

KINETIC RELATIONSHIPS BETWEEN PHOTOSYNTHESIS AND RESPIRATION IN THE ALGAL FLAGELLATE, *OCHROMONAS MALHAMENSIS*¹

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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 (+CO₂/−O₂) would be decreased and the photosynthetic quotient (+O₂/−CO₂) would be increased. The rate of photosynthetic O₂ 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 O₂ produced in light to the sum: CO₂ consumed + light-induced extra O₂ uptake + light-induced deficit in respiratory CO₂ 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*.

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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 × G; they were washed once with a solution containing the major minerals of the culture medium; finally they were resuspended in

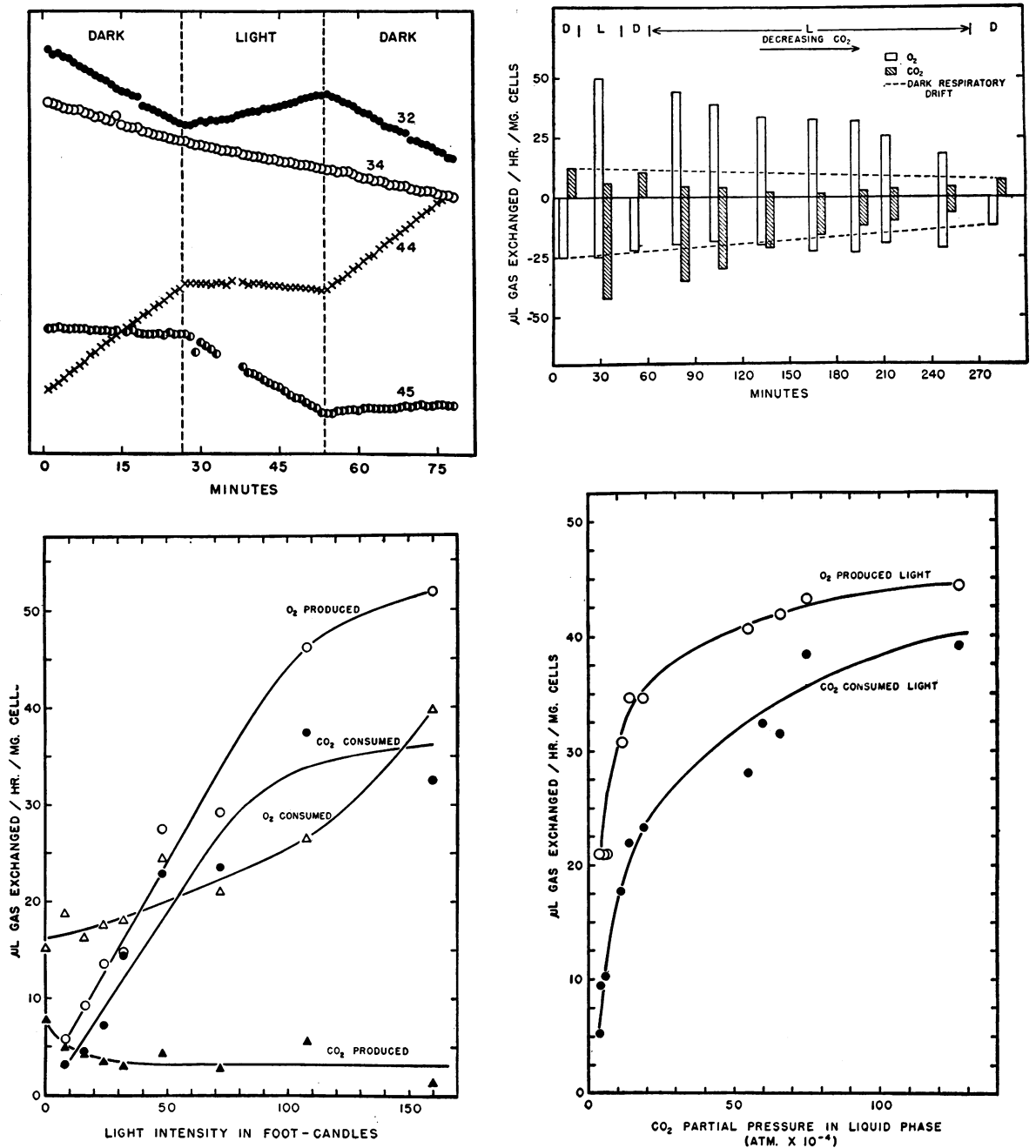


FIG. 1 (top, left). Example of mass spectrometric data for 2 isotopic forms of CO₂ (mass 44 and 45) and 2 of O₂ (mass 32 and 34). Ordinate: relative partial pressures of gas.

FIG. 2 (bottom, left). The effect of light intensity on gas exchanges by starved cells. Gas phase, CO₂ : O₂ : He (2 : 3 : 95). Cells starved 24 hours.

FIG. 3 (top, right). The effect of decreasing concentration of CO₂ 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: CO₂ : O₂ : 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 (C¹³O₂) was used. The isotopic O₂ was enriched with mass 34 (O¹⁸O₂).

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 O₂ and CO₂ 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 CO₂ production and O₂ consumption

in the light brought about by starvation, by changes of light intensity, and by altering concentrations of O₂ and CO₂ 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 saturating 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 I

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

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

* Starvation period, 24 hours dark. Gas phase, O₂ : CO₂ : He (2 : 2 : 96).

** Units, μl O₂ consumed per mg dry wt per hour.

TABLE II

EFFECTS OF LIGHT ON GAS EXCHANGES OF OCHROMONAS *

	UNSTARVED CELLS**	STARVED CELLS**
Deficit in CO ₂ evolution	16	7
Enhancement of O ₂ uptake	— 1	8
CO ₂ consumption	23	27
	38	42
O ₂ production	36	45

* Same conditions as for table I.

** Units, μl mg⁻¹ hr⁻¹.

quired in order that photosynthesis in starved *Ochromonas* cells proceed without CO₂ limitation. A quotient, +O₂/-CO₂, 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 × 10⁻³ atmospheres it was 1.6; at 1 × 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 O₂ production rate, P_{O₂}, should be equal to the sum: deficit in rate of CO₂ evolution, Δ P_{CO₂}, enhancement of O₂ uptake rate, Δ U_{O₂}, and the rate of CO₂ utilization, U_{CO₂}.

$$\Delta P_{CO_2} + \Delta U_{O_2} + U_{CO_2} = P_{O_2} \quad (1)$$

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 CO₂ 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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