons is as follows  Chl (PS-I)-FRS-Fd-Cyt. b6-Cyt. f-PC- Chl (PS-I) | The flow of electrons is as follows:  PS-II-Q-PQ-Cyt.f-PC-PS-I-FRS-NADP+ |

### CALVIN CYCLE / DARK REACTION

During the dark reactions of photosynthesis carbon dioxide is reduced to form carbohydrates. The term dark reaction implies that the reaction is not dependent on light. Synthesis of carbohydrates proceeds in the dark. The dark reaction has been worked out mainly by Calvin, Bensn and Bassham. The pathway by which carbon dioxide is fixed into carbohydrates is called the Calvin-Benson cycle or Calvin-Bassham cycle. Carbon dioxide and water are used to generate carbohydrate in the presence of ATP and NADPH. (Fig.5.8).

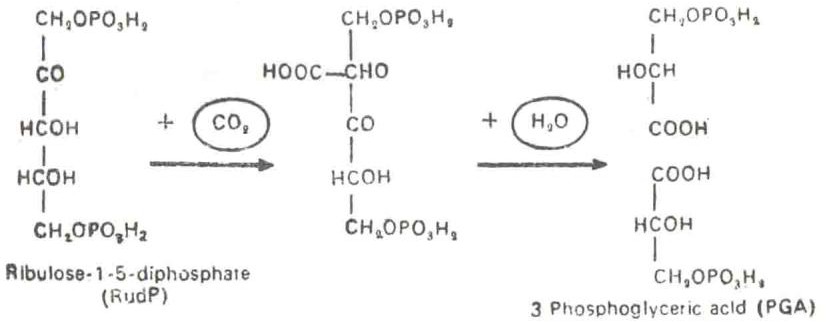


**Fig.5.8 Outline of the dark reactions**

###### Production of PGA

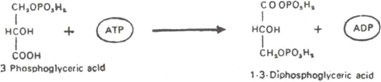
Calvin and his co-workers found that the first product to accumulate during photosynthesis was phosphoglyceric acid (PGA). This arises as follows.

Carbon dioxide is first attached to ribulose-1-5-diphosphate (RudP) a 5-carbon atom compound, to from an intermediate 6-carbon compound. Each molecule of this compound then splits to form two molecules of PGA. Radioactive carbon dioxide (14CO2) was used in the experiment, and this CO2 contributed one carbon atom of the two PGA molecules formed only one has radi oactive carbon. Thus only one free molecule of PGA is formed per molecule of CO2 entering the cycle. Actually, 6 molecules of RudP and 6 molecules of CO2 react to produce 12 molecules of PGA.

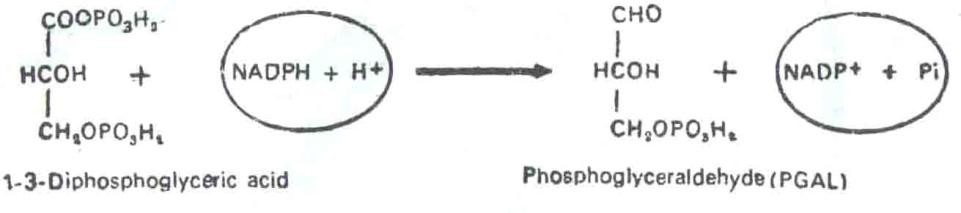


###### Production of PGAL

Phosphoglyceric acid (PGA) is reduced to phosphoglyceraldehyde (PGAL). This Process is the reverse of the oxidation step in glycolysis when PGA is oxidized to PGAL. In all 12 molecules of PGAL are produced from the 6 molecules of RudP. The reaction takes place in two steps. Firstly, PGA is phosphorylated by ATP to 1, 3-diphosphoglyceric acid.

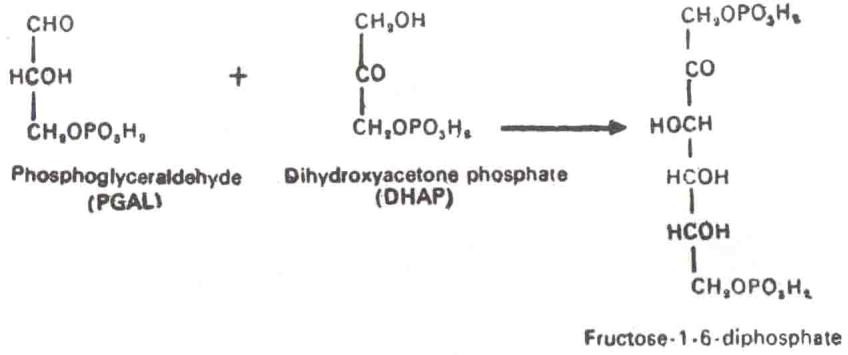


Secondly, 1, 3- diphosphoglyceric acid is reduced by NADPH + H+ to Phosphoglyceraldehyde (PGAL)



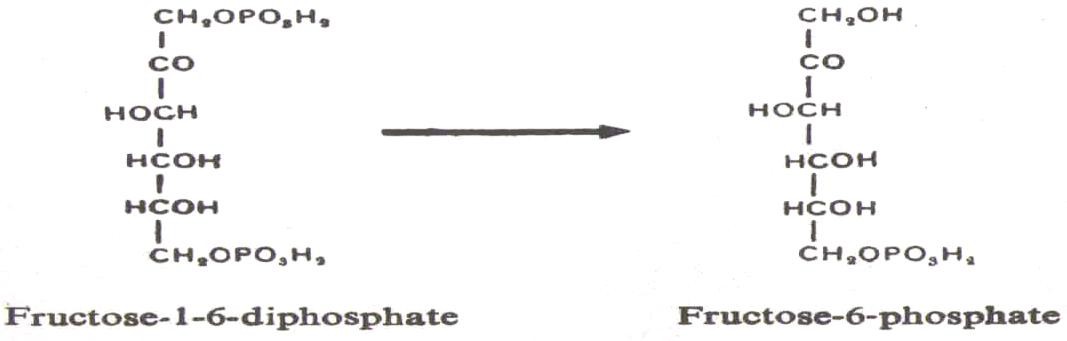
###### Production of Fructose-6- phosphate

PGAL is converted into its isomer dihydroxyactone phosphate (DHAP), as in glycolysis. DHAP condense with PGAL to from fructose-1-6-diphosphate.



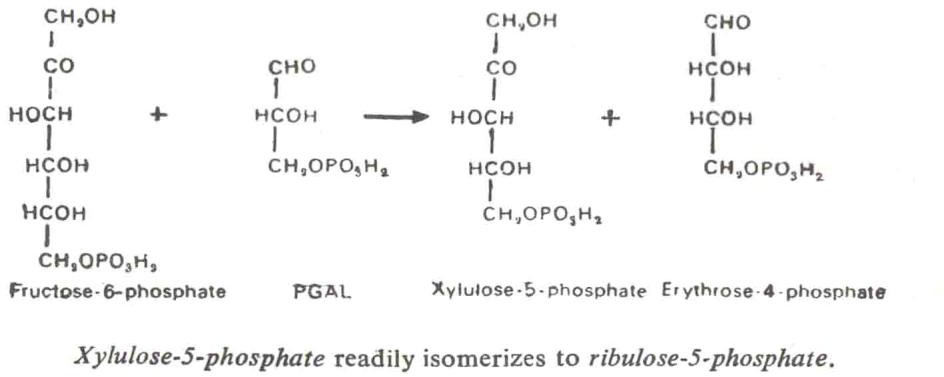
This process is the reverse of the breakdown of fructose-1-6-diphosphate in glycolysis.

One phosphate group is removed from fructose 1-6 diphosphate (dephosphorylation) resulting in the formation of fructose-6-phosphate.



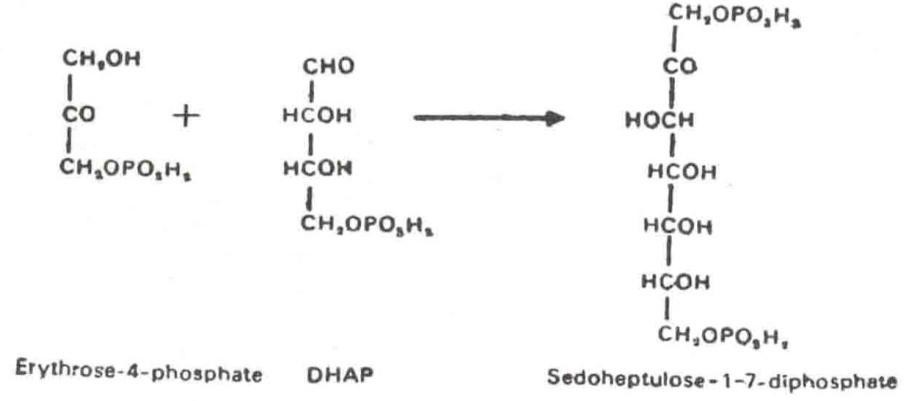
###### Production of Xylulose Phosphate

Fructose-6-phosphate reacts with PGAL to yield a pentose (5C), xylulose-5-phosphate, and a tetrose (4C), erythrose-4-phosphate the reaction is catalysed by the enzyme transketolase.



###### Formation of Sedoheptulose-1, 7-diphosphate

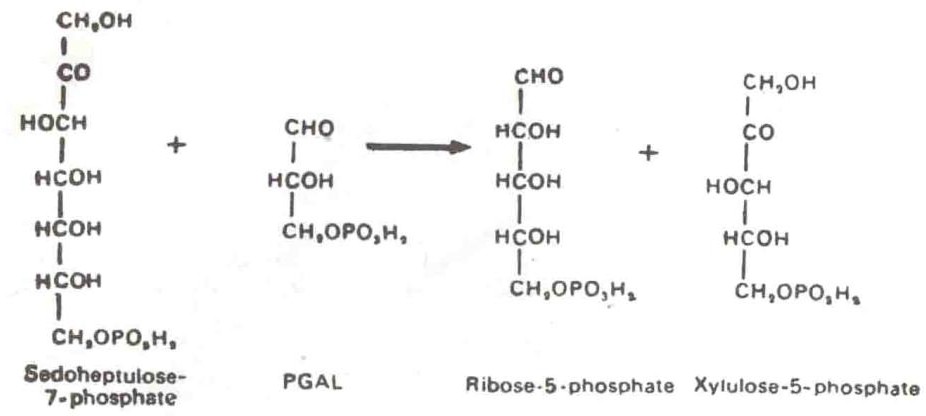
Erythrose-4-phosphate reacts with DHAP to from sedoheptulose-1-7 disposphate, the reaction being catalysed by the enzyme transaldolase.



Sedoheptulose 1-7-diphosphate loses one phosphate (dephosphorylation) and becomes sedoheptulose-7-diphosphate.

###### Formation of Ribose-5-phosphate

Sedoheptulose-7-phosphate reacts with another molecule of PGAL to from ribose-5-phosphate and xyluose-5-phosphate. Both these products readily isomerizes to ribulose-5-phosphate or ribulose monophosphate (RumP)



### C4 PATHWAYS

Most of the C4 plants are monocots. There are also about 300 species of C4 plants in dicots. About 900 species belonging to 19 families are C4 plants. The primary objective of the Hatch Slack cycle is to trap atmospheric CO2 in the maximum amount and to transport it into the bundle sheath cells so that the rate of Calvin cycle is enhanced.

Till 1965 the mechanism of the photosynthetic CO2 fixation was believed to occur only by means of what is popularly known as Calvin cycle. In 1965 H.P. Kortschak, C.E. Hart can G.O. Burr working with C14O2 on sugar cane leaves found highly efficient photosynthesis and C4 dicarboxylic acid, malate and aspartate to be the major labeled products (80% of radio activity) in very short periods of photosynthesis. Working on grasses this observation was confirmed by

M.D. Hatch and C.R. Slack of David North Plant Research Centre, Queensland, Australia in 1967. The Hatch Slack pathway, as this alternative CO2 fixation is called, has been found to occur in tropical and subtropical grasses and some dicotyledons. Some of the important C4 plants are sugarcane, maize, sorghum etc. It is interesting to note that even within a single genus a sub- tropical species *Atriplex rosea* exhibits Hatch Slack pathway whereas the temperate species *Atriplex hastata* has only the Calvin cycle.

There are 4 distinct stages in the C4 cycle:

1. CO2 fixation in the mesophyll by phosphoenol pyruvate to form C4 acids: malic and aspartic acids.
2. The transfer of C4 acids into the bundle sheath through plasmodesmata.
3. Decarboxylation of the C4 acids in the bundle sheath.
4. The diffusion of pyruvate or alanine back into the mesophyll for the regeneration of CO2 acceptor pyruvate.

In Hatch Slack cycle the first step occurs in the cytoplasm of the mesophyll where PEP Carboxylase (PEPcase) brings about the following reaction in the presence of water.



The oxaloacetate then moves into the chloroplast where it is reduced to malate by the enzyme malic acid dehydrogenase which is present inside the chloroplast.



The oxaloacatate is also transaminated into aspartate. The malate diffuses into the cells of bundle sheath through plasmodesmata. (Fig.5.9).The malate is decarboxylated within the cells of the bundle sheath by NADP+ dependent malic enzyme to produce CO2 and pyruvate. While CO2 is taken up by RuBP to accelerate the calvin cycle the pyruvate diffuses back to the mesophyll cell. The pyruvic acid reacts with ATP and an inorganic phosphate in the presence of an enzyme present in the chloroplast known as pyruvate phosphate dikinase to produce phosphoenolpyruvic acid, AMP and pyrophosphate.

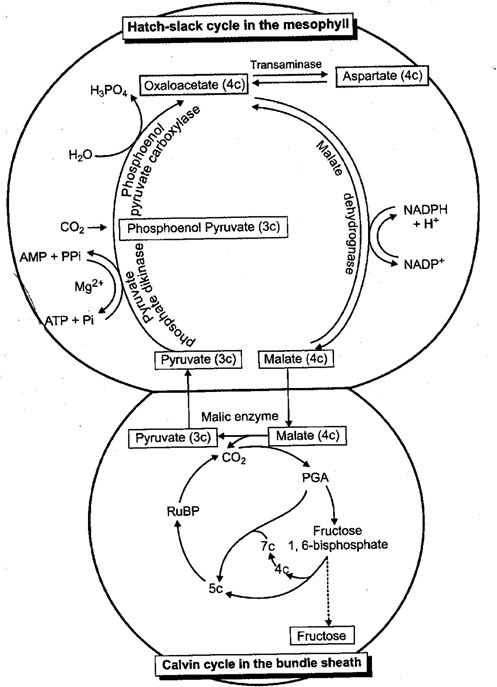


The number of ATP molecules required to synthesis one hexose is, therefore, much more than that of the Calvin cycle. The enzyme pyruvate orthophosphate dikinase brings about the conversation of pyruvic acid into phosphoenol pyruvic acid by breaking up of two energy rich bonds of ATP. The conversion of AMP back into ATP requires expenditure of t wo ATP

molecules. While in Calvin cycle the requirement is 18 ATP in C4 plants, 12 extra ATP are required because two additional ATP per CO2 are essential for regenerating ATP from AMP. The reactions of the Hatch-Slack cycle are given in Table 5.

Phosphophenolpyruvate carboxylase, pyruvate phosphate dikinase and the NADP+ specific malate dehydrogenase are present in the chloroplasts of the mesophyll cells, whereas RUBP carboxylase NADP specific malic enzyme , and the remaining Calvin cycle enzymes have been found to be present in the chloroplasts of a layer of paranchymatous cells which form a sheath around the vascular bundle. Plasmodesmata have been observed to connect adjacent cells of the bundle sheath and mesophyll layer in maize and sugarcane.

##### Table: 5

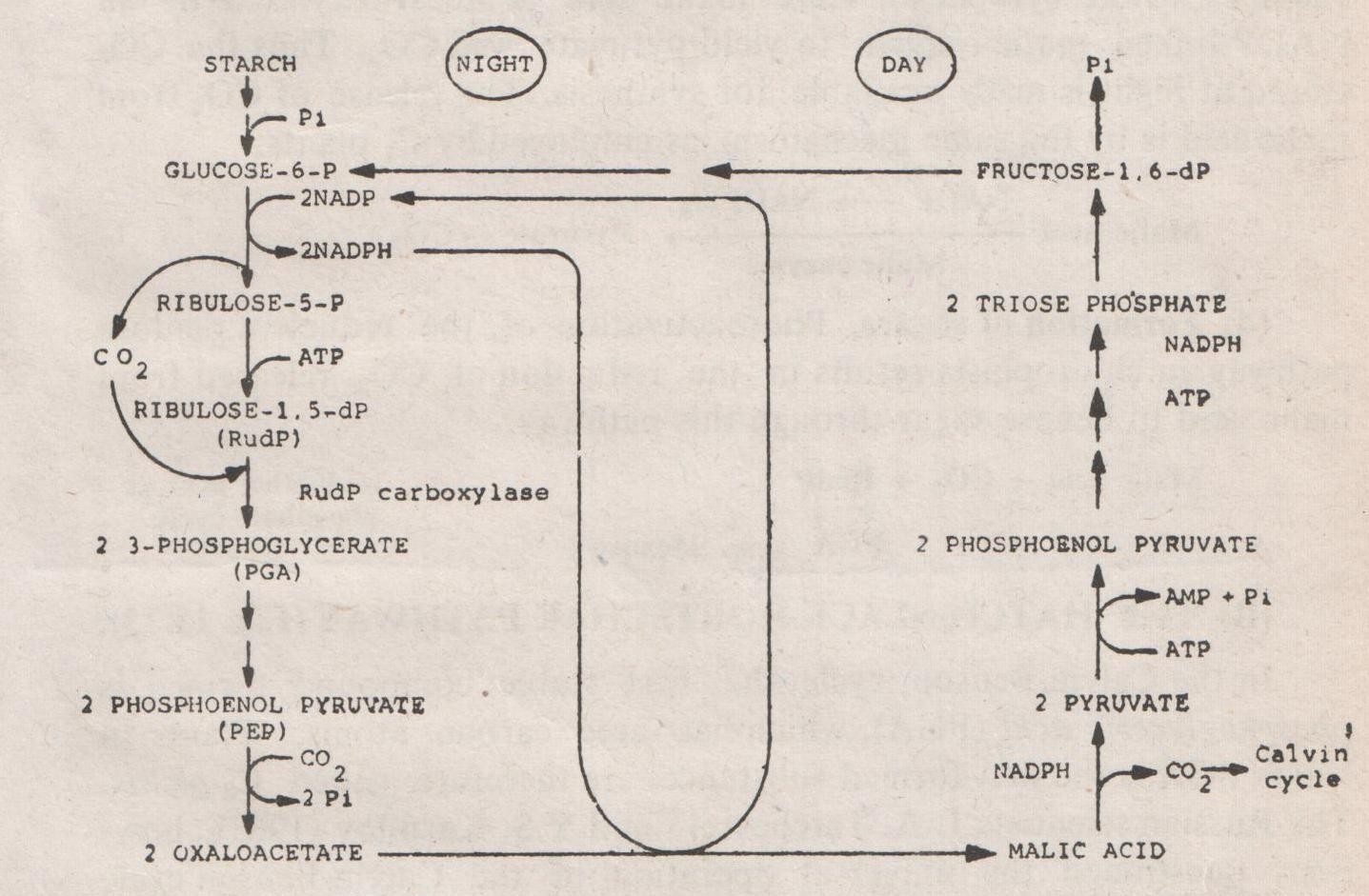


**Fig.5.9 Hatch-Slack cycle and Calvin cycle as they occur within the mesophyll and bundle sheath of the C4 plants**

### CAM PLANTS

The members of the family Crassulaceae have a special type of metabolism called Crassulacean acid metabolism (CAM) which is also exhibited by member of other families. CAM plant are succulents with thick fleshy leaves, or, when leaves are absent, a swollen photosynthetic stem. CAM plant can synthesize large amounts of malic and isocitric acids at night. Photosynthesis occurs during the day and these acids disappear. The stomata of the leaves remain closed during

the day and open only at night. This is an adaptation to conserve water, since succulents exhibiting CAM are found in dry habitats. During the night Co2 is taken into the leaves through the open stomata. Because photosynthesis is limited by the storage pool of organic acid and carbohydrate, CAM plants are generally slow growing. (Fig.5.10)



**Fig.5.10 Crassulaecean acid metabolism**

The CAM mechanism shows various modifications. Well watered *Agave americana* shows some normal daytime photosynthesis along with some CO2 fixation at night. In watered *Agave deserti*, however, dark carboxylation stops and is replaced by normal C3 daytime photosynthesis.

#### Reactions of CAM

1. **Formation of oxaloacetate**. The requirements for formation of oxaloacetate are CO2 and phosphoenol pyruvic acid (PEP). PEP is formed from stored carbohydrate in leaves, particularly starch. In CAM species the stomata remain open at night and CO2 entering the leaves are fixed by PEP to form oxaloacetate.

CO2+PEP Oxaloacetate + Pi

1. **Formation of Malic acid**- Oxaloacetate is reduced by malic dehydrogenase to malic acid, which accumulates in the vacuoles of leaf cells. During this step NADPH2 formed in the pentose phosphate pathway is utilized , and the NADP formed enters the pentose phosphate pathway.



1. **Release of CO2 from malic acid.** – During the day ATP and NADPH are abundantly available from the photosynthesis reactions. The stomata of the leaves are closed and malate is transported back out of the vacuoles to the cytoplasm. Here malic acid is decarboxylated by an NADP-linked malic enzyme to yield pyruvate and CO2. Thus the CO2 stored at night is made available for synthesis. The release of CO2 from malic acid is by the same mechanism as employed by C4 plants.



1. **Formation of sugar** – Photoactivation of the reductive pentose pathway in chloroplasts results in the reduction of CO2 released from malic acid to hexose sugar

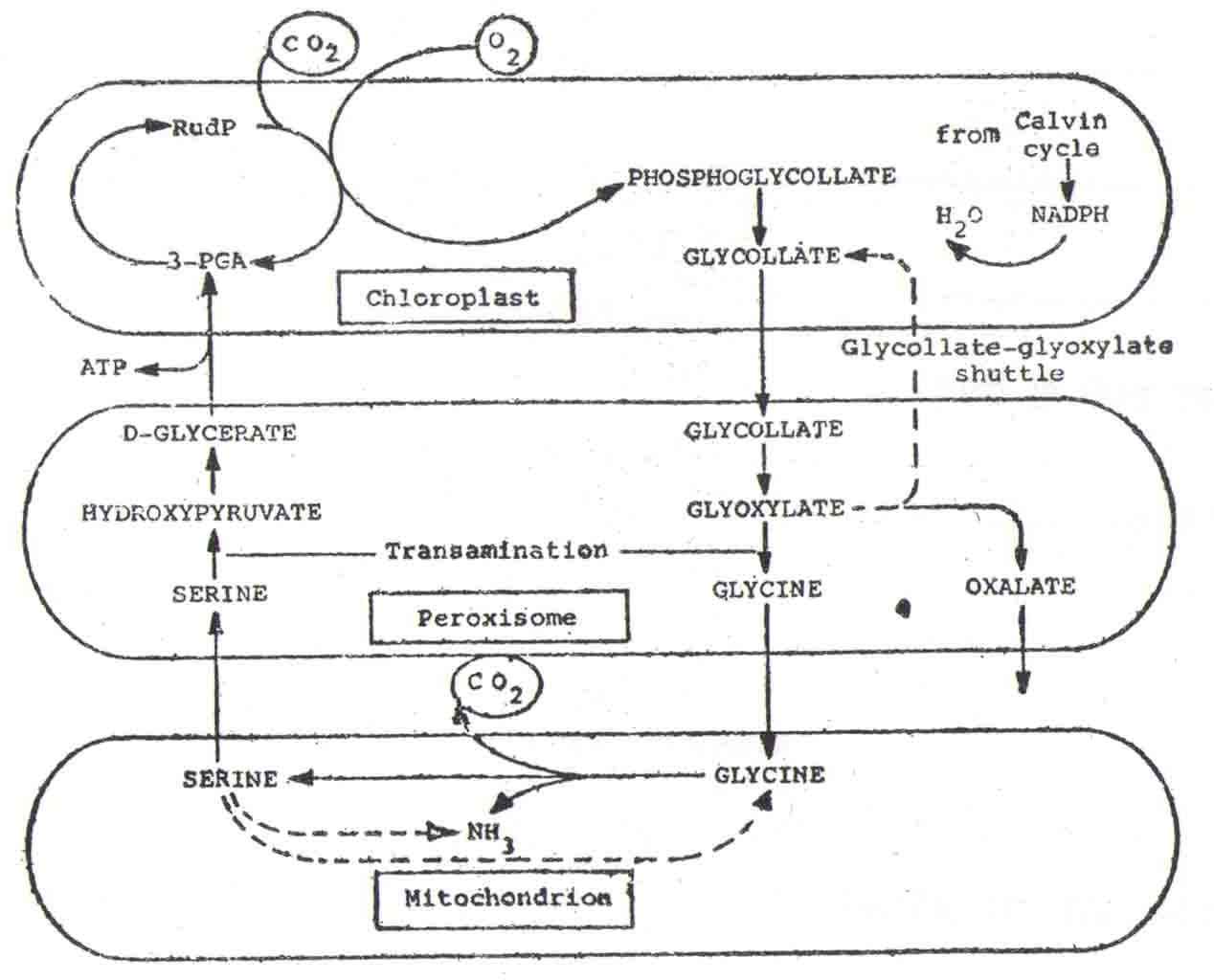
through this pathway.



### PHOTORESPIRATION

Although C3 plants respire in the dark, the rate of oxygen utilization increases markedly when the plants are illuminated. Photorespiration is a light driven efflux of CO2 which proceeds alongside with net CO2 influx during photosynthesis. Photorespiration may attain 50% of the net rate of photosynthesis. Photorespiration results in CO2 evolution in light. This has the net effect of decreasing photosynthesis which takes up CO2 in light. It is therefore a wasteful process which prevents plants from achieving a maximum yield in photosynthesis. In crop species the yield would be greater if photorespiration did not occur. The substrate for photorespiration is glycollate. Breeding of plants with lower photorespiration rates, or inhibiting glycollate synthesis, would be means of increasing crop yields.

Photorespiration is exhibited by crop plants like wheat, rice, other cereals, many legumes and sugar beets, while crops like corn, sorghum and sugarcane do not have photorespiration. (Fig. 5. 11)



**Fig.5.11 Some pathways in glycollate metabolism**

The CO2 compensation point is the CO2 concentration at a given constant light intensity at which there is a balance between photosynthetic assimilation and respiration. The enzymes of the photo respiratory pathways are present in both C3 and C4 plants. The carbondioxide compensation point for common C3 crop plants is about 40-60 ppm at 25O C, while that for C4 plants is often less than 10 ppm. The CO2 generated in C4 plants during photorespiration is trapped and re-cycled internally by cytoplasmic PEP carboxylase of mesophyll cells. Thus CO2 efflux is prevented.

Glycollic acid is a 2-carbon compound which is formed in large quantities in the chloroplasts of C3 plants, from where it moves out into the cytosol.