CO_2 is reduced to carbohydrate in dark and this phase is purely a chemical or dark phase.

The two phases of the photosynthetic process are described here, separately.

MECHANISM OF PHOTOSYNTHESIS: I

LIGHT REACTION

(Activities found in thylakoids or grana)

Until 1930s it was thought that photosynthetic reaction is reverse of respiration and oxygen evolved during the process comes from CO_2 and water combines with carbon dioxide to produce carbohydrate.

$$CO_2 + H_2O \underset{\text{Respiration}}{\overset{\text{Photosynthesis}}{\rightleftharpoons}} (CH_2O) + O_2$$

or

$$6CO_2 + 6H_2O \rightleftharpoons C_6H_{12}O_6 + 6O_2$$

(carbohydrate)

In 1937, Robert Hill demonstrated that isolated chloroplasts evolved oxygen when they were illuminated in the presence of suitable electron acceptor, such as ferricyanide. The ferricyanide is reduced to ferrocyanide by photolysis of water. This reaction is now called **Hill Reaction** and it explains that water is used as a source of electrons for CO_2 fixation and oxygen is evolved as a by product.

Ruben, Randall and Kamen (1941) using heavy isotope of oxygen (O^{18}) in their experiments provided the direct proof that oxygen evolved in photosynthesis comes from water and not from carbon dioxide. When photosynthesis is allowed to proceed in presence of H_2O^{18} and normal CO_2 the evolved oxygen contains the heavy isotope.

$$2H_2O^{18} + CO_2 \xrightarrow[\text{Light}]{\text{Green Plants}} (CH_2O) + H_2O + O_2^{18}$$

(Carbohydrate)

And if photosynthesis is allowed to proceed in presence of CO_2^{18} and normal water (H_2O), heavy oxygen is not evolved.

$$2H_2O + CO_2^{18} \xrightarrow[\text{Light}]{\text{Green plants}} O_2 + (CH_2O^{18}) + H_2O^{18}$$

(Carbohydrate)

Thus, the fate of different molecules can be summarised as follows:

$$6CO_2 + 12H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} C_6H_{12}O_6 + 6H_2O + 6O_2\uparrow$$

The reaction also allows to assume with reasonable certainty that hydrogen necessary for CO_2 reduction is provided by water.

According to recent studies, the light reaction phase of photosynthesis is a considerably complicated process with several important events. It may be briefly discussed with the help of following subheadings:

I. Red drop, Emerson effect and two pigment systems.

(a) Red drop and Emerson effect.

(b) Two pigment systems.

II. Production of assimilatory powers.

(a) Electron transport system in photosynthesis or reduction of NADP.

(b) Photophosphorylation

(i) Non-cyclic Photophosphorylation.

(ii) Cyclic Photophosphorylation.

(iii) Pseudocyclic Photophosphorylation.

III. Energy relationships and efficiency of photosynthesis.

IV. Interrelationship between light and dark reactions.

I. Red Drop and Emerson Effect and Two Pigment Systems

(a) **Red drop and Emerson Effect**. Photosynthesis is considered as a two quanta process, i.e. it takes two light quanta energy to drive each electron. Since four electrons are required for the reduction of one molecule of CO_2, eight quanta will be required to reduce it or to evolve one molecule of oxygen.

$$CO_2 + 4H^+ \longrightarrow CH_2O + H_2O$$

$$[4H_2O \xrightarrow[\text{energy}]{\text{Radiant}} 4(H^+ + e^-) + 2H_2O + O_2]$$

Number of O_2 molecules released can be used to determine the quantum yield of the process. **Quantum yield** is defined as the number of O_2 molecules released per light quanta absorbed. In the process, one mol. of O_2 is evolved utilizing eight quanta energy, i.e. quantum yield is 1/8 or 12%.

Emerson and Lewis (1943) worked on quantum yield of photosynthesis in monochromatic (single colour) light of different wavelengths. They observed that quantum yield declined sharply at wavelength greater than 680 nm in the red zone. This decline is called **red drop**.

Eight years later, Emerson and Chalmers found that the sharp decline in the quantum yield of photosynthesis beyond 680nm can be brought to full efficiency by simultaneously providing shorter wavelengths of light. The effect of two superimposed beams of light on the rate of photosynthesis exceeds the sum effect of both beams of light used separately. This photosynthetic enhancement is referred to as **Emerson effect** (for reason *see* cyclic photophosphorylation).

(b) **Two Pigment Systems**. With the discovery of red drop and Emerson effect it was concluded that at least two pigment systems are involved in photosynthesis (Fig. 12.1). These two pigment systems have been referred to as pigment system I (PS I) and pigment system II (PS II). The presence of two such systems has been supported by studies based on chloroplast fractionation process which showed two types of particles within the chloroplast membrane, smaller and lighter particles of PS I and larger and heavier particles of PS II.

PS I complex consists of ~ 200 chlorophylls, ~ 50 carotenoids, a mol. of P 700, one cyt. *f*, one plastocyanin, two cyt. b 563, FRS (ferredoxin reducing substance), one or two membrane bound ferredoxin molecules etc. It is rich in chl *a*, iron and copper. Now it's chl. *a* is called chl. *a* I. PS I controls the process of producing a strong reductant to reduce NADP into $NADPH+H^+$.

PS II Complex consists of ~ 200 chlorophylls, ~ 50 carotenoids, a mol. of P 680, *a* primary electron acceptor Q, a plastoquinone, four plastoquinone equivalents, four Mn molecules bound to one or more proteins, two cyt. *b* 559, one cyt. b_6 and chloride. It's chl. *a* is now called chl. *a* II. PS II is concerned with generation of strong oxidant and weak reductant coupled with the release of oxygen.

Salisbury and Ross (1986) proposed that grana mainly contain PS II while stroma lamellae PS I. For PS I, light energy is gathered by chl. *a* (P700) and possibly by some chl. *b* and some carotenoids, while for PS II light energy is collected by chl. *a* (P 673), chl. *b* and xanthophylls. The carotenoids collect light energy for both the systems. Reduced carotenoids such as carotenes are found in PS I while more oxidised forms as xanthophylls, violaxanthin and *r* – neoxanthin are found in PS II. Park (1970) proposed that four subunits of PS II are found attached to one subunit of PS I. It is thought that in quantasome, P700 acts as a photochemical reaction centre and the energy absorbed by the large number of chl. *a* 683 molecules is funnelled to P 700 to keep it continuously in excited state. In PS II, primary electron acceptor is colourless chl. *a* that lacks Mg and called pheophytin (Pheo). Closely associated with Pheo is a quinone called Q because of its ability to quench fluorescence of P 680 by accepting its excited electron.

II. Production of Assimilatory Powers in Photosynthesis

Arnon (1956) used the term assimilatory powers to refer ATP (adenosine triphosphate) and $NADPH_2$ (reduced Nicotinamide adeninedinucleotide phosphate). The process of reduction of NADP into $NADPH+H^+$ may be denoted as *Electron transport system* in photosynthesis or *Reduction of NADP* while the process of formation of ATP from ADP and inorganic phosphate (Pi) utilising light energy is called *Photophosphorylation, i.e.*

(*a*) Electron transport system in photosynthesis or reduction of NADP (transport of electron from water to NADP).

(*b*) Photophosphorylation or formation of ATP in photosynthesis.

(*a*) Electron Transport System (ETS) or Reduction of NADP

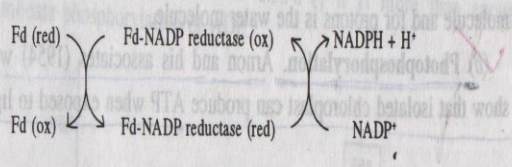
When a photon is absorbed by a pigment in the light harvesting complex associated with PS II, its energy is transferred by inductive resonance to P680 which is removed by Pheo (Pheophytin). So Pheo is the first electron acceptor. Loss of electrons from P680 causes it to become positively charged. It then attracts an electron from an adjacent Mn-protein. As the Mn-protein becomes oxidised by losing one electron, in turn it strongly attracts an electron from water. This pulling apart of electron from water molecule has been imperfectly referred to as *Photolysis of water*. On the other hand, electron from Pheo is used in reduction of plastoquinone (PQ to PQH_2). This reduction step requires two electrons and two protons

$$\left[PQ \underset{-2e^-,\ -2H^+}{\overset{+2e^-,\ +2H^+}{\rightleftharpoons}} PQH_2 \right]$$

At least four types of plastoquinones have been reported from chloroplast,

three are tocopheryl quinones and one is vit. K (1, 4-naphthoquinone acetate). From PQH_2, electrons move, one at a time, to cytochrome b_6 to cytochrome *f* to plastocyanin (PC). PC moves along the edge of membrane to PS I where P700 accepts the electron. P700 cannot accept the electron unless it has previously lost one which can occur by light excitation.

Excited P700 gives its electron to Fe^{3+} in one of the Fe-S proteins which may be called ferredoxin reducing substance (FRS). The exact reducing potential and chemical composition of FRS is still unknown. However, electron from P700 either through FRS or directly reduces ferredoxin. So the electron required to reduce ferredoxin (Fd) is provided by photoexcited P700 and thus ferredoxin (or FRS) becomes the primary electron acceptor of PS I.



Ferredoxin reduces $NADP^+$ by providing two electrons, one at a time.

All this can be better explained using Z-scheme (Fig. 12.1) model as proposed by Hill and Bendal (1960). In view of recent researches, it has been partly modified.

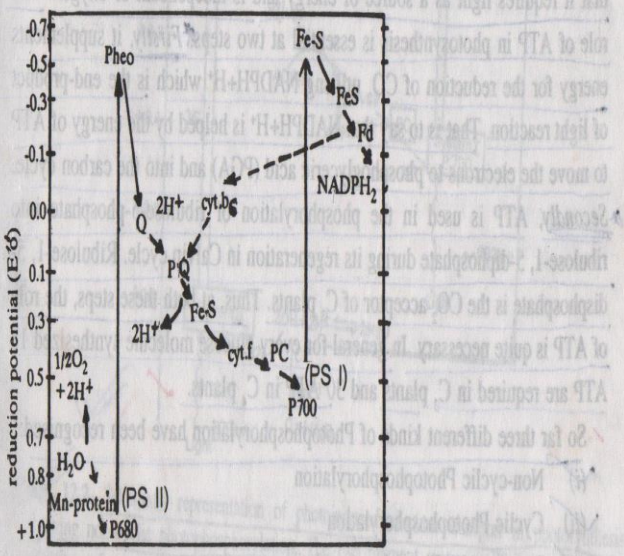


Fig. 12.1. Z-scheme (partly modified by Cogdell 1983). Steps between cyt. b_6 to cyt. *f* and P700 to Fe-S are called down Hill and up Hill reaction, respectively. On the left reduction potential (E_0) values are given.

In earlier literature, the term photolysis of water has been used but it is confusing as photolysis is not the primary photochemical reaction occurring under the direct influence of light. It is actually due to pulling apart of electrons by the oxidised PS II from OH ions. The protons (H^+) are released in the system and are used for the reduction of NADP while OH radicals react to form H_2O_2 which because of its unstable nature breaks up to produce water and evolves oxygen.

$$4OH \rightarrow 2H_2O_2 \rightarrow 2H_2O+O_2 \uparrow$$

Thus, in the reduction of NADP, the source of electrons is the chlorophyll molecule and for protons is the water molecule.

(*b*) **Photophosphorylation.** Arnon and his associates (1954) were first to show that isolated chloroplast can produce ATP when exposed to light, *i.e.*

$$ADP+Pi \xrightarrow[\text{chlorophyll}]{\text{light}} ATP$$

[ADP=adenosine diphosphate; Pi=inorganic phosphate and ATP=adenosine triphosphate]

They called this kind of phosphorylation as photophosphorylation which is different from the oxidative phosphorylation found in mitochondria in respect that it requires light as a source of energy and is independent of oxygen. The role of ATP in photosynthesis is essential at two steps. *Firstly*, it supplements energy for the reduction of CO_2 utilisng NADPH+H^+ which is the end-product of light reaction. That is to say that NADPH+H^+ is helped by the energy of ATP to move the electrons to phosphoglyceric acid (PGA) and into the carbon cycle. *Secondly*, ATP is used in the phosphorylation of ribulose-5-phosphate into ribulose-1, 5-diphosphate during its regeneration in Calvin cycle. Ribulose-1, 5-disphosphate is the CO_2 acceptor of C_3 plants. Thus, at both these steps, the role of ATP is quite necessary. In general for every glucose molecule synthesized 18 ATP are required in C_3 plants and 30 ATP in C_4 plants.

So far three different kinds of Photophosphorylation have been recognised:

(i) Non-cyclic Photophosphorylation

(ii) Cyclic Photophosphorylation

(iii) Pseudocyclic Photophosphorylation

(i) Non-cyclic Photophosphorylation. It occurs among the green plants and involves both PSI and PS II.

As the energy-rich electron passes through the electron transport system, at every step it releases energy generally equal to the difference between the energy levels of donor and acceptor. At steps of transfer where the released energy is not sufficient to bind inorganic phosphate with ADP, it is wasted in the form of heat or fluorescent light but at steps where this released energy is sufficient to bind inorganic phosphate with ADP, the ATP synthesis is coupled with energy released. During the passage of excited electrons from PS II to PS I, the ATP synthesis is presumed to occur between cytochrome b_6 (E'_0=0.03 V) and cytochrome f (E'_0=0.36 V). As the difference between the redox potentials of the two cytochromes amounts to 0.33 e V, it is more than enough to accommodate phosphorylation of ADP.

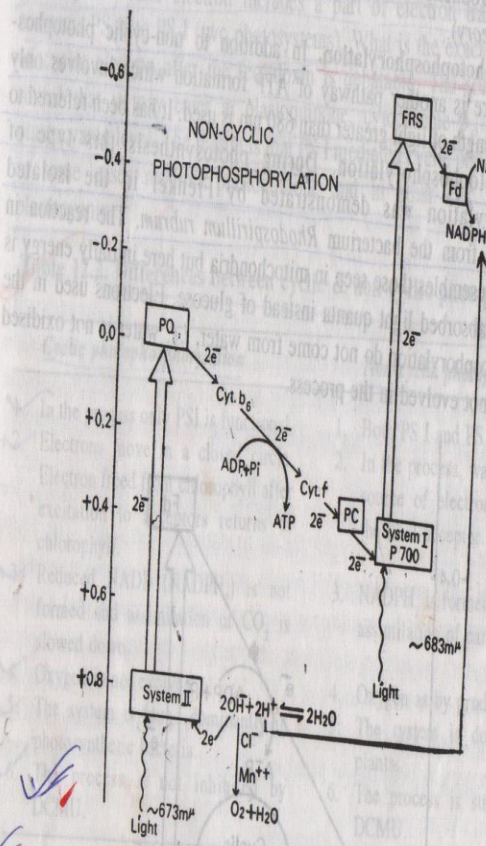


Fig. 12.2. Schematic representation of photoinduced electron transport in photosynthesis showing non-cyclic photophosphorylation. The two pigment systems –PS I and PS II, the splitting of water molecule and generation of assimilatory powers are also shown. (PQ=plastoquinone; PC=plastocyanin; FRS=ferredoxin reducing substance and Fd=ferredoxin). Cyt. b_3 is also found in between PS II and PS I but its function is so far unknown.

In non-cyclic photophosphorylation, the flow of electron is unidirectional, that is, electron donated by PS II after passing through plastoquinones, cyt. b_6, cyt. f, plastocyanin and PS I eventually reaches ferredoxin which in turn donates to reduce NADP. The reduced NADP (NADPH+H^+) is utilised for the reduction of CO_2 to carbohydrate level. The electron does not complete the cycle. It starts from PS II and is drained off in the carbohydrates produced by CO_2 reduction.

So the ATP synthesis resulting from this type of non-cyclic electron transport chain is known as non-cyclic photophosphorylation. Water molecule is utilised as a source of electron in this system. In the process, two molecules of ATP are formed per two molecules of $NADP^+$ reduced or one molecule of oxygen evolved or two molecules of water oxidised.

$$2ADP + 2Pi + 2NADP^+ + 2H_2O \rightarrow 2ATP + 2NADPH + H^+ + O_2$$

During light reaction, the protons (H^+) accumulate inside the thylakoid membrane resulting in a PROTON GRADIENT. The energy released by the protons when they diffuse across the thylakoid membrane into the stroma (along the proton concentration gradient) is used to produce ATP. The process is similar to the production of ATP by F_0—F_1 particles of mitochondria (see *chemiosmotic theory*).

(ii) **Cyclic photophosphorylation.** In addition to non-cyclic photophosphorylation, there is another pathway of ATP formation which involves only PS I and wavelength of light greater than 680 nm is used. It has been referred to as cyclic photophosphorylation. During photosynthesis this type of photophosphorylation was demonstrated by Frenkel in the isolated chromatophores from the bacterium *Rhodospirillum rubrum*. The reaction in cyclic process resembles those seen in mitochondria but here initially energy is provided from absorbed light quanta instead of glucose. Electrons used in the cyclic photophosphorylation do not come from water, *i.e.* water is not oxidised and oxygen is not evolved in the process.

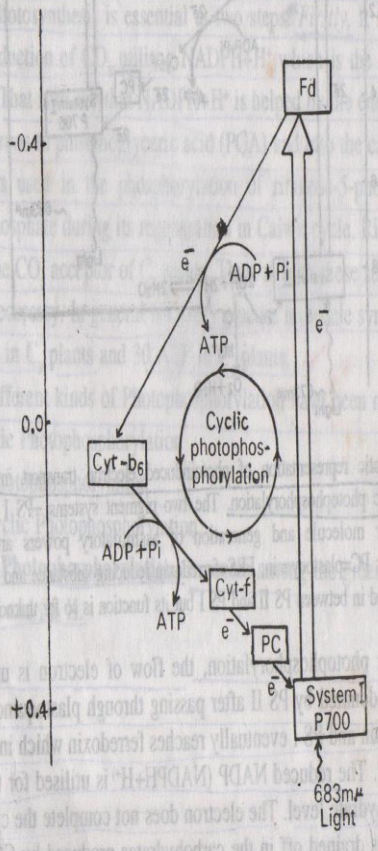


Fig. 12.3. Schematic representation of photoinduced electron transport in photosynthesis showing cyclic photophosphorylation. (Fd=ferredoxin; PC=plastocyanin.)

In the cyclic photophosphorylation, light lifts the electron from P 700 (E'_0 = 0.42 V) to FRS (E'_0 = -0.6 V) or Ferredoxin (E'_0 = -0.417 V). The excited electron returns to P 700 through two to three transfer steps to decreasing redox potentials. It is during such a downhill migration of the electron that enough energy is released for ATP synthesis. The potential gap between FRS to P 700 is nearly one volt which is sufficient to produce at least 2 ATP molecules per electron transfer, or four ATP molecules per two electrons transfer. In the process, ATP can be formed between cytochrome b_6 (E'_0=0.03 V) and cytochrome f (E'_0=0.36 V) and between ferredoxin and cytochrome b_6, the potential gap between these two being more than ~ 0.32 V (See Fig. 12.3).

The concept about its site, rate and mechanism is not yet very clear. The pathway of cyclic electron includes a part of electron transport chain that connects PS II to PS I (two photosystems). What is the exact point of entrance of cyclic electron after the ferredoxin is debatable. The studies so far made reveal that it may enter at plastoquinone, cytochrome b_6, cytochrome f or plastocyanin level. The reason is that PS I mediated cyclic photophosphorylation in some cases requires plastoquinone and in other cyt. b_6 or cyt. f or plastocyanin.

Table 12.2. Differences between cyclic & non-cyclic photophosphorylation

Cyclic photophosphorylation	*Non-cyclic photophosphorylation*
1. In the process only PSI is functional.	1. Both PS I and PS II are functional.
2. Electrons move in a closed circle. Electron freed from chlorophyll after excitation to acceptors returns to chlorophyll.	2. In the process, water is the ultimate source of electrons and $NADP^+$ is the final acceptor.
3. Reduced NADP ($NADPH_2$) is not formed and assimilation of CO_2 is slowed down.	3. $NADPH_2$ is formed which is used in assimilation of carbon dioxide.
4. Oxygen is not evolved.	4. Oxygen as by product is evolved.
5. The system is found dominantly in photosynthetic bacteria.	5. The system is dominant in green plants.
6. The process is not inhibited by DCMU.	6. The process is stopped by use of DCMU.

However, when cyclic photophosphorylation operates, the CO_2 assimilation drops down because of the shortage of reduced NADP ($NADPH+H^+$) due to lack of protons (H^+). But at the same time it can explain the phenomena of Red drop and Emerson enhancement effect. When wavelength of light above 680 nm is provided non-cyclic photophosphorylation is blocked which results in shortage of $NADPH+H^+$ necessary for CO_2 assimilation and fall of rate of photosynthesis. And when non-cyclic photophosphorylation is restored by supplementary wavelength of light below 680 nm, the $NADPH+H^+$ production starts and more efficient use of ATP produced in cyclic photophosphorylation is made and the rate of photosynthesis increases. It explains the enhancement phenomenon.

Certain algae can grow in a hydrogen atmosphere in the complete absence of

required. The total free energy would be

136,800+631,200=768,000 cal

whereas free energy required for the synthesis of one hexose molecule from CO_2 is 673,000 cal; that means the utilisation of 18 ATP and 12 NADPH+H^+ would be more than sufficient for the purpose. Light reaction thus can be summarised as follows:

$$12\,H_2O + 12NADP + 18ADP + 18Pi \xrightarrow[\text{chlorophyll}]{\text{light}} 12\,NADPH + H^+ + 18\,ATP + 6O_2\uparrow$$

Efficiency of Photosynthesis. Consideration about energy efficiency of photosynthesis requires other methods of calculation.

In **one way** it may be calculated as follows. The two photosystems harvest energy in the form of ATP and NADPH+H^+. ATP stores energy nearly 7.6 Kcal. (7,600 cal.) and NADPH+H^+ about 52 Kcal. (52,000 cal.). Light energy utilised is four light quanta (two per photosystem), each of 40 Kcal. of light energy. Now if we measure the efficiency of photosynthetic conversion of light energy in terms of ATP and NADPH+H^+, it will come to [(52+7.6)100/160]=37.25% or nearly 37 per cent. Thus a major portion of absorbed light energy is wasted.

For the **second way** of measuring efficiency of photosynthesis, calculation in terms of CO_2 assimilated or molecule of oxygen evolved is made. For every CO_2 molecule fixed, one molecule of oxygen is evolved and nearly 112 Kcal. of light energy is stored, i.e. 673 Kcal. light energy for each molecule of carbohydrate or for reduction of six molecules of CO_2. Since a molecule of CO_2 consumes eight light quanta, the total consumption will be 48 quanta per hexose molecule formed. Assuming 40 Kcal. energy present in red light (most efficient light for photosynthesis), the energy efficiency for the process will be 673×100/48×40=35.052 per cent or nearly 35 per cent.

The difference between the two methods in photosynthetic efficiency may be explained on the basis that a part of light energy stored in ATP and NADPH + H^+ may be lost during the participating in the process of CO_2 assimilation.

Salisbury and Ross (1986) proposed that instead of 8 photons and 3 ATP, 12 photons and more than 3 ATP are required to reduce each mol. of CO_2. This would further change the efficiency of process.

IV. Interrelationships between Light and Dark Reaction

Light reaction is commonly called *photostage* and dark reaction *synthesis stage*. The two phases may also be called *hydrogen transfer phase* (light reaction) and *carbon assimilation phase* (dark reaction).

Levitt (1969) proposed a scheme showing interrelationships between the two phases. It explains that the reduction of each molecule of CO_2, 3 molecules of ATP and 2 molecules of NADPH + H^+ (for C_3 plants) are utilised. Both ATP and $NADPH_2$ act as assimilatory powers to provide sufficient energy to carry out the process. Twelve molecules of ATP are utilised for uplifting the twelve molecules of $NADPH_2$ because the redox potential of $NADPH_2$ is much lower (E'_0 = −0.32V) than required (E'_0 = −0.4V) to act as hydrogen donor during the process. Another six molecules of ATP are utilised during the phosphorylation of ribulose-5-phosphate to regenerate ribulose-1,5-diphosphate. Similarly twelve

molecules of $NADPH_2$ are utilised because for the reduction of each molecule of CO_2 four electrons (hydrogen atoms) are required.

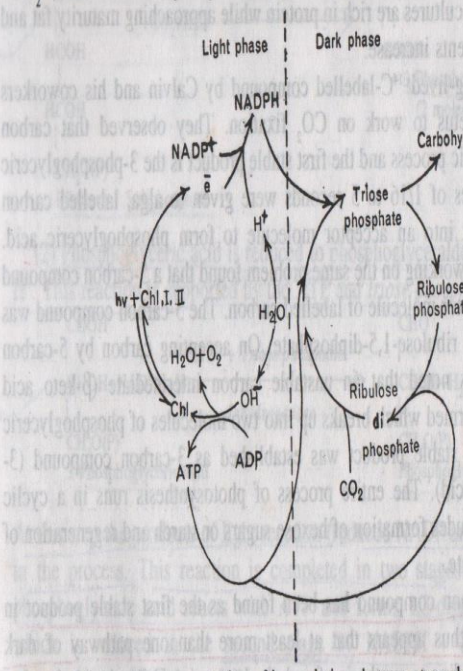


Fig. 12.4. Schematic representation of interrelations between two phases of photosynthesis.

Thus, in all 18 ATP molecules and 12 $NADPH_2$ molecules are required for the synthesis of each molecule of glucose from CO_2.

$$CO_2 + 2NADPH + H^+ \xrightarrow[\text{ATP molecules}]{\text{utilising energy}} CH_2O + H_2O + 2NADP$$

or

$$6CO_2 + 12NADPH + H^+ + 18ATP \longrightarrow C_6H_{12}O_6 + 6H_2O + 12NADP + 18ADP + 18Pi$$

MECHANISM OF PHOTOSYNTHESIS: II

DARK REACTION

(Activities found in Stroma)

The fact that non-photochemical process (dark reaction process) is involved in photosynthesis was established by Blackman (1905). But it was only after 1946 that usage of radioactive and other modern sophisticated techniques could elucidate recent concept of photosynthesis and Calvin and his co-workers proposed the pathway of CO_2 reduction. They advanced their studies while working on unicellular green alga *Chlorella pyrenoidosa*. The alga offered many advantages and has served as a classical material for studies on photosynthesis. It was used in the study for the first time giving consideration to following points:

(i) It grows luxuriantly in different environmental conditions and avoids contamination.

(ii) The entire plant behaves like a single chloroplast.

(iii) It is a rapidly dividing alga.

(iv) Its synchronous culture is easy to maintain which is essential for physiological studies.

(v) It contains Photosynthetic pigments similar to the higher plants and, therefore, the end products of photosynthesis are also similar.

(vi) The young cultures are rich in protein while approaching maturity fat and carbohydrate contents increase.

The use of long-lived ^{14}C-labelled compound by Calvin and his coworkers gave a new impetus to work on CO_2 fixation. They observed that carbon reduction is a cyclic process and the first stable product is the 3-phosphoglyceric acid. When flashes of 1/16 to 5 seconds were given to alga, labelled carbon (^{14}C) entered first into an acceptor molecule to form phosphoglyceric acid. However, Benson working on the same problem found that a 5-carbon compound was the first acceptor molecule of labelled carbon. The 5-carbon compound was soon identified as ribulose-1,5-diphosphate. On accepting carbon by 5-carbon compound, it was noted that an unstable carbon intermediate (β-keto acid intermediate) is formed which breaks up into two molecules of phosphoglyceric acid. So the first stable product was established as 3-carbon compound (3-phosphoglyceric acid). The entire process of photosynthesis runs in a cyclic fashion which includes formation of hexose sugars or starch and regeneration of ribulose diphosphate.

Recently 4-carbon compound has been found as the first stable product in certain plants. It thus appears that at least more than one pathway of dark fixation of CO_2 leading to synthesis of carbohydrate exist. Following three types are now well established:

1. Calvin cylce (C_3 plants)
2. Hatch and Slack cycle (C_4 plants)
3. CAM cycle (CAM plants)

The three types of plants are differentiated on the basis that in C_3 plants the first stable product of photosynthesis is 3-carbon compound (phosphoglyceric acid) and in C_4 plants, 4-carbon compound (oxaloacetic acid or malic acid) is the first stable compound. In CAM cycle also, the first product is malic acid.

The three processes are discussed here.

1. CALVIN CYCLE (C_3 Plants)

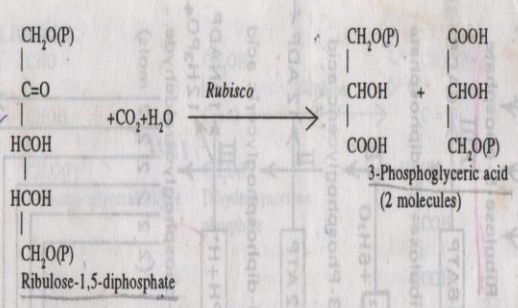
The dark reaction process of photosynthesis has been named variously such as-Calvin cylce, Bassham and Calvin cycle, Blackman reaction, Carbon assimilation, Path of carbon in photosynthesis, Reductive pentose phosphate cycle etc. Calvin cycle consists of two important parts.

(a) Synthesis of carbohydrate

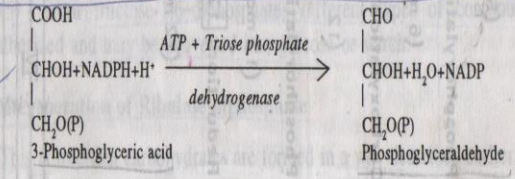
(b) Regeneration of ribulose diphosphate.

(a) Synthesis of Carbohydrate

(1) Carbon dioxide is first accepted by ribulose diphosphate and forms an unstable 6-carbon compound from which two molecules of phosphoglyceric acid are formed. The reaction is regulated by enzyme called *carboxydismutase* or *RuDP carboxylase (Rubisco)*.

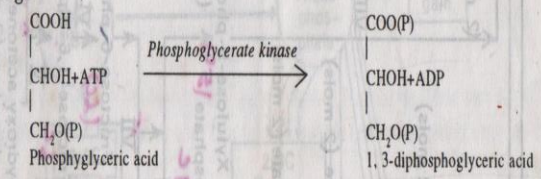


(2) Phosphoglyceric acid is reduced to phosphoglyceraldehyde by NADPH + H^+. This reaction is supported by the ATP and *triose phosphate dehydrogenase*.

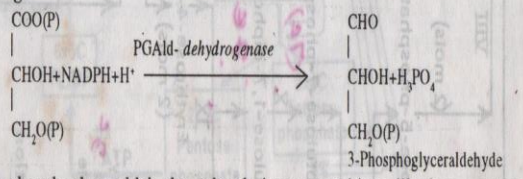


At this step the products of light reaction (reduced NADP and ATP) are used up in the process. This reaction is completed in two stages. In the first stage, phosphoglyceric acid reacts with ATP and forms 1,3-diphosphoglyceric acid which is reduced in the second stage by NADPH + H^+ to give rise to phosphoglyceraldehyde as follows:

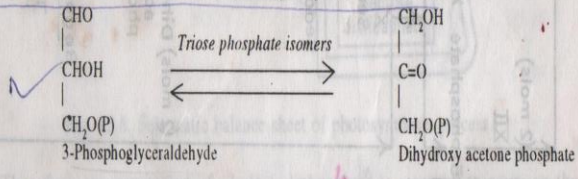
First Stage



Second Stage



(3) The phosphoglyceraldehyde molecule is converted into dihydroxyacetone phosphate in presence of enzyme *triose phosphate isomerase*.



(4) Phosphoglyceraldehyde and dihydroxyacetone phosphate (one molecule each) unite to form fructose-1,6-diphosphate. The enzyme *aldolase* regulates

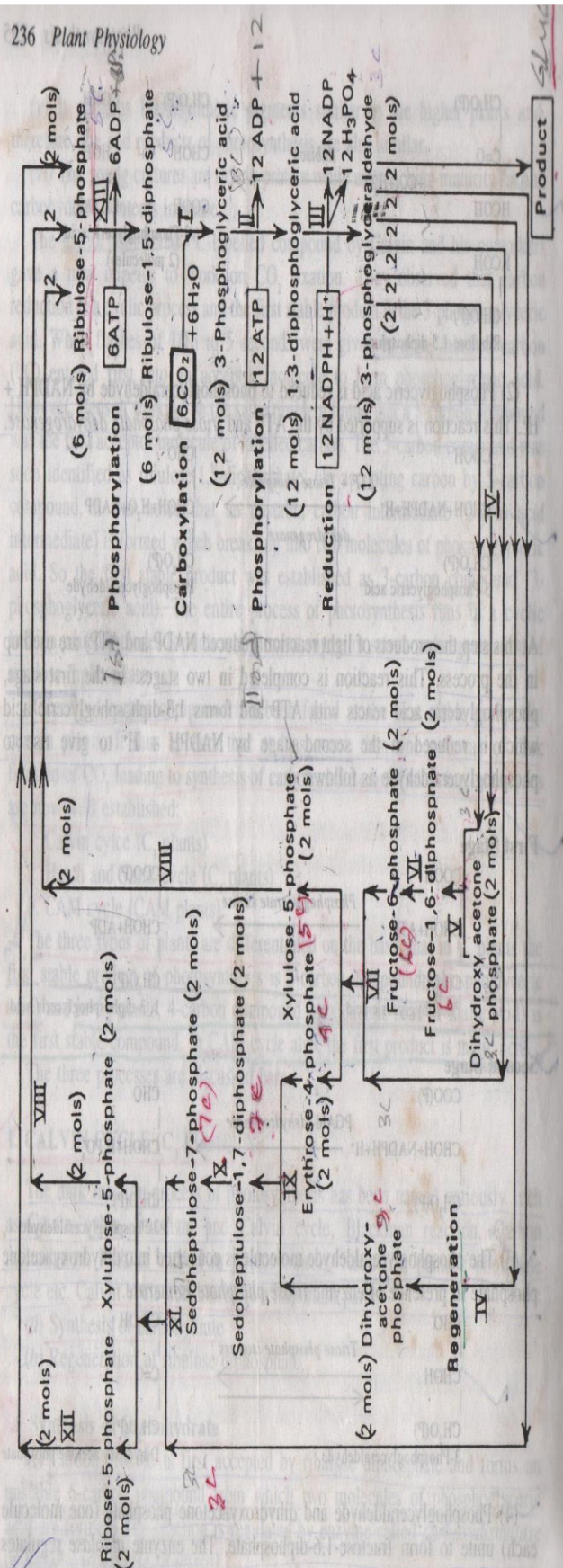
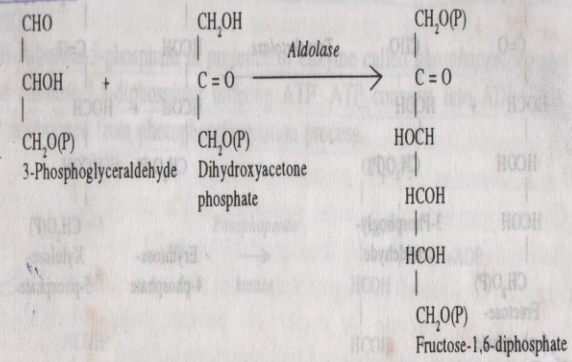


Fig. 12.5. Calvin cycle. I to XIII enzymes. I-Rubisco, II- 3-phosphoglycerate kinase, III-PGAld-dehydrogenase, IV-Triose-phosphate isomerase, V-Aldolase, VI-Phosphatase, VII-Transketolase, VIII-Epimerase, IX-Aldolase, X-Phosphatase, XI-Transketolase, XII-Isomerase and XIII-Phosphopentokinase.

this reaction.



(5) From fructose-1,6-diphosphates different types of compounds are synthesized and may be converted into glucose or starch.

(*b*) Regeneration of Ribulose diphosphate

This is true that carbohydrates are formed in a way described earlier, but the process utilises one ribulose diphosphate molecule. Therefore, its regeneration is essential in order to say that carbohydrates are synthesized by utilising CO_2 and H_2O. In this cycle only one molecule of CO_2 is utilised at a time, thus cycle will run for 6 times so as to synthesize carbohydrate directly using CO_2 and H_2O. A schematic balance sheet is described in Fig. 12.6 and the cycle in Fig. 12.5.

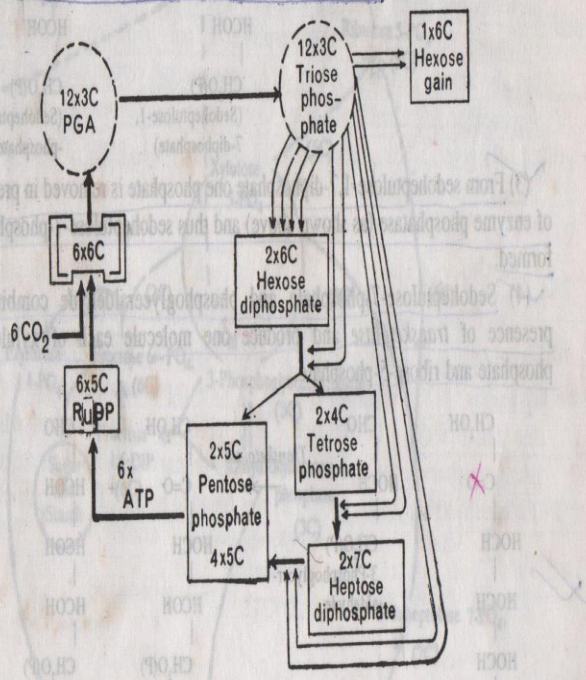
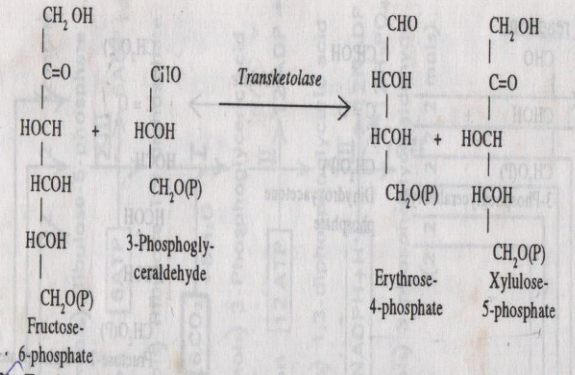


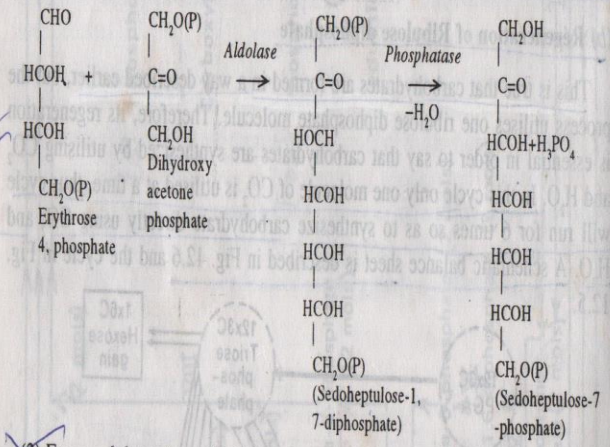
Fig. 12.6. Schematic balance sheet of photosynthesis process.

Therefore, the generation of ribulose diphosphate is essential to carry on the process of photosynthesis which takes place as follows:

(1) The so formed fructose-6-phosphate and phosphoglyceraldehyde combine and break into 4-carbon compound (erythrose-4-phosphate) and 5-carbon compound (xylulose-5-phosphate) in presence of enzyme *transketolase*.

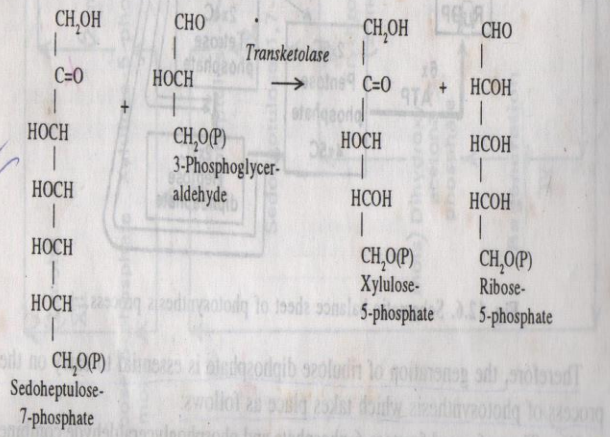


(2) Erythrose-4-phosphate combines with a molecule of dihydroxyacetone phosphate to form sedoheptulose-1,7-diphosphate in presence of enzyme *aldolase*.



(3) From sedoheptulose-1,7-diphosphate one phosphate is removed in presence of enzyme phosphatase (as shown above) and thus sedoheptulose-7-phosphate is formed.

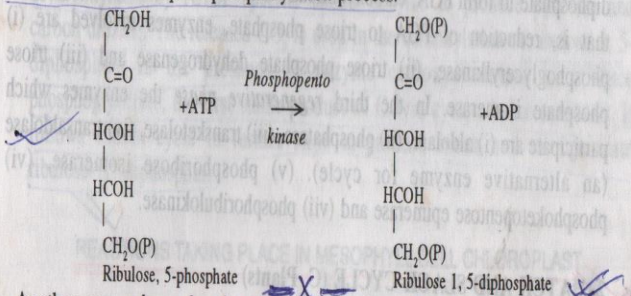
(4) Sedoheptulose-7-phosphate and phosphoglyceraldehyde combine in presence of *transketolase* and produce one molecule each of xylulose-5, phosphate and ribose-5-phosphate.



(5) Both these compounds convert into ribulose-5-phosphate in presence of enzyme *phosphopentose isomerase*.

Thus ribulose-5-phosphate is formed from three different ways (described earlier).

(6) Ribulose-5-phosphate in presence of enzyme called *phosphopentokinase* forms ribulose-1,5-diphosphate utilising ATP. ATP converts into ADP. This ATP is obtained from photophosphorylation process.



Another easy scheme has been proposed in Fig. 12.7. It is little modified from Calvin's cycle and is a sort of summary of the process.

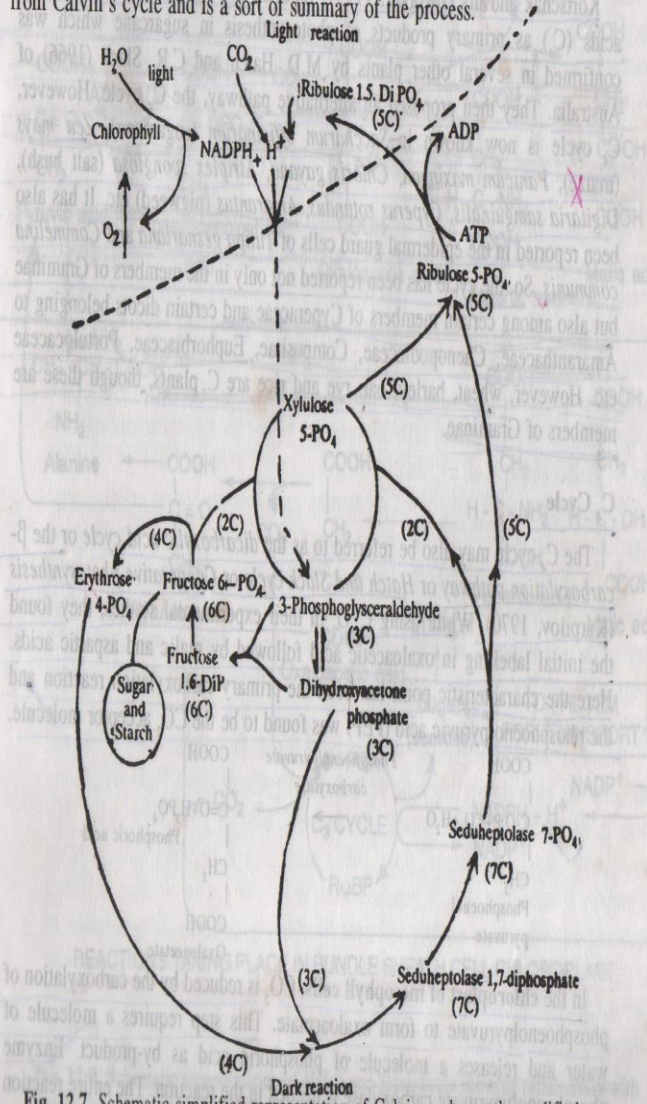


Fig. 12.7. Schematic simplified representation of Calvin cycle, partly modified.

Enzymes of CO_2 Reduction Cycle

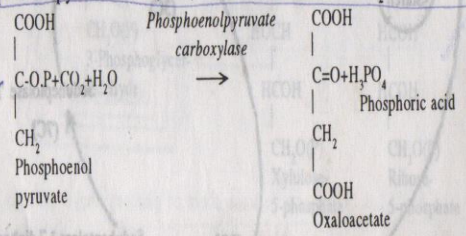
A look at the Calvin cycle indicates that it can be conveniently divided into three phases–the *carboxylative phase*, the *reductive phase* and the *regenerative phase*. In the first *carboxylative phase* that is carboxylation of ribulose-1,5-diphosphate to form PGA, carboxydimutase is involved. In the *reductive phase*, that is, reduction of PGA to triose phosphate, enzymes involved are (i) phosphoglycerylkinase, (ii) triose phosphate dehydrogenase and (iii) triose phosphate isomerase. In the third *regenerative phase* the enzymes which participate are (i) aldolase, (ii) phosphatase, (iii) transketolase, (iv) transaldolase (an alternative enzyme for cycle). (v) phosphoribose isomerase, (vi) phosphoketopentose epimerase and (vii) phosphoribulokinase.

2. HATCH AND SLACK CYCLE (C_4 Plants)

Kortschak and his coworkers (1954) reported the formation of dicarboxylic acids (C_4) as primary products of photosynthesis in sugarcane which was confirmed in several other plants by M.D. Hatch and C.R. Slack (1966) of Australia. They then proposed an alternative pathway, the C_4 cycle. However, C_4 cycle is now known in–*Saccharum officinarum* (sugarcane), *Zea mays* (maize), *Panicum maximum, Chloris gayana, Atriplex spongiosa* (salt bush), *Digitaria samguinalis, Cyperus rotundus, Amarantus* (pigweed) etc. It has also been reported in the epidermal guard cells of *Tulipa gesnariana* and *Commelina communis*. So, the cycle has been reported not only in the members of Graminae but also among certain members of Cyperaceae and certain dicots belonging to Amaranthaceae, Chenopodiaceae, Compositae, Euphorbiaceae, Portulocaceae etc. However, wheat, barley, oat, rye and rice are C_3 plants, though these are members of Graminae.

C_4 Cycle

The C_4 cycle may also be referred to as the *dicarboxylic acid cycle* or the β-*carboxylation pathway* or *Hatch and Slack cycle* or *Cooperative photosynthesis* (Karpilov, 1970). While using $C^{14}O_2$ in their experimental studies, they found the initial labelling in oxaloacetic acid followed by malic and aspartic acids. Here the characteristic point has been the primary carboxylation reaction and the phosphoenolpyruvic acid (PEP) was found to be the CO_2 acceptor molecule.



In the **chloroplast of mesophyll cells**, CO_2 is reduced by the carboxylation of phosphoenolpyruvate to form oxaloacetate. This step requires a molecule of water and releases a molecule of phosphoric acid as by-product. Enzyme phosphoenolypyruvate carboxylase is needed in the reaction. The entire reaction

may be expressed as in Fig. 12.8. The oxaloacetate is readily converted into malate or aspartate depending upon species. Malate is derived from oxaloacetate by reduction with NADPH +H^+ in the presence of malic dehydrogenase. Malate is then transported to the **chloroplasts of bundle sheath cells** where it is decarboxylated by NADP specific malate enzyme to produce pyruvate and carbon dioxide. The released CO_2 is used in the carboxylation of ribulose-1, 5-diphosphate in the presence of enzyme carboxydismutase to produce phosphoglycerate, the first stable product of Calvin cycle of photosynthesis. It follows Calvin cycle in further steps to produce starch and to regenerate ribulose-1,5-diphosphate.

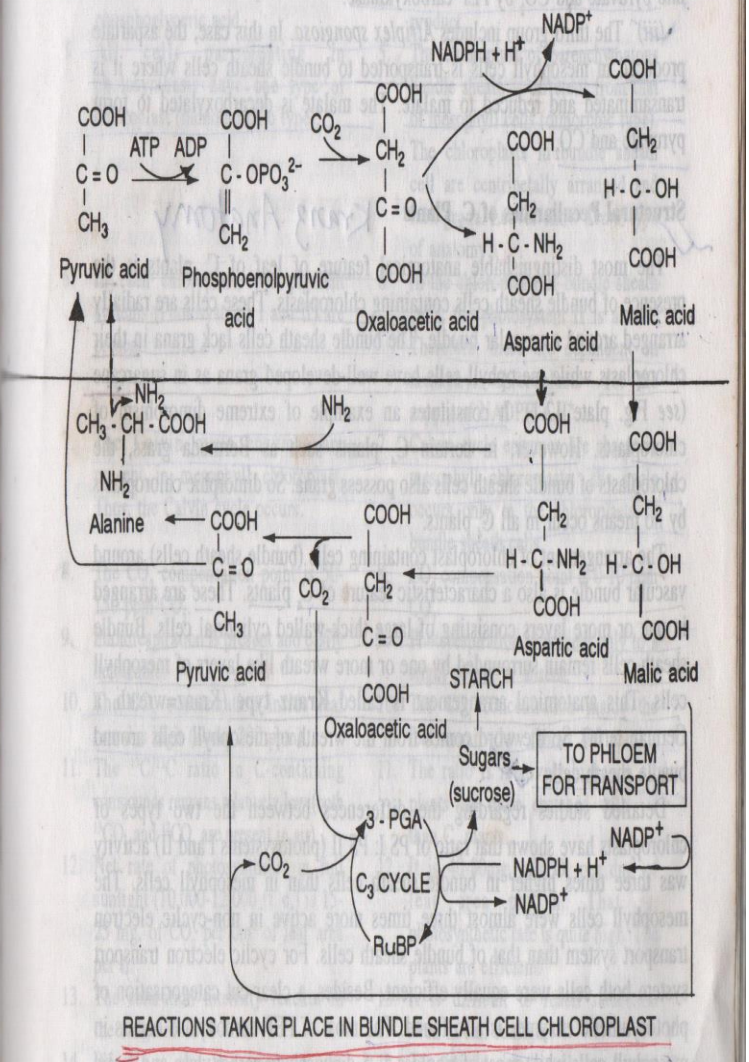


Fig. 12.8. Schematic representation of Hatch and Slack cycle showing relationship with Calvin cycle (modified from Hatch and Slack cycle, 1974).

Simultaneously, the pyruvate is transported back to the chloroplast of

mesophyll cells where it is reconverted into the phosphoenolpyruvate by utilising energy of ATP of light phase in the presence of enzyme pyruvate phosphate dikinase. ATP is converted to AMP in the process. Since, the conversion results in the form of AMP, the requirement to regenerate ATP from AMP is 2 ATP. This is how 12 additional ATP are needed in the C_4 pathway.

Chollet and Ogren (1975) recognised three categories of C_4 plants.

(i) The first group includes maize and sugarcane where CO_2 is initially fixed by phosphoenolpyruvate and oxaloacetate is formed. The malate produced from it is transported to bundle sheath cells.

(ii) Second group includes plants such as *Panicum maximum* and *Chloris gayana*, in which case it is aspartate, rather than malate, transported to bundle sheath cells. There it is transaminated to oxaloacetate which becomes converted into pyruvate and CO_2 by PEP carboxykinase.

(iii) The third group includes *Atriplex spongiosa*. In this case, the aspartate produced in mesophyll cells is transported to bundle sheath cells where it is transaminated and reduced to malate. The malate is decarboxylated to form pyruvate and CO_2.

Structural Peculiarities of C_4 Plants

The most distinguishable anatomical feature of leaf of C_4 plants is the presence of bundle sheath cells containing chloroplasts. These cells are radially arranged around a vascular bundle. The bundle sheath cells lack grana in their chloroplasts while mesophyll cells have well-developed grana as in sugarcane (*see* Fig. plate 12.1). It constitutes an example of extreme dimorphism of chloroplasts. However, in certain C_4 plants such as Bermuda grass, the chloroplasts of bundle sheath cells also possess grana. So dimorphic chloroplasts by no means occur in all C_4 plants.

The arrangement of chloroplast containing cells (bundle sheath cells) around vascular bundle is also a characteristic feature of C_4 plants. These are arranged in one or more layers consisting of large thick-walled cylindrial cells. Bundle sheath cells remain surrounded by one or more wreath like layers of mesophyll cells. This anatomical arrangement is called **Kranz type** (Kranz=wreath, a German term). So the word comes from the wreath of mesophyll cells around bundle sheath cells.

Detailed studies regarding the differences between the two types of chloroplasts have shown that ratio of PS I: PS II (photosystems I and II) activity was three times higher in bundle sheath cells than in mesophyll cells. The mesophyll cells were almost three times more active in non-cyclic electron transport system than that of bundle sheath cells. For cyclic electron transport system both cells were equally efficient. Besides, a clear-cut categorisation of photosynthetic enzymes is also found. The most of PEP carboxylase occurs in mesophyll cells while most of ribulose-1, 5-diphosphate carboxylase and malic enzymes in bundle sheath cells.

A comparative account of C_3 and C_4 plants is given in Table 12.3. However, it should always be kept in mind that such findings are still in the early stage and a large team of scientists is now engaged in resolving this intricacy.

Table 12.3 Differences between C_3 and C_4 Plants

C_3 Plants (Calvin cycle)	*C_4 Plants (Hatch and Slack cycle)*
1	*2*
1. Calvin cycle is found in all photosynthetic plants.	1. C_4 cycle is found only in certain tropical plants.
2. The efficiency of CO_2 absorption at low concentration is far less. So they are less efficient.	2. The efficiency of CO_2 absorption from low concentration is quite high. So they are more efficient plants.
3. The CO_2 acceptor is Ribulose-1, 5-diphosphate.	3. The CO_2 acceptor is phosphoenolpyruvate.
4. The first stable product is phosphoglyceric acid.	4. Oxaloacetate is the first stable product.
5. All cells participating in photosynthesis have one type of chloroplast (monomorphic type).	5. The chloroplast of parenchymatous bundle sheath is different from that of mesophyll cells (dimorphic type). The chloroplasts in bundle sheath cell are centripetally arranged and lack grana. Leaves show *Kranz type* of anatomy.
6. In each chloroplast, two pigment systems (Photosystems I and II) are present.	6. In the chloroplasts of bundle sheath cells, the photosystem II is absent. Therefore, these are dependent on mesophyll chloroplasts for the supply of NADPH + H^+.
7. The Calvin cycle enzymes are present in mesophyll chloroplast. Thus, the Calvin cycle occurs.	7. Calvin cycle enzymes are absent in mesophyll chloroplasts. The cycle occurs only in the chloroplasts of bundle sheath cells.
8. The CO_2 compensation point is 50-150 ppm CO_2.	8. CO_2 compensation point is 0-10 ppm CO_2.
9. Photorespiration is present and easily detectable.	9. Photorespiration is present only to a slight degree or absent.
10. The CO_2 concentration inside leaf remains high (about 200 ppm).	10. The CO_2 concentration inside the leaf remains low (about 100 ppm).
11. The $^{13}C/^{12}C$ ratio in C-containing compounds remains relatively low (both $^{13}CO_2$ and $^{12}CO_2$ are present in air).	11. The ratio is relatively high, i.e. C_4 plants are more enriched with ^{13}C than C_3 plants.
12. Net rate of photosynthesis in full sunlight (10,000-12,000 ft. c.) is 15-25 mg. of CO_2 per dm^2 of leaf area per h.	12. It is 40-80mg. of CO_2 per dm^2 of leaf area per h. That is, photosynthetic rate is quite high. The plants are efficient.
13. The saturation intensity reaches in the range of 1000-4000 ft. c.	13. It is difficult to reach saturation even in full sunlight.
14. Bundle sheath cells are unspecialised.	14. The bundle sheath cells are highly developed with unusual construction of organelles.
15. Ony C_3 cycle is found.	15. Both C_4 and C_3 cycles are found.