UNIT 13 PHOTOSYNTHESIS

Structure

13.1 INTRODUCTION

Our concern in this Unit will be with photosynthesis — the process by which plants utilise energy from sunlight to convert carbon dioxide and water for the synthesis of sugar. This sugar can then be converted to other carbohydrates or other food materials like fats and proteins. The general importance of the process was recognised as long ago as 2000 years. The biblical saint, Isaiah, who lived between 700-600 B.C., said *"All flesh is grass"* recognising that all food chains are finally traced to plants. Plants are also responsible for the fossil fuels such as petroleum, oil and coal, which represent products of photosynthesis carried out millions of years ago in the carboniferous era. It is through this process that plants continuously purify air during daytime and thus allow animals to breathe.

The overall importance of this process is best expressed in the words of Eugene Rabinowitch, one of the great authors and researchers of photosynthesis, who said *"Physiologically speaking, all the animals on land and in the sea, including man, are but a small brood of parasites living off the great body of the plant kingdom"*, and *"if plants could express themselves, they would probably have the same low opinion of animals as we have of fleas and tapeworms — organisms that must lazily depend on others for survival ."*

The photosynthetic products are utilised by humans and other animals to provide energy. He proceeded to state that *"without them no heart could beat, no amoeba could swim, no sensation could speed along a nerve, no thought could flash in the human brain"*. Clearly, for all these activities we are dependent on plants.

In sheer magnitude, too, the process dwarfs any other chemical activity on earth. It has been estimated that photosynthesis gives 200×10^9 tonnes of solid plant material per year which comes to about 70 to 80 tonnes of sugar equivalent per person! Clearly, photosynthesis represents the greatest chemical factory on earth. Unravelling the mechanism of the process has, therefore, been one of the most important tasks of plant biology.

The subject matter in this unit you may find quite unique and interesting. Here, we give in a story form an historical account of major experiments that led to the detailed knowledge of photosynthesis such as we have today. We have particularly emphasised how the various key concepts in photosynthesis were formulated.

The various sections and subsections are arranged in a chronological order. Beginning with experiments that led to the formulation of basic equation, we tell you about the light and dark reactions of photosynthesis, that is, photolysis of water and synthesis of ATP by photophosphorylation, the role of pigments in these processes and then to fixation of carbon dioxide by the C_3 and C_4 pathways. The intricate details of photosynthetic machinery which have been unravelled partly in the last two decades are discussed later.

A section on photorespiration, and the relevance of photosynthesis to agriculture and human welfare is also included. Finally, we briefly discuss the evolutionary aspects of the origin of chloroplast.

Objectives

After studying this unit you should be able to :

- outline the scientific developments that led to recognition of the necessary raw materials of photosynthesis and the important end products,
- list the main photosynthetic pigments and describe their functions,
- list the evidences that led first to the discovery of light and dark reactions and later to the two photochemical reactions,
- outline the path of electrons in electron transport chain from water to the final electron acceptor,
- outline the C_3 or the Calvin cycle and illustrate its connection with the energy capturing reactions in the thylakoid membrane,
- draw and label the structure of a chloroplast and its membranes and show the sites where PS I and II and the components of electron transport chain are located and various processes such as photolysis of water, photoreduction of $NADP^+$, photophosphorylation, and carbon dioxide fixation go on,
- outline the reactions that result in the loss of carbon dioxide during photorespiration,
- compare the fixation of CO_2 in C_3, C_4 and CAM plants and explain why C_4 plants are photosynthetically more efficient than C_3 plants,
- gain an idea of the future prospects of increasing photosynthetic efficiency through biotechnology,
- give reasons for considering the present day chloroplast as one time free-living prokaryote which became an endosymbiont during the course of evolution.

Study Guide

Since this is a double unit, you will find it very lengthy. It is important that you spend more time in learning it.

13.2 FORMULATION OF BASIC CONCEPTS

13.2.1 The Beginnings

We can trace the beginnings of research on photosynthesis to about 300 years ago. The idea that water is an important reactant came from experiments of a Dutch alchemist, Van Helmont, performed in 1648 but published posthumously in 1740 under the title *"By Experiments, that All Vegetable Matter is Totally and Materially of Water Alone"*. He grew a 5 lb sapling of the willow tree (*Salix*) in an earthenware pot containing soil which he carefully dried and weighed — it was 200 lbs. He watered the sapling regularly with distilled water, if rain failed, and at the end of five years decided to take stock of the experiment. He found that the weight of the tree increased to about 169 lbs, but the weight of the soil was nearly the same, decreasing by only 2 ounces (Fig. 13.1). He concluded, therefore, 164 lbs of wood, bark and roots were formed from water **alone**. Of course, we know today that Van Helmont was only partially right and was totally unaware of the contribution of carbon dioxide.

Fig. 13.1 : Van Helmont's experiment in which it was concluded that a plant grows from water alone (see text for further details).

The knowledge that gases also participate in the process of photosynthesis came from studies of an English clergyman Joseph Priestley, who was intensely interested in the process by which bad air could be purified. In a contribution entitled *"Observations on Different Kinds of Air"* he wrote that *"I have been so happy as by accident to hit upon a method of restoring air which has been injured by the burning of candles, and that to have discovered at least one of the restoratives which nature employs for this purpose. It is vegetation"*. He had found that animals such as mice, vitiated the common air and a candle no longer burnt in it (Fig. 13.2). He conducted a series of experiments in 1771 and showed that plants had the remarkable ability of turning impure air into pure air. In his own words. *"Accordingly, on the 17th of August, 1771, I put a sprig of mint into a quantity of air, in which a wax candle had burned out, and found that, on the 27th of the same month, another candle burned perfectly well in it"*. He added *"I generally found that five or six days were sufficient to restore this air, when the plant was in its vigour"*. At that time, chemists were obsessed with the idea of phlogiston, then considered a principle of flammability. According to Priestley, plants dephlogisticated the foul air. Further, the pure air had properties similar to the gas which he had discovered and was released by focusing sunrays on the red oxide of mercury with the help of a huge lens — he had procured one almost a foot in diameter.

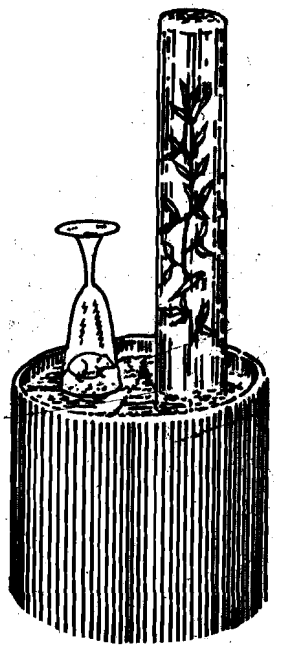


Fig. 13.2 : Priestley grew small twigs of mint in an inverted tube and piped air to a jar containing mouse. By such experiments, he proved that plants have the capacity of purifying air.

Priestley's experiments excited the interest of Jan Ingen-Housz in Vienna who was a court physician to Empress Maria Theresa of Austria. In 1778, on a visit to England for a three-month vacation he rented a villa and conducted some 500 experiments. He confirmed that not only mint but even other plants purified air; but, more importantly, he found that the process will proceed only in the presence of sunlight and plants could purify air significantly even in a few hours. To quote from a book *"Experiments on Vegetables Discovering Their Great Power of Purifying the Common Air in Sunshine, and of Injuring at Night"* he said *"I was not long engaged in this enquiry before I saw a most important scene opened to my view: I observed, that plants not only have a faculty to correct bad air in six or ten days, by growing in it, as the experiments of Dr. Priestley indicate, but that they perform this important office in a compleat (sic) manner in a few hours; that this wonderful operation is by no means owing to the vegetation of the plant, but to the influence of the light of the sun upon the plant"*. He also found that only the green parts of the plant purified air and not the non-green parts and that so long as the plants were green, the *"acrid, ill-scented, and even the most poisonous plants perform this office in common with the mildness and the most salutary"*.

Phlogiston : The alchemists thought that when metals rust or a candle burns something was lost. This something they called phlogiston. Today, we know that actually nothing is lost and, in fact, a burning substance unites with oxygen. Similarly, rusted metal combines with oxygen and actually weighs more than the pure metals.

Neither Priestley nor Ingen-Housz knew the true chemical nature either of impure or of pure air and it was the brilliant French chemist Antoine Lavoisier who discovered the principle of combustion and identified the "pure" component of air as oxygen (O_2) and the "impure" air as carbon dioxide (CO_2). (Unfortunately, Lavoisier was guillotined by terrorists during the French revolution but that is the way fate overcomes occasionally the best of men). Another important advance was made by a Genevan, Jean Senebier, who found that the quantity of pure air (O_2) generated depended on the presence of noxious or vitiated air (CO_2) at the start of an experiment.

SAQ 1

a) Match the experimental findings related to photosynthesis (given in column 1) with the names of scientists (given in column 2) who were responsible for the findings.

Column 1		Column 2	
i)	A sprig of mint can purify air injured by breathing of animals	a)	Antoine Lavoisier
ii)	Plants are made of water alone	b)	Jan Ingen-Housz
iii)	All kinds of plant purify bad air, but light is necessary for such purification	c)	Van Helmont
iv)	Identified pure air as O_2 and impure air CO_2	d)	Joseph Priestley

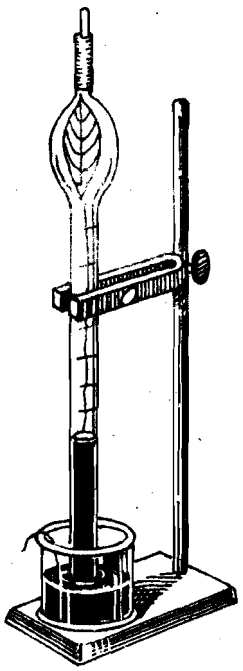


Fig. 13.3 : An apparatus for determining gas exchange during the process of photo synthesis. This type of set-up was employed by Nicolas Theodore de Saussure. A leaf or other plant parts can be put in the area of the bulb with a suitable support. The com position of the air can be determined by use of an alkali water and pyrogallol at the end of the experiment.

13.2.2 Formulation of the Equation of Photosynthesis

By 1804, methods of quantitative measurements of gases were well established. By the use of an eudiometer (Fig. 13.3) followed by simple methods of gas analysis Nicolas Theodore de Saussure, also a Genevan, confirmed the equivalence of release of O_2 to consumption of CO_2 in the process of photosynthesis. But much more important, he once again drew attention to the role of water, an ingredient whose participation in photosynthesis had been totally ignored after Van Helmont. In 1837 chloroplasts were also described. By the end of the last century, the stage was thus set for formulating the following equation of photosynthesis:

$$6CO_2 + 6H_2O \xrightarrow[\text{green colouring matter (chlorophyll pigments)}]{\text{sunlight}} C_6H_{12}O_6 + 6O_2$$

Another very important development which took place at the end of the last century was the determination of the action spectrum of photosynthesis. Earlier, the use of spectroscopy easily established that chlorophyll absorbed strongly in the blue and red regions of the spectrum (it had hardly any absorption in the green region, explaining why plants appear green). However, there was one uncertainty, although researchers of that time associated, "the green colouring matter" with the process of photosynthesis, there was no conclusive evidence that chloroplasts were the site of photosynthesis and the pigments in them participated in this important reaction. The German botanist Theodore Engelmann (1882) determined the action spectrum of photosynthesis and showed that it indeed closely matched the absorption spectrum of the chlorophyll pigments.

Absorption spectrum : A graph depicting absorption as a function of wavelength is called absorption spectrum. The absorption spectra of chl *a* and chl *b* indicate that very little of green and yellow green light between 500 to 600 nm are absorbed but violet, blue, orange and red wavelengths are absorbed strongly.

By way of explanation, it can be said that the **absorption spectrum** is an optical property of a solution. With the help of a spectroscope one learns about the wavelengths absorbed by a plant extract or a solution such as of chloroplast pigments in acetone. Now with a modern spectrophotometer one can even know the degree to which they are absorbed. An **action spectrum**, on the other hand, tells us about the **relative activity** of a physiological process in different parts of the spectrum (to obtain an action spectrum one must illuminate the living cell, tissue or the organism with monochromatic light in different regions of the spectrum). Obviously, to associate a photoreceptor convincingly — to a certain process or action, the two spectra must match (Fig. 13.4).

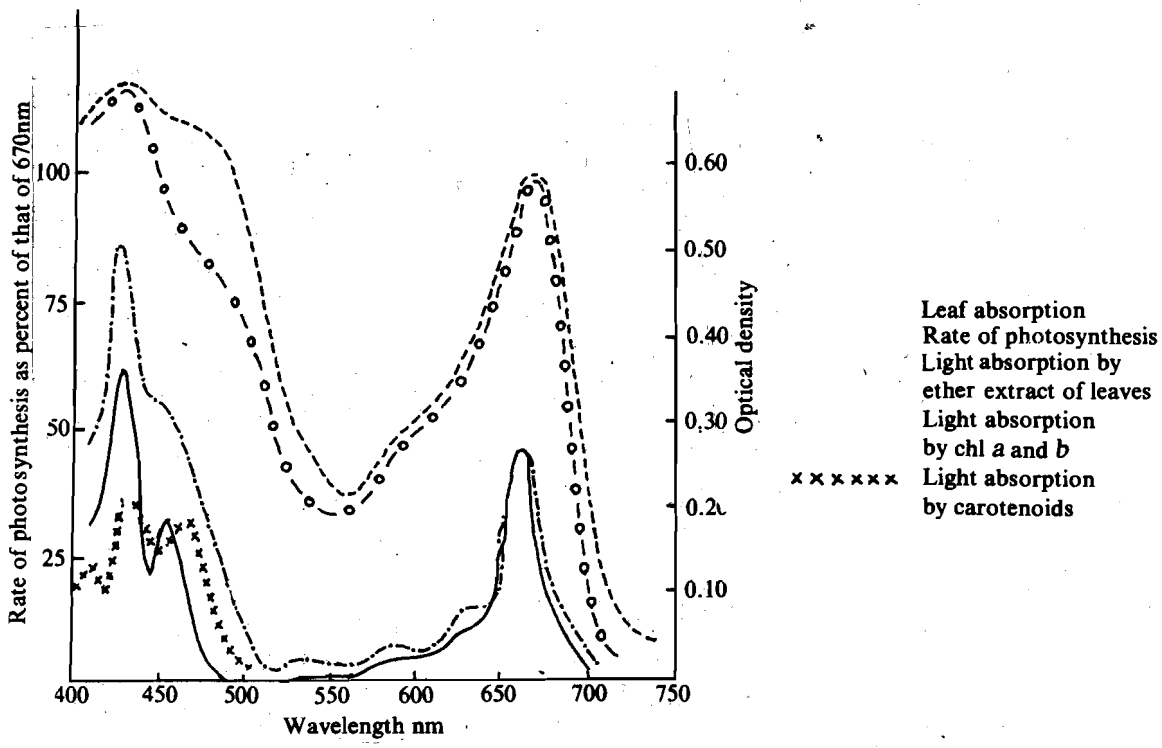


Fig. 13.4 : Comparison of action spectrum for the rate of photosynthesis by *Elodea densa* leaf with absorption spectra of the intact leaf and with extracted pigments. (Adapted from G. Ray Noggle and George V. Fritz, Plant physiology, 1989, Prentice-Hall of India)

Engelmann's experiments — which are among the most ingenious ever devised — were carried out with filamentous algae such as *Cladophora*. The algal filament was laid on a slide on the stage of a microscope. In the optical path of the microscope a prism was inserted such that a small spectrum illuminated the algal filament and the spectrum could be seen readily through the eyepiece by the viewer. To determine which wavelengths of light were effective in evolving oxygen, Engelmann made use of a species of highly aerobic motile bacteria. A drop of the bacterial suspension was introduced over the algal filament, whereupon the bacteria clustered around those regions of the algal filament which received the red and blue light (see Fig. 13.5).

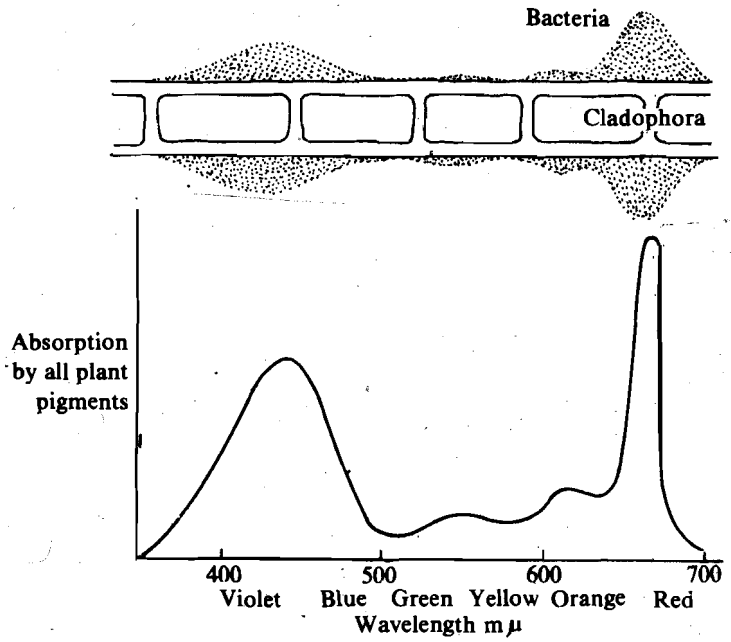


Fig. 13.5 : Engelmann's experiment to determine the wavelengths of light that are most effective for photosynthesis (see text for further details). On the lower side is given an absorption spectrum of all plant pigments extracted in a solvent. (Adapted from John W. Kimball, Biology, Addison-Wesley, 1965)

Thus, the density of distribution of bacteria provided an idea of the relative effectiveness of various spectral regions of visible light, depicting the action spectrum.

To summarise, by the end of the 19th century it was clear that water and carbon dioxide were converted to sugar and oxygen with the help of chlorophyll pigments and light. Meanwhile, in 1842, Julius Mayer, a surgeon in Germany, also formulated the "Law of Conservation of Energy" and expounded the theme that photosynthesis, in the main, represented a process in which physical energy was conserved as chemical energy. He wrote in 1845 *"Nature has put itself the problem how to catch in flight light streaming to the earth and to store the most elusive of all powers in rigid form. To achieve this aim, it has covered the crust of earth with organisms which in their life processes absorb the light of the sun and use this power to produce a continuously accumulating chemical difference .... . These organisms are the plants; the plant kingdom forms a reservoir in which the fleeting sun rays are fixed and skillfully stored for future use; an economic provision to which the physical existence of mankind is inexorably bound. The plants take in one form of power, light; and produce another power : chemical difference".*

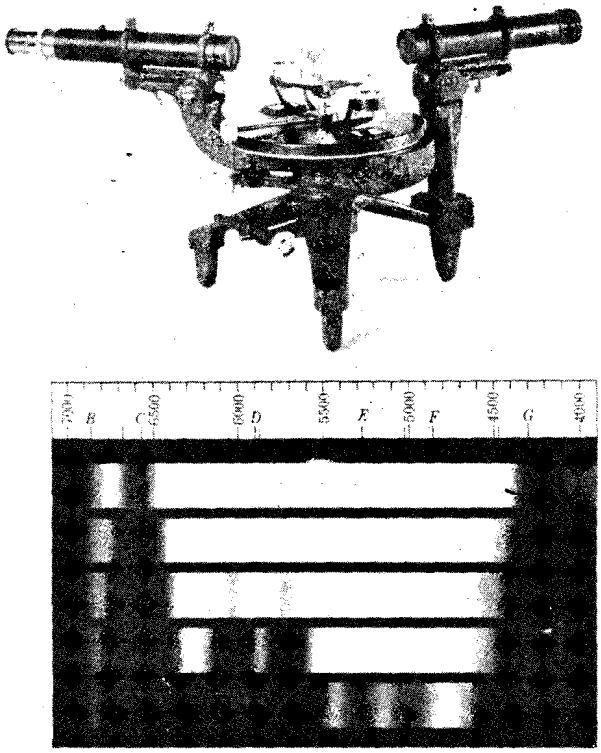


Fig : a): A spectroscope. b): Absorption spectra of five different concentrations of chlorophyll a The wavelengths in $m\mu$ are indicated above.

Spectroscope

The figure above (a) illustrates a spectroscope. Light is passed through a slit at one end and a spectrum emerges at the other end. If a solution containing photosynthetic pigments is interposed between the viewer and the light, dark bands are seen — in the blue and red regions of the spectrum in case of chlorophyll pigments and the blue and violet regions in case of carotene and xanthophyll. If the concentration of chlorophyll pigment is high some absorption can also be seen in the other regions. If photographs are taken of the absorption spectra at various dilutions and placed one below the other, one can determine absorption peaks more exactly. Absorption spectra of this type were obtained by the German chemist, Willstätter as seen in Fig. b. They are similar to the absorption spectra which are obtained by a modern spectrophotometer (which essentially represents a spectroscope fitted with accessories such that the emerging light can be measured electrically by having it fall on a photocell or a photomultiplier). For obtaining an absorption spectrum, the prism has to be rotated to allow scanning of absorption values from the blue end of the spectrum to the red end. The slow rotation can be accomplished by a motor.

Measurement of Photosynthesis

The rate of photosynthesis can be measured in many different ways and, with time, more and more sophisticated techniques have been developed so that results can be had almost instantly or at the most within a few seconds.

Fig. a represents the simplest method of measuring photosynthesis which can be employed for aquatic plants. Oxygen bubbles, escaping from cut stems or

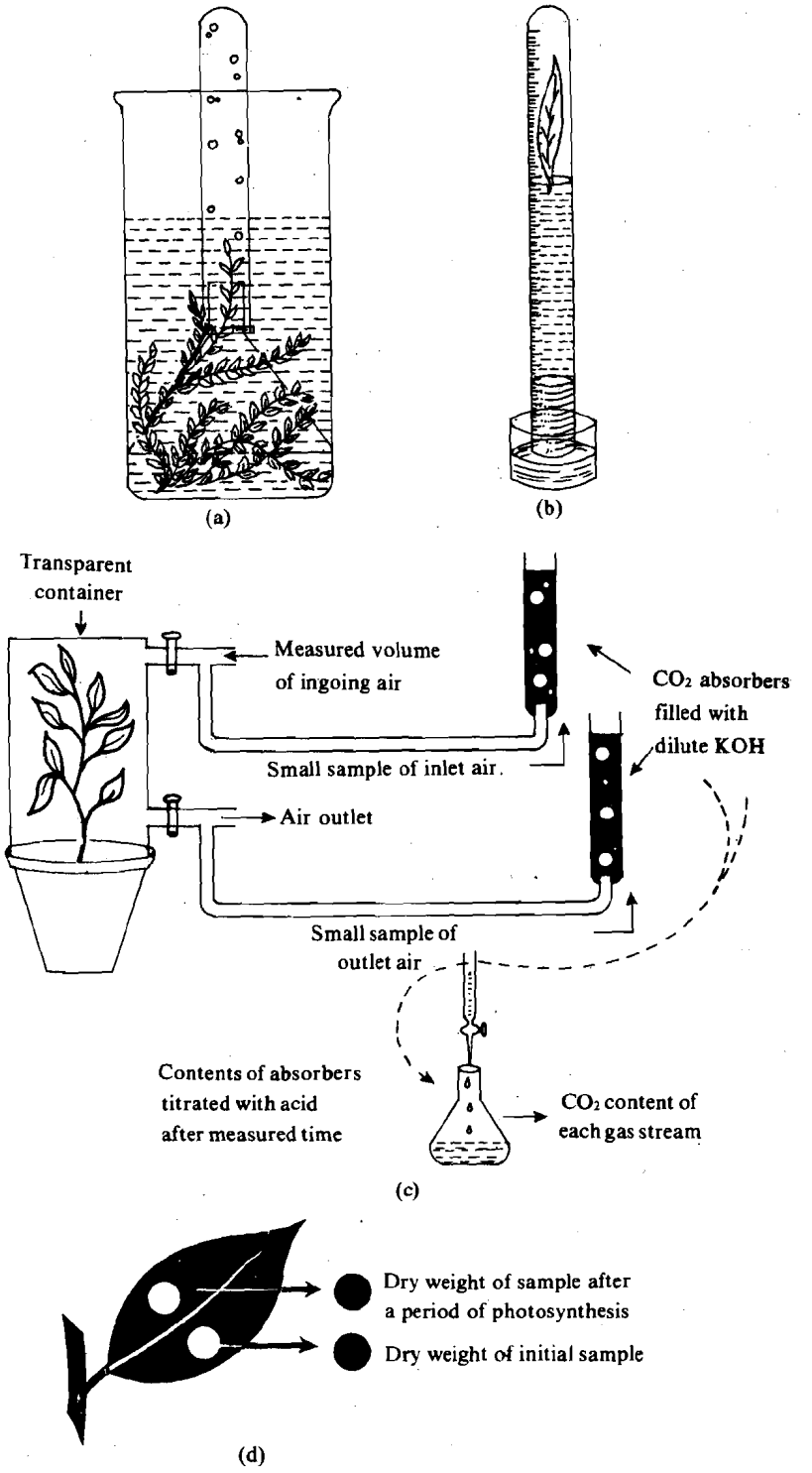
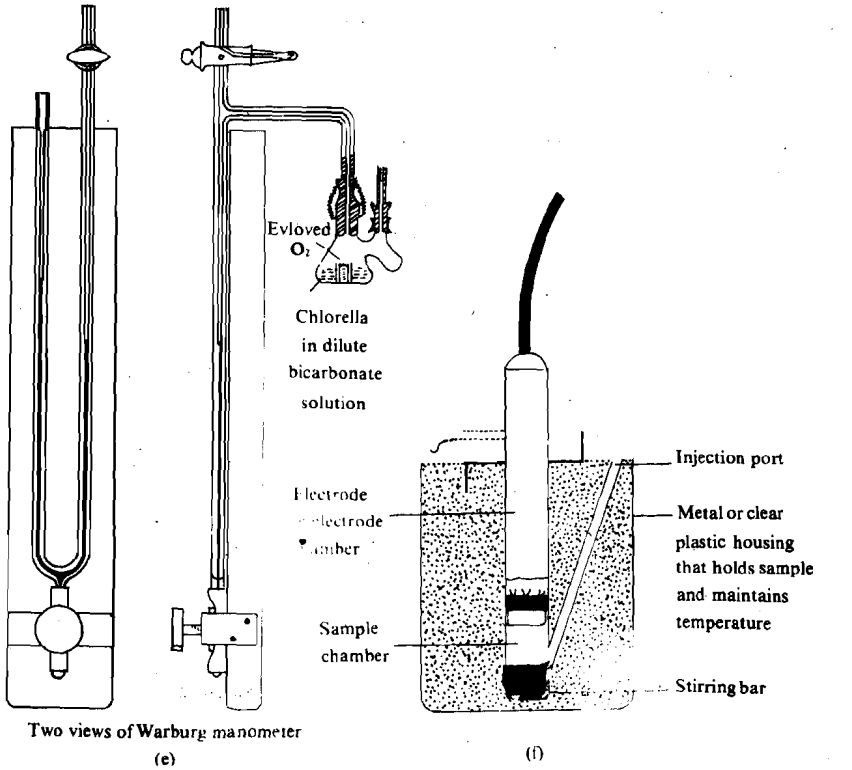


Fig. a): Collection of oxygen from water plants in light.
b): Leaf in position in a measuring tube, for demonstration of absorption of carbon dioxide and elimination of oxygen during photosynthesis.
c): Determinations of photosynthetic rate, based on measurement of CO_2 uptake from a moving gas stream, depend on analyses of the CO_2 content of small samples of the inlet and outlet air.
d): The net photosynthetic rate may be roughly determined from the dry weight increase of a measured area of leaf surface during a period of CO_2 assimilation.

other parts of a plant can be collected in an inverted test tube. For more precision, the composition of the gas can be checked by chemical methods of analysis such as through use of pyrogallol or KOH. Fig. b represents another technique adopted by early workers for determining the rate of photosynthesis in leaves or other similar organs of land plants which can be enclosed in a graduated tube. The tube is inverted over mercury and gas exchange after a period of time determined by chemical methods of analysis. Fig. c depicts the gas train method. This method came into wide use in the early part of the century (this was employed by Blackman for determining the Q_{10} of the photosynthesis reaction). The air is led into a chamber containing the plant material and the amount of carbon dioxide assimilated from the air can be measured by titrating the carbon dioxide absorbing solution i.e. the alkali (e.g. barium hydroxide) with a standard solution of acid after a measured time. A blank run without plant material gives the quantity of carbon dioxide in air sample; but when there is an experimental run, less $BaCO_3$ is precipitated and the difference in titration values in the control and experimental runs gives us a measure of photosynthesis.

Another method popular in the early days was to take samples of leaf material as shown in Fig. d and determine their dry weights — at start and after a period of photosynthesis. The difference in weights gives us a measure of photosynthesis. It is important to realise that all these methods suffer from the drawback that what one measures is net photosynthesis, since respiration also takes place simultaneously and losses of carbon fixed do occur. In order to estimate the real rate of photosynthesis, the rate of respiration should also be measured in darkness and appropriate corrections made.



e): The Warburg manometer and reaction vessel. f) Features of a typical oxygen electrode system.

In the manometric technique, developed by Warburg in 1920s and which remained extremely popular till the sixties, the volume of oxygen evolved is measured directly by reading against a graduated scale attached to the manometer (Fig. e). The technique is specially suited for algae or other small samples of green tissue which are suspended in a dilute solution of sodium bicarbonate (which gives HCO_3^- or CO_2). Lowering the level of the manometric fluid in the closed arm is due to oxygen evolution and consequent rise in pressure. A high degree of sensitivity in estimation of oxygen output can be obtained by employing a thin-diameter glass tubing for fabrication of the manometer. The reaction flask is always kept submerged in constant temperature water bath to maintain constant conditions and up to 14

manometers can be used simultaneously in a single apparatus. However, one of the manometers is left without any plant material so that it can serve as a barometer and appropriate corrections can be made for the estimation of the true volume of gas exchange.

The most recent method, however which has come into wide use is the oxygen electrode technique (Fig. f). The oxygen electrode is sensitive to concentration of oxygen and the output of oxygen can be measured electrically.

SAQ 2

a) Describe the experiment which led to the discovery of:

i) the role of chlorophyll in photosynthesis.

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ii) the wavelengths of visible spectrum that are effective in photosynthesis.

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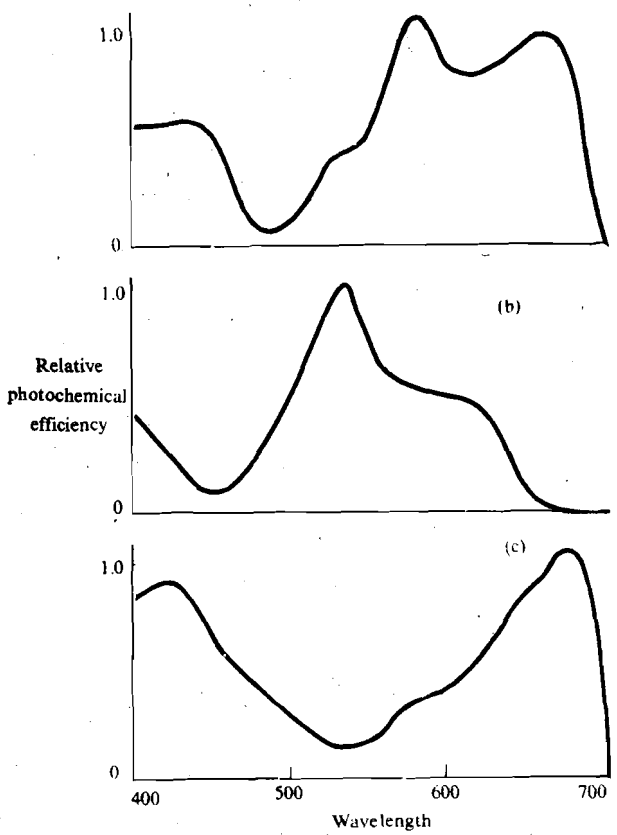
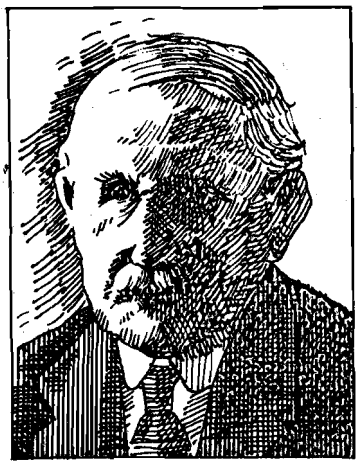
b) The following three action spectra relate to photosynthesis in blue green algae, purple sulphur bacteria and barley leaves. Identify the spectrum that most likely corresponds to a particular organism.





F.F. Blackman

13.3 UNDERSTANDING THE MECHANISM OF PHOTOSYNTHESIS

13.3.1 Evidence for the Existence of Light and Dark Reactions

As discussed above, the process of photosynthesis was known in its bare outline already at the beginning of this century. But the phenomenon was still much like a mysterious black box and scientists had little idea of the events that proceeded inside the box. Unfortunately, the box could also not be opened then since photosynthesis ceased immediately if one broke up the cell or tissue where photosynthesis was going on. For a long time there was no clue, till at the turn of the century, an English plant physiologist, F.F. Blackman, working in Cambridge, started his experiments. He was the first to give the idea that photosynthesis consists of at least two kinds of reactions — the light and dark reaction(s) (Fig. 13.6). He estimated Q_{10} of the photosynthetic reaction and found it about 2.5, provided that photosynthesis was studied under optimal conditions, specially of adequate light and supply of carbon dioxide. That

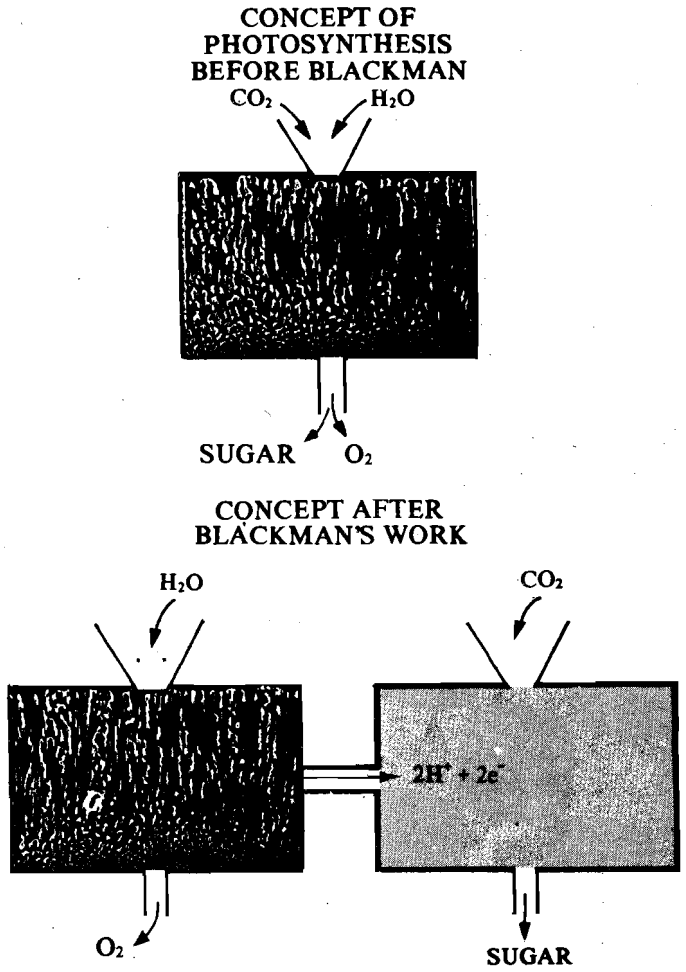


Fig. 13.6 : Diagram to show evolution of concepts concerning the mechanism of photosynthesis. The box above represents the concept at the end of the last century. After Blackman conducted the experiments to determine the Q_{10} of the photosynthetic reaction (see text for further details), it was concluded that the reactions in the "black magic box" consisted at least of two sub-sets of reactions, the light and the dark reactions. The actual roles, that in the light box there is photolysis of water and in the dark box there is reduction of carbon dioxide was established much later.

photosynthesis involved some photochemical reaction(s) was of course obvious because of the necessity of light and chlorophyll pigments. However, the derivation of Q_{10} value gave a unique insight into the complexity of the reactions. The Q_{10} of photochemical reaction is 1, whereas for chemical reactions it is generally 2-3. By a simple study of the effect of temperature and analysis of results indicated that photosynthesis consists also of chemical reaction(s) often called 'dark' reactions since they do not need light. To put in other words, one learnt immediately that instead

Q_{10}

For chemical and biochemical reactions it is commonly observed that increase of temperature by 10°C, increases the rate of thermal reaction by twofold. The minimum energy which the reactants must possess for a reaction to proceed is called activation energy. The increase in temperature increases molecular collisions and so the proportion of molecules possessing activation energy for reaction to occur increases. Hence, the reaction rate increases.

The ratio of the rates of reactions at t°C and (t + 10°C) is denoted by Q_{10}.

$$Q_{10} = \frac{\text{Reaction rate at } (t + 10)°C}{\text{Reaction rate at } t°C}$$

$$= 2$$

(Q denotes Quotient and 10 stands for 10°C rise in temperature.)

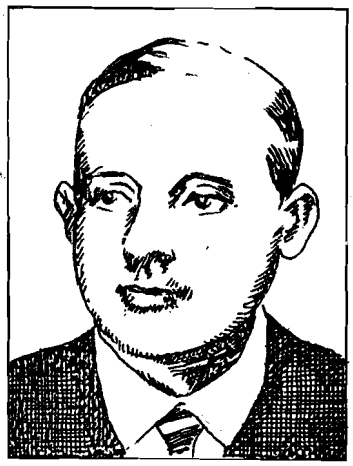
For physical processes, the Q_{10} is in the range of 1.2 to 1.4. Enzymatic and physiological processes have Q_{10}, 2 to 3.

The rate of photochemical reaction is not affected by temperature increase because the activation energy to substrate is supplied by photons of required energy and the energy for bond breaking process is also given by photons. Photochemical reactions have a Q_{10} of 1.

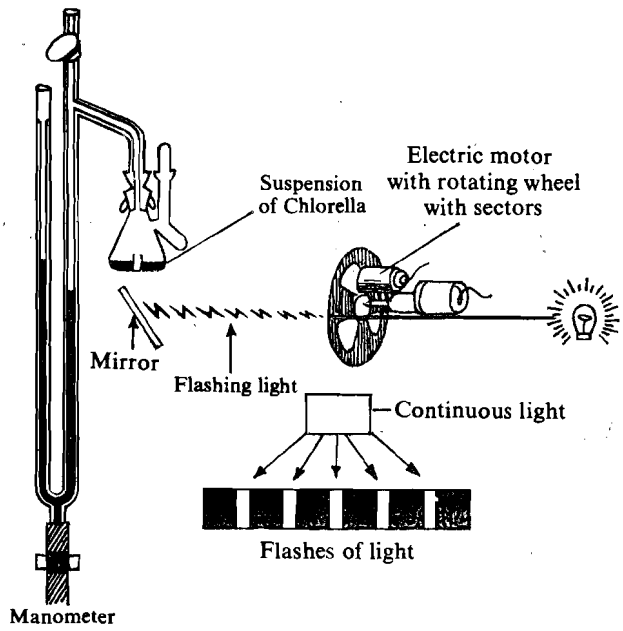
of the single magic box (alluded to earlier and representing the photosynthetic process), there were really two boxes (Fig. 13.6):

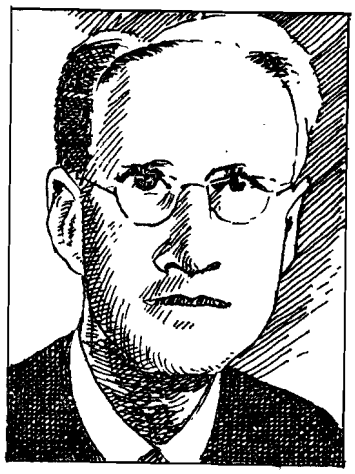
1) One box contained the chlorophyll pigments on which light shone and which represented the light reactions, and
2) the other was a "dark" box which represented the dark reactions.

The existence of dark and light reactions was further confirmed when the German Nobel laureate, Otto Warburg, and later his American pupils, Robert Emerson and William Arnold — all of them with a deep understanding of biochemistry, biophysics and cell biology — carried out experiments with alternating flashes of light and darkness given to the unicellular green alga, *Chlorella*. A mechanical device, consisting of an electric motor to which was attached a rotating disc with sectors cut in different sizes (see Fig. 13.7) was set in the light path. With this they could obtain intermittent light and darkness. In later experiments the mechanical flashing device was replaced by electronic flash discharges. It was shown that, if light and dark periods were separated by appropriate intervals, the oxygen yield per unit light shone increased by as much as 400% over the control where the same amount of light was given continuously. Thus, it was confirmed that photosynthesis consisted of *both* light



Otto Warburg





Robert Emerson

Fig. 13.7 : The experimental set-up employed for the flashing light experiments (see text for further details).

Cornelius B. Van Niel

and dark reactions. By experiments with different durations of light and darkness, it was found further that dark reactions were much slower than light reactions and that was the reason why light given continuously was utilised less efficiently.

13.3.2 The Role of Light Reaction

Photolysis of Water

The identities and roles of light and dark reactions were not clear until this time. In 1930s a dutch microbiologist, Cornelius B. Van Niel, began experiments on the photosynthesis of green sulphur bacteria. These contain bacteriochlorophyll (a slightly different form of chlorophyll) and survive on hydrogen sulphide (H_2S) but release elemental sulphur (in nature wherever these bacteria grow there are picturesque deposits of sulphur). He found that photosynthesis in these organisms followed the reaction as per the equation given below:

$$6CO_2 + 12H_2S \xrightarrow[\text{bacteriochlorophyll}]{\text{light}} C_6H_{12}O_6 + 6H_2O + 12S$$

The equation indicated that the primary role of H_2S was that of a reductant to supply electrons. By simple analogy, he reasoned that H_2O, too, in case of higher plants, must serve as reductant and the O_2 evolved in photosynthesis must come entirely from H_2O rather than from both H_2O and CO_2 or CO_2 alone, thus clearly contradicting the ideas that existed before. In fact, he predicted that the equation for photosynthesis of higher plants would be:

$$6CO_2 + 12H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

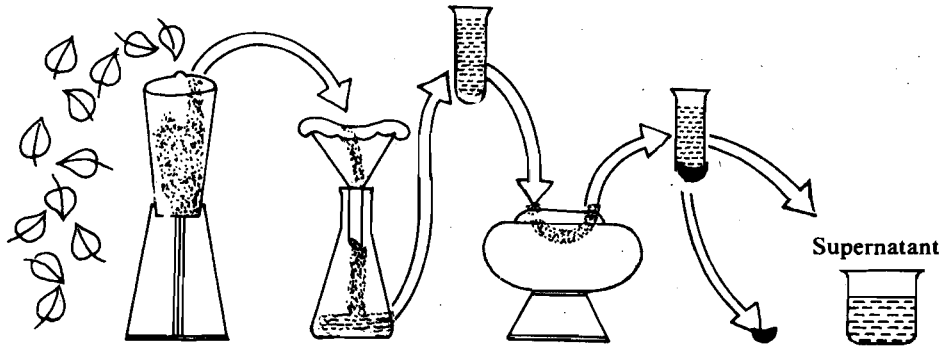
Equally important, he also proposed on simple, purely thermodynamic consideration that the role of the light reaction would be to split H_2O (a process for which he coined the term "photolysis") since this was the step that would require the maximum input of energy. The role of H_2O or H_2S, thus could be depicted by the following equation:

$$H_2S \longrightarrow 2H^+ + 2e^- + S$$
$$H_2O \longrightarrow 2H^+ + 2e^- + {}^1/_2O_2$$

The proof that O_2 is indeed evolved from H_2O came soon after from experiments of two American chemists, Samuel Ruben and Martin D. Kamen, who worked for the U.S. Atomic Energy Commission. These workers used water labelled with O^{18}, the heavy isotope of oxygen. Employing a mass spectrometer for analysis they showed that — if H_2O^{18} was used — the oxygen evolved was largely O^{18} rather than O^{16}, confirming thereby that the new equation for photosynthesis formulated by Van Niel was the correct one. Photosynthesis had now to be viewed as a chemical process in which the central reaction was a "redox" reaction (i.e. consisting of a reduction and another an oxidation event). It involves transfer of hydrogen or electrons from a suitable raw material such as H_2S or H_2O to CO_2. Overall, a reductant such as H_2O or H_2S was oxidised and an acceptor such as CO_2 was reduced.

Photoreduction — Production of Reducing Power — NADPH

The work with the heavy isotope of oxygen (O^{18}) was followed by another significant discovery. The English biochemist Robin Hill working in Cambridge, U.K., found that even isolated chloroplasts could evolve O_2 from H_2O, if they were provided with light and a suitable electron acceptor such as ferroxalate or ferricyanide (Fig. 13.8). Later workers have shown that many other electron donors such as benzoquinone and 2,6-dichlorophenol indophenol can also be used (see reaction below the Figure). The general reaction is now called Hill reaction in honour of its discoverer. Since O_2 evolved by isolated chloroplast was rather in small quantities, Hill adopted a clever strategy : he put some reduced haemoglobin in the chloroplast suspension which was readily oxidised by the O_2 released and which he monitored by a spectroscope. Clearly, by the end of the first half of this century, the basic role of at least one of the two "boxes", the box in which electrons are produced from H_2O for reducing CO_2, was established beyond doubt



$$2H_2O + 2\,O{=}\bigcirc{=}O \xrightarrow[\text{Isolated chloroplasts}]{\text{Light}} 2HO{-}\bigcirc{-}OH + O_2$$

Benzoquinone — Hydroquinone

Fig. 13.8 : A common procedure for the isolation of chloroplasts from leaves for demonstrating Hill's reaction given in the lower part of the figure.

The role of the other box was clarified by the American biochemists Wolf Vishniac and Severo Ochoa. It was shown that photolysis of H_2O by illuminated chloroplasts led to the reduction of TPN to $TPNH_2$ (now referred to as $NADPH_2$) which could be coupled directly to fixation of CO_2 by reactions such as carboxylation of pyruvic acid to malic acid—as shown per equations below. Earlier in the thirties and forties, parallel work on respiration of animal cells and yeast had firmly established the role in living cells of NAD^+ and $NADP^+$ as electron acceptors and NADH and NADPH as donors. The moral of the new experiment was that fixation of CO_2, by itself, required no light: indeed, parallel work in various laboratories showed that if reduced $NADP^+$ was provided, fixation of carbon into organic acids could be carried out even by extracts of animal cells.

(i)
$$H_2O \longrightarrow 2\,H^+ + 2\,e^- + {}^1/_2\,O_2$$
$$NADP^+ + 2\,H^+ \longrightarrow NADPH + H^+$$

(ii)
$$\begin{matrix}COOH\\|\\C{=}O\\|\\CH_3\end{matrix} + CO_2 + NADPH + H^+ \longrightarrow \begin{matrix}COOH\\|\\CH_2\\|\\CHOH\\|\\COOH\end{matrix} + NADP^+$$

Pyruvic acid — malic acid — Dark reactions

or to put more generally

$$CO_2 + NADPH + H^+ \longrightarrow [CH_2O] + NADP^+$$

The actual acceptor of CO_2, as you may know is a phosphorylated pentose sugar, ribulose bisphosphate (RuBP), but this matter will be discussed in more detail later.

Photophosphorylation — Production of ATP

We have just learnt that one major role of light in photosynthesis is to produce reducing power in the form of NADPH However, the photosynthetic process also requires considerable energy in the form of ATP molecules.

Daniel I. Arnon, working at the University of California, Berkeley made an outstanding discovery in the fifties. By using radioactive phosphate, labelled with P^{32} he showed that isolated chloroplasts when exposed to light could synthesise ATP even without oxygen.

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

The process is called photosynthetic phosphorylation or **photophosphorylation**. The discovery made good sense, since photosynthesis is generally 10-20 times faster than respiration. Obviously, the requirement for ATP in dark reactions of photosynthesis (for example in phosphorylating ribulose to RuBP) could not be met by the ATP supplied by respiration via the ordinary pathway of oxidative phosphorylation. By this time a general scheme for primary and secondary processes as shown in Fig. 13.9 was formulated.

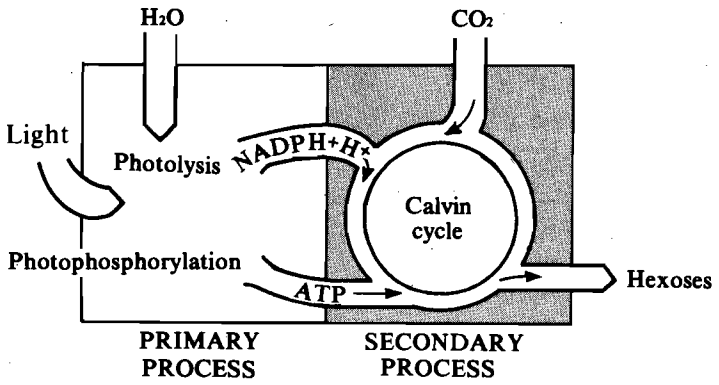


Fig. 13.9 : A scheme of primary and secondary processes of photosynthesis. Through light reactions, not only electrons are transferred from water to $NADP^+$ to make NADPH, but ATP is also synthesised. Both NADPH and ATP are then used for driving the Calvin cycle through which CO_2 is fixed.

SAQ 3

a) i) Which of the experimental findings in photosynthesis gave the clue that the process consists of both light and dark reactions?

.....

ii) What exactly is the role of the photochemical reaction?

.....

.....

b) Fill in the blanks in the following statements with appropriate words.

i) In photosynthesis the O_2 is released because of ..... of ..... . This was reasoned by ..... who observed analogous splitting of H_2S in photosynthetic ..... bacteria.

ii) Light splits ..... was shown by using an isotope of H_2O containing ..... .

iii) The action of light leads finally to reduction of ..... to ..... as also synthesis of ..... from ..... and ..... .

iv) Reduction of an electron acceptor and evolution of O_2 by isolated chloroplast on illumination is called ..... .

13.4 CHEMISTRY OF CHLOROPLAST PIGMENTS

Before we proceed to the more modern ideas of the mechanism of photosynthesis it is necessary for us to understand some other fundamentals, for example, the chemistry and role of photosynthetic pigments. Anyone could infer from yellowing leaves that they have more than one pigment. Mikhail Tswett, a Russian botanist, at the beginning of the century, separated the chloroplast pigments by chromatography — a technique he developed — and showed that leaves have four types of photosynthetic pigments :

Pigment	Colour	Range of Maximal Absorption	
		Blue Region	Red Region
1) Chloophyll *a*	Blue green	(400-500 nm) Peak at 430 nm	(600-700 nm) Peak at 670 nm
2) Chlorophyll *b*	Green	(400-500 nm) Peak at 470 nm	(600-700 nm) Peak at 650 nm
3) Carotenoids	Yellow or Orange	(400-500 nm) Peak at 450 nm	

Note : Absorption peak changes with the solvent used.

The carotenes and xanthophylls are basically hydrocarbons with two aromatic rings at the ends joined together with a long polyene aliphatic chain — because of their structure they are highly non-polar or hydrophobic (Fig. 13.10a). On the other hand, the chlorophylls are comparatively less hydrophobic and more polar on account of the existence of a porphyrin "head" and charged N atoms. The "head" or the "flag" is borne on a long hydrocarbon "tail" or "pole" of an aliphatic alcohol (Fig. 13.10b). The difference in chl *a* and chl *b* is minor in nature — in chl *b*, a methyl group in the porphyrin "head" is replaced by $-CHO$, an aldehyde group. In the centre of the porphyrin ring there is a Mg ion. The haem of haemoglobin has similar structure, but there the metal ion in the porphyrin ring is Fe^{3+}/Fe^{2+} rather than Mg^{2+}.

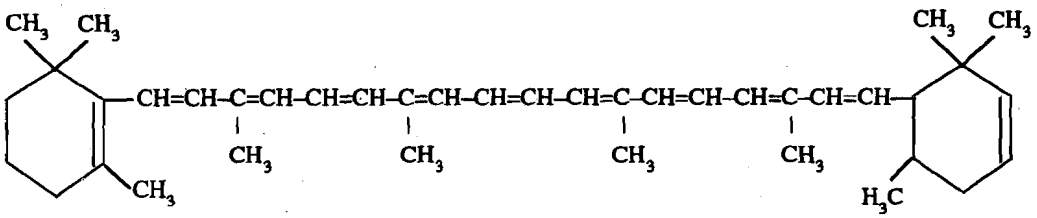


Fig. 13.10 b) : Structure of carotene.

A unique feature of both carotenoids and chlorophylls is the presence of a system of alternating double bonds with resonating electrons which are rather easily excited by photons of the visible light, specially at the blue and red ends (Fig. 13.4). In fact, the chloroplast pigments are among the most intensely light absorbing molecules in nature.

We should now have a look at the chemical mechanism by which the pigments help in transferring electrons from H_2O to CO_2 via $NADP^+$. The most critical role is played by a special pair of chl *a* molecules (P_{680} and P_{700}) which may be called "daddy" molecules. By virtue of the fact that chl *a* is the longest wavelength absorbing pigment, it can receive energy from an excited chl *b* molecule, provided it is sufficiently close. The chl *b* molecule can, in turn, absorb energy from excited molecules of carotenes and xanthophylls. The direction of transfer of electrons is always from a pigment which absorbs photons of higher energy to one that can be excited with those of lower energy, as given below:

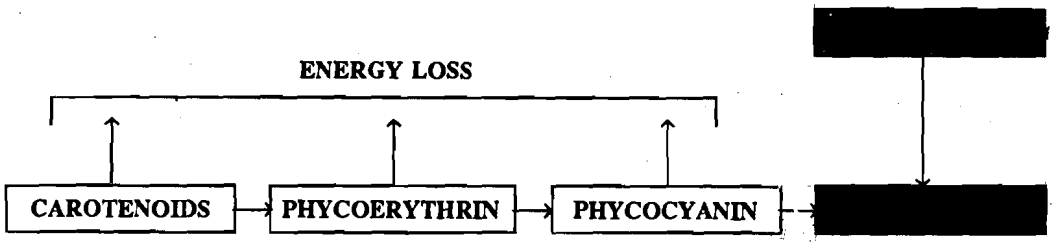


Fig. 13.11 : Scheme of energy transfer between various pigments.

Since the energy of light varies inversely with wavelength of a photon this means that its transfer is always from a lower wavelength (but higher energy photon) absorbing

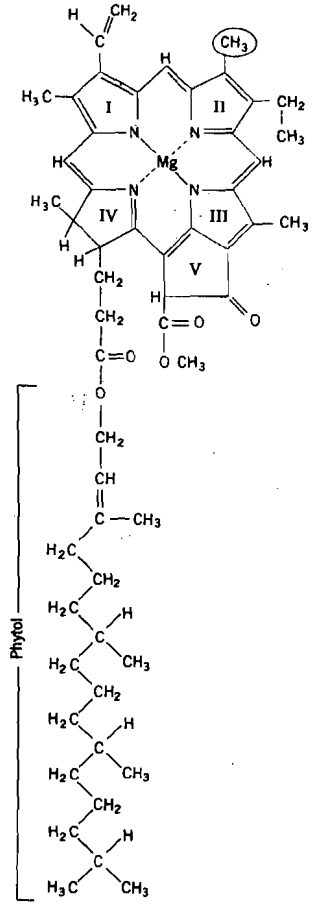


Fig. 13.10 a) : Structure of chlorophyll molecule. In chl *a*, the circled methyl group is replaced by an aldehyde group (CHO) in chlorophyll *b* as shown here.

The chemistry of chlorophyll *a* and *b* as also carotene and xanthophyll was worked out by German chemist Richard Willstätter and his associates. Willstätter was given a Nobel Prize.

molecule (like carotenoids), to a higher wavelength (low energy photon) absorbing molecule (for example chl *a*). Of course, the photon may marginally lose some energy in the process of transfer and that is why energy can never be transferred in the reverse direction.

Light is absorbed in discrete packets referred to as photon or quanta (sing. quantum) of light, the energy of a photon is given by

$$E = h \frac{c}{\lambda}$$

where h = Planck's constant (6.626×10^{-34} J*-s)

c = velocity of light (3.0×10^{8} m s^{-1}),

λ = wavelength of light (in m)

$$E = \frac{6.626 \times 10^{-34} \times 3.0 \times 10^{8}}{\lambda} \text{ J photon}^{-1}$$

$$= \frac{1.988 \times 10^{-25}}{\lambda} \text{ J photon}^{-1} \quad \ldots (1)$$

For example E for blue light (λ = 450 nm) is

$$E = \frac{1.988 \times 10^{-25}}{450 \times 10^{-9}} \text{ J photon}^{-1}$$

$$= 4.42 \times 10^{-19} \text{ J photon}^{-1}$$

The energy for 1 mole of photon (i.e. for 1 einstein) would be

$$E = N \times h \frac{c}{\lambda}$$

N = Avogadro's number (6.022×10^{23} photons mol^{-1} or 1 einstein)

From equation (1) $E = \frac{6.022 \times 10^{23} \times 1.988 \times 10^{-25}}{\lambda}$

$$= \frac{0.1197}{\lambda} \text{ J einstein}^{-1}$$

So, we can calculate energy of one einstein of photons by dividing 0.1197 by wavelength of light.

* J = joule

4.184 J = 1 calorie

Now let us enquire into the precise mode by which chlorophyll molecules help transfer electrons from H_2O to $NADP^+$. According to the laws of physics, an electron electron hit by a photon — of a certain minimal energy — can pass from a lower atomic orbital to the next higher atomic orbital. In fact, with a higher energy photon — as in case of blue light — the electron can jump even to the next orbital. Nonetheless, since the excited states are short-lived, the electron tends to readily pass its energy to a neighbouring molecule and especially so to molecules that absorb longer wavelengths. The exact mechanism of transfer is still a matter of debate among physicists, but a particle called "exciton" may mediate this process. Ultimately, a special chlorophyll molecule in the reaction centre of a photosynthetic unit acts as the final trapping centre for the energy of the photon.

Although the evidence for the existence of the organisation of the photosynthetic machinery in the form of photosynthetic units of two kinds of photosystems (PS I and PS II embedded in the thylakoid membrane) will be discussed in the following section, we can leave for a while the matter of the history of evolution of the concept and consider a bit more closely the arrangement of the chlorophyll molecules in them. Each of the photosynthetic unit contains some 200-300 chlorophyll molecules. The photosynthetic units in fact consist also of several proteins (about 2 dozen in each photosynthetic unit). The peripheral components of each unit are the so called Light Harvesting Complexes (LHC), where the large majority of chlorophyll molecules are bound. The Light Harvesting Complex surrounds an inner core and at the heart of each Core Complex is the Reaction Centre. The light harvesting complexes also

contain the accessory pigments and absorb light throughout the range of photosynthetically active radiation (PAR). They are popularly referred to as the antenna since they function much like the ones we have on roof-tops connected to the TV sets inside our homes. The energy in photons captured by the antennae, is passed on to the special (daddy) chlorophyll *a* molecules which constitute the heart of the photosynthetic machinery.

The difference in chl *a* at the reaction centre and other chl *a* molecules—for example in the antenna—is that when it is excited, the electron is lost altogether from the orbit causing **photoionisation**. This electron finally passes to $NADP^+$. The reaction centre chl *a* — now positively charged — regains an electron from water, and setting free in this process protons as well as oxygen (we shall discuss the fate of the protons later). To put it very simply, chlorophyll acts as a pump for transferring electrons and light provides the energy for this process. And as someone has observed the jump of the electron from chl *a* is more significant for life on earth than the highest "jump" of any object that humans can effect!

$$\text{Chl} \xrightarrow{\text{light}} \text{Chl}^* + e^-$$

$$NADP^+ + 2H^+ + 2e^- \longrightarrow NADPH + H^+$$

13.5 DISCOVERY OF TWO LIGHT REACTIONS

After our short diversion in matters of pure chemistry (essential, nonetheless, to gain proper understanding), let us now enquire into detailed mechanism of both the light as well as the dark reactions. We shall start this section with the light "box" — and discuss one of the great conceptual advances in photosynthesis i.e. the discovery that there are **two light** reactions (or boxes), and not one as was originally believed.

13.5.1 Quantum Requirement of Photosynthesis

In the earlier section on Understanding the Mechanism of Photosynthesis, while we were discussing flashing light experiments (page 65), the emphasis was laid on confirmation of the idea of the existence of light and dark reactions. But a second objective of doing these experiments was to determine the quantum requirement for photosynthesis to obtain an overall idea of the efficiency of the process and its mechanism. Since oxygen evolution is a measure of the photosynthetic process, the question that needed to be resolved experimentally was "How many quanta of light are necessary for evolving one molecule of oxygen"?

With the development of the Atomic theory in the early part of this century as also the new equation of photosynthesis (that a minimum of 4 electrons are needed to evolve a molecule of oxygen) and Einstein's Law of Photochemical Equivalence, the studies of quantum requirement of photosynthesis took an entirely new turn. According to the concept advanced by Einstein, in a photoelectric effect one hit by a photon leads to expulsion of only one electron at a time from a molecule. Thus, the overall quantum requirement (number of quanta required for evolution of one oxygen molecule) could serve as an indicator of the number of hits needed for an electron to be pulled up via chlorophyll from water to a higher energy state as in NADPH.

As per the new equation of photosynthesis, four electrons need to be extracted from a H_2O molecule for evolution of one oxygen molecule. Thus, if the quantum requirement is 4, this may mean that to drive a single electron only one photochemical reaction is involved in photosynthesis. However, if the quantum requirement is 8, it would mean that for each electron to move up two steps may be required and so on (it should be understood that quantum requirement has to be in simple multiples and so one has to choose a figure from amongst 4, 8, 12 and so on Fig. 13.12). Although Warburg—who initiated these studies—found a quantum requirement of 4 (giving a rather misleading idea of the extraordinary efficiency of the photosynthetic process), Emerson and Arnold and later many other workers found that the quantum requirement was about 8, meaning thereby that the overall photochemical process involved a two-step reaction.

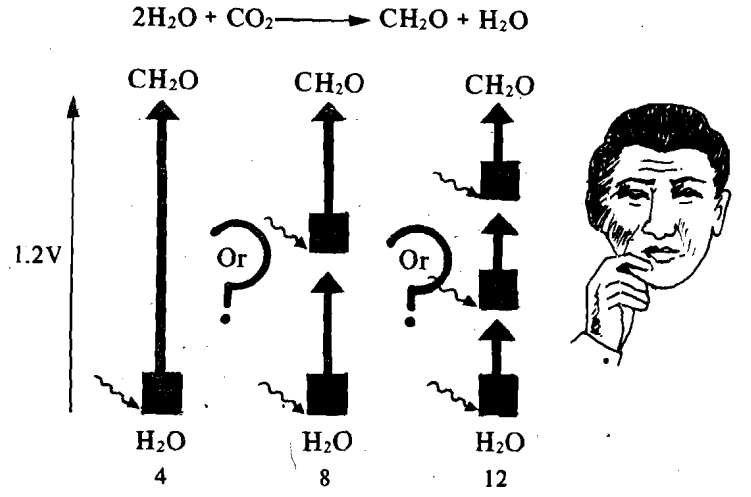


Fig. 13.12 : a) The diagram illustrates the significance of studies on quantum requirement to evolve oxygen during photosynthesis. As shown in the equation above, four electrons have to be released from water by photons hitting chlorophyll molecules. Square boxes represent chlorophyll molecules at the reaction centres. A quantum requirement of 8 indicates that at least two photochemical reactions may be involved in the transfer of each electron in photosynthesis.

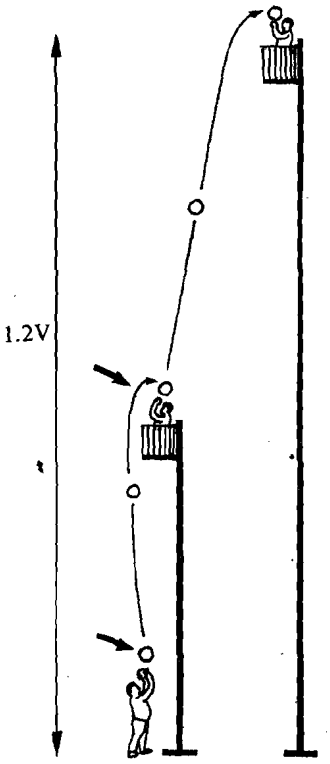


Fig. 13.12 : b) Cartoon

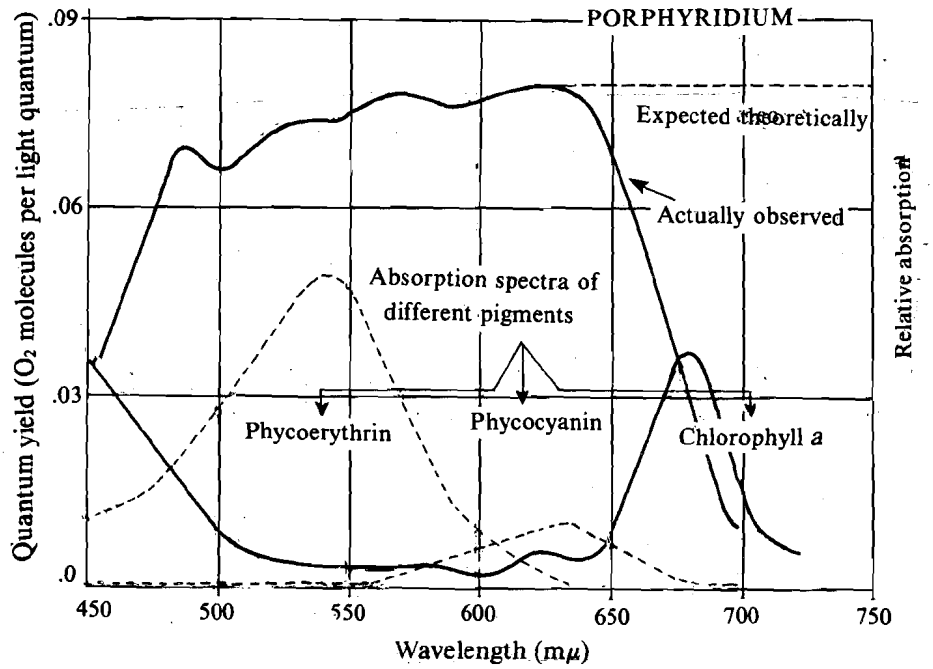
13.5.2 Red Drop

Another hint that there are two photochemical reactions — and which proceed in two distinct photosystems came from studies of quantum requirement as a function of wavelength of light. Since quantum requirement is calculated in terms of the amount of oxygen evolved per unit of light energy **actually absorbed**, it was expected that the curve for quantum requirement should stay more or less constant from 400 to 700 nm, i.e. covering both ends of the spectrum (until some absorption of light by one or other photosynthetic pigment still occurred). Strangely, the quantum yield (this is simply the number of oxygen molecules evolved per quantum of light absorbed) dropped precipitously when O_2 yield was determined employing red light for photosynthesis (and even when chl a still absorbed). The "red drop" specially clear in experiments with red algae (Fig. 13.13) was highly perplexing since chl *a* has been considered as the most important of all photosynthetic pigment molecules and to which in fact all energy is ultimately transferred from the other pigments.

13.5.3 Emerson Enhancement Effect

When Emerson continued his experiments to unravel the mystery of red drop, he found that if simultaneously a weak beam i.e. of low intensity light of any wavelength in the visible spectrum was shone, the "red drop" was abolished. Indeed, *the oxygen yield with two light beams shining together was more than the sum of yields obtained from two lights given individually.* By this experiment we now know that there is not one but two photochemical reactions in photosynthesis which need to be driven simultaneously.

Ultimately, finer work led to the realisation that chl *a* itself existed in at least two forms — one absorbing at longer and the other at shorter wavelengths. Thus it was established that there are two energy-capturing reactions, each using a different cluster of pigments and a reaction centre chlorophyll. They are now termed photosystems (PS) I and II and Robin Hill proposed the 'Z' scheme of photosynthesis, now universally accepted by all (Fig. 13.14). Ingenious experiments by L.N.M. Duysens, a dutch biophysicist, showed in the sixties that the photosystem II is driven by the shorter wavelength light and accepts electrons from H_2O whereas photosystem I by longer wavelength light provides elec' ons to $NADP^+$. We cannot go in a description of the experiment and you may like to co.sult more advanced texts. Let us, however now learn a little more of PS I and PS II.



g. 13.13 : An action spectrum of the quantum yield for photosynthesis, i.e. the yield of oxygen evolution *per quantum absorbed* as a function of wavelength. The straight dotted line on top represents the quantum yield as expected theoretically, through different regions of the spectrum. The solid line shows that actually there is a fall in the red region even though chl *a* is still absorbing. There is, however, restoration of the quantum yield if weak light of a lower wavelength is shone simultaneously.

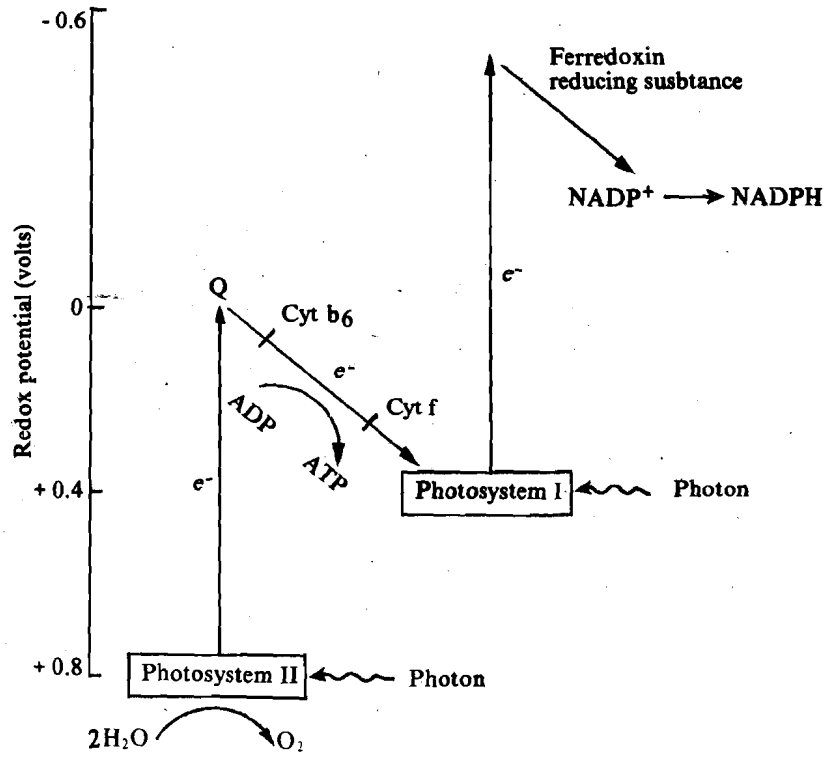


Fig. 13.14 : The simplified 'Z' scheme of photosynthesis and the pathway of electron transfer from H_2O to $NADP^+$ via PS I and PS II essentially in the form first proposed by Hill. Q is an unidentified electron acceptor of PS II.

13.5.4 Photosystems I and II

Since the seventies, biochemical methods have been developed which enable isolation of the two photosystems by density gradient centrifugation. Many studies have naturally been done on the properties of the two photosystems. By isolating each system and carrying out appropriate experiments, it is clear that photosystem II (heavier) is responsible for the evolution of O_2 from H_2O — it has a shorter wavelength absorbing form of chl *a* (P_{680}). In contrast, photosystem I is responsible for the reduction of $NADP^+$ to NADPH and has a longer wavelength absorbing form of chl *a* (P_{700}). The ratio of chl *a* to *b* is also different in the two photosystems. In a plant, as a whole, there are about 3 molecules of chl *a* for 1 of chl *b*. However, chl *a* (the longer wavelength absorbing pigment) may be 5 times as much in photosystem I and only 2.0-2.5 times the concentration of chl *b* in photosystem II. This explains why a mixture of both longer and shorter wavelength lights drives photosynthesis better than if only one kind of light is employed. Our current understanding of the two photosystems is shown in Fig. 13.15, which is a modern version of the 'Z' scheme in which all the reactions are arranged against the redox scale to illustrate as to how an electron from a lower energy level (as in water) is being raised to a higher energy state (as in NADPH).

A principal feature of the electron transport pathway is (see Fig. 13.15) that there several electron carriers involved in electron transfer from H_2O to $NADP^+$. Two of them, cyt b_6 and cyt f, bear some similarity to cytochromes involved in respiration (however, the cytochromes in the green leaf are unique). In addition, there are others such as plastoquinone, plastocyanin and ferredoxin, which too have similarity with respiratory electron transfer carriers (refer to Unit 11 of Cell Biology). It can also be seen that the two photosystems are connected by a "dark bridge" in which, for a short distance, electrons move from one carrier to another without any need of light. This is made possible by the fact that in each photochemical act, the electron is

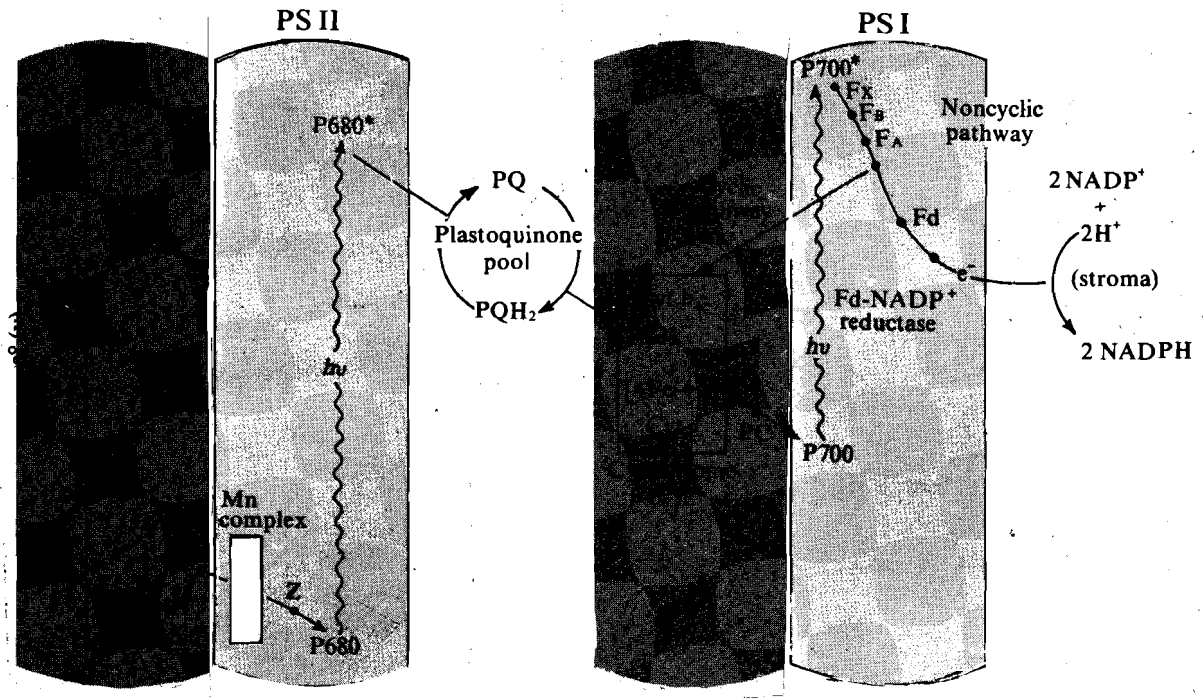


Fig. 13.15 : A modern version of the 'Z' scheme. Since Hill first proposed the 'Z' scheme of photosynthesis, a number of electron carriers and intermediates in the electron transfer chain have been discovered. It has been established that Mn-centre plays an important role in the transfer of electrons from H_2O to P_{680}, but there is some evidence that an unknown electron carrier —Z may exist between the Mn-centre and P_{680}. An excited molecule of P_{680} transfers electron to phaeophytin (Ph) which is a modified form of chl *a*. Phaeophytin, in turn, transfers electrons to a plastoquinone (PQ) which is reduced to PQH_2 through a step generating a semiquinone and requiring protons. From plastoquinone, which is a mobile carrier the electron goes to the cyt b_6/f complex which, then, transfers it to plastocyanin (PC), again a mobile carrier. From PC the electron moves to P_{700}. In the excited state, the electron is transferred to a Fe-S complex, ferredoxin (Fd), and finally to $NADP^+$ via the enzyme Fd-$NADP^+$ reductase. It is believed that there may be three other forms of Fe-S clusters (F_X, F_B and F_A) between P_{700} and Fd complex yet to be completely characterised.

excited to a level slightly more than that required for the next photosystem to be excited. It is believed that the "downhill" journey of electrons provides a mechanism of synthesis of extra ATP molecules.

Redox Reactions

Reactions that involve transfer of electrons from one molecule to another are called oxidation-reduction reactions or redox reactions. The molecule that loses electron is oxidised and the molecule that gains electron is reduced. In a complete redox reaction the two would occur simultaneously.

$$y_{(red)} \longrightarrow y_{(ox)} + e^-$$
$$x_{(ox)} + e^- \longrightarrow x_{(red)}$$
$$x_{(ox)} + y_{(red)} \longrightarrow x_{(red)} + y_{(ox)}$$

The oxidised and reduced forms of x, are called a **redox couple** written as $x_{(ox)}/x_{(red)}$. The energy associated with the transfer of electrons (under standard biological conditions) is expressed as standard reduction potential, E_0', in units of volts. Since the energy associated with electrons of different redox couple varies, it can be arranged on a scale taking an arbitrary standard, the hydrogen half-reaction $2H^+/H_2$ (under standard conditions, pH = 0, $E_0' = 0.00$ volt, at pH = 7 its value is E_0' −0.42 volts). The E_0' values for redox couple involving electrons with higher energy than $2H^+/H_2$ are assigned a negative sign and those involving electrons with lower energy than $2H^+/H_2$ are assigned a positive sign. The redox couples with highest energy electrons (most negative) are arranged at the top and the one with least energy (most positive) at the bottom of redox scale. The spontaneous transfer of electrons can occur only in downward direction. Some important redox couples of biological systems are given in Table 13.1.

Table 13.1 : Some Standard Redox Couple Potentials of Biological Interest

Redox Couple	Number of electrons transferred	E_0' (volts)
acetate + CO_2 + $2H^+$/pyruvate + H_2O	2	−0.70
chlorophyll : $P_{\ddagger}^+/P^*$	1	−0.6
ferredoxin ox/red	1	−0.43
$2H^+/H_2$	2	−0.42
S + $2H^+/H_2S$	2	−0.23
chlorophyll : P_{II}^+/P^*_{II}	1	−0.2
FAD (flavin adenine dinucleotide) $2H^+/FADH_2$ (Free)	2	−0.18
Standard hydrogen half cell ($2H^+/H_2$)	2	$E_0' = 0.00$
cytochrome b (Fe^{3+}/Fe^{2+})	1	0.06
ubiquinone ox/red	2	0.10
haemoglobin (Fe^{3+}/Fe^{2+})	1	0.17
cytochrome c (Fe^{3+}/Fe^{2+})	1	0.22
cytochrome a (Fe^{3+}/Fe^{2+})	1	0.29
$2H^+ + O_2/H_2O_2$	2	0.30
chlorophyll : P_I^*/P_I^o	1	0.4
Fe^{3+}/Fe^{2+}	1	0.77
$2H^+ + 1/2\ O_2/H_2O$	2	0.82
chlorophyll : P_{II}^+/P_{II}^o	1	0.9

P_I^*, P_I^+ and P^o represent the excited, the electron-deficient, and the ground state of chlorophyll at photoreactive centres, PS I and PS II.

SAQ 4

a) List the evidences for the involvement of two light reactions instead of one in photosynthesis.

b) In the following statements fill in the blanks with appropriate words:

i) The photosensitive pigments transfer ..... electron at a time, while a molecule of $NADP^+$ requires ..... electrons for its reduction.

ii) Light energy captured by chlorophyll is used to produce a strong .... from a weak one.

iii) Light energy absorbed by the .... pigments is funnelled to a single molecule of ....

iv) The expelled electron from chl P..... travels through the electron transfer chain and reaches chl P..... The final acceptor of electron is ..... .

c) In the following statements choose the correct word from the alternate given in parenthesis:

i) The relative quantum efficiency of photosynthesis (drops/increases) sharply in the red region of the spectrum.

ii) When a beam of blue light is given along with red light to a photosynthesizing cell, there is (enhancement/red drop) in the quantum yield.

iii) The reaction centre chl *a* molecule at PS I absorbs a (higher/lower) energy photon than the one at PS II.

d) Which among the following statements are true. Write T for true and F for false in given boxes.

i) Photoreduction of $NADP^+$ requires CO_2. [ ]

ii) Electron can move from a redox couple with positive redox potential to another couple with negative redox potential. [ ]

iii) ATP is formed when high energy electrons move down from negative redox potential to a positive one in an electron transfer chain. [ ]

iv) The reaction centre chl *a* in PS I absorbs photon of a higher energy than the reaction centre chl *a* in PS II. [ ]

v) The pigment molecules absorbing at yellow region can transfer their energy to the ones absorbing in orange region of the visible spectrum. [ ]

vi) Evolution of a molecule of O_2 requires 8 quanta of light. It means a total of 8 electrons are expelled from the two chl *a* molecules in the reaction centres of PS I and II. [ ]

Melvin Calvim

The introduction of *Chlorella* as a material of choice for photosynthetic studies and the development of the manometric technique for the estimation of oxygen evolution (for which the alga was eminently suited) greatly advanced the studies.

13.6 THE DARK REACTIONS

13.6.1 The Calvin Cycle

It is time now to consider the dark reactions of photosynthesis. The process of fixation of CO_2 was elucidated by Melvin Calvin, who was a Professor of Chemistry in the University of California at Berkeley. By the use of the radioactive isotope of carbon, C^{14}, he discovered what is now called the Calvin cycle in his honour and the unravelling of which merited a Nobel Prize.

A suspension of *Chlorella* cells was fed radioactive sodium bicarbonate ($NaHC^{14}O_3$), which results in the production of $C^{14}O_2$). The suspension was placed in a glass container that looked like a "Lollipop" (Fig. 13.16) and then the contents were extracted in hot alcohol after brief period of illumination and concentrated by evaporation. Its constituents were.then separated by chromatography, and those that were radioactive identified by the technique of radioautography. Once a certain spot was identified on the autoradiogram, one could go back to the original chromatogram, cut it out, elute the compound and determine its structure or investigate other properties.

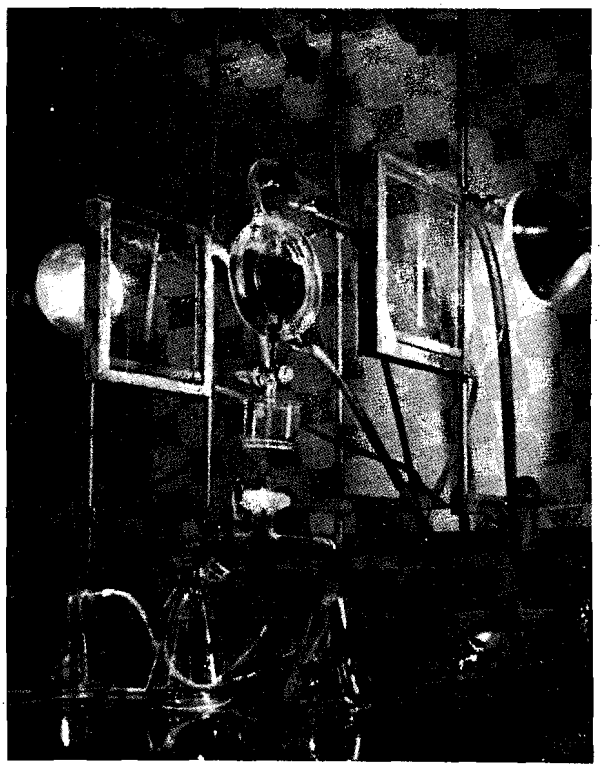


Fig. 13.16 : The 'lollipop' apparatus employed by Calvin for experiments to elucidate the pathway of CO_2 fixation.

Being a chemist, Calvin went a step further and employed a technique of "molecular dissection", whereby the terminal carbon atom in a radioactive intermediate was oxidised to carbon dioxide which was trapped as $BaCO_3$ by passing it through a barium hydroxide solution. The radioactivity in the carbon atoms was then determined by a Geiger-Müller counter. He could thus not only tell whether a particular carbon atom was radioactive, but also to what extent in a given sample. It was found that the first compound to become radioactive, already within a few seconds of photosynthesis, was the three-carbon compound, 3-phosphoglyceric acid (3-PGA see Fig. 13.17). Since only the terminal carbon atom was radioactive, it was clear that CO_2 was added to a pre-existing acceptor molecule. Initially, thought to be a 2-C molecule, later the acceptor was found to be a 5-C sugar, ribulose bisphosphate (RuBP) and the corresponding enzyme ribulose-

bisphosphate (RuBP) and the corresponding enzyme ribulose-bisphosphate carboxylase (called Rubisco) that fixes CO_2 was also discovered. Since no 6-carbon intermediate has ever been detected, the intermediate obviously fragments immediately into two 3-carbon compounds: phosphoglyceric acid and dihydroxy acetone phosphate, and both of which are well-known as intermediates arising also via the glycolytic pathway in respiration.

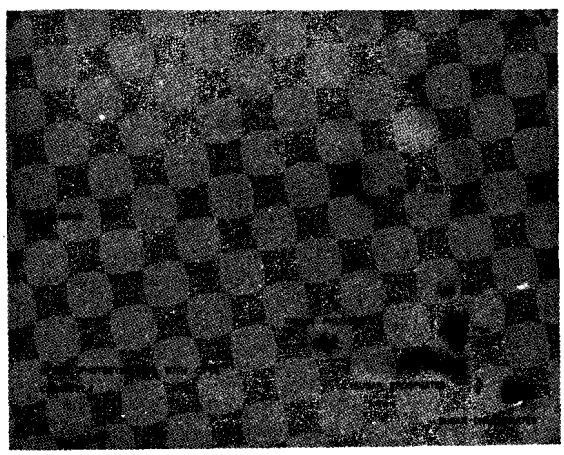


Fig. 13.17 : Radioautograph showing products of CO_2 fixation (see text for the details of experiment). It can be seen here that most of the radioactivity is localised in PGA.

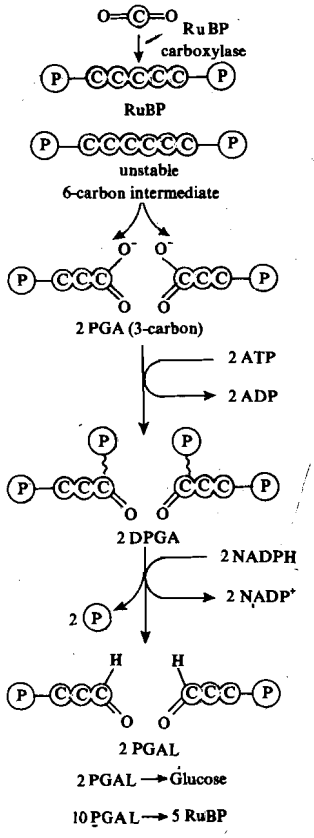


Fig. 13.18 : Carbon-fixation via Calvin cycle. Carbon dioxide combine with ribulose bisphosphate and forms an unstable 6-carbon intermediate which splits into two phosphoglycerates (PGA). Phosphorylation of PGA by ATP forms diphosphoglycerate (DPGA), which is reduced by NADPH to phosphoglycer-aldehyde (PGAL) . Out of 12 PGALS produced by fixation of 6 CO_2 molecules, 2 combine to produce a molecule of glucose, whereas the remaining 10 PGAL molecules recombine to regenerate the 6 molecules of RuBP that are needed to start the Calvin cycle. Each PGA requires 1 ATP and 1 NADH to form PGAL. A third ATP is required for the regeneration of RuBP. In all, a total of 12 NADPH and 18 ATP molecules are needed for producing a molecule of glucose.

Obviously, in order for photosynthesis to proceed to some significant level, an acceptor has to be continuously generated. The Calvin cycle illustrated in Fig. 13.18 shows in brief the mechanism by which RuBP, the acceptor molecule is regenerated. If we start with 6 RuBP, then out of 12 PGA molecules that are formed, in reality only two are available to go into the pool for the synthesis of a molecule of glucose,

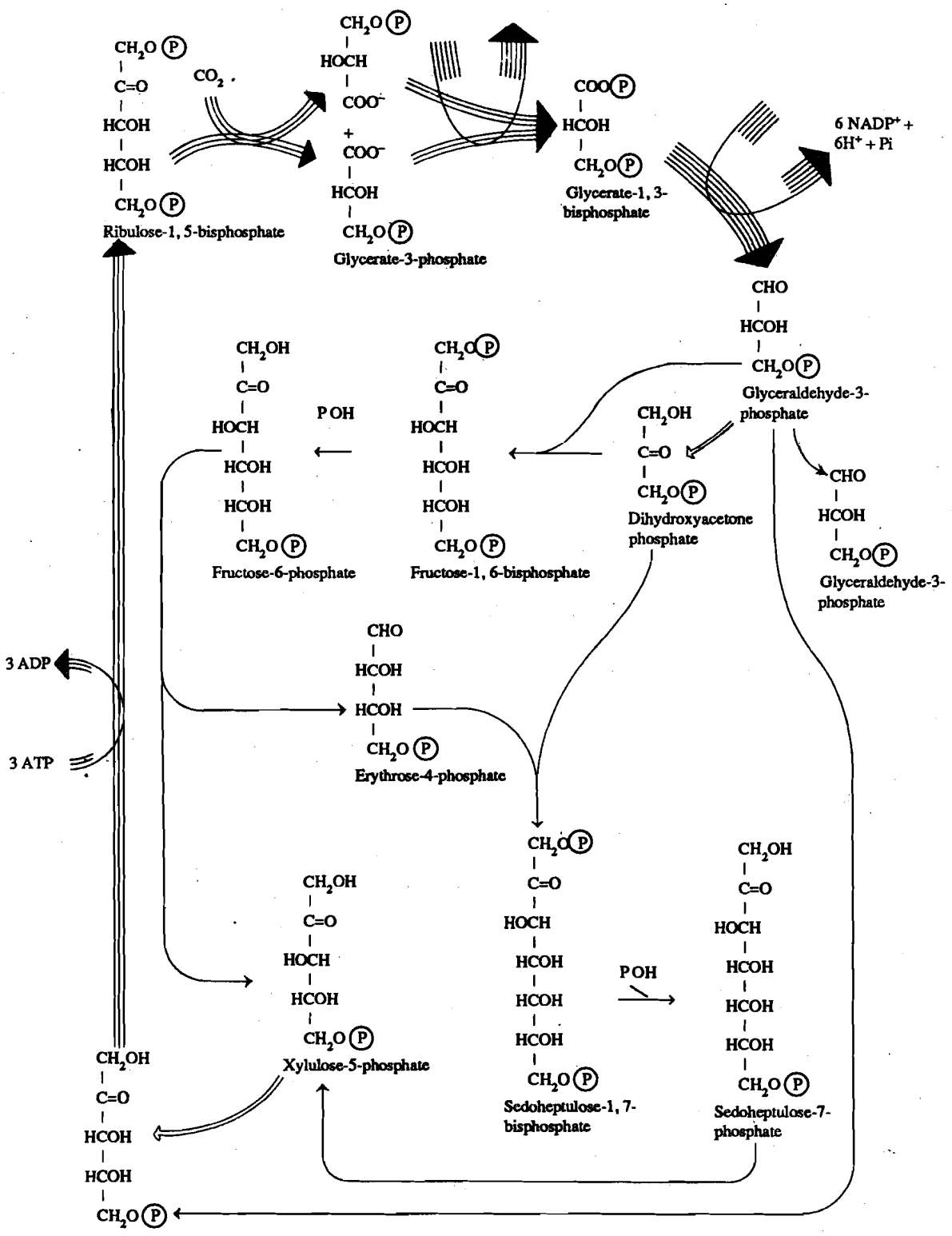


Fig. 13.19 : Detailed reactions of calvin cycle. The number of arrows drawn at each step in the diagramme indicates the number of molecules proceeding through that step for every three molecules of CO^3 that enter the cycle. The entry of three molecules of CO_2 results In the formation of one molecule of glyceraldehyde-3-phosphate (box on right), and requires the oxidation of six molecules of NADPII to $NAPD^+$ and the breakdown of nine molecules of ATP to ADP.

and from it to other substances such as starch, cellulose and so on. The remaining 10 molecules recycle through a series of reactions involving 4–, 5– and 7-C intermediates to regenerate the five molecules of RuBP from 10 PGA. Confirmation of the existence of the cycle has come not only by an analysis of distribution of radioactivity in the C atom in different sugars, but also by isolation of various enzymes involved in the process (other than Rubisco already mentioned). Figure 13.19 also illustrates that ATP and NADPH molecules are also required to run the cycle. The overall reaction of carbon fixation is given below:

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

SAQ 5

a) Melvin Calvin elucidated the path of carbon in fixation of CO_2. The following statements highlight his approach. Fill in the missing words in the statements:

i) ..... $NaHCO_3$ was used for the production of CO_2.

ii) ..... was the technique used in tracing the pathway of radioactive carbon.

iii) The technique of ..... converted the terminal carbon atom to CO_2.

iv) The radioactivity in carbon atoms was detected by ..... counter.

b) Give one word answer:

i) The first compound in which the radioactivity appeared was .....

ii) The CO_2 acceptor molecule in Calvin Cycle is .....

iii) The final product of CO_2 fixation is ..... .

iv) The number of ATP molecules required for the fixation of six molecules of CO_2 is ..... .

v) The number of ATP molecules used for the production of a molecule of glucose is ..... .

vi) The number of NADPH used for fixation of 6 CO_2 is ..... .

vii) The number of molecules of ATP required for the regeneration of one RuDP is ..... .

13.7 PHOTORESPIRATION AND THE C4 PLANTS

13.7.1 Photorespiration

Since photosynthesis has been evolving for millenia, it might be expected that the development of the machinery of carbon dioxide assimilation may have reached a stage of perfection. Yet, one of the great problems in photosynthesis lies with ribulose bisphosphate carboxylase itself, the key enzyme concerned with CO_2 fixation. Its catalytic site is such that the enzyme cannot make an absolute distinction between CO_2 and O_2. Thus, O_2 also competes with molecules of CO_2 for binding at the catalytic site, and often fragments ribulose bisphosphate into phosphoglycolic acid (a 2-carbon compound) and phosphoglyceric acid (Fig. 13.20), instead of two 3-carbon fragments of PGA which should be normally produced. After the discovery of this reaction, the enzyme is now commonly called ribulose bisphosphate carboxylase/

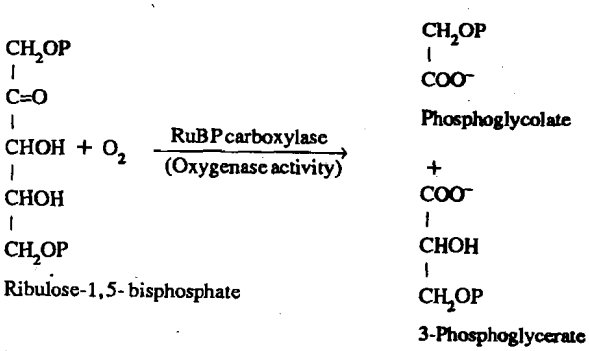


Fig. 13.20 : Reaction showing the oxygenase activity of the enzyme RuBP carboxylase.

oxygenase. The oxygenase activity is very significant because in nature molecules of O_2 are far in excess to those of CO_2. Locally, i.e. in the interior of leaf, photosynthesis can lead to accumulation of even higher concentration of O_2 than is present in the atmosphere.

Production of glycolic acid is a disadvantage since, apart from loss of a potential carbon atom that could have been fixed, the state of oxidation becomes higher than had been existing before as a result of the oxygenation reaction. Eventually, one of the two carbon atoms constituting glycolate is respired away as CO_2. Thus, instead of fixation of CO_2, plants under conditions of high intensity light actually evolve CO_2, a process which is called "photorespiration" and yet in this process no energy is released. It is estimated that if oxygenase activity and photorespiration could be avoided, plants would have fixed 30% more carbon.

Long ago, Otto Warburg himself had noted that with increasing O_2 concentration, photosynthesis in *Chlorella* was inhibited. Later, in the sixties, botanists started experiments with higher plants. They observed that if CO_2 output was monitored continuously in light and then upon transfer to darkness, immediately there was a transient "burst" in the net CO_2 output in certain plants. Curiously, the "burst" became more intense if O_2 concentration in atmosphere was higher. Thus, the existence of photorespiration has been suspected for long. However, definitive evidence came only by the use of O^{18} which allowed measurement of oxygen uptake even upon exposure of a plant to constant illumination. When the consumption of O^{18} was monitored by mass spectrometry in the surrounding atmosphere, it was discovered that the rate of uptake of O^{18} became significantly higher after illumination.

The detailed mechanism whereby CO_2 is evolved has been investigated by American biochemists and is illustrated in Fig. 13.21. According to this scheme, phosphoglycolic

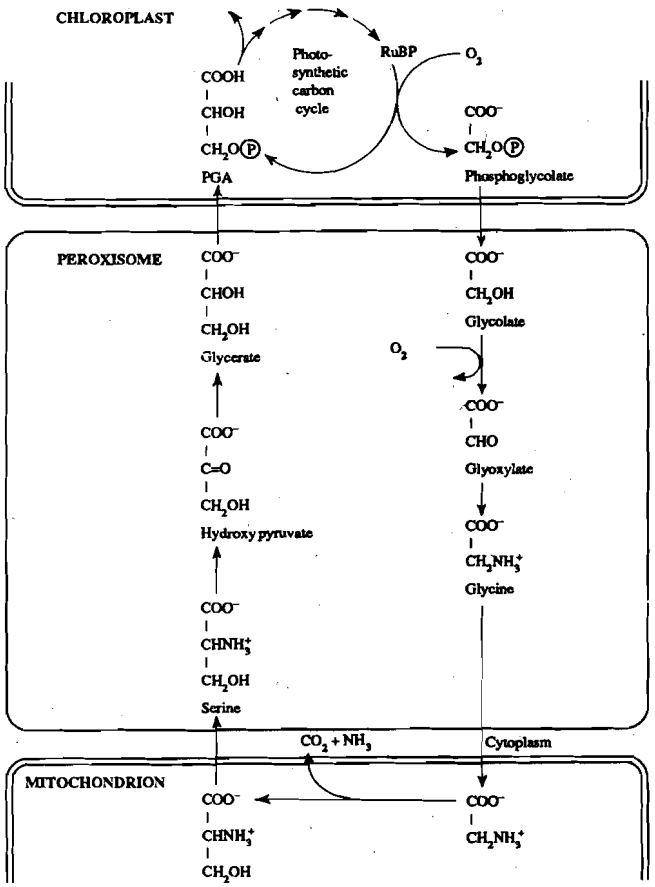


Fig. 13.21 : A simplified scheme indicating the reactions involved in the glycolate metabolism. Only the oxygenase activity of the enzyme RuBP carboxylase is shown, which results in the formation of glycolic acid. These molecules move out through the cytoplasm, to organelles called peroxisomes and glyoxysomes which convert glycolate to glyoxylate and then to glycine, which then moves into the mitochondria. Although *photorespiratory* CO_2 is ultimately believed to be liberated from mitochondria, as can be readily seen, this mechanism of respiration is quite different from that which normally occurs in mitochondria.

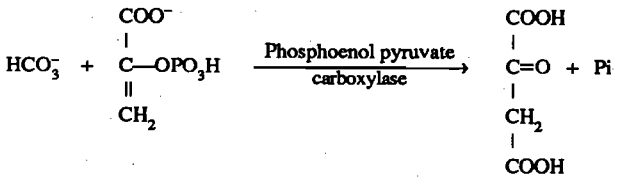
acid enters peroxisomes where it is converted to glycolate and gets further oxidised to glyoxylate. The transamination of glyoxylate, yields glycine (a 2-C amino acid). Glycine then enters the mitochondrion, where one molecule of serine is formed from two molecules of glycine with the release of a molecule each of CO_2 and NH_3. The amino acid serine (a 3-carbon compound) then reenters peroxisomes and gets deaminated to form glyceric acid, which is again converted to phosphoglyceric acid in chloroplasts. The pathway obviously requires close cooperation of biochemical activities among three organelles — the chloroplasts, the peroxisomes and the mitochondria. Remarkably, electron micrographs do show these three organelles very closely appressed to each other indicating that there is indeed some important functional relationship among them.

As would be clear, the pathway serves to *recycle three carbon atoms (ending up as PGA) out of the 4 carbon atoms i.e. 2 molecules of glycolate.* There is loss of one of them as CO_2. Also, of the two $-NH_2$ groups donated in the transamination reaction, one is given up as NH_3.

At first sight, the loss of CO_2 and NH_3 does appear as a wasteful process. However, there is another way of looking at the cycle. At least, by loss of one atom of carbon (and another of nitrogen), the three other carbon atoms are conserved, and one molecule of phosphoglycerate is formed, ready to be reduced to phosphoglyceraldehyde and enter the Calvin cycle, or be converted to sugar directly. Viewed this way, photorespiration is of benefit to plants — one should then look upon it as a "necessary evil".

13.7.2 The C_4 Plants

Another interesting mechanism plants have developed to capture CO_2 (over and above what is possible through C_3 cycle) is via another enzyme, phosphoenol pyruvate carboxylase (PEP carboxylase), according to the following reaction:



Phosphoenol pyruvate (PEP) Oxaloacetic acid (OAA)

Carbon dioxide is accepted by a 3-carbon compound, phosphoenol pyruvic acid (PEP), and fixed in the form of a 4-carbon acid, oxaloacetic acid (OAA), which is readily reduced with the help of NADH to malic acid (an amination reaction also leads to production of aspartic acid). Since in this pathway a 4-C acid is the first product of CO_2 fixation, it is called the C_4 cycle to distinguish it from Calvin cycle where fixation of CO_2 takes place in the form of a 3-C acid. The discovery of the new cycle took place rather accidentally, when Kortshak and co-workers began work in 1965 on mode of fixation of CO_2 in sugarcane at the Sugarcane Experimental Station in Hawaii (where they were employed). They were puzzled to find that in sugarcane the first stable compound of photosynthesis was not PGA. Instead, the radioactivity appeared in acids such as oxaloacetate, malate and aspartate, following a pattern quite different from that discovered by Calvin in *Chlorella* and later confirmed in many higher plants like spinach. Further work by M.D. Hatch and C.R. Slack in Australia showed that this represented an additional pathway of CO_2 assimilation.

Despite the novelty of the C_4 cycle, a point to note about this pathway is that it is not totally independent of the Calvin cycle. By a mechanism, yet to be explained satisfactorily, the 4-carbon acid, i.e. oxaloacetate, immediately gives up the CO_2 to be refixed by ribulose bisphosphate carboxylase via the Calvin cycle. In the process, the phosphoenol pyruvate, which is the acceptor for C_4 cycle, regenerates. The C_4 cycle has, therefore, to be viewed not as an independent cycle, but as one adjunct to the Calvin cycle. Nonetheless, plants do not fix CO_2 in photosynthetic cells by both C_3 and C_4 pathways in any one cell. Indeed, in plants, like the grasses, which have the C_4 cycle, there is a spatial separation. As is well-known, these plants have the so-called Kranz (German, wreath-like) anatomy (Fig. 13.22) — there being a central

Some common examples of photorespiring (C_3) and non-photorespiring (C_4) plants

Photo respiring C_3	Non-photo respiring C_4
Wheat	Corn
Rice	Sugarcane
Legumes	Sorghum
Potato	Sugar-beet
Tomato	Bajra

bundle sheath region consisting of a ring of large chlorophyll containing cells, surrounded by more loosely arranged spongy mesophyll cells. The enzyme PEP carboxylase abounds in the outer mesophyll cells whereas ribulose bisphosphate carboxylase is restricted to the bundle sheath cells.

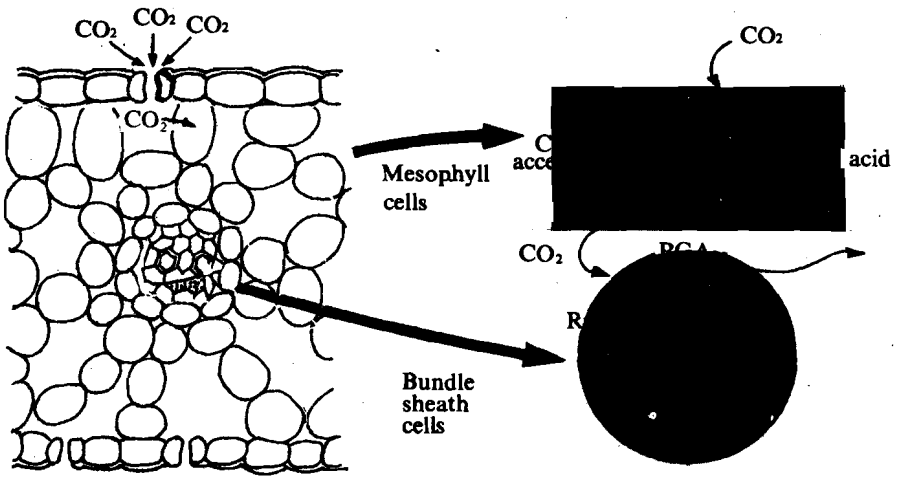


Fig. 13.22 : The diagram illustrates the anatomy of plants which apparently do not photorespire and the C-4 pathway of carbon dioxide fixation with which they are endowed. Here, CO_2 is captured initially, that is, from the atmosphere by the enzyme PEP carboxylase, instead of by RuBP carboxylase as in the Calvin cycle plants, which are now commonly referred to as the C_3 plants since the first product of CO_2 fixation in this cycle is the 3-carbon containing molecule of phosphoglyceric acid rather than oxaloacetic acid. If some photorespiration does occur in the C_4 plants in the bundle sheath cells, the CO_2 so released would probably be captured and cycled back.

Another interesting point is that PEP carboxylase has a much higher affinity for CO_2 than RuBP carboxylase has. It would seem, therefore, that PEP carboxylase is ideally suited to fix CO_2 from the atmosphere. As we have learnt above, the CO_2, so fixed, has to enter the Calvin cycle. Electron micrographs show that the bundle sheath cells on their outer surface have a peculiar network of membranes — this may provide the extra absorptive surface for 4-C acids, like malate, to be transported into the bundle sheath cells to give off their carbon to the Calvin cycle. The more successful plants, then, have a rather unique and interesting mechanism to capture CO_2 from the atmosphere by a more efficient enzyme.

One cannot fail to appreciate also another advantage to the plants by virtue of the Kranz anatomy. Oxygen tension is likely to be high in the outer areas and low in the innermost parts of the leaf, thus, diminishing oxygenase activity of Rubisco. However, if there is some unavoidable photorespiration in the inner bundle sheath cells, the CO_2 so formed can be trapped again by PEP carboxylase, as it tries to escape. No wonder, then, the C_4 plants are highly efficient photosynthesisers. Grasses are widely considered to be one of the most photosynthetically efficient species colonising the earth and this may be due to the C_4 pathway they possess. Many of our important crops like corn, besides sugarcane mentioned above, are also C_4 plants. On the other hand, the dicots are by and large C_3 plants. However, there are some notable exceptions among dicots, for example, sugarbeet and members belonging to the family Chenopodiaceae and Portulacaceae. It is of interest that these plants also have the "Kranz" anatomy — much like monocots — instead of a palisade layer and spongy parenchyma typical of dicotyledonous leaves. Overall, C_4 pathway is an adaptation to enable plants to survive better under conditions of higher temperatures and poor water supply — it is of interest that if one studies the distribution of C_3 and C_4 species geographically, the former dominate in the temperate regions, but as one approaches the equator, the C_4 species dominate.

13.7.3 The CAM Plants

Exceptional among dicots from the viewpoint of CO_2 fixation are also *Bryophyllum*. *Kalanchoe*, the cacti, and some members belonging to the Euphorbiaceae that grow in the desert. These members have basically the C_4 pathway of C-fixation, but they

are called CAM plants (standing for Crassulacean Acid Metabolism) since they have some additional unique features.

Botanists have known for long that many plants with fleshy leaves have a rather sour taste on account of large quantities of organic acids such as malic and oxalic. However, it is only now that the role of these acids has been appreciated. Many of these plants fix their carbon largely in the form of 4-C organic acids which represents a unique and truly clever adaption for survival in their desert surroundings (where the day temperatures are high and water must be conserved). The strategy these plants have adopted, therefore, is to fix CO_2 at night and reduce the organic acids *via* the Calvin cycle pathway, using NADPH formed during the day (Fig. 13.23 and 13.24). The plants, thus, close their stomates during the day (to avoid transpiration) but open them at night (to permit inflow of CO_2)! The CAM plants have, therefore, separated the function of CO_2 fixation and electron transfer for reduction of organic acids (to aldehyde and alcoholic groups as in sugar) in a temporal sense i.e. one process occurring during the day and the other at night.

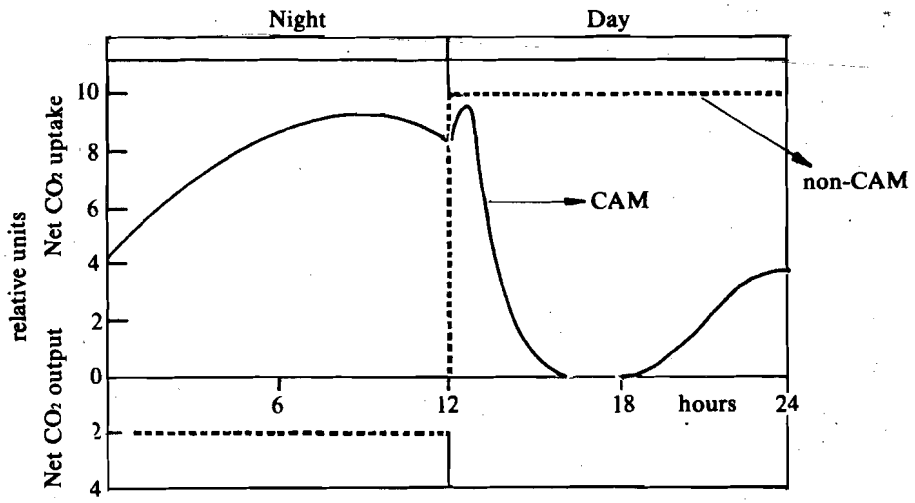


Fig. 13.23 : Typical pattern of gas exchange in a CAM plant. Uptake of CO_2 occurs largely at night. During the day, there is decline in CO_2 as it is reabsorbed for production of organic acids. Dashed line shows the CO_2 exchange curve for a non-CAM plant.

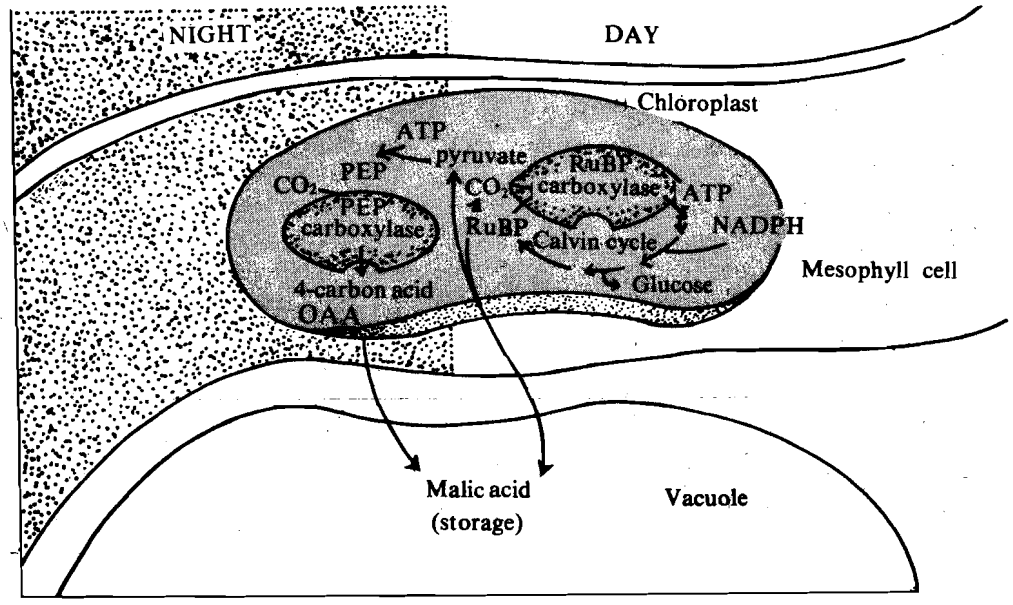


Fig. 13.24 : CAM synthesis. The reactions are identical to C_4 synthesis reactions except that they take place at different times within the same mesophyll cell. At night, stomate pores open to take up CO_2. The CO_2 is fixed to PEP by PEP carboxylase producing oxaloacetate. The oxaloacetate is then converted to malic acid, which is then stored in the cell's vacuole. During the daytime, CAM plants close their stomates, conserving water. The stores of malic acid are gradually moved back to the chloroplasts and split to release CO_2 and pyruvate. The released CO_2 is then fixed to RuBP by RuBP carboxylase and is introduced into the Calvin cycle, while the pyruvate is converted into PEP with energy from ATP.

SAQ 6

a) List the conditions required for photorespiration:

.....

.....

b) Match the reactions of photorespiration in column 1 with the site of their occurrence in column 2.

	Column 1		Column 2
i)	Evolution of CO_2 from two molecules of glycine	a)	Chloroplast
ii)	Oxidation of RuBP	b)	Peroxisomes
iii)	Formation of serine	c)	Mitochondria
iv)	Formation of glyoxylate		
v)	Regeneration of PGA		

c) Complete the following statements about C_4 plants and the C_4 pathway.

i) The acceptor of CO_2 is a three-carbon compound .....

ii) The enzyme for fixation of CO_2 is ..... .

iii) A molecule of ..... is used per molecule of CO_2 fixed.

iv) The pathway occurs in the..... of mesophyll cells.

v) Plants that use C_4 pathway also use .... which operates in .... cells.

vi) PEP carboxylase has higher affinity for CO_2 than ..... .

vii) Bundle sheath cells of C_4 plants have peculiar .....of..... for CO_2 absorption.

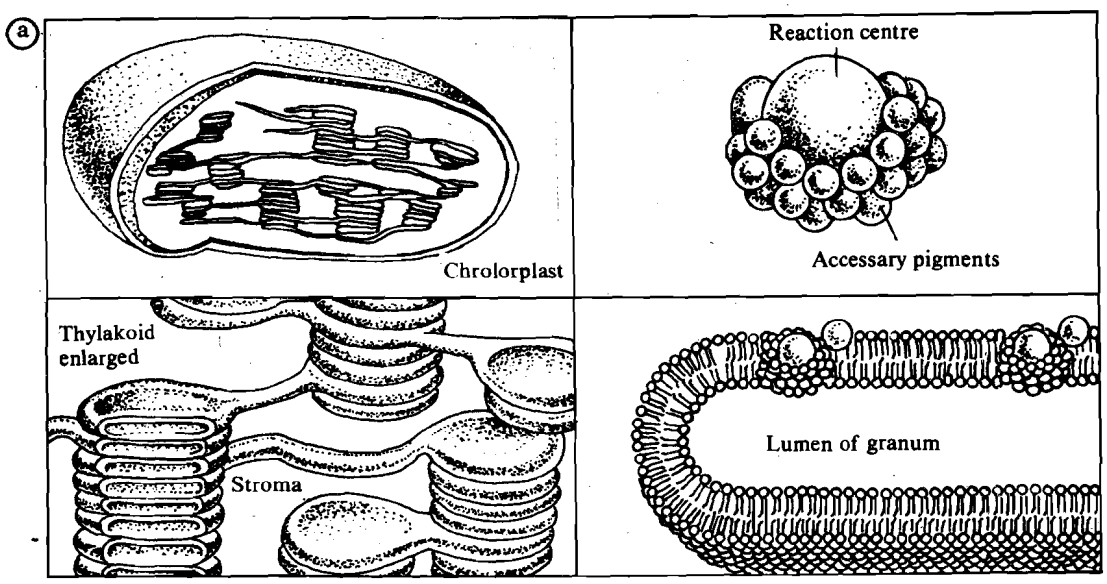
viii) C_4 plants are ....than C_3 plants.

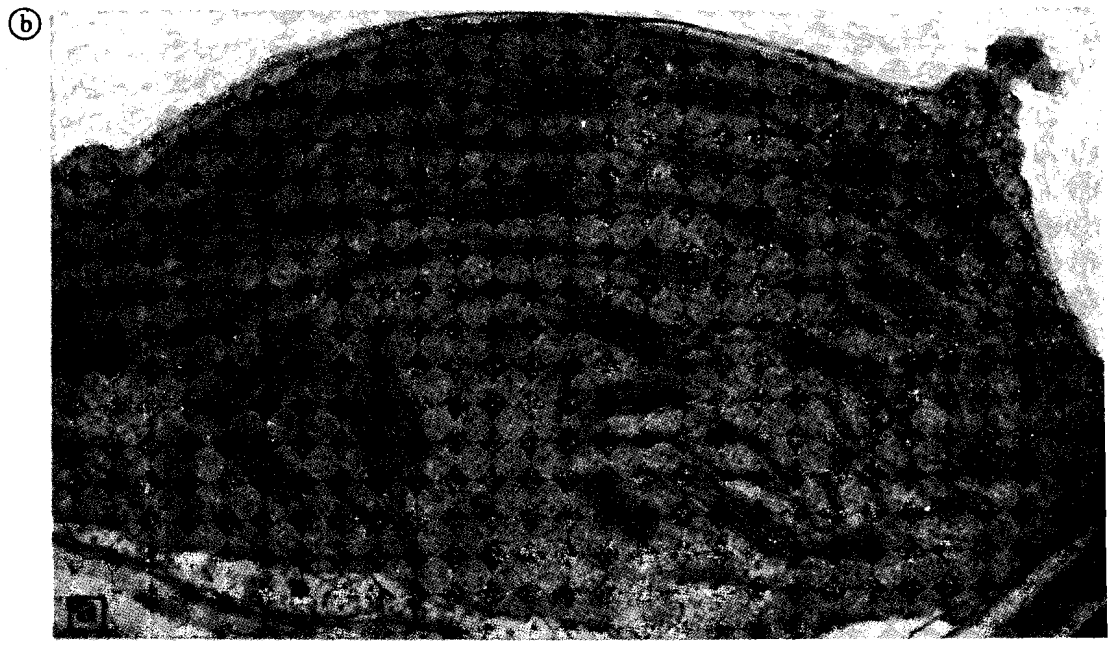
13.8 THE CHLOROPLASTS — ULTRASTRUCTURE AND ORGANISATION OF PHOTOSYNTHETIC MACHINERY

We can now have a close look at the structure of chloroplast and the photosynthetic machinery inside (Fig. 13.25). Since the chloroplast was first described by the German botanist von Mohl in 1837, and Engelmann in 1882 could definitively correlate photosynthesis with this organelle, we have come a long way towards the understanding of the chloroplast structure. The major breakthrough came in the fifties when the techniques of preparing ultrathin sections and electron microscopy came into existence. The chloroplast was found to have an extensive internal membrane system comprising the thylakoids (Thyla, Greek = sacs; thylakoid = sac-like) connected to each other through tubular connections and all embedded within the stroma which contains ribosomes and other soluble components in addition to DNA. Thylakoids pile upon one another to produce grana which are discoid in shape. Grana are much like a pile of coins. The techniques of disruption of cells and organelles by lysis or sonication and of high speed centrifugation allow grana to be separated—and from experiments with purified grana it is clear that it is here that the real photosynthetic machinery lies.

More sophisticated specimen preparation techniques for electron microscopy developed in the sixties and seventies, i.e. use of freeze-fracturing and shadowing methods (refer LSE-01, Section 2.3.3), have revealed also the presence of smaller granular particles which may represent the ultimate units of the photosynthetic machinery. This is discussed below further.

The idea that the photosynthetic machinery is not distributed randomly in the chloroplast, but organised in small units first came from the experiments of Emerson,





Chloroplast enevelop (CE),
Stroma (S)
Stroma lamellae (SL)
Grana (G)

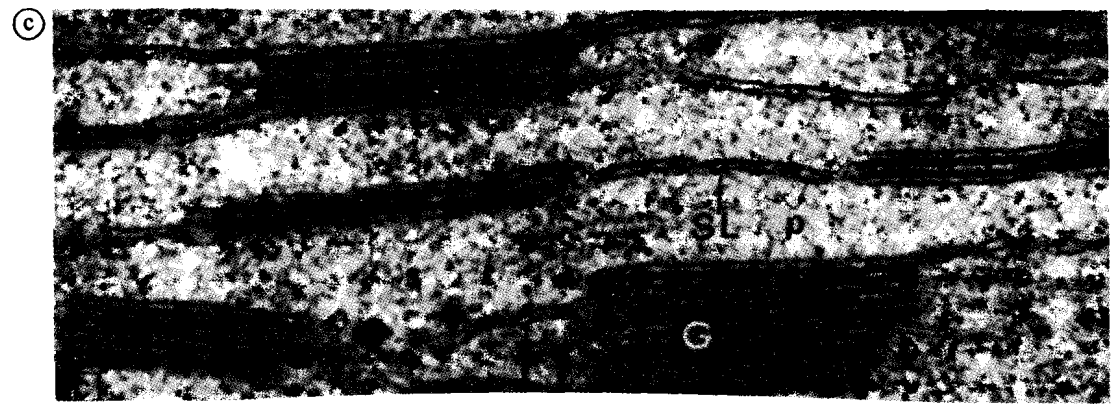


Fig. 13.25 : Organisation for photosynthesis. Photosynthesis takes place in the chloroplasts of plant cells. The thylakoid system of the chloroplast is the site of the light reactions, whereas the stroma is the site of the synthesis reactions. Photosynthetic pigments are precisely arranged in the thylakoid membrane to form light-harvesting photosystems. The reaction centre of each photosystem receives energy from surrounding antenna pigments. (b) E M photograph of chloroplast (c) Thylakoids enlarged (by Swadesh Taneja, 1973)

some fifty years ago, when he carried out the flashing light experiments and measured O_2 output per flash. Emerson became interested in discovering as to how many chlorophyll molecules are required to evolve one oxygen molecule. He found that even when experiments were conducted under the best conditions the ratio of number

of chlorophyll molecules to a molecule of O_2 evolved was always between 2,000 and 2,500 corresponding to a value of about 250-300 per quantum of light absorbed (this calculation assumes that 8 quanta are required for one O_2 molecule to evolve). Clearly, chlorophyll molecules are in great excess and if the photosynthetic unit was made, let us say of 250 chlorophylls, for one chlorophyll molecule (of the special pair) actively participating in electron transfer, 249 molecules were "standby" molecules. This immediately led to the concept of existence of photosynthetic units comprising one chlorophyll molecule as part of a "reaction centre" and the others forming an antenna (Fig. 13.26).

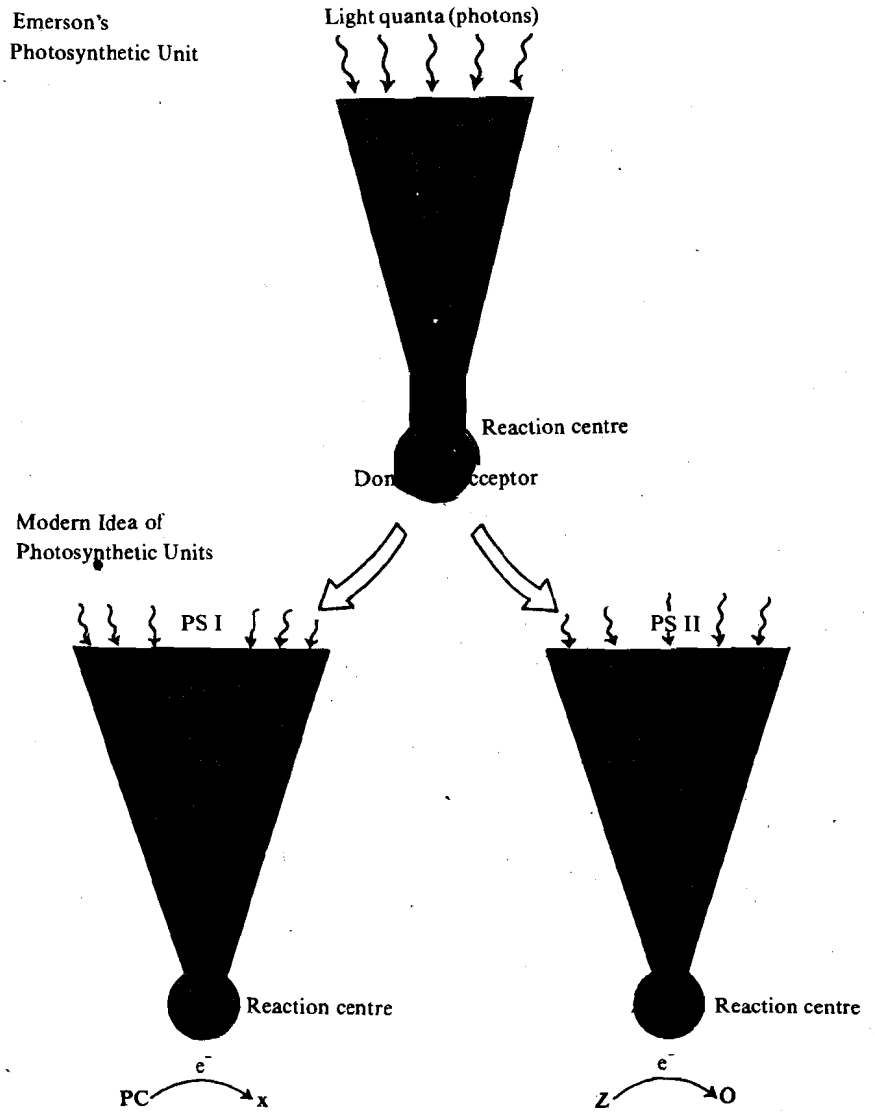


Fig. 13.26 : The photosynthetic unit. At the top is a simplified view of the photosynthetic unit as conceived by Emerson and coworkers and representing the antenna pigments and the reaction centre. Below is a modern version of the photosynthetic unit. The diagrams depict only the contrast and distribution of the photosynthetic pigments. It is well established now that there are two photosynthetic units, each containing carotenes, chl *b* and various forms of chl *a*. The chlorophylls at reaction centres are called P_{700} and P_{680}.

The new electron microscopic observations employing the freeze-fracturing technique show that particles of approximately the predicted size of photosynthetic units do exist in the membranes. The first such observations with clear images of particles came in the late sixties. The particles were called "quantasomes" as it was thought. that each can catch one quantum of light energy (Fig. 13.27). Since it is now recognised that there are two photosystems, the term quantasome is no longer in much use. Instead, now one refers to them as "photosystems" and further electron microscopy studies show that the particles are not homogenous but are at least of two types, corresponding to the currently accepted concept of two photochemical reactions. Photosystem II is the larger and shows rather regular crystalline structure when viewed from the luminal side. On the other hand, the smaller particles are

thought to represent photosystem I. Other particles may represent the cytochrome b_6f complex as also the ATP synthetase complex which carries out photophosphorylation (Fig. 13.28).

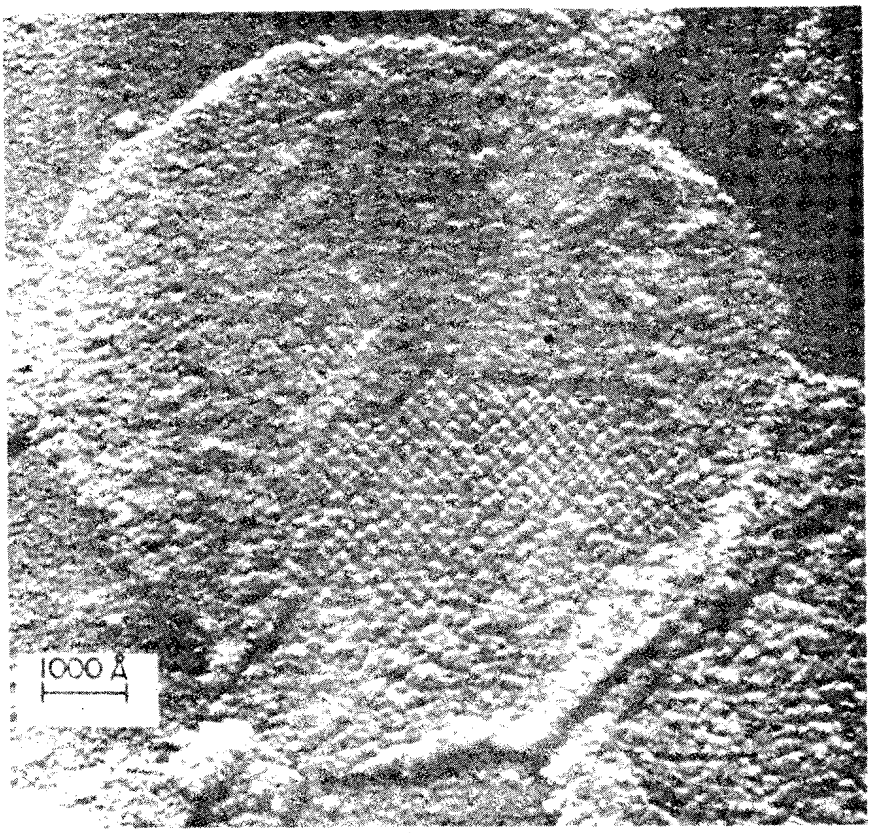


Fig. 13.27 : Electron micrographs made by employing the freeze-fracture and freeze-etching methods to show the photosynthetic units. The photograph on top was made by Roderic Park and Biggins and they called the units "quantasomes".

Unfortunately, at present, even the best electron microscopic techniques do not have sufficient power to resolve the inner sub-structure of these particles. Therefore, biochemical techniques are being relied upon to elucidate the structure. The arrangement illustrated in Fig. 13.29 is due largely to the work of biochemists and molecular biologists. The basic feature of the structure of photosystems I and II is that there is an inner core complex (called the reaction centre) which contains the "daddy" chlorophyll molecules and which participates in all electron transfer reactions (the daddy chlorophyll molecules here undergo photoionisation). The core is, however, surrounded by the antenna or the light harvesting complex, where extra pigment molecules are located. A photon is more likely to be absorbed by an outer chlorophyll molecule, but according to the principles of physics we have discussed earlier, the energy is ultimately trapped by the reaction centre chlorophyll molecules. The movement of electron across the thylakoid membrane is shown in Fig. 13.30

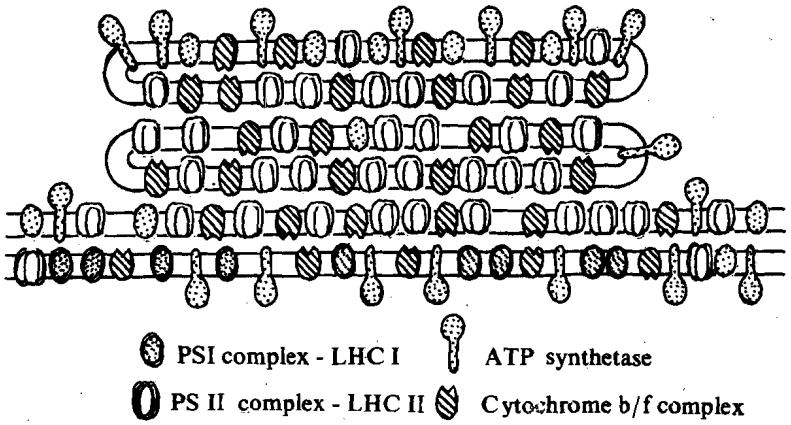


Fig. 13.28 : Schematic diagram to show the location of PS I, PS II systems in the thlakoid membrane. Shown also are cyt b_6/f and ATP synthetase complexes. Note that PS II is located mostly in appressed membranes.

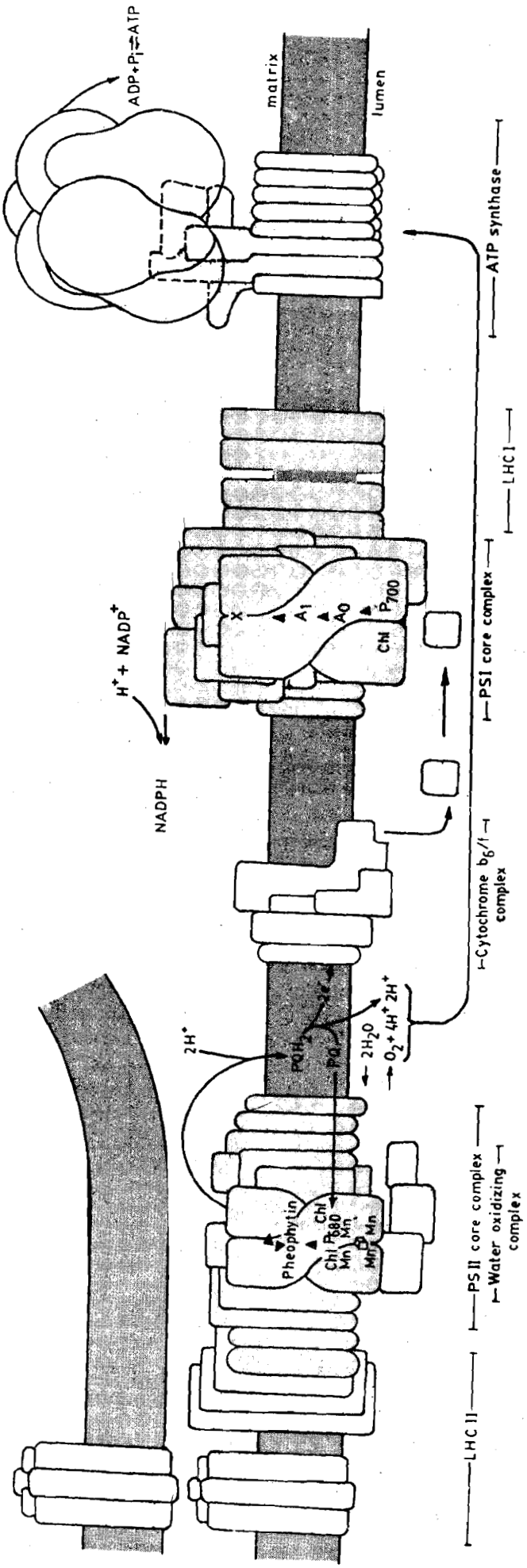


Fig. 13.29 : A more detailed representation of the structure of the photosynthetic machinery. The complexes from left to right are photosystem II, Cyt b_6/f complex, photosystem I and ATPase complex Each complex consists of various polypeptides, the precise orientation of which still remains to be determined. Electrons are transferred from photosystem I to Cyt b_6/f complex via a pool of plastoquinone (not shown) and from the Cyt b_6/f complex to photosystem I via plastocyanin which is thought to be mobile.

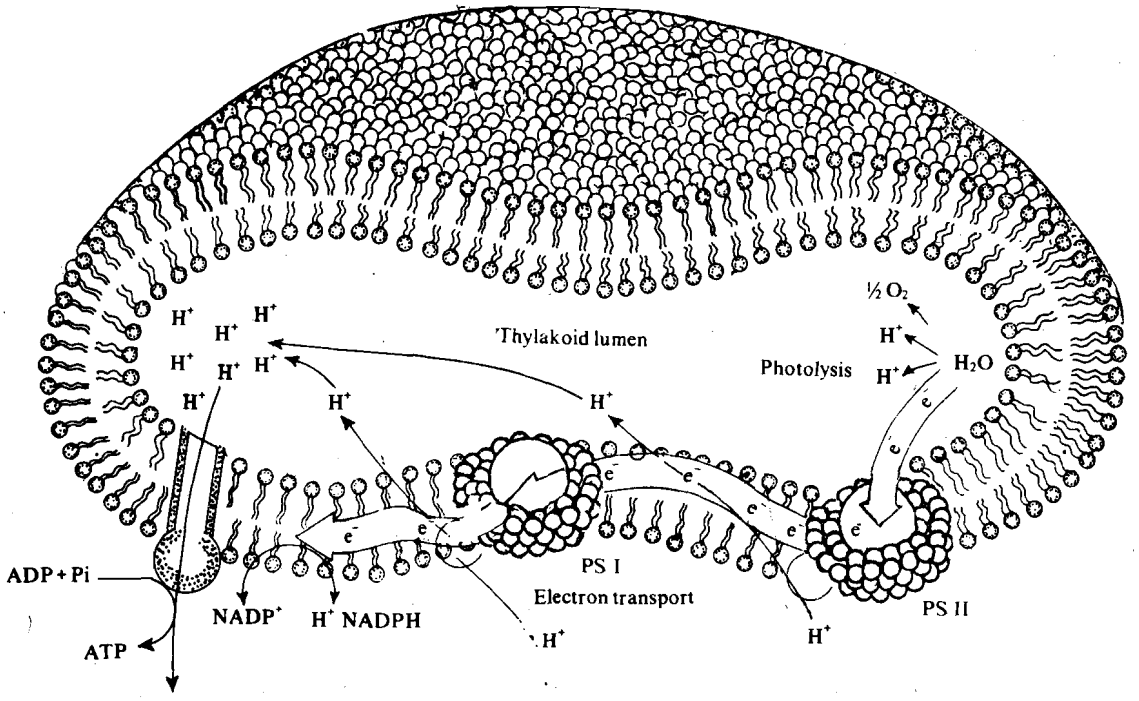


Fig. 13.30 : Illustrates how electrons are transferred from water to $NADP^+$ with the help of light energy captured in photosystems I and II. The diagram also illustrates how ATP is synthesized as a result of a concentration gradient of protons which develops due to splitting of water. As the protons escape through the proton tunnel of ATPase, ATP is synthesized.

While the basic design is similar for both photosystems, the detailed structures are naturally different. Indeed, the structure of each photosystem is unique and rather complex. By electrophoresis of purified photosystems, but pretreated in such a way as to disassemble all components, many unique polypeptides have been resolved in each photosystem. For example, in photosystem II a few polypeptides are concerned with H_2O splitting complex which are absent in photosystem I. Similarly, certain polypeptides in photosystem I have no corresponding components in photosystem II. However, it must be emphasised that the evidence for detailed structure is mostly indirect and highly speculative. The technique most extensively employed by biochemists for unravelling detailed structure of macromolecules or their complexes is X-ray crystallography. However, researchers of photosynthesis have faced considerable difficulty in crystallising the photosystems, essential for such work. Nevertheless, three German scientists, Hartmut Michel, Johann Deisenhofer and Robert Huber have, recently provided a detailed structure by X-ray crystallography of the bacterial photosystem, fetching them a Nobel prize. Similar work has yet to be done in higher plants.

SAQ 7

In column 1 we have listed processes or components related to photosynthesis, match them with their locations given in column 2.

	Column 1	Column 2
i)	The "heart" of photosynthetic machinery	a) stroma
ii)	C_3 cycle	b) grana
iii)	P700	c) thylakoid membrane
iv)	P680	d) in the thylakoid membrane towards the lumen
v)	Electron carriers	e) from stroma to the lumen of thylakoid
vi)	Water splitting apparatus	f) transmembrane protein more towards stroma
vii)	Light harvesting complexes	
viii)	Site of release of NADPH	
ix)	Site of release of ATP	
x)	ATPase	

13.9 PHOTOSYNTHESIS, AGRICULTURE AND HUMAN WELFARE

Finally, let us have a look at the relationship of studies on the mechanism of photosynthesis to agriculture and productivity. We shall first take up the question: What is the theoretical, maximal efficiency of photosynthesis. We shall then look into the actual efficiency prevailing in the field and in nature. And, if it is low, we shall enquire into the reasons and examine if anything can be done about it.

13.9.1 Efficiency of Photosynthesis

Many investigators have looked for an answer to the question of theoretical efficiency, from the time Warburg first undertook an enquiry into it in the twenties of this century. Warburg found a quantum requirement of 4 for every CO_2 molecule reduced (or O_2 evolved). From a purely theoretical angle, $4 \times 6 = 24$ quanta should be more than sufficient for reduction of 6 molecules of CO_2 since one mole of glucose contains 686 Kcal of energy, whereas 24 mole quanta of red light will have $24 \times 41 = 984$ Kcal, well above the maximal energy requirement. But if we consider the true quantum requirement for photosynthesis as 8, as established by later workers, the efficiency should be about 35%.

Nonetheless, in stark contrast to the above figure of even 35% in natural conditions, the efficiency of photosynthesis is rarely more than 1 to 2% of the total solar receipt of energy, which speaks poorly of man's ability to tap free energy from sunlight. There are many reasons for this. Firstly, of the total light energy incident upon a leaf, more than 50% consists of radiation which is not available for photosynthesis — for example UV, infrared and other such rays etc. Even of the photosynthetically active band of radiation (PAR) from 400 to 800 nm — for example, green light — is reflected from leaf surface (only certain algae have the ability to use this light). Some is simply transmitted through the leaf and a great deal is just converted to heat — in point of fact, nearly 20% of even the PAR is wasted Fig. 13.31. If we consider total

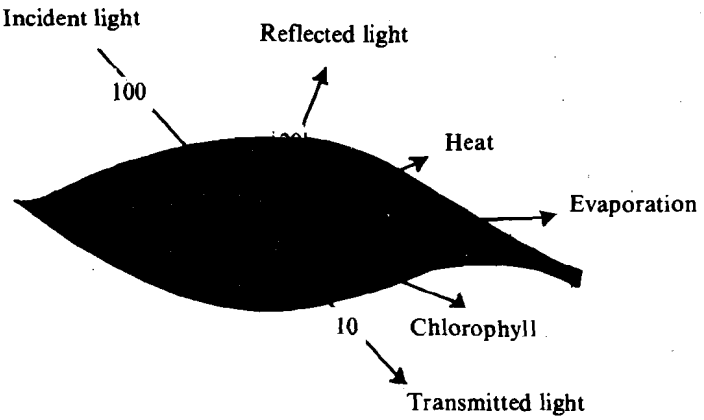


Fig. 13.31 : A diagram to show that distribution of light energy consumed or wasted at various steps shown as the ratio of incident light falling on a typical leaf directly.

plant productivity for example of a crop through a whole year, many other wastages are immediately apparent. Thus, there are times when land is lying barren without any plant cover and even if the land is fertile, most crops are seasonal and it is rather a short span of time when a plant has a fully developed leaf surface. Further, in a crop plant, not every part is edible or useful. If we consider only the usable parts, the efficiency becomes still lower. But then all tracts are not fertile and if we consider the drought affected areas and the deserts — on very large tracts of the surface of the earth — there is no plant cover to intercept sunlight.

Of the various types of plants, the maximum efficiency is attained by trees (5%), because they provide a permanent cover in terms of leaf surface. For crops, the efficiency calculated for a season or on an yearly basis is much lower, of the order of only 1-2% or so. However, under the best conditions of irrigation, optimum use of fertilisers, and if photosynthesis is measured over a short period of time, the efficiency may reach up to 12% which is about one-third of the maximal theoretical

efficiency. Such levels of efficiency have, however, been possible in countries like Switzerland and Denmark, with advanced crop husbandry techniques. But the world average is much lower.

Many estimates have been made of photosynthetic productivity considered in the world as a whole. The total photosynthetically active radiation available is around 0.17%, equivalent to energy capable of fixing 0.4×10^{11} tons of carbon per annum. One important point to know is that perhaps more than about one-third of the total photosynthesis is carried out in oceans. The average efficiency of photosynthesis in oceans is lower (almost half as much as on land) on a per square meter basis. However, since oceans occupy nearly two-third of the surface of the earth, their total contribution is quite significant.

13.9.2 Environment and Photosynthesis

We turn now to the question as to how man can have increased productivity via photosynthesis, in view of the fact that the world productivity is much lower than attainable theoretically. One of the pressing needs of agriculture is improvement of the microenvironment.

Many studies have been made of the role of environmental factors affecting photosynthesis. One of the most critical factors is CO_2 concentration which is only 0.03% in natural atmosphere. Blackman's experiments showed that if CO_2 concentration is increased, the photosynthetic rate increases, and even 10-20 fold higher concentration of CO_2 is by no means injurious to plants, at least in the short-term. Unfortunately, however, there is not much that can be done to increase the CO_2 concentration in large tracts of agricultural land or of forest areas, though CO_2 concentration is rising in the atmosphere because of use of fossil fuels and industrial activity. Very low or high temperatures, too, limit photosynthesis : whereas high temperature results in photobleaching and destruction of chlorophyll, low temperatures, although not destructive slow down the thermochemical dark reactions. Similarly, although water too seriously limits photosynthesis in many regions of the world (and stomates often close during the day preventing CO_2 from entering the leaf cell), again not much can be done. Irrigation can certainly help and schemes like Indira Gandhi Canal in the Western Rajasthan desert is fast changing the scenario converting barren lands into rich fields of bumper crops. Other better husbandry practices and better way of nutrition especially of nitrogen and phosphorus which are limiting in the environment, if corrected, can markedly improve overall photosynthesis. Another important research areas could concern with improvement of plant architecture, structure of canopy so that the light interception is more efficient. The erect leaves are also better than those that stretch out horizontally. One could also have dwarf varieties as in wheat since such varieties tend to have higher chlorophyll in the ears as compared to stems and stalks, other than the obvious advantage the dwarfs have. The second area of improvement is development of early maturing varieties so that over the years there is higher proportion of plant cover on the earth.

13.9.3 Agricultural Biotechnology

It would seem from the foregoing discussion that there is little scope for increasing efficiency of photosynthesis under existing conditions, unless the environment itself is improved in terms of increasing water supply or providing fertilisers etc. A question that we ask now is can we do anything to adapt a plant better to existing conditions. There is much hope that, with the rise of modern molecular biology and biotechnology, plants can indeed be modified such that the efficiency of photosynthesis or the yield can be increased in a permanent way.

At this point some brief remarks are appropriate on the molecular biology of chloroplast, also from the viewpoint of fundamental knowledge. Work in the sixties and seventies has shown that the chloroplasts, as also the mitochondria, have not only their own DNA, but they also have RNA and protein synthesising machinery. However, the size of chloroplast DNA is relatively small and is just enough to code for about 100 genes. The actual number of proteins that exist in chloroplasts is much larger, in thousands — and a great majority of the proteins therefore are encoded by the nucleus. There are, in fact, many interesting cases, for example in case of multi-subunit proteins or enzymes (such as Rubisco), where some of the polypeptides are encoded by the nucleus and others by the chloroplast, which demands a very high degree of synchronisation between the two cellular constituents. The unique origin

of such proteins provides an excellent opportunity for studying the biochemistry of such interaction and general regulation of chloroplast development. The various genes are gradually being identified both in the chloroplast and the nuclear genome. Since the chloroplast genome is smaller, it has been the focus of much work. In fact, it has been recently completely sequenced by Sugiura and co-workers in Japan in certain plants, namely *Marchantia,* tobacco and rice and most of the genes already identified. The work on nuclear genes encoding chloroplast proteins is now just beginning. When the important genes have been identified, one can expect research to modify them. How to make them more efficient, however, remains a task for the future.

From an agricultural viewpoint, one could give a few exmaples of future directions of research. One can possibly make Rubisco more efficient such that it does not accept oxygen and thus the process of photorespiration is avoided altogether. One could even attempt to transfer characteristics of C_4 plants into C_3 such as an active PEP carboxylase. Thinking of still other ways of enhancing photosynthesis perhaps one could engineer chloroplasts to live longer or divide more rapidly. Perhaps one could change the metabolic pathways so that one can enhance the synthesis of amino acids rather than largely manufacture carbohydrates. This modification can be of far-reaching consequence in management of global nutrition. Also, one could engineer plants resistant to herbicides by altering proteins in chloroplasts (which are often the target sites for their action), and without impairing electron transfer functions. Some genetically engineered herbicide resistant crops have in fact already been developed in the West, and it is likely to be big business.

The techniques for transferring foreign genes into nuclei are already will-established and, when nuclear-encoded genes are involved, their modification should not be too difficult. However, the stable transformation of the chloroplast genome poses a more serious challenge since the usual vectors — such as the Ti plasmid — do not stably integrate into chloroplast genome. Another difficulty is that the chloroplast genome is very compactly organised — often two genes reside in the same region of DNA and transcribe in opposite directions. It may therefore, be difficult to insert genes in the chloroplast without inactivating some already existing useful gene, even though some success in transferring a gene — purely as a test case, for example to correct a gene deliberately mutated — directly in chloroplasts by the particle gun technique has been reported. Perhaps a more useful strategy will be to transfer a required gene in the nucleus in such a way that the protein product ultimately finds its way into the chloroplast. For this, one can attach a region of DNA coding for signal polypeptides, which have the ability to lead a polypeptide chain smoothly into the chloroplast. In fact, most of the nuclear-encoded chloroplast polypeptides have some kind of a signal polypeptide chain attached to them, which makes it possible for them to enter the chloroplast. Man could adopt this strategy for *directed* genetic changes.

13.10 EVOLUTIONARY ASPECTS OF THE CHLOROPLAST

Finally, one can touch briefly on the evolutionary aspect of the photosynthetic process and of chloroplast itself. It is believed that photosynthesis preceded aerobic respiration. According to current evidence, in the primitive world there was not much of free oxygen. Organisms survived by virtue of anaerobic respiration, and gradually thus there was accumulation of carbon dioxide in the atmosphere. It is only then that photosynthesis started. Finally, when O_2 started accumulating in the atmosphere, aerobic respiration evolved.

Another fact of great interest is that the modern-day chloroplast probably represents a prokaryotic unicellular alga which invaded ordinary non-photosynthetic eukaryotic cells. There are indeed many pieces of evidence pointing towards a prokaryotic origin of chloroplasts e.g. the circularity of the chloroplast genome, existence of smaller ribosomes such as in bacteria, or of an extra set of tRNAs unique to this organelle. When the unicellular alga became an endo-symbiont, most of the genes (not essential any more for corporate existence) were gradually lost; and others — still essential — gradually moved to the nucleus. Some evidence that genes are still in the process of movement comes from a study of some lower plants. Here certain genes have been found in chloroplasts which have moved to the nucleus in the higher land plants. It appears that it is basically the genes for the photochemical machinery which continue

to reside in the chloroplast. Perhaps this is because proteins are highly hydrophobic and it is thus easier to synthesise and assemble them in the thylakoid machinery rather than move them through the aqueous environment of the cytoplasm.

13.11 CONCLUSIONS

To conclude, photosynthesis is the most basic of all phenomena in the living world. Photosynthesis is a subject which has attracted the attention of the scientists from all disciplines such as from physics, chemistry, biochemistry, molecular biology, genetics, botany, zoology, and evolution. A great deal has been learnt and the outline of the process is now sufficiently clear. Despite so much work, however, the real structure of the photosystems, and the placement of chlorophyll and other constituents, remains uncertain. This remains a task for the future.

Biotechnology and genetic engineering have opened up a new approach towards increasing yield. However, increasing efficiency of the plants by these techniques remains, also, a task for the future since we need to know a lot more about the molecular biology of chloroplasts, before we can intelligently modify the photosynthetic machinery to our advantage. What is certain is that because of the rich interaction of physics, chemistry, biochemistry and molecular biology, photosynthesis will continue to remain a subject to great interest of scientists for decades to come.

13.12 SUMMARY

- Photosynthesis is the process by which carbon dioxide and water are assimilated into carbohydrates with the help of energy of sunlight. The reaction is carried out by all green plants including the algae and is of the greatest significance — since not only animals derive their food through this process, but also our energy requirements are largely met by fossil fuels like coal and petroleum.
- The photosynthetic process is known to consist of two types of reactions — the light and the dark reactions. In the former, the key role is played by four pigments — chl *a*, chl *b*, xanthophylls and carotenoids — all located in thylakoid membranes of chloroplast. Carotenoids and xanthophylls absorb the blue region of the spectrum whereas chlorophylls absorb both blue and red regions. Since chl *a* absorbs light of the longest wavelength, it can collect the energy captured by all other pigments. From chl *a* the energy is transferred finally to a special form of chl *a*.
- The capture of photons by chlorophyll pigments ultimately leads to the photolysis of the water molecule resulting in the release of electrons, protons and oxygen. Splitting of a water molecule follows ionization of the special chl *a* molecule, since on release of the electron chl *a* becomes positively charged (chl a^+). An electron is regained from water; the chl *a* molecule, therefore, acts as a pump that drives electrons from H_2O to reduce CO_2 and synthesise carbohydrates. The energy for driving the pump comes from sunlight. The common electron carrier $NADP^+$ serves as the final acceptor of electrons (and it is through NADPH that reduction of CO_2 occurs).
- In reality, the transfer of electrons from H_2O to NADPH is mediated by two separate photochemical reactions through two sets of pigment molecules organised in the form of photosynthetic units, representing the PS I and PS II. Two special kinds of chl *a* molecules exist in these photosystems. The special chl *a* molecule in PS II maximally absorbs at 680 nm and is called P_{680} whereas the special chl *a* molecule in PS I absorbs at 700 nm and is called P_{700}. Transfer of light energy to P_{680} and P_{700} is greatly facilitated by the existence of antennae attached to the photosystems and each of which comprises about 250-300 chlorophyll molecules bound in special pigment-protein complexes. Photolysis of water takes place in PS II and the electron is transferred via a cytochrome b_6/f complex, to complex, to PS I. Here, an electron can gain further energy by absorbing energy from an additional photon and then reach $NADP^+$ to reduce it to NADPH.
- To drive the dark reactions of photosynthesis, apart from NADPH, ATP is also necessary. Since photosynthesis proceeds at a much faster rate than respiration, green plants are able to synthesise ATP by a process known as photophos-

phorylation, independent of oxidative phosphorylation carried on in mitochondria. This is made possible by the release of protons during splitting of H_2O, and which results in the generation of a proton gradient (with a high concentration of H^+ in the luminal space of thylakoids). As the gradient dissipates — by the movement of protons through the channel of an ATPase complex outside the stroma — ATP is synthesised by the "chemiosmotic" mechanism.

- The fixation of carbon dioxide occurs in most plants via the Calvin cycle through ribulose bisphosphate carboxylase. Ribulose bisphosphate (RuBP, a 5-carbon sugar) serves as the primary acceptor. Since the intermediate is unstable, the first stable product is a 3-carbon compound, phosphoglyceric acid, which is reduced to phosphoglyceraldehyde with the help of NADPH generated in the photochemical reactions. Two molecules of phosphoglyceraldehyde combine to form a molecule of glucose by reversal of reactions in glycolysis. Some of the phosphoglyceraldehyde molecules, however, serve to regenerate the acceptor, RuBP, through reactions of the Calvin cycle.

- The C_4 plants are a special group of plants (mainly represented by the grasses) where the primary acceptor of carbon dioxide is a 3-carbon acid, phosphoenol pyruvic acid, and the first product is a 4-carbon compound, oxaloacetic acid, which is reduced to malic acid. The enzyme which fixes CO_2 in these plants is called PEP-carboxylase. Since this enzyme has higher affinity of CO_2 (than RuBP carboxylase), the C_4 plants carry out photosynthesis more efficiently. The 4-carbon acids cannot, however, be converted to carbohydrates directly. Instead, the fixed CO_2 reenters the Calvin cycle by a reaction which is still not understood properly. But, it is well established that in a typical monocot leaf, the outer cells have PEP carboxylase; on the other hand, the bundle sheath cells — more centrally located — have RuBP carboxylase. The organic acids are believed to be transported to bundle sheath cells, when the CO_2 is released to be refixed by the C_3 cycle. The CAM plants (a group to which cacti and many other succulents belong) essentially constitute a variant group of C_4 plants in which stomates open at night to allow CO_2 fixation by PEP-carboxylase whereas the process of reentry of CO_2 into Calvin cycle and reduction of PGA to phosphoglyceraldehyde by NADPH takes place during the day.

- The enzyme RuBP carboxylase, is unable to discriminate totally CO_2 from O_2, leading to an oxygenase activity and photorespiration. Often, therefore, instead of two molecules of PGA only one molecule of PGA is formed — the other product being a molecule of 2-carbon glycolic acid. Two molecules of glycolic acid can be recycled to yield another molecule of phosphoglyceric acid with the loss of molecule of CO_2. However, because of the more efficient process of CO_2 fixation in C_4 plants, any CO_2 released in light is wholly recaptured by PEP-carboxylase. Hence, they do not show photorespiration. One of the goals researchers have is to convert C_3 plants into C_4 plants and eliminate photorespiration so as to conserve fixed carbon lost during the process.

- In recent years, the techniques of electron microscopy and X-ray diffraction crystallography have allowed us to understand the structure of the photosynthetic machinery in surprising detail. The photosynthetic units — originally proposed on purely theoretical grounds — can be seen in electron micrographs. In bacteria even a detailed model of the reaction centre of the photosynthetic machinery has become recently available. It is expected that in the near future, a detailed model will become available also of the organisation of the photosynthetic machinery in higher plants.

- The area of molecular biology of chloroplast is also developing at a rapid rate and not only proteins, but each gene related to photosynthesis is being identified. Simultaneously, genetic engineering techniques to transform the photosynthetic machinery in chloroplasts are also being developed and it is possible that photosynthesis can be made more efficient in future in selected economically useful plants.

- Finally, the origin of the chloroplast is very intriguing subject from the viewpoint of evolution and taxonomy. It is widely believed that chloroplasts represent primitive prokaryotic cells which were capable of carrying out photosynthesis and which got entrapped in the eukaryotic cell giving rise to higher plants such as we know today.

13.13 TERMINAL QUESTIONS

1. The photochemical reactions in grana capture light energy and convert it into chemical energy as ATP and NADPH. What is the necessity of carbon fixation?

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2. Give the raw materials and end-products of the following reactions of photosynthesis.

Photosynthesis Reactions	Raw Material	End-Product
i) PS I		
ii) PS I followed by electron transport		
iii) PS II		
iv) PS II followed by electron transport		
v) Cyclic phosphorylation		
vi) Carbon fixation		

3. Compare photorespiration and mitochondrial respiration.
(**Hint** : Compare with respect to substrate, enzyme, waste products, gain of energy and loss of carbon.)

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13.14 ANSWERS

Self-assessment Questions

1) a) i) d, ii) c, iii) b, iv) a

2) a) i) Determination of relative photochemical efficiency of different wavelength of visible spectrum. It closely matched with the absorption spectrum of chlorophyll.

ii) Exposure of portions of algal filament to different wavelengths and measurement of photochemical efficiency (O_2 evolution was measured by differential accumulation of aerobic motile bacteria on the filament).

b) The colour of an object is due to the light it reflects. The three organisms — purple sulphur bacteria, blue green algae and leaves of barley reflect purple, blue-green and green light respectively and hence they appear purple, blue-green and green. They absorb the colours of visible spectrum other than the colours they reflect. Since the action spectrum and absorption spectrum generally overlap, therefore, the spectra represent a) leaves, b) blue green algae, and c) purple sulphur bacteria.

3) a) i) The value of Q_{10} was more than 1.

ii) Splitting of water, formation of ATP and reducing power.

b) i) Photolysis, H_2O, Van Niel, sulphur ii) H_2O, O^{18}

iii) $NADP^+$, NADPH, ATP, ADP, Pi. iv) Hill reaction

4) a) 1) Quantum requirement (number of light quanta required for each molecule of O_2 produced was 8 instead of 4.

2) The quantum yield drops at higher wavelengths (red region) but it is abolished when a beam of shorter wavelength was simultaneously given.

b) i) one, two
ii) electron donor
iii) antenna complex, reaction centre chl *a*
iv) 680, 700, $NADP^+$

c) i) drops, ii) enhancement, iii) lower

d) i) F, ii) F, iii) T, iv) F, v) T, vi) F

5) a) i) Radioactive, ii) Radioautography, iii) molecular dissection, iv) Geiger-Müller

b) i) PGA, ii) RuBP, iii) Glucose, iv) 18, v) 12, vi) 12, vii) one

6) a) i) Light, ii) RuBP, iii) higher concentration of O_2 in leaves, iv) high intensity of light, v) C_3 plants, vi) peroxisomes

b) i) c, ii) a, iii) c, iv) b, v) a

c) i) phosphoenol pyruvate ii) pyruvate carboxylase
iii) ATP iv) chloroplasts
v) C_3 pathway, bundle sheath cells vi) RuBP carboxylase
vii) network, membranes viii) photosynthetically more efficien

7) i) b, ii) a, iii) c, iv) c, v) c, vi) d, vii) c, viii) a, ix) a, x) f,

Terminal Questions

1) Firstly, the storage of energy in the form of carbohydrates is much more convenient and lot more energy can be stored in this form. Secondly, carbon skeleton of carbohydrates is needed for various biosynthesis.

2)

	Photochemical Reaction	Raw Material	End Product
i)	Excitation of PS I	$h\nu$ + light harvesting pigment complexes + P_{700}	excited electrons
ii)	PS I followed by electron transport	$h\nu$ + (light harvesting pigment complexes + P_{700}) + $NADP^+$	NADPH
iii)	Excitation of PS II	$h\nu$ + light harvesting pigment complexes, P_{680}	electrons
iv)	Excitation of PS II followed by electron transport	$h\nu$ + light harvesting pigment complexes + P_{680} + ADP + Pi	ATP + electrons
v)	Cyclic photo-phosphorylation	H^+ reservoir, ADP + Pi	ATP
vi)	Carbon fixation	RuBP + CO_2 + ATP + NADPH	Sugars + ADP + Pi + $NADP^+$

3)

Item	Respiration	Photorespiration
Substrate	Carbohydrates, fats, proteins or their monomeric units + O_2	RuBP
Enzymes	Various enzymes of glycolysis, TCA cycle, electron transfer chain	RuBP carboxylase/ oxygenase
Loss	Loss of carbon	Loss of carbon
Gain	Energy 36 ATP/glucose	No ATP
Waste products	CO_2, H_2O	CO_2, NH_3