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INTERACTIONS BETWEEN PHOTOSYNTHESIS AND RESPIRATION IN CHLORELLA

I. TYPES OF TRANSIENTS OF OXYGEN EXCHANGE AFTER SHORT LIGHT EXPOSURES

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SUMMARY

1. Synchronous *Chlorella fusca* was exposed to light for periods of 0.1 sec–15 min. Subsequent to the illumination, the rate of O_2 exchange passes through a series of transient phenomena. These transients vary greatly with cellular age and depend also upon conditions prior to and during the experiment. However, the individual maxima and minima of O_2 exchange specified as T_1, T_2, T_3..., occur at reproducible times provided that algae of the same developmental stage and pretreatment are used.

2. At least 5 individual components are involved in the complex changes: (a) A sudden photosynthetic O_2 evolution (T_1) which, after darkening, declines rapidly and without oscillations. (b) A brief and distinct O_2 uptake (T_2) immediately after switching off the light, which is only observed under special conditions. (c) The next maxima and minima T_3, T_4, T_5, and partly also T_6 obviously are damped oscillations of the respiratory O_2 uptake, started by an inhibition of some respiratory step by photosynthesis. (d) A slow declining stimulation of O_2 uptake superimposed on the foregoing transients occurs after longer light exposures. (e) A very strong stimulation of respiratory O_2 consumption (T_8), culminating not until 6 to 8 min after the start of illumination, is induced only by irradiation with short-wave light (< 540 nm) after longer dark periods.

3. The individual components differ with respect to their dependence on intensity, wavelength and duration of the light exposure and their sensitivity to several inhibitors. According to their characteristics all transients except T_8 depend on, but in different ways, the function of the photosynthetic apparatus. The components b and c seem to be closely connected with System I.

INTRODUCTION

In Chlorella, a single light exposure of a few seconds or less may, under some conditions, result in a long sequence of complex changes in the rate of O_2 exchange[1].

Abbreviations: CCCP, *m*-chlorocarbonyl cyanide phenylhydrazone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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These may last up to 1 h. Most of these transients have already been described[2], but little is known about their nature and the conditions needed to produce each one. One question is whether these transients are really different in nature or whether they merely reflect one basic process which varies with the conditions of the experiment, the physiological or developmental stage of the cells, and so on.

The aim of this work was to investigate the nature of all these transients in Chlorella grown under controlled conditions and to characterize the individual transients. The conditions required for their appearance, their dependence on intensity, wavelength, and duration of the light exposure, and the influence of some inhibitors were determined.

MATERIAL AND METHODS

Chlorella fusca Shihira et Kraus (culture collection at Göttingen, No 211-8b, = *Chlorella pyrenoidosa* Chick) was grown synchronously at 30° in N5 medium[3] aerated with 2.5 % CO_2 in air. A 24-h cycle of 16 h light (from 6 fluorescent tubes, GE 40 W cool white in a white box) and 8 h dark was used as in previous experiments to get complete synchronization. All experiments described in this paper were performed with young cells, harvested 1 to 3 h after the start of the light period. This is about 1 to 3 h after average time for the release of the autospores.

Relative rates of O_2 exchange were measured polarographically with an electrode as described by FORK[4]. The platinum surface was covered with a Teflon membrane about 6 μ thick. A thin, homogeneous layer of Chlorella cells over the Teflon was separated, by a dialysing membrane, from a volume of 50 or 100 ml of N5 medium, which was aerated with 5 % CO_2 in air and recirculated by a centrifugal pump as described by HART[5].

All experiments were performed at room temperature (21 to 23°). The desired wavelengths were isolated from the light of an incandescent lamp by Balzer interference filters (half-band width about 9 nm). Light intensity was measured with a calibrated silicon photocell of the same size as the platinum electrode. Since most of the transients described change markedly with age and pretreatment of the cells, standard light signals were given at short intervals to assure the comparability of the measurement within a series of light exposures or to make possible a recalculation of the individual measurements on a common basis.

RESULTS AND DISCUSSION

The time sequence of transients caused by a single light flash

Fig. 1a shows the complete series of transients that may result from a short light exposure. The individual maxima and minima are called T_1, T_2, T_3, *etc.*, regardless of whether they may be separate effects or represent oscillations of only one system. The even numbers symbolize minima (enhanced O_2 uptake), the odd numbers, positive spikes or maxima (reduced O_2 uptake or O_2 evolution). The peak of photosynthetic O_2 evolution during a short light period is included in this enumeration system as T_1.

As a first approximation, the transients can be identified by the time, t_m, which elapses between the end of the light period and the appearance of the maxima or

minima. When Chlorella cells of the same developmental stage and of similar pretreatment are used, these t_m values vary only over a limited range and in a regular manner with experimental conditions. There is almost no overlapping of t_m values of successive positive or negative transients, even if cells of different developmental stages (0–5 h after the beginning of the light period) are used and when preteatment of the cells was markedly different (Table I).

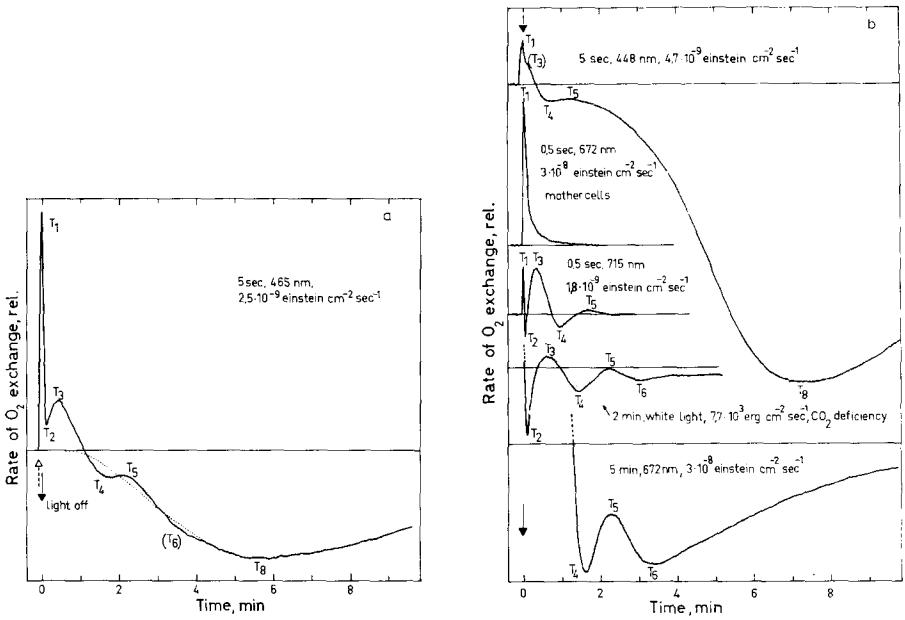

Fig. 1. Transients in O_2 exchange following light exposures chosen to show the whole series of transients (a) or to maximize some of the separate effects (b). Experimental data are given at the single curves. If not mentioned otherwise, the measurements were made with young cells, and the medium was aerated with 5% CO_2. The time scale starts at the end of a brief light exposure. The base line is the initial rate of O_2 uptake. O_2 evolution or a reduction in rate of uptake makes the curve ascend.

However, the shape of the curves, that is the relative magnitudes of the different maxima, varies greatly with experimental conditions (Fig. 1b). In this respect, the individual maxima and minima are influenced by external factors in a completely different manner. From the data in Fig. 1 it can be concluded that these deflections of O_2 exchange do not represent oscillations of only one system caused by a single impulse. By systematic variation of the experimental conditions and by using several inhibitors, it was possible to characterize several individual components of the whole complex of transients. Some of them could be partly isolated. At least 5 components can be distinguished:

Component a (T_1): Photosynthetic O_2 evolution. It declines rapidly after darkening and without any oscillations to zero.

Component b (T_2): A short-lived enhancement of O_2 uptake immediately after switching off the light. It is observed especially under two entirely different sets of experimental conditions. It cannot be decided whether under both conditions the

TABLE I

THE TIME (t_m) FROM THE END OF A LIGHT EXPOSURE TO EACH MAXIMUM OR MINIMUM IN 200 RECORDED CURVES

Values from cells harvested 0 to 5 h after beginning of the light period, obtained after different pretreatment. The light time varied from 0.025 sec to 15 min and light intensity of different wavelengths varied from $9 \cdot 10^{-10}$ to $6 \cdot 10^{-8}$ Einstein $\cdot cm^{-2} \cdot sec^{-1}$.

Transient	t_m *(sec)*		
	Shortest	*Longest*	*Mean*
T_2 minimum	3	26	13
T_3 maximum	10	53	25
T_4 minimum	54	166	98
T_6 minimum	155	285	207
T_8 minimum	360	600	485

same process is observed or, alternatively, whether two quite different O_2-consuming processes may produce a similar effect on O_2 exchange.

Component c (T_3/T_4): The following maxima and minima, T_3, T_4, T_5, partly also T_6, obviously belong to one system. They are thought to be damped oscillations of the respiratory O_2 uptake, started by an inhibition of some respiratory step by a photosynthetic reaction.

Component d: After longer light periods, the foregoing transients are superimposed with a slowly declining stimulation of O_2 uptake which can be measured together with the second minimum (T_6) of the oscillating system (Component c).

Component e (T_8): Only after irradiation with short-wave light < 540 nm a prominent rise of O_2 uptake is observed, culminating 6 to 8 min after the onset of illumination. This effect is marked only after long dark periods of several hours.

The individual components and their characteristics

Photosynthetic O_2 evolution (Component a). Photosynthetic O_2 evolution can be isolated from almost all transients enumerated above when the respiratory O_2 uptake is completely suppressed by removing O_2 or by adding antimycin A. The same result can be obtained by stimulation of O_2 uptake to a maximum rate by adding glucose as already shown by BRACKETT, OLSON AND CRICKARD[6] (Fig. 3). Transients are also absent when endogenous O_2 uptake is maximal during cytokinesis (Fig. 1b). Under these conditions photosynthetic steady-state O_2 evolution as well as the pre-a spike (the fast O_2 output during the first sec of illumination)[7] are nearly normal. Therefore it is concluded that the transients in O_2 exchange after darkening are caused by temporary or delayed stimulation or inhibition of O_2-consuming processes not essentially involved in normal photosynthesis.

This conclusion is supported by the reverse possibility of suppressing almost totally photosynthetic O_2 production (T_1) during short illumination periods of 1 sec or less without impairing the following transients. Using short exposures to red light, the damped oscillations T_3/T_4 (T_5) can be isolated from all other transients (except T_2) by either (1) the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) at a concentration which inhibits photosynthesis by less than 50 % or (2) the addition of 2,4-dinitrophenol at a concentration which produces maximal stimulation of dark

O_2 uptake with only slight inhibition of the steady-state O_2 evolution (Fig. 4). In the absence of inhibitors, similar curves can be obtained under special physiological conditions by short irradiations (<0.5 sec) with far red light (> 700 nm, Fig. 4). It

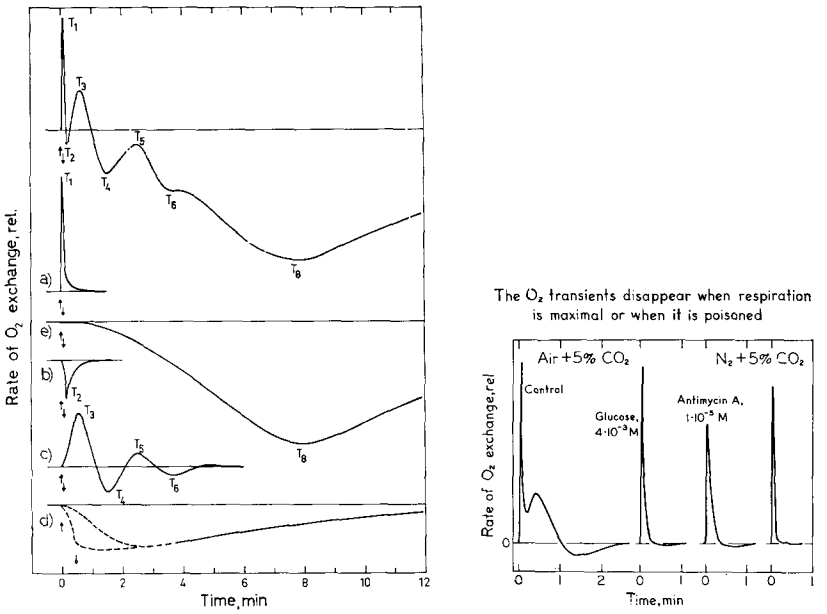

Fig. 2. Idealized transients in O_2 exchange following a short light exposure. The upper curve shows all transients combined. The separate time course curves (Components a–e), believed to comprise the composite curve, are shown individually.

Fig. 3. All transients except O_2 evolution, T_1, are suppressed by maximizing O_2 uptake with glucose or by reducing O_2 uptake either with antimycin A or removal of O_2. Time course curves of O_2 exchange from 0.55-sec, 672-nm flashes at an intensity of $3 \cdot 10^{-8}$ Einstein$\cdot$cm$^{-2}\cdot$sec^{-1}. The faster decay of O_2 evolution after darkening under N_2 may be caused, at least in part, by the higher concentration difference for O_2 inside and outside the cells.

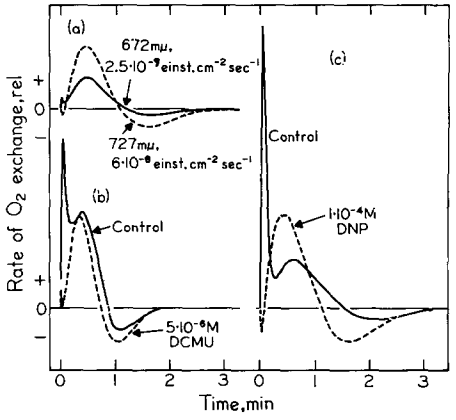

Fig. 4. Comparison of the second maximum, T_3, and photosynthetic O_2 evolution, T_1, from 0.55-sec flashes. (a) Wavelength dependence. Light intensities at 672 nm and 727 nm gave about the same steady-state O_2 evolution. (b,c) Effect of DCMU and dinitrophenol (DNP) on T_1 and T_3. Light intensity, $3 \cdot 10^{-8}$ Einstein$\cdot$cm$^{-2}\cdot$sec^{-1}; wavelength, 672 nm.

appears that the first O_2 outburst during the induction period of photosynthesis differs from steady-state O_2 evolution not only in its sensitivity to hydrostatic pressure[7], and in its action spectrum (having a spectrum like System II, see ref. 8), but also in its sensitivity to photosynthetic inhibitors. The results are consistent with the idea that System II is exclusively responsible for the occurrence of the pre-a spike (T_1). The transitory uncoupling of O_2 evolution from System I can be explained by the replenishing of some pool(s) of electron carrier(s)[9,10].

The oscillatory system (Component c). In contrast to the pre-a spike, T_3 and T_4 seem to be more or less independent of System II, at least after short exposures ($<$ 1 sec). The different sensitivity of T_3 and true photosynthetic O_2 evolution to DCMU was previously observed by D. C. FORK (personal communication). Considered with the fact that T_1 and T_3 depend differently on intensity, duration, and wavelength of the light flash, this result confirms the concept that T_3 is caused by a process quite different from true photosynthetic O_2 evolution. It can hardly be the result of a slow, lingering O_2 evolution, separated from T_1 by a rapid overlapping, but short-lived, O_2 consumption.

After illuminations of several seconds or after shorter light fla shes with light-adapted algae, T_3 appears as a shoulder on the declining slope of O^2 evolution after darkening. Subsequent to still longer irradiation periods with higher light intensities, T_3 is obscured by lingering O_2 evolution. After light exposures shorter than 1 sec, or in dark-adapted algae also after longer exposure times, T_3 is a well-pronounced peak, separated from T_1 by a more or less deep inflection of the curve, T_2. Doubtless it is identical with the effect described by FRENCH[11] from experiments with Scenedesmus, probably also with a similar effect observed in Ulva by VIDAVER AND FRENCH[2]. D. C. FORK also (personal communication) observed the same effect in Chlorella.

T_3 and T_4 coincide nearly completely in their dependence on the intensity (Fig. 5) and wavelength of the incident light, on irradiation time (Fig. 6), and in their responses to some inhibitors (Fig. 4, Table II). Therefore they are assumed to be damped oscillations of the same system. This assumption is supported by the occassional occurrence of a second sinusoidal oscillation (T_5, T_6) of the same period length (about 110 sec at 21°).

T_3 as well as T_4 can be obtained by irradiations with light of any wavelength in the visible range (investigated between 397 and 727 nm). However, as may be

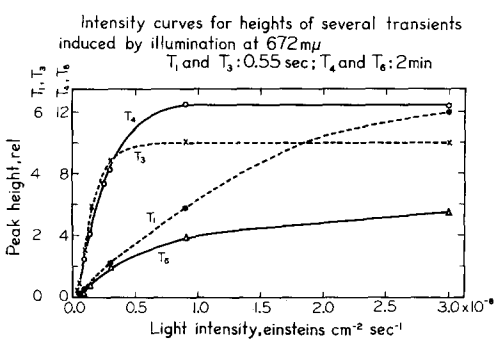

Fig. 5. The magnitudes of the transients as functions of light intensity. T_1 and T_3 were measured after 0.55-sec exposures, T_4 and T_6 after 2-min exposures to monochromatic light of 672 nm.

seen from Fig. 4a, their appearance is more marked after irradiation with wavelengths > 690 nm, when light intensities at different wavelengths are chosen to give nearly the same O_2 evolution as in the steady state. They are saturated at less than 10 % of the light intensity necessary for saturation of the pre-a spike and of steady-state photosynthesis (Fig. 5). Also in this respect they resemble known special activities of System I, for example cyclic phosphorylation or the 'photostimulation of respiration' observed in Porphyridium by FRENCH AND FORK[12]. A dependence of these transients on System I is also suggested by their low sensitivity to DCMU.

T_3 and T_4 not only disappear when respiration is inhibited or is stimulated to a maximum rate (Fig. 3), but between these extreme situations, the amplitude of this oscillation and the t_m values depend on the respiratory activity. The t_m values rise strongly with declining respiratory activity (O_2 consumption), whereas the amplitude is greatest at medium activity, when respiration is thought to be most effectively controlled by the adenylate system (or by P_i).

Therefore it is assumed that the transients T_3/T_4 (and T_5/T_6) imply an inhibition of the respiratory turnover by some activity of System I, possibly by cyclic phos-

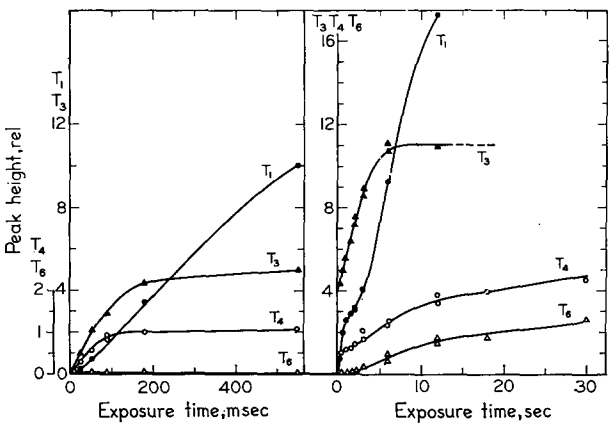

Fig. 6. The magnitudes of the transients as functions of the duration of 672-nm flashes. The intensity was $3 \cdot 10^{-8}$ Einstein $\cdot cm^{-2} \cdot sec^{-1}$.

TABLE II

EFFECT OF INHIBITORS

Symbols: ++ = nearly complete inhibition (80 to 100 %); + = partial inhibition (50 % or less); ○ = almost no inhibition; ● = stimulation; ◑ = inconstant stimulation; ⊕ = inconstant, weak inhibition; and — = not tested.

Transient	*DCMU* ($5 \cdot 10^{-6}$ *M*)	*2,4-Dinitrophenol* (10^{-4} *M*)	*CCCP* (10^{-5} *M*)	*Antimycin A* (10^{-5} *M*)
T_1	++	++	○	○
T_2	●	●	—	++
T_3	○	●	++	++
T_4	●	●	++	++
T_6	+	○	—	++
T_8	◑	—	—	++
Steady state	+	○	+	⊕

phorylation. This inhibition reaches its maximum about 15 to 50 sec after the start of the light period (or after a light flash), depending on the rate of turnover. Thereafter in the dark, respiration returns to its steady state with one, or occasionally two, damped oscillations.

Such a conclusion is compatible with many observations indicating a partial inhibition of respiratory steps during photosynthesis[13–18]. The assumption that there is a respiratory control by photophosphorylation *via* the adenylate system (or the P_i level) is strengthened by the effectiveness of *m*-chlorocarbonyl cyanide phenylhydrazone (CCCP) (uncoupling both oxidative and photophosphorylation) in suppressing these transients, whereas 2,4-dinitrophenol does not influence or even enlarge them (Table II). The latter effect can be understood since 2,4-dinitrophenol, uncoupling only oxidative phosphorylation, does not impair the control of glycolysis by substrate level phosphorylation or by activation or inhibition of phosphofructokinase. It appears likely that there is a fundamental similarity between this phenomenon and the oscillatory control of glycolysis in yeast cells as investigated by CHANCE and co-workers[19–21].

The relationship between the magnitude of T_3 and T_4 and the duration of irradiation is complicated. The curves for T_3 and T_4 show a sharp bend after exposure times of 0.1 to 0.2 sec (Fig. 6). With lengthening exposure times (up to about 10 sec) the amplitudes of T_3 and T_4 increase only slowly to nearly the threefold values observed after 0.2 sec. There is no obvious correlation to the initial slope of photosynthesis (which reaches the steady state only after about 1 min or more). The marked dependence of T_3 and T_4 on System I, as reported here, can only be observed at exposure times $<$ 1 sec. However, at exposure periods of several seconds (that is, when the increase in peak height connected with the second rise of the curve predominates over the initial effect after 0.2 sec), the dependence of T_3 and T_4 on environmental factors and on inhibitors resembles more and more that of steady-state photosynthesis. The continued rise of T_4 with illumination periods longer than 10 sec seems to be due to a superimposed effect described as Component d.

The dip, T_2, between T_1 and T_3 (Component b). The depression designated as T_2 is caused, at least in part, by the time lag between O_2 evolution, T_1, and the light-induced inhibition of O_2 uptake, T_3. Under some conditions, however, the curve drops distinctly below the baseline between T_1 and T_3. Such an enhanced O_2 uptake may be the first response to a light flash (Fig. 4). This actual, light-stimulated, net O_2 uptake was always observed when dark-adapted algae were exposed to short flashes ($<$ 1 sec) of long-wavelength light which excited predominantly System I. The effect is also observed with light-adapted algae and/or exciting with shorter wavelengths when T_1 is suppressed by addition of DCMU (Fig. 4). This net O_2 uptake shows characteristics similar to the 'negative spike' reported by FRENCH AND FORK[12] in the red alga Porphyridium, and by VIDAVER AND FRENCH[2] in the marine green alga Ulva. All these effects are thought to be the result of the same process.

The question arises as to whether this O_2-consuming process is necessarily linked to photosynthesis and, consequently, always involved in T_2, or whether it is only a casual effect, restricted to special conditions. In the absence of inhibitors and at wavelengths $<$ 680 nm, there is no evidence for such a light-dependent O_2 uptake, at least in the case of light-adapted Chlorella cells. Under these conditions, T_2 can be explained sufficiently as a result of an overlapping between the lingering photo-

synthetic O_2 evolution and the retarded start of the inhibition of the respiratory O_2 uptake.

A similar, short-lived, enhanced O_2 uptake is observed immediately after darkening of cells that have been exposed for many seconds (up to minutes) to high light intensities and CO_2 deficiency. This effect appears to be more prominent at wavelenghts preferentially absorbed by System II and might be of a completely different nature.

The background stimulation of respiration (Component d). After longer exposures to red light of medium or high intensity, the oscillations T_3, T_4, (T_5, T_6) are superimposed on a slowly and continually declining stimulation of O_2 consumption, which obviously begins during the light time. This effect can not be isolated from other transients after exposure times shorter than 5 min. It may be measured most accurately along with the minimum of the second oscillation (T_6) about 4 min after darkening. After longer irradiation with strong light however, usually only this effect can be observed. It resembles steady-state photosynthesis in its dependence on environmental factors and in its sensitivity to several inhibitors (Table II). However, it is nearly linear with time over a fairly long period and after 15 min of irradiation at medium intensity (10^{-9} Einstein·cm^{-2}·sec^{-1}), it is still far from the point of saturation. From these characteristics, it may be concluded that this effect is linked to photosynthesis by an end product of photosynthesis or, more likely, by an energy-consuming process following photosynthesis.

The blue-light-dependent stimulation of O_2 uptake, T_8 (Component e). The most conspicuous and most extended transient, T_8, appears only after illumination with

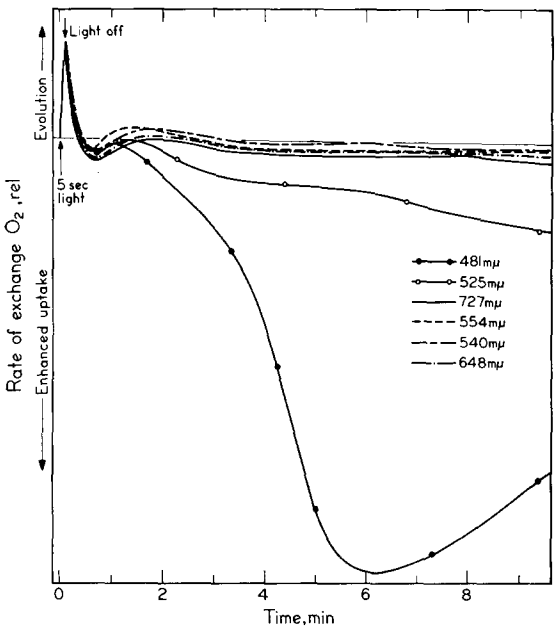

Fig. 7. Effectiveness of different wavelengths in producing T_8. Light intensities between $9.5 \cdot 10^{-9}$ (481 nm) and $6 \cdot 10^{-8}$ Einstein·cm^{-2}·sec^{-1} (727 nm) were chosen to give about the same height of T_1. Exposure time was 5 sec. All records were made immediately after a dark time of $>$ 10 h or after irradiation periods when wavelengths not effective in producing T_8 were used.

wavelengths < 540 nm and reaches an appreciable extent only after a dark period of several hours. Under optimal conditions, the additional O_2 uptake caused by a short-wavelength flash may be more than 500 times as great as the photosynthetic production of O_2 during the light period (Fig. 7). The rate of O_2 uptake in the dark is enhanced up to 100 %. The maximal effect can be obtained only once. It almost disappears after a few repetitions of the light flash. Because of the long regeneration time, it was not possible to get a complete enough action spectrum to determine the peak wavelength exactly. As shown in Fig. 3, illumination with wavelengths >540 nm (at intensities resulting in approximately the same values for T_1 and steady state), has no detectable effect in evoking T_8. The preliminary action spectrum obtained so far is in fairly good accordance with the spectrum reported by PICKETT AND FRENCH[22].

The effect is completely insensitive to DCMU. It is certainly not dependent on photosynthesis. When the light exposure is extended to several minutes, the effect is observed during the light period as a strong decay of O_2 evolution which becomes visible after a lag phase of 1 to 2 min. At moderately low intensities of blue light, the O_2 evolution may drop below the compensation point and finally result in an enhanced O_2 uptake in the light.

The long lag time between a short blue-light exposure and the maximal stimulation of O_2 uptake, as well as the long duration of this effect, clearly show that it cannot consist of a simple photooxidation. Rather, it may involve an activation of regular respiration, as suggested by interaction with the oscillating system (Component c), as will be reported in another paper.

Obviously, this is the same effect as reported by EMERSON AND LEWIS[23] for wavelengths near 470 nm. It also may be identical to the blue-light effect on respiration described by KOWALLIK AND GAFFRON[24], which seems to be correlated with the stimulation of protein synthesis by blue light[25].

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