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.

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 Filis (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 photorespiration 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 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 batches were aerated 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 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 CO$_2$ partial pressure on O$_2$ production and CO$_2$ 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 collimating 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 (C⁵O₂) was used. The isotopic O₂ was enriched with mass 34 (O³⁴O⁰⁻).

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**

<table>
<thead>
<tr>
<th>Successive periods</th>
<th>Unstarved cells**</th>
<th>Starved cells**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>Light</td>
<td>33</td>
<td>28</td>
</tr>
<tr>
<td>Dark</td>
<td>34</td>
<td>17</td>
</tr>
</tbody>
</table>

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

---

**Table II**

<table>
<thead>
<tr>
<th></th>
<th>Unstarved cells**</th>
<th>Starved cells**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficit in CO₂ evolution</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Enhancement of O₂ uptake</td>
<td>− 1</td>
<td>8</td>
</tr>
<tr>
<td>CO₂ consumption</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>O₂ production</td>
<td>38</td>
<td>42</td>
</tr>
</tbody>
</table>

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

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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 \( O_2 \) consumption. At light intensities up to about compensation for \( O_2 \), light failed to exert a marked effect. Above that compensation point, an increasing photosimulation of \( O_2 \) uptake rate was observed. This acceleration approached 100 % at light saturation and was then about equivalent to the maximal stimulation of \( O_2 \) 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 \( O_2 \) production over \( CO_2 \) consumption became more pronounced only at the higher intensities. That even slight photosynthetic activity depressed the \( CO_2 \) evolution rate implies that light generated reductant can compete very favorably with substrate electrons, maintaining nearly the same overall rate of \( O_2 \) reduction with photochemical reductant in lieu of substrate electrons. A maximal (ca. 50 %) photoinhibition of \( CO_2 \) 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 \( CO_2 \). Such behavior would be expected of a system having 2 mechanisms for \( CO_2 \) evolution with approximately equal functioning capacity, if one is directly accessible to a depressing influence of photosynthetic reactions and the other is not. Respiratory \( CO_2 \) production by 2 chemically distinct pathways may be involved. Alternatively intra- and extra-chloroplastic systems of \( CO_2 \) evolution 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]).

![Diagram](https://via.placeholder.com/150)

If photosynthetic reductant competes with the transport of substrate electrons at reaction a, depression of \( CO_2 \) evolution would occur without the rate of \( O_2 \) 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 predominate influence is on \( CO_2 \) 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 \( O_2 \) 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, \(+ O_2 /- CO_2 \) 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 \( CO_2 \), a greater fraction of the photochemical reductant should be accounted for not in photosynthesis (\( CO_2 \) utilization) but by the alternative fate of reducing components of the respiratory system. Figure 3 shows the results from an experiment in which \( CO_2 \) tension in the experimental vessel was gradually depleted by photosynthesizing Ochromonas cells. Respiratory drift was apparent as the dark \( O_2 \) uptake declined with time (onset of starvation). However, the rate of \( O_2 \) 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 \( CO_2 \)) was observed and was greater the lower the \( CO_2 \) 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 re-
quired in order that photosynthesis in starved Ochro-
monas cells proceed without CO₂ limitation. A quoti-
et, +O₂/-CO₂, of about 1.1 usually was observed.
As shown in figure 4, at 10 × 10⁻¹ atmospheres dis-
solved CO₂ the quotient was 1.2 to 1.4; at 2 × 10⁻¹
atmospheres it was 1.6; at 1 × 10⁻¹ atmospheres it rose
0.4. 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 concentra-
tion of CO₂, the higher the photosynthetic quotient.
A greater fraction of photosynthetic reductant is not
involved in CO₂ assimilation but reacts with the res-
piratory system to enhance O₂ consumption or to de-
press CO₂ production in accordance with the model
employed. In this sense a competition is revealed
between CO₂ and the respiratory system of Ochro-
monas.

It was noted earlier that the model used here to
explain the several interactions between light gener-
ated reductant and respiration demands, under all
conditions in the light, that O₂ production rate, P₀₂,
should be equal to the sum: deficit in rate of CO₂
evolution, ΔP₀₂, enhancement of O₂ uptake rate,
ΔU₀₂, and the rate of CO₂ utilization, U₀₂:

\[ \Delta P_{O_2} + \Delta U_{O_2} + U_{CO_2} = P_{O_2} \]  
(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 photo-
chemical origin exerts an influence could be an im-
mediate result of respiratory production and photo-
synthetic 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 ex-
perimental vessel. Simultaneous production and con-
sumption 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 sub-
strate. Quantitatively the behavior of Ochromonas
was consistent with a model which accounts for an
influence of light on respiration mediated by a photo-
chemically generated reductant. At low light the O₂
consumption rate was maintained while the photo-
synthetic reductant competes with reductant of respira-
tory origin. At higher light a second type of inter-
action enhancing the O₂ uptake rate, was superimposed
on the first effect.

Support from the Kettering Foundation in the
form of a fellowship for one of us (D.W.) is grate-
fully acknowledged.

This work was aided by a contract between the
Office of Naval Research, Department of the Navy,
and the University of Minnesota (NR104-030) and
also was supported by a grant from the Graduate
School.

The authors are grateful for the isotopically en-
riched O₂ which was supplied by Prof. A. O. Nier.
It was prepared under a grant from the American
Cancer Society through the Committee on Growth
of the National Research Council.

Some of the work reported here was taken from
a thesis presented by one of us (D.W.) in partial
fulfillment of requirements of the Ph. D. degree, Yale
University, 1955.

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