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# Chloroplasts and Mitochondria

*Michael Tribe and  
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# Chloroplasts and Mitochondria

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# General Preface to the Series

It is no longer possible for one textbook to cover the whole field of Biology and to remain sufficiently up to date. At the same time students at school, and indeed those in their first year at universities, must be contemporary in their biological outlook and know where the most important developments are taking place.

The Biological Education Committee, set up jointly by the Royal Society and the Institute of Biology, is sponsoring, therefore, the production of a series of booklets dealing with limited biological topics in which recent progress has been most rapid and important.

A feature of the series is that the booklets indicate as clearly as possible the methods that have been employed in elucidating the problems with which they deal. Wherever appropriate there are suggestions for practical work for the student. To ensure that each booklet is kept up to date, comments and questions about the contents may be sent to the author or the Institute.

1971

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## Preface

The subject matter of this booklet has been chosen in a particularly active area of research, in which rapid changes have taken place in the last ten years. We have not attempted to cover every aspect of photosynthesis and respiration, as these are dealt with in more detail elsewhere (see references at the back of the book), but we have tried to emphasize the problems of energy conservation in relation to the structure and function of organelles. Although we have tried to present an up-to-date outline of our subject, there is considerable speculation about certain problems, and many differences of opinion. The student should appreciate that this is a healthy, thought-provoking situation, even though confusing at times. In addition, he or she should appreciate that many of the currently accepted ideas present here, may in five or ten years time have changed considerably. In view of this we have included an historical evaluation of events leading up to the present state of knowledge to provide perspective to our theme.

We have given some practical details for making crude preparations of chloroplasts and mitochondria, which we hope may be useful as a starting point for those students wishing to pursue special studies in this field.

Brighton, 1971

M.T.  
P.W.

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# The Need for an Energy Conserving System

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## 1.1 Why do we need an energy conserving system?

The Principle of the Conservation of Energy is one of the most important known to science. It states that energy is neither created nor destroyed, but can nevertheless be transformed from one form to another. It is paramount in living systems, because energy must be available to do work. However, if we try to light a 12 V bulb with a 1 V accumulator, the bulb will not light because the potential of chemical energy in this case is not high enough to power the process. In other words, we require an accumulator with more cells or a higher potential energy source. Again, if we use the same bulb with a 12 V accumulator, light will be produced, but not indefinitely unless the accumulator is frequently recharged. This analogy brings home the point that living systems have to be frequently 'recharged' in order to maintain themselves in the higher energy state. However, there is the difference that living systems are recharged more or less continuously and this can be likened to an accumulator doing work while on charge.

From another important physical law, that of entropy, we know that all systems whether living or non-living tend towards the lowest energy state; i.e. there is an increase in the randomness of systems or disorder, and hence an increase in entropy. To put it bluntly, most living things are in the lowest (and stablest) energy state when they die and decompose, thus dispersing their component atoms, with the result that there is an increase in entropy.

Living organisms are certainly unusual, but they do not possess, as was once thought, some mysterious 'vital force' which enables them to do work contrary to physical laws. The real difference between the living and the non-living is that the processes which go on in living cells enable the organism to maintain a higher energy state. Such a state is more orderly, and thus gives rise to a decrease in entropy.

To get water from an underground reservoir into a storage tank above ground, the engineer uses a pump. The pump performs work and expends energy in maintaining the head of water, but once the fuel supply to the pump, or the pump itself, breaks down, the water cannot be raised to the new level. The situation is very similar in living systems. Here again an energy input (e.g. sunlight or food) is essential to generate energy in cells, so that the input of energy at least balances output. Once this system breaks down, the organism is no longer able to maintain the higher energy state and death ensues.

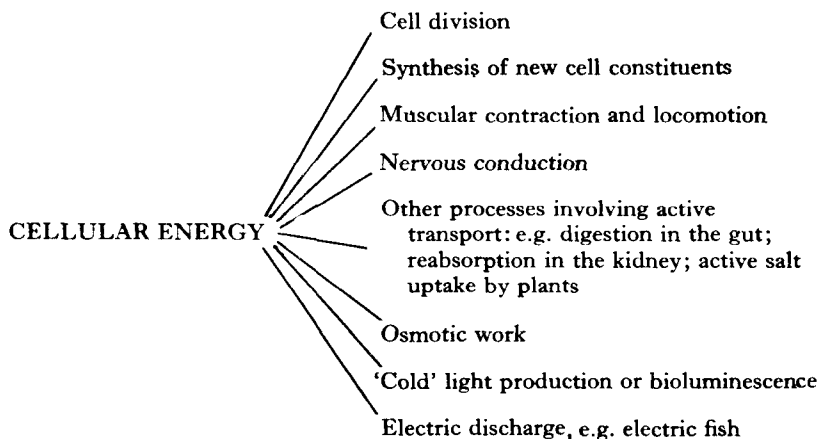
Thus we can see the need for an energy conserving system. It is the purpose of later chapters in this book to examine how cells are structurally and

chemically adapted to raise their lowest energy state, and to maintain this metastable state once acquired.

### 1.2 Uses of energy in cells

It has been stated that energy must be available in living systems to power the various processes associated with them. Table 1 summarizes the requirements for energy in cells.

**Table 1**



### 1.3 What type of energy is most useful in cellular systems?

In order to answer this question, it is necessary to ask further questions about the 'usefulness' of forms of energy as they apply to living organisms, and then to consider the six major forms of energy in relation to them.

(1) Can it be **TRANSFERRED** easily?

The form of energy must be transferable between cells and within cells so that it can be moved to any site within the organism as required.

(2) Can it be **TRANSFORMED** easily?

The energy currency must be readily convertible into a variety of forms as demanded by those processes shown in Table 1.

(3) Can it be made **AVAILABLE** easily?

Any living organism must be able to obtain its energy currency easily, or generate its own supply from precursors which may in the first instance be more readily available.

## (4) Can it be STORED easily?

It is obviously desirable that energy should be stored and made available when required. This means that the form of energy must be compatible with the life of the organism, but must not be easily lost. The form of the energy too, must be relatively stable, yet capable of rapid release when required.

## (5) Can it be USED by the organism?

The energy must be in such a form that its entry into living organisms is compatible with life, and the organism itself must be structurally adapted to harness the energy once it becomes available to the organism.

Table 2 shows how the six major forms of energy measure up to the five critical questions proposed above. An S denotes 'suitable', an X denotes 'unsuitable'.

Table 2

<i>Forms of energy</i>	<i>Suitability in relation to questions 1-5 (§ 1.3)</i>				
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>
Chemical	S	S	S	S	S
Light	S	S	S	X	S
Electrical	S	S	S	X	S
Mechanical	X	X	S	X	X
Heat	S	X	S	X	X
Sound	S	X	S	X	X

From Table 2, it can be seen that chemical energy is the most suitable form of energy for use by living cells. Chemical energy is also advantageous compared with most of the other forms, in that carefully controlled amounts of energy can be released accurately and economically.

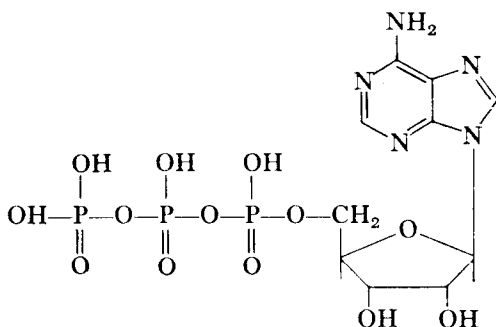
Considering the other forms of energy for a moment, electrical energy could be quite useful apart from its inability to be stored in living systems, and where electrical energy is used, as in nervous conduction, it is generated chemically. Again, green plants have the structural adaptations necessary for trapping light, but almost immediately light energy is converted into chemical energy. Occasionally, this process is reversed, as in certain bioluminescent organisms, producing a 'cold' light. The chemical pathway involved, however, is not a reversal of photosynthesis.

Heat energy is common to most living and non-living systems, but is unsuitable for a number of reasons. Firstly, high temperatures (energy in the form of heat) are incompatible with living systems, since excessive heat

denatures proteins, particularly enzymes. Secondly, heat is far too easily lost or dissipated, and hence is useless for storage purposes. There are similar objections which can be raised for both mechanical and sound as possible forms of energy in living systems. All things considered, it is now obvious why chemical energy has become the common energy source for living organisms.

#### 1.4 ATP—a high energy compound

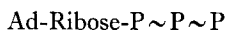
The energy currency of living cells is a chemical compound called adenosine triphosphate (ATP). As a chemical energy source it exhibits all the properties outlined in the last section. ATP consists of a nitrogenous base, adenine, linked to the five-carbon sugar, ribose. A string of three phosphate residues is attached to the sugar molecule.



ATP can be hydrolysed to adenosine diphosphate (ADP) and inorganic phosphate ( $P_i$ ) according to the equation:



Removal of the terminal phosphate residue from ATP thus releases 30 Kjoules of heat/mole. Hydrolysis of normal organic phosphates such as glucose phosphates releases only 13 Kjoules/mole. For this reason ATP has become known as a 'high-energy' phosphate and the formula for ATP is often written as

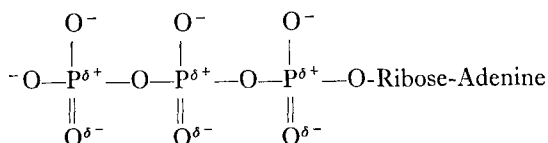


The  $\sim$  (squiggle bond) represents a high-energy linkage between phosphate residues, or more correctly, tells us that breakage of this linkage by hydrolysis yields more free energy that one would expect to obtain on hydrolysis of a normal organic phosphate. The term 'high energy' is however, only relative and there are some organic phosphates (e.g. phosphoenolpyruvate) which liberate more free energy than ATP on hydrolysis.

The reason why ATP is a high-energy compound is not clear, but it is



almost certainly a result of the distribution of charge within the molecule. One possible explanation is that ATP may ionize as follows:



If this were the case, the ATP molecule must contain more chemical energy in order to maintain the integrity of the molecule in the face of opposed positive and negative charges on the oxygen and phosphorus atoms respectively. The chemical energy released on hydrolysis of the terminal phosphate of ATP is therefore greater than the amount released from conventional organic phosphates, where such opposed charges are absent.

Although this explanation of how ATP is a 'high-energy' compound may not be accurate, it does emphasize the important fact that the 'high energy' is not contained in a single bond but is a function of the molecule as a whole.

As the hydrolysis of ATP into ADP and  $P_i$  releases so much energy, it follows that the synthesis of ATP from these components requires at least an equal amount of energy. Thus the biochemical processes and cellular structures involved in the elaboration of ATP are of great importance in a discussion of energy metabolism in living cells.

## 1.5 The sources of energy for living organisms

Living organisms obtain energy for the synthesis of ATP by breaking down organic compounds such as sugars, fats or amino acids. The organism's source of these compounds allows us to place it into one of two categories:

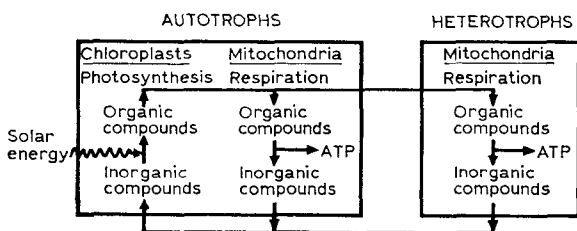
- (i) Heterotrophic organisms (animals and non-green plants). These assimilate the organic energy sources from the breakdown products of other organisms—either living or dead.
- (ii) Autotrophic organisms (green plants). These are able to manufacture their own organic energy sources from simple inorganic compounds.

Consequently the synthesis of organic from inorganic compounds which occurs in autotrophs is the starting point for the energy flow through the living world.

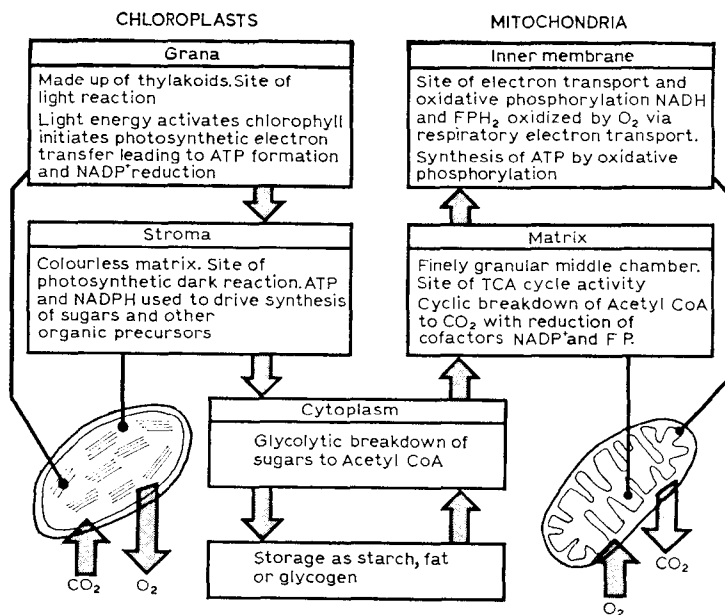
The majority of autotrophic organisms are photosynthetic. They use sunlight energy to drive the synthesis of sugars and amino acids from carbon dioxide, water, and simple nitrogenous compounds. However, some autotrophic bacteria (the chemosynthetic bacteria) do not need to trap light energy as they obtain the energy required for the synthesis of sugars and amino acids from the oxidation of inorganic substances such as ammonia,

hydrogen, or sulphur. The biochemistry of chemosynthetic processes is poorly understood and as they occur only in certain species of bacteria, and as bacteria do not possess chloroplasts or mitochondria, we consider that they are outside the scope of this book.

Figure 1-1 summarizes the relationships between the biochemical processes of heterotrophs and autotrophs.



**Fig. 1-1** Solar energy drives the synthesis of organic compounds from inorganic. The organic compounds may be broken down to inorganic in the mitochondria of autotrophs and heterotrophs. The energy released is used to drive ATP synthesis. Inorganic end products of respiration can be used in photosynthesis.



**Fig. 1-2**

**1.6 Cellular location of photosynthetic and respiratory processes**

Photosynthesis takes place in subcellular organelles called chloroplasts. These are present in the green cells of autotrophic organisms (although the green colour may be masked by other pigments).

Respiration and the bulk of ATP synthesis occurs in the mitochondria which are present in almost all the living cells of both autotrophic and heterotrophic organisms. Notable exceptions are the bacteria, blue-green algae, and mammalian erythrocytes.

# Sunlight to ATP: The Flow of Energy 2

## 2.1 Introduction

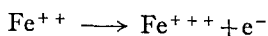
The processes whereby living organisms transform light energy into chemical energy and make it available for ATP synthesis, can be conveniently divided into four phases.

The first two phases are what we normally refer to as photosynthesis and occur within the chloroplast. The *photosynthetic light reaction*, the capture of light energy and its conversion to chemical energy occurs in the grana (solid regions) of the chloroplasts and produces the co-factors necessary to drive the *photosynthetic dark reaction*. This 'reaction' which occurs in the chloroplast stroma (fluid region) is a cyclic series of reactions which converts simple inorganic substances into carbohydrates and other organic compounds. Carbohydrates are relatively stable and, with the exception of storage carbohydrates, relatively mobile compounds. They can therefore be translocated to other parts of the plant or they may become assimilated by animals. In both plants and animals the *breakdown of carbohydrates* and utilization of the energy for ATP synthesis begins in the cell cytoplasm (glycolysis) and continues in the matrix of the mitochondrion (TCA—tricarboxylic acid cycle). The synthesis of ATP (*oxidative phosphorylation*) occurs on the mitochondrial cristae (inner-membrane).

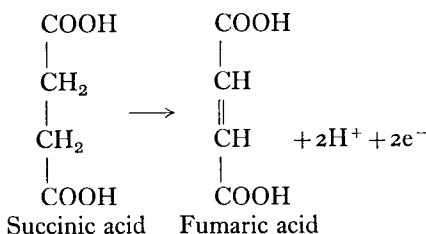
## 2.2 Oxidation-Reduction reactions—controlled release of energy

If you drop a match into the petrol tank of your car it will probably cause the car to move, but hardly in the direction you would like it to! If on the other hand, you treat your car in a more conventional manner and allow the petrol to be burned in successive small quantities you should have little trouble in moving the car in a controlled fashion. Similarly, in biological systems, energy is made available a little at a time to give maximum efficiency of ATP synthesis. Both the photosynthetic light reaction and oxidative phosphorylation processes involve organized series of oxidation-reduction reactions releasing energy at each step in the sequence.

Oxidation is always an energy-yielding process and can be considered to be the loss of an electron or electrons. For example oxidation of ferrous ions to ferric ions occurs by loss of a single electron.

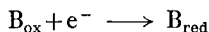
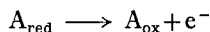


In another type of oxidation hydrogen ions (protons) may be given up as well as the electrons. An example of this is the oxidation of succinic acid to fumaric acid.



Conversely reduction is always an energy-requiring process involving the gain of electrons (or electrons and protons). If the directions of the two oxidation reactions given above are reversed, then ferric ions may be reduced to ferrous ions by gaining a single electron, or fumaric acid may be reduced to succinic acid by gaining two protons and two electrons.

If one substance becomes oxidized, another must be reduced. The electrons released in the oxidation are used to bring about the reduction. The following is a general example involving two compounds A and B capable of oxidation-reduction (i.e. *electron carriers*):

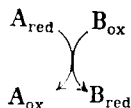


(*red* and *ox* signify the reduced and oxidised states of the carriers respectively)

The electrons, however, will be passed directly from A to B. Therefore it is more meaningful to sum these two equations and eliminate  $\text{e}^-$ :



This equation can be rewritten as follows in a form which is more convenient for describing the series of oxidation-reduction reactions used to control the release of the energy for ATP synthesis in biological processes.



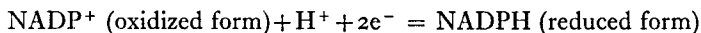
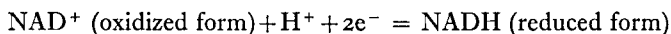
In biological oxidation-reduction systems the electron carriers are organized in such a way that the oxidation of a carrier releases more energy than is required to reduce the next carrier in the sequence. If there is a sufficient surplus of energy released at any stage this can be used to drive the synthesis of ATP from ADP and  $\text{P}_i$ .

There are four major classes of electron carriers involved in these energy conservation processes.

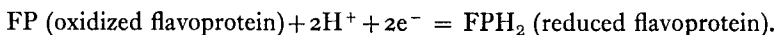
(i) *Pyridine nucleotides*

Nicotinamide adenine dinucleotide ( $\text{NAD}^+$ )\* and nicotinamide adenine dinucleotide phosphate ( $\text{NADP}^+$ )\*.

These are electron and proton carriers undergoing oxidation-reduction in the following manner:

(ii) *Flavoproteins*

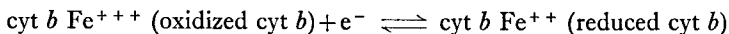
These are enzymes with prosthetic groups derived from riboflavin (Vitamin  $\text{B}_2$ ). The riboflavin derivative undergoes an oxidation-reduction involving protons and electrons.

(iii) *Cytochromes*

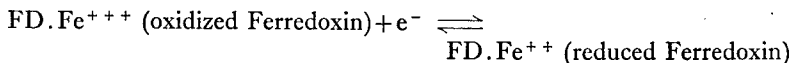
These are also enzymes with prosthetic groups capable of oxidation-reduction. In this case the prosthetic groups are haem molecules, similar in structure to the haem of haemoglobin (see CHAPMAN, *Animal Body Fluids* in this series). The cytochromes are classified primarily on the basis of the precise structure of the haem part of the molecule, into cytochromes *a*, *b*, *c*, etc. Differences within each class in structure of the protein part of the enzymes are indicated by use of a subscript, e.g. cytochrome  $c_1$ , cytochrome  $a_3$ , etc.

The cytochromes of importance in biological energy conversion processes are cytochromes *a* and  $a_3$ , *b* and  $b_6$ , *c* and  $c_1$ , and *f*.

The cytochromes are electron carriers, the haem iron being capable of undergoing oxidation-reduction between ferric and ferrous forms. e.g.

(iv) *Ferredoxin*

A further electron carrier of importance in the photosynthetic system is ferredoxin, a protein containing iron which is not part of a haem residue. The iron can undergo oxidation-reduction in a similar manner to that of the cytochromes.



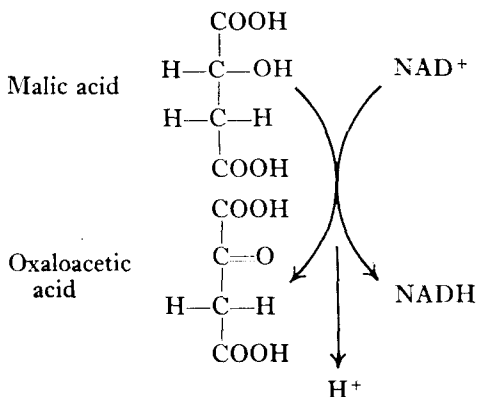
## 2.3 Source of reducing power and electron transfer processes

Because of the overall passage of electrons along a series of oxidation-reduction carriers during the photosynthetic light reaction and oxidative

\* Still referred to by some authors as  $\text{DPN}^+$  and  $\text{TPN}^+$ .

phosphorylation, the processes are referred to as the photosynthetic electron transfer system, and the respiratory electron transfer system respectively. The precise sequence of electron carriers, particularly in photosynthesis, is not firmly established. The schemes we describe are the ones that we feel best fit the available experimental evidence. The initiation of electron transfer obviously requires the reduction of the first carrier in the series. In the photosynthetic electron transfer system, the first carrier to become reduced is ferredoxin. This is reduced by an energized chlorophyll electron. Chlorophyll can absorb visible light in the wavelength regions of 430 nanometres (violet) and 650 nm (red). If a photon (discrete unit) of light in these wavelength regions is absorbed, a chlorophyll electron is activated from its ground energy state to a higher energy state. The energized state of the chlorophyll electron is indicated in subsequent diagrams by an asterisk. Under normal circumstances the excited electron would fall back to its ground state, emitting the energy lost as heat or light (fluorescence). In the chloroplast, however, the energy may be trapped by using the excited electron to reduce ferredoxin.

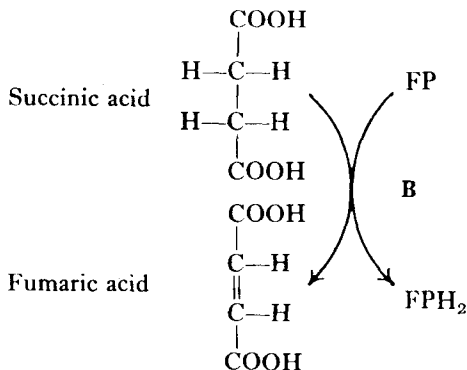
In the respiratory electron transfer system the first carrier reduced is generally  $\text{NAD}^+$ .  $\text{NAD}^+$  is reduced either by pyruvic acid or by one of the TCA cycle substrates, isocitric acid,  $\alpha$ -ketoglutaric acid, or malic acid. Consequently, these reducing compounds become oxidized (see formulæ A).



However, although  $\text{NAD}^+$  is generally the first carrier reduced, there is one major exception. Succinic acid oxidation is coupled to the reduction of a flavoprotein (see formulæ B p. 12).

The first carrier in either system having been reduced, the linked sequences of oxidation-reduction reactions begin. The photosynthetic system is the more complex than the respiratory system as there are probably two separate electron transfer routes

(i) cyclic electron transfer—geared to the generation of ATP, and



(ii) non-cyclic electron transfer geared to the production of NADPH and ATP.

NADPH and ATP are the two co-factors which are essential for driving the photosynthetic dark reaction. Figures 2-1 and 2-2 outline the reaction sequences of the cyclic and non-cyclic processes.

In the cyclic system oxidation-reduction reactions occur in an unbroken sequence as far as cytochrome *f*. The reduced cytochrome *f* is reoxidized by

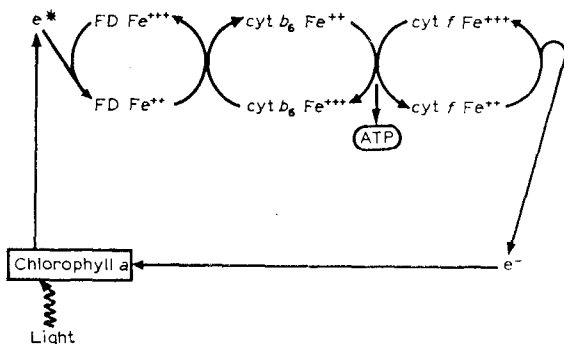


Fig. 2-1 Cyclic photosynthetic electron transport.

donating an electron back to chlorophyll. This electron will enter the ground state, ready for a further activation by a photon of light. There is at least one point in the cycle where sufficient energy is released to drive ATP synthesis—this, as shown in Fig. 2-1, is between *cyt b<sub>6</sub>* and *cyt f*.

As will be observed from Fig. 2-2 the non-cyclic system is somewhat more complex. This is because the system has a dual role, i.e. the reduction of NADP<sup>+</sup> to NADPH and ATP synthesis. The non-cyclic system is probably not physically divorced from the cyclic system. It is likely that



the FD and  $\text{cyt } b_6$  of Fig. 2-2 can react as shown for the cyclic system in Fig. 2-1. The two schemes are drawn separately merely for clarity. In the non-cyclic system the oxidation of reduced ferredoxin is used to drive the reduction of  $\text{NADP}^+$ . The hydrogen ions required for this process come from the splitting of water (photolysis). The hydroxyl ions remaining are

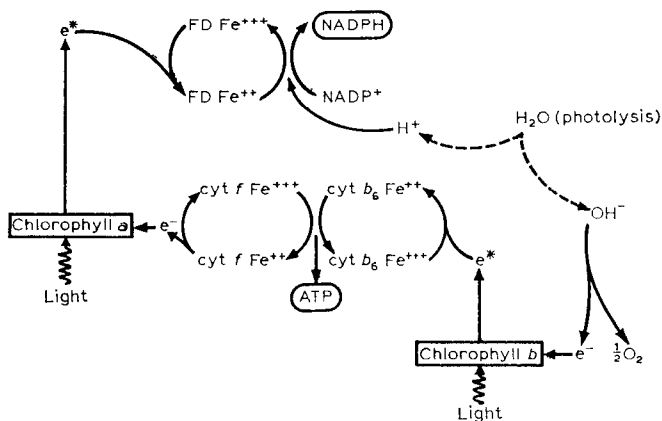
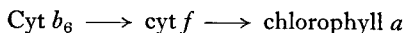


Fig. 2-2 Non-cyclic photosynthetic electron transport.

used to generate  $\text{O}_2$  and electrons according to an equation which is slightly more complex than shown in Fig. 2-2.



This is the only source of oxygen evolved during photosynthesis. The electrons released in the process are passed to chlorophyll. This chlorophyll is probably chlorophyll *b* (different in structure from the chlorophyll *a* which is involved in the initial electron activation). The chlorophyll *b* electron can now be activated by a photon of light and used to reduce  $\text{cyt } b_6$ . The oxidation-reduction reactions completing the non-cyclic system follow the last part of the cyclic system i.e.



As in the cyclic system there is probably an ATP synthesizing site between  $\text{cyt } b_6$  and  $\text{cyt } f$ .

Thus the overall result of photosynthetic electron transfer is (i) ATP production, (ii)  $\text{NADP}^+$  reduction, and (iii)  $\text{O}_2$  evolution. It is unlikely that the ATP and NADPH formed can be passed outside the chloroplast and so these can only be used to drive the synthesis of organic compounds from  $\text{CO}_2$  in the dark reaction.

The mitochondrial respiratory electron transfer system has one major