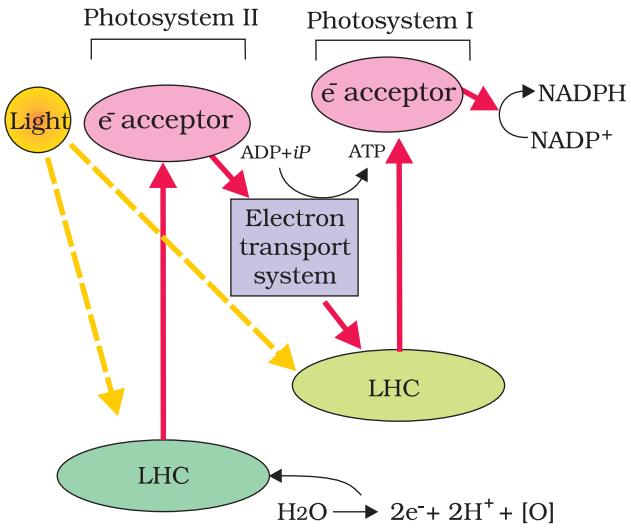

Figure 13.5 Z scheme of light reaction

system consisting of cytochromes (Figure 13.5). This movement of electrons is downhill, in terms of an oxidation-reduction or redox potential scale. The electrons are not used up as they pass through the electron transport chain, but are passed on to the pigments of photosystem PS I. Simultaneously, electrons in the reaction centre of PS I are also excited when they receive red light of wavelength 700 nm and are transferred to another accepter molecule that has a greater redox potential. These electrons then are moved downhill again, this time to a molecule of energy-rich $NADP^+$. The addition of these electrons reduces $NADP^+$ to NADPH + H^+. This whole scheme of transfer of electrons, starting from the PS II, uphill to the acceptor, down the electron transport chain to PS I, excitation of electrons, transfer to another acceptor, and finally down hill to $NADP^+$ causing it to be reduced to NADPH + H^+ is called the **Z scheme**, due to its characterstic shape (Figure 13.5). This shape is formed when all the carriers are placed in a sequence on a redox potential scale.

13.6.1 Splitting of Water

You would then ask, *How does PS II supply electrons continuously?* The electrons that were moved from photosystem II must be replaced. This is achieved by electrons available due to splitting of water. The splitting of water is associated with the PS II; water is split into H^+, [O] and electrons. This creates oxygen, one of the net products of photosynthesis. The electrons needed to replace those removed from photosystem I are provided by photosystem II.

$$2H_2O \longrightarrow 4H^+ + O_2 + 4e^-$$

We need to emphasise here that the water splitting complex is associated with the PS II, which itself is physically located on the inner side of the membrane of the thylakoid. *Then, where are the protons and O_2 formed likely to be released – in the lumen? or on the outer side of the membrane?*

13.6.2 Cyclic and Non-cyclic Photo-phosphorylation

Living organisms have the capability of extracting energy from oxidisable substances and store this in the form of bond energy. Special substances like ATP, carry this energy in their chemical bonds. The process through which

ATP is synthesised by cells (in mitochondria and chloroplasts) is named phosphorylation. Photo-phosphorylation is the synthesis of ATP from ADP and inorganic phosphate in the presence of light. When the two photosystems work in a series, first PS II and then the PS I, a process called non-cyclic photo-phosphorylation occurs. The two photosystems are connected through an electron transport chain, as seen earlier – in the Z scheme. Both ATP and NADPH + H^+ are synthesised by this kind of electron flow (Figure 13.5).

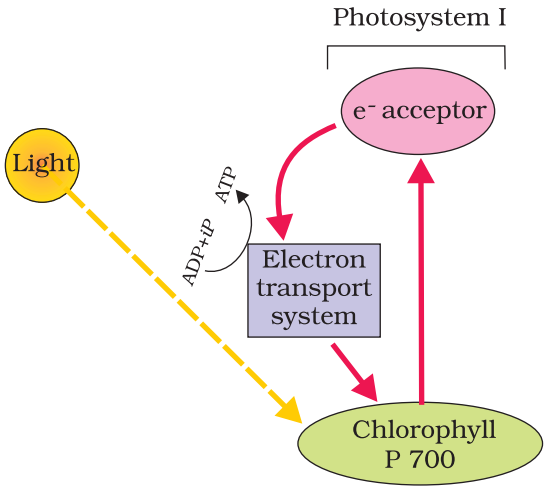

Figure 13.6 Cyclic photophosphorylation

When only PS I is functional, the electron is circulated within the photosystem and the phosphorylation occurs due to cyclic flow of electrons (Figure 13.6). A possible location where this could be happening is in the stroma lamellae. While the membrane or lamellae of the grana have both PS I and PS II the stroma lamellae membranes lack PS II as well as NADP reductase enzyme. The excited electron does not pass on to $NADP^+$ but is cycled back to the PS I complex through the electron transport chain (Figure 13.6). The cyclic flow hence, results only in the synthesis of ATP, but not of NADPH + H^+. Cyclic photophosphorylation also occurs when only light of wavelengths beyond 680 nm are available for excitation.

13.6.3 Chemiosmotic Hypothesis

Let us now try and understand how actually ATP is synthesised in the chloroplast. The chemiosmotic hypothesis has been put forward to explain the mechanism. Like in respiration, in photosynthesis too, ATP synthesis is linked to development of a proton gradient across a membrane. This time these are membranes of the thylakoid. There is one difference though, here the proton accumulation is towards the inside of the membrane, i.e., in the lumen. In respiration, protons accumulate in the intermembrane space of the mitochondria when electrons move through the ETS (Chapter 14).

Let us understand what causes the proton gradient across the membrane. We need to consider again the processes that take place during the activation of electrons and their transport to determine the steps that cause a proton gradient to develop (Figure 13.7).

(a) Since splitting of the water molecule takes place on the inner side of the membrane, the protons or hydrogen ions that are produced by the splitting of water accumulate within the lumen of the thylakoids.

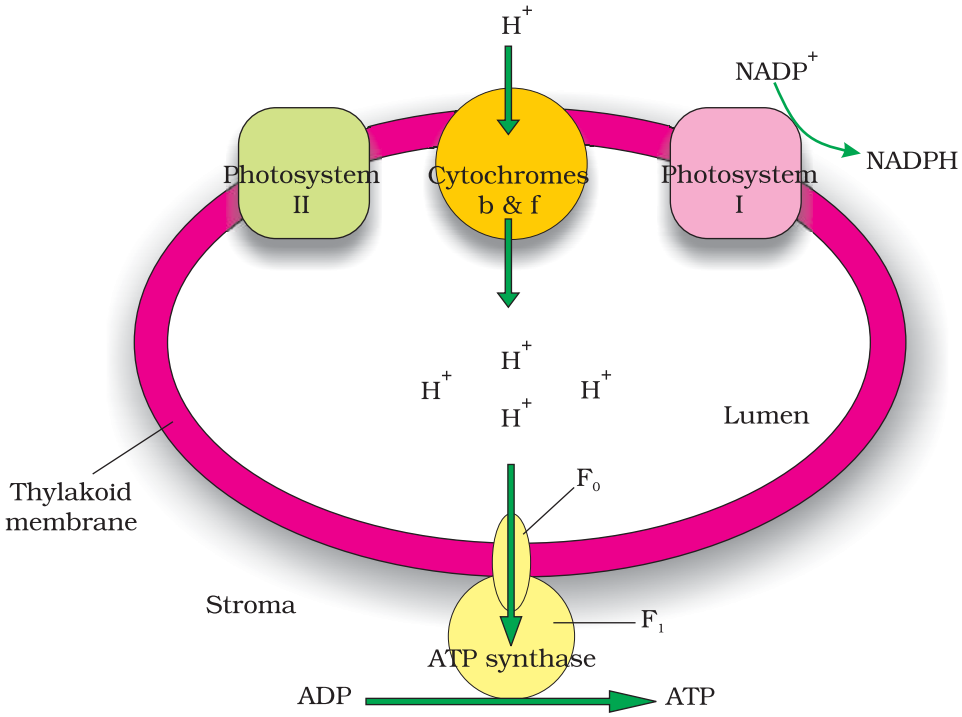

Figure 13.7 ATP synthesis through chemiosmosis

(b) As electrons move through the photosystems, protons are transported across the membrane. This happens because the primary accepter of electron which is located towards the outer side of the membrane transfers its electron not to an electron carrier but to an H carrier. Hence, this molecule removes a proton from the stroma while transporting an electron. When this molecule passes on its electron to the electron carrier on the inner side of the membrane, the proton is released into the inner side or the lumen side of the membrane.

(c) The NADP reductase enzyme is located on the stroma side of the membrane. Along with electrons that come from the acceptor of electrons of PS I, protons are necessary for the reduction of $NADP^+$ to $NADPH + H^+$. These protons are also removed from the stroma.

Hence, within the chloroplast, protons in the stroma decrease in number, while in the lumen there is accumulation of protons. This creates a proton gradient across the thylakoid membrane as well as a measurable decrease in pH in the lumen.

Why are we so interested in the proton gradient? This gradient is important because it is the breakdown of this gradient that leads to release of energy. The gradient is broken down due to the movement of protons across the membrane to the stroma through the transmembrane channel

of the F_0 of the ATPase. The ATPase enzyme consists of two parts: one called the F_0 is embedded in the membrane and forms a transmembrane channel that carries out facilitated diffusion of protons across the membrane. The other portion is called F_1 and protrudes on the outer surface of the thylakoid membrane on the side that faces the stroma. The break down of the gradient provides enough energy to cause a conformational change in the F_1 particle of the ATPase, which makes the enzyme synthesise several molecules of energy-packed ATP.

Chemiosmosis requires a membrane, a proton pump, a proton gradient and ATPase. Energy is used to pump protons across a membrane, to create a gradient or a high concentration of protons within the thylakoid lumen. ATPase has a channel that allows diffusion of protons back across the membrane; this releases enough energy to activate ATPase enzyme that catalyses the formation of ATP.

Along with the NADPH produced by the movement of electrons, the ATP will be used immediately in the biosynthetic reaction taking place in the stroma, responsible for fixing CO_2, and synthesis of sugars.

13.7 WHERE ARE THE ATP AND NADPH USED?

We learnt that the products of light reaction are ATP, NADPH and O_2. Of these O_2 diffuses out of the chloroplast while ATP and NADPH are used to drive the processes leading to the synthesis of food, more accurately, sugars. This is the **biosynthetic phase** of photosynthesis. This process does not directly depend on the presence of light but is dependent on the products of the light reaction, i.e., ATP and NADPH, besides CO_2 and H_2O. You may wonder how this could be verified; it is simple: immediately after light becomes unavailable, the biosynthetic process continues for some time, and then stops. If then, light is made available, the synthesis starts again.

Can we, hence, say that calling the biosynthetic phase as the ***dark reaction*** *is a misnomer? Discuss this amongst yourselves.*

Let us now see how the ATP and NADPH are used in the biosynthetic phase. We saw earlier that CO_2 is combined with H_2O to produce $(CH_2O)_n$ or sugars. It was of interest to scientists to find out how this reaction proceeded, or rather what was the first product formed when CO_2 is taken into a reaction or fixed. Just after world war II, among the several efforts to put radioisotopes to beneficial use, the work of Melvin Calvin is exemplary. The use of radioactive ^{14}C by him in algal photosynthesis studies led to the discovery that the first CO_2 fixation product was a 3-carbon organic acid. He also contributed to working out the complete biosynthetic pathway; hence it was called **Calvin cycle** after him. The first product identified was **3-phosphoglyceric** acid or in short **PGA**. *How many carbon atoms does it have?*

Scientists also tried to know whether all plants have PGA as the first product of CO_2 fixation, or whether any other product was formed in other plants. Experiments conducted over a wide range of plants led to the discovery of another group of plants, where the first stable product of CO_2 fixation was again an organic acid, but one which had 4 carbon atoms in it. This acid was identified to be **oxaloacetic acid** or OAA. Since then CO_2 assimilation during photosynthesis was said to be of two main types: those plants in which the first product of CO_2 fixation is a C_3 acid (PGA), i.e., the **C_3 pathway,** and those in which the first product was a C_4 acid (OAA), i.e., the **C_4 pathway**. These two groups of plants showed other associated characteristics that we will discuss later.

13.7.1 The Primary Acceptor of CO_2

Let us now ask ourselves a question that was asked by the scientists who were struggling to understand the 'dark reaction'. *How many carbon atoms would a molecule have which after accepting (fixing) CO_2, would have 3 carbons (of PGA)?*

The studies very unexpectedly showed that the acceptor molecule was a 5-carbon ketose sugar – ribulose bisphosphate (RuBP). *Did any of you think of this possibility?* Do not worry; the scientists also took a long time and conducted many experiments to reach this conclusion. They also believed that since the first product was a C_3 acid, the primary acceptor would be a 2-carbon compound; they spent many years trying to identify a 2-carbon compound before they discovered the 5-carbon RuBP.

13.7.2 The Calvin Cycle

Calvin and his co-workers then worked out the whole pathway and showed that the pathway operated in a cyclic manner; the RuBP was regenerated. Let us now see how the Calvin pathway operates and where the sugar is synthesised. Let us at the outset understand very clearly that the Calvin pathway occurs in **all photosynthetic plants**; it does not matter whether they have C_3 or C_4 (or any other) pathways (Figure 13.8).

For ease of understanding, the Calvin cycle can be described under three stages: carboxylation, reduction and regeneration.

1. **Carboxylation** – Carboxylation is the fixation of CO_2 into a stable organic intermediate. Carboxylation is the most crucial step of the Calvin cycle where CO_2 is utilised for the carboxylation of RuBP. This reaction is catalysed by the enzyme RuBP carboxylase which results in the formation of two molecules of 3-PGA. Since this enzyme also has an oxygenation activity it would be more correct to call it RuBP carboxylase-oxygenase or **RuBisCO**.

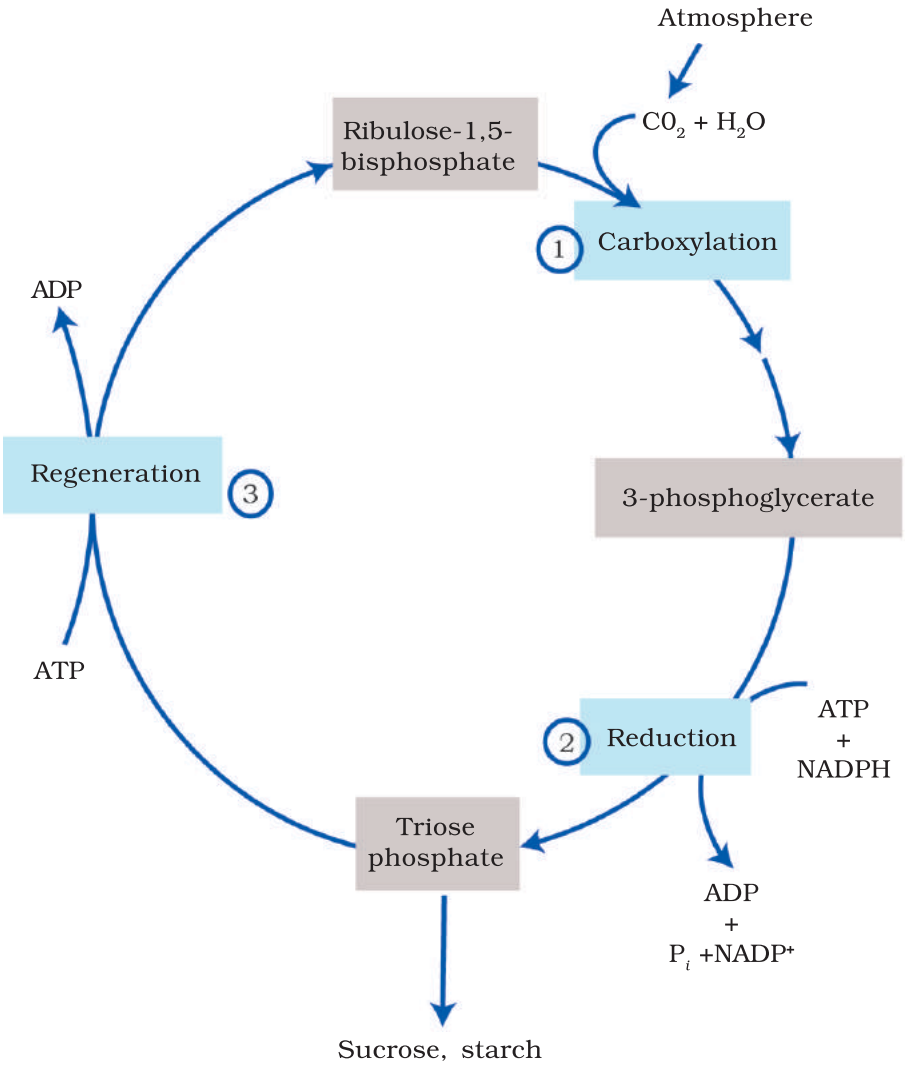

Figure 13.8 The Calvin cycle proceeds in three stages : (1) carboxylation, during which CO_2 combines with ribulose-1,5-bisphosphate; (2) reduction, during which carbohydrate is formed at the expense of the photochemically made ATP and NADPH; and (3) regeneration during which the CO_2 acceptor ribulose-1,5-bisphosphate is formed again so that the cycle continues

2. **Reduction** – These are a series of reactions that lead to the formation of glucose. The steps involve utilisation of 2 molecules of ATP for phosphorylation and two of NADPH for reduction per CO_2 molecule fixed. The fixation of six molecules of CO_2 and 6 turns of the cycle are required for the removal of one molecule of glucose from the pathway.

3. **Regeneration** – Regeneration of the CO_2 acceptor molecule RuBP is crucial if the cycle is to continue uninterrupted. The regeneration steps require one ATP for phosphorylation to form RuBP.

Hence for every CO_2 molecule entering the Calvin cycle, 3 molecules of ATP and 2 of NADPH are required. It is probably to meet this difference in number of ATP and NADPH used in the dark reaction that the cyclic phosphorylation takes place.

To make one molecule of glucose 6 turns of the cycle are required. *Work out how many ATP and NADPH molecules will be required to make one molecule of glucose through the Calvin pathway.*

It might help you to understand all of this if we look at what goes in and what comes out of the Calvin cycle.

In	Out
Six CO_2	One glucose
18 ATP	18 ADP
12 NADPH	12 NADP

13.8 The C_4 Pathway

Plants that are adapted to dry tropical regions have the C_4 pathway mentioned earlier. Though these plants have the C_4 oxaloacetic acid as the first CO_2 fixation product they use the C_3 pathway or the Calvin cycle as the main biosynthetic pathway. Then, in what way are they different from C_3 plants? This is a question that you may reasonably ask.

C_4 plants are special: They have a special type of leaf anatomy, they tolerate higher temperatures, they show a response to high light intensities, they lack a process called photorespiration and have greater productivity of biomass. Let us understand these one by one.

Study vertical sections of leaves, one of a C_3 plant and the other of a C_4 plant. *Do you notice the differences? Do both have the same types of mesophylls? Do they have similar cells around the vascular bundle sheath?*

The particularly large cells around the vascular bundles of the C_4 pathway plants are called **bundle sheath cells**, and the leaves which have such anatomy are said to have **'Kranz' anatomy**. 'Kranz' means 'wreath' and is a reflection of the arrangement of cells. The bundle sheath cells may form **several layers** around the vascular bundles; they are characterised by having a large number of chloroplasts, thick walls impervious to gaseous exchange and no intercellular spaces. You may like to cut a section of the leaves of C_4 plants – maize or sorghum – to observe the Kranz anatomy and the distribution of mesophyll cells.

It would be interesting for you to collect leaves of diverse species of plants around you and cut vertical sections of the leaves. Observe under the microscope – look for the bundle sheath around the vascular bundles. The presence of the bundle sheath would help you identify the C_4 plants.

Now study the pathway shown in Figure 13.9. This pathway that has been named the Hatch and Slack Pathway, is again a cyclic process. Let us study the pathway by listing the steps.

The primary CO_2 acceptor is a 3-carbon molecule **phosphoenol pyruvate (PEP)** and is present in the mesophyll cells. The enzyme responsible for this fixation is **PEP carboxylase** or PEPcase. It is important to register that the mesophyll cells lack RuBisCO enzyme. The C_4 acid OAA is formed in the mesophyll cells.

It then forms other 4-carbon compounds like malic acid or aspartic acid in the mesophyll cells itself, which are transported to the bundle sheath cells. In the bundle sheath cells these C_4 acids are broken down to release CO_2 and a 3-carbon molecule.

The 3-carbon molecule is transported back to the mesophyll where it is converted to PEP again, thus, completing the cycle.

The CO_2 released in the bundle sheath cells enters the C_3 or the Calvin pathway, a pathway common to all plants. The bundle sheath cells are

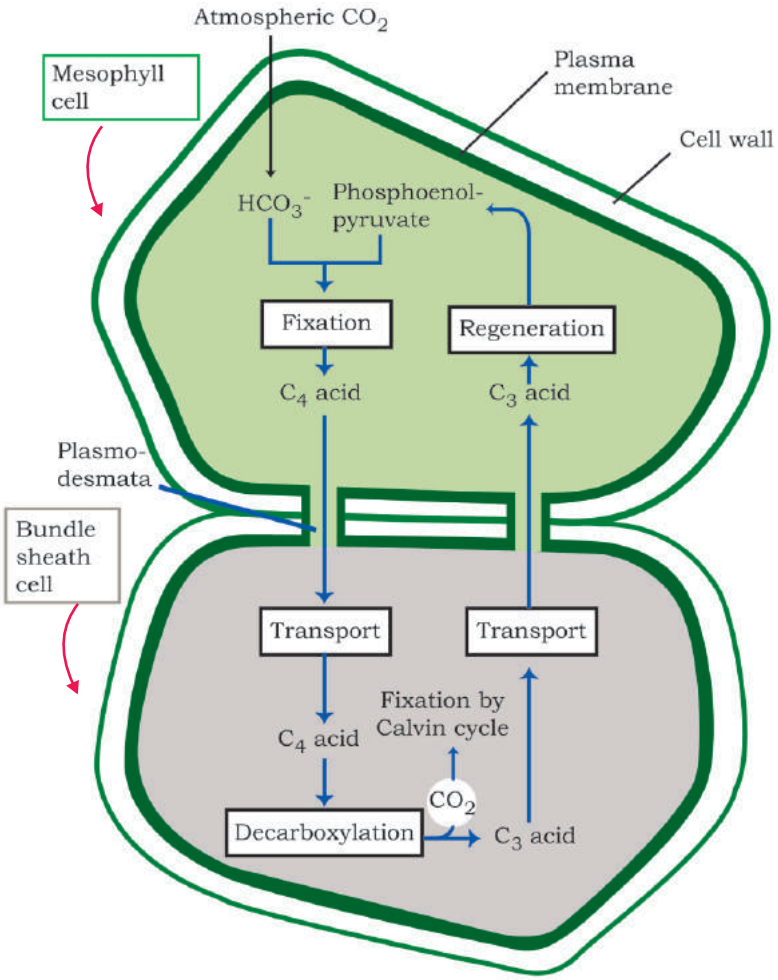

Figure 13.9 Diagrammatic representation of the Hatch and Slack Pathway

rich in an enzyme Ribulose bisphosphate carboxylase-oxygenase **(RuBisCO)**, but lack PEPcase. Thus, the basic pathway that results in the formation of the sugars, the Calvin pathway, is common to the C_3 and C_4 plants.

Did you note that the Calvin pathway occurs in all the mesophyll cells of the C_3 plants? In the C_4 plants it does not take place in the mesophyll cells but does so only in the bundle sheath cells.

13.9 PHOTORESPIRATION

Let us try and understand one more process that creates an important difference between C_3 and C_4 plants – **Photorespiration**. To understand photorespiration we have to know a little bit more about the first step of the Calvin pathway – the first CO_2 fixation step. This is the reaction where RuBP combines with CO_2 to form 2 molecules of 3PGA, that is catalysed by RuBisCO.

$$RuBP + CO_2 \xrightarrow{RuBisCo} 2\times 3PGA$$

RuBisCO that is the most abundant enzyme in the world (Do you wonder why?) is characterised by the fact that its active site can bind to both CO_2 and O_2 – hence the name. *Can you think how this could be possible?* RuBisCO has a much greater affinity for CO_2 than for O_2. Imagine what would happen if this were not so! This binding is competitive. It is the relative concentration of O_2 and CO_2 that determines which of the two will bind to the enzyme.

In C_3 plants some O_2 does bind to RuBisCO, and hence CO_2 fixation is decreased. Here the RuBP instead of being converted to 2 molecules of PGA binds with O_2 to form one molecule of phosphoglycerate and phosphoglycolate in a pathway called photorespiration. In the photorespiratory pathway, there is neither synthesis of sugars, nor of ATP. Rather it results in the release of CO_2 with the utilisation of ATP. In the photorespiratory pathway there is no synthesis of ATP or NADPH. Therefore, photorespiration is a wasteful process.

In C_4 plants photorespiration does not occur. This is because they have a mechanism that increases the concentration of CO_2 at the enzyme site. This takes place when the C_4 acid from the mesophyll is broken down in the bundle sheath cells to release CO_2 – this results in increasing the intracellular concentration of CO_2. In turn, this ensures that the RuBisCO functions as a carboxylase minimising the oxygenase activity.

Now that you know that the C_4 plants lack photorespiration, you probably can understand why productivity and yields are better in these plants. In addition these plants show tolerance to higher temperatures.

Based on the above discussion can you compare plants showing the C_3 and the C_4 pathway? Use the table format given and fill in the information.

TABLE 13.1 Fill in the Columns 2 and 3 in this table to highlight the differences between C_3 and C_4 Plants

Characteristics	C_3 Plants	C_4 Plants	Choose from
Cell type in which the Calvin cycle takes place			Mesophyll/Bundle sheath/both
Cell type in which the initial carboxylation reaction occurs			Mesophyll/Bundle sheath /both
How many cell types does the leaf have that fix CO_2.			Two: Bundle sheath and mesophyll One: Mesophyll Three: Bundle sheath, palisade, spongy mesophyll
Which is the primary CO_2 acceptor			RuBP/PEP/PGA
Number of carbons in the primary CO_2 acceptor			5 / 4 / 3
Which is the primary CO_2 fixation product			PGA/OAA/RuBP/PEP
No. of carbons in the primary CO_2 fixation product			3 / 4 / 5
Does the plant have RuBisCO?			Yes/No/Not always
Does the plant have PEP Case?			Yes/No/Not always
Which cells in the plant have Rubisco?			Mesophyll/Bundle sheath/none
CO_2 fixation rate under high light conditions			Low/ high/ medium
Whether photorespiration is present at low light intensities			High/negligible/sometimes
Whether photorespiration is present at high light intensities			High/negligible/sometimes
Whether photorespiration would be present at low CO_2 concentrations			High/negligible/sometimes
Whether photorespiration would be present at high CO_2 concentrations			High/negligible/sometimes
Temperature optimum			30-40 C/20-25C/above 40 C
Examples			Cut vertical sections of leaves of different plants and observe under the microscope for Kranz anatomy and list them in the appropriate columns.

13.10 FACTORS AFFECTING PHOTOSYNTHESIS

An understanding of the factors that affect photosynthesis is necessary. The rate of photosynthesis is very important in determining the yield of plants including crop plants. Photosynthesis is under the influence of several factors, both internal (plant) and external. The plant factors include the number, size, age and orientation of leaves, mesophyll cells and chloroplasts, internal CO_2 concentration and the amount of chlorophyll. The plant or internal factors are dependent on the genetic predisposition and the growth of the plant.

The external factors would include the availability of sunlight, temperature, CO_2 concentration and water. As a plant photosynthesises, all these factors will simultaneously affect its rate. Hence, though several factors interact and simultaneously affect photosynthesis or CO_2 fixation, usually one factor is the major cause or is the one that limits the rate. Hence, at any point the rate will be determined by the factor available at sub-optimal levels.

When several factors affect any [bio] chemical process, Blackman's (1905) **Law of Limiting Factors** comes into effect. This states the following:

If a chemical process is affected by more than one factor, then its rate will be determined by the factor which is nearest to its minimal value: it is the factor which directly affects the process if its quantity is changed.

For example, despite the presence of a green leaf and optimal light and CO_2 conditions, the plant may not photosynthesise if the temperature is very low. This leaf, if given the optimal temperature, will start photosynthesising.

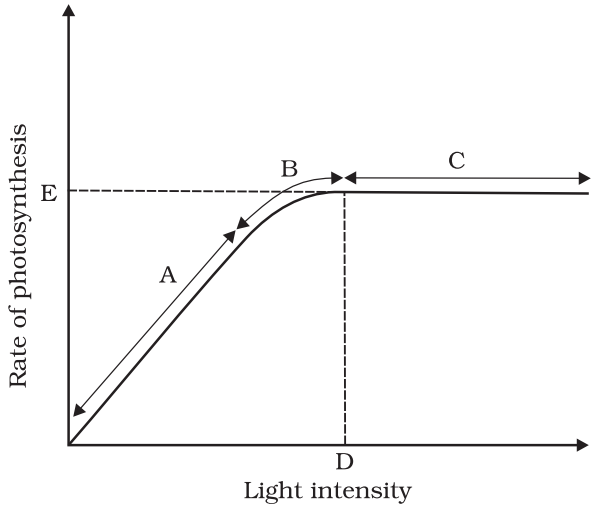

Figure 13.10 Graph of light intensity on the rate of photosynthesis

13.10.1 Light

We need to distinguish between light quality, light intensity and the duration of exposure to light, while discussing light as a factor that affects photosynthesis. There is a linear relationship between incident light and CO_2 fixation rates at low light intensities. At higher light intensities, gradually the rate does not show further increase as other factors become limiting (Figure 13.10). What is interesting to note is that light saturation occurs at 10 per cent of the full sunlight. Hence, except for plants in shade or in dense forests, light is rarely a limiting factor in nature. Increase in

incident light beyond a point causes the breakdown of chlorophyll and a decrease in photosynthesis.

13.10.2 Carbon dioxide Concentration

Carbon dioxide is the major limiting factor for photosynthesis. The concentration of CO_2 is very low in the atmosphere (between 0.03 and 0.04 per cent). Increase in concentration upto 0.05 per cent can cause an increase in CO_2 fixation rates; beyond this the levels can become damaging over longer periods.

The C_3 and C_4 plants respond differently to CO_2 concentrations. At low light conditions neither group responds to high CO_2 conditions. At high light intensities, both C_3 and C_4 plants show increase in the rates of photosynthesis. What is important to note is that the C_4 plants show saturation at about 360 $\mu l L^{-1}$ while C_3 responds to increased CO_2 concentration and saturation is seen only beyond 450 $\mu l L^{-1}$. Thus, current availability of CO_2 levels is limiting to the C_3 plants.

The fact that C_3 plants respond to higher CO_2 concentration by showing increased rates of photosynthesis leading to higher productivity has been used for some greenhouse crops such as tomatoes and bell pepper. They are allowed to grow in carbon dioxide enriched atmosphere that leads to higher yields.

13.10.3 Temperature

The dark reactions being enzymatic are temperature controlled. Though the light reactions are also temperature sensitive they are affected to a much lesser extent. The C_4 plants respond to higher temperatures and show higher rate of photosynthesis while C_3 plants have a much lower temperature optimum.

The temperature optimum for photosynthesis of different plants also depends on the habitat that they are adapted to. Tropical plants have a higher temperature optimum than the plants adapted to temperate climates.

13.10.4 Water

Even though water is one of the reactants in the light reaction, the effect of water as a factor is more through its effect on the plant, rather than directly on photosynthesis. Water stress causes the stomata to close hence reducing the CO_2 availability. Besides, water stress also makes leaves wilt, thus, reducing the surface area of the leaves and their metabolic activity as well.

SUMMARY

Green plants make their own food by photosynthesis. During this process carbon dioxide from the atmosphere is taken in by leaves through stomata and used for making carbohydrates, principally glucose and starch. Photosynthesis takes place only in the green parts of the plants, mainly the leaves. Within the leaves, the mesophyll cells have a large number of chloroplasts that are responsible for CO_2 fixation. Within the chloroplasts, the membranes are sites for the light reaction, while the chemosynthetic pathway occurs in the stroma. Photosynthesis has two stages: the light reaction and the carbon fixing reactions. In the light reaction the light energy is absorbed by the pigments present in the antenna, and funnelled to special chlorophyll *a* molecules called reaction centre chlorophylls. There are two photosystems, PS I and PS II. PS I has a 700 nm absorbing chlorophyll *a* P700 molecule at its reaction centre, while PS II has a P680 reaction centre that absorbs red light at 680 nm. After absorbing light, electrons are excited and transferred through PS II and PS I and finally to NAD forming NADH. During this process a proton gradient is created across the membrane of the thylakoid. The breakdown of the protons gradient due to movement through the F_0 part of the ATPase enzyme releases enough energy for synthesis of ATP. Splitting of water molecules is associated with PS II resulting in the release of O_2, protons and transfer of electrons to PS II.

In the carbon fixation cycle, CO_2 is added by the enzyme, RuBisCO, to a 5-carbon compound RuBP that is converted to 2 molecules of 3-carbon PGA. This is then converted to sugar by the Calvin cycle, and the RuBP is regenerated. During this process ATP and NADPH synthesised in the light reaction are utilised. RuBisCO also catalyses a wasteful oxygenation reaction in C_3 plants: photorespiration.

Some tropical plants show a special type of photosynthesis called C_4 pathway. In these plants the first product of CO_2 fixation that takes place in the mesophyll, is a 4-carbon compound. In the bundle sheath cells the Calvin pathway is carried out for the synthesis of carbohydrates.

EXERCISES

1. By looking at a plant externally can you tell whether a plant is C_3 or C_4? Why and how?
2. By looking at which internal structure of a plant can you tell whether a plant is C_3 or C_4? Explain.
3. Even though a very few cells in a C_4 plant carry out the biosynthetic – Calvin pathway, yet they are highly productive. Can you discuss why?

4. RuBisCO is an enzyme that acts both as a carboxylase and oxygenase. Why do you think RuBisCO carries out more carboxylation in C_4 plants?
5. Suppose there were plants that had a high concentration of Chlorophyll *b*, but lacked chlorophyll *a*, would it carry out photosynthesis? Then why do plants have chlorophyll *b* and other accessory pigments?
6. Why is the colour of a leaf kept in the dark frequently yellow, or pale green? Which pigment do you think is more stable?
7. Look at leaves of the same plant on the shady side and compare it with the leaves on the sunny side. Or, compare the potted plants kept in the sunlight with those in the shade. Which of them has leaves that are darker green ? Why?
8. Figure 13.10 shows the effect of light on the rate of photosynthesis. Based on the graph, answer the following questions:

   (a) At which point/s (A, B or C) in the curve is light a limiting factor?
   (b) What could be the limiting factor/s in region A?
   (c) What do C and D represent on the curve?
9. Give comparison between the following:

   (a) C_3 and C_4 pathways
   (b) Cyclic and non-cyclic photophosphorylation
   (c) Anatomy of leaf in C_3 and C_4 plants

Chapter 14
Respiration in Plants

All of us breathe to live, but why is breathing so essential to life? What happens when we breathe? Also, do all living organisms, including plants and microbes, breathe? If so, how?

All living organisms need energy for carrying out daily life activities, be it absorption, transport, movement, reproduction or even breathing. Where does all this energy come from? We know we eat food for energy – but how is this energy taken from food? How is this energy utilised? Do all foods give the same amount of energy? Do plants 'eat'? Where do plants get their energy from? And micro-organisms – for their energy requirements, do they eat 'food'?

You may wonder at the several questions raised above – they may seem to be very disconnected. But in reality, the process of breathing is very much connected to the process of release of energy from food. Let us try and understand how this happens.

All the energy required for 'life' processes is obtained by oxidation of some macromolecules that we call 'food'. Only green plants and cyanobacteria can prepare their own food; by the process of photosynthesis they trap light energy and convert it into chemical energy that is stored in the bonds of carbohydrates like glucose, sucrose and starch. We must remember that in green plants too, not all cells, tissues and organs photosynthesise; only cells containing chloroplasts, that are most often located in the superficial layers, carry out photosynthesis. Hence, even in green plants all other organs, tissues and cells that are non-green, need food for oxidation. Hence, food has to be translocated to all non-green parts. Animals are heterotrophic, i.e., they obtain food from plants

directly (herbivores) or indirectly (carnivores). Saprophytes like fungi are dependent on dead and decaying matter. What is important to recognise is that ultimately all the food that is respired for life processes comes from photosynthesis. This chapter deals with **cellular respiration** or the mechanism of breakdown of food materials within the cell to release energy, and the trapping of this energy for synthesis of ATP.

Photosynthesis, of course, takes place within the chloroplasts (in the eukaryotes), whereas the breakdown of complex molecules to yield energy takes place in the cytoplasm and in the mitochondria (also only in eukaryotes). The breaking of the C-C bonds of complex compounds through oxidation within the cells, leading to release of considerable amount of energy is called **respiration**. The compounds that are oxidised during this process are known as **respiratory substrates.** Usually carbohydrates are oxidised to release energy, but proteins, fats and even organic acids can be used as respiratory substances in some plants, under certain conditions. During oxidation within a cell, all the energy contained in respiratory substrates is not released free into the cell, or in a single step. It is released in a series of slow step-wise reactions controlled by enzymes, and it is trapped as chemical energy in the form of ATP. Hence, it is important to understand that the energy released by oxidation in respiration is not (or rather cannot be) used directly but is used to synthesise ATP, which is broken down whenever (and wherever) energy needs to be utilised. Hence, ATP acts as the energy currency of the cell. This energy trapped in ATP is utilised in various energy-requiring processes of the organisms, and the carbon skeleton produced during respiration is used as precursors for biosynthesis of other molecules in the cell.

14.1 DO PLANTS BREATHE?

Well, the answer to this question is not quite so direct. Yes, plants require O_2 for respiration to occur and they also give out CO_2. Hence, plants have systems in place that ensure the availability of O_2. Plants, unlike animals, have no specialised organs for gaseous exchange but they have stomata and lenticels for this purpose. There are several reasons why plants can get along without respiratory organs. First, each plant part takes care of its own gas-exchange needs. There is very little transport of gases from one plant part to another. Second, plants do not present great demands for gas exchange. Roots, stems and leaves respire at rates far lower than animals do. Only during photosynthesis are large volumes of gases exchanged and, each leaf is well adapted to take care of its own needs during these periods. When cells photosynthesise, availability of O_2 is not a problem in these cells since O_2 is released within the cell. Third, the

distance that gases must diffuse even in large, bulky plants is not great. Each living cell in a plant is located quite close to the surface of the plant. 'This is true for leaves', you may ask, 'but what about thick, woody stems and roots?' In stems, the 'living' cells are organised in thin layers inside and beneath the bark. They also have openings called lenticels. The cells in the interior are dead and provide only mechanical support. Thus, most cells of a plant have at least a part of their surface in contact with air. This is also facilitated by the loose packing of parenchyma cells in leaves, stems and roots, which provide an interconnected network of air spaces.

The complete combustion of glucose, which produces CO_2 and H_2O as end products, yields energy most of which is given out as heat.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy}$$

If this energy is to be useful to the cell, it should be able to utilise it to synthesise other molecules that the cell requires. The strategy that the plant cell uses is to catabolise the glucose molecule in such a way that not all the liberated energy goes out as heat. The key is to oxidise glucose not in one step but in several small steps enabling some steps to be just large enough such that the energy released can be coupled to ATP synthesis. How this is done is, essentially, the story of respiration.

During the process of respiration, oxygen is utilised, and carbon dioxide, water and energy are released as products. The combustion reaction requires oxygen. But some cells live where oxygen may or may not be available. *Can you think of such situations (and organisms) where O_2 is not available?* There are sufficient reasons to believe that the first cells on this planet lived in an atmosphere that lacked oxygen. Even among present-day living organisms, we know of several that are adapted to anaerobic conditions. Some of these organisms are facultative anaerobes, while in others the requirement for anaerobic condition is obligate. In any case, all living organisms retain the enzymatic machinery to partially oxidise glucose without the help of oxygen. This breakdown of glucose to pyruvic acid is called **glycolysis.**

14.2 GLYCOLYSIS

The term glycolysis has originated from the Greek words, *glycos* for sugar, and *lysis* for splitting. The scheme of glycolysis was given by Gustav Embden, Otto Meyerhof, and J. Parnas, and is often referred to as the EMP pathway. In anaerobic organisms, it is the only process in respiration. Glycolysis occurs in the cytoplasm of the cell and is present in all living organisms. In this process, glucose undergoes partial oxidation to form two molecules of pyruvic acid. In plants, this glucose is derived from sucrose, which is the end product of photosynthesis, or from storage

carbohydrates. Sucrose is converted into glucose and fructose by the enzyme, invertase, and these two monosaccharides readily enter the glycolytic pathway. Glucose and fructose are phosphorylated to give rise to glucose-6-phosphate by the activity of the enzyme hexokinase. This phosphorylated form of glucose then isomerises to produce fructose-6-phosphate. Subsequent steps of metabolism of glucose and fructose are same. The various steps of glycolysis are depicted in Figure 14.1. In glycolysis, a chain of ten reactions, under the control of different enzymes, takes place to produce pyruvate from glucose. While studying the steps of glycolysis, please note the steps at which utilisation or synthesis of ATP or (in this case) NADH + H^+ take place.

ATP is utilised at two steps: first in the conversion of glucose into glucose 6-phosphate and second in the conversion of fructose 6-phosphate to fructose 1, 6-bisphosphate.

The fructose 1, 6-bisphosphate is split into dihydroxyacetone phosphate and 3-phosphoglyceraldehyde (PGAL). We find that there is one step where NADH + H^+ is formed from NAD^+; this is when 3-phosphoglyceraldehyde (PGAL) is converted to 1, 3-bisphosphoglycerate (BPGA). Two redox-equivalents are removed (in the form of two hydrogen atoms) from PGAL and transferred to a molecule of NAD^+. PGAL is oxidised and with inorganic phosphate to get converted into BPGA. The conversion of BPGA to 3-phosphoglyceric acid (PGA), is also an energy yielding process; this energy is trapped by the formation of ATP. Another ATP is synthesised during the conversion of PEP to pyruvic acid. *Can you then calculate how many ATP molecules are directly synthesised in this pathway from one glucose molecule?*

Pyruvic acid is then the key product of glycolysis. What is the metabolic fate of pyruvate? This depends on the cellular need.

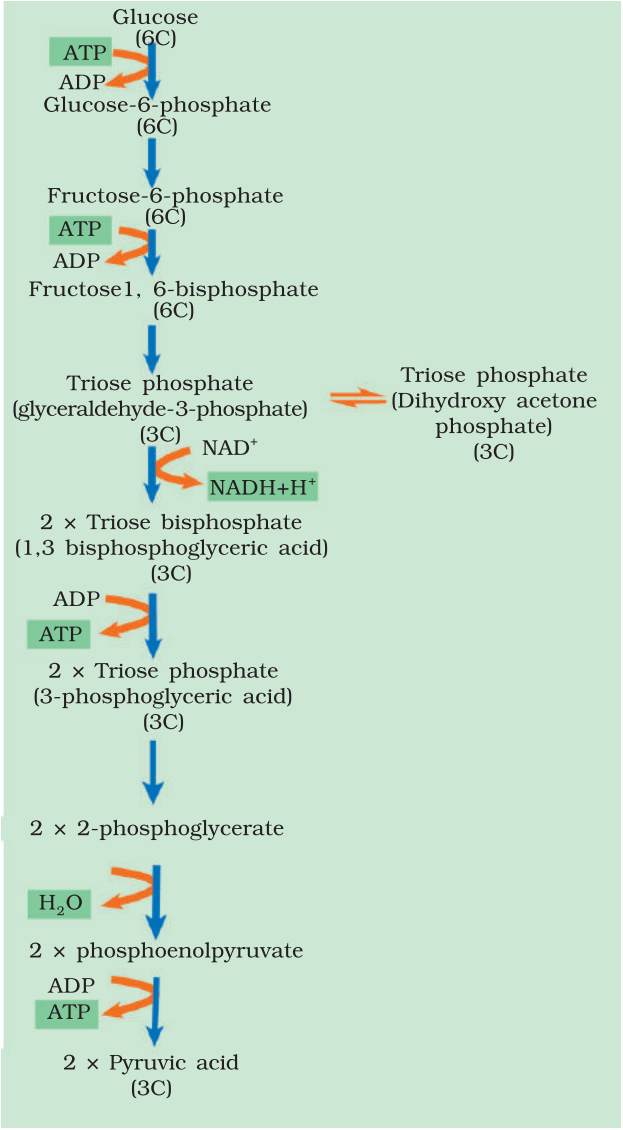

Figure 14.1 Steps of glycolysis

There are three major ways in which different cells handle pyruvic acid produced by glycolysis. These are lactic acid fermentation, alcoholic fermentation and aerobic respiration. Fermentation takes place under anaerobic conditions in many prokaryotes and unicellular eukaryotes. For the complete oxidation of glucose to CO_2 and H_2O, however, organisms adopt Krebs' cycle which is also called as aerobic respiration. This requires O_2 supply.

14.3 FERMENTATION

In fermentation, say by yeast, the incomplete oxidation of glucose is achieved under anaerobic conditions by sets of reactions where pyruvic acid is converted to CO_2 and ethanol. The enzymes, pyruvic acid decarboxylase and alcohol dehydrogenase catalyse these reactions. Other organisms like some bacteria produce lactic acid from pyruvic acid. The steps involved are shown in Figure 14.2. In animal cells also, like muscles during exercise, when oxygen is inadequate for cellular respiration pyruvic acid is reduced to lactic acid by lactate dehydrogenase. The reducing agent is $NADH+H^+$ which is reoxidised to NAD^+ in both the processes.

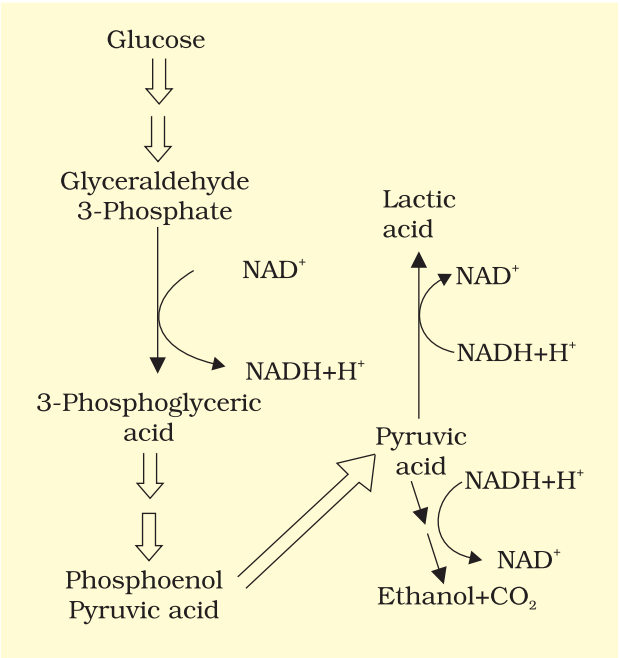

Figure 14.2 Major pathways of anaerobic respiration

In both lactic acid and alcohol fermentation not much energy is released; less than seven per cent of the energy in glucose is released and not all of it is trapped as high energy bonds of ATP. Also, the processes are hazardous – either acid or alcohol is produced. What is the net ATPs that is synthesised (calculate how many ATP are synthesised and deduct the number of ATP utilised during glycolysis) when one molecule of glucose is fermented to alcohol or lactic acid? Yeasts poison themselves to death when the concentration of alcohol reaches about 13 per cent. *What then would be the maximum concentration of alcohol in beverages that are naturally fermented?* How do you think alcoholic beverages of alcohol content greater than this concentration are obtained?

What then is the process by which organisms can carry out complete oxidation of glucose and extract the energy stored to synthesise a larger number of ATP molecules

needed for cellular metabolism? In eukaryotes these steps take place within the mitochondria and this requires O_2. **Aerobic respiration** is the process that leads to a complete oxidation of organic substances in the presence of oxygen, and releases CO_2, water and a large amount of energy present in the substrate. This type of respiration is most common in higher organisms. We will look at these processes in the next section.

14.4 AEROBIC RESPIRATION

For aerobic respiration to take place within the mitochondria, the final product of glycolysis, pyruvate is transported from the cytoplasm into the mitochondria. The crucial events in aerobic respiration are:

- The complete oxidation of pyruvate by the stepwise removal of all the hydrogen atoms, leaving three molecules of CO_2.
- The passing on of the electrons removed as part of the hydrogen atoms to molecular O_2 with simultaneous synthesis of ATP.

What is interesting to note is that the first process takes place in the matrix of the mitochondria while the second process is located on the inner membrane of the mitochondria.

Pyruvate, which is formed by the glycolytic catabolism of carbohydrates in the cytosol, after it enters mitochondrial matrix undergoes oxidative decarboxylation by a complex set of reactions catalysed by pyruvic dehydrogenase. The reactions catalysed by pyruvic dehydrogenase require the participation of several coenzymes, including NAD^+ and Coenzyme A.

$$\text{Pyruvic acid} + \text{CoA} + \text{NAD}^+ \xrightarrow[\text{Pyruvate dehydrogenase}]{\text{Mg}^{2+}} \text{Acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

During this process, two molecules of NADH are produced from the metabolism of two molecules of pyruvic acid (produced from one glucose molecule during glycolysis).

The acetyl CoA then enters a cyclic pathway, tricarboxylic acid cycle, more commonly called as Krebs' cycle after the scientist Hans Krebs who first elucidated it.

14.4.1 Tricarboxylic Acid Cycle

The TCA cycle starts with the condensation of acetyl group with oxaloacetic acid (OAA) and water to yield citric acid (Figure 14.3). The reaction is catalysed by the enzyme citrate synthase and a molecule of CoA is released. Citrate is then isomerised to isocitrate. It is followed by two successive steps of decarboxylation, leading to the formation of α-ketoglutaric acid

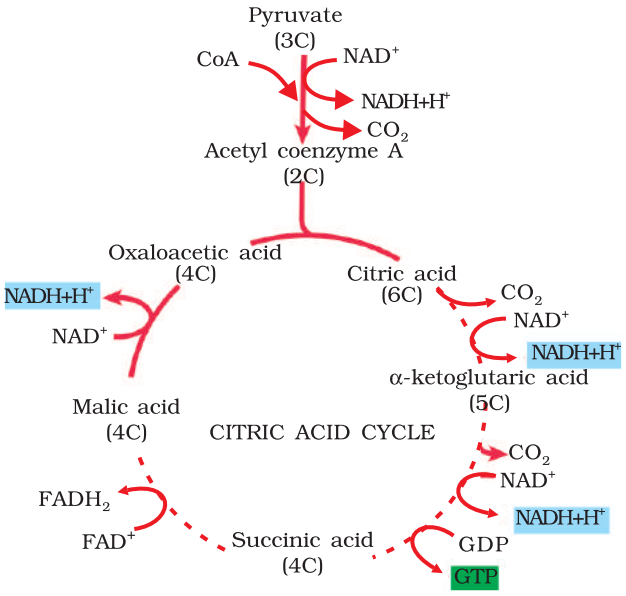

Figure 14.3 The Citric acid cycle

and then succinyl-CoA. In the remaining steps of citric acid cycle, succinyl-CoA is oxidised to OAA allowing the cycle to continue. During the conversion of succinyl-CoA to succinic acid a molecule of GTP is synthesised. This is a substrate level phosphorylation. In a coupled reaction GTP is converted to GDP with the simultaneous synthesis of ATP from ADP. Also there are three points in the cycle where NAD^+ is reduced to NADH + H^+ and one point where FAD^+ is reduced to $FADH_2$. The continued oxidation of acetyl CoA via the TCA cycle requires the continued replenishment of oxaloacetic acid, the first member of the cycle. In addition it also requires regeneration of NAD^+ and FAD^+ from NADH and $FADH_2$ respectively. The summary equation for this phase of respiration may be written as follows:

$$\text{Pyruvic acid} + 4NAD^+ + FAD^+ + 2H_2O + ADP + Pi \xrightarrow{\text{Mitochondrial Matrix}} 3CO_2 + 4NADH + 4H^+ + FADH_2 + ATP$$

We have till now seen that glucose has been broken down to release CO_2 and eight molecules of NADH + H^+; two of $FADH_2$ have been synthesised besides just two molecules of ATP. You may be wondering why we have been discussing respiration at all – neither O_2 has come into the picture nor the promised large number of ATP has yet been synthesised. Also what is the role of the NADH + H^+ and $FADH_2$ that is synthesised? Let us now understand the role of O_2 in respiration and how ATP is synthesised.

14.4.2 Electron Transport System (ETS) and Oxidative Phosphorylation

The following steps in the respiratory process are to release and utilise the energy stored in NADH+H^+ and $FADH_2$. This is accomplished when they are oxidised through the electron transport system and the electrons are passed on to O_2 resulting in the formation of H_2O. The metabolic pathway through which the electron passes from one carrier to another, is called the **electron transport system** (ETS) (Figure 14.4) and it is present in the inner mitochondrial membrane. Electrons from NADH

produced in the mitochondrial matrix during citric acid cycle are oxidised by an NADH dehydrogenase (complex I), and electrons are then transferred to ubiquinone located within the inner membrane. Ubiquinone also receives reducing equivalents via $FADH_2$ (complex II) that is generated during oxidation of succinate in the citric acid cycle. The reduced ubiquinone (ubiquinol) is then oxidised with the transfer of electrons to cytochrome *c* via cytochrome bc_1 complex (complex III). Cytochrome *c* is a small protein attached to the outer surface of the inner membrane and acts as a mobile carrier for transfer of electrons between complex III and IV. Complex IV refers to cytochrome *c* oxidase complex containing cytochromes *a* and a_3, and two copper centres.

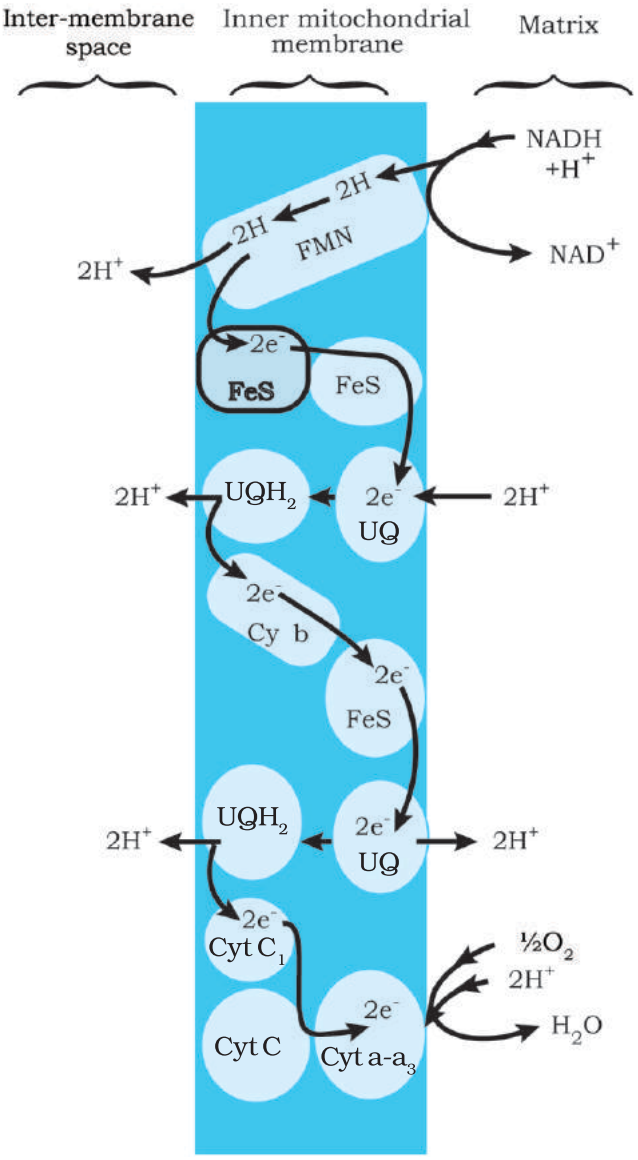

Figure 14.4 Electron Transport System (ETS)

When the electrons pass from one carrier to another via complex I to IV in the electron transport chain, they are coupled to ATP synthase (complex V) for the production of ATP from ADP and inorganic phosphate. The number of ATP molecules synthesised depends on the nature of the electron donor. Oxidation of one molecule of NADH gives rise to 3 molecules of ATP, while that of one molecule of $FADH_2$ produces 2 molecules of ATP. Although the aerobic process of respiration takes place only in the presence of oxygen, the role of oxygen is limited to the terminal stage of the process. Yet, the presence of oxygen is vital, since it drives the whole process by removing hydrogen from the system. Oxygen acts as the final hydrogen acceptor. Unlike photophosphorylation where it is the light energy that is utilised for the production of proton gradient required for phosphorylation, in respiration it is the energy of oxidation-reduction utilised for the same process. It is for this reason that the process is called oxidative phosphorylation.

You have already studied about the mechanism of membrane-linked ATP synthesis as explained by chemiosmotic hypothesis in the earlier chapter. As mentioned earlier, the energy released during the electron

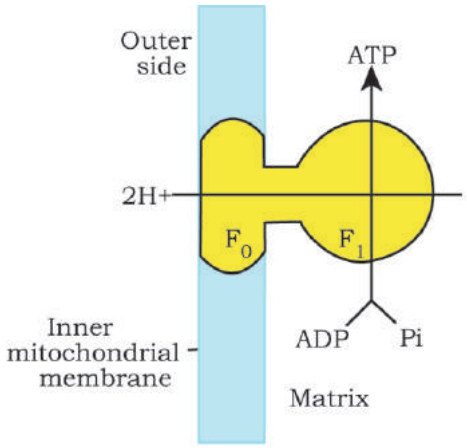

Figure 14.5 Diagramatic presentation of ATP synthesis in mitochondria

transport system is utilised in synthesising ATP with the help of ATP synthase (complex V). This complex consists of two major components, F_1 and F_0 (Figure 14.5). The F_1 headpiece is a peripheral membrane protein complex and contains the site for synthesis of ATP from ADP and inorganic phosphate. F_0 is an integral membrane protein complex that forms the channel through which protons cross the inner membrane. The passage of protons through the channel is coupled to the catalytic site of the F_1 component for the production of ATP. For each ATP produced, $2H^+$ passes through F_0 from the intermembrane space to the matrix down the electrochemical proton gradient.

14.5 The Respiratory Balance Sheet

It is possible to make calculations of the net gain of ATP for every glucose molecule oxidised; but in reality this can remain only a theoretical exercise. These calculations can be made only on certain assumptions that:

- There is a sequential, orderly pathway functioning, with one substrate forming the next and with glycolysis, TCA cycle and ETS pathway following one after another.
- The NADH synthesised in glycolysis is transferred into the mitochondria and undergoes oxidative phosphorylation.
- None of the intermediates in the pathway are utilised to synthesise any other compound.
- Only glucose is being respired – no other alternative substrates are entering in the pathway at any of the intermediary stages.

But this kind of assumptions are not really valid in a living system; all pathways work simultaneously and do not take place one after another; substrates enter the pathways and are withdrawn from it as and when necessary; ATP is utilised as and when needed; enzymatic rates are controlled by multiple means. Yet, it is useful to do this exercise to appreciate the beauty and efficiency of the living system in extraction and storing energy. Hence, there can be a net gain of 36 ATP molecules during aerobic respiration of one molecule of glucose.

Now let us compare fermentation and aerobic respiration:

- Fermentation accounts for only a partial breakdown of glucose whereas in aerobic respiration it is completely degraded to CO_2 and H_2O.
- In fermentation there is a net gain of only two molecules of ATP for each molecule of glucose degraded to pyruvic acid whereas many more molecules of ATP are generated under aerobic conditions.
- NADH is oxidised to NAD^+ rather slowly in fermentation, however the reaction is very vigorous in case of aerobic respiration.

14.6 AMPHIBOLIC PATHWAY

Glucose is the favoured substrate for respiration. All carbohydrates are usually first converted into glucose before they are used for respiration. Other substrates can also be respired, as has been mentioned earlier, but then they do not enter the respiratory pathway at the first step. See Figure 14.6 to see the points of entry of different substrates in the respiratory pathway. Fats would need to be broken down into glycerol and fatty acids first. If fatty acids were to be respired they would first be degraded to acetyl CoA and enter the pathway. Glycerol would enter the pathway after being converted to PGAL. The proteins would be degraded by proteases and the individual amino acids (after deamination) depending on their structure would enter the pathway at some stage within the Krebs' cycle or even as pyruvate or acetyl CoA.

Since respiration involves breakdown of substrates, the respiratory process has traditionally been considered a catabolic process and the respiratory pathway as a catabolic pathway. But is this understanding correct? We have discussed above, at which points in the respiratory pathway different substrates would enter if they were to be respired and used to derive energy. What is important to recognise is that it is these very compounds that would be withdrawn from the respiratory pathway for the synthesis of the said substrates. Hence, fatty acids would be broken down to acetyl CoA before entering the respiratory pathway when it is used as a substrate. But when the organism needs to synthesise fatty acids, acetyl CoA would be withdrawn from the respiratory pathway for it. Hence, the respiratory pathway comes into the picture both during breakdown and synthesis of fatty acids. Similarly, during breakdown and synthesis of protein too, respiratory intermediates form the link. Breaking down processes within the living organism is catabolism, and synthesis is anabolism. Because the respiratory pathway is involved in both anabolism and catabolism, it would hence be better to consider the respiratory pathway as an **amphibolic pathway** rather than as a catabolic one.

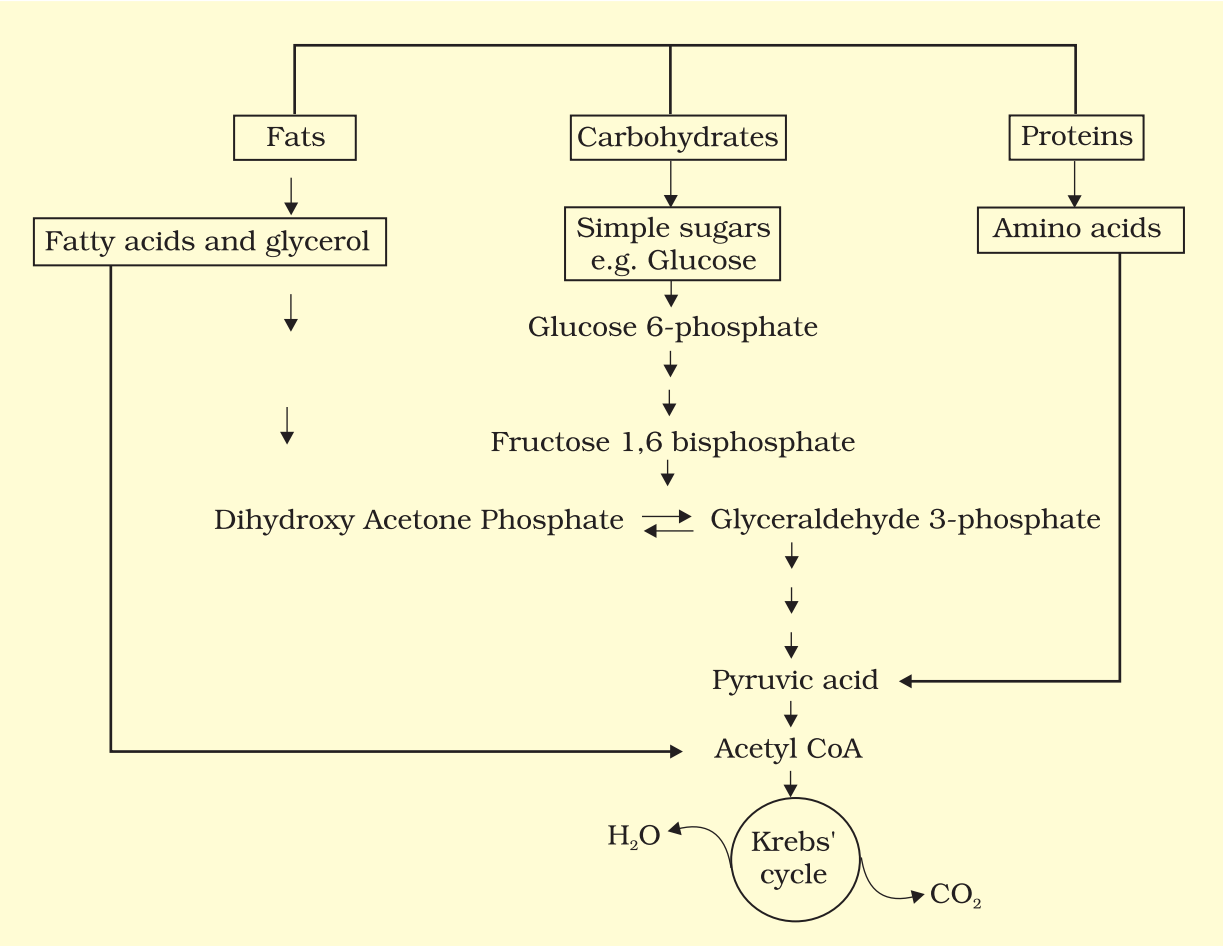

Figure 14.6 Interrelationship among metabolic pathways showing respiration mediated breakdown of different organic molecules to CO_2 and H_20

14.7 Respiratory Quotient

Let us now look at another aspect of respiration. As you know, during aerobic respiration, O_2 is consumed and CO_2 is released. The ratio of the volume of CO_2 evolved to the volume of O_2 consumed in respiration is called the **respiratory quotient** (RQ) or respiratory ratio.

$$RQ = \frac{\text{volume of } CO_2 \text{ evolved}}{\text{volume of } O_2 \text{ consumed}}$$

The respiratory quotient depends upon the type of respiratory substrate used during respiration.

When carbohydrates are used as substrate and are completely oxidised, the RQ will be 1, because equal amounts of CO_2 and O_2 are evolved and consumed, respectively, as shown in the equation below :