INTRODUCING GLYCOLYSIS

Cell respiration involves the production of ATP using energy released by the oxidation of glucose, fat or other substrates. If glucose is the substrate, the first stage of cell respiration is a metabolic pathway called **glycolysis**. The pathway is catalysed by enzymes in the cytoplasm. Glucose is partially oxidized in the pathway and a small amount of ATP is produced. This partial oxidation is achieved without the use of oxygen, so glycolysis can form part of both aerobic and anaerobic respiration.

OXIDATION AND REDUCTION IN CELL RESPIRATION

Cell respiration involves many oxidation and reduction reactions. The figure (top right) compares the ways in which chemical substances can be oxidized and reduced. Hydrogen carriers accept hydrogen atoms removed from substrates in cell respiration. The most commonly used hydrogen carrier is NAD^+ (nicotinamide adenine dinucleotide). Hydrogen atoms consist of one proton and one electron. When two hydrogen atoms are removed from a respiratory substrate, NAD^+ accepts the electrons from both atoms and the proton from one of them.

$$NAD^+ + 2H \longrightarrow NADH + H^+$$

The figure (right) shows equations for some of the chemical changes that are part of cell respiration. It is possible to use the information in the figure (top right) to deduce whether each of them is an oxidation, a reduction or both.

Comparison of oxidation and reduction

Oxidation reactions	Reduction reactions
Addition of oxygen atoms to a substance.	Removal of oxygen atoms from a substance.
Removal of hydrogen atoms from a substance.	Addition of hydrogen atoms to a substance.
Loss of electrons from a substance.	Addition of electrons to a substance.

Examples of oxidations and reductions in cell respiration

$$Fe^{3+} + \text{electron} \longrightarrow Fe^{2+}$$

$$Fe^{2+} \longrightarrow Fe^{3+} + \text{electron}$$

$$\text{succinate} + FAD \longrightarrow \text{fumarate} + FADH_2$$

$$\text{malate} + NAD^+ \longrightarrow \text{oxaloacetate} + NADH + H^+$$

$$\text{pyruvate} + NADH + H^+ \longrightarrow \text{lactate} + NAD^+$$

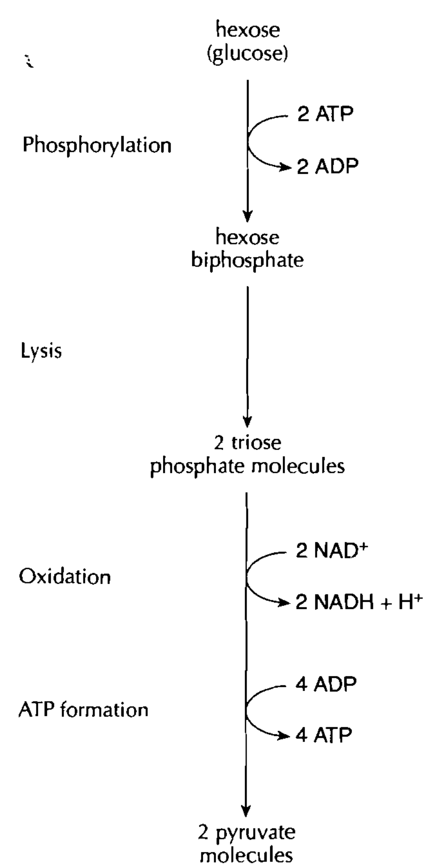
CONVERTING GLUCOSE TO PYRUVATE IN GLYCOLYSIS

There are four main stages in glycolysis.

1. Two phosphate groups are added to a molecule of glucose to form hexose biphosphate. Adding a phosphate group is called **phosphorylation**. Two molecules of ATP provide the phosphate groups. The energy level of the hexose is raised by phosphorylation and this makes the subsequent reactions possible.
2. The hexose biphosphate is split to form two molecules of triose phosphate. Splitting molecules is called **lysis**.
3. Two atoms of hydrogen are removed from each triose phosphate molecule. This is an **oxidation**. The energy released by this oxidation is used to link on another phosphate group, producing a 3-carbon compound carrying two phosphate groups. NAD^+ is the hydrogen carrier that accepts the hydrogen atoms.
4. Pyruvate is formed by removing the two phosphate groups and by passing them to ADP. This results in **ATP formation**.

The figure (right) shows the main stages of glycolysis

Stages of glycolysis



SUMMARY OF GLYCOLYSIS

- One glucose is converted into two pyruvates.
- Two ATP molecules are used per glucose but four are produced so there is a net yield of two ATP molecules.
- Two NAD^+ are converted into two $NADH + H^+$

Krebs cycle

Enzymes in the matrix of the mitochondrion catalyse a cycle of reactions called the **Krebs cycle**. These reactions can only occur if oxygen is available and so are part of aerobic, but not anaerobic cell respiration.

THE CENTRAL ROLE OF ACETYL COA IN METABOLISM

Acetyl groups (CH_3CO) are the substrate used in the Krebs cycle. A carrier called CoA (Coenzyme A) accepts acetyl groups produced in metabolism and brings them for use in the cycle.

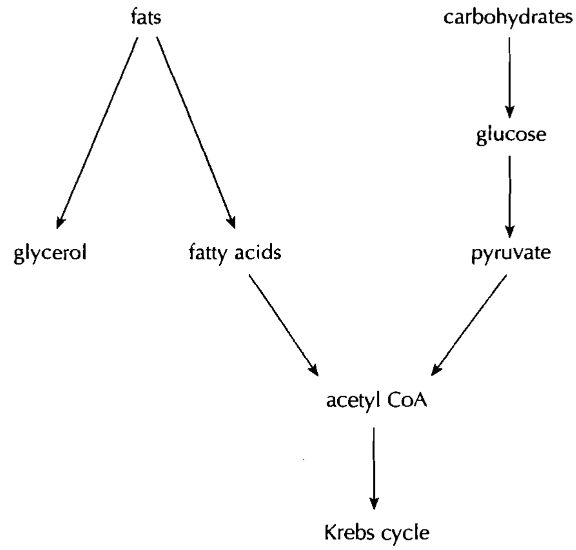
acetyl group + CoA → acetyl CoA

Acetyl CoA is formed in both carbohydrate and fat metabolism.

- Carbohydrates are converted into pyruvate and the pyruvate is converted to acetyl CoA by a reaction that is often called the link reaction, as it links glycolysis and the Krebs cycle.
- Fats are broken down into fatty acids and glycerol. The hydrocarbon tails of the fatty acids are then broken down into two-carbon fragments and oxidized to form acetyl CoA.

Acetyl CoA is therefore the connection between the metabolism of carbohydrates and fats and the Krebs cycle is used whether glucose or fats are the substrate for respiration.

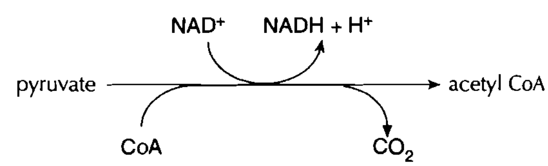
Summary of metabolic pathways involving acetyl CoA



THE LINK REACTION

Pyruvate from glycolysis is absorbed by the mitochondrion. Enzymes in the matrix of the mitochondrion remove hydrogen and carbon dioxide from the pyruvate. The hydrogen is accepted by NAD^+. Removal of hydrogen is oxidation. Removal of carbon dioxide is decarboxylation. The whole conversion is therefore **oxidative decarboxylation**. The product of oxidative decarboxylation of pyruvate is an acetyl group, which is accepted by CoA (right).

Summary of the link reaction



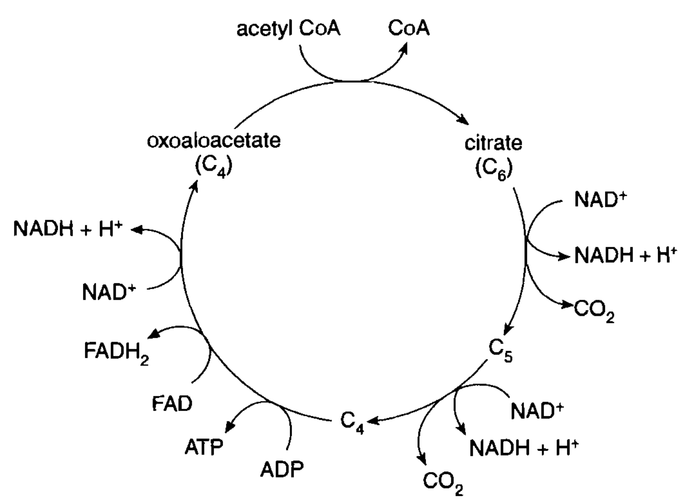
THE KREBS CYCLE

In the first reaction of the cycle an acetyl group is transferred from acetyl CoA to a four-carbon compound (oxaloacetate) to form a six-carbon compound (citrate). Citrate is converted back into oxaloacetate in the other reactions of the cycle. Three types of reaction are involved.

- Carbon dioxide is removed in two of the reactions. These reactions are **decarboxylations**. The carbon dioxide is a waste product and is excreted together with the carbon dioxide from the link reaction.
- Hydrogen is removed in four of the reactions. These reactions are **oxidations**. In three of the oxidations the hydrogen is accepted by NAD^+. In the other oxidation FAD accepts it. These oxidation reactions release energy, much of which is stored by the carriers when they accept hydrogen. This energy is later released by the electron transport chain and used to make ATP.
- ATP is produced directly in one of the reactions. This reaction is **substrate-level phosphorylation**.

The figure (right) is a summary of the Krebs cycle.

Summary of the Krebs cycle



Oxidative phosphorylation

THE ELECTRON TRANSPORT CHAIN

The electron transport chain is a series of electron carriers, located in the inner membrane of the mitochondrion. NADH supplies two electrons to the first carrier in the chain. The electrons come from oxidation reactions in earlier stages of cell respiration. The two electrons pass along the chain of carriers because they give up energy each time they pass from one carrier to the next. At three points along the chain enough energy is given up for ATP to be made by ATP synthase. As this ATP production relies on energy released by oxidation it is called **oxidative phosphorylation**. ATP synthase is also located in the inner mitochondrial membrane.

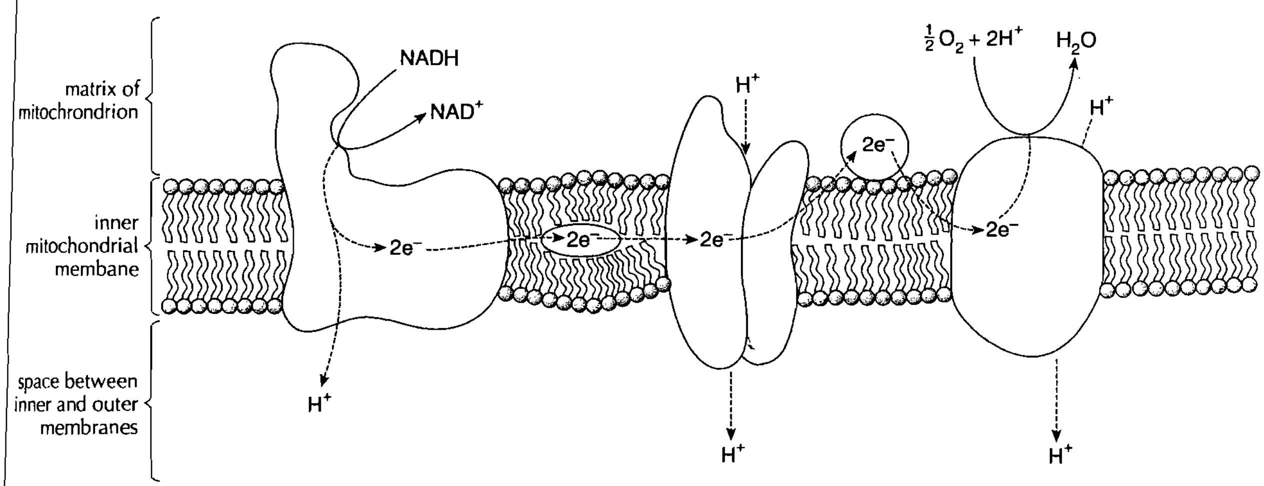
$FADH_2$ also feeds electrons into the electron transport chain, but at a slightly later stage than NADH and at only two stages is sufficient energy released for ATP production by electrons from $FADH_2$.

THE ROLE OF OXYGEN

At the end of the electron transport chain the electrons are given to oxygen. At the same time oxygen accepts hydrogen ions, to form water. This happens in the matrix, on the surface of the inner membrane. This is the only stage at which oxygen is used in cell respiration. If oxygen is not available, electron flow along the electron transport chain stops and NADH + H^+ cannot be reconverted to NAD^+. Supplies of NAD^+ in the mitochondrion run out and the link reaction and Krebs cycle cannot continue. Glycolysis can continue because conversion of pyruvate into lactate or ethanol and carbon dioxide produces as much NAD^+ as is used in glycolysis. However, whereas aerobic cell respiration gives a yield of about 30 ATP molecules per glucose, glycolysis produces only two. Oxygen thus greatly increases the ATP yield.

The figure (below) shows the electron transport chain and the role of oxygen as the terminal electron acceptor.

The electron transport chain of mitochondria

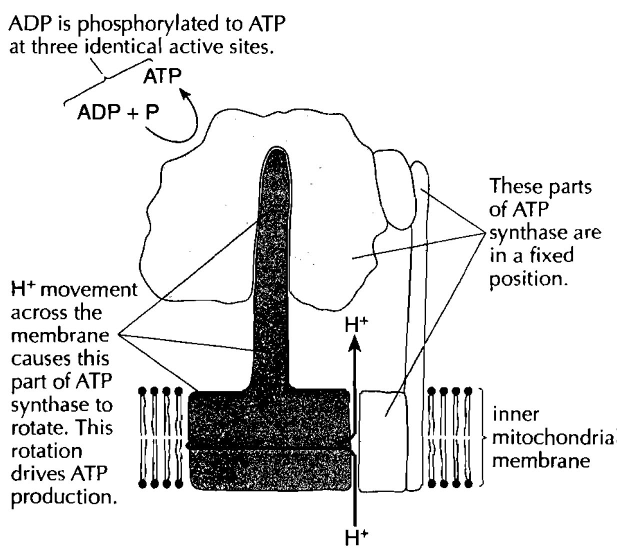


THE COUPLING OF ELECTRON TRANSPORT TO ATP SYNTHESIS

Energy released as electrons pass along the electron transport chain is used to pump protons (H^+) across the inner mitochondrial membrane into the space between the inner and outer membranes. A concentration gradient is formed, which is a store of potential energy. ATP synthase, located in the inner mitochondrial membrane, transports the protons back across the membrane down the concentration gradient. As the protons pass across the membrane they release energy and this is used by ATP synthase to produce ATP. The coupling of ATP synthesis to electron transport via a concentration gradient of protons is called **chemiosmosis**.

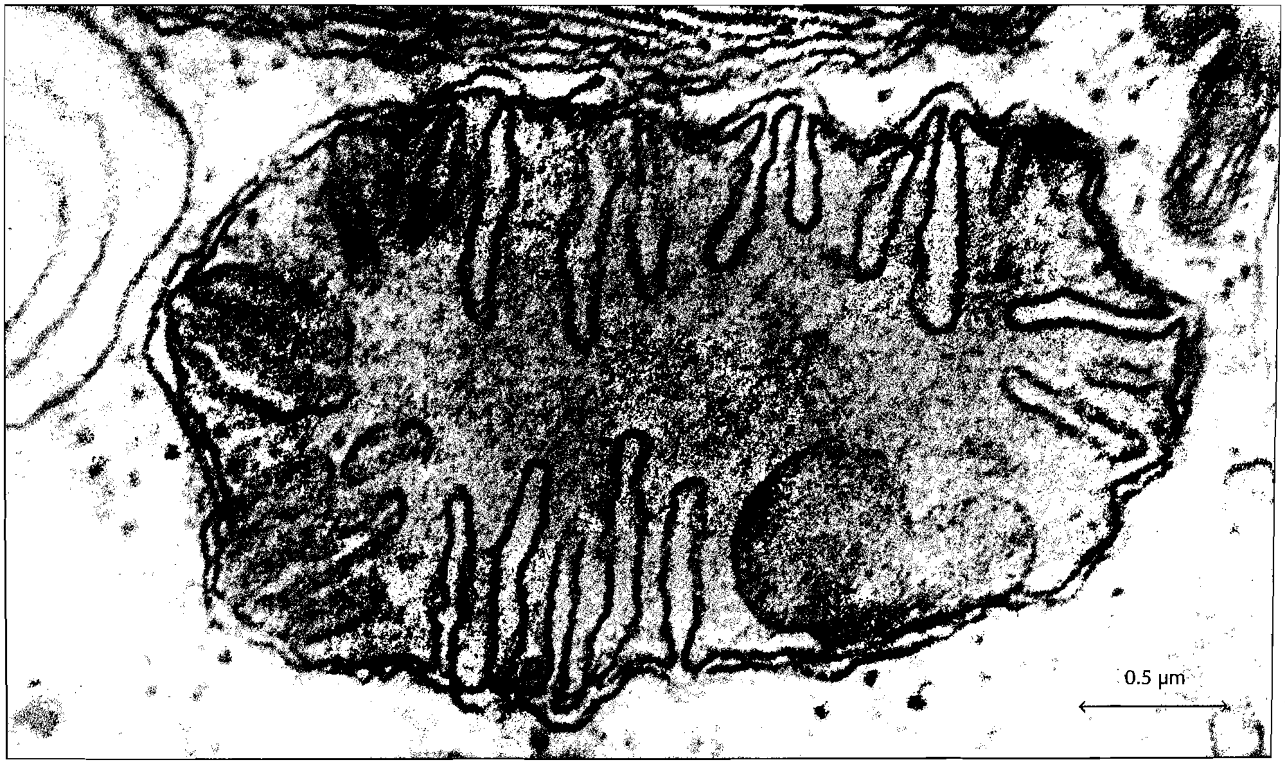
The figure (right) shows some features of ATP synthase.

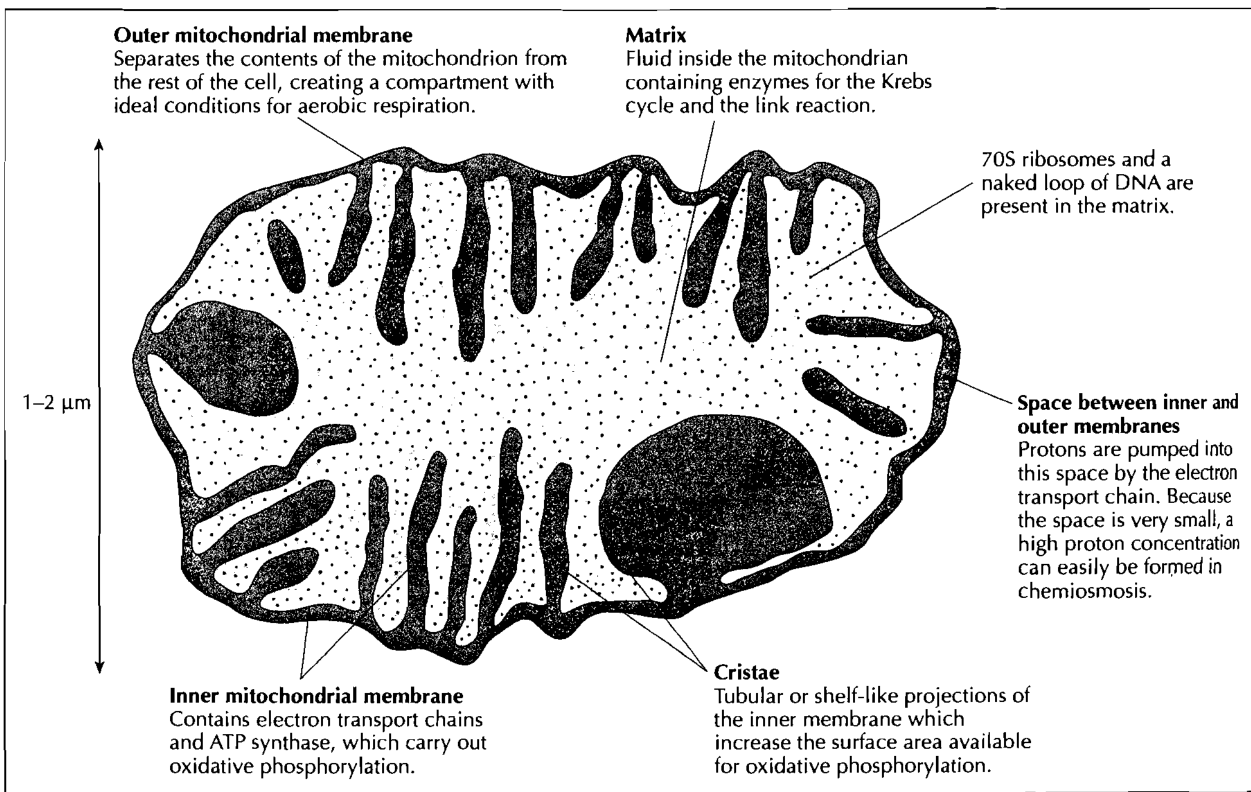
Structure of ATP synthase



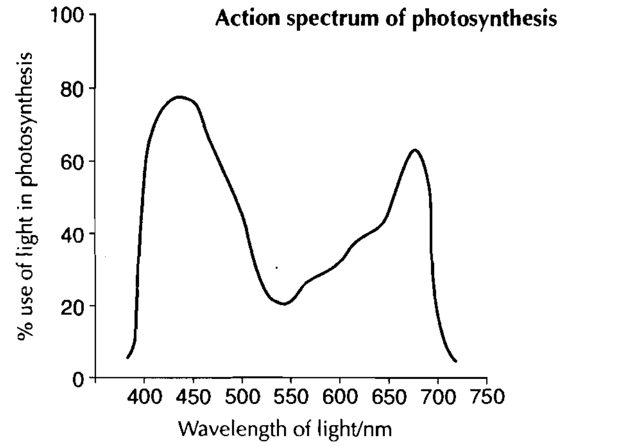
Mitochondria

The mitochondrion is an excellent example of the relationship between structure and function.
The figure (below) is an electron micrograph of a whole mitochondrion.
The figure (bottom) is a drawing of the same mitochondrion, labelled to show how it is adapted to carry out its function.





Photosynthesis is the process that plants, algae and some bacteria use to produce all of the organic compounds that they need.
Photosynthesis involves many chemical reactions. Some of them need a continual supply of light and so are called **light-dependent reactions**.
Other reactions need light indirectly, but can carry on for some time in darkness. These are called **light-independent reactions**.
Glucose, amino acids and other organic compounds are produced in the light independent reactions. The light-dependent reactions produce intermediate compounds that are used in the light-independent reactions. In darkness these intermediate compounds are gradually used up.

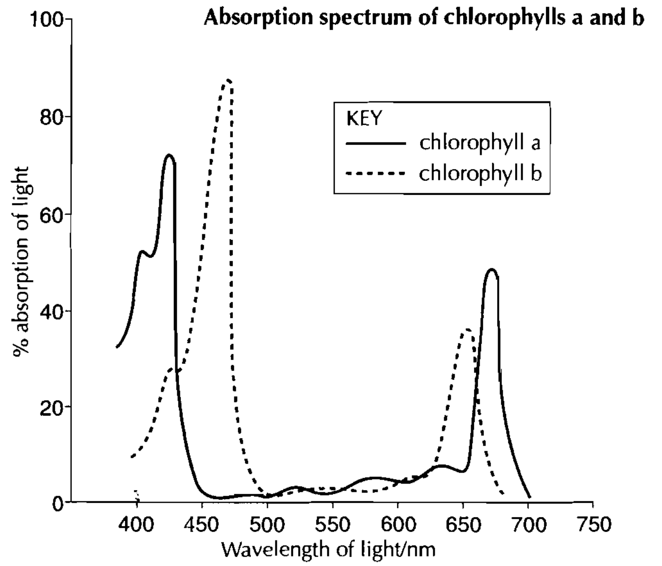


THE ACTION SPECTRUM OF PHOTOSYNTHESIS

A spectrum is a range of wavelengths of electromagnetic radiation. The spectrum of light is the range of wavelengths from 400 nm to 700 nm. Each wavelength is a pure colour of light:

400–525 violet–blue
525–625 green–yellow
625–700 orange–red

The efficiency of photosynthesis is not the same in all wavelengths of light. The efficiency is the percentage of light of a wavelength that is used in photosynthesis. The figure (top right) is a graph showing the percentage use of the wavelengths of light in photosynthesis. This graph is called the **action spectrum** of photosynthesis. The graph shows that violet and blue light are used most efficiently and red light is also used efficiently. Green light is used much less efficiently.



THE ABSORPTION SPECTRA OF PHOTOSYNTHETIC PIGMENTS

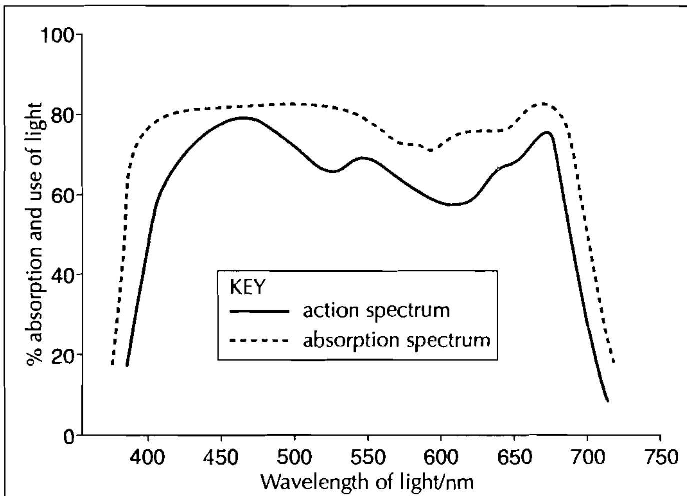
The action spectrum of photosynthesis is explained by considering the light-absorbing properties of the photosynthetic pigments. Most pigments absorb some wavelengths better than others. The figure (centre right) shows the percentage of the wavelengths of visible light that are absorbed by two common forms of chlorophyll. This graph is called the **absorption spectrum** of these pigments. The graph shows strong similarities with the action spectrum for photosynthesis.

- The greatest absorption is in the violet–blue range.
- There is a also a high level of absorption in the red range of the spectrum
- There is least absorption in the yellow–green range of the spectrum. Most of this light is reflected.

There are some differences between the action spectrum and the absorption spectra. Whereas little light is absorbed by chlorophylls in the green to yellow range there is some photosynthesis. This is due to accessory pigments, including xanthophylls and carotene, which absorb wavelengths that chlorophyll cannot.

Action and absorption spectra of an alga

Some algae contain large amounts of accessory pigments. For example, kelp (*Laminaria saccharina*) contains carotene and fucoxanthin in addition to chlorophylls and so can absorb and use all wavelengths of light with about the same efficiency in photosynthesis. The graph below shows the action and absorption spectra for kelp. The colour of kelp can be deduced from the data.



Light-dependent reactions

LIGHT ABSORPTION

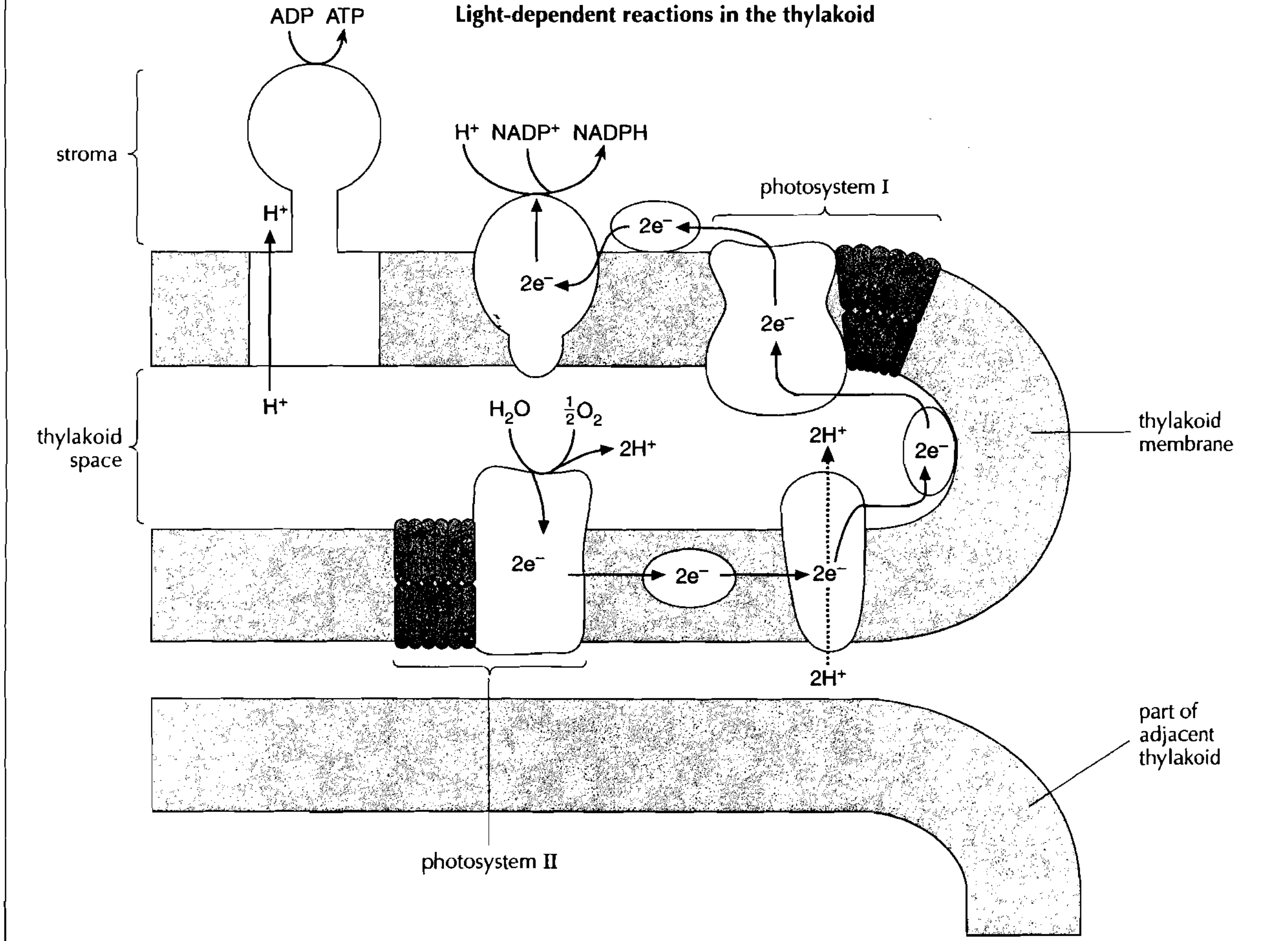
Chlorophyll absorbs light and the energy from the light raises an electron in the chlorophyll molecule to a higher energy level. The electron at a higher energy level is an **excited electron** and the chlorophyll is **photoactivated**. In single chlorophyll molecules the excited electron soon drops back down to its original level, re-emitting the energy. Chlorophyll is located in thylakoid membranes and is arranged in groups of hundreds of molecules, called **photosystems**. There are two types of photosystem – photosystems I and II. Excited electrons from absorption of photons of light anywhere in the photosystem are passed from molecule to molecule until they reach a special chlorophyll molecule at the reaction centre of the photosystem. This chlorophyll passes the excited electron to a chain of electron carriers.

PRODUCTION OF ATP

An excited electron from the reaction centre of photosystem II is passed along a chain of carriers in the thylakoid membrane (below). It gives up some of its energy each time that it passes from one carrier to the next. At one stage, enough energy is released to make a molecule of ATP. The coupling of electron transport to ATP synthesis is by chemiosmosis, as in the mitochondrion. Electron flow causes a proton to be pumped across the thylakoid membrane into the fluid space inside the thylakoid. A proton gradient is created. ATP synthase, located in the thylakoid membranes, lets the protons across the membrane down the concentration gradient and uses the energy released to synthesize ATP.

The production of ATP using the energy from an excited electron from Photosystem II is called **non-cyclic photophosphorylation**. An alternative method of photophosphorylation is shown on page 81.

Light-dependent reactions in the thylakoid



PRODUCTION OF NADP

After releasing the energy needed to make ATP, the electron that was given away by photosystem II is accepted by photosystem I. The electron replaces one previously given away by photosystem I. With its electron replaced, photosystem I can be photoactivated by absorbing light and then give away another excited electron. This high-energy electron passes along a short chain of carriers to $NADP^+$ in the stroma. $NADP^+$ accepts two high-energy electrons from the electron transport chain and one H^+ ion from the stroma, to form NADPH.

PRODUCTION OF OXYGEN

Photosystem II needs to replace the excited electrons that it gives away. The special chlorophyll molecule at the reaction centre is positively charged after giving away an electron. With the help of an enzyme at the reaction centre, water molecules in the thylakoid space are split and electrons from them are given to chlorophyll. Oxygen and H^+ ions are formed as by-products. The splitting of water molecules only happens in the light, so is called **photolysis**. The oxygen produced in photosynthesis is all the result of photolysis of water. Oxygen is a waste product and is excreted.

Light-independent reactions

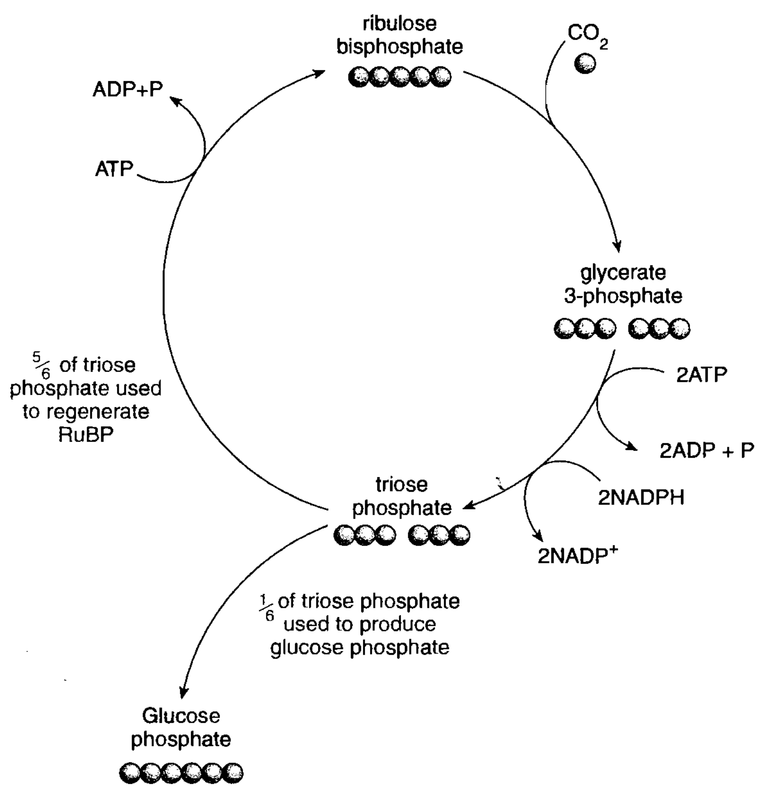
THE CALVIN CYCLE

The light-independent reactions take place within the stroma of the chloroplast. The first reaction involves a five-carbon sugar, ribulose bisphosphate (RuBP). RuBP is also a product of the light independent reactions, which therefore form a cycle, called the **Calvin cycle**. There are many alternative names for the intermediate compounds in the Calvin cycle. Glycerate 3-phosphate is sometimes also called 3-phosphoglycerate. Glycerate 3-phosphate is sometimes abbreviated as GP, which could be confused with glyceraldehyde 3-phosphate, which is a form of triose phosphate or with glucose phosphate. The abbreviation GP should therefore be avoided!

CARBON FIXATION

Carbon dioxide is an essential substrate in the light-independent reactions. It enters the chloroplast by diffusion. In the stroma of the chloroplast carbon dioxide combines with ribulose bisphosphate (RuBP), a five-carbon sugar, in a carboxylation reaction. The reaction is catalysed by the enzyme ribulose bisphosphate carboxylase, usually called **rubisco**. Large amounts of rubisco are present in the stroma, because it works rather slowly and the reaction that it catalyses is a very important one. The product of the reaction is a six-carbon compound, which immediately splits to form two molecules of glycerate 3-phosphate. This is therefore the first product of carbon fixation.

Summary of the Calvin cycle



REGENERATION OF RUBP

For carbon fixation to continue, one RuBP molecule must be produced to replace each one that is used. Triose phosphate is used to regenerate RuBP. Five molecules of triose phosphate are converted by a series of reactions into three molecules of RuBP. This process requires the use of energy in the form of ATP. The reactions can be summarized using equations where only the number of carbon atoms in each sugar molecule is shown.

$$C_3 + C_3 \longrightarrow C_6$$
$$C_6 + C_3 \longrightarrow C_4 + C_5$$
$$C_4 + C_3 \longrightarrow C_7$$
$$C_7 + C_3 \longrightarrow C_5 + C_5$$

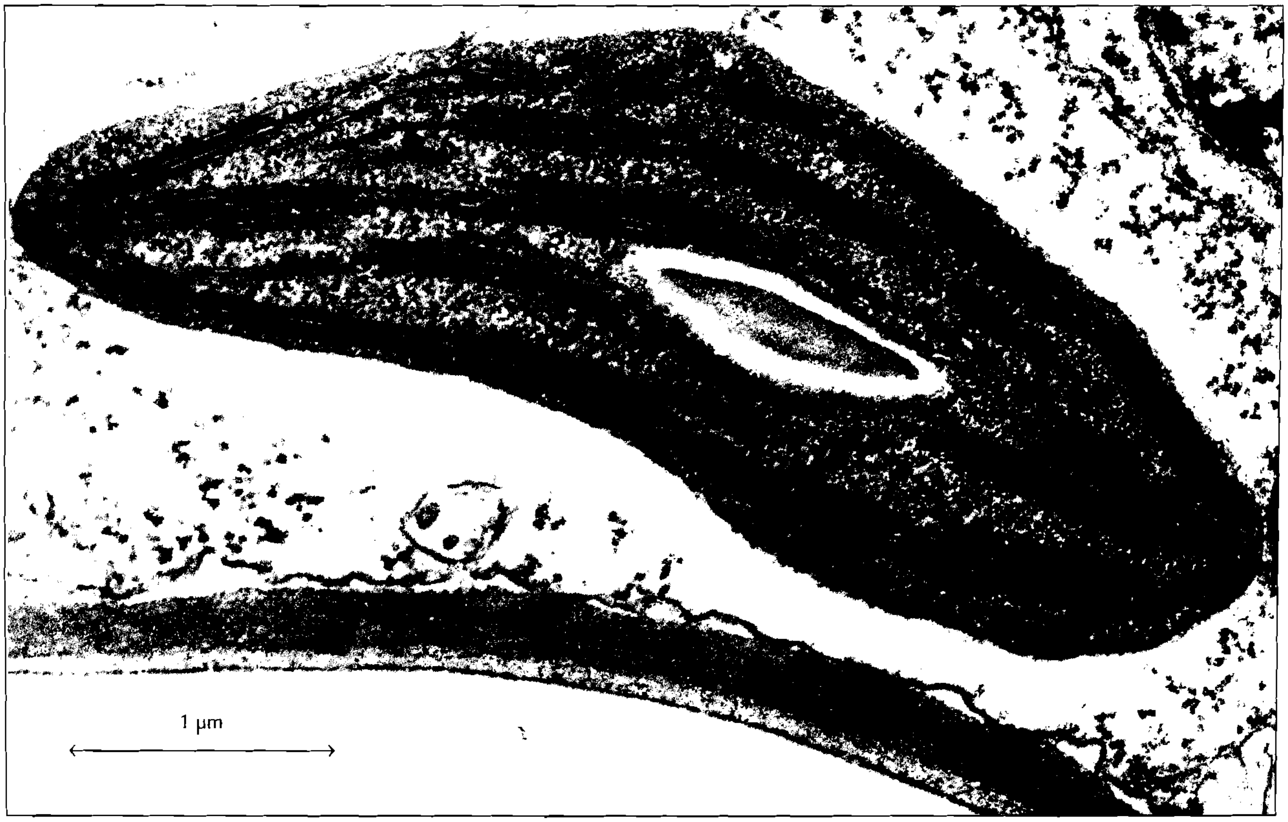
For every six molecules of triose phosphate formed in the light-independent reactions, five must be converted to RuBP.

SYNTHESIS OF CARBOHYDRATE

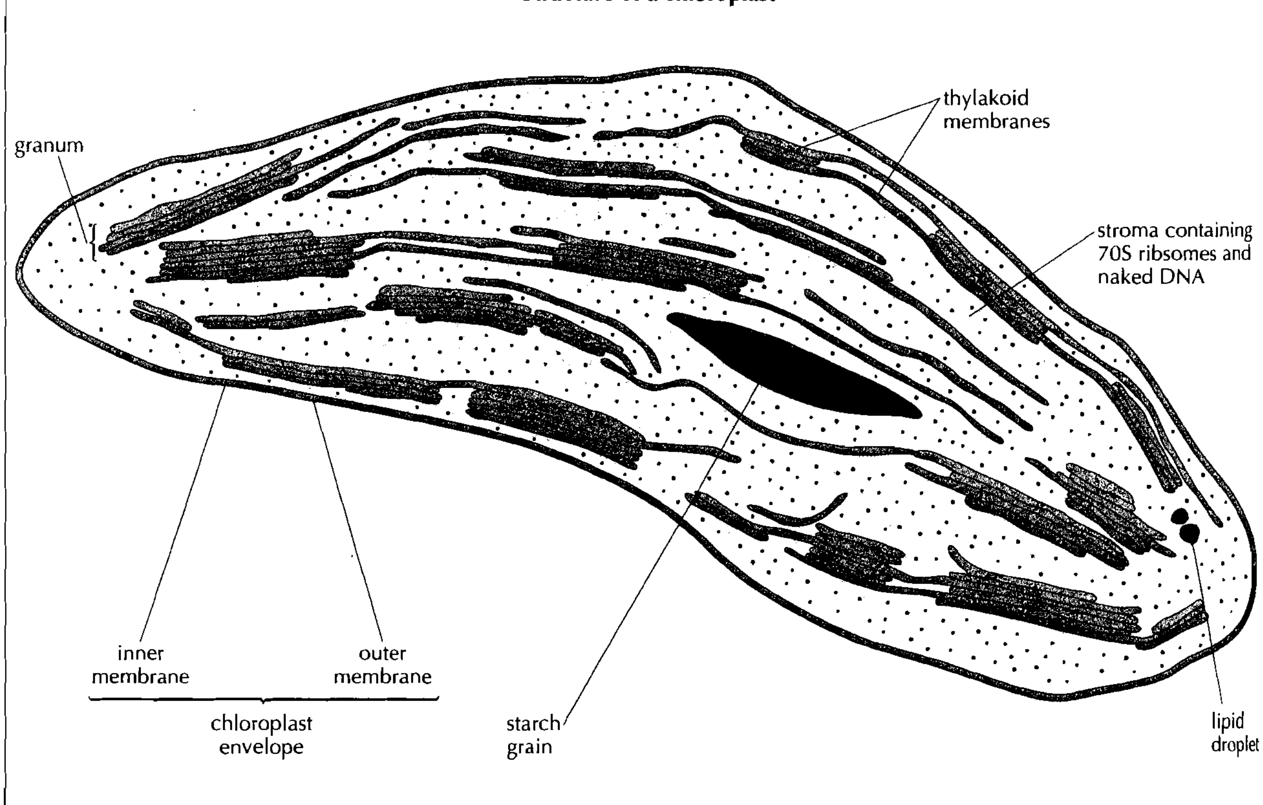
Glycerate 3-phosphate, formed in the carbon fixation reaction, is an organic acid. It is converted into a carbohydrate by a reduction reaction. Hydrogen is needed to carry out this reaction and is supplied by NADPH. Energy is also needed and is supplied by ATP. NADPH and ATP are produced in the light-dependent reactions of photosynthesis. Glycerate 3-phosphate is reduced to a three-carbon sugar, triose phosphate (TP). Linking together two triose phosphate molecules together produces glucose phosphate. Starch, the storage form of carbohydrate in plants, is formed in the stroma by condensation of many molecules of glucose phosphate.

Chloroplasts

The chloroplast is another example of close relationship between structure and function. The figure (below) is an electron micrograph of a chloroplast. The figure (bottom) is a drawing of the same chloroplast, labelled to show how it is adapted to carry out its function.



Structure of a chloroplast



THE CONCEPT OF LIMITING FACTORS

Light intensity, carbon dioxide concentration and temperature are three factors that can determine the rate of photosynthesis. If the level of one of these factors is changed, the rate of photosynthesis changes. Usually, only changes to one of the factors will affect the rate of photosynthesis in a plant at a particular time. This is the factor that is nearest to its minimum and is called the **limiting factor**. Changing the limiting factor increases or decreases the rate, but changes to the other factors have no effect. This is because photosynthesis is a complex process involving many steps. The overall rate of photosynthesis in a plant is determined by the rate of whichever step is proceeding most slowly at a particular time. This is called the **rate-limiting step**. The three limiting factors affect different rate-limiting steps.

The figures on page 17 show the relationship between each of the limiting factors and the rate of photosynthesis.

THE EFFECT OF LIGHT INTENSITY

At low light intensities, there is a shortage of the products of the light-dependent reactions – NADPH and ATP. The rate-limiting step in the Calvin cycle is the point where glycerate 3-phosphate is reduced.

At high light intensities some other factor is limiting.

Unless a plant is heavily shaded, or the sun is rising or setting, light intensity is not usually the limiting factor.

THE EFFECT OF CO_2 CONCENTRATION

At low and medium CO_2 concentrations, the rate-limiting step in the Calvin cycle is the point where CO_2 is fixed to produce glycerate 3-phosphate. RuBP and NADPH accumulate.

At high CO_2 concentrations some other factor is limiting.

Because the level of carbon dioxide in the atmosphere is never very high, carbon dioxide concentration is often the limiting factor.

THE EFFECT OF TEMPERATURE

At low temperatures, all of the enzymes that catalyse the reactions of the Calvin cycle work slowly. NADPH accumulates.

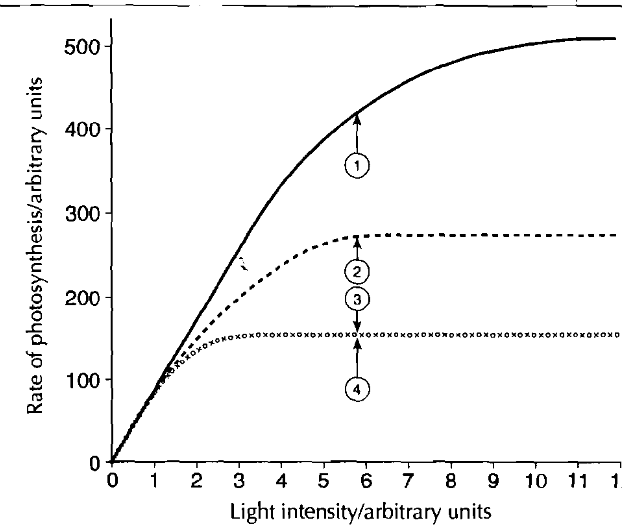
At intermediate temperatures, some other factor is limiting.

At high temperatures, RuBP carboxylase does not work effectively, so the rate-limiting step in the Calvin cycle is the point where CO_2 is fixed. NADPH accumulates.

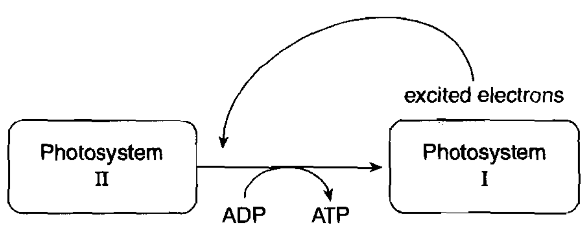
Results of an investigation into limiting factors

The figure (right) shows the effects of light intensity on the rate of photosynthesis at two different temperatures and two carbon dioxide concentrations. It is possible to deduce which is the limiting factor at the point marked with an arrow (① – ④) on each curve.

KEY
- ——— 30 °C and 0.15% CO_2
- - - - - - 20 °C and 0.15% CO_2
- × × × × × 30 °C and 0.035% CO_2
- ∘ ∘ ∘ ∘ ∘ 20 °C and 0.035% CO_2



Summary of cyclic photophosphorylation



CYCLIC PHOTOPHOSPHORYLATION

When light is not the limiting factor, NADPH tends to accumulate in the stroma and there is a shortage of $NADP^+$. The normal flow of electrons in the thylakoid membranes is inhibited because $NADP^+$ is needed as a final acceptor of electrons. An alternative route can be used that allows ATP production when $NADP^+$ is not available.

This pathway is called **cyclic photophosphorylation**.

- Photosystem I absorbs light and is photoactivated.
- Excited electrons are passed from photosystem I to a carrier in the chain between photosystem II and photosystem I.
- The electrons pass along the chain of carriers back to photosystem I.
- As the electrons flow along the chain of carriers they cause pumping of protons across the thylakoid membrane.
- A proton gradient is formed and this allows production of ATP by ATP synthase.

The figure (left) shows the pathway used in cyclic photophosphorylation.