

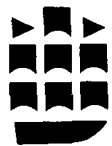
J N Prebble

**MITOCHONDRIA
CHLOROPLASTS
AND BACTERIAL
MEMBRANES**

MITOCHONDRIA CHLOROPLASTS AND BACTERIAL MEMBRANES

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Preface

Books on mitochondria and on photosynthesis abound. Thus the addition of yet another book on these subjects requires some justification. Most of the books on mitochondria fall into two categories, those which are research monographs, many of them being the proceedings of a scientific meeting, and those which form an introduction to the subject at about the first- or second-year University level. A not dissimilar situation applies to photosynthesis. The chloroplast as an organelle receives rather less emphasis. Although the cross-fertilisation of ideas between the fields of chloroplast and mitochondrial biochemistry is widely acknowledged, treatment of both subjects together is less common. In the present work, I have endeavoured to bridge the gap between the introductory and the advanced research-orientated works by attempting to provide a broad coverage of the main aspects of mitochondria and chloroplasts with a view to providing a jumping-off point for the extensive literature of reviews. Each chapter terminates with a short list of recent reviews.

The book is written as a textbook with the intention of conveying concepts and information and is aimed primarily at third-year undergraduate and graduate students. The background knowledge of students tends to be very variable, even among those of the same institution, and an attempt has been made to limit the knowledge required to understand the major concepts discussed. The more elementary material has been presented in a historical manner in order to demonstrate the development of the subject. The number of references has been kept to a minimum not only for economy of space, but also because references can create a forbidding distraction to the text. A major objective is to present a balanced account of the organelles although the notion of balance is inevitably subjective. Thus, the mitochondrion has been viewed both as the organelle responsible for oxidative phosphorylation and as a particle possessing a complex pattern of metabolic pathways and permeation systems some of which are not directly related to its energetic function. In recent years, the study of bacterial biochemistry has contributed significantly to an understanding of chloroplasts and mitochondria; two chapters summarising energetics of bacterial membranes have therefore been included.

I would like to acknowledge my indebtedness to many friends at Bedford College for their advice, help and encouragement. In particular I wish to thank Professor D. F. Cheesman who earlier encouraged my interest in biochemistry

and who has kindly read the manuscript and made many valuable suggestions. My thanks go to Dr K. E. Howlett for advice on Chapter 1, Sarah Chapman and Sonia Copeland who typed the manuscript and to the staff of Longman for their help. Finally, I would also like to add my appreciation of the patience and support of my wife and family.

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Abbreviations

$\Delta\psi$	membrane potential
Δp	proton motive force (PMF)
λ	wavelength
ADP	adenosine diphosphate
ALA	γ -aminolaevulinic acid
Ala	alanine
AMP	adenosine monophosphate
Asp	aspartic acid
ATP	adenosine triphosphate
CAM	crassulacean acid metabolism
CCCP	carbonylcyanide- <i>m</i> -chlorophenylhydrazone
Chl	chlorophyll
CoA	coenzyme A
CP	chlorophyll-protein complex
ctDNA	chloroplast DNA
cyt	cytochrome
DABS	<i>p</i> -(diazonium)-benzenesulphonate
DBTQ	dibromothymoquinone
DCCD	<i>N,N</i> -dicyclohexylcarbodiimide
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DCPIP	dichlorophenol indophenol
DNA	deoxyribose nucleic acid
DNP	dinitrophenol
E_m	mid-point potential (pH included as subscript: $E_m 7.0$)
EDTA	ethylenediaminetetra-acetic acid
EPR	electron paramagnetic resonance
<i>F</i>	Faraday
FAD	flavin adenine dinucleotide
FCCP	carbonylcyanide- <i>p</i> -trifluoromethoxyphenylhydrazone
Fd	ferredoxin
Fe-S	iron-sulphur centre or protein
FMN	flavin mononucleotide
Fp	flavoprotein
GDP	guanosine diphosphate
GTP	guanosine triphosphate
His	histidine
Ile	isoleucine
kb	kilobase (= 1000 bases)
Lys	lysine
Met	methionine

MK	menaquinone
mRNA	messenger RNA
mtDNA	mitochondrial DNA
NAD, NADH ₂	nicotinamide adenine dinucleotide (oxidised and reduced respectively)
NADP, NADPH ₂	nicotinamide adenine dinucleotide phosphate (oxidised and reduced respectively)
OSCP	oligomycin sensitivity conferring protein
PGA	3-phosphoglyceric acid
Phe	phenylalanine
Pi	inorganic phosphate
PP	pyrophosphate
PQ	plastoquinone
Pro	proline
PS I, PS II	pigment system or photosystem I and II respectively
Q	ubiquinone (also used for the primary acceptor of PS II)
RNA	ribonucleic acid
Ser	serine
SMP	submitochondrial particle
Thr	threonine
TMPD	tetramethylphenylenediamine
TPP	thiamin pyrophosphate
Try	tryptophan
Tyr	tyrosine
UDP	uridine diphosphate
UTP	uridine triphosphate
Val	valine

Contents

<i>Preface</i>	xi
<i>Abbreviations</i>	xiii
Chapter 1 <i>Mitochondria and chloroplasts: basic concepts</i>	1
1.1 The nature of the subcellular particles	1
(a) Introduction (b) The mitochondrion – a metabolic system for ATP synthesis (c) The chloroplast – an energy transducing system (d) Distribution of particles (e) Isolation of mitochondrial and chloroplast fractions	
1.2 Energetic considerations – ATP	13
1.3 Oxidation–reduction systems	18
Chapter 2 <i>Development of ideas on oxidation and phosphorylation</i>	24
2.1 Early studies on cell oxidation	24
2.2 The discovery of cytochromes	26
2.3 A simple respiratory chain	27
(a) Keilin's work on the cytochrome chain (b) The discovery of cytochrome c_1 (c) <i>b</i> -Cytochromes (d) Isolation of flavoprotein dehydrogenases (e) Ubiquinone (f) The use of difference spectra (g) Inhibitors and cross-over points	
2.4 The cytochromes	33
2.5 The respiratory chain and mitochondrial oxidation	38
2.6 Phosphorylation coupled to respiration	41
(a) Types of phosphorylation (b) Discovery of oxidative phosphorylation (c) Measurement of P/O ratios (d) Phosphorylation associated with regions of the respiratory chain	
2.7 Coupled mitochondria, respiratory control	45
2.8 Sites of phosphorylation	46
2.9 The link between oxidation and phosphorylation	47
(a) The mitochondrial ATPase (b) Reverse electron flow driven by ATP hydrolysis (c) The inorganic phosphate–ATP exchange reaction (d) The intermediate high-energy state	
2.10 Inhibitors of oxidative phosphorylation	48
2.11 Phosphorylation and control of metabolism	50

Chapter 3	<i>Mitochondrial oxidative metabolism</i>	52
3.1	The formulation of the citric acid cycle	52
3.2	The oxidation of pyruvate	56
	(a) The role of acetyl coenzyme A (b) Cofactors and prosthetic groups (c) Pyruvate dehydrogenase (d) Regulation	
3.3	Citric acid cycle reactions	63
	(a) Tricarboxylate metabolism (b) Conversion of oxoglutarate to succinate (c) Dicarboxylate metabolism	
3.4	The glyoxylate cycle	68
3.5	Carboxylation reactions	70
3.6	Regulation of the citric acid cycle	72
3.7	Gluconeogenesis	75
3.8	Mitochondrial nitrogen metabolism	75
3.9	Fatty acid oxidation	78
3.10	Fatty acid activation	81
3.11	The β -oxidation pathway	83
3.12	Metabolism of the products of β -oxidation	87
	(a) Oxidation of acetyl coenzyme A (b) Synthesis and metabolism of ketone bodies (c) Propionyl coenzyme A metabolism (d) Gluconeogenesis in plants	
3.13	Ethanol metabolism	89
3.14	The relationship between carbohydrate and lipid metabolism	90
3.15	Appendix: fatty acid elongation	90
Chapter 4	<i>The structure of the mitochondrion</i>	93
4.1	The mitochondrion: development of ideas	93
4.2	Mitochondrial structure	95
	(a) General structure (b) Membrane surfaces (c) Freeze-fracture studies (d) Membrane structure	
4.3	Disruption of mitochondria	103
	(a) Submitochondrial particles, sidedness of mitochondrial membranes (b) Separation of inner and outer membranes	
4.4	Permeability of mitochondrial membranes	106
4.5	Properties of mitochondrial membranes	107
4.6	Mitochondrial enzymes	108
	(a) Distribution (b) Metabolic functions of the mitochondrion	
Chapter 5	<i>Mitochondrial biogenesis</i>	125
5.1	Early approaches to mitochondrial biogenesis	125
5.2	The nucleic acids of the mitochondrion	126
	(a) Mitochondrial DNA (b) Mitochondrial RNA	
5.3	Protein synthesis	130
	(a) Transcription of mtDNA: synthesis of mtRNA (b) Translation of mRNA: synthesis of protein (c) Cytochrome oxidase (d) The ATPase (e) Cytochrome <i>b</i> (f) Cytoplasmic synthesis of mitochondrial protein (g) Regulation of the synthesis of mitochondrial protein	
5.4	Mitochondrial genetics: mapping the mitochondrial DNA	134

Chapter 6	<i>Mitochondrial water movement and substrate transport</i>	137
6.1	Compartmentalisation	137
6.2	Water movement: mitochondrial swelling	137
6.3	Permeability to substrates	138
	(a) Factors influencing substrate transport (b) Early studies of substrate transport	
6.4	Mitochondrial translocation systems	142
	(a) Phosphate (b) Dicarboxylate and tricarboxylate transport (c) Pyruvate (monocarboxylate) transport (d) Amino acid transport (e) Adenine nucleotide translocation (f) Carriers for other substrates	
6.5	Transfer of reducing equivalents across the inner membrane	152
	(a) Maintenance of the $\text{NAD}^+/\text{NADH}_2$ ratios (b) Malate-aspartate shuttle (c) α -Glycerophosphate shuttle (d) Fatty acid cycle (e) Shuttles concerned with the export of reducing equivalents (f) Other shuttles	
Chapter 7	<i>Mitochondrial cation transport</i>	157
7.1	Mitochondrial cations	157
7.2	Calcium uptake	157
	(a) 'Massive loading' experiments (b) 'Limited loading' experiments (c) Relationship between calcium transport, phosphorylation and proton transport (d) Calcium-binding proteins and the calcium translocase (e) Calcium efflux (f) Physiological significance of calcium transport	
7.3	Magnesium and iron transport	163
7.4	Monovalent cation transport	164
	(a) Non-induced transport (b) Ionophore-induced transport (c) Role of ionophores	
7.5	Proton translocation	167
	(a) Demonstration of proton transport (b) Stoichiometry (c) Reversible relationship between ion transport and the ATPase reaction	
7.6	Models of cation transport	169
Chapter 8	<i>Theories of phosphorylation</i>	171
8.1	The high-energy intermediate	171
8.2	The chemical theory	171
8.3	The chemiosmotic theory	172
8.4	Conformational theories	177
8.5	Which theory?	178
Chapter 9	<i>Resolution of the respiratory chain and oxidative phosphorylation</i>	180
9.1	The mitochondrial adenosine triphosphatase (ATP synthase)	180
	(a) General properties and exchange reactions (b) Coupling factors (c) F_1 ATPase (d) Reconstitution studies	
9.2	Coupling and uncoupling	186
	(a) Properties of coupled mitochondria (b) Brown adipose tissue mitochondria	
9.3	Isolation of complexes of the respiratory chain of beef heart	188

9.4	NADH ₂ -ubiquinone reductase, NADH dehydrogenase	189
	(a) Properties and types of enzyme (b) Prosthetic groups: FMN and Fe (c) Iron-sulphur proteins (d) Iron-sulphur centres in NADH-ubiquinone reductase (e) Lipids (f) Resolution of Complex I (g) Reconstitution studies (h) Membrane orientation	
9.5	Transhydrogenase	196
9.6	Succinate-ubiquinone reductase	196
	(a) Properties (b) Resolution into subunits (c) Catalytic activity	
9.7	Ubiquinol-cytochrome <i>c</i> reductase	198
	(a) Composition (b) Cytochromes (c) iron-sulphur protein (d) Anti-mycin A (e) Cleavage of the complex (f) Reconstitution studies and ion translocation	
9.8	Cytochrome oxidase	201
	(a) General properties (b) Composition (c) Mechanism (d) Proton translocation (e) Conformational changes	
9.9	Ubiquinone	203
9.10	The respiratory chain	204
	(a) General (b) Plant mitochondrial respiration (c) Micro-organisms	
9.11	Thermodynamic considerations	206
	(a) Membrane potentials and ion gradients (b) Redox potentials and phosphorylation (c) Phosphorylation and standard potentials	
Chapter 10	<i>Bacterial energy transformation</i>	211
10.1	Bacterial energetics	211
10.2	Bacterial membranes	211
10.3	Bacterial respiratory chain	215
	(a) Components of the chain (b) Dehydrogenases (c) Quinones (d) Cytochromes (e) Cytochrome oxidases (f) Environmental effects (g) Anaerobic electron transport	
10.4	Energy conservation	221
10.5	Bacterial ATPases	226
10.6	Chemolithotrophic bacteria	227
10.7	Bacterial transport	227
10.8	Evolution of the mitochondrion from prokaryotes	230
Chapter 11	<i>Photosynthesis: the fixation of carbon dioxide</i>	235
11.1	The Calvin pentose phosphate pathway	235
	(a) Introduction (b) Role of phosphoglyceric acid (c) The Calvin cycle (d) Ribulose diphosphate carboxylase (e) Photorespiration (f) Factors affecting Calvin cycle activity	
11.2	Starch and sucrose synthesis	247
11.3	The dicarboxylic acid pathway (C ₄ pathway)	249
	(a) Outline of the pathway (b) Regulation	
11.4	Other carboxylation pathways	254
	(a) Malate formation in C ₃ plants (b) The CAM pathway	
Chapter 12	<i>The chloroplast: structure, properties and biogenesis</i>	257
12.1	Structure	257
12.2	Chemical composition	258
	(a) Envelope (b) Thylakoid membranes	

12.3	Thylakoid membrane structure	259
12.4	Chloroplast fractionation	263
12.5	Permeability	266
	(a) Permeability of chloroplast membranes (b) Specific membrane translocators	
12.6	Chloroplast enzymes	268
	(a) Systems involved in carbon fixation (b) Carbohydrate metabolism and galactolipid synthesis (c) Fatty acid synthesis (d) Isoprenoid synthesis (e) Porphyrin synthesis (f) Other reactions	
12.7	Chloroplast biogenesis	276
	(a) A system for protein synthesis (b) Gene products (c) Mapping the chloroplast DNA	
12.8	Evolution of chloroplasts	278
Chapter 13	<i>Chloroplast photochemistry</i>	280
13.1	Light energy	280
13.2	Excitation of organic molecules	281
	(a) Excited singlet states (b) Higher excited states (c) Energy transfer (d) Triplet states	
13.3	Pigments	283
	(a) Pigment distribution (b) Forms of chlorophyll (c) Chlorophyll proteins	
13.4	Development of concepts of pigment function	288
13.5	Thylakoid photochemistry	289
	(a) Antenna molecules and reaction centres (b) Energy transfer (c) Action spectra (d) Evidence for energy transfer (e) Transfer of energy in pigment systems (f) 'Puddles' and 'lakes' (g) The 'spillover' or 'separate package' hypothesis	
13.6	Photochemical reactions	294
13.7	Summary	295
Chapter 14	<i>The chloroplast electron transport chain</i>	297
14.1	Development of the idea of a chloroplast electron transport chain	297
	(a) Oxygen evolution and the Hill reaction (b) NADP reduction (c) Cytochromes (d) Electron transport between the photochemical systems (e) Photophosphorylation	
14.2	Oxidation-reduction reactions associated with PS I	303
	(a) P700 (b) Primary acceptor for P700 (c) Electron donor to P700 (d) Algal <i>c</i> -type cytochromes	
14.3	Electron transport associated with photosystem II	306
	(a) P 680 (b) Fluorescence studies; Q the primary electron acceptor for PS II (c) Plastoquinones, secondary electron acceptors for PS II (d) The identities of Q, X320 and C550 (e) Oxygen evolution and the electron donor to PS II (f) Delayed fluorescence	
14.4	The <i>b</i> -cytochromes	314
	(a) Cytochrome <i>b</i> ₅₅₉ (b) Cytochrome <i>b</i> ₆	
14.5	Orientation of photosystems in the thylakoid membrane	315
	(a) Transmembrane orientation of the electron transport chain (b) Location of PS II electron acceptors (c) Electron donors to PS I: plastocyanin and cytochrome <i>f</i> (d) Electron acceptor for PS I	

14.6	Artificial electron donors and acceptors for electron transport	317
14.7	Proton translocation and membrane potentials	318
14.8	The chloroplast ATPase	319
	(a) Discovery (b) Catalytic activity (c) CF_1	
14.9	The ATPase and theories of photophosphorylation	321
	(a) Chemiosmotic evidence (b) Conformational evidence	
14.10	Photophosphorylation	322
	(a) Estimates of the P/2e ratio (b) Sites of phosphorylation (c) Mechanism of phosphorylation (d) Cyclic photophosphorylation	
14.11	Energetics	328
	(a) Quantum yield (b) Thermodynamic considerations (c) Proton gradients and membrane potentials	
Chapter 15	<i>Bacterial photosynthesis</i>	332
15.1	Groups of photosynthetic prokaryotes	332
	(a) Cyanobacteria (b) Rhodospirillales (c) Halobacterium	
15.2	Organelles of photosynthetic bacteria (Rhodospirillales)	334
	(a) Purple bacterial chromatophores (b) Organelles of green sulphur bacteria (c) Photosynthetic pigments	
15.3	Photochemistry	337
15.4	Reaction centres	340
15.5	Electron transport chain	341
	(a) Electron donor to P870 (b) Cyclical electron transport: cytochrome b_{50} (c) Proton translocation	
15.6	Photophosphorylation	344
15.7	Carbon dioxide fixation	344
15.8	Respiratory metabolism	345
15.9	Photosynthesis without chlorophyll	346
	(a) The purple membrane (b) Photochemistry (c) Function of the purple membrane (d) Ecological and theoretical significance of bacteriorhodopsin-catalysed photophosphorylation	
	<i>References</i>	354
	<i>Index</i>	365

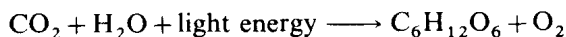
Chapter 1

Mitochondria and chloroplasts: basic concepts

1.1 The nature of the subcellular particles

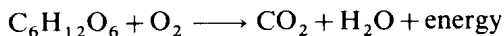
(a) Introduction

The primary source of energy available for life on our planet is solar radiation. This may be converted to chemical energy by organisms possessing suitable photochemical pigments and associated systems. In green plants the process, photosynthesis, is concerned with the synthesis of organic compounds (e.g. hexose sugars) from carbon dioxide and water and may be represented as:



The fossil fuels, coal, oil and natural gas which are now being rapidly consumed, are ultimate products of this process and represent the photosynthetic activity of plants in earlier periods of geological time.

Just as technology harnesses the energy of organic compounds by their combustion in the presence of oxygen, so living cells are able to use the energy in organic compounds for life processes such as the synthesis of fresh cellular material and muscular movement. For hexoses, this oxidative process may be represented:



The two processes above are thermodynamically but not mechanistically the reverse of one another. However, as we shall see, they have similarities, for example in regard to ATP synthesis. Except in simple organisms (prokaryotes) the major part of both processes takes place in discrete organelles within the cell. The photosynthetic system is housed within the chloroplast, while the essential oxidative systems are the property of the mitochondrion. Both organelles are surrounded by a double membrane which is selectively permeable to cell constituents, so that the organelles constitute metabolic compartments separate from the rest of the cell but connected to it in a manner controlled by the permeation systems.

The reason for discussing both the chloroplast and the mitochondrion within the compass of a single book lies in the fact that both possess similar chains of oxidation – reduction reactions which are linked to ATP synthesis. In addition, the oxidation – reduction systems are also linked to complex metabolic

cycles. Further, both are semi-autonomous in possessing a partial hereditary system, a small 'chromosome' coding for some of the particle's proteins which can be totally synthesised within the particles.

(b) The mitochondrion – a metabolic system for ATP synthesis

The major functions of the mitochondrion can be conveniently listed as:

- (i) the oxidation of pyruvate to acetyl coenzyme A
- (ii) the oxidation of fatty acids to acetyl coenzyme A
- (iii) the oxidation of acetyl coenzyme A to CO_2 and reduced cofactors (e.g. NADH_2)
- (iv) the oxidation of reduced cofactors (NADH_2) by oxygen forming water
- (v) synthesis of ATP coupled to NADH_2 oxidation, oxidative phosphorylation.

All five of these processes are more or less intimately linked (see Fig. 1.1) and may be considered as part of the oxidative system for ATP synthesis. Thus the *raison d'être* of the mitochondrion is to be the major supplier of ATP for the cell. However, the mitochondrion also has a significant role in other processes such as nitrogen metabolism and various biosynthetic systems, for example in porphyrin synthesis and in steroid hormone synthesis in the adrenal cortex.

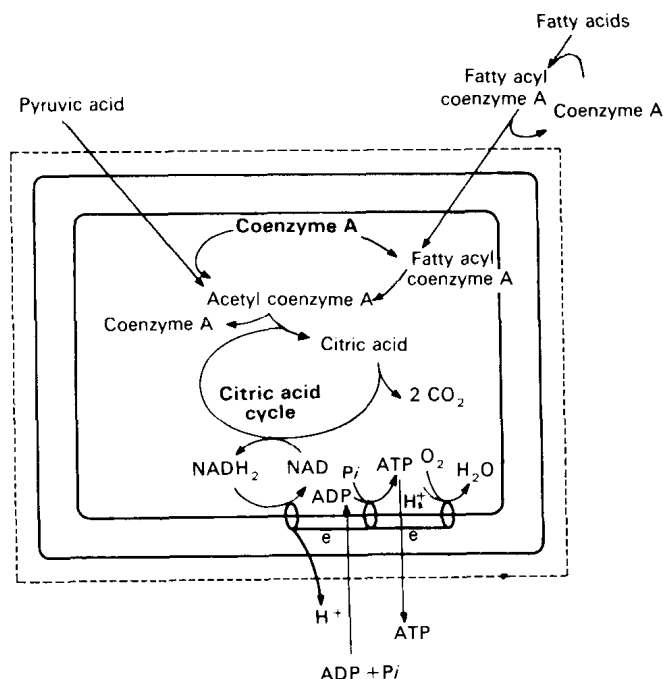


Fig. 1.1 Outline of the major metabolic pathways of the mitochondrion.

The study of the biochemistry of the mitochondrion has proceeded by several almost separate lines which have slowly converged on the particle as the main focus of investigation. Workers such as Szent-Györgyi who were interested in the chemistry of respiration demonstrated the catalytic effect of four-carbon

dicarboxylic acids on oxygen uptake. This work was complemented by earlier studies on dehydrogenases by Wieland and others and was brought to fruition by Krebs with the formulation of the citric acid cycle. Later Lehninger showed the cycle to be a property of the mitochondrion.

A second line of investigation was initiated by Warburg, who showed, in his studies on carbon monoxide inhibition of respiration, that an iron porphyrin was involved in oxygen metabolism. Subsequently Keilin rediscovered the cytochromes and isolated cytochrome *c*. From observations of the absorption spectra of tissues, he was able to construct a scheme for the respiratory chain.

A third line of study was developed by Kalckar and by Belitzer and Tsybakova leading to the concept of oxidative phosphorylation in which ATP synthesis is coupled to respiratory activity.

Starting with the observations of Knoop, the system of fatty acid oxidation was investigated and this also was eventually seen as a property of the mitochondrion.

Cytologists in the late nineteenth century had become aware of the mitochondrial particles in cells. A number of attempts were made to study these particles either *in situ* with dyes or by isolating them. However, it was not until 1940, when Claude began a systematic investigation of large granules (mitochondria) and small granules (microsomal fraction) isolated from liver homogenates by differential centrifugation, that a biochemical understanding of mitochondria became possible. By 1950, it had become clear that the fatty acid oxidising system, the citric acid cycle and the respiratory chain with its associated phosphorylation system were all located in the mitochondrion. With the advent of the electron microscope, the detailed structure of the mitochondrion was formulated, particularly by Palade. This led to a study of enzyme distribution within the structure and to the problem of transport of metabolites across the membranes. A further area of investigation developed as workers became aware of the questions associated with the biogenesis of the mitochondrion leading to studies of the synthesis of mitochondrial protein.

Although bacteria are too small to possess mitochondria (*Escherichia coli*, a short rod $0.8 \times 1.2 \mu\text{m}$ has dimensions similar to mitochondria) many are capable of similar oxidative metabolism and possess comparable systems of respiration and phosphorylation. Investigations of these systems in bacteria have augmented the studies on the mitochondrion.

(c) The chloroplast – an energy-transducing system

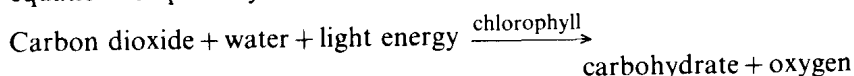
The prime function of the chloroplast is photosynthesis. The earliest ideas on this subject were developed in the eighteenth century by a colourful group of amateur naturalists. Stephen Hales (1677–1761) was the first of several workers to study photosynthesis as a process of gas exchange. In his essay *Vegetable Staticks*, he wrote 'Plants very probably draw through their leaves some part of their nourishment from the air . . . may not light also, by freely entering surfaces of leaves and flowers, contribute much to the ennobling principles of vegetables?'

The eighteenth century was also the era of development of ideas on the chemistry of gases associated with the names of Black, Scheele, Priestley, Cavendish, Lavoisier and others. The new insights had an impact on the understanding of respiration and particularly of photosynthesis. Joseph Priestley demonstrated the purification of air in the light in the presence of a photosynthesising system – a piece of mint. Air in a glass jar in which a candle had burned out, was restored so that the candle would again burn in it. The full significance of these experiments was not realised by Priestley who interpreted them in terms of the current phlogiston theory. However, later work showed convincingly the evolution of oxygen from photosynthesising algae. Priestley's experiments, published in the Proceedings of the Royal Society in 1772, attracted the attention of a Dutch physician, Ingen-Housz, who became physician to the Empress Maria Theresa. The experiments of Ingen-Housz convinced him of the importance of light in the air-restoration process and the active production of oxygen in the light. In his book *Food of Plants and Renovation of Soil*, he claimed that plants obtained their carbon by decomposition of the carbonic acid of air and that they evolved oxygen. Thus towards the end of the eighteenth century a coherent concept of photosynthesis began to develop, namely the synthesis of organic matter from carbon dioxide in the light with the evolution of oxygen.

These views were supported by the careful weighing experiments on plants by de Saussure (1767–1845). He showed that plants obtain most of their elements from the soil, but all the carbon from the CO_2 of the atmosphere. He also found that the increase in dry weight of plants was considerably greater than the increase in carbon and concluded that water was also assimilated. His data showed that the assimilation of water was linked to the photosynthetic process although he did not realise this. An understanding of the role of water in photosynthesis had to wait until later.

The later part of the nineteenth century saw many developments in ideas on photosynthesis. Three only will be mentioned here. First, Robert Mayer, who formulated the law of conservation of energy, drew attention to the energetic aspects of photosynthesis as a process in which light energy is converted into chemical energy. Secondly, J. Sachs observed the development of starch grains in photosynthesising chloroplasts. Thirdly, Engelmann carried out the first action spectrum for photosynthesis. Engelmann illuminated a filamentous alga with light passed through a prism. Motile bacteria sensitive to the level of dissolved oxygen moved to those parts of the filament where the greatest oxygen evolution occurred. This was in the parts illuminated with the blue and red regions of the spectrum, providing confirmation for the role of chlorophyll in photosynthesis.

Thus at the beginning of the twentieth century it was possible to write a simple equation for photosynthesis:



The first step in analysis of this equation was the demonstration that the photosynthetic process consisted of separable light and dark reactions. A variety