RELATIONSHIP OF PHOTOSYNTHETIC EFFECTIVENESS OF DIFFERENT KINDS OF LIGHT TO CHLOROPHYLL CONTENT AND CHLOROPLAST STRUCTURE IN GREENING WHEAT AND IN IVY LEAVES

By J. T. O. KIRK* and D. J. GOODCHILD*

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Abstract

A polarographic technique for the study of photosynthesis in higher plant leaves, using a bare platinum electrode of the Haxo and Blinks type, is described.

The development of photosynthetic activity in dark-grown wheat leaves exposed to light has been studied. Photosynthetic oxygen evolution typically appears after illumination for 2–3 hr. To detect photosynthesis at early stages it is necessary to use high actinic light intensities: there appears to be a threshold light intensity below which there is no oxygen evolution. At saturating white light intensity, leaves early in the greening process show almost as much photosynthetic activity as fully green leaves. The ability of the leaves to use white light of moderate intensity shows a marked increase as chlorophyll content increases during greening. Also, the relative photosynthetic effectiveness of green light, compared to red light increases dramatically during greening. It is concluded that in the early stages of chloroplast development it is the light-gathering systems which are rate-limiting at low and intermediate light intensities.

Chloroplast differentiation and increase in leaf chlorophyll content in ivy leaves grown under natural day–light conditions are accompanied by a marked increase in the ability of the leaf to utilize green light compared to red light, and to utilize moderate intensity white light. The mature chloroplasts show a great proliferation of thylakoid membranes compared to the young chloroplasts. The young leaves have a higher chlorophyll *a*/chlorophyll *b* ratio than the old. The relative photosynthetic effectiveness of green and red light is in reasonable agreement with the ratio of percentage absorption green to percentage absorption red light obtained by spectroscopic measurements on living material from both young and mature leaves.

Calculations suggest that the increase in relative percentage absorption, and relative photosynthetic effectiveness, of green light compared to red light can in very large part be explained in terms of the increase in chlorophyll concentration per unit area, with some intensification of the absorption resulting from light scattering within the tissue. The "bunching" (or "sieve") phenomenon appears to have little effect on the green/red absorption ratio.

I. INTRODUCTION

When etiolated dark-grown plants are exposed to light, the yellow non-photosynthetic etioplasts develop, over a period of 1–2 days, into green photosynthetically functional chloroplasts. For investigation of the development of photosynthetic activity in greening plants, some use has been made of the polarographic method of measuring oxygen evolution. Schiff (1963) studied the time course of development of ability to evolve oxygen in dark-grown cells of *Euglena gracilis* exposed to light:

* Division of Plant Industry, CSIRO, P.O. Box 1600, Canberra City, A.C.T. 2601.

he found that activity was first detectable at about 4 hr, and increased progressively with further illumination. Litvin and Ho I-t'an (1967) measured the action spectrum of photosynthetic oxygen evolution in the 650–695 nm wavelength range in etiolated rice leaves exposed to light for various periods. Oxygen evolution was first detectable just after 2 hr. Action spectra taken at 4, 6, and 28 hr showed the red peak to be at about 670 nm at the earliest time, but shifting to about 680 nm by 28 hr. Kirk and Reade (1970) measured the action spectrum of photosynthetic oxygen evolution in the 400–740 nm wavelength range in dark-grown cells of *E. gracilis* exposed to light. Activity was first detected at about 4 hr. Early in chloroplast development the action spectra showed peaks in the blue and red regions but there was no activity in the green. Oxygen evolution in green light was first detectable after about 12 hr of illumination, and the relative activity with green light, compared to that with red or blue light, increased markedly with further chlorophyll accumulation, reaching 50–80% of the activity given by red light by the time chloroplast development was complete.

In the case of plants growing on a natural day–night regime chloroplasts normally develop from proplastids without the intermediate formation of etioplasts (Kirk and Tilney-Bassett 1967). To our knowledge no studies have been reported on the development, in such plants, of ability to utilize light of varying wavelength and intensity, for photosynthesis.

In the present work a newly devised procedure for polarographic measurement of oxygen exchange in leaves of terrestrial plants has been utilized in a study of the ability of dark-grown wheat leaves, illuminated for various periods, and of ivy leaves growing under a natural day–night regime to make use of white light of various intensities and of red and green light for the photosynthetic evolution of oxygen. The ivy leaves have also been used to investigate the relationship between the ability to utilize different kinds of light and chlorophyll concentration, chloroplast structure, and spectroscopic properties of the leaf.

II. Methods

Wheat seedlings (*Triticum aestivum* cv. Olympic) were grown for 8–10 days at 24°C, in the dark, in vermiculite watered with nutrient solution. The shoots were then cut off at the base and placed with their cut ends in water in 50-ml Erlenmeyer flasks. The flasks were illuminated with fluorescent light, intensity 750 f.c., at 23–27°C. For chlorophyll and photosynthesis measurements an apical 3-in. segment was taken from one of the shoots. This was cut into three separate 1-in. segments. The second segment was used for photosynthesis measurements and the first and third for chlorophyll analysis. The middle segment was lightly abraded up to six times on its upper surface with moistened emery paper and then placed in position on the platinum electrode as described in Appendix 1. The ivy (*Hedera helix*) leaves used in this work were taken from a plant growing at the base of a south-facing wall, at various times in the midspring to midsummer period: the plant was exposed to ample diffuse daylight but received direct sunlight only for a short time in the evening. This particular variety has the property that the young leaves, although grown in the light, form chlorophyll very slowly, so that a leaf can be almost, or completely, fully expanded, but still be pale green. The slow accumulation of chlorophyll to reach the final high concentration takes place over a period of at least 2 months. The young leaves chosen for these experiments were yellow-green to very pale green in colour, and ranged in area from about 30 to nearly 100% of that typical of fully grown leaves. The mature leaves chosen were dark green in colour. For the photosynthesis measurements, strips about 7 by 20 mm were cut from the

leaf, adjoining and parallel to the midrib. The lower surface of the strip was abraded 12 times with a piece of emery paper and the tissue was placed with its abraded surface in contact with the platinum electrode of the polarographic system.

The wheat or ivy leaf sections were allowed to equilibrate on the electrode for at least 4 min in the dark and then illuminated with an Aldis QI 24 slide projector (150 W quartz iodine lamp) at a light intensity of 6400 f.c. at the leaf surface. When the steady state of photosynthesis had finally been achieved (i.e. after completion of the transients described in Appendix I) the light intensity was reduced by placing a neutral density interference filter (Balzer) in the light beam. After a period of time—within a minute or two at high light intensities, but sometimes as much as 15–20 min at low light intensities—the rate of oxygen evolution settled down to a new steady-state value. The light intensity was then lowered again by interposition of another neutral density interference filter with lower transmission than the first one. In this way a series of values for the rate of steady-state photosynthesis at a series of light intensities at the leaf surface from 6400 down to 80 f.c. was obtained. After the last illumination the light was switched off for a period of 1–2 min to obtain the dark steady-state recorder signal. The difference (ΔV) between a given signal in the light and the steady-state dark signal is approximately proportional to the rate of oxygen evolution, as already discussed (Kirk and Reade 1970): it should be noted that no correction has been made for a possible increased oxygen uptake in the light, due to photorespiration.

After the 1–2 min dark period following the measurements of photosynthesis at different white light intensities the leaf segment was illuminated with green light obtained by placing a Balzer Filtraflex B-40 interference filter (wavelength of maximum transmission 551 nm; half bandwidth *c.* 4 nm) in the slide projector light beam. The intensity at the surface of the leaf segment was 5900 ergs/cm^2/s, which is equivalent to $1{\cdot}64 \times 10^{15}$ quanta/cm^2/s. With this monochromatic light beam given after a relatively short dark period, the various induction transients observed with intense white light after longer dark periods (Appendix I) were not observed: the rate of photosynthetic oxygen evolution (except on the occasions when this was entirely absent) rose relatively slowly over a period of 3–8 min and then levelled off at the final steady-state value. The green filter was then replaced with a red filter (Balzer Filtraflex B-40 interference filter, wavelength of maximum transmission 676 nm; half bandwidth *c.* 5 nm) giving red light of intensity 3350 ergs/cm^2/s ($1{\cdot}143 \times 10^{15}$ quanta/cm^2/s) at the leaf surface. The rate of oxygen evolution then rose, within 1–3 min, to a new, usually higher, steady-state value. In other experiments the leaf was illuminated with high intensity white light (6400 f.c.) until photosynthesis had reached its steady state. The white light was followed by a series of monochromatic irradiations, alternately green and red, of diminishing intensity (achieved by combining the green and red filters with appropriate neutral density filters) and the steady-state rate of photosynthesis at each intensity was noted.

White light intensities (in foot-candles) were measured with a Weston light-meter. The energies of the red and green light beams (in ergs/cm^2/s) were measured with a Yellow Springs model 65 radiometer: values were converted to quanta/cm^2/s by calculation. The total light intensity incident on the leaf is higher than that on the surface facing the light beam because some light is transmitted through the leaf and reflected back again by the electrode. To make approximate allowance for this effect the light intensity values were increased by an amount corresponding to the intensity initially impinging on the leaf, multiplied by the transmittance of the leaf at the wavelength in question and also by the reflectivity of the platinum electrode (Haxo and Blinks 1950). Transmittance values in red and green light were obtained using the Cary spectrophotometer with the scatter transmission attachment (see later). Approximate transmittance values in white light were obtained with the help of a piece of aluminium foil, with a 20 by 2 mm slit, placed on the sensor of a Weston light-meter. The white light intensity (using the QI 24 projector) was measured with or without a suitable piece of leaf placed behind the slit, the slit being also covered with a drop of water and a glass coverslip.

For chlorophyll analyses the first and third 1-in. wheat leaf sections were weighed, finely chopped with a razor-blade, stirred with 1·5 ml of 80% acetone plus a little calcium carbonate with a glass rod in a centrifuge tube, subjected to ultrasonic vibrations for 30 s at 90 W with a Branson B-12 sonifier, and the suspension centrifuged.

In the case of ivy leaves, pieces (1 cm^2 from mature leaves, 2–4 cm^2 from young leaves) were taken from the corresponding area, on the opposite side of the midrib to that from which the strip for photosynthesis measurements had been removed. Chlorophyll was extracted by grinding in 5 ml of 80% acetone plus a little calcium carbonate. After centrifugation the extinction values of the supernatant solutions at 663 and 645 nm were measured using the Beckman DU or Gilford 2400 spectrophotometer (for routine total chlorophyll analyses), or the Cary 14R spectrophotometer (for accurate chlorophyll *a*/chlorophyll *b* ratio determinations). Chlorophyll *a*, chlorophyll *b*, and total chlorophyll concentrations were calculated using a nomogram (Kirk 1968) based on the published extinction coefficients (MacKinney 1941).

To measure absorption spectra of ivy leaves, pieces of young or mature leaf were placed in a cuvette assembly of the type described by Bonner (1961), modified to suit the optical system of the Cary spectrophotometer. To provide a blank with suitable scattering properties, pieces of Scotties facial tissue were used: a sufficient number of tissue pieces was used to balance the light-scattering properties of the leaf at 750 nm. Spectra were recorded in the Cary model 14R spectrophotometer, fitted with a model 1462 scatter transmission attachment, and an EMI 9558 QB photomultiplier. The optical density trace (approximately flat and horizontal) in the 750–770 nm region (in which the chlorophylls do not absorb) was assumed to correspond to the baseline. Percentage absorption values at 550 and 676 nm were calculated from the measured extinction values at these wavelengths.

For electron microscopy several leaf pieces approximately 1 mm^2 were cut from young and mature leaves and fixed in 3% glutaraldehyde in 0·025M sodium phosphate buffer at pH 7·2 for 1·5 hr at 20°C. They were then washed in four changes of buffer for a total of 1 hr and post-fixed in a 2% solution of osmium tetroxide in the 0·025M phosphate buffer for 2 hr at 20°C. After washing in buffer and dehydration in ethanol and propylene oxide the leaf pieces were embedded in an Araldite–Epon mixture. The resin was vacuum-infiltrated at 85°C and polymerized at the same temperature for 24 hr before sectioning with a Reichert ultramicrotome. Sections were stained with saturated uranyl acetate in 50% ethanol for 1 hr followed by lead citrate before examination in a Philips EM 200 electron microscope.

III. Results

(*a*) *Inception of Photosynthetic Oxygen Evolution in Greening Wheat*

To determine at what stage of greening photosynthetic oxygen evolution could first be detected, wheat leaves were taken after various periods of illumination and tested on the platinum electrode system, using white light, of intensity 6400 f.c. Etiolated leaves had no activity, nor did leaves illuminated for 1 hr. After 2 hr greening, activity was not detected in five experiments, was barely detectable in two experiments, and was clearly detectable in one experiment. After 3 hr greening, activity was detected in nine experiments and not detected in one experiment. Activity was always present in leaves illuminated for 4 hr or more. Thus, the "typical" situation seems to be that in 7–9-day-old etiolated wheat leaves, greening-up in white light at an intensity of 750 f.c., at about 25°C, photosynthetic oxygen evolution becomes detectable between 2 and 3 hr, by which time the chlorophyll content of the leaves has risen to 20–80 μg per gram fresh weight (*c.* 2–8% of the final chlorophyll content reached after about 30 hr illumination).

(*b*) *Photosynthetic Oxygen Evolution at Varying White Light Intensities at Different Stages during Greening of Wheat*

In some of the early experiments of the present series, a relatively low white light intensity—about 400 f.c.—was used in attempts to detect photosynthetic oxygen evolution after short periods of greening. It was found that with low intensities

of actinic light during the photosynthesis measurements, longer periods of greening (more than 3 hr) were required before activity could be detected. However, when such a piece of leaf—greened for, say, 3 hr and showing no oxygen evolution at 400 f.c.—was illuminated with light of intensity 6400 f.c., there was immediate oxygen evolution. These results suggested that at early stages of greening the leaves possessed the ability to photosynthesize but that this ability was only manifest at high light intensities. To clarify the nature of this phenomenon further, experiments were carried out in which, after various periods of greening, the rate of photosynthetic oxygen evolution was measured, at a series of different light intensities. Figure 1

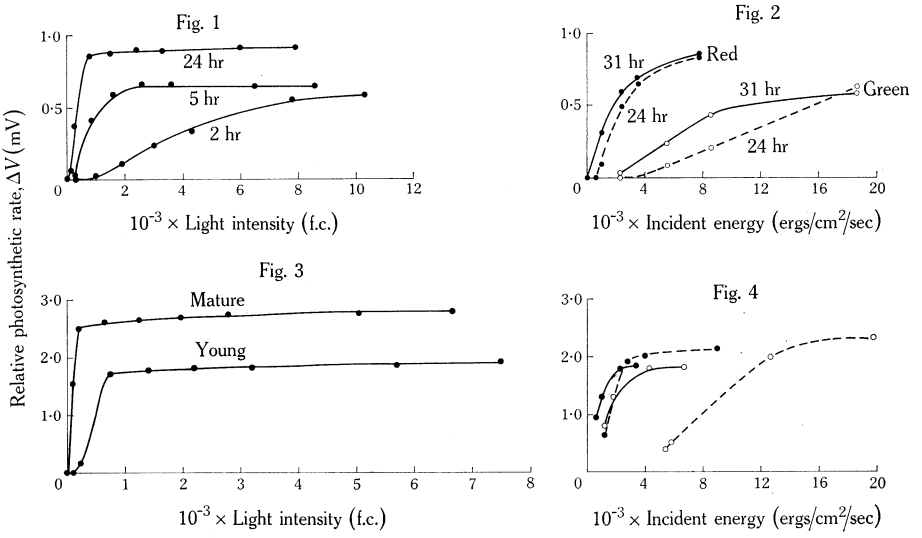


Fig. 1.—Dependence of photosynthetic rate on white light intensity in dark-grown wheat seedlings after various periods of greening. Intensities were corrected for reflection from the electrode. The leaves contained 42, 120, and 496 μg chlorophyll/g fresh weight at 2, 5, and 24 hr respectively.

Fig. 2.—Variation of photosynthetic rate with monochromatic light intensity in greening wheat leaves. The leaves greened for 24 hr contained 500 μg chlorophyll/g fresh weight, those greened for 31 hr contained 1053 μg chlorophyll/g fresh weight. Intensity values were corrected for reflection from the electrode. The relative photosynthetic effectiveness (measured at subsaturating intensities, about 8500 ergs/cm^2/s at 551 nm, and 2400 ergs/cm^2/s at 676 nm) of green light as a percentage of that of red light, increased from 14·1 to 25·0% between 24 and 31 hr.

Fig. 3.—Dependence of photosynthetic rate on white light intensity in young and mature ivy leaves. Intensities were corrected for reflection from the electrode. The young leaf contained 750 μg chlorophyll/dm^2; the mature leaf contained 5350 μg chlorophyll/dm^2.

Fig. 4.—Variation of photosynthetic rate with monochromatic light intensity in young (– – –) and mature (——) ivy leaves. Intensities were corrected for reflection from the electrode. The young leaf contained 875 μg chlorophyll/dm^2; the mature leaf contained 4950 μg chlorophyll/dm^2. ○ Green light. ● Red light.

shows the results obtained in such an experiment. The results show that as the chlorophyll concentration rises during greening, the leaf becomes very much better at utilizing the lower light intensities for photosynthesis. For instance, in the experiment illustrated, at a light intensity of 800–1000 f.c., the 2-hr greened leaf

shows negligible activity, the 5-hr leaf shows about 60% of the activity at saturating light intensity, and the 24-hr leaf is approximately at light saturation. At an intensity of 240 f.c. the 2- and the 5-hr leaves show no activity but the 24-hr leaf has about 40% of the activity obtained at light saturation. By contrast, at the higher light intensities, in the region of 8,000–10,000 f.c., the differences between the three types of leaf become comparatively small.

(c) *Relative Photosynthetic Effectiveness of Red and Green Light at Different Stages during Greening of Wheat*

In view of the earlier finding (Kirk and Reade 1970) that the rate of photosynthesis in green light, relative to that in red light, increased markedly during chloroplast development in *E. gracilis*, it was considered of interest to determine whether a similar relationship would be found during greening of etiolated wheat leaves. Accordingly, the steady-state rates of photosynthetic oxygen evolution in green and red light (at intensities below the saturation levels) in etiolated wheat leaves illuminated for various periods were measured. The results in Table 1 show

TABLE 1

CHANGES IN RELATIVE PHOTOSYNTHETIC EFFECTIVENESS OF GREEN AND RED LIGHT DURING CHLOROPLAST DEVELOPMENT IN WHEAT LEAVES

The rate of photosynthesis, expressed as the change (light minus dark) in the oxygen electrode signal, of dark-grown wheat leaves after different periods of greening was measured in green (551 nm, $1 \cdot 64 \times 10^{15}$ quanta/cm^2/s) and in red (676 nm, $1 \cdot 143 \times 10^{15}$ quanta/cm^2/s) light. In the calculations of photosynthetic effectiveness (P.E., expressed as mV/10^{15} quanta/cm^2/s) these intensities were corrected for reflection of transmitted light from the electrode

Time of greening (hr)	Chlorophyll content (μg/g fresh wt.)	Green light		Red light		$\frac{\text{P.E.}_{\text{green}}}{\text{P.E.}_{\text{red}}}$ (%)
		ΔV (mV)	P.E.	ΔV (mV)	P.E.	
$4\frac{3}{4}$	88	0·0	0·0	0·12	0·08	0·0
$7\frac{1}{4}$	236	0·0	0·0	0·39	0·30	0·0
24	673	0·23	0·10	0·72	0·60	16·7
31	1064	0·56	0·24	0·71	0·60	40·0

that the photosynthetic effectiveness (the photosynthetic rate for a given number of incident quanta/cm^2/s) of green light compared to that of red light does indeed increase very markedly as the chloroplasts develop. In this particular experiment red light induced oxygen evolution at all four times of testing, the actual rate increasing as greening proceeded. Green light brought about no detectable photosynthesis at $4\frac{3}{4}$ or $7\frac{1}{4}$ hr, but by 24 hr induced oxygen evolution about one-sixth as effectively as red light, and by 31 hr was 40% as effective as red light. Figure 2 shows the results of a separate experiment in which the variation of photosynthetic rate with green or red light intensity was measured in etiolated wheat leaves illuminated for 24 or 31 hr. The results again show an increase in the photosynthetic effectiveness of green light relative to that of red light as chlorophyll concentration increases.

(*d*) *Photosynthetic Oxygen Evolution of Young and Mature Ivy Leaves at Varying White Light Intensities*

To determine whether the rate of photosynthesis by ivy leaves developing under normal conditions showed a similar relationship to light intensity to that found for greening wheat, the rates of oxygen evolution by young and mature leaves were measured at a series of different intensities of white light. The results (Fig. 3) showed that there was only a relatively small difference between the two types of leaf in the rates of photosynthesis at intensities from about 740 f.c. upwards, these intensities being saturating in both cases. However, at lower intensities there was a very marked difference. For instance, at 200 f.c. the mature leaf was still approximately at the saturation rate, but the young leaf evolved oxygen at only about 8% of the saturation rate. At 80 f.c. the mature leaf was still photosynthesizing at 55% of the saturation rate (or even nearly at the saturation rate in other experiments) but the young leaf gave no detectable oxygen evolution. It should be noted that the rates in Figure 3 correspond to rates per unit area, since the same area of leaf was in contact with the electrode in both cases. Since the chlorophyll content of the young leaves is very much lower than that of the old, this means that the rate of photosynthesis per unit chlorophyll, at saturating light intensities, is about 4–5 times as high in the young leaves as in the old.

(*e*) *Efficiency of Utilization of Red and Green Light by Young and Mature Ivy Leaves*

To determine whether ivy leaves at early and late stages of chloroplast development differed in their ability to utilize green light (compared to red light) the rates of oxygen evolution were measured at a series of intensities of red (676 nm) and green (551 nm) light. In the young leaf green light is much less effective than red light at intensities below saturation: in the mature leaf the difference between the effectiveness of red and green light is considerably less (Fig. 4).

In order to compare the photosynthetic effectiveness of red and green light on a quantitative basis, the rates of photosynthesis (at intensities below saturation in all cases) were divided by the corresponding incident intensities (in quanta/cm^2/s). The ratios of the photosynthetic effectiveness (rate of photosynthesis per unit incident intensity) of green light to that of red light for a series of young and mature ivy leaves are listed in Table 2. The results show that the relative photosynthetic effectiveness of green light, compared to that of red light, is much greater in the mature leaves with high chlorophyll content than in the young leaves with low chlorophyll content.

(*f*) *Chlorophyll* a/*Chlorophyll* b *Ratios of Young and Mature Ivy Leaves*

When an etiolated leaf is exposed to light, only chlorophyll *a* is formed at first, and although chlorophyll *b* formation begins after several minutes (Thorne and Boardman 1971*b*), the chlorophyll *a*/chlorophyll *b* ratio is higher during the early stages of chloroplast development, when stacking of thylakoids into grana has occurred only to a small extent, than in the mature chloroplast. The situation in the ivy cultivar used in this work is different from the greening of a dark-grown bean, pea, or wheat leaf, because the ivy, unlike these other plants, requires many weeks on a normal daylight regime, rather than a couple of days, for full chloroplast develop-

ment to occur. Although the young, pale green ivy leaves used here have been growing for several days in the light, the thylakoid membranes have only reached a stage of organization [see Section III(*h*)] corresponding to that of, say, an etiolated

TABLE 2

RELATIVE PHOTOSYNTHETIC EFFECTIVENESS OF RED AND GREEN LIGHT IN YOUNG AND MATURE IVY LEAVES

The rate of photosynthesis, expressed as the change in oxygen electrode signal (ΔV, in millivolts) is divided by the incident energy (E, in quanta/cm^2/s), corrected for reflection from the electrode, to give a measure of the photosynthetic effectiveness of light at that wavelength

Chlorophyll content (μg/dm^2)	Chlorophyll content (μg/g fresh wt.)	$\frac{(\Delta V/E)_{green}}{(\Delta V/E)_{red}}$	Chlorophyll content (μg/dm^2)	Chlorophyll content (μg/g fresh wt.)	$\frac{(\Delta V/E)_{green}}{(\Delta V/E)_{red}}$
Young leaves			Mature leaves		
720	457	0·133	7320	3064	0·595
850	511	0·228	6450	2650	0·644
875	555	0·345	4950	2250	0·639
712	364	0·105	5350	2250	0·555

oat leaf given about 10 hr illumination (Gunning and Jagoe 1967). It therefore seemed possible that despite their long exposure to light these chloroplasts might still have a chlorophyll *a*/chlorophyll *b* ratio higher than the value (*c.* 2·7) characteristic of a fully developed chloroplast. Measurements (Table 3) showed that this is indeed the case.

TABLE 3

CHLOROPHYLL *a*/CHLOROPHYLL *b* RATIOS OF YOUNG AND MATURE IVY LEAVES

Chlorophyll content (μg/dm^2)	$\frac{\text{Chlorophyll } a}{\text{Chlorophyll } b}$	Chlorophyll content (μg/dm^2)	$\frac{\text{Chlorophyll } a}{\text{Chlorophyll } b}$
Young leaves		Mature leaves	
362	3·83	7800	2·53
725	5·44	5260	2·65
638	3·64	5380	2·71
727	5·26	7520	2·78

The mature leaves have a relatively constant chlorophyll *a*/chlorophyll *b* ratio in the region of 2·7. The young leaves have a more variable ratio but it is distinctly higher than in the mature leaves, being usually in the range 3·6–5·5.

(*g*) In vivo *Light Absorption Properties of Young and Mature Ivy Leaves*

In view of the observed difference between the photosynthetic effectiveness of green light (relative to red light) in young ivy leaves and that in mature leaves it was of interest to attempt to measure the percentage absorption of green and red light by the living leaves. Ideally, such measurements should be carried out with the sample at the centre of an integrating sphere so that all the light, no matter in what

direction it is scattered, is collected and measured. Since such a device was not available, the spectra were recorded on a Cary spectrophotometer with the sample as close to the photomultipler as possible, so that a large proportion of the scattered light was collected. The measured values given in Table 4 are inevitably somewhat approxi-

TABLE 4

MEASURED AND CALCULATED VALUES OF PERCENTAGE ABSORPTION OF GREEN AND RED LIGHT BY YOUNG AND MATURE IVY LEAVES

Values of percentage absorption were measured using the scatter transmission attachment of the Cary spectrophotometer as described in Section II. The calculated values were obtained by first calculating the extinction values expected on the basis of the weight of chlorophyll per unit area, assuming the system to obey Beer's law. The extinction is given by the equation, $E = \alpha C_A \times 10^{-3}$, where C_A represents the weight of chlorophyll per unit area (μg/cm^2) and α is the appropriate specific absorption coefficient, this being assumed to have the same value as for chlorophyll in diethyl ether solution. From the published values for chlorophyll *a* and chlorophyll *b* (French 1960) it can be calculated that the specific absorption coefficients at 550 nm (green light) and 662 nm (676 nm *in vivo*) (red light), respectively, are 4·03 and 83·6 litres/g/cm for a 4·5 : 1 mixture of chlorophyll *a* and chlorophyll *b* (young leaves), and 4·36 and 75·3 litres/g/cm for a 2·7 : 1 mixture (mature leaves)

Chlorophyll content (μg/dm^2)	Measured values of percentage absorption			Calculated values of percentage absorption		
	Green light	Red light	Ratio*	Green light	Red light	Ratio*
			Young leaves			
435	12·7	59·4	0·214	4·0	56·7	0·071
727	21·7	78·9	0·275	6·5	75·4	0·086
			Mature leaves			
6250	71·6	98·1	0·730	46·6	100·0	0·466
8270	87·8	99·1	0·886	56·4	100·0	0·564

* Percentage absorption in green light to percentage absorption in red light.

mate but clearly show that there is a very marked increase in the ratio of percentage absorption of green (550 nm) light to percentage absorption of red (676 nm) light during development of the young, to the mature, leaf. The measured values obtained for the green/red absorption ratios in Table 4 are roughly in agreement with the values of the ratio of photosynthetic effectiveness of green light to that of red light given in Table 2. It is interesting to compare these measured values of percentage absorption with values calulated on the basis of the known chlorophyll contents, making the simplifying (and very approximate) assumptions that the chlorophyll is uniformly distributed throughout the leaf and has the same absorption properties as if it were dissolved in diethyl ether. Comparison of calculated values with measured values (Table 4) shows that the ratio of percentage absorption of green light to percentage absorption of red light *in vivo* is considerably higher than might be expected on the basis of chlorophyll content alone.

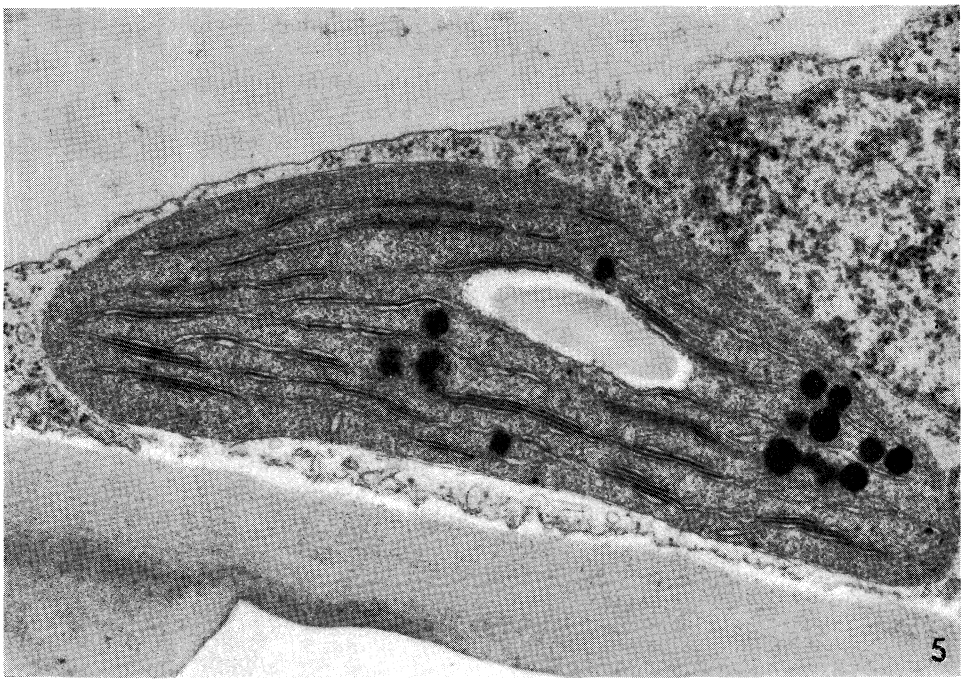
As the chloroplasts develop, not only does the chlorophyll content rise, but there is an increase in the proportion of chlorophyll *b* relative to chlorophyll *a* (Table 3): this change in relative composition will also bring about an increase in the green/red absorption ratio. The specific absorption coefficient (French 1960) of chlorophyll *b* at 550 nm (7·07 litres/g/cm) is rather more than twice the value for chlorophyll *a* (3·35 litres/g/cm). At the red absorption peak (662 nm in ether solution) of chlorophyll *a*, however, chlorophyll *b* has a very much lower specific absorption coefficient than chlorophyll *a*: 6·2 litres/g/cm compared to 101·0 litres/g/cm. The change in the chlorophyll *a*/chlorophyll *b* ratio has been taken into account in the calculations in Table 4, and makes a small contribution to the predicted increase in green/red absorption ratio in the development of the young to the mature leaf. Consider, for example, the first and third lines of calculated values in Table 4: if the chlorophyll *a*/chlorophyll *b* ratio remained, during development, at the value of about 4·5 (typical of the young leaves) instead of falling to 2·7, the calculated green/red absorption ratio would rise from 0·071, not to 0·466 as in the table but to 0·440.

(*h*) *Chloroplast Structure and Light-absorption Properties in Young and Mature Ivy Leaves*

The visible absorption (extinction) spectrum of a leaf is predominantly a function of the concentration of chlorophylls and carotenoids in the tissue but is also markedly affected by the segregation of the pigments into discrete particles, the chloroplasts [see Section III(*i*)]. The extent to which the spectrum is distorted is in large part determined by the light-absorbing properties of the individual chloroplasts which in turn depend on the amount and spatial disposition of the chlorophyll-bearing membrane of the plastids. The structure of the plastids of young and mature ivy leaves was therefore investigated by electron microscopy. The internal membranes of young chloroplasts (Fig. 5) are organized into what might be described as compound lamellae (in section, having the appearance of bands), 8–11 in number, extending the full width of the chloroplasts. These compound lamellae vary in complexity from one place to another: at any given point they may be one, two, or three thylakoids thick. The young ivy chloroplasts have some resemblance to the chloroplasts of certain orders of algae—the Chrysophyta, Pyrrophyta, Phaeophyta, and Euglenophyta: these too have compound lamellae containing two to four thylakoids (Kirk and Tilney-Bassett 1967). However, a difference is that in the algae the thylakoids are much longer and single thylakoids commonly extend nearly the full width of the chloroplast.

The mature chloroplasts are characterized by a vast increase in the number of thylakoids (Fig. 6), most of which are organized into relatively distinct grana, although a significant proportion exist as single stroma thylakoids extending out from the grana. These grana are unusually deep, some containing as many as 60–70 stacked thylakoids.

In order to obtain some idea of the change in absorption properties of the chloroplasts resulting from this increase in the amount of chlorophyll-bearing membranes, some very approximate calculations have been carried out on the basis of the structural information derived from electron microscopy. If the simplifying, and very approximate, assumption is made that the chlorophyll molecules *in vivo*



Figs. 5 and 6.—Chloroplast in young (Fig. 5) and mature (Fig. 6) ivy leaf.

obey Beer's law, then it can be calculated (see Appendix II) that if the specific absorption coefficient (in litres/g/cm) of the chlorophyll is α, and the area (in Å^2) of thylakoid membrane occupied per chlorophyll molecule is A, then for a stack of n thylakoids the extinction value at the wavelength in question is given by the equation

$$E = 0{\cdot}0298\alpha n/A.$$

To obtain approximate values of n for young and mature ivy chloroplasts the number of thylakoids traversed by a line drawn through the middle of the plastid, at right angles to the average plane of the thylakoids, was determined on electron micrographs of 12 young and 10 mature chloroplasts. The mean values of n obtained were 16 for young chloroplasts and 64 for mature chloroplasts.

In order to calculate extinction and percentage absorption values for the two kinds of plastid, it was assumed (see subheading to Table 4) that the specific absorption coefficients of the chlorophylls at the red peak and in the green trough are the same in the thylakoid as in ether solution.

Estimates of the area of thylakoid membrane available per chlorophyll molecule were obtained by Thomas, Minnaert, and Elbers (1956) for seven different plant species: the estimates ranged from 90 to 380 Å^2, but the mean value for all the species was about 260 Å^2, and this is the value we shall assume in our calculations.

Using the above equation we may now calculate that for a typical young chloroplast ($n = 16$), at 550 nm the extinction is 0·007, the percentage absorption is 1·7; at 676 nm the extinction is 0·153, the percentage absorption is 29·7. The ratio of percentage absorption in the green to that in the red is 0·057. For a typical mature chloroplast ($n = 64$), at 550 nm the extinction is 0·032, the percentage absorption is 7·1; at 676 nm the extinction is 0·552, the percentage absorption is 71·9. The ratio of percentage absorption in the green to that in the red is 0·099, which is substantially higher than the corresponding value for young chloroplasts.

(*i*) *Factors Governing the Relative Absorption of Green and Red Light*

It was pointed out previously (Kirk and Reade 1970) that an inevitable consequence of the increase in chlorophyll concentration during greening is an increase in the ratio of percentage absorption of green light to percentage absorption of red. The ratio of extinction values in the green and the red, is, by contrast, independent of concentration (at least for chlorophyll in free solution). Since the photosynthetic activity of light of a given wavelength depends upon percentage absorption rather than extinction, this effect will increase the relative photosynthetic effectiveness of green light compared to red light, as the chlorophyll concentration rises.

The average chlorophyll concentration throughout the leaf is not the only factor which determines the relative percentage absorption of green and red light: this ratio is also affected by the detailed spatial distribution of pigment within the tissue. Duysens (1956) and Rabinowitch (1956) showed by a mathematical analysis that when, in a light-absorbing system, the pigment is organized into discrete particles, the extinction values for the suspension are lower, at all wavelengths, than the corresponding extinction values for the same amount of pigment in free solution: the lowering effect is greater at the peaks than at the troughs, and so the shape of the

spectrum becomes flattened. This is known as the "sieve" or "bunching" effect (Rabinowitch 1956).

To determine to what extent the bunching effect will alter the action spectrum it is necessary to ascertain in what way it will alter the variation with wavelength of percentage absorption rather than extinction. The degree of distortion of the percentage absorption spectrum of the leaf at any given wavelength turns out to be a function of the percentage absorption of individual particles and of the extinction value that the total leaf pigments would have if they were present in free solution. The calculation has been carried out for green and red light absorption by a model greening leaf, the overall chlorophyll concentration of which varies from 5 to 100% of the final values. The theoretical basis for the calculations is outlined in Appendix III, and the results are given in Table 5. The values obtained for $A_{sol.,green}/A_{sol.,red}$

TABLE 5

CALCULATION OF PERCENTAGE ABSORPTION OF GREEN AND RED LIGHT BY GREENING LEAVES IN THE PRESENCE OR ABSENCE OF THE BUNCHING EFFECT

$A_{sol.}$ is the percentage absorption by the leaf calculated on the assumption that the pigments are uniformly distributed in free solution throughout the leaf. $A_{sus.}$ is the percentage absorption calculated on the assumption that the pigments are confined to particles—the chloroplasts: the leaf is treated as a suspension of these particles. The final chlorophyll concentration of the leaf is 6 mg/dm². Further details are given in the text. The last column shows the green/red absorption ratio of the suspension (A.R.$_{sus.}$) as a proportion of the green/red absorption ratio of the solution (A.R.$_{sol.}$), i.e. it gives a measure of the extent to which the bunching effect distorts the green/red absorption ratio

Percentage of final chlorophyll concn.	$A_{sol.}$ (%)		$\frac{A_{sol.,green}}{A_{sol.,red}}$	$A_{sus.}$ (%)		$\frac{A_{sus.,green}}{A_{sus.,red}}$	$\frac{\text{A.R.}_{sus.}}{\text{A.R.}_{sol.}}$
	Green light	Red light		Green light	Red light		
5	3·0	41·4	0·072	3·0	40·3	0·074	1·03
10	5·8	65·6	0·088	5·8	63·6	0·091	1·03
20	11·1	88·2	0·126	11·1	85·3	0·130	1·03
30	16·2	95·9	0·169	16·2	93·4	0·173	1·02
40	21·1	98·6	0·214	21·1	96·8	0·218	1·02
50	25·7	99·5	0·258	25·7	98·3	0·261	1·01
60	29·9	99·8	0·299	29·2	99·1	0·295	0·99
70	33·9	99·9	0·339	33·3	99·4	0·335	0·99
80	37·8	100·0	0·378	37·2	99·7	0·373	0·99
90	41·3	100·0	0·413	40·2	99·8	0·403	0·98
100	44·7	100·0	0·447	43·6	99·8	0·437	0·98

(the green/red absorption ratio that the pigments would have if they were in free solution) show the very marked increase that might be expected during greening on the basis of chlorophyll content alone. From the data in the last column it can be seen that the bunching effect brings about only a slight change in the green/red absorption ratio, not more than about 3% in this particular system. The ratio is slightly increased at the lower chorophyll concentrations and decreased at the higher. It is interesting that the green/red extinction ratio is, by contrast, quite markedly influenced by the bunching effect; for instance, for the fully greened leaf it can

readily be calculated that this ratio is 0·0555 for the pigments in solution and 0·0890 for the leaf, an increase of 60%. Thus, percentage absorption turns out to be much less sensitive to the bunching effect than is extinction.

Similar calculations have been carried out for young and mature ivy leaves, making use of the absorption values for individual ivy chloroplasts calculated on the basis of structural data in the previous section, and total pigment extinction values (for the pigments in free solution) calculated from the mean chlorophyll contents in Table 4. For the young leaf the percentage absorption turns out to be 5·2% at 550 nm and 61·1% at 676 nm, giving a green/red absorption ratio of 0·085. The corresponding values calculated purely on the basis of pigment content alone (Table 4), ignoring the bunching effect, are 5·25% at 550 nm and 66·0% at 676 nm, giving a green/red absorption ratio of 0·0795. For the mature leaf, the percentage absorption is 50·45% at 550 nm and 99·92% at 676 nm, giving a green/red absorption ratio of 0·505. The corresponding values calculated from the pigment content alone (Table 4) are 51·5% at 550 nm and 100·0% at 676 nm, giving a green/red absorption ratio of 0·515.

These calculations for ivy indicate, in agreement with the conclusions reached for the greening leaf, that the bunching effect does not greatly alter the shape of the percentage absorption versus wavelength spectrum. Also, again in agreement with the previous finding, the sign of the change induced by the bunching effect depends on the chlorophyll concentration: in the young leaves the effect increases the green/red absorption ratio by about 7%, in the mature leaves it lowers the ratio by about 2%. What emerges quite clearly from all the calculations is that even when the bunching effect is taken into account, the major factor determining the green/red absorption ratio is still the overall chlorophyll concentration in the system.

IV. Discussion

While there is general agreement that etiolated leaves not previously exposed to light are unable to carry out photosynthesis when first illuminated, there is a considerable amount of variation in the values found for the period of greening required before photosynthesis can be detected. Smith (1954), using an extremely sensitive procedure for detecting oxygen (its ability to quench phosphorescence), reported that in dark-grown barley leaves exposed to light, oxygen evolution was first detectable at 30 min and increased rapidly after this. However, even after 100 min of illumination the rate was only about 3·0 μl oxygen evolved per gram of leaf per hour. Since a fully green leaf can easily evolve 2000–4000 μl of oxygen per gram fresh weight per hour, it will be appreciated that these very low rates observed by Smith (which would be quite undetectable by the procedures normally used for measuring photosynthesis), while of considerable theoretical interest, represent for all practical purposes, negligible photosynthesis.

Gabrielsen, Madsen, and Vejlby (1961) found that when dark-grown wheat seedlings were exposed to continuous light there was no uptake of carbon dioxide for at least 25 min. With alternating light and dark periods, carbon dioxide uptake in the light was first detected 36–48 min after the beginning of the first light treatment. These workers did not, however, measure oxygen evolution, and so while

it is entirely possible that the light-dependent CO_2 uptake was accompanied by a corresponding oxygen evolution, more direct evidence seems desirable. Biggins and Park (1966) detected incorporation of $^{14}CO_2$ into phosphoglyceric acid and hexose phosphate in etiolated barley leaves after illumination for 1 hr; here too, oxygen evolution was not studied. Litvin and Ho I-t'an (1967) found that in dark-grown rice seedlings exposed to light, oxygen evolution only became detectable after 2 hr. In dark-grown maize leaves Inman (1935) found that oxygen evolution made its appearance after $2\frac{1}{4}$ hr of illumination. In greening barley, Rhodes and Yemm (1966) detected light-dependent CO_2 fixation after illumination for 3 hr. Tolbert and Gailey (1955) observed that in dark-grown wheat seedlings, significant light-dependent $^{14}CO_2$ fixation developed only after 5 hr of illumination. In dark-grown bean (*Phaseolus vulgaris*) leaves, Bradbeer (1969) found that photosynthetic carbon dioxide fixation and oxygen evolution were first detectable after about 16 hr of illumination.

It seems that the finding reported here, that photosynthesis (as indicated by oxygen evolution) in greening wheat usually makes its appearance between 2 and 3 hr, occupies an intermediate position in the previously reported range of times for higher plants, and is in good agreement with the only one of these previous reports (that of Litvin and Ho I-t'an 1967) which also made use of the polarographic procedure. However, there seems no reason to doubt that the apparent large variation between one plant species or variety, and another, in time required for photosynthesis to develop, is real. The causes of this variability remain obscure.

The data reported in this paper show quite clearly that as chloroplast development proceeds the ability of the leaf to utilize white light of moderate intensity (compared to high intensity white light) and to utilize green light (compared to red light) both increase very markedly. The curves in Figure 1 show a number of points of interest. The first is that after short periods of greening there appears to be a threshold light intensity above which oxygen evolution is readily detectable and below which it appears to be absent. This should not be confused with the compensation point. In the apparatus used here the signal at any moment corresponds to the rate at which oxygen molecules are reaching the electrode. In the dark this rate is equal to the rate of diffusion of oxygen from the flowing medium, through the dialysis membranes and leaf tissue, minus that component which is taken up in respiration as the oxygen passes through the tissue. This means that even a relatively low rate of photosynthetic oxygen evolution, which does not exceed respiratory oxygen uptake, will increase the signal because it decreases the net amount of oxygen taken up by the tissue and therefore allows an increased rate of oxygen flow to the electrode. Consequently, if all intensities of light can produce oxygen evolution by the chloroplasts, approximately in proportion to the intensity used, the curve of oxygen evolution versus light intensity as measured with the present apparatus, should increase more or less linearly, from the origin. The results therefore suggest that chloroplasts in the early stages of greening require a certain minimum light intensity before they can evolve oxygen. This effect might possibly be connected with the two-dimensional distribution of chlorophyll molecules on the thylakoid membranes in the early stages of chloroplast development. The etioplast already contains a substantial area of membrane, which to begin with exists as the tubules of the prolamellar body. During

the first 2 hr or so of greening most of the membrane material is reorganized into a number of large, single thylakoids (Gunning and Jagoe 1967). This means that early on in development, the average concentration of chlorophyll per unit area of membrane is likely to be much lower than that achieved later on, which in turn may mean that the number of chlorophyll molecules per photosynthetic unit is low. Eight quanta are required for the liberation of one molecule of oxygen and the reduction of two molecules of NADP. It may be that for a molecule of oxygen to be evolved, these eight quanta have to be absorbed by the photosynthetic unit within a certain minimum period of time. One possible mechanism is that unless the full quota of photons is received within this minimum time, the labile transitory intermediates may decay before liberation of the oxygen molecule is completed. An alternative or additional mechanism is that hydrogen atoms are withdrawn from water but that the reduced components of photosystem II are rapidly reoxidized by molecular oxygen before they can be taken up by photosystem I: this would result in there being no net release of oxygen. Some recent studies by Thorne and Boardman (1971*a*) on the effects of oxygen on the fluorescence kinetics of spinach chloroplasts suggest that reduced intermediates in photosystem II can indeed readily be reoxidized under some circumstances. Whatever the detailed mechanism, it seems possible that at low chlorophyll concentration (particularly if the chlorophyll molecules are widely separated from one another so that intermolecular energy transfer is reduced) the rate at which quanta are absorbed and reach the reaction centres may simply be below the minimum rate required to achieve oxygen evolution. As the number of chlorophyll molecules per photosynthetic unit increases, or as the quantal flux (light intensity) is raised, more photons are absorbed and a stage will be reached at which quanta are arriving at the reaction centres at the requisite minimum rate: from this point on oxygen evolution will appear and will increase as the pigment concentration rises, or the light intensity is further increased.

Another interesting feature of the data in Figure 1 is the relatively small difference between the rates of photosynthesis at saturating light intensities achieved by leaves greened for different periods. This suggests that in the case of wheat the enzymes of the dark reactions of photosynthesis (or at least those which are rate-limiting), and also the actual numbers of photosynthetic units, have reached more than half their final levels in leaves at very early stages of greening. It is interesting in this connection that Wilstätter and Stoll (1918, as quoted by Smith 1949) observed that in etiolated bean leaves, early in the greening process, the photosynthetic rate (per unit fresh weight) at high light intensity (4500 f.c.) was 40–50% of that of fully green leaves, even though their chlorophyll content was only about 5% of the final level. A similar situation can be brought about by mutation: a yellow variety of *Sambucus* (having 5–10% of the normal chlorophyll content) was found to have a much lower photosynthetic rate (per unit fresh weight) than the green variety at low light intensities, but at high intensity the photosynthetic rate of the yellow approached that of the green (Wilstätter and Stoll 1918, as quoted by Rabinowitch 1956). A yellow mutant of tobacco was found to give a lower rate of photosynthesis (per unit area) than the wild type at low light intensity, but gave an even higher rate than the wild type at high intensity (Schmid and Gaffron 1967).

The low rates of photosynthesis found at moderate light intensities in leaves greened for short times, and also in young ivy leaves, indicate that in such leaves it is the light-gathering systems which are rate-limiting: it cannot simply be the electron carriers because these clearly work very much faster when the light intensity is increased. The threshold effect, discussed above, does not account for this rate limitation: indeed in Figure 1 we can, in effect, correct for this phenomenon by simply moving the 2- and 5-hr curves to the left so that they pass through the origin—the light reactions are still rate-limiting at the intermediate light intensities.

The most plausible explanation would seem to be the obvious one that at the lower light intensities the chloroplasts with low chlorophyll levels absorb relatively few quanta (per unit time) and so supply electrons to the electron-transfer and CO_2-fixation systems at a rate much lower than the maximum these systems are capable of handling. The very marked increase in the rate of photosynthesis at the lower light intensities during chloroplast development is no doubt due to the increase in the proportion of incident quanta absorbed by the chloroplast as the chlorophyll concentration rises. This increase in the number of quanta absorbed has two aspects. One is simply the increase in the height of the red and blue absorption peaks as the chlorophyll concentration increases. The other is a disproportionate increase in the absorption of light in that spectral band which lies between the blue and red peaks. Approximate calculations (Table 4) show that the chlorophyll concentration in the ivy leaves are in the range in which changes in chlorophyll level can be expected to produce big changes in the green/red absorption ratio (it can be shown that at much lower levels of chlorophyll this ratio would be relatively insensitive to changes in chlorophyll content). The same is likely to be true of the wheat leaves. Direct spectroscopic measurements on the intact ivy leaves (Table 4) demonstrate that there is indeed a substantial increase in the green/red absorption ratio as the chlorophyll content rises which is of much the same magnitude as the increase in photosynthetic effectiveness of green light relative to red (Table 2). A small contribution to this increase in green/red absorption ratio is made by the increase in the proportion of the chlorophyll which is chlorophyll *b*. The bunching effect, however, as we have seen, does not contribute to the increase in the absorption ratio during leaf maturation: in fact, it appears to cause a slight diminution in the ratio. In short, we believe that the leaves with a higher chlorophyll content photosynthesize in moderate white light intensities much more rapidly than the paler leaves, mainly because they are so much better at absorbing the green and yellow components of the white light, these being largely wasted by the leaves with low chlorophyll content. There does not seem to be any necessity in the present work to postulate, as Lundegardh (1969) did, a direct excitation of the cytochromes, in order to explain the high photosynthetic effectiveness of green light.

One remaining puzzle is that the measured values of percentage absorption, particularly of green light, are so much higher than those expected on the basis of the chlorophyll content (Table 4). The simplest and most plausible explanation is that this is a consequence of the scattering of the light beam within the leaf tissue: this will increase the effective path length of the beam through the tissue, and so will increase the average number of chloroplasts traversed by the beam. Since this is

equivalent to an increase in the concentration of chloroplasts, and therefore of chlorophyll, in the tissue, this effect should increase the green/red absorption ratio still further.

V. References

Biggins, J., and Park, R. B. (1966).—CO_2 assimilation by etiolated *Hordeum vulgare* seedlings during the onset of photosynthesis. *Pl. Physiol., Lancaster* **41**, 115–18.

Blinks, L. R., and Skow, R. K. (1938).—The time course of photosynthesis as shown by a rapid electrode method for oxygen. *Proc. natn. Acad. Sci. U.S.A.* **24**, 420–27.

Bonner, W. D. (1961).—The cytochromes of plant tissues. In "Haematin Enzymes". (Eds. J. E. Falk, R. Lemberg, and R. K. Morton.) Pt. 2. pp. 479–97. (Pergamon Press: Oxford.)

Bradbeer, J. W. (1969).—The activities of the photosynthetic carbon cycle enzymes of greening bean leaves. *New Phytol.* **68**, 233–45.

Duysens, L. N. M. (1956).—The flattening of the absorption spectrum of suspensions as compared to that of solutions. *Biochim. Biophys. Acta* **19**, 1–12.

Egneus, H. (1967).—Primary photosynthetic induction phenomena in wheat. *Physiologia Pl.* **20**, 463–76.

Egneus, H. (1968).—Action spectra for the transient and the normal photosynthetic oxygen evolution in wheat leaves. *Physiologia Pl.* **21**, 602–14.

French, C. S. (1960).—The chlorophylls *in vivo* and *in vitro*. In "Encyclopaedia of Plant Physiology". (Ed. W. Ruhland.) Vol. V. Pt. 1. pp. 252–97. (Springer-Verlag: Berlin.)

Gabrielsen, E. K. (1948).—Effects of different chlorophyll concentrations on photosynthesis in foliage leaves. *Physiologia Pl.* **1**, 5–37.

Gabrielsen, E. K., Madsen, A., and Vejlby, K. (1961).—Induction of photosynthesis in etiolated leaves. *Physiologia Pl.* **14**, 98–110.

Govindjee, and Govindjee, R. (1964).—Induction transients in O_2 evolution by *Porphyridium cruentum* in monochromatic light. *Yb. Carnegie Instn Wash.* **63**, 468–72.

Gunning, B. E. S., and Jagoe, M. P. (1967).—The prolamellar body. In "Biochemistry of Chloroplasts". (Ed. T. W. Goodwin.) Vol. II. pp. 655–75. (Academic Press: London.)

Haxo, F. T., and Blinks, L. R. (1950).—Photosynthetic action spectra of marine algae. *J. gen. Physiol.* **33**, 389–422.

Inman, O. L. (1935).—Formation of chlorophyll and the beginning of photosynthesis. *Pl. Physiol., Lancaster* **10**, 401–3.

Kirk, J. T. O. (1968).—Studies on the dependence of chlorophyll synthesis on protein synthesis in *Euglena gracilis*, together with a nomogram for determination of chlorophyll concentration. *Planta* **78**, 200–7.

Kirk, J. T. O., and Reade, J. A. (1970).—The action spectrum of photosynthesis in *Euglena gracilis* at different stages of chloroplast development. *Aust. J. biol. Sci.* **23**, 33–41.

Kirk, J. T. O., and Tilney-Bassett, R. A. E. (1967).—"The Plastids." 608 pp. (W. H. Freeman and Co: London and San Francisco.)

Kouchkovsky, Y. de (1964).—Kinetics of enhancement. *Yb. Carnegie Instn Wash.* **63**, 447–53.

Litvin, F. F., Ho I-t'an, and Efimtsev, E. I. (1965).—A polarographic method for studying the kinetics and action spectra of photosynthesis. *Fiziol. Rast.* **12**, 313–19. (Consultants Bureau translation.)

Litvin, F. F., and Ho I-t'an (1967).—Action spectrum of photosynthesis, the Emerson and induction phenomena in higher plants. *Fiziol. Rast.* **14**, 186–94. (Consultants Bureau translation.)

Lundegardh, H. (1969).—Relative quantum efficiency of photosynthetic oxygen production in chloroplasts of spinach. *Nature, Lond.* **221**, 280–1.

MacKinney, G. (1941).—Absorption of light by chlorophyll solutions. *J. biol. Chem.* **140**, 315–23.

Menke, W. (1966).—In "Biochemistry of Chloroplasts". (Ed. T. W. Goodwin.) Vol. 1. pp. 3–18. (Academic Press: London.)

Rabinowitch, E. I. (1956).—"Photosynthesis." Vol. II. Pt. 2. (Interscience: New York.)

RHODES, M. J. C., and YEMM, E. W. (1966).—The development of chloroplasts and photosynthetic activities in young barley leaves. *New Phytol.* **65**, 331–42.

SCHIFF, J. A. (1963).—Oxygen exchange by *Euglena* cells undergoing chloroplast development. *Yb. Carnegie Instn Wash.* **62**, 375–8.

SCHMID, G., and GAFFRON, H. (1967).—Chloroplast structure and the photosynthetic unit. *Brookhaven Symp. Biol.* **19**, 380–92.

SMITH, J. H. C. (1949).—The relationship of plant pigments to photosynthesis. *J. Chem. Ed.* **26**, 631–8.

SMITH, J. H. C. (1954).—The development of chlorophyll and oxygen-evolving power in etiolated barley leaves when illuminated. *Pl. Physiol., Lancaster* **29**, 143–48.

THOMAS, J. B., MINNAERT, K., and ELBERS, P. F. (1956).—Chlorophyll concentrations in plastids of different groups of plants. *Acta bot. neerl.* **5**, 315–21.

THORNE, S. W., and BOARDMAN, N. K. (1971*a*).—The effect of temperature on the fluorescence kinetics of spinach chloroplasts. *Biochim. Biophys. Acta* **234**, 113–25.

THORNE, S. W., and BOARDMAN, N. K. (1971*b*).—Formation of chlorophyll *b* and the fluorescence properties and photochemical activities of isolated plastids from greening pea seedlings. *Pl. Physiol. Lancaster* **47**, 252–61.

TOLBERT, N. E., and GAILEY, F. B. (1955).—Carbon dioxide fixation by etiolated plants after exposure to white light. *Pl. Physiol., Lancaster* **30**, 491–9.

VEEN, R. VAN DER (1960).—Induction phenomena. In "Encyclopaedia of Plant Physiology". (Ed. W. Ruhland.) Vol. V. Pt. 1. pp. 675–88. (Springer-Verlag: Berlin.)

VIDAVER, W. (1963).—Effects of hydrostatic pressure on induction transients of oxygen evolution. In "Photosynthesis Mechanisms in Green Plants". pp. 726–32. [Publ. 1145, Natl. Acad. Sci.–Nat. Res. Council.]

VIDAVER, W., and FRENCH, C. S. (1965).—Oxygen uptake and evolution following monochromatic flashes in *Ulva* and an action spectrum for system I. *Pl. Physiol., Lancaster* **40**, 7–12.

APPENDIX I

APPLICATION OF POLAROGRAPHY TO MEASUREMENT OF PHOTOSYNTHESIS BY LEAF TISSUE

Although the first application of polarography to measurement of photosynthetic oxygen evolution made use of a higher plant leaf as the experimental material (Blinks and Skow 1938), the method has, in the intervening years, been applied to the study of algal photosynthesis much more commonly than to photosynthesis by leaves of terrestrial higher plants. Apart from the early work of Blinks and Skow, the present writer is aware of only two other studies on terrestrial higher plant photosynthesis utilizing polarography: those of Litvin and co-workers on bean and rice leaves (Litvin, Ho I-t'an, and Efimtsev 1965; Litvin and Ho I-t'an 1967), and of Egneus (1967, 1968) on wheat leaves. By contrast, innumerable papers have appeared describing polarographic investigations of algal photosynthesis. In view of the advantages of polarographic measurements with a small platinum electrode—rapid response permitting observation of fast induction transients, small area of tissue required permitting the use of narrow, highly monochromatic beams of actinic light—it may be wondered why it has hardly ever been applied to studies of photosynthesis in higher plants. One possible reason is that it is necessary when using this technique to immerse the tissue or cells in an aqueous medium, and workers may well have been reluctant to study photosynthesis of higher plants under conditions which are rather

unnatural for these organisms. Another possible reason is that the response of terrestrial higher plant tissue on a platinum electrode to actinic light is much slower than the response of a layer of algal cells: this sluggishness has been attributed to the slow diffusion of oxygen from the leaf (Litvin, Ho I-t'an, and Efimtsev 1965).

Despite these drawbacks it seemed possible that the polarographic technique might yield useful information about photosynthesis in terrestrial higher plants. Accordingly, an attempt has been made to adapt the polarographic technique for the study of photosynthesis in higher plant leaves, and in particular to increase the speed of the response. Some of the characteristics of the experimental system devised are described.

The polarographic apparatus used was the bare platinum electrode system, of a modified Haxo and Blinks type, described previously (Kirk and Reade 1970). The following slight alterations to the arrangements were made. The electrode system was immersed centrally in a 100-ml beaker, instead of the 50-ml beaker previously used. Instead of the medium previously described, the beaker contained 75 ml of a medium with the composition 10 mM KCl, 2 mM $KHCO_3$, 1mM KH_2PO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, pH 7·4–7·5. The circulation of medium through an external reservoir by means of a siphon and a pump was dispensed with: instead, a teflon-covered flea, 1 in. long, was placed at the bottom of the beaker and driven at about 200 cycles per minute by a magnetic stirrer underneath (this stirrer should have a grounded metal casing to avoid generating electrical noise in the recorder). The stirring produces a steady, circular movement of medium around the electrode system: this ensures that the medium remains in equilibrium with atmospheric oxygen, and also that any exchange of oxygen between the medium and the tissue (held flat against the 6·5 mm square platinum electrode by a length of dialysis tubing and elastic bands as previously described) takes place steadily and continuously.

With this polarographic system, the recorder signal at any time in the light minus the steady-state dark signal ($V_L - V_D$, or ΔV) is approximately porportional to the rate of photosynthesis. The reasoning on which this conclusion is based follows that of Haxo and Blinks (1950) and is given in the earlier paper (Kirk and Reade 1970). It should be noted that the steady-state dark signal corresponds to the point at which the consumption of oxygen by the electrode and by tissue respiration becomes equal to the rate at which oxygen is diffusing through the membrane and the tissue from the flowing medium. Use of the bare platinum electrode for direct rate measurement in this work, as in the work of Haxo and Blinks (1950) and Litvin and co-workers (1965, 1967), is to be distinguished from the use of a teflon-covered electrode (as in the work of Egneus 1967), which measures oxygen concentration in the medium.

Except where otherwise stated wheat (cv. Olympic), pea (cv. Greenfeast), and cucumber (cv. Marketer) seedlings were grown in vermiculite watered with nutrient solution for 1–2 weeks, on a south-facing window sill (consequently receiving ample diffuse daylight but not direct sunlight). In a few experiments wheat or cucumber seedlings were grown for 7–9 days at 23–25°C in the dark, the excised shoots were stood with their cut ends in water in 50-ml Erlenmeyer flasks, and illuminated with fluorescent light at an intensity of 750 f.c. and a temperature of 23–27°C for varying

periods. Photosynthesis measurements were carried out in an air-conditioned dark room, maintained at a temperature of 23–25°C.

An untreated piece of wheat leaf placed with either its upper or its lower surface in contact with the platinum electrode shows only a very small and slow oxygen evolution when illuminated [Fig. 7(*a*)]. It seemed possible that the sluggishness of the response might be due to the slowness of the diffusion of oxygen evolved photosynthetically in the mesophyll and palisade cells through the stomata and the cuticle to the electrode or slowness of diffusion of CO_2/HCO_3^- from the medium to the cells or both. It was reasoned that physical destruction of the diffusion barriers by making holes or cuts in the epidermis should greatly facilitate diffusion. A number of methods of treating the leaf surface were tried, including making pinholes in a square pattern at 1-mm intervals, scoring parallel lines at 1-mm intervals, and gently scraping with a razor-blade. Although all these methods worked, the simplest and most satisfactory procedure was found to be very gentle abrasion of the lower or upper leaf surface with fine emery paper (Primex abrasive paper, No. 400) moistened with medium. For most types of leaf up to six gentle strokes were found to be sufficient: however, this is likely to be a matter of trial and error for each experimenter. A sufficient degree of abrasion can usually be recognized by the leaf's taking on a darker green, rather wet, appearance. The treated piece of leaf is then placed on the electrode with the abraded surface in contact with the platinum. After abrasion the wheat leaf shows a much larger response on illumination [Fig. 7(*b*)].

Fig. 7

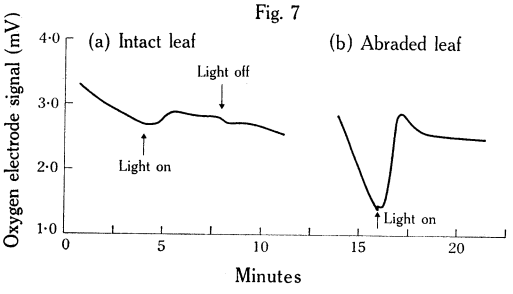


Fig. 7.—Effect of abrasion of leaf surface on apparent photosynthetic rate as detected by the oxygen electrode. (*a*) A piece of wheat leaf was placed with its upper surface in contact with the electrode. (*b*) The leaf segment was removed, abraded gently on its upper surface with emery paper, and replaced with the abraded surface in contact with the electrode.

With leaves of pea seedlings the results are rather more variable. In some cases the untreated leaf shows very little activity but develops a high level of activity on abrasion. In other cases, the leaf already has substantial activity, but this is markedly increased, and the rate at which it responds to light speeded up, by abrasion. The reason for the variability of the untreated pea leaves is not known. It seems possible that in the case of a very soft tissue such as a young pea leaflet, the surface may sometimes receive sufficient damage in the course of handling and placing on the electrode to give a substantial response without deliberate abrasion.

Activity has also been obtained with untreated cucumber cotyledons placed with their upper surfaces in contact with the platinum: however, in this case too the speed and extent of the response is markedly increased by abrasion of the surface. The photosynthetic activity of the abraded leaf sections immersed in medium is surprisingly stable. A given piece of treated leaf shows only slight diminution of

activity throughout the day, and about half or more of the activity can survive immersion overnight.

The induction phenomena observed when this polarographic technique is applied to leaf tissue are very much the same as those which have been described in other photosynthetic systems, both higher plants (Blinks and Skow 1938; van der

Fig. 8

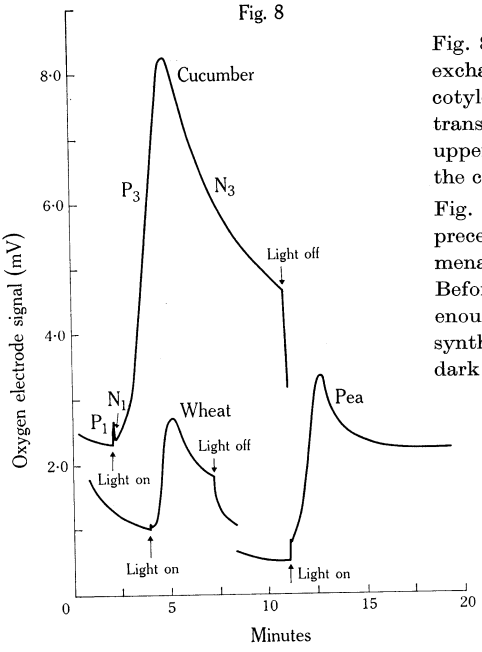
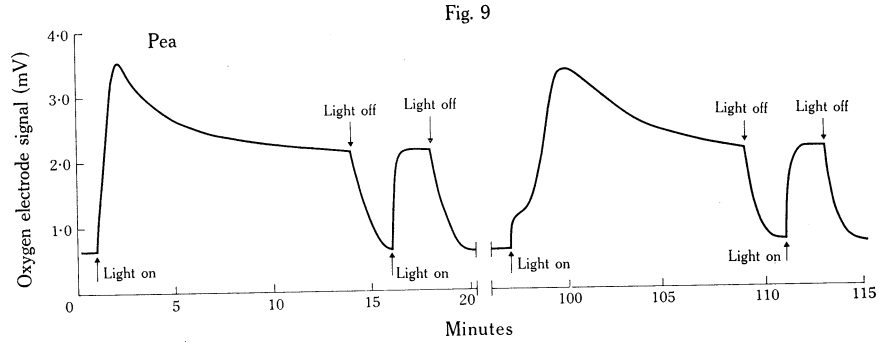


Fig. 8.—Time courses of photosynthetic oxygen exchange in wheat and pea leaf, and cucumber cotyledon, showing the P_1, N_1, P_3, and N_3 transients. The wheat leaf was abraded on the upper surface, the pea leaf on the lower surface, the cucumber cotyledon on both surfaces.

Fig. 9.—Effect of varying the length of the preceding dark period, on the induction phenomena. Pea leaf abraded on lower surface. Before time 0 the leaf was illuminated for long enough to reach the steady state of photosynthesis, and this was followed by a 20-min dark period.

Fig. 9



Veen 1960) and algae (Blinks as quoted by Vidaver 1963; Vidaver 1963; Govindjee and Govindjee 1964; de Kouchkovsky 1964). A typical sequence of events is that immediately on illumination there is a small, very rapid, but transient (lasting 1 or 2 s) evolution of oxygen, variously called the oxygen "gush", or "pre-*a*" transient. To systematize the nomenclature of the various transients of oxygen exchange, in the present paper the letter P is used for positive transients, and the letter N for negative transients.

The oxygen gush is therefore referred to as P_1. The oxygen gush is almost immediately overtaken by an equally rapid oxygen uptake (Vidaver and French 1965), which we shall refer to as N_1. Usually the N_1 transient is followed by a relatively rapid rise in the rate of photosynthesis to a peak, the "*a* spike" in the nomenclature of Vidaver (1963), which is reached after $\frac{1}{4}$–1 min of illumination. The *a* spike is followed by a relatively fast decrease in rate (Vidaver's "*b* downslope"). The *a* spike and *b* downslope are here referred to as transients P_3 and N_3 (the symbols P_2 and N_2 are reserved for another pair of transients which under some conditions show up before the *a* spike—see later). With the abraded-leaf system, N_3 is generally followed by a levelling-off to give the steady state of photosynthesis. In the case of algae (Vidaver 1963) the *b* downslope was commonly followed by a slow rise (the "*c* rise") before the steady state was finally achieved. This rise is only rarely observed with abraded leaves; it is given the symbol P_4. Figures 7(*b*) and 8 show examples of the sequence of induction transients typically obtained with higher plant leaves. The P_1 and N_1 transients are not always seen as a distinct spike in the trace because the P_3 transient often commences early enough to hide them (Fig. 9). The height of the *a* spike, or P_3 transient, increases as the time in the dark before illumination increases, but increasing the length of the dark period beyond 15–20 min causes little further increase in the size of the transient. In the case of pea leaf (Fig. 9), if a period of illumination is interrupted with a dark period as short as 1–2 min, then on re-illumination the photosynthetic rate rapidly rises to the steady-state value again within 1 min or less, and levels off with little or no overshoot. Increasing the length of this dark period to 10–15 min or more will increase the height of the subsequent P_3 transient, so that once again it overshoots the steady-state level: a P_1 transient may in addition become detectable (Fig. 9).

Blinks and Skow (1938) observed a distinct lag period lasting up to 1 min in which the signal fell to the dark level, after the oxygen gush and before the P_3 transient, in a *Ricinus* leaf. This has not been observed in leaves grown on a normal light–dark regime in the present work. However, a lag phase is often, but not invariably, observed with wheat leaves or cucumber cotyledons grown in the dark and then allowed to green up for 24–30 hr in the light. The lag phase may be up to about 1 min long in the case of wheat (Fig. 10) and 3 min in the case of cucumber. Even longer lag phases, up to about 4 and 6 min, respectively, are often observed in leaves and cotyledons at earlier stages of greening (3–7 hr of illumination).

Egneus (1967), using wheat leaves, observed a small peak of oxygen evolution to occur between the oxygen gush and the P_3 transient. This has not been observed in the present work in leaves grown on a light–dark regime, but is sometimes, although rarely, seen in leaves grown in the dark and greened up in the light (Fig. 11). This peak consists of a positive, followed by a negative, transient to which we give the symbols, P_2 and N_2, respectively.

In this series of experiments the technique of gentle abrasion of the leaf surface to facilitate diffusion in and out of the tissue has proved to be of considerable value in ensuring the polarographic detection of photosynthetic oxygen evolution in terrestrial plant leaf material. Without such treatment the very rapid oxygen gush, or P_1 transient, is undetectable, and the main (P_3) oxygen evolution transient is severely damped. The ease of detection of photosynthetic activity in pieces of abraded leaf

immersed in our medium (rates tend to be as high as or higher than those given by aquatic plants such as *Elodea* or *Ulva*) on the platinum electrode, the relative stability of the activity during the day, and the overall similarity of the pattern of induction phenomena to those observed by other workers in other green plants encourage the belief that the polarographic technique used in the manner described here may indeed be a useful tool for studying photosynthesis in leaves of terrestrial plants.

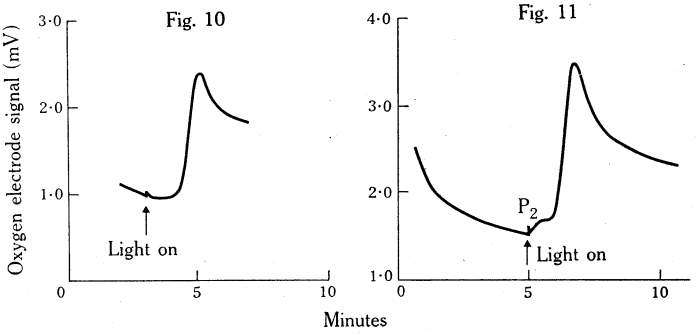


Fig. 10.—A time course of oxygen evolution showing a distinct lag phase. The primary leaf of a dark-grown wheat seedling was exposed to light for 31 hr; a segment of the leaf, abraded on the upper surface, was used for measurements of photosynthesis.

Fig. 11.—Time course of photosynthetic oxygen exchange in wheat leaf, showing the P_2 and N_2 transients. The primary leaf of a dark-grown wheat seedling was exposed to light for 24 hr: a segment of the leaf, abraded on the upper surface, was used for measurements of photosynthesis. The N_2 transient shows only as a shoulder following the P_2 transient.

Appendix II

Calculation of the light absorption properties of a stack of thylakoids

It is intended to derive an expression relating the light-absorption properties of a stack of thylakoids to the number of thylakoids in the stack. It is assumed that the pigments in the stack obey Beer's law. The light beam, the absorption of which is being considered, is at right angles to the plane of the thylakoids. There are n thylakoids in the stack and the stack is d cm thick. The extinction at any particular wavelength is given by

$$E = \alpha dc, \tag{1}$$

where α is the specific absorption coefficient (litre/g/cm) of chlorophyll at that wavelength and c is the average concentration in grams per litre of chlorophyll thoughout the volume occupied by the stack of thylakoids.

Let us assume that the area of thylakoid membrane occupied by each chlorophyll molecule is A Å^2, i.e. $A \times 10^{-16}$ cm^2. It follows that the number of chlorophyll molecules per square centimetre of a single thylakoid membrane is $10^{16}/A$. Each thylakoid consists of two such membranes separated by a loculus, therefore the number of chlorophyll molecules per square centimetre of thylakoid is $2.10^{16}/A$.

Assuming a chlorophyll *a*/chlorophyll *b* ratio of 3·0, the average molecular weight is 897. The weight W in grams of chlorophyll per square centimetre of thylakoid is $[(2.10^{16}/A)\times 897/(6.02\times 10^{23})]$. Since there are n thylakoids in the stack, the weight of chlorophyll per square centimetre of stack is nW. The volume of the thylakoid stack per square centimetre of cross-sectional area is d cm^3, i.e. $10^{-3}d$ litres. Therefore the average concentration, c, of chlorophyll throughout the thylakoid stack is given by $10^3\, nW/d$ which equals $0{\cdot}0298n/Ad$ g/l. The extinction may now be calculated by substituting this expression for c into equation (1):

$$E = \alpha d(0{\cdot}0298n/Ad) = 0{\cdot}0298\alpha n/A. \tag{2}$$

Appendix III

Effect of the "bunching" or "sieve" phenomenon on the photosynthetic action spectrum of a leaf

Duysens (1956) and Rabinowitch (1956) showed that for a suspension of pigmented, cubical particles the following relationship holds:

$$E_{\text{sus.}}/E_{\text{sol.}} = (1-t)/\ln(1/t), \tag{3}$$

where $E_{\text{sus.}}$ and $E_{\text{sol.}}$ are the extinction values, at some given wavelength, for the suspension and solution, respectively, and t is the transmission (as a proportion of 1·0) of the light by a single particle. These authors pointed out that this diminution of the extinction values will increase as the absorption of the individual particles is increased, i.e. the lowering effect is greater at the peaks than at the troughs, and so the shape of the spectrum becomes flattened. That this will be a consequence of the "bunching effect", or "sieve effect" (Rabinowitch uses both terms) may perhaps be more readily appreciated if equation (3) is modified slightly:

$$E_{\text{sus.}}/E_{\text{sol.}} = (1-t)/\ln(1/t) = (1-t)/-\ln t = a/-\ln(1-a), \tag{4}$$

where $a = (1-t)$ and is the absorption (as a proportion of 1·0) of a single particle. This may be expressed in the form:

$$E_{\text{sus.}}/E_{\text{sol.}} = a/[a+\tfrac{1}{2}a^2+\tfrac{1}{3}a^3+\ldots] = 1/[1+\tfrac{1}{2}a+\tfrac{1}{3}a^2+\ldots]. \tag{5}$$

From equation (5) it can immediately be seen that $E_{\text{sus.}}$ is less than $E_{\text{sol.}}$ and that the ratio falls as the value of a increases.

To determine to what extent the bunching effect will alter the action spectrum it is necessary to determine in what way it will alter the percentage absorption of the suspension at different wavelengths. The percentage transmission of the system is

$$T_{\text{sus.}} = 100.10\exp(-E_{\text{sus.}}).$$

The percentage absorption of the system is

$$A_{\text{sus.}} = 100-T_{\text{sus.}} = 100[1-10\exp(-E_{\text{sus.}})].$$

From equation (4) it follows that

$$A_{\text{sus.}} = 100\Big(1-10\exp\{[a/\ln(1-a)].E_{\text{sol.}}\}\Big). \tag{6}$$

For the equivalent amount of pigment in solution

$$A_{\text{sol.}} = 100[1-10\exp(-E_{\text{sol.}})]. \tag{7}$$

Therefore

$$\frac{A_{\text{sus.}}}{A_{\text{sol.}}} = \frac{1-10\exp\{[a/\ln(1-a)].E_{\text{sol.}}\}}{1-10\exp(-E_{\text{sol.}})}. \tag{8}$$

For a given suspension, equation (8) may be used to calculate the degree of distortion of the percentage absorption spectrum, or action spectrum, at any wavelength. $A_{\text{sus.}}$ is always less than $A_{\text{sol.}}$, i.e. the bunching effect lowers the percentage absorption just as it lowers the extinction. However, it should be noted that whereas the extent of flattening of the extinction spectrum ($E_{\text{sus.}}/E_{\text{sol.}}$) is a function only of the absorption per particle, a, the extent of flattening of the percentage absorption spectrum ($A_{\text{sus.}}/A_{\text{sol.}}$) is a function not only of a, but also of $E_{\text{sol.}}$. That is, $A_{\text{sus.}}/A_{\text{sol.}}$, unlike $E_{\text{sus.}}/E_{\text{sol.}}$, depends not only on the absorption properties of single particles but also on the overall concentration of pigment, and therefore of particles, in the system. This means that in order to predict the consequences of the bunching effect on the percentage absorption, and action, spectra, mere inspection of equation (8) is not enough: it is necessary to work the results out in full.

In order to determine to what extent the bunching effect will modify the green/red absorption ratio of a leaf at different stages of greening we shall use equations (6) and (7) to calculate the percentage absorptions of green and red light for the leaf (regarded as equivalent to a suspension of pigment-bearing particles—the chloroplasts), and for the corresponding amount of pigment in free solution (but the same concentration per unit area). We shall assume that the fully green leaf contains 6 mg chlorophyll/dm^2. Gabrielsen (1948) showed that increasing the chlorophyll concentration above this causes little increase in the amount of energy absorbed: furthermore, his value for the chlorophyll concentration in a fully green wheat leaf—6·7 mg/dm^2—is close to this. Assuming that the chlorophylls in the leaf have the same specific absorption coefficients in the green (550 nm) and the red (676 nm *in vivo*, 662 nm *in vitro*) as they do in ether solution, and that the chlorophyll *a*/chlorophyll *b* ratio is 3·0, then we may calculate that, in the fully green leaf, $E_{\text{sol.}}$ is 0·257 at 550 nm and 4·638 at 676 nm; $A_{\text{sol.}}$ is 44·7% at 550 nm and 100·0% at 676 nm. At any intermediate stage of development, when the overall chlorophyll concentration has reached some given fraction of the final value, the appropriate values of $E_{\text{sol.}}$ can be obtained by proportion, and the corresponding values of $A_{\text{sol.}}$ can then be calculated.

In order to simplify the calculation of a at different stages of development we shall make the simplifying assumption that there is little change in size and number of the plastids during greening. This means that the extinction value of a chloroplast at any stage, when the overall chlorophyll concentration has reached some given fraction of the final value, can be calculated as the appropriate proportion of the extinction value of the mature chloroplast. The value of a may then be calculated from the extinction. Values of extinction of the right order of magnitude for a mature chloroplast may be derived from a consideration of the known dimensions and crude chemical composition of chloroplasts. These plastids, in higher plant leaves, are typically lens-shaped bodies with diameter commonly in the region of 5 μm and about 2 μm thick: we shall therefore regard the chloroplast as being approximately

equivalent to a shallow cylinder, 5 μm in diameter, 2 μm high. We shall assume that it has a dry weight of $24{\cdot}6\times10^{-12}$ g (Nickel 1966 as quoted by Menke 1966) and a chlorophyll content which is 5% of the dry weight (Kirk and Tilney-Bassett 1967). It may now be calculated that for a light beam traversing the chloroplast at right angles to its plane, the extinction values are 0·0268 at 550 nm and 0·484 at 676 nm. This means that the light absorption (as a proportion of 1·0) of the mature chloroplast is 0·06 at 550 nm and 0·672 at 676 nm. These results may be compared with the corresponding values of 0·071 and 0·719, derived for mature ivy chloroplasts on the basis of structural considerations in Section III(h).

We are now in a position to calculate a and $E_{\mathrm{sol.}}$ for green and red light at any intermediate stage of greening: from the values so obtained we can, using equations (6) and (7), derive the appropriate values of $A_{\mathrm{sus.}}$ and $A_{\mathrm{sol.}}$. Table 5 lists the results of such calculations for greening leaves the overall chlorophyll concentration of which varies from 5 to 100% of the final value.

Equation (6) has also been used to calculate the percentage absorption values of young and mature ivy leaves in green and red light.