

The Mechanism of Photosynthesis

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CONTEMPORARY BIOLOGY



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Preface

The study of the mechanism of photosynthesis has attracted the interest of chemists, physicists and biologists. This book provides an introduction to present research in such a way that each specialist will be interested and led to appreciate the contribution of the others. The book does not attempt to consider all the relevant literature but rather to provide a perspective for the university undergraduate and the young research worker.

Rothamsted 1973

C.P.W.



Abbreviations

ADP	adenosine-5'-diphosphate
ATP	adenosine-5'-triphosphate
CoA	Coenzyme A
(CH ₂ O)	A generalized formula for sugars
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DPIP	2,6-dichlorophenol indophenol
EDTA	ethylene diamine tetra-acetate
INH	isonicotinylhydrazide (isoniazid)
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
P _i	inorganic phosphate
PGA	phosphoglyceric acid
PMS	N-methylphenazonium sulphate (Phenazine methosulphate)
PQ	plastoquinone
Q	a hypothetical quencher of fluorescence

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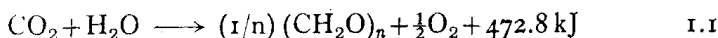
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Physiology of Photosynthesis

Photosynthesis is the process by which green plants provide our principal foodstuffs through the conversion of carbon dioxide and water to carbohydrates and oxygen. The reaction was first identified by the ability of the green leaf to produce oxygen, as shown by Joseph Priestley in 1771, very shortly after he had discovered the nature of the gas oxygen itself. Priestley demonstrated the complementary relationship between plants and animals by placing a mouse and a piece of mint together in a closed space under a bell jar. Even after one month the mouse survived without harm because the carbon dioxide produced by the breathing of the mouse was consumed during photosynthesis by the plant and converted back to oxygen. In later years Priestley was unable to repeat some of his earlier findings and it was Ingen-Housz who demonstrated that plants produce oxygen only when illuminated and that in darkness they behave like animals, consuming oxygen and producing carbon dioxide. Man is dependent on plants not only to supply food but to maintain his oxygen supply in the atmosphere. Similar experiments have been made in recent times to develop a biologically balanced system of man and plants on a microscale in a sealed submarine or spaceship.

By 1800 the basic metabolism of the green plant was understood. The process of photosynthesis producing food in the light was separated from that of respiration, in which foodstuffs were oxidized in the dark and which, overall, effected the opposite reaction. The process of photosynthesis requires a supply of energy whereas respiration releases energy. The energy contained within the organic carbon compounds produced in photosynthesis may be released either in the process of respiration or long after the plant has died by combustion of fossil fuels such as coal, petrol or oil. The

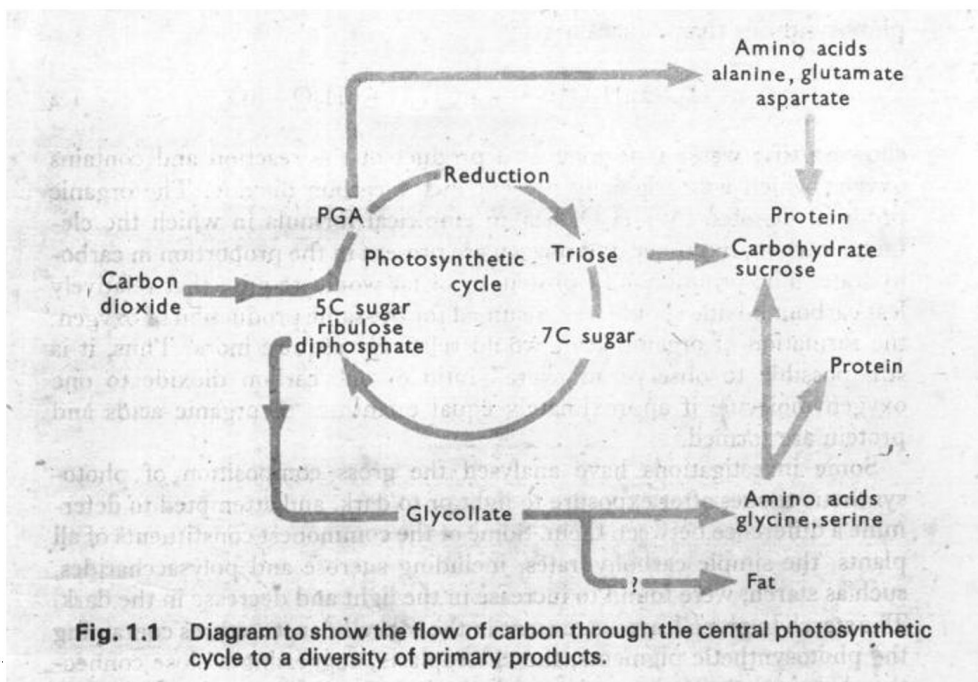
green plant represents the most efficient energy converter known and converts solar energy into chemical energy. The radiation energy reaching the earth's surface from the sun is of the order of 2.0×10^{24} J per year, of which 6.0×10^{23} J is absorbed by plant vegetation. It has been estimated that 2-3% of the energy incident on, or 1 to 2% of the energy absorbed by, the plant is used in photosynthesis (say 1×10^{22} J), yet this suffices for the natural conversion of 3×10^{11} tonnes of carbon each year from the form of carbon dioxide to organic compounds, thus fixing approximately $10^{22}/3 \times 10^{11}$ J per tonne. According to the overall equation:



for each 1 g of carbon in the form of CO_2 converted to the equivalent quantity (0.68 g) of carbohydrate, 472.8/12 or 40 kJ free energy are required. So that for each tonne of carbon (10^6 g) the theoretical requirement is 4.0×10^{10} J, in good agreement with the above estimate. The average annual yield of photosynthesis by crop plants has been well established as of the order of 5-10 g C/m²/day corresponding to 40 to 80 tonnes per hectare per year, or 16-32 English tons per acre.¹⁴³ According to equation I.1 this would require an energy input of 2.0 to 4.0×10^8 J/m²/day. The energy falling on unit area of the earth's surface can be measured and in full sunlight the incident intensity may rise to 8.0 J/cm²/min, i.e. 6.0×10^7 J/m²/12 h day, although on dull days it will fall to one-third of this. Only the uppermost leaves of the plant will be exposed to the full intensity, many of the leaves lower down the plant being at least partially shaded although the total area of leaf per unit area of soil can rise in a crop plant at maturity to four or five. However, only part of the plant, i.e. the grain in a cereal plant, is harvested, so that the overall efficiency of approximately 0.5% is not unreasonable.

In the sea or in lakes and rivers, carbon dioxide is supplied to photosynthesis from dissolved salts of bicarbonate or carbonate in the medium bathing the plants. Most photosynthetic plants can use only the free carbon dioxide or undissociated carbonic acid, but some are capable of using bicarbonate ions as a direct source of carbon in photosynthesis. Terrestrial plants must obtain their carbon dioxide from the atmosphere and they do so largely through small holes in the epidermis of the leaf, the stomata. These holes permit not only the entry of carbon dioxide but also the escape of water vapour from the wet walls of the cells inside the leaf. The resulting loss of water by the plant in the process of transpiration is an inevitable consequence of the need by the leaf to absorb carbon dioxide. When the stomata are completely closed, a supply of carbon dioxide will be available for photosynthesis from the oxidation of compounds within the plant in the process of respiration and from the relatively slow diffusion through the waxy layer (cuticle) which covers the leaf surface.

In the broadest sense all the organic materials of the green plant may be considered to be the products of photosynthesis. In photosynthesis carbon taken up in the form of carbon dioxide from the air (or from solution in water) is incorporated into organic carbon compounds, and these are the starting point for all other biosyntheses (Fig. 1.1). The incorporation of

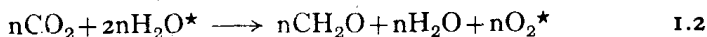


carbon dioxide involves a reduction reaction which is balanced by a corresponding oxidation reaction in which oxygen is liberated from water. It is not always easy to distinguish clearly between the organic products formed directly by incorporating the carbon dioxide taken up in photosynthesis and those which are the result of subsequent metabolic transformations. But it is desirable that this distinction should be made.

The assimilatory quotient and the nature of the product of photosynthesis

The earliest observations on photosynthesis included quantitative determinations of the amount of carbon dioxide taken up relative to that of oxygen liberated as a reaction product. Recent experiments have shown that under a wide range of conditions, provided the oxygen concentration

was low, the volume of oxygen liberated was almost equal to that of carbon dioxide absorbed (Fock *et al.*⁴²); when the oxygen concentration was high the O₂ evolved was less than the carbon dioxide consumed. Using water distinguished by isotopic 'labelling' with ¹⁸O it could be shown that almost all the oxygen produced in photosynthesis came from water (Dole and Jenks³²). Thus the following equation is a more correct representation of photosynthesis than equation 1.1:



showing that water is formed as a product of the reaction and contains oxygen which was originally present in the carbon dioxide. The organic product, denoted by CH₂O, has an empirical formula in which the elements carbon, hydrogen and oxygen are present in the proportion in carbohydrate. The production of protein or of fat would require that relatively less carbon dioxide should be consumed for the same production of oxygen; the formation of organic acids would relatively require more. Thus, it is still possible to observe an overall ratio of one carbon dioxide to one oxygen molecule if approximately equal quantities of organic acids and protein are formed.

Some investigations have analysed the gross composition of photosynthetic tissues after exposure to light or to dark, and attempted to determine a difference between them. Some of the commonest constituents of all plants, the simple carbohydrates, including sucrose and polysaccharides, such as starch, were found to increase in the light and decrease in the dark. The starch in green leaves is present in the subcellular structures containing the photosynthetic pigments, the chloroplasts, suggesting a close connection between starch formation and photosynthesis. Green leaves have a high protein content and in variegated forms the green areas contain more nitrogen than the less green. Analysis of isolated chloroplasts shows that they are unusually rich in protein and the protein is different in type from cytoplasmic protein. However, whether a leaf produces more protein in the light than it does in the dark is not clear from the evidence. In many photosynthetic organisms, especially in some algae, there is a high content of fat; but again the existence of a significant difference in content between light and dark grown cells has not been established.

One approach to the problem of the nature of the primary product of photosynthesis has been to measure the increase in the heat of combustion of a given area of leaf consequent upon a period of photosynthesis. In general it is found that the increase in heat of combustion per gram of carbon dioxide absorbed (10.8 kJ/g) is about intermediate between that calculated for sucrose or glucose synthesis (approximately 14 kJ/g CO₂ as in equation 1.1) and that for protein synthesis (1 g CO₂ forms about 0.5 g

protein + 12.6 kJ/g) but it is considerably less than that required for fat synthesis (1 g CO₂ forms about 0.4 g fat + 15.1 kJ/g).

The clearest evidence that protein could be formed during photosynthesis came from observations by Burström²⁰ using detached wheat leaves. Young leaves containing various quantities of nitrate were allowed to photosynthesize at low and at high intensities of light. Leaves containing little nitrate when they were illuminated at low intensities converted all the carbon dioxide absorbed into sugar. If the nitrate content was higher, more carbon dioxide was absorbed and the excess was used to convert the nitrogen to protein. The two kinds of product were additive and did not compete with one another at high light intensity, so that the total assimilation of carbon dioxide was greater and the formation of sugar relatively diminished in the leaves with higher nitrate content. This work suggested a relationship between nitrate assimilation and the uptake of carbon in photosynthesis, but it did not establish that the relationship was necessarily a direct one. By contrast, J. H. C. Smith¹²¹ showed with sunflower leaves that almost the whole of the carbon dioxide absorbed during photosynthesis could be accounted for by the formation of carbohydrate. Many workers concluded that carbohydrate was the only primary product of photosynthesis and this view was supported by the observation that albino maize plants could be grown almost to maturity on sucrose as their only source of carbon.

The diffusion of water vapour and carbon dioxide in leaves

In the terrestrial plant which consumes carbon dioxide during photosynthesis, there must be a fall in concentration of carbon dioxide between the ambient air and the place of reaction in the chloroplasts of the leaf cell. Since carbon dioxide enters the leaf by a process of diffusion it moves from a position of higher to one of lower concentration. From the outside carbon dioxide diffuses in a gaseous phase through the stomata and the intercellular spaces of the leaf, then in an aqueous phase through the cell wall and within the cell up to the chloroplast. Since the cell walls of the mesophyll cells are wet the air immediately adjacent to them in the intercellular spaces will be very nearly saturated with water vapour at the temperature of the leaf. Under almost all conditions the atmosphere outside the leaf will not be saturated so that water will diffuse out from the mesophyll cells through the air spaces of the leaf and beyond the leaf surface into the atmosphere. The rate of exchange of carbon dioxide inwards or of water vapour outwards between the leaves and the surrounding atmosphere is given according to Fick's Law for transfer by molecular diffusion, that is the rate of flow is related to the difference in concentration between the supply and the point of consumption and inversely to the distance separating them. The quantity

S flowing per unit time t , when the difference in concentration is δc and the distance apart of source and sink is δx , is expressed by:

$$\frac{\delta S}{\delta t} = -KA \frac{\delta c}{\delta x}$$

This proportionality involves a coefficient of diffusion, K , whose dimensions are cm^2/s if S is in cm^3 , A in cm^2 , x in cm and δc cm^3/cm^3 ; its value varies according to the nature of the substance-diffusing and the medium through which it diffuses. When this equation is applied to the process of transpiration we assume that the rate of transpiration is proportional to the difference in vapour pressure immediately adjacent to the wet cell wall within the leaf and that in the outside atmosphere above the leaf, an area term A and a length term δx . From determinations of the rate of transpiration under measured conditions of relative humidity, it is possible to derive from this expression a value for $K/\delta x$, provided A is known. The early workers Brown and Escombe¹⁷ assumed that A could be taken as the total area of stomatal pore*, and δx the average length. Then substituting the known value for K , the coefficient of diffusion of water vapour in air, they calculated the rate of transpiration. The calculated rate proved to be 3-5 times larger than the actual value measured for a sunflower leaf in the field on a bright sunny day. It follows that the diffusion path for transpiration must be greater than that assumed and include additional components which account for the total distance from the wet mesophyll cell wall up to the leaf surface and beyond, including a layer of air on the surface of the leaf called the 'shearing' layer. Three components of the transpiration path have been identified:

1. The path inside the leaf starting at the wet mesophyll cell wall and extending up to the stoma r_m ,
2. the stomatal path r_s and
3. the depth of still air upon the leaf surface, the 'shearing' layer r_a .

The rate of transpiration can then be written as:

$$T = \frac{K[(\text{H}_2\text{O})_{\text{leaf}} - (\text{H}_2\text{O})_{\text{air}}]}{r_a + r_s + r_m}$$

where $(\text{H}_2\text{O})_a$ represents the concentration of water vapour at a . Note that the symbols r_a etc. are sometimes expressed relative to the diffusion constant, i.e. as $R_a = r_a/K$ with the dimensions of s/cm . Brown and Escombe's observations suggest that the stomatal path r_s can account for only about one-third to one-fifth of the total path, i.e. $r_a + r_s + r_m$.

* They ignored diffusion through the waxy layer on the leaf surface, i.e. cuticular transpiration, which in many plants probably accounts for about 10% of the total flow.

There is a thin layer of air adjacent to the leaf surface which is relatively stationary; this is referred to as the boundary or 'shearing' layer, mentioned above, and represents a stationary region where air adheres to the leaf surface. Diffusion between the leaf and the external air must take place through this layer. When the wind speed above the leaf is relatively slow the layer is thick and diffusion of water vapour or carbon dioxide across it correspondingly slow; but increase in wind velocity will decrease the depth of the 'shearing' layer increasing the rate of flow and hence the rate of transpiration. There is an empirical relationship between the depth of the 'shearing' layer and the velocity of the wind across the leaf surface,¹⁰¹ namely

$$R_a = 1.3 \sqrt{\frac{l}{u}},$$

where l = leaf width (cm), 1.3 = constant ($\text{s}^{\frac{1}{2}}/\text{cm}$),
 u = wind speed (cm/s).

It will be noted the depth of the layer is also a function of leaf size because the 'shearing' layer is smaller at the edge of the leaf than towards the centre. For a wind speed of 4 m.p.h. or 200 cm/s and a leaf 2 cm wide

$$R_a = 1.3 \sqrt{\frac{2}{200}} = 0.13 \text{ s/cm} \quad \text{or} \quad r_a = 0.03 \text{ cm.}$$

It has been calculated that normally on a bright sunny day with only a gentle breeze the shearing layer accounts for between $\frac{1}{2}$ and $\frac{1}{4}$ of the total transpiration path. It follows that under these conditions, changes in the stomatal aperture, due for example to changes in light intensity, water regime, or in carbon dioxide partial pressure surrounding the leaf, are not going to have very significant effects upon the rate of transpiration.

Similar considerations apply to photosynthesis. The average rate of photosynthesis for a sunflower leaf under good conditions is of the order of $100 \text{ mm}^3 \text{CO}_2/\text{cm}^2/\text{h}$, or $3.3 \times 10^{-5} \text{ cm}^3 \text{CO}_2/\text{cm}^2/\text{s}$. From this we can deduce a value for $\sum R = \delta x/K$ (per unit area of leaf surface) of the order of 10 s/cm,

$$\text{for } P = K \cdot A \frac{\delta c}{\delta x}, \quad \text{i.e. } \frac{\delta x}{K} = \frac{\delta c}{P/A}.$$

Since $\delta c = (\text{CO}_2)_{\text{air}} - (\text{CO}_2)_{\text{chloroplast}} = 3 \times 10^{-4} \text{ cm}^3/\text{cm}^3$,

when $(\text{CO}_2)_{\text{air}} = 0.03\% = 3 \times 10^{-4} \text{ cm}^3/\text{cm}^3$

and $(\text{CO}_2)_{\text{chloroplast}} = 0$ (assumed),

$$\text{therefore } \sum R = \frac{3 \times 10^{-4}}{3.3 \times 10^{-5}} \approx 9 \text{ s/cm.}$$

From the known value of the coefficient of diffusion K for CO_2 diffusing in air, $K_{\text{CO}_2}^{\text{air}} = 0.14 \text{ cm}^2/\text{s}$, it follows that the actual physical length of air diffusion path equivalent to the complex leaf structure is of the order of 1.5 cm. If the rate of photosynthesis is double that given above, then $\delta x/K$ becomes of the order of 5 s/cm and the equivalent diffusing path is approximately 0.75 cm.

For a given leaf the simplest assumption is that the equivalent diffusion path for transpiration is identical with that for photosynthesis up to the mesophyll cell wall; it follows that the rate of transpiration should bear some relationship to the rate of photosynthesis and is given in the simplest case by the following expression:

$$\frac{T_{\text{Transpiration}}}{P_{\text{Photosynthesis}}} = \frac{K_1(\text{H}_2\text{O}' - \text{H}_2\text{O}'')}{K_2(\text{CO}_2'' - \text{CO}_2')} \simeq 1.85 \frac{[\text{H}_2\text{O}' - \text{H}_2\text{O}'']}{[\text{CO}_2'' - \text{CO}_2']}$$

when K_1 is the coefficient of diffusion for water vapour in air, $\text{H}_2\text{O}' - \text{H}_2\text{O}''$ represents the difference in vapour pressure inside and outside the leaf, K_2 is the coefficient of diffusion for carbon dioxide in air and $\text{CO}_2'' - \text{CO}_2'$ the equivalent concentration gradient. At 20°C $K_1 = 0.257 \text{ cm}^2/\text{s}$ and $K_2 = 0.14 \text{ cm}^2/\text{s}$. Penman and Schofield¹¹² found that turf during the summer lost 11 000 tonnes of water per 0.405 hectares and produced 2040 kilogrammes of dry matter. Values of the transpiration ratio, i.e. g water lost/g carbohydrate produced, range from 150 to 1500 so that the parameter is determined by many variables. Maize leaves produce a relatively high ratio of dry matter per water loss and during a day with bright illumination and high temperature lose 35 mg H_2O per mg CO_2 fixed.¹⁹

By use of such an expression we can calculate the concentration of carbon dioxide $[\text{CO}_2']$ at a distance along the diffusion path equal to the length of diffusion path for transpiration. This will provide an indication of the concentration of carbon dioxide near the mesophyll cell wall; it can be calculated as of the order of $\frac{2}{5}$ ths that of the concentration in the external atmosphere. In the process of photosynthesis the total diffusion path is, however, longer and more complex than for transpiration; since not only does it include the three terms already discussed for transpiration but it must include also diffusion through the wet mesophyll cell wall up to the chloroplast surface and further into the chloroplast to the point at which it reacts. Approximately $\frac{3}{4}$ ths to $\frac{5}{6}$ ths of the equivalent air diffusion path for photosynthesis is internal to the mesophyll cell wall. Possibly $\frac{1}{3}$ rd of this distance is due to penetration through the wet mesophyll cell wall, since the coefficient of diffusion for carbon dioxide through water is 1/10 000 that through air. Consequently, the physical distance through the *wet* cell wall assumes a much greater magnitude when it is converted to an equivalent *air* diffusion path. The remaining $\frac{3}{4}$ ths, i.e. about $\frac{5}{6}$ ths of the total path, is within the mesophyll cell. The average distance which a molecule of

carbon dioxide must diffuse within the chloroplast for certainty of reaction i.e. the distance to the point at which the average concentration is decreased to zero, is referred to as the reaction or carboxylation resistance.

Since the stomatal path is an even smaller fraction of the total diffusion path for photosynthesis than that for transpiration, changes in stomatal aperture have even less effect on photosynthesis than on transpiration. It is only when the stomata close appreciably and their contribution to the total diffusion path becomes relatively large that they exert a significant influence on the rate of transpiration; even greater closure is required before they can exert a significant effect on the rate of photosynthesis. This can be demonstrated by the application of chemical inhibitors which primarily affect the stomatal mechanism. For example, Slatyer and Bierhuizen¹²⁰ showed that the stomata of cotton leaves sprayed with 10^{-4} M phenylmercuric acetate remained closed for many days but growth of the plants

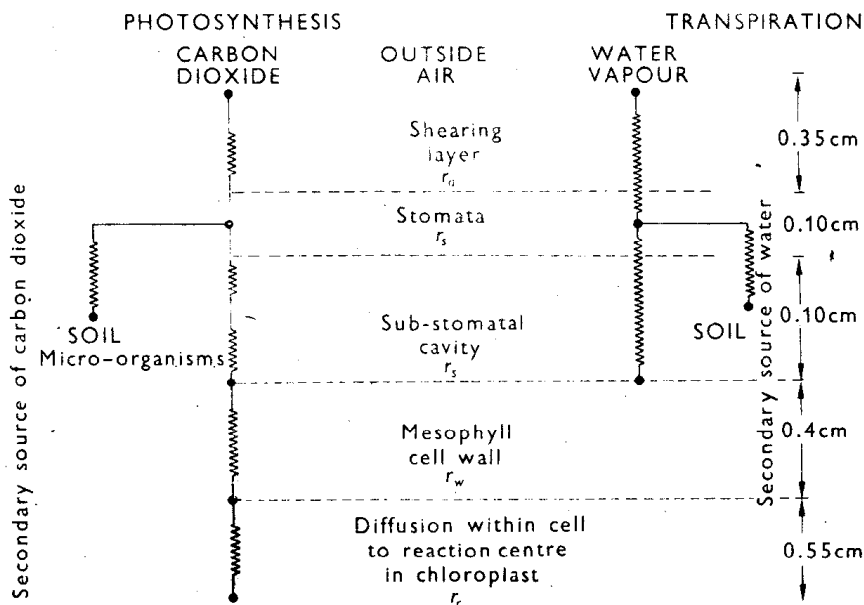


Fig. 1.2 Diagram of diffusion through stomata of the leaf. The tinted line indicates diffusion through the aqueous phase. The values on the right hand side give the approximate equivalent air diffusion path in cm for a leaf on a sunny day with relatively still air. According to the velocity of the wind r_a may vary from 0.50 to zero, according to stomatal aperture r_s from 0.1 to 1.0 and, according to reaction conditions, r_r from 0.4 to 1.0. The rate of photosynthesis observed will be in the ratio of 0.1:1.5, i.e. 1:15 to that predicted if the stomata constituted the total diffusion path and the rate of transpiration 0.1:0.55, i.e. 1:5.5. Brown and Escombe¹⁷ calculated from their data ratios of 1:16 and 1:5 respectively.

was not greatly affected; transpiration was decreased 40% but photosynthesis only 10%.

When such considerations are applied to a crop growing in the field, other factors complicate the situation; for example, there is a significant contribution of carbon dioxide (possibly up to one-third of the total) from the activity of micro-organisms in the soil so that an additional supply must be entered in parallel to the supply from the external atmosphere. Similarly in considerations of transpiration, there is an additional supply of water vapour from the soil to the leaf in parallel with the supply from the air above the leaf. These relationships have been formalized by Penman and are shown diagrammatically in Figure 1.2.

The rate of photosynthesis and the concentration of carbon dioxide

Thus the concentration of carbon dioxide outside the leaf surface, and that available to the photosynthetic reaction centre in the chloroplast, do not bear a simple relationship to each other. Differences in structure and of internal anatomy between different types of leaf may have significant effects on the relationships between these two quantities. In Chapter 2 reference will be made to tropical grass species the leaves of which have large intercellular spaces between the mesophyll cells which have relatively few chloroplasts and the closely packed cells of the bundle sheath which are densely packed with chloroplasts arranged around the veins (Fig 1.3); both these factors may increase the equivalent diffusion resistance of the leaf for photosynthesis. It follows that the relationship between the rate of photosynthesis and the concentration of carbon dioxide in the outside atmosphere will vary in leaves of different species and under different environmental conditions. To deduce from the relationship observed between the rate of photosynthesis and the concentration of carbon dioxide in the atmosphere the true relationship between the rate of photosynthesis and the concentration at the reaction centre within the chloroplast involves complex calculation. Failure to appreciate this point led to considerable confusion between 1920 and 1936 when different investigators sought to maintain that a particular relationship observed by them experimentally had greater validity than that of some other observer. For this reason investigators later turned away from the study of such effects in leaves and preferred to investigate the kinetics of photosynthesis in an aqueous suspension of unicellular algae.

Even in the case of a uniform suspension of small microscopic cells, such as of the alga *Chlorella*, there must be some difference between the concentration of carbon dioxide in the bulk of the medium and that immediately adjacent to the chloroplast surface. The diffusion equation given earlier in this chapter can be applied, remembering that the diffusion path