The Institute of Biology's Studies in Biology no. 37

Photosynthesis

Third edition

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D. O. Hall
Ph.D.
Professor of Biology,
King's College, University of London

K. K. Rao
Ph.D.
Honorary Lecturer,
King's College, University of London

Edward Arnold

First published 1972
by Edward Arnold (Publishers) Limited,
41 Bedford Square, London, WC1B 3DQ

Reprinted with additions 1974
Second edition 1977
Reprinted 1978
Third edition 1981

ISBN: 0 7131 2827 5

Printed and bound in Great Britain at
The Camelot Press Ltd, Southampton

General Preface to the Series

Because it is no longer possible for one textbook to cover the whole field of biology while remaining sufficiently up to date the Institute of Biology proposed this series so that teachers and students can learn about significant developments. The enthusiastic acceptance of 'Studies in Biology' shows that the books are providing authoritative views of biological topics.

The features of the series include the attention given to methods, the selected list of books for further reading and, wherever possible, suggestions for practical work.

Readers' comments will be welcomed by the Education Officer of the Institute.

1981

Institute of Biology
41 Queen's Gate
London SW7 5HU

Preface to the Third edition

Hardly a day goes by without the importance of photosynthesis being brought to our attention. All our food and our fossil and biological fuels (biomass) are derived from the process of photosynthesis. Increasingly the products of photosynthesis are being sought to feed and fuel the world and also in the future to provide chemicals and fibres. Thus an understanding of the fundamental and applied aspects of photosynthesis is now essential to a wide range of scientists and technologists – from agriculture and forestry through ecology and biology to chemistry and engineering. It is this universality that attracts varied approaches to studying photosynthesis and makes it such an exciting field of work to so many different types of people. We hope that this becomes evident in our book.

In this third edition we have retained the inherent characteristics of the previous editions which seem to appeal to students and teachers alike, viz. basic descriptive information on the processes of photosynthesis, how this was obtained and where modern research is leading. We have updated sections on chloroplast membrane structure, electron transport and phosphorylation in plants and bacteria, C_4 and CAM photosynthesis; also the chapter on research in photosynthesis has been extended to include recent developments presented at the 1980 Photosynthesis Congress. The figures have been updated and reference list has been completely revised.

The suggestions for improvements by readers and reviewers have been most welcome. We look forward to further comments and, as we have said before, we are always open to queries and possible problem solving.

London, 1981

D.O.H.
K.K.R.

Contents

1 Importance and Role of Photosynthesis

1.1 Ultimate energy source

The term photosynthesis literally means building up or assembly by light. As used commonly, photosynthesis is the process by which plants synthesize organic compounds from inorganic raw materials in the presence of sunlight. All forms of life in this universe require energy for growth and maintenance. Algae, higher plants and certain types of bacteria capture this energy directly from the solar radiation and utilize the energy for the synthesis of essential food materials. Animals cannot use sunlight directly as a source of energy; they obtain the energy by eating plants or by eating other animals which have eaten plants. Thus the ultimate source of all metabolic energy in our planet is the sun and photosynthesis is essential for maintaining all forms of life on earth.

We use coal, natural gas, petroleum, etc. as fuels. All these fuels are decomposition products of land and marine plants or animals and the energy stored in these materials was captured from the solar radiation millions of years ago. Solar radiation is also responsible for the formation of wind and rain and hence the energy from windmills and hydro-electric power stations could also be traced back to the sun.

The major chemical pathway in photosynthesis is the conversion of carbon dioxide and water to carbohydrates and oxygen. The reaction can be represented by the equation,

$$CO_2 + H_2O \xrightarrow[\text{plants}]{\text{sunlight}} \underset{\text{Carbohydrate}}{[CH_2O]} + O_2$$

The carbohydrates formed possess more energy than the starting materials, viz. CO_2 and H_2O. By the input of the sun's energy the energy-poor compounds, CO_2 and H_2O, are converted to the energy-rich compounds, carbohydrate and O_2. The energy levels of the various reactions which lead up to the above overall equation can be expressed on an oxidation-reduction scale ('redox potential' given in volts) which tells us the energy available in any given reaction – this will be discussed later in Chapter 4. Photosynthesis can thus be regarded as a process of converting radiant energy of the sun to chemical energy of plant tissues.

1.2 The carbon dioxide cycle

The CO_2 content of the atmosphere remains almost constant in spite of its depletion during photosynthesis. All plants and animals carry out the process of respiration (in mitochondria) whereby oxygen is taken from the atmosphere by living tissues to convert carbohydrates and other tissue constituents eventually to carbon dioxide and water, with the simultaneous liberation of energy. The energy is stored in ATP (adenosine triphosphate) and is utilized for the normal functions of the organism. Respiration thus causes a decrease in the organic matter and oxygen content and an increase in the CO_2 content of the planet. Respiration by living organisms and combustion of carbonaceous fuels consume on an average about 10 000 tonnes of O_2 every second on the surface of the earth. At this rate all the oxygen of the atmosphere would have been used up in about 3000 years. Fortunately for us the loss of organic matter and atmospheric oxygen during respiration is counter-balanced by the production of carbohydrates and oxygen during photosynthesis. Under ideal conditions the rate of photosynthesis in the green parts of plants is about 30 times as much as the

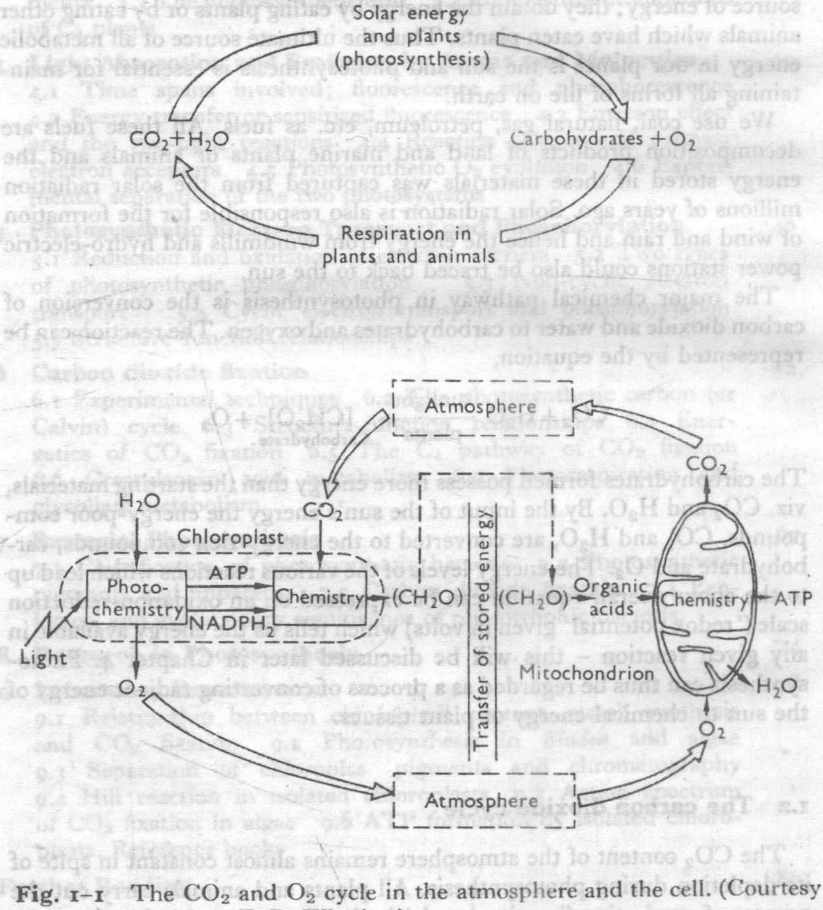

Fig. 1–1 The CO_2 and O_2 cycle in the atmosphere and the cell. (Courtesy Professor F. R. Whatley.)

rate of respiration in the same tissues. Thus photosynthesis is very important in regulating the O_2 and CO_2 content of the earth's atmosphere. The cycle of operations can be represented as shown in Fig. 1–1. All the CO_2 in the atmosphere is cycled through plants, via photosynthesis, every 300 years and all the O_2 is cycled every 2000 years.

It should be made clear that the energy liberated during respiration is finally dissipated from the living organism as heat and is not available for recycling. Thus from millions of years past energy is constantly removed from the sun and wasted as heat in the earth's atmosphere. But there is still enough energy in the sun's atmosphere for photosynthesis to continue for millions of years to come.

1.3 Efficiency and turnover

The solar energy striking the earth's atmosphere every year is equivalent to about 56×10^{23} joules of heat. Of this roughly half is reflected back by the clouds and by the gases in the upper atmosphere. Of the remaining radiation that reaches the earth's surface only 50% is in the spectral region of light that could bring about photosynthesis, the other half being weak infrared radiation. Thus the annual influx of energy of photosynthetically active radiation, i.e. from violet to red light, to tne earth's surface is equivalent to about 15×10^{23} joules. However, some 40% of this is reflected by ocean surface, deserts, etc. and only the rest can be absorbed by the plant life on land and sea. Recent estimates of the total annual amount of biomass produced by autotrophic plants are about 2×10^{11} tonnes of organic matter which is equivalent to about 3×10^{21} joules of energy. About 40% of this organic matter is synthesized by phytoplankton, minute plants living near the surface of the oceans. The annual food intake by the earth's human population (assuming the population to be 4300 million) is approximately 800 million tonnes or 13×10^{18} joules. Thus the average coefficient of utilization of the incident photosynthetically active radiation by the entire flora of the earth is only about 0.2% $(3 \times 10^{21}/15 \times 10^{23})$ and of this less than 0.5% $(13 \times 10^{18}/3 \times 10^{21})$ is consumed as nutrient energy by mankind. It is interesting that the consumption of energy by the world in 1976 was 3×10^{20} joules – this was only one-tenth of the energy stored by photosynthesis! In fact, the energy content of the biomass standing on the earth's surface today (90% trees) is equivalent to all our proven reserves of fossil fuel, i.e. oil, gas and coal; also the total resources of fossil fuel stored below the earth's surface only represents about 100 years of net photosynthesis.

1.4 Spectra

Light is a form of electromagnetic radiation. All electromagnetic radiation has wave characteristics and travels at the same speed of 3×10^8 m s^{-1} (c, the speed of light). But the radiations differ in wavelength, the distance between two successive peaks of the wave. Gamma rays and X-rays have very small wavelengths (less than 1000 millionth of a centimetre) while radio waves are in the order of 10^4 cm. Wave lengths of visible light are conveniently expressed by a unit called nanometre. One nanometre is 1000 millionth of a metre (1 nm $= 10^{-9}$ m). It has been known since the time of Isaac Newton that white light can be separated into a spectrum, resembling the rainbow, by passing light through a prism. The visible portion of this spectrum ranges from the violet at about 380 nm to the red at 750 nm.

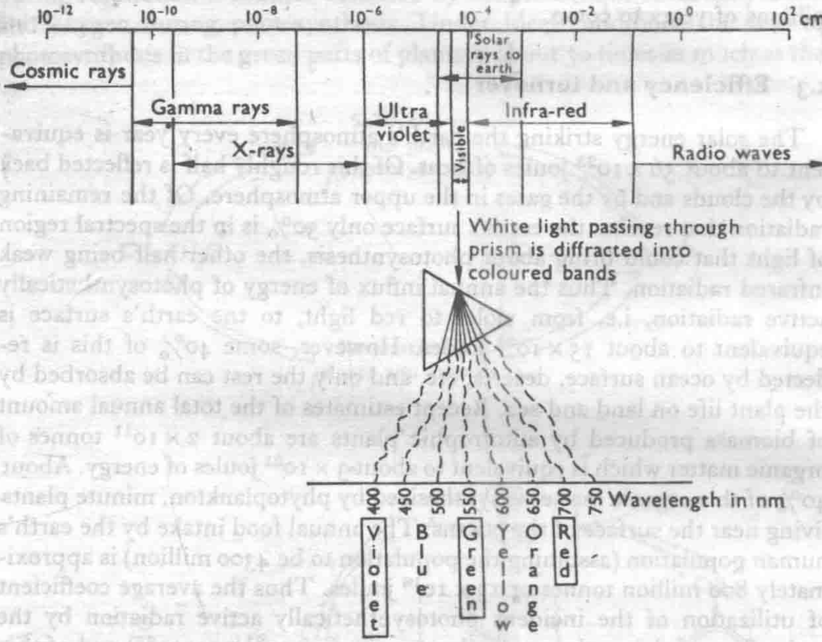

Fig. 1–2 Spectra of electromagnetic radiation.

The atmosphere of the sun consists mainly of hydrogen. The energy of the sun is derived from the fusion of four hydrogen nuclei to form a helium nucleus. $4\,{}^1_1\text{H} \rightarrow {}^4_2\text{He} + 2e^- + h\nu$ (energy). The energy liberated during the nuclear fission maintains the surface temperature of the sun around 6000 K. The sun radiates energy representing the entire electromagnetic spectrum but the earth's atmosphere is transparent only to part of the infra-red and ultraviolet light and all the visible light. The ultraviolet waves which are somewhat shorter than the shortest visible light waves are absorbed by the oxygen and ozone of the upper atmosphere. This is fortunate since ultraviolet radiations are harmful to living organisms. At 6000 K, the temperature of the sun, the maximum intensity of emitted light lies in the orange part of the visible spectrum, around 600 nm.

1.5 Quantum theory

In 1900 Max Planck enunciated the theory that the transfer of radiation energy within a hot object involved discrete 'units' of energy called quanta. Planck's quantum theory can be expressed mathematically as $E=h\nu$ where E is the energy of a single quantum of radiation, ν is the frequency of the radiation (frequency is the number of waves transmitted in unit time), and h a constant. The Planck's constant (h) has the dimensions of the product of energy and time and its value in the c.g.s. system is 6.626×10^{-34} J s. Planck's theory proposes that an oscillator of fundamental frequency (ν) would take up energy $h\nu$, $2h\nu$, $3h\nu \rightarrow nh\nu$, but it could not acquire less than a whole number of energy quanta. Five years later Albert Einstein extended Planck's theory to light and proposed that light energy is transmitted not in a continuous stream but only in individual units or quanta. The energy of a single quantum of light or *photon* is the product of the frequency of light and Planck's constant, i.e. $E=h\nu$. Since frequency is inversely related to wavelength, it follows that photons of short wave light are more energetic than photons of light of longer wavelength, i.e. at one end of the spectrum photons of blue light are more energetic than those of red light at the other end.

For photosynthesis to take place the pigments present in plant tissues should absorb the energy of a photon at a characteristic wavelength and then utilize this energy to initiate a chain of photosynthetic chemical events. We will learn later that an electron is ejected from the pigment immediately after the absorption of a suitable quantum of light. It should be emphasized that a photon cannot transfer its energy to two or more electrons nor can the energy of two or more photons combine to eject an electron. Thus the photon should possess a critical energy to excite a single electron from the pigment molecule and initiate photosynthesis. This accounts for the low efficiency of infra-red radiation in plant photosynthesis since there is insufficient energy in the quantum of infra-red light. Certain bacteria, however, contains pigments which absorb infra-red radiation and carry out photosynthesis which is quite different from plant-type photosynthesis in that no O_2 is evolved during the process (see Chapter 7).

1.6 Energy units

According to Einstein's law of photochemical equivalence a single molecule will react only after it has absorbed one photon of energy ($h\nu$). Hence one mole (gram-molecule) of a compound must absorb N ($N=6.023 \times 10^{23}$, the Avogadro number) photons of energy, i.e. $Nh\nu$, to start a reaction. The total energy of photons absorbed by one mole of a compound is called an Einstein.

Let us calculate the energy of a mole (or Einstein, i.e. 6.023×10^{23}

quanta) of red light of wavelength 650 nm (6.5×10^{-7} m). The frequency, $\nu = c/\lambda$ = speed of light/wavelength of light

$$\nu = 3.0 \times 10^{8}/6.5 \times 10^{-7} = 4.61 \times 10^{14}$$

$E = Nh\nu$, i.e. Energy = number of molecules × Planck's constant × frequency

$$\therefore E = 6.023 \times 10^{23} \times 6.626 \times 10^{-34} \times 4.61 \times 10^{14}$$

$$= 18.40 \times 10^{4} \text{ joules} = \text{energy of one Einstein of red light}$$

$$\text{or } E = 18.40 \times 10^{4}/4.184 \times 10^{3} = 43.98 \text{ kcal}$$

(One kilocalorie, kcal, is equal to 4.184×10^{3} joules.) Thus 1 mole of red light at 650 nm contains 18.40×10^{4} joules of energy.

The energy of photons can also be expressed in terms of electron volts. An electron volt, eV, is the energy acquired by an electron when it falls through a potential of 1 volt, which is equal to 1.6×10^{-19} joules. If 1 mole of a substance acquires an average energy of 1 eV the total energy of the mole (6.023×10^{23} molecules) can be calculated to be 9.64×10^{4} joules. Thus the energy of 1 mole of 650 nm light is equal to 1.91 eV (18.40×10^{4}/ 9.64×10^{4}.

Table 1 Energy levels of visible light.

Wavelength	*Colour*	*Joules per mole*	*kcal per mole*	*Electron volts per mole*
700 nm	Red	17.10×10^{4}	40.87	1.77
650 nm	Orange-red	18.40×10^{4}	43.98	1.91
600 nm	Yellow	19.95×10^{4}	47.68	2.07
500 nm	Blue	23.95×10^{4}	57.24	2.48
400 nm	Violet	29.93×10^{4}	71.53	3.10

2 History and Progress of Ideas

2.1 Early discoveries

In the early half of the seventeenth century the Flemish physician van Helmont grew a willow tree in a bucket of soil feeding the soil with rain water only. He observed that after five years the tree had grown to a considerable size though the amount of soil in the bucket had not diminished significantly. Van Helmont naturally concluded that the material of the tree came from the *water* used to wet the soil. In 1727 the English botanist Stephen Hales published a book in which he observed that plants used mainly *air* as the nutrient during their growth. Between 1771 and 1777 the famous English chemist Joseph Priestley (who was one of the discoverers of oxygen) conducted a series of experiments on combustion and respiration and came to the conclusion that green plants were able to reverse the respiratory processes of animals. Priestley burnt a candle in an enclosed volume of air and showed that the resultant air could no longer support burning. A mouse kept in the residual air died. A green sprig of mint, however, continued to live in the residual air for weeks. At the end of this time Priestley found that a candle could burn in the reactivated air and a mouse could breathe in it. We now know that the burning candle used up the *oxygen* of the enclosed air which was replenished by the photosynthesis of the green mint. A few years later the Dutch physician, Jan Ingenhousz, discovered that plants evolved oxygen only in *sunlight* and also that only the *green* parts of the plant carried out this process.

Jean Senebier, a Swiss minister, confirmed the findings of Ingenhousz and observed further that plants used as nourishment *carbon dioxide* 'dissolved in water'. Early in the nineteenth century another Swiss scholar, de Saussure, studied the quantitative relationships between the CO_2 taken up by a plant and the amount of organic matter and O_2 produced and came to the conclusion that *water* was also consumed by plants during assimilation of CO_2. In 1817 two French chemists, Pelletier and Caventou, isolated the green substance in leaves and named it *chlorophyll*. Another milestone in the history of photosynthesis was the enunciation in 1845 by Robert Mayer, a German physician, that plants transform energy of sunlight into chemical *energy*. By the middle of the last century the phenomenon of photosynthesis could be represented by the relationship

$$CO_2 + H_2O + \text{light} \xrightarrow[\text{plant}]{\text{green}} O_2 + \text{organic matter} + \text{chemical energy}$$

Accurate determinations of the ratio of CO_2 consumed to O_2 evolved during photosynthesis were carried out by the French plant physiologist Boussingault. He found in 1864 that the photosynthetic ratio – the volume of O_2 evolved to the volume of CO_2 used up – is almost unity. In the same year the German botanist Sachs (who also discovered plant respiration) demonstrated the formation of *starch* grains during photosynthesis. Sachs kept some green leaves in the dark for some hours to deplete them of their starch content. He then exposed one half of a starch-depleted leaf to light and left the other half in the dark. After some time the whole leaf was exposed to iodine vapour. The illuminated portion of the leaf turned dark violet due to the formation of starch-iodine complex; the other half did not show any colour change.

The direct connection between oxygen evolution and chloroplasts of green leaves and also the correspondence between the action spectrum of photosynthesis and the absorption spectrum of chlorophyll (see Chapter 4) were demonstrated by Engelmann in 1880. He placed a filament of the

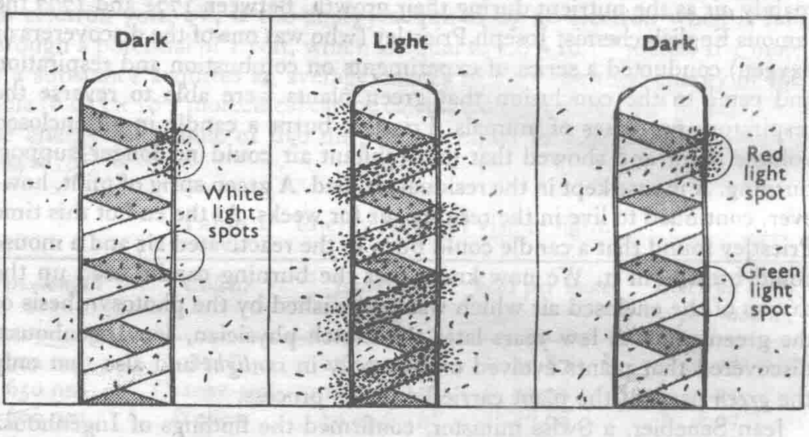

Fig. 2–1 Summary of Engelmann's experiment for studying photosynthesis using the alga *Spirogyra* and motile bacteria. The alga has a spiral chloroplast and the bacteria migrate towards regions of higher O_2 concentration. Left: Illumination with a spot of white light. Centre: Complete illumination with white light. Right: Illumination with spots of red and green light. Note the lack of O_2 evolution in green light.

green alga *Spirogyra*, with its spirally arranged chloroplasts, on a microscope slide together with a suspension of an oxygen-requiring, motile bacteria. The slide was kept in a closed chamber in the absence of air and illuminated. Motile bacteria would move towards regions of greater O_2 concentration. After a period of illumination the slide was examined under a microscope and the bacterial population counted. Engelmann found that the bacteria were concentrated around the green bands of the algal filament. In another series of experiments he illuminated the alga with a spectrum of light by interposing a prism between the light source and the microscope stage. The largest number of bacteria surrounded those parts of the algal filament that were in the blue and red regions of the spectrum. The chlorophylls present in the alga absorbed blue and red light; since light has to be absorbed to bring about photosynthesis Engelmann concluded that chlorophylls are the active photoreceptive *pigments* for photosynthesis. The state of knowledge on photosynthesis at the beginning of this century could be represented by the equation:

$$(CO_2)_n + H_2O + \text{light} \xrightarrow[\text{plant}]{\text{green}} (O_2)_n + \text{starch} + \text{chemical energy}$$

2.2 Further work related to techniques

Though by the beginning of this century the overall reaction of photosynthesis was known, the discipline of biochemistry had not advanced enough to understand the mechanism of reduction of carbon dioxide to carbohydrates. It should be admitted that even now we know very little about certain aspects of photosynthesis. Attempts were made early to study the effects of light intensity, temperature, carbon dioxide concentration, etc. on the overall yields of photosynthesis. Though plants of divergent species were used in these studies, most of the determinations were carried out with unicellular green algae, *Chlorella* and *Scenedesmus*, and the unicellular flagellate *Euglena*. Unicellular plants are more suitable for quantitative research since they can be grown in all laboratories under fairly standard conditions. They can be suspended uniformly in aqueous buffer solutions and aliquots of the suspension can be transferred with a pipette as though they were true solutions. Chloroplasts are best prepared and studied from leaves of higher plants, the most common being spinach leaves as they are usually available fresh in the market and can be grown quite easily; peas and lettuce are also sometimes used.

Since CO_2 is fairly soluble and O_2 is relatively insoluble in water, during photosynthesis in a closed system there will be a change in gas pressure. The Warburg respirometer (adapted by Otto Warburg in 1920) is often used in studies involving the action of light on photosynthetic systems by measuring the changes in the O_2 volume of the system (see *Manometric Techniques*, UMBREIT, BURRIS and STAUFFER, Burgess Publ. Co., U.S.A., for details).

The oxygen electrode is a more convenient instrument to measure uptake or liberation of O_2 during a reaction. The electrode works on the principle of polarography and is sensitive enough to detect O_2 concentrations of the order of 10^{-8} moles cm^{-3} (0.01 millimolar). The apparatus consists of platinum wire sealed in plastic as cathode, and an anode of circular silver wire bathed in a saturated KCl solution. The electrodes are separated from the reaction mixture by an O_2 gas-permeable teflon membrane. The reaction mixture in the plastic (or glass) container is stirred constantly with a small magnetic stirring rod. When a voltage is applied across the two electrodes, with the platinum electrode negative to the reference electrode, the oxygen in the solution undergoes electrolytic reduction. The flow of current in the system between 0.5 and 0.8 V varies in a linear relationship to the partial pressure of the oxygen in solution. The instrument is usually operated at a voltage of about 0.6 V. The current liberated is measured by connecting the electrode set up to a suitable recorder. The whole apparatus is kept at a constant temperature by circulating water from a controlled

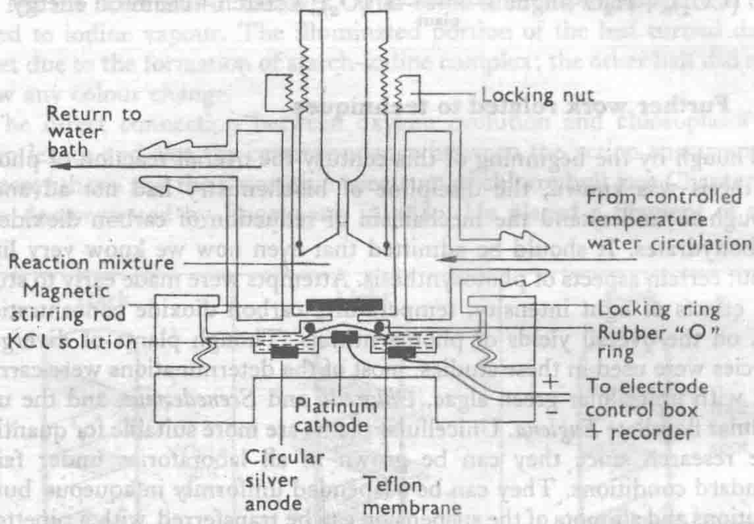

Fig. 2–2 The oxygen electrode (Rank Bros., Bottisham, Cambridge).

temperature water source. The effects of light and of various chemicals on photosynthesis are measured using the oxygen electrode. The O_2 electrode has the advantage over the Warburg method in that rapid and continuous measurements of O_2 evolution can be made. However, the Warburg apparatus can measure up to 20 reaction mixtures simultaneously while the O_2 electrode measures reactions one at a time.

2.3 Limiting factors

The extent of photosynthesis performed by a plant depends on a number of internal and external factors. The chief internal factors are the structure of the leaf and its chlorophyll content, the accumulation within the chloroplasts of the products of photosynthesis, the influence of enzymes and the presence of minute amounts of mineral constituents. The external factors are the quality and quantity of light incident on the leaves, the ambient temperature and the concentration of carbon dioxide and oxygen in the surrounding atmosphere.

2.3.1 The effect of light intensity

The effect of light intensity on the photosynthetic activity of a healthy suspension of *Chlorella* cells is illustrated in Fig. 2–3. At low light intensities the rate of photosynthesis, as measured by oxygen evolution, increases linearly in proportion to light intensity. This region of the curve, marked X, is known as the light-limiting region. With more and more light intensity, photosynthesis becomes less efficient until after about 10 000 lux

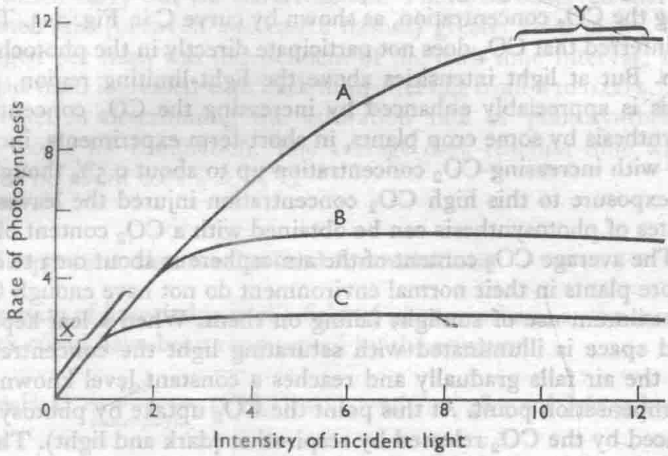

Fig. 2–3 Effect of external factors on rate of photosynthesis. A, Effect of light intensity at 25 °C and 0.4% CO_2; B, at 15 °C and 0.4% CO_2; C, at 25 °C and 0.01% CO_2. All units in the graph are arbitrary.

(1000 foot candles) increasing light intensity produces no further effect on the rate of photosynthesis. This is indicated by the horizontal parts of the curves in the figure. This plateau region designated Y is the light saturation region. If the rate of photosynthesis is to be raised in this region, factors other than light intensity would have to be adjusted. The amount of sunlight falling on a clear summer day in many places on the earth is about 100 000 lux, also equal to about 1000 Wm^{-2}. Thus, except for plants growing in thick forests and in shade, there is often sufficient sunlight incident on the plants to saturate their photosynthetic capacity. The energy of the extreme blue (400 nm) and red (800 nm) light quanta differs only by a factor of two and the photons in this wavelength range are qualitatively efficient to start photosynthesis though, as we shall later see, the leaf pigments preferentially absorb light of certain definite wavelengths.

2.3.2 *Effect of temperature*

A comparison of the curves A and B in the figure shows that at low light intensities the rate of photosynthesis is the same at 15 °C and at 25 °C. The reactions in the light-limiting region, like true photochemical reactions, are not sensitive to temperature. At higher light intensities, however, the rate of photosynthesis is much higher at 25 °C than at 15 °C. So, factors other than mere photon absorption influence photosynthesis in the light-saturation region. Most temperate climate plants function well between 10 °C and 35 °C, the optimum temperature being around 25 °C.

2.3.3 *Effect of CO_2 concentration: CO_2 compensation point*

In the light-limiting region the rate of photosynthesis is not affected by lowering the CO_2 concentration, as shown by curve C in Fig. 2-3. Thus, it can be inferred that CO_2 does not participate directly in the photochemical reaction. But at light intensities above the light-limiting region, photosynthesis is appreciably enhanced by increasing the CO_2 concentration. Photosynthesis by some crop plants, in short-term experiments, increased linearly with increasing CO_2 concentration up to about 0.5% though continued exposure to this high CO_2 concentration injured the leaves. Very good rates of photosynthesis can be obtained with a CO_2 content of about 0.1%. The average CO_2 content of the atmosphere is about 0.03 to 0.04%. Therefore plants in their normal environment do not have enough CO_2 to make maximum use of sunlight falling on them. When a leaf kept in an enclosed space is illuminated with saturating light the concentration of CO_2 in the air falls gradually and reaches a constant level known as the CO_2 compensation point. At this point the CO_2 uptake by photosynthesis is balanced by the CO_2 released by respiration (dark and light). The CO_2 compensation point varies with species; it is very low (less than 10 ppm) for the C_4 plants (Chapter 6) whereas for the C_3 photosynthetic species the value is greater than 50 ppm (0.005%).

2.4 Light and dark reactions; flashing light experiments

As early as 1905 the British plant physiologist F. F. Blackman interpreted the shape of the light saturation curves by suggesting that photosynthesis is a two-step mechanism involving a photochemical or light reaction and a non-photochemical or dark reaction. The dark reaction which is enzymic is slower than the light reaction and hence at high light intensities the rate of photosynthesis is entirely dependent upon the rate of the dark reaction. The light reaction has a low or zero temperature coefficient while the dark reaction has a high temperature coefficient, characteristic of enzymic reactions. It should be clearly understood that the so-called dark reaction can proceed both in light and in darkness.

The light and dark reactions can be separated by using flash illuminations lasting fractions of a second. Light flashes lasting less than a millisecond (10^{-3} s) can be produced either mechanically by placing a slit in a rotating disc in the path of a steady light beam, or electrically by loading up a condenser and discharging it through a vacuum tube. Ruby lasers emitting red light at 694 nm are also used as a radiation source. In 1932 Emerson and Arnold illuminated suspensions of *Chlorella* cells with condenser flashes lasting about 10^{-5} s. They measured the rate of oxygen evolution in relation to the energy of the flashes, the duration of the dark intervals between the flashes, and the temperature of the cell suspension. Flash satura-