THE INHIBITION OF PHOTOSYNTHESIS BY OXYGEN

I. COMPARATIVE PHYSIOLOGY OF THE EFFECT

By J. S. TURNER,* MARY TODD,* and E. G. BRITTAIN*

[*Manuscript received July* 23, 1956]

Summary

(i) A comparative summary is presented of new and published data on the inhibition of photosynthesis by high oxygen concentration.

(ii) An oxygen inhibition of photosynthesis is demonstrated for 25 species, representing the following major plant groups: Angiospermae—land and aquatic forms, both monocotyledons and dicotyledons, Filicales, Bryophyta, Chlorophyceae, Phaeophyceae, Rhodophyceae.

(iii) The inhibition is rapidly produced and equally rapidly reversible in all the species so investigated.

(iv) In the great majority of experiments at high light intensity and high CO_2 concentration the inhibition is not significant until the oxygen concentration exceeds 20 per cent. The effect is absent or small between 0 and 20 per cent., large and variable between 20 and 100 per cent.

(v) The inhibitory effect of oxygen on photosynthesis cannot be accounted for by a higher rate of dark respiration accompanying photosynthesis at the higher oxygen concentrations.

(vi) In two angiosperms, a reduced rate of photosynthesis resulted on transfer of the tissue from 20 to 5 per cent. oxygen. This could have been due to stomatal control of the process, but requires further investigation.

I. INTRODUCTION

Although oxygen is a product of the photosynthetic reaction, there has been comparatively little study of the effect of the partial pressure of oxygen on the rate of the reaction. When the present work was initiated, the major publication on this aspect of photosynthesis was that of Warburg (1920). He demonstrated for *Chlorella* a well-marked depression in the rate of photosynthesis when the external gas phase was changed from 2 to 100 per cent. oxygen. Similar oxygen inhibition in *Chlorella* was described 18 years later by Wassink *et al.* (1938). It was also described in the brown alga *Nereocystis* (Surbeck, Holt, and Lund 1925), in *Scenedesmus* (Gaffron 1939, 1940), and in the wheat leaf (McAlister and Myers 1940). Moyse (1953) reported unusual effects of oxygen on the photosynthesis of a crassulacean, *Bryophyllum*. The first thorough study of the typical oxygen inhibition of photosynthesis was that of Tamiya and Huzisige (1949) who worked with *Chlorella ellipsoidea*, but it is to be noted that their conclusions on the mechanism of the effect have been radically altered as a result of further work on the effect of oxygen on the CO_2-fixing capacity of *Chlorella* cells (Miyachi, Izawa, and Tamiya 1955). In the present paper we summarize the published results and relate them to new data gathered over a period of years, in order to show the very widespread occurrence of the effect in the plant kingdom, its extent, and its reversibility.

* Botany School, University of Melbourne.

The oxygen effect dealt with here apparently bears no relation to the effect of the complete lack of oxygen on photosynthesis. There is evidence (Gaffron 1940, Hill and Whittingham 1953) that even a short dark period of complete anaerobiosis may suppress photosynthesis, alter the lag phase, or change the assimilatory quotient.

We use the following symbols and conventions:

rP = rate of real photosynthesis,
aP = rate of apparent photosynthesis,
Rd = rate of dark respiration.

The concentrations of CO_2 and oxygen quoted are those at the beginning of an experimental period. Unless otherwise stated, percentage inhibition figures are based on the rate in the lower or lowest oxygen concentration applied, e.g.

$$\text{Percentage inhibition (real)} = \{(rP_{N_2} - rP_{O_2})/rP_{N_2}\} \times 100,$$

and

$$\text{Percentage inhibition (apparent)} = \{(aP_{air} - aP_{O_2})/aP_{air}\} \times 100.$$

II. Oxygen Effects in Various Species

(a) *Fresh Water Algae*

(i) Chlorella *Sp.* (*probably* C. pyrenoidosa).—Warburg (1920) recorded only 30-min readings in each gas, and did not demonstrate reversibility of the inhibition. He published the results of five such readings in 100 per cent. oxygen, one in air, five in 2 per cent. oxygen. Light intensity was saturating (10,000–20,000 lux, $aP = 20\ Rd$) and CO_2 concentration was high (the cells suspended in carbonate-bicarbonate buffer (No. 9)). In Table 1 and Figure 1, the rates in 2 per cent. oxygen are all converted to 100, and the corresponding figures for higher oxygen concentrations are given. As the effect here is so variable, the single result for 21 per cent. oxygen does not allow one to draw any conclusions as to the form of the aP *v.* $[O_2]$ curve. Percentage inhibition by pure oxygen ranged from 29 to 42.

Table 1

DATA OF WARBURG FOR CHLORELLA SP.

Temperature 25°C; $[CO_2] = 91 \times 10^{-6}$ mole/l; light intensity 10,000–20,000 lux

Oxygen Concentration (%)	Comparative Rates of Apparent Photosynthesis
100	57·8, 60·9, 67·9, 69·2, 71·1
21	80·2
2	100 (five values, range 45–65, converted)

It was immaterial whether nitrogen or hydrogen was the diluting gas. Warburg stated that there was no clear effect of oxygen concentration on the rate of photosynthesis in low light intensity (400–800 lux, $aP = Rd$), but quoted only two results in support of this. The oxygen effect could not have been due to an acceleration of the normal respiration, for there was no change in the rate of the dark respiration when the oxygen concentration was raised from 2 to 100 per cent.

(ii) Chlorella vulgaris *var.* viridis.—Wassink *et al.* (1938) confirmed some of Warburg's results. At high CO_2 concentration (91×10^{-6} mole/l) and high, but not

saturating, light from a sodium lamp ($1{\cdot}45 \times 10^4$ ergs/cm^2/sec) they obtained oxygen inhibition of photosynthesis, the rate in oxygen being between 33 and 47 per cent. lower than that in pure nitrogen. Contrary to Warburg's experience, the rates in nitrogen and air were not significantly different (Fig. 1). They used "extra pure nitrogen freed from the last traces of oxygen by.....double reduction over heated copper" but did not incubate the cells in nitrogen previous to illumination (which may increase the lag phase (Gaffron 1940)); they stated that oxygen was evolved

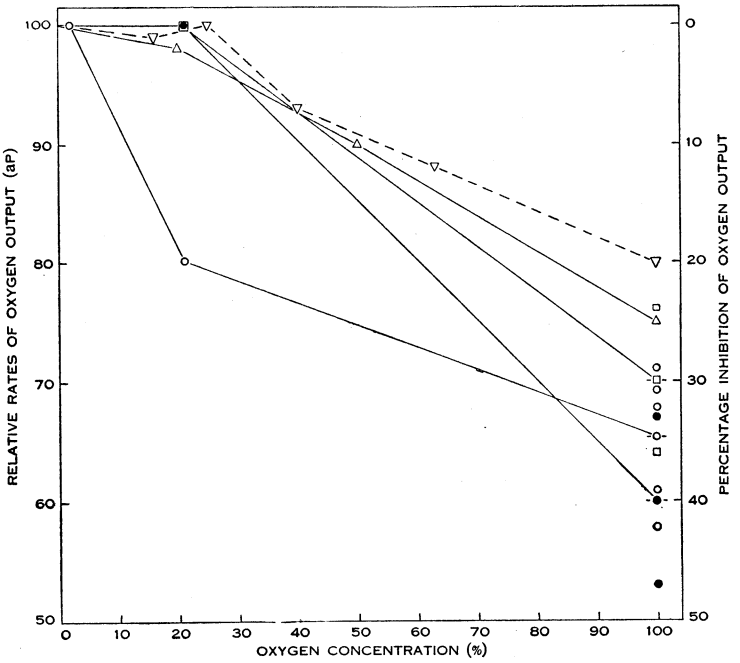

Fig. 1.—Oxygen inhibition of photosynthesis in *Chlorella* as a function of oxygen concentration. ○ (mean —○—) Warburg (1920); ● (mean —●—) Wassink *et al.* (1938); △ ▽ Tamiya and Huzisige (1944); □ (mean — □—) Briggs and Whittingham (1952).

after a few minutes illumination, and that the rate of its production remained constant during the experiments. The lack of an oxygen effect between air and nitrogen can hardly be ascribed, therefore, to the extra complications that might ensue as a result of using pure nitrogen. Dark metabolism was taken into account and was very small under all conditions.

(iii) Chlorella ellipsoidea.—Tamiya and Huzisige (1949) worked throughout at saturating light intensity (25,000 lux). They referred all inhibitions caused by oxygen to the rate in nitrogen (which was purified by passage over copper at 450°C).

At high light intensity and high CO_2 concentration (91×10^{-6} mole/l) they obtained the results shown in Figure 1. On passage from nitrogen to oxygen, there was between 20 and 25 per cent. decrease in the rate of oxygen output. In agreement with Wassink *et al.*, and differing from Warburg, they obtained only a small oxygen

effect (2 per cent., of doubtful significance) between air and nitrogen. They reported a much larger percentage oxygen inhibition at low CO_2 concentration (which will be discussed elsewhere) and it is to be emphasized that under these conditions the inhibition due to the change from air to oxygen was five or six times that due to the change from nitrogen to air.

Tamiya and Huzisige (1949) were the first to publish results showing the reversibility of the oxygen inhibition. Initial rates in air were not recorded, but quantitatively similar effects (but of different sign) were obtained on passage from oxygen to nitrogen (three determinations) or from nitrogen to oxygen (three determinations). Moreover, the rate in nitrogen was the same whether passage was from pure oxygen, 50 per cent. oxygen, or from air. No results were given showing a double change (e.g. oxygen to nitrogen to oxygen) and there were no rate/time curves given for any gas mixture. In a personal communication, however, Professor Tamiya writes that their manometer readings were made at 10–20-min intervals for periods of 60–80 min, during which time the rates were steady.

(iv) Chlorella pyrenoidosa (*Emerson strain*).—Briggs and Whittingham (1952) reported that in this species at high light intensity (40,000 lux) and high CO_2 concentration ($= 91 \times 10^{-6}$ mole/l) there was no oxygen inhibition of aP over the range 0·5–21 per cent. oxygen; there was marked inhibition (30 per cent.) by oxygen over the range 21–100 per cent. oxygen (Fig. 1). The slightly different results for cells "adapted" by a period of illumination in low concentrations of CO_2 will be discussed elsewhere.

(v) Chlorella vulgaris *and* C. pyrenoidosa.—In our experiments we have used the Warburg manometric method but with a different experimental technique from that used in work already reported. Some comparisons have been made between separate, comparable samples shaken in different gas mixtures; in each experiment, however, each sample also served as its own control, being shaken and illuminated successively under two or sometimes three gas mixtures (e.g. 2·5–20–2·5 per cent. oxygen). The light source was a tubular sodium discharge lamp (Osram SD/H82). Typical experiments are illustrated in Figure 2. In such long experiments (2–3 hr), the rate of photosynthesis (aP) rarely remains constant and allowance must be made for drifts in calculating inhibition by oxygen. The details of calculation are indicated in the legend to Figure 2.

All gas exchanges were made with the vessels out of the bath, and gas mixtures were previously equilibrated over the appropriate carbonate-bicarbonate buffer in order to minimize pressure changes due to equilibration in the closed vessel. Only those results obtained at high CO_2 concentration and high light intensity are reported here. In most experiments only apparent photosynthesis rates were measured; the effects of oxygen on dark respiration were small when the gas change was from 20 to 100 per cent. (where the oxygen effect on photosynthesis is greatest), and in these experiments $Rd < aP/10$.

C. vulgaris: Temperature 25°C, $[CO_2] = 37{\cdot}5 \times 10^{-6}$ mole/l, light intensity 30,000 lux (sodium lamp) (Fig. 2(*a*)). A change from air to 100 per cent. oxygen or from 100 per cent. oxygen to air gave 27 and 24 per cent. inhibition of aP by the higher oxygen concentration, the inhibition being reversible.

C. pyrenoidosa: Temperature 25°C, $[CO_2] = 91 \times 10^{-6}$ mole/l, light intensity 30,000 lux (sodium lamp). Three series of experiments were carried out:

(1) Nitrogen to 100 per cent. oxygen (12 expts.): the oxygen inhibition of *aP* was very variable; in two experiments it was as low as 9 and 11 per cent. but in the others it ranged from 22 to 100 per cent., with an overall mean of 35 per cent.

(2) 20 to 100 per cent. oxygen (7 expts.): the oxygen inhibition ranged from 20 to 42 per cent. the mean (30 per cent.) not being significantly different from the mean in (1).

(3) 2·5 to 20 per cent. oxygen (5 expts.): the oxygen inhibition ranged from 0–9 per cent., and was of doubtful significance.

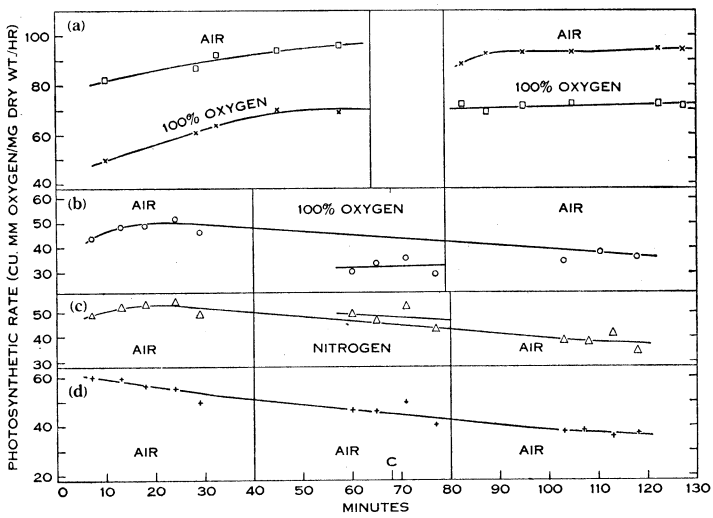

Fig. 2.—Representative experimental results with *Chlorella*. (*a*) *C. vulgaris:* showing reversible inhibition of photosynthesis by oxygen. Percentage effects for comparable samples calculated from the curves at 60 and 100 minutes. Temperature 25°C; $[CO_2] = 37{\cdot}5 \times 10^{-6}$ mole/l, light intensity 30,000 lux. (*b*), (*c*), (*d*) *C. pyrenoidosa:* The drift of apparent photosynthetic rate in air, showing the effect of interpolating short periods of oxygen, nitrogen, or air (renewed). Gas changes made at 40 and 80 minutes. Percentage inhibition calculated from the curves at time *C*. Temperature 25°C; $[CO_2] = 91 \times 10^{-6}$ mole/l; light intensity 30,000 lux.

The oxygen inhibition of *aP* was clearly reversible, but owing to rate/time drifts it is not possible in these experiments to be certain that reversibility was always complete—as it appears to be in Figure 2(*a*).

The above 24 experiments included nine in which the *Chlorella* was grown in low oxygen concentration (0·5 per cent.), five in high oxygen concentration (95 per cent.), and 10 in 21 per cent. oxygen, all gases being enriched with 5 per cent. CO_2. All samples showed clear oxygen inhibition of *aP* on transfer from low to high oxygen concentration. The possibility that cultural conditions and especially oxygen concentration modify the extent of the oxygen inhibition and the growth rate will be discussed in a later paper.

(vi) Scenedesmus "D_3".—Gaffron (1940), in the course of a paper on photosynthetic induction, provides two results only which demonstrate an oxygen effect for *Scenedesmus*. At 20·5°C, high light intensity (10,000 lux, $aP = 10Rd$), and high CO_2 concentration (4 per cent., two-vessel method, plant in phosphate solution), he obtained a 43 per cent. increase in the rate of oxygen output (rP) by transfer from oxygen to nitrogen, the assimilation quotient remaining unchanged. Later in the same experiment the inhibition was only 11 per cent. and he states, "The influence of the partial pressure of oxygen varies with the internal conditions of the cell."

(vii).—Cladophora.—Our experiments on oxygen inhibition of photosynthesis were initiated by an exploratory series carried out by the senior author and Mr. (now Professor) G. E. Briggs, at Cambridge. The plant used was *Cladophora glomerata*.

Methods: The Warburg direct method was used, with four vessels, all illuminated from below by light reflected by a mirror from filament lamps of 100–500 W, each one opposite a vessel. As with more recent experiments described here, each gas mixture was equilibrated with the appropriate buffer solution before being passed through the manometer vessel.

The alga was gathered from the river Cam on the day prior to the experiment, cut into short branched pieces, and kept overnight in tap water. Branches carrying epiphytic algae were discarded. The activity was such that only a small number of filaments was required in each vessel and shading by overlapping was thus avoided. The shaking (100–120 oscillations/min) did not appear to damage the filaments.

The dry weight of the samples used varied from 6 to 25 mg. As it is difficult to obtain closely comparable samples of such a filamentous alga, each sample served as its own control. It was suspended in the buffer solution under a given atmosphere and readings were taken every 10 min until the rate became steady, or until a drift line was established. The atmosphere above the buffer was then changed; measurements were continued and usually the experiment was concluded after a second gas change, which restored the original conditions.

The main objection to this procedure is that the photosynthetic rate rarely remains constant for long, usually, but not always, drifting downwards. In early experiments we used a Warburg buffer (No. 9) made up in distilled water, and the rapid drift in the rate during the experimental period (2–3 hr) could be shown to be associated with reduction in the CO_2 concentration. The drifts were in part, however, due to other causes, for when buffer was made up in river water, the rate of downward drift was reduced. In most experiments it was possible to keep the basic rate fairly constant over a period of some hours, (1) by using less plant material in proportion to the volume of buffer (e.g. 6 mg/4 ml), and (2) by using 1 per cent. sodium bicarbonate made up in river water and filtered. This, when used, approximated in composition to Warburg buffer No. 11, but contained rather more bicarbonate. Calculations show that during experiments lasting 230–250 min, with a high rate of photosynthesis, the CO_2 concentration fell approximately from 350 to 90×10^{-6} mole/l, both of which are high concentrations, unlikely to limit the rate. In one experiment the rate in 100 per cent. oxygen remained almost constant over 6 hr.

Results: The following is a summary of the results obtained under conditions of high light intensity, (near saturation) and high CO_2 concentration ($>91\times10^{-6}$ mole/l, $Rd<aP/10$) (see Table 2):

When the concentration of oxygen in equilibrium with the aqueous medium surrounding *Cladophora* was decreased from 100 to 2·5 per cent. there was a consistently rapid and marked increase in *aP*. This effect persisted as long as the plant was maintained at the low concentration of oxygen, but was completely reversible. A gas change from 2·5 to 100 per cent. oxygen caused a rapid and marked decrease in *aP*.

TABLE 2

OXYGEN INHIBITION OF PHOTOSYNTHESIS AND RESPIRATION OF CLADOPHORA GLOMERATA

High CO_2 concentration ($>91\times10^{-6}$ mole/l); high light intensity; temperature 25°C

Expt. No.	100% Oxygen			2·5% Oxygen			Percentage Effects (based on higher of two rates)		
	Rd	*aP*	*rP*	*Rd*	*aP*	*rP*	*Rd*	*aP*	*rP*
C1A	4·4	52·5	56·9	3·9	72·5	76·4	−11	+28	+26
C7	2·3	11·5	13·8	1·9	21·0	22·9	−17	+45	+40

In six experiments, in each of which two closely matched samples of *Cladophora* were exposed, one to 2·5 per cent. oxygen, and the other to 100 per cent. oxygen, the differences in *aP* between paired samples were always large and in the same direction, i.e. the rate was higher in 2·5 per cent. oxygen.

In experiments with individual samples of *Cladophora*, the atmosphere over the plants was changed 57 times, either from 2·5 to 100 per cent. oxygen, or in the reverse direction. Oxygen inhibition was shown clearly in 56 of these exchanges, which were made thus:

(i) In 18 changes, from 100 to 2·5 per cent. oxygen, a marked rise in *aP* occurred, followed by a fall to the expected rate (allowing for time drifts) in 17 of the samples when 100 per cent. oxygen was reintroduced (see Figs. 3(*a*), 3(*b*), 4).

(ii) In five changes from 2·5 to 100 per cent. oxygen, a marked fall in *aP* occurred, followed by reversal on return of the plant to 2·5 per cent. oxygen (see Figs. 3(*c*), 3(*d*)).

(iii) In six simple changes, from 100 to 2·5 per cent. oxygen, all showed a marked rise in *aP*.

(iv) In five simple changes, from 2·5 to 100 per cent. oxygen, all showed a marked fall in *aP*.

The reversibility and the time-scale of the effect are shown in Figures 3 and 4. The interpolated lines joining the rates before and after the gas changes are drawn visually, and no claim is made as to any accuracy in their actual form. While the

drifts of the rate with time make it difficult to be certain that the reversibility is always complete, experiments of the type shown in Figure 3 (expts. C13, C19) indicate

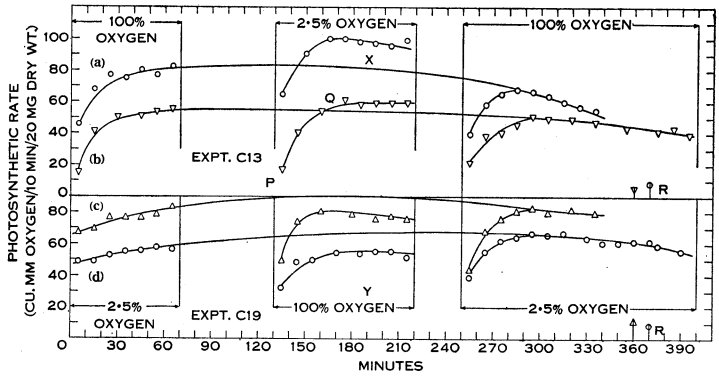

Fig. 3.—*Cladophora glomerata:* Drift of apparent photosynthetic rate with time, showing reversible inhibition of photosynthesis by high oxygen concentration. Temperature 25°C, $[CO_2] > 91 \times 10^{-6}$ mole/l, high light intensity (near saturation) for both experiments. *R*, mean respiration rates at conclusion of the experiments.

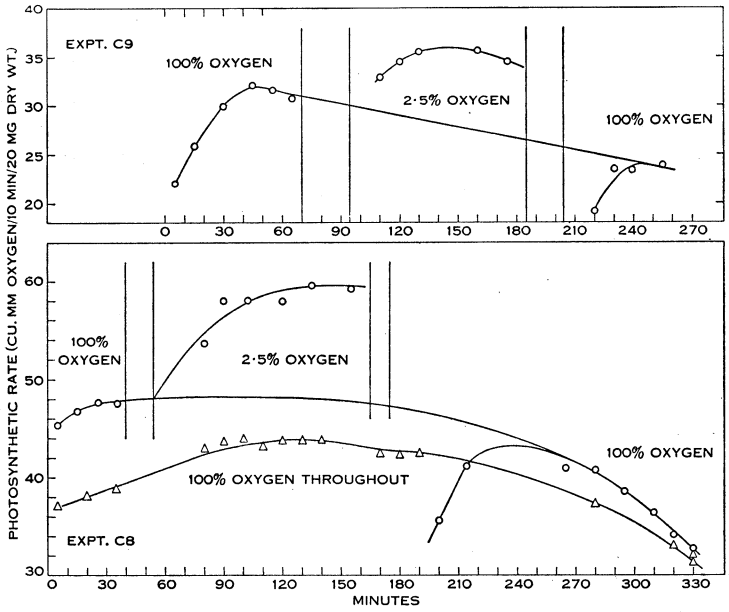

Fig. 4.—*Cladophora glomerata:* Drifts in apparent photosynthetic rates in oxygen with time with and without interpolation of periods in low oxygen concentration. Temperature 25°C; $[CO_2] > 91 \times 10^{-6}$ mole/l; high light intensity, near saturation.

complete reversibility. This is made clearer in Figure 4 (expts. C9, C8), where a control sample was kept in one gas throughout the experiment and showed a drift in rate parallel to that suggested for the treated plant.

The oxygen inhibition was large and (as Warburg (1920) noted) variable, a feature which is accentuated in this series of experiments because of a lack of uniformity in plant samples and experimental treatment. Taking expt. C13, Figure 3,

TABLE 3

OXYGEN INHIBITION OF APPARENT PHOTOSYNTHETIC RATE IN MARINE ALGAE

Except where otherwise stated, all experiments were carried out at 20°C and high CO_2 concn. ($= 36 \times 10^{-6}$ mole/l)

Species	Light Source	Light Intensity (lux)	Changes in Oxygen Concn. (%)	Inhibition of aP (as % of rate in low oxygen concn.)	Reversal of Inhibition	Comments on Rate–Time Curves (see Fig. 5)
Green algae						
Enteromorpha	Sodium	17,000	20–100–20	18	+	Upward drift, then steady
Caulerpa scalpelliformis	Sodium	17,000	20–100–20	62	+	Downward drift
Cladophora sp.	Sodium	17,000	20–100–20	43	+	Rapid downward drift
Bryopsis sp.	Sodium	17,000	20–100–20	5	+	Rapid downward drift
Red algae						
Nitzemia sp.	Mercury	9,500	5–100–5	20	+	Downward drift
Phacelocarpus complanatus	Mercury	9,500	5–100–5	36	+	Rate steady
Chylocladia muelleri	Mercury	9,500	5–100–5	34	+	Rapid downward drift
Polysiphonia abscissa	Sodium	17,000	5–100–5	100,† 88	+	Rapid downward drift
Unidentified sp.	Mercury	9,500	5–100–5	48	+	Rapid downward drift
*Porphyridium cruentum**	Mercury	9,500	1–100–1	100†	+	Upward drift

* Temperature 25°C, $[CO_2] = 1 \times 10^{-6}$ mole/l.

† Oxygen uptake in the light in 100 per cent. oxygen.

as an example, we chose to compare the rates at time XY using the hypothetical drift lines and avoiding use of the initial lag phase (PQ) after the plant was transferred to a new atmosphere. For all experiments the percentage inhibition (based on the rate in low oxygen) ranged from 5 to 74 per cent. The mean was 30 per cent. and standard deviation 15·1.

In some experiments the oxygen effect on the dark respiration rate (*Rd*) was measured. Change of oxygen concentration from 2·5 to 100 per cent. increased *Rd* by as much as 20 per cent. However, as *Rd* itself was low in comparison with photosynthetic rate, the oxygen inhibition of photosynthesis cannot be explained in terms of the oxygen effect on the dark respiration (cf. Table 2 and Fig. 3). The same conclusion has been reached by those working with *Chlorella*.

(*b*) *Marine Algae*

In the following series of experiments the existence of a typical oxygen effect on photosynthesis was established for some marine algal species. The manometric technique was similar to that used for *Cladophora*. The algae were maintained in a photosynthetically-active condition for a few days in aerated sea-water, in the diffuse light of the laboratory. They were washed as free as possible of epiphytic organisms and were suspended, for the duration of each experiment, in the saline buffer solution E recommended by Emerson and Green (1934). This had an initial pH of 7·6 and a CO_2 concentration of 36×10^{-5} mole/l. All gases were equilibrated over the bicarbonate-carbonate buffers in saline solution before being passed into the vessels. For experiments with green algae, tubular sodium discharge lamps (Osram SD/H82) mounted horizontally under the glass window of the water-bath were used. For the red algae, mercury discharge lamps (Osram MA/H, 250 W) held vertically were used, the light being reflected upwards by a mirror. The bath temperature was controlled to $\pm 0{\cdot}04$°C.

In all these experiments the light intensity and the CO_2 concentration were high but not saturating. For most species the rate of photosynthesis declined during the 3-hr course of the experiment; these drifts, however, appear to be due to factors other than the CO_2 concentration, and they were absent for some species with a high photosynthetic rate.

Results are summarized in Table 3 and some data are plotted in Figure 5. Oxygen effects are calculated from the curves (as for *Cladophora*) on the basis of the rate in low oxygen concentration. For all species used there was a clear and reversible depression of photosynthesis by high oxygen concentration. In two experiments the high oxygen concentration depressed the photosynthesis below the compensation point, there being gas uptake in the light. As respiration was not measured in these experiments we have recorded those data as 100 per cent. inhibition of the apparent photosynthesis rate.

(*c*) *Bryophyta and Pteridophyta*

The moss *Funaria hygrometrica* has proved particularly suitable for photosynthesis experiments by the Warburg direct method, aerial leaves being very thin and capable of survival for many hours in carbonate-bicarbonate buffers. The moss was grown on soil in a glasshouse, and before each experiment the plants in a small tuft were carefully separated and washed in the appropriate buffer solution. From each plant, pieces of the green leafy stem from 2 to 5 mm long were cut, blotted dry, and weighed out in groups. Each Warburg vessel contained 0·2–0·4 g fresh weight in 10 ml buffer and was illuminated from below with light from a sodium discharge lamp (Osram SD/H82).

Here we deal only with experiments at high light intensity (30,000–33,000 lux) and high CO_2 concentration. Typical experimental data are plotted in Figure 6. The dark respiration was measured at the beginning and the end of each experiment in whichever gas mixture was used for the initial and final light periods, but gas changes in the course of the experiment were made only for samples in the light.

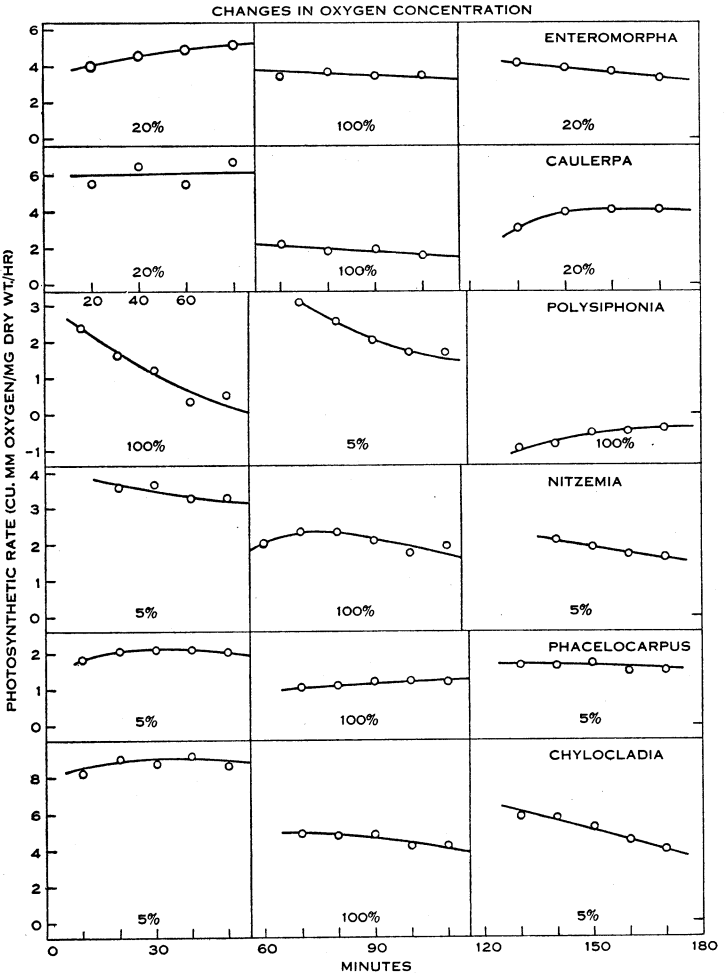

Fig. 5.—Oxygen inhibition of apparent photosynthesis in some marine algae. Details of each experiment will be found in Table 3.

On changing from low oxygen concentration (approximately 0·5 per cent.) to 100 per cent. oxygen there was a marked and reversible inhibition of aP. Judging from the respiration rates of two comparable samples in each gas, there was also a marked increase of respiration at the higher oxygen level, varying with time from 72 to 55 per cent. of the rate in oxygen. It is probable that the dark respiration rates are best given by the second set of dark readings at BB' (Fig. 6), after the low oxygen concentration has been raised by the evolution of oxygen in the light. Nevertheless, we have

estimated *Rd* from the interpolated lines *AB*, *A'B'*. The effect of oxygen on the rate of true photosynthesis has then been estimated from these curves in two ways:

(i) We have assumed equality in leaf area of the paired samples and calculated percentage inhibitions of true photosynthesis from the paired samples at points *C*, *D*, *E* of the rate/time drift (Fig. 6). On the basis of the rate in low oxygen these are 14, 20·8, and 21·9 per cent. respectively.

(ii) We have also used the interpolated lines *YZ* and *Y'Z'* at time *D* to calculate the oxygen inhibition for each sample separately, thus obtaining two results for the whole experiment. The figures are 20 and 17 per cent. respectively, indicating complete reversibility within limits of experimental error.

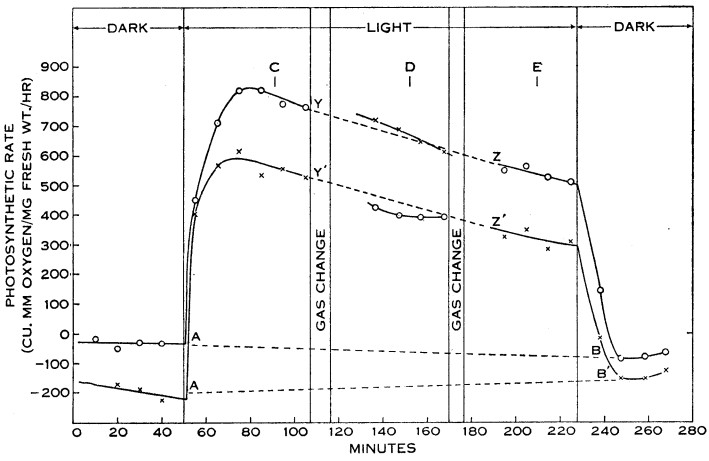

Fig. 6.—*Funaria hygrometrica:* Comparable samples initially in nitrogen (○) and oxygen (×) respiring in the dark, illuminated at 50 minutes. Gas phase reversed at YY′ and again at ZZ′. Calculation of percentage inhibition as in the text. Temperature 25°C; $[CO_2] = 91 \times 10^{-6}$ mole/l; light intensity 30,000 lux.

As these percentage effects are calculated for the true rate of photosynthesis, it is clear that the inhibitory effect of oxygen on the photosynthesis is not explicable in terms of the effect of oxygen on the dark respiration.

Results of all experiments with *Funaria* are given in Table 4. As will be seen in the last line of this table, the effect of oxygen on photosynthesis at high CO_2 concentration is not significant over the range 5–20 per cent. oxygen.

In one experiment with a species of filmy fern (*Hymenophyllum*) it was possible to show a clear oxygen effect although the rate of photosynthesis drifted rapidly downwards reaching half its initial value in 4 hr. The fronds evidently suffered from immerson in bicarbonate-carbonate buffer, turning black if left in it for 12 hr. Thus at 25°C, with a light intensity of 6500 lux (sodium lamp), and a CO_2 concentration of 91×10^{-6} mole/l, and with gas changes from 20 to 100 to 20 per cent. oxygen, the oxygen inhibition of *aP* was 35 per cent. for this fern.

(*d*) *Angiosperms*

The only published record of the oxygen inhibition of photosynthesis in a higher plant is that of McAlister and Myers (1940); this is also the first record of the oxygen inhibition of CO_2 uptake. For young wheat leaves, at nearly saturating light intensity (50×10^4 ergs/cm^2/sec) and low CO_2 concentration (0·03 per cent. in the gas phase), these authors found that the rate of CO_2 assimilation in air was 23 per cent. lower than that in 0·5 per cent. oxygen. The effects of higher oxygen concentrations were not studied.

We have found that high oxygen concentrations inhibit photosynthetic oxygen production in a wide variety of angiosperms.

TABLE 4

OXYGEN INHIBITION OF PHOTOSYNTHESIS IN FUNARIA HYGROMETRICA

In all these experiments the rate of photosynthesis drifted downwards over a period of 4 hr (see Fig. 6). Percentage effects based on the rate in the lower oxygen concentration. $[CO_2] = 91 \times 10^{-6}$ mole/l; light intensity (sodium lamp) = 30,000–33,000 lux; temperature 25°C

Changes in Oxygen Concentration (%)	Percentage Inhibition		Reverse of Inhibition
	aP	*rP*	
0*–100–0*	34	30	+
0*–100–0*	36	19	+
100–0*–100	33	15	+
100–0*–100	21	18	+
100–5†–100	25	16	+
5†–100–5†	29	26	+
5†–100	45	34	
5†–100	53	35	
5†–100	67	44	
20†–100–20†	90	74	+
20†–5–20†	7	0	

* Indicates hydrogen.

† The 5 and 20 per cent. oxygen mixtures contain nitrogen.

(i) *Aquatic species.*—A number of submerged or floating aquatic angiosperms are suitable for experiments by the Warburg direct method: the leaves are thin, the cuticle thin or absent, and they are not obviously harmed by immersion for periods of up to 6 hr in carbonate-bicarbonate buffers. In the submerged species the possible complications of stomatal control are absent.

Method: The Warburg direct method was used, portions of the leafy shoots being either immersed in the buffer (*Vallisneria, Cabomba*) or partly immersed, partly floating (*Callitriche*). For *Lemna*, whole fronds were used, floating on the surface. For *Nuphar*, circular disks were cut from mature leaves and a single disk was floated on the buffer in each vessel. For all species the photosynthetic rate drifted downwards over the experimental period, even at high CO_2 concentration; the rate of drift was least in *Cabomba*.

Results: At high light intensity and high CO_2 concentration there were clearly-marked oxygen effects between 20 and 100 per cent. oxygen, and in some cases smaller effects between 2·5 and 20 per cent. oxygen (Table 5). The inhibition of oxygen output by oxygen was reversible. For comparison with other species all oxygen effects are

TABLE 5

OXYGEN EFFECTS IN SOME AQUATIC ANGIOSPERMS

Sodium lamps were used for the light source except for *Cabomba caroliniana* when filament lamps were used. $[CO_2] = 91 \times 10^{-6}$ mole/l

Species	Temp. (°C)	Saturating Light Intensity (lux)	Changes in Oxygen Concn. (%)	Inhibition of aP† (as % of rate in lower oxygen concn.)	Reversal of Inhibition	Comments on Rate–Time Curves
Vallisneria spiralis	30	9,000	20–100–20 20–5–20	+23 0	+	Steady downward drift
Lemna minor	25	35,000	20–100–20 20–100–20 20–5–20 20–5–20	+58 +52 −9 −19	+ +	Linear downward drift
Nuphar sp.	25	35,000 (below satn.)	20–100–20	+43	+	Marked downward drift
Cabomba caroliniana	25		20–2·5–20 20–5–20 20–100–20	+16 +5 +47	+ + +	Drift slight or absent
Callitriche sp.	30*	30,000	20–100–20 20–5–20	+18 +16	+ +	Steady downward drift
	25*	30,000	20–5–20 20–100 20–100 20–5 20–100 2·5–20	+16 +34 +35 0 +33 +5	+ + + + +	Steady downward drift

* $Rd = aP/5$ at 30°C and $aP/7$ at 25°C.

† The + sign indicates inhibition by the higher of the two oxygen concentrations.

calculated on the basis of the rate in air, the + sign indicating inhibition by the higher of the two oxygen concentrations (Table 5). *Lemna* gave the only anomalous result (duplicated), an inhibition of oxygen output on passing from air to 5 per cent. oxygen, as well as the usual inhibition on passing from air to 100 per cent. oxygen. In this plant the frond was floating and uncut. Although it has been reported

that the stomata of *Lemna* are immobile (Reuter 1948), it is possible, in view of Williams' work (1954), that the stomata close at low oxygen tension. This could affect the rate if CO_2 uptake is mainly from the gas phase.

(ii) *Land Plants.*—For exploratory experiments on the oxygen effect in the photosynthesis of land plants the continuous method is not convenient, if only by reason of the need for very careful matching of the CO_2 concentration in the two gas streams; or, alternatively, the need for using high CO_2 concentrations with consequent absorption problems. A modification of the manometric technique has proved suitable, although it has definite limitations. Portions of aerial leaves cannot usefully be immersed in carbonate-bicarbonate buffers, but, in spite of diffusion difficulties, data of value can be obtained if the leaf is illuminated directly in a closed vessel containing the shaken buffer solution in a separate compartment.

Methods: In this experiment, the Dickens-Simer manometric vessels were used (Dixon 1943). The buffer solution was placed in the outer rim and a leaf disk, approximately 1 in. in diameter, was placed flat with the abaxial surface downwards facing the source of light. The edges of the disk were moistened before each experiment, but injection of the air spaces with water was guarded against. The vessels were shaken at a speed of 100 excursions per minute.

The disadvantages of this technique are well known, and it would be useless for some types of study. At the beginning of illumination or change of gas phase the apparent photosynthetic rate is reduced because of the slow diffusion of the CO_2 from the buffer, as compared with its rapid uptake by the leaf. In preliminary studies it was not found possible to increase the rate of equilibration of the CO_2 between the buffer and the gas phase. When a bicarbonate solution (M/10 or M/1) is shaken with CO_2-free air in a closed manometer vessel, equilibration is reached in about 1 hr, the half-time being 10 min. These periods are not significantly altered by the addition to the buffer of glass beads, powdered glass, charcoal, colloidal ferric bicarbonate, or by exposing the buffer to the atmosphere on filter paper. No advance in the technique seems possible along these lines.

When a leaf disk of *Ampelopsis* is placed in the manometric vessel as described above, with M/10 bicarbonate in the rim, and shaken under high light intensity, the rate of increase of pressure in the vessel rises steadily to a maximum value reached after 1 hr; thereafter the rate remains constant in this species for several hours. In other species tried the high rate is reached much more quickly and subsequently drifts slowly downwards. If, at any time, the leaf disk is darkened, the pressure continues to rise but at a rapidly decreasing rate, until at about 1 hr after darkening an uptake of gas is recorded due to respiration. The half-time for the fall in darkness from the maximum rate to zero pressure change is about 10 min.

Under such conditions, we assume that the pressure increase during the illumination is the net effect of three processes: (1) the uptake of CO_2 by the leaf; (2) the equal output of oxygen by the leaf, assuming an assimilation quotient of 1·0; and (3) the output of CO_2 from the buffer solution. The increase in the rate is therefore simply due to the increase in the concentration gradient of CO_2 between air and buffer. When this is sufficiently great, however, (when the CO_2 may be limiting photosynthesis), we assume that rate (1) equals rate (3) and the pressure change in the

steady state will be a measure of the oxygen output in photosynthesis. Even if maximum possible rates of photosynthesis cannot be measured by this method, owing to the diffusion lag, it should be possible to demonstrate oxygen inhibition of photosynthesis if such occurs. Its major defect is that conditions bringing about a change in the rate of photosynthesis must alter the CO_2 concentration in the gas phase. Thus oxygen inhibition so measured would not be maximal, as it is to be expected that

TABLE 6

OXYGEN INHIBITION OF PHOTOSYNTHESIS IN SOME LAND PLANTS

Sodium lamps were used for the light source, the intensity being approx. 35,000 lux; CO_2 concentration unknown, probably limiting; temperature 30°C

Species	Buffer Used (in rim of vessel)	Changes in Oxygen Concn. (%)	Inhibition (as % of rate in lower oxygen concn.)		Reversal of Inhibition
			aP	*rP*	
Hydrangea hortensis	Warburg No. 11	5–20–5*	11		+
		5–20–5*	7		+
		5–100–5†	30		+
		5–100–5†	40		+
	Warburg No. 9	5–100	25		
		100–0‡–100	18		+
		0‡–100–0‡	19		+
		100–20–100	32	24	+
Nicotiana tabacum	Warburg No. 9	20–100–20	41		+
		20–5–20	0		
Ampelopsis veitschii	M/1 $KHCO_3$	20–100–20	64		−
		20–100–20	39		−
		20–100–20	41		+
		20–100–20	78		50%
		20–100–20	77		50%
		20–5–20	(−25)		−
		20–5–20	(−45)		Partial
		20–5–20	(−20)		Partial

*† Same disks used on successive days.

‡ Indicates nitrogen.

after a fall in *aP* the CO_2 concentration in the gas phase would rise and, if limiting, increase the rate of *aP* again. The method is entirely unsuitable for the study of induction or of transitional effects but it has advantages of its own. The apparatus is very simple and it is possible to use two closely comparable small samples, one from each half of the same leaf. In our experiments these gave almost identical rates in the steady state, but differing from leaf to leaf.

A test of the method was made with *Hymenophyllum* fronds. Some were submerged in the buffer (as described in Section II (*c*)), others were floated on a thin film of water and supplied with CO_2 from the buffer in the annular rim of the vessel. Closely similar curves for oxygen output were obtained and the percentage oxygen effect was 35 per cent. in both types of experiment.

Results: The results obtained by these means (Table 6) indicate that the oxygen effect on photosynthesis occurs in the aerial leaves of angiosperms. The results for *Ampelopsis* show the anomaly noted for *Lemna*, i.e. although the normal oxygen inhibition occurs on passage from air to 100 per cent. oxygen, there is also, in some experiments, an inhibition when the change is made from air to 5 per cent. oxygen. As these anomalous effects have only been found with organs carrying stomata, it is tempting to suggest that they are due to stomatal closure, in spite of the fact that the leaves used had circular cut edges.

In these higher plant leaves, after a period in oxygen, reversal of inhibition was not always obtained, but in such experiments it was noticed that the leaf disks showed signs of injection with water. It is proposed to investigate oxygen effects with the higher plants with more satisfactory techniques.

The results reported in this paper will be discussed in a second publication dealing with the effect of various factors on the oxygen inhibition of photosynthesis.

III. References

Briggs, G. E., and Whittingham, C. P. (1952).—*New Phytol.* **51**: 236.
Dixon, M. (1943).—"Manometric Methods." 2nd Ed. p. 94. (Cambridge Univ. Press.)
Emerson, R., and Green, L. (1934).—*J. Gen. Physiol.* **17**: 817.
Gaffron, H. (1939).—*Cold Spr. Harb. Sym. Quant. Biol.* **7**: 377.
Gaffron, H. (1940).—*Amer. J. Bot.* **27**: 204.
Hill, R., and Whittingham, C. P. (1953).—*New Phytol.* **52**: 133.
McAlister, E. D., and Myers, J. (1940).—*Smithson. Misc. Coll.* **99** (Publ. No. 3591): 1.
Miyachi, S., Izawa, S., and Tamiya, H. (1955).—*J. Biochem., Tokyo* **42**: 221.
Moyse, A. (1953).—*C. R. Acad. Sci., Paris* **236**: 111.
Reuter, L. (1948).—*Phyton (Austria)* **1**: 76.
Surbeck, E., Holt, V., and Lund, E. J. (1925).—*Proc. Soc. Exp. Biol., N.Y.* **23**: 681.
Tamiya, H., and Huzisige, H. (1949).—*Acta Phytochim., Tokyo* **15**: 83.
Warburg, O. (1920).—*Biochem. Z.* **103**: 188.
Wassink, E. C., Vermeulen, D., Reman, G. H., and Katz, E. (1938).—*Enzymologia* **5**: 100.
Williams, W. T. (1954).—*J. Exp. Bot.* **5**: 343.