KINETIC RELATIONSHIPS BETWEEN PHOTOSYNTHESIS AND RESPIRATION IN THE ALGAL FLAGELLATE, OCHROMONAS MALHAMENSIS' DALE WEIS' AND ALLAN H. BROWN

BOTANY DEPARTMENT, UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MINNESOTA

Studies on the influence of light on respiration have yielded different results with different organisms. In some instances results were interpreted as evidence for light stimulation of respiration (3, 4, 5, 6), in other cases for photoinhibition (8, 13), and in still other examples for a negligible effect (1). With a given species simultaneous measurements of respiratory and photosynthetic O₂ metabolism have shown that the influence of light on respiratory processes varied with experimental conditions (3, 4). The fact that conditions have not always been strictly comparable in different investigations probably accounts for some contradictory results.

In the preceding paper, which also dealt with the problem of accounting for manifold influences of light -or photosynthetic metabolism—on respiratory processes, a very simple model was proposed (figure 1, page 226. In that model photosynthesis is considered as an oxidation-reduction reaction yielding oxidant and reductant at equal rates. The oxidant is the precursor of molecular O2; the reductant serves ultimately to reduce CO₂. It is assumed that the reductant, but not the oxidant, may react also with components of the respiratory mechanism. Such interaction could result in either an increased O2 consumption rate, a decreased CO2 production rate, or both. Interaction of some of the reductant with respiratory intermediates would result in diversion of this amount of reductant from its photosynthetic role of CO2 assimilation.

The photosynthetic-respiratory interactions proposed in the model have several specific consequences which should be experimentally observable. The respiratory quotient $(+CO_2/-O_2)$ would be decreased and the photosynthetic quotient $(+O_2/-CO_2)$ would be increased. The rate of photosynthetic O_2 production would be unaffected whether or not the postulated interactions occur, since by assumption the photosynthetic oxidant is not involved. A further consequence of the model is the stoichiometric equivalence of O_2 produced in light to the sum: CO_2 consumed + light-induced extra O_2 uptake + light-induced deficit in respiratory CO_2 production.

Perhaps only because of its simplicity, this model was able to explain quantitatively some light effects on respiration in an arbitrarily selected organism, Ankistrodesmus (4). The present paper describes similar experiments on the physiologically heterodox genus, Ochromonas.

² Present address : The College, University of Chicago. Chicago 37, Ill.

MATERIALS AND METHODS

The Chrysophyte flagellate, Ochromonas malhamensis isolated by Chen and described by Pringsheim (10), was used in these experiments. A defined medium allowing physiological experimentation was devised by Hutner, Provasoli and Filfus (7) and a number of physiological studies have since been carried out. The role of photosynthesis in the metabolism of the organism has been studied by Myers and Graham (9) and by Weis (14) and dark metabolism was studied by Reazin (11). The ability of this flagellate to carry out photoreduction was investigated by Vishniac and Reazin (12). An investigation of the effect of culture conditions on the development of enzymes required for CO₂ reduction was carried out by Reazin and Fuller (personal communication).

A characteristic of Ochromonas, advantageous for the present work, is its relatively rapid rate of respiration and low maximal rate of photosynthesis. In past experiments from this laboratory rather low rates of gas exchange (i.e., relatively low light intensities) were employed for technical reasons. Reliable results were confined to the lower (nearly linear) portion of the light intensity—photosynthetic rate curve. With Ochromonas, even at saturating light intensities, accurate measurements of both respiration and photosynthesis were possible.

Another feature of Ochromonas which proved useful was the ease with which its respiratory rate could be reduced by starvation and subsequently enhanced by exogenous substrate. This permitted greater flexibility in the design of experiments to examine the kinetic interrelations between photosynthetic and respiratory metabolism, since rates of both processes were subject to experimental control over a considerable range.

Cells were cultured at 23° C in a defined medium (7) modified by the substitution of ammonium sulfate for ammonium citrate and calcium chloride for calcium carbonate. Light intensity was maintained at 25 ft-c supplied by fluorescent tubes and filtered through orange glass (Corning no. 348). Cultures containing 30 ml in 125 ml cotton stoppered Erlenmeyer flasks were aerated by shaking once daily. More vigorous aeration and higher light intensity were avoided because of evidence that such treatment prevents complete development of the photosynthetic apparatus (14).

After 4 to 5 days growth, cells were harvested by centrifugation at $500 \times G$: they were washed once with a solution containing the major minerals of the culture medium; finally they were resuspended in

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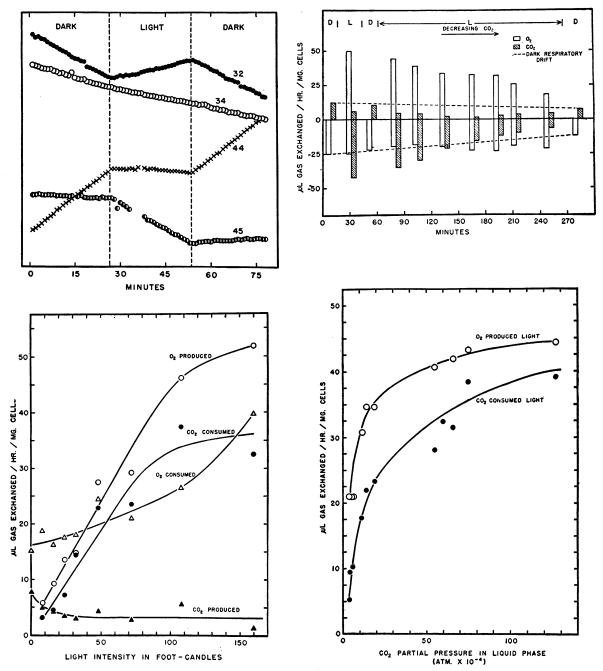


FIG. 1 (top, left). Example of mass spectrometric data for 2 isotopic forms of CO_2 (mass 44 and 45) and 2 of O_2 (mass 32 and 34). Ordinate: relative partial pressures of gas.

FIG. 2 (bottom, left). The effect of light intensity on gas exchanges by starved cells. Gas phase, $CO_2 : O_2 :$ He (2 : 3 : 95). Cells starved 24 hours.

FIG. 3 (top, right). The effect of decreasing concentration of CO_2 on gas exchanges by starved cells in the light. Red light from 250-watt tungsten lamp filtered through Corning no. 2403 red glass filter. Initial gas phase: CO_2 : O_2 : He (1 : 3 : 96). Cells starved 18 hours.

FIG. 4 (bottom, right). The effect of CO₂ partial pressure on O₂ production and CO₂ consumption by starved cells in the light. Experimental conditions as in figure 3.

0.02 M phosphate buffer (pH 5.5) and either used directly or starved prior to measurements of gas exchange. Starvation was carried out in the dark at 23° C, sterility being maintained throughout the starvation period.

All experiments were carried out in a rectangular reaction flask attached to the gas inlet system of a mass spectrometer. The bath in which the flask was immersed was at 28° C. The adaptation of the mass spectrometer for use in such experiments has been described (1) and the spectrometer leak housing to which the vessel was attached has been illustrated (8).

Light was supplied from a 250-watt tungsten filament projector lamp through appropriate columnating lenses and was introduced into the constant temperature bath to illuminate the reaction flask as described previously (2). Red light was obtained by placing a glass filter (Corning no. 2403) in the bath between the light source and the reaction flask.

In those experiments in which data on metabolic exchanges of CO2 were sought, CO2 enriched with respect to mass 45 ($C^{13}O_2$) was used. The isotopic O_2 was enriched with mass 34 (O¹⁶O¹⁸).

Dry weight was used as a measure of cell material. To calculate rate of gas exchange in tracer experiments it was necessary to correct for diffusion lag across the liquid-gas interface. The reason for this correction and the manner of making it was explained in the preceding paper (4).

At the beginning of an experiment the experimental suspension with appropriate addenda was pipetted into the reaction flask and the latter, attached to the mass spectrometer gas inlet assembly, was placed on a shaking device in the constant temperature bath. Isotopically enriched CO2 and O2 were introduced into the gas phase which was primarily He. Data were recorded continuously on the several isotopic forms of the metabolic gases. Computations of production and consumption rates of O_2 and CO_2 were made from results such as those of figure 1. In general the experimental procedures and methods of handling the data were essentially the same as have been described previously (4).

Results and Discussion

The metabolic relationships of interest are the effects on rates of CO_2 production and O_2 consumption

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EFFECTS OF STARVATION AND LIGHT ON THE RATE OF OXYGEN CONSUMPTION OF OCHROMONAS*

SUCCESSIVE PERIODS	UNSTARVED CELLS**	Starved cells**
Dark	35	15
Light Dark	33	28
Dark	34	17

* Starvation period, 24 hours dark. Gas phase, $O_2 : CO_2 : He (2:2:96)$.

** Units, $\mu l O_2$ consumed per mg dry wt per hour.

in the light brought about by starvation, by changes of light intensity, and by altering concentrations of O_2 and CO_2 in the milieu.

STARVATION: After starvation in the dark the endogenous respiratory rate was reduced. Addition of glucose enhanced the O2 consumption rate of starved cells up to the same level as that of unstarved cells. The respiratory rate of unstarved cells was found to be relatively insensitive to illumination whereas the rate of O₂ uptake by starved cells was nearly doubled by saturing light intensities. The light effect was reversible. These light relations are illustrated by the example given in table I. The data in the table were computed from experiments in which tracer O₂ was employed. Thus these data represent actual O₂ consumption rather than net O2 change.

With respect to CO₂ production, both starved and unstarved cells were observed to be light sensitive. A significant reduction in rate of respiratory output of CO₂ was induced by light in both cases. The 1st line in table II and figure 2 furnish examples of these effects. These data and all data to follow were taken from experiments in which both tagged O2 and tagged CO₂ were used and thus represent total rather than merely net rates.

We may think of respiration in terms of a flow of "substrate electrons" toward O2. Starvation may be considered to deplete the supply of endogenous respiratory substrate thus reducing the rate of electron transport. Should photosynthetic reductant compete with substrate electrons, such competition would be observed as a light induced deficit in the rate of CO₂ evolution. This evidence of competition was found with both starved and unstarved cells (table II). If. on the other hand, photosynthetic reductant only results in an increased rate of electron transport to O₂, no effect on CO₂ production would be expected; only the rate of O2 utilization would be enhanced. This latter effect was observed with starved cells in which the electron transport system presumably was not functioning at maximal capacity. Since, with unstarved cells, a light induced change in O2 consumption was not observed, it may be suggested that the electron transport system already was operating at full capacity in the dark; addition of further reductant (of photochemical origin) could not produce an increase pro-

TABLE II

EFFECTS OF LIGHT ON GAS EXCHANGES OF OCHROMONAS *

	Unstarved cells**	Starved cells**
Deficit in CO_2 evolution Enhancement of O_2 uptake CO_2 consumption	$-{16 \\ -1 \\ 23}$	7 8 27
O ₂ production	38 36	42 45

* Same conditions as for table I. ** Units, μ l mg⁻¹ hr⁻¹.

vided the point of influence lay below (with respect to O_2) whatever redox reaction was rate limiting. A direct photochemical reduction of O_2 itself thus seemed improbable in the case of Ochromonas, as this could occur regardless of the degree of saturation of the electron transport mechanism by substrate electrons.

LIGHT INTENSITY: Using starved cells, since photo-effects on respiration were found to be optimal in them, the production and consumption rates of O_2 and CO_2 were studied over a light intensity range from darkness to light saturation (fig 3). Production of O_2 and uptake of CO_2 proceeded more or less linearly with light intensity below saturation. Respiratory CO_2 production was affected at even the lowest light intensities and an inhibition in the neighborhood of 30 to 50 % was established even at a light intensity approximating O_2 compensation. Above this relatively low light intensity no further reduction of CO_2 evolution occurred.

A very different pattern was found for O₂ consumption. At light intensities up to about compensation for O₂, light failed to exert a marked effect. Above that compensation point, an increasing photostimulation of O₂ uptake rate was observed. This acceleration approached 100 % at light saturation and was then about equivalent to the maximal stimulation of O₂ uptake rate which was observed when glucose was supplied to starved cells.

Over the entire range of light intensities the photosynthetic quotient was somewhat above unity although excess of O₂ production over CO₂ consumption became more pronounced only at the higher intensities. That even slight photosynthetic activity depressed the CO₂ evolution rate implies that light generated reductant can compete very favorably with substrate electrons, maintaining nearly the same overall rate of O2 reduction with photochemical reductant in lieu of substrate electrons. A maximal (ca. 50 %) photoinhibition of CO₂ production was achieved by rather little light and was not increased by several-fold further increase of intensity. This suggests a dual source of respiratory CO2. Such behavior would be expected of a system having 2 mechanisms for CO₂ evolution with approximately equal functioning capacity, if one is directly accessible to a depressing influence of photosynthetic reactions and the other is not. Respiratory CO₂ production by 2 chemically distinct pathways may be involved. Alternatively intra- and extra-chloroplastic systems of CO2 evolutions may operate in Ochromonas.

The fact that light of low intensity had little effect on the O_2 consumption rate yet, at higher intensity, considerable stimulation occurred can be interpreted as evidence for 2 types of interaction between the photosynthetic reductant and the respiratory process. For the light generated reductant to affect the rate of O_2 consumption it must react, if not with O_2 directly (which was considered improbable), then with a member of the coenzyme-carriers-oxidase system at some point beyond (on the way toward O_2) the rate limiting reaction step in the overall redox sequence (b in the example below, where components of the electron transport system are referred to only by symbols since they remain unidentified in Ochromonas. Photosynthetic reductant is represented by [H]).

$$A \xrightarrow{a} B \xrightarrow{b}_{rate limiting} C \xrightarrow{c} D \xrightarrow{d} O_{2}$$

$$[H] \qquad [H]$$

If photosynthetic reductant competes with the transport of substrate electrons at reaction a, depression of CO₂ evolution would occur without the rate of O₂ uptake being enhanced, because the point of competition is below the rate limiting step. This is characteristic of the effects observed at low light intensity where the predominating influence is on CO₂ production. If photosynthetic reductant competes with substrate electrons at some point beyond b, the later stages of the transport sequence could react still more rapidly and an increased rate of O₂ uptake would result. This is characteristic of the effects observed at the higher light intensities.

The above suggestion provides a unified explanation for 2 apparently diverse effects of light on Ochromonas respiration. At both high and low light intensities the postulated influence is one of competition between substrate electrons and photosynthetic reductant for components of the respiratory electron transport system. The different manifestations of this competition at different light intensities arise because the predominant influence is in one case below—in the other case also above—the rate limiting redox reaction. This second point of interaction comes into play only at high light intensity.

PHOTOSYNTHETIC QUOTIENT: With increasing light intensity the quotient, $+O_2/-CO_2$, was found always to increase. Since the rate of photochemical reductant generation increases with increasing light intensity, correlation between increased photosynthetic quotient and higher light intensity suggests that the CO_2 is in some kind of indirect competition with an alternative oxidant such as the respiratory electron transport system. By depleting CO₂, a greater fraction of the photochemical reductant should be accounted for not in photosynthesis (CO2 utilization) but by the alternative fate of reducing components of the respiratory system. Figure 3 shows the results from an experiment in which CO2 tension in the experimental vessel was gradually depleted by photosynthesizing Ochromonas cells. Respiratory drift was apparent as the dark O2 uptake declined with time (onset of starvation). However, the rate of O₂ consumption in the light did not change significantly. Therefore a light stimulation of respiration (effect of photochemical reductant reducing the electron transport system rather than CO₂) was observed and was greater the lower the CO₂ tension.

A series of experiments were carried out in order to demonstrate in a more definitive manner the competition between the oxidants, CO_2 and O_2 . It was found, unexpectedly, that from 1 to 2 % CO_2 was required in order that photosynthesis in starved Ochromonas cells proceed without CO2 limitation. A quotient, $+O_2/-CO_2$, of about 1.1 usually was observed. As shown in figure 4, at 10×10^{-3} atmospheres dissolved CO₂ the quotient was 1.2 to 1.4; at 2 \times 10⁻³ atmospheres it was 1.6; at 1 \times 10⁻³ atmospheres it rose to 4.2. For technical reasons the data taken at lower CO2 tensions are less reliable, but an obvious trend is revealed in figure 4. The lower the concentration of CO₂, the higher the photosynthetic quotient. A greater fraction of photosynthetic reductant is not involved in CO2 assimilation but reacts with the respiratory system to enhance O2 consumption or to depress CO₂ production in accordance with the model employed. In this sense a competition is revealed between CO2 and the respiratory system of Ochromonas.

It was noted earlier that the model used here to explain the several interactions between light generated reductant and respiration demands, under all conditions in the light, that O_2 production rate, P_{O_2} , should be equal to the sum : deficit in rate of CO_2 evolution, ΔP_{CO_2} , enhancement of O_2 uptake rate, ΔU_{O_2} , and the rate of CO_2 utilization, U_{CO_2} .

 $\Delta P_{CO2} + \Delta U_{O2} + U_{CO2} = P_{O2}$ (1) Throughout this study where the above 4 quantities were determined, equation 1 was found valid within experimental error. Examples of the equivalence are noted in table II.

The readiness with which reductant of photochemical origin exerts an influence could be an immediate result of respiratory production and photosynthetic consumption of CO_2 being mediated by the same enzyme system or by bound enzymes in close juxtaposition.

SUMMARY

Gas exchanges of Ochromonas malhamensis were studied in dark and in light using a recording mass spectrometer to analyze the partial pressure changes of isotopically enriched CO2 and O2 within the experimental vessel. Simultaneous production and consumption rates of both CO2 and O2 were determined. Light intensity, partial pressure of CO₂, and state of nutrition of the cells were varied. At light intensities below compensation, illumination had slight influence on rate of O2 consumption; at higher intensities uptake was stimulated. CO2 production was inhibited even at very dim light but with increasing intensity no further depression of CO2 production rate occurred. Light had a more pronounced effect on respiration in starved cells than in cells with ample endogenous substrate. Quantitatively the behavior of Ochromonas was consistent with a model which accounts for an influence of light on respiration mediated by a photochemically generated reductant. At low light the O2 consumption rate was maintained while the photosynthetic reductant competes with reductant of respiratory origin. At higher light a second type of interaction enhancing the O2 uptake rate, was superimposed on the first effect.

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LITERATURE CITED

- BROWN, A. H. The effects of light on respiration using isotopically enriched oxygen. Amer. Jour. Bot. 40: 719-729. 1953.
- BROWN, A. H. and GOOD, N. Photochemical reduction of oxygen in chloroplast preparations and in green plant cells. I. The study of oxygen exchanges in vitro and in vivo. Arch. Biochem. Biophys. 57: 340-354. 1955.
- BROWN, A. H. and WEBSTER, G. C. The influence of light on the rate of respiration of the blue-green alga, Anabaena. Amer. Jour. Bot. 40: 753-759. 1953.
- BROWN, A. H. and WEIS, D. S. Relation between respiration and photosynthesis in the green alga, Ankistrodesmus braunii. Plant Physiol. 34: 224-234. 1959.
- DECKER, J. P. A rapid, postillumination deceleration of respiration in green leaves. Plant Physiol. 30: 82-84. 1955.
- DECKER, J. P. Further evidence of increased carbon dioxide production accompanying photosynthesis. Jour. Solar Energy Science and Eng. 1: 30-33. 1957.
- HUTNER, S. H., PROVASOLI, L., and FILFUS, J. Nutrition of some phagotrophic fresh-water chrysomonads. Ann. New York Acad. Sci. 56: 852-862. 1953.
- JOHNSTON, J. A. and BROWN, A. H. The effect of light on the oxygen metabolism of the photosynthetic bacterium, *Rhodospirillum rubrum*. Plant Physiol. 29: 117-182. 1955.
- MYERS, J. and GRAHAM, JO-RUTH. The role of photosynthesis in the physiology of Ochromonas. Jour. Cell. and Comp. Physiol. 47: 397–414. 1956.
- PRINGSHEIM, E. G. On the nutrition of Ochromonas. Quart. Jour. Microscop. Sci. 93: 71-96. 1952.
- REAZIN, G. H., JR. On the dark metabolism of a golden-brown alga, Ochromonas malhamensis. Amer. Jour. Bot. 41: 771-777. 1954.
- VISHNIAC, W. and REAZIN, G. H., JR. Photoreduction in Ochromonas malhamensis. In: Research in Photosynthesis, H. Gaffron, ed. Pp. 239-242. Interscience, New York 1957.
- WEIGL, J., WARRINGTON, P. M. and CALVIN, M. The relation of photosynthesis to respiration. Jour. Amer. Chem. Soc. 73: 5058-5063. 1951.
- WEIS, D. S. The relation of photosynthesis to respiration in Ochromonas malhamensis. Ph. D. Thesis, Yale University, New Haven 1955.