$$\left. \frac{\nu_1 v_f}{e_1 k_1 - \nu_1 v_f} \exp(-e_1 k_1 t/v_f) \right]$$

if ν is known for both ρ_1 and ρ_2 we should have 2 equations

$$G_1 + G_2 = \frac{R_1}{K_1} a + \frac{R_2}{K_2} \beta$$

$$g_1 + g_2 = \frac{R_1}{k_1} a' + \frac{R_2}{k_2} \beta'$$

where a, β, a', and β' stand for the respective bracketed terms. These equations can be solved for R_1 and R_2. If ρ_1 and ρ_2 vary with time in an unknown way then a, β, etc. cannot be evaluated and we cannot solve the equations.

If the lag due to resistance to interchange between gaseous and liquid phases is ignored, that is a, etc. are assumed to be unity, and the value for ρ_1 calculated as c_1.

$$c_1 = \frac{K_2(G_1 + G_2) - k_2(g_1 + g_2)}{K_2/K_1 - k_2/k_1}$$

$$\text{then } c_1 = \frac{R_1(aK_2/K_1 - a'k_2/k_1) + R_2(\beta - \beta')}{K_2/K_1 - k_2/k_1}$$

If subsequent to illumination oxygen production proceeded at a steady rate ρ_1 and carbon dioxide was consumed at the same steady rate, that is $\rho_2 = -\rho_1$, then R_1 and R_2 in the above equation are replaced by ρ_1 and $-\rho_1$ and

$$a = \left\{ 1 - \exp(-E_1 K_1 t/v_F) \right\}$$

$$a' = \left\{ 1 - \exp(-e_1 k_1 t/v_f) \right\}$$

$$\beta = \left\{ 1 - \exp(-E_2 K_2 t/v_F) \right\}$$

$$\beta' = \left\{ 1 - \exp(-e_2 k_2 t/v_f \right\}$$

and c_1 would be an underestimate of ρ_1 at least during the early stages of equilibration lag. If the vessels were paired for oxygen (1) then $a = a'$ and

$$c_1 = \rho_1 \left\{ a - \frac{\beta - \beta'}{K_2/K_1 - k_2/k_1} \right\}$$

If, however, there was a big enough burst of CO_2 during this period then c_1 could exceed β_1. That is there could be an apparent burst of oxygen.

Sufficient has been said to show that estimates of rates of gas production from manometer readings over a relatively short period, subsequent to a change of conditions which result in a change in rate, can be misleading.

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RELATION BETWEEN RESPIRATION AND PHOTOSYNTHESIS IN THE GREEN ALGA, ANKISTRODESMUS BRAUNII[1]

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In what way does light affect the respiration of photosynthetic cells? In more than a century since the reaction of photosynthesis was identified in its overall aspect this question has been asked repeatedly. The earliest concern was a purely kinetic one in that "apparent" photosynthesis required correction for respiration if "true" photosynthetic rates were to be calculated and measured as a function of environmental parameters. The possible influence of light on the photosynthetic rate again became of paramount importance when quantum yield determinations were made on photosynthesis beginning some 30 years ago. Encouraged by what indirect evidence was available, investigators of photosynthetic efficiency generally have come to rely on the assumption that, if rates of dark metabolism were the same before and after a light period, then during illumination this same respiratory rate could be used for correcting the rate of apparent photosynthesis.

Less than 20 years ago the basic question was posed not in kinetic but in chemical terms. Thimann (18), in a speculative note, asked in effect if photosynthesis could not be the reverse of respiration in respect to its intrinsic mechanism as well as in its overall equation. Since then our rapidly expanding biochemical knowledge of respiratory pathways has several times called for a rephrasing of the question. The existence of chemical intermediates common to both photosynthetic CO_2 reduction and to glycolysis and respiration made it difficult to feel complacent about their kinetic independence. Also, within the last decade, has come the recognition that at least some coenzymes and electron carriers, ubiquitous in the catabolic schemes which modern biochemistry uses as working models, may be

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photochemically reduced by preparations from green cells and furthermore that the pyridine nucleotide pools of chemical potential energy, so interrelated with anaerobic and aerobic stages of respiration, are subject to similar photochemical regeneration. All this makes it seem rather unlikely that strict kinetic independence of respiratory and photosynthetic gas exchanges should in most cases obtain, and the kineticists' assumption that respiration and photosynthesis proceed almost without reciprocal influence (except in relatively long term experiments) is hardly to be anticipated from modern biochemical considerations. In any case, the opposing processes of respiration and photosynthesis cannot very well employ potentially rate controlling reaction steps in common without there being a most intimate dependence of the rate of the one on that of the other. Should this dependence not be observed, we are almost compelled to assume either that the mechanisms are truly chemically independent or that they chiefly operate in separate morphological compartments of the cell. Since we are dealing with systems which are heterogeneous in both gross and fine aspects, perhaps whatever kinetic independence of photosynthesis and respiration may be observed is but the fortuitous consequence of a cellular morphology which confines the photosynthetic apparatus to organelles which account for only a minor fraction of the cell's respiratory activity.

Whether or not biochemical compartmentalization on morphological grounds needs to be invoked can only be determined from kinetic observations, and the basic question—to what extent does light affect the respiratory rate of photosynthetic cells?—is one to be settled only by quantitative observations. Also, physiological variation between species being what it is, an answer to the question for one plant may not form a secure basis of generalization.

Recognizing that in the respiratory process as well as in photosynthesis gas exchange quotients may depart from unity, it seems that an unequivocal answer, if it can be given at all, must be provided with respect to both metabolic gases simultaneously. Also, if the answer is to be direct, it appears that the only method for simultaneous measurement of production and consumption of a gas within a single experimental system is by the employment of isotopic tracers.

The essential contribution made possible by the use of tracers is just this separation of concurrent processes of consumption and production of the same gas. Throughout the present paper, when we speak of gas *consumption*, we are referring to that process alone without regard to whether the same gas also is being produced. Similarly *production* refers to the evolution of gas whether or not gas uptake occurs as well. These terms thus do not have here the overall characteristic which is conventional in considerations of *net* gas change.

Some tracer work having a direct bearing on this matter already has been published. It was shown in this laboratory, for *Chlorella pyrenoidosa* Chick. and for some other photosynthetic species, that illumination at low to moderate intensities had only minor effects on the endogenous respiratory rate insofar as O_2 was concerned (2). These results, obtained under a necessarily limited range of experimental conditions, strongly suggested that respiratory and photosynthetic processes functioned quite independently. Nevertheless evidence from tracer studies was by no means unilateral. In certain species, cases of extreme photoinhibition or of moderate photostimulation of tracer O_2 uptake were observed (4, 13). Results from radiocarbon labelling experiments by Calvin and associates (1, 5) were interpreted as evidence that the Krebs cycle does not operate in bright light and, from kinetic measurements with tracer CO_2 (21), it was concluded that CO_2 evolution was reduced by intense light to about one half its value in darkness. This latter finding was of special interest when contrasted with measurements using tagged O_2 (19) on the same kind of plant (barley) and under rather similar conditions; there a marked photoinhibition of O_2 was not observed.

While the various pieces of direct experimental evidence are somewhat fragmentary, it is tempting nevertheless to assume that CO_2 production is mediated in photosynthetic cells by oxidative decarboxylations such as occur in the Krebs cycle or the pentose phosphate cycle, to accept at face value the purported photoinhibition of CO_2 production and to reconcile the reduced CO_2 evolution with the relatively unchanged O_2 consumption by proposing a substitution, during the light, of photochemically generated reductant for the "substrate hydrogen" which serves alone to reduce O_2 in dark metabolism. Speculating that photosynthesis and respiration are related in this manner, we may examine the salient features of a general scheme illustrating the postulated interrelation and determine what consequences of its operation ought to be observable. Such a model is shown in figure 1. Much more detailed schemes could be employed suggesting possible interplay between breakdown and synthesis through, let us say specifically, control of the DPN^+/DPNH ratio (leading to enhanced respiratory activity in the light), or through a downward adjustment of the ADP/ATP ratio by photophosphorylation (to produce respiratory inhibition), or perhaps even more directly via regulation of the concentration of a rate limiting common intermediate such as phosphoglyceric acid. However, our limited kinetic as well as biochemical information about the systems with which we are dealing hardly justifies a model more detailed than is necessary for illustrative purposes. The operation of the model (fig 1) is as follows:

If no interrelations between respiratory and photosynthetic metabolism are postulated except those arising from the existence of a common pool of compounds which collectively represent the ultimate respiratory substrate and at the same time the ultimate sink for the organic products of photosynthesis, then only the solid lines in figure 1 apply. Respiration and photosynthesis are seen as independent processes. Photochemically produced reductant is then accounted for quantitatively by CO_2 assimilation (reaction 1). On

the other hand, if photochemically generated reductant is capable of reducing directly (or, more likely, after its reducing potential has been degraded to more or less the appropriate level) any of the respiratory components as indicated by the dashed lines, then photosynthesis must, at least under some conditions, affect respiration. If the reductant, generated photochemically, is capable of reducing O_2 directly (reaction 2), this would tend to increase O_2 consumption in the light but would not alter the rate of respiratory CO_2 production. If the reductant can react directly with one of the cytochrome chain components (reaction 3), it would tend to stimulate O_2 consumption via the respiratory oxidase system. If the carrier-oxidase chain were running at capacity, substitution of photochemically produced reductant for the "substrate hydrogen" normally serving as respiratory reductant would necessitate a decrease in the rate of oxidative decarboxylation. In consequence CO_2 production would be reduced. If, by photochemical reduction (reaction 4), the pyridine nucleotide redox potential should be forced so low that coenzyme molecules cannot easily be loaded with "respiratory" electrons, similar inhibition of CO_2 evolution (accompanied by unaltered or even stimulated O_2 consumption) would be expected. If the glycolytic intermediate, phosphoglyceric acid, were to be reduced directly (reaction 5), conceivably this could limit the supply of pyruvate to the Krebs cycle so that, in strong light, slowing down of the cycle might lead to reduced O_2 uptake as well as a proportionate inhibition of CO_2 production.

Figure 1 is of course not the only model which might be suggested to illustrate possible light effects on respiratory processes. However other models lead to the same or similar expectations. They have in common the feature that, if photosynthesis and respiration are related through the photochemically produced reductant, this relation may become apparent either as a light induced deficiency in respiratory CO_2 production or as enhancement of O_2 consumption or both. Assuming for convenience that the quotients of both respiration and photosynthesis normally are unity, then the rate of photochemical generation of reductant corresponds stoichiometrically to the rate of O_2 evolution. The operation of any of the reactions 2, 3, 4, or 5 in figure 1 will account for the fate of some of the reductant by a path other than CO_2 reduction (reaction 1). The rate of photosynthetic reduction of CO_2 would diminish and the respiratory portion of the gas exchange would be altered—effects which would be detectable as enhanced O_2 consumption or reduced CO_2 production or both. These effects should bear a stoichiometric relation to the inhibition of photosynthetic CO_2 uptake rate.

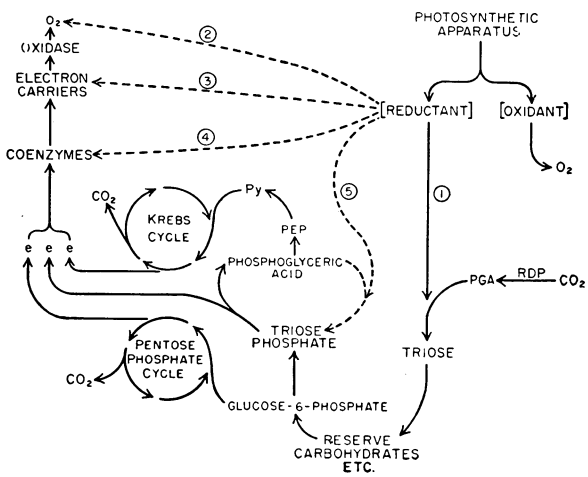


FIG. 1. Schematic representation of possible influences on respiratory processes by means of a reductant produced by a partial reaction of photosynthesis.

If, instead of a reductant, it is the photochemically produced oxidant (vide Gaffron, (10)) which is postulated as influencing the respiratory rate, any component with which it reacts—directly or indirectly—must become oxidized, and in such a model, analogous with figure 1, it is readily shown that the influence of light would be to depress both production and consumption of O_2 and to accelerate CO_2 evolution—changes in respiratory gas exchange which are opposite from those predicted above. Only when O_2 uptake *and* CO_2 *evolution* remain unchanged in the light can we conclude that photosynthesis and respiration are truly kinetically independent.

What has been said in qualitative terms about the interrelations suggested by figure 1 may be made quantitative as follows: Let P_{CO_2} and U_{CO_2} designate respectively rates of production and uptake of CO_2. Similarly P_{O_2} and U_{O_2} are rates of production and consumption of O_2. In the light an increase in O_2 uptake rate as compared with the dark value is referred to as $\Delta\ U_{O_2}$ and a decrease in CO_2 evolution as $\Delta\ P_{CO_2}$. For every 4 units (electron equivalents) of photo-reductant formed in the light there must be generated 4 units of oxidant corresponding to the production of 1 O_2 unit. The 4 units of reductant may be accounted for by the uptake of 1 unit of CO_2, or by the enhancement of U_{O_2} to the extent of 1 unit, or by the depression of P_{CO_2} to the extent of 1 unit, or by any combination of U_{CO_2}, $\Delta\ P_{CO_2}$, and $\Delta\ U_{O_2}$ which adds up to 1 unit. Thus in the light according to our model,

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

Another way of looking at the matter is to say that only as long as equation 1 is obeyed will the chemical reduction level of the cell remain unaltered.

The purpose of the present study was to test the validity of equation 1 by evaluating its 4 component terms simultaneously for each of a series of experiments. These experiments were carried out over a range of light intensities both above and below photosynthetic compensation. Agreement of the data with equation 1 implies consistency with the general model shown in figure 1.

Materials and Methods

In a series of contributions from this laboratory, respiration rates of photosynthetic cells have been measured in the light by a method which employed tracer O_2 in the gas phase of the experimental vessel. By thus tagging the environmental O_2 consumed by the cells, the rate of O_2 uptake was measured independently of the photosynthetic production of unlabelled O_2 from water. In principle, analogous experiments can be performed with tracer CO_2. However, in preliminary studies carried out several years ago, CO_2 isotope exchange in the dark was observed to be of such a magnitude that large corrections for this exchange were required (18). The uncertainty of these corrections rendered the interpretation of results obtained with tracer CO_2 much more difficult than in the case of tracer O_2 experiments where isotope exchange in the dark was not detectable under the conditions prevailing in biological experiments. Numerous studies carried out subsequent to our earlier publications and on divers photosynthetic species have demonstrated that CO_2 isotope exchange is not as serious a drawback to the use of the tracer method as we at first thought, for it has been found that the earlier results showing excessive CO_2 exchange were atypical; high exchange rates have since been the exception rather than the rule. In short, we usually are able to effect a kinetic separation of simultaneous CO_2 uptake and production without prohibitive corrections for isotope exchange. Fortuitously, certain second order corrections which apply to such isotope data (to be described below) are even of much smaller magnitude with tracer CO_2 than with tracer O_2 measurements.

The organism chosen for the present work was *Ankistrodesmus braunii* (Naegeli) Collins. This is a green alga similar morphologically and physiologically to the genus, Scenedesmus. The cells were cultured autotrophically in medium V of Norris et al (15) at 26 to 29° C. Culture flasks, illuminated from below by a bank of white fluorescent lights with a red-orange filter (Corning, no. 348) were agitated by a stream of air enriched with 5 % carbon dioxide and by occasional shaking. Cultures were harvested after 3 to 5 days while the growth rate was still high. Cells collected by slow centrifugation (< 1000 G) were washed once and were resuspended in media to be used in the experiment. Ordinarily this was 0.05 M potassium phosphate buffer (pH 5.8) containing 2 % glucose.

A Nier-Consolidated mass spectrometer (Model 21-201) continuously monitored the gas phase of the reaction vessel which was a rectangular Warburg-type vessel of 15.8 ml total volume when attached to the joint which was in turn connected to the mass spectrometer inlet leak. The design of this joint and the method of shaking the vessel in a constant temperature water bath did not differ in any essential manner from what was illustrated previously (13). The use of the mass spectrometer as adapted for the required measurements also has been described (3). Labelled O_2 and labelled CO_2 were introduced initially into the gas phase of the experimental vessel and continuous monitoring of the gas phase with the mass spectrometer was carried on thereafter.

When cells were suspended in inorganic buffer only, the respiratory rate was at first high but declined over several hours to a small fraction of the initial rate. Addition of 2 % glucose to the experimental medium prevented this decline. Since the smaller the respiratory rate the greater was the percent error in its measurement, data on glucose stabilized respiration was more reliable than were measurements on endogenous gas exchange. Also it was convenient to work with cells whose dark metabolism was not changing greatly with time. Therefore, critical light-dark comparisons were made only with cell suspensions containing glucose.

For present purposes it is necessary to reconsider the principles involved in the tracer method of measuring independently both uptake and output of a given gas. Whether O_2 or CO_2 is considered, the presence of isotopically enriched gas in the environment insures that gas produced will have an isotopic composition different from that of gas consumed. If isotopic enrichment is large, it is possible to ignore the influence of the small natural concentrations of rare isotopes of O_2 and of CO_2. It is readily seen that the actual rates of production, P, and of consumption, U, are functions of the rates of metabolism of both isotopes. These "true" rates are given by the equations,

$$P = h\ (R - R^* \frac{M}{M^*}) \qquad (2)$$

$$U = h\ R^*\ (1 + \frac{M}{M^*}) \qquad (3)$$

where R and R* are rates of change of the 2 isotopic forms of the gas being metabolized, M and M* are the mean concentrations of the respective isotopes which prevail during the period of measurement at the site of gas consumption, and h is a dimensional constant. While it is approximately correct to consider U a linear function of R*, as was done in some of the earlier work from this laboratory, when only relative rates of gas exchange are required, it should be kept in mind that this simplification is valid only as long as M/M* remains essentially constant during the experiment. This ratio, measured in the gas phase, can be expected to remain adequately constant only if the total tension of the gas is relatively high and the rate of change of either isotopic form of the gas relatively low.

Furthermore, it is the ratio, M/M*, at the site of reaction which is critical rather than the ratio which prevails in the gas phase where the mass spectrometer can measure it. Under any single set of steady state conditions a difference in this ratio on passing from the gas phase to the reaction site is of minor consequence for relative measurements. But, if it is necessary to compare data taken under different steady state conditions (viz. light vs. darkness), the M/M* ratio at the reaction site can be appreciably different under the separate conditions. With photosynthesiz-

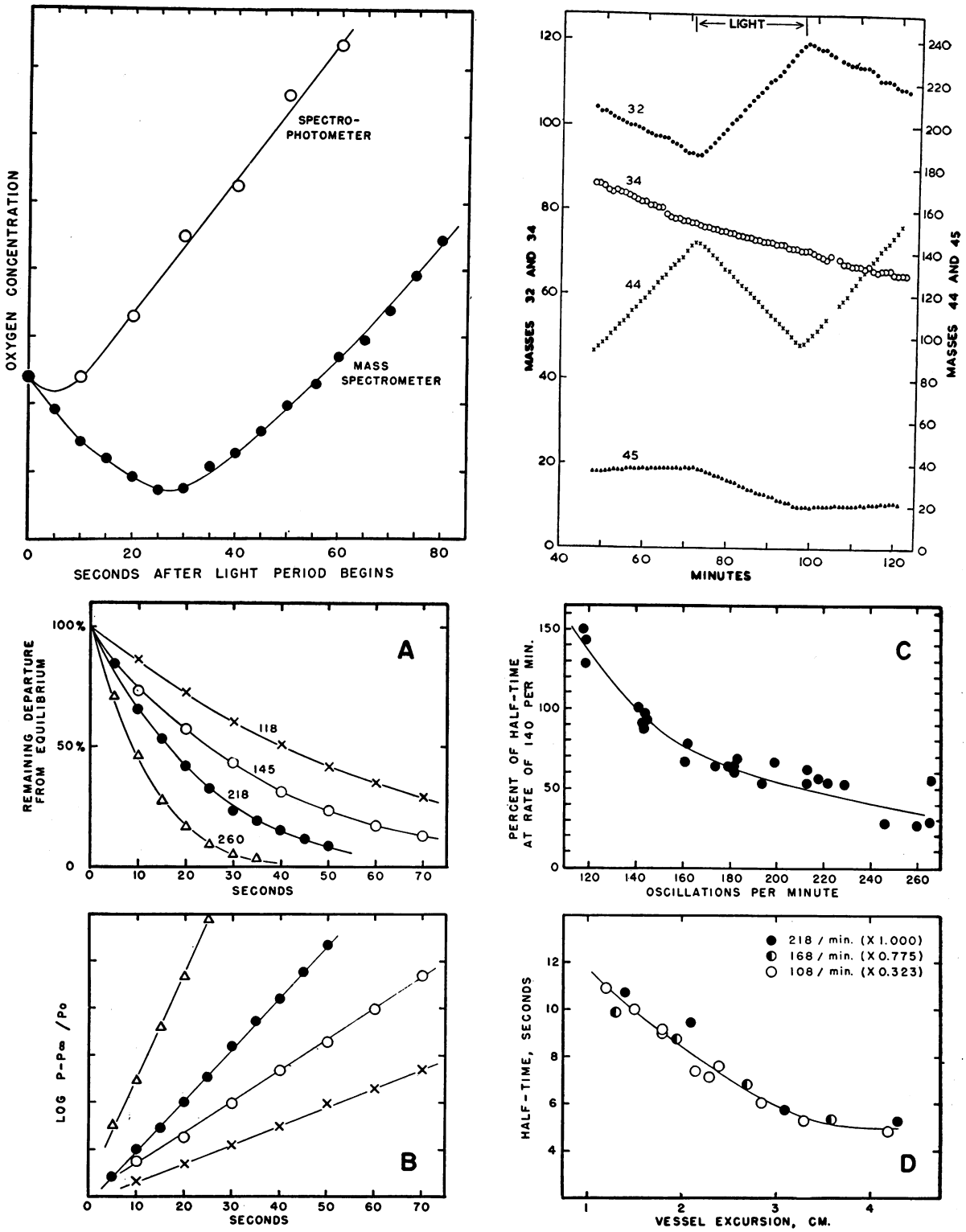


FIG. 2 (*top, left*). Apparent induction period for photosynthesis measured in liquid and in the gas phase.

FIG. 3 (*below*). A. Time course of equilibration of O_2 in a rectangular Warburg type vessel (15.8 ml) containing 3 ml 0.1 M KH_2PO_4. Gas phase: He, O_2, H_2O vapor. The parameter of the 4 curves was shaking rate as indicated in oscillations per minute. B. Semilogarithmic plot of data above. C. Relation between equilibration and shaking rates. D. Relation between equilibration rate and vessel excursion distance for 3 different shaking rates. Ordinate values were multiplied by the factors shown so as to superimpose curves.

FIG. 4 (*top, right*). Example of mass spectrometer data for 2 isotopic forms of CO_2 (masses 44 and 45) and 2 of O_2 (masses 32 and 34). Expt. 2607. 8.4 μl cells. 26.0° C. Gas phase, CO_2 : O_2 : He (1 : 4 : 328). Tungsten illumination through red filter (Corning no. 2403) at intensity corresponding to 2.6 times compensation.

ing cells the ratio will be smaller in the gas phase than at the site of reaction so that the O_2 utilization rate as calculated by equation 3 must be considered a minimal value; the true rate may be higher. A qualitative way of looking at this discrepancy between calculated and true rates is to think of O_2, as it is evolved but before it can escape into the gas phase, being utilized by the respiratory enzymes in preference to the O_2 of the gaseous environment which must cross a significant diffusion barrier before the enzymes have access to it. The use of the tracer method for experiments such as ours has been criticized along these lines by Warburg (20). Decker (6) has also raised objections which are more easily considered in these terms.

The difference between M/M* ratios in the gas phase and at the site of reaction can be minimized by various devices but never eliminated and, when the difference is large, it leads to what has been termed "preferential reutilization" of the gas produced (2). In previous studies, keeping the metabolic rate low and the prevailing tension of the gas being measured relatively high (insofar as technical considerations would permit) offered assurance only that the errors in relative metabolic rate determinations were as small as possible. It now seems that errors from this cause were sometimes larger than we had thought and, when either more precise or absolute measurements are needed, corrections for "preferential reutilization" usually are mandatory.

In the present work, absolute rather than merely relative measurements were required. Also, as can be seen from inspection of equations 2 and 3, computed changes in production or consumption of the 2 metabolic gases depended upon 4 essentially simultaneous measurements of concentrations of different mass components of the gas phase and upon 4 measurements of the rates of change of these components as compared with the result of similar computations from a comparable set of 8 experimentally determined values taken from another condition being tested. Thus the conclusion to be arrived at from a single experiment (e.g., an individual point in fig 7) is sensitive to error in any of 16 items of data. Under such circumstances the necessity to reduce all known errors to a minimum was unusually compelling. Therefore corrections of M/M* in equations 2 and 3 have been introduced so that the computed values approach as closely as possible the ratios prevailing at the site of reaction. If these corrections are ignored, when relatively high metabolic rates are determined by the tracer method, the estimated rates can be in error by over 100 % in the case of O_2 at least. This error arises from the interrelated effects of diffusion gradients which exist in the experimental system and the corrections are based on an approximate evaluation of these gradients.

Mixing within the liquid is so rapid in a well shaken vessel that appreciable diffusion gradients are not established within the liquid proper and, for several reasons, it seems likely that, of the various diffusion barriers which must exist between gas phase and enzyme, that which is by far the most important exists at the liquid-gas interface (shearing layer) in the reaction vessel.

The effect of this barrier alone is illustrated in figure 2 in which time course data from gas phase mass spectrometer measurements are compared with liquid phase determinations obtained by a different method (21). Chlorella cells were utilizing O_2 in the dark until, at zero time, the suspensions were illuminated. The much greater response lag in the case of the mass spectrometric data is attributable almost entirely to the diffusion gradient across this barrier for which we must make correction.

Provisionally, we shall assume that with small cells the barrier at the cell-liquid interface generally is unimportant in comparison with the barrier which exists at the gas-liquid interface.

The effects of the interfacial barrier in manometric experiments have been considered both theoretically and experimentally by Roughton (16) and by Myers and Matsen (14) in terms of the usual stationary film theory. Only a brief explanation is in order here. The influence of the barrier can be described quantitatively by an equilibration constant, K, which characterizes the rate at which a concentration difference spontaneously disappears across the interfacial film according to the equation,

$$(p - p_x) = e^{-Kt} \qquad (4)$$

in which p is the partial pressure of the gas at any given time, t, and p_x is the final value of p after equilibration. The constant, K, is a function of the chemical nature of the 2 phases, the surface area, the amplitude and frequency of shaking the vessel, and the volumes of liquid and gas space.

In order to determine K experimentally, one makes a sequence of measurements of the partial pressure of a gas which is undergoing equilibration across the interfacial diffusion barrier. Plotted semilogarithmically, the data on departure from equilibrium are linear with time. The value of K which applies to the conditions under which the measurements were made can be computed readily from the slope of this linear plot as described by Roughton. If desired, values of K are easily converted to the half-times for gas phase response to liquid phase changes—the familiar way of expressing equilibration lag. Using this procedure variables such as vessel shape, liquid volume and composition, presence of surface active materials, gas phase composition, vessel shaking rate, and excursion distance were studied systematically to determine their repective influences on K—i.e., on equilibration lag. Figure 3 illustrates by example this method of observing effects of the last two variables mentioned.

When making measurements of K using a mass spectrometer instead of a manometer, one gains the significant advantage that, of the several gases which may be involved in complete pressure equilibration, data are taken only on the single gas with which one is concerned. However, 2 additional points must be

considered; instrument response time and gas phase mixing.

The rate of response of a manometer to an instantaneous pressure change is limited by manometer fluid viscosity and several other minor factors. In practice the Warburg manometer responds so fast to gas phase pressure change that, for nearly all ordinary uses, the inherent instrumental response lag is negligible. On the other hand, most commercially available mass spectrometers have gas inlet systems which offer major resistance to diffusion either on the high pressure side of the leak or in the low pressure region where molecular diffusion occurs. In the present adaptation of a Nier-Consolidated mass spectrometer these instrumental response lags have been minimized by reducing the diffusion barriers as much as was feasible by altering the design of the leak and gas inlet system to remove unnecessary constrictions. The speed of the mass spectrometer diffusion pump is high and the pump-out time of the mass spectrometer tube (time for half replacement of the gas in the ion source region) was in our case 2.5 seconds which is small compared with the half-time for equilibration across the gas-liquid interface in the reaction vessel.

The response of a mass spectrometer is somewhat slower than that of a manometer also because gas phase mixing is a much more important factor in the former. The most rapid response was observed at near vacuum pressures in the reaction vessel. Various background or carrier gases slowed down the response. The effect was a function of the carrier gas viscosity. As might be expected, the O_2 and CO_2 equilibration constants depended on the total pressure in the gas phase but not on the partial pressure of the gas being measured nor on the direction of diffusion (into or out of solution). Helium was chosen (in preference to nitrogen) as the carrier gas to be used in all experiments reported here. Measurements were carried out in a gas phase which was 96 to 98 % He and the remainder was a mixture of CO_2 and O_2, both isotopically enriched. The total pressure was approximately 1 atmosphere.

All experiments were run in the same rectangular Warburg-type reaction flask under essentially the same conditions except as regards light intensity. The volume of the cell suspension was 2 ml with a cell concentration of about 0.5 %. The shaking rate was 172 complete oscillations per minute at a vessel excursion of 17 mm. The value of the constant, K, in equation 4 was, for O_2 in this system, 0.06. This corresponds to a half-equilibration time of about 11 seconds.

As was anticipated above, knowledge of the K value characteristic of the system used in our experiments makes it possible to compute the ratio, M/M*, in solution from measurements of the ratio made in the gas phase. For a particular gas being measured the diffusion rate, R, across the interface is a linear function of the concentration difference through the barrier. The proportionality constant, referred to as the interface transfer constant, is k which is related to K by

$$K = k\left(\frac{a\,v + V}{v\,V}\right) \qquad (5)$$

where v and V are liquid and gas volumes respectively and a is the solubility coefficient. Therefore k is easily computed from K as determined experimentally and the concentration of dissolved gas, M_s, is given by

$$M_s = a\,M - \frac{R}{k} \qquad (6)$$

in which R is the steady state rate of gas transport across the interface and M is the concentration of the gas prevailing in the gas phase. The values of k for our experimental conditions were, for O_2, 0.13 and, for CO_2, 0.072 vol. units sec^{-1}.

Parenthetically, another method of evaluating k may be mentioned. If the density of the cell suspension is increased until R reaches a maximal value, it may be assumed that this condition prevails only when M_s in equation 6 becomes vanishingly small. Thus $k = R/aM$ approximately. It was noted that the 2 methods led to essentially the same value of k in spite of the fact that very different cell densities were employed. While this was the case for Ankistrodes-

TABLE I

SAMPLE DATA AND COMPUTATION RESULTS*

	EXPERIMENTAL PERIODS		
	DARK 45 MIN	LIGHT 29 MIN	DARK 31 MIN
R_{32}	−0.49	2.7	−0.68
M_{32}	143	278	318
R_{34}	−0.22	−0.04	−0.22
M_{34}	121	111	102.5
R_{44}	1.1	−1.2	1.3
M_{44}	115	125	127
R_{45}	0	−0.92	0.05
M_{45}	84.5	72	59.5
P_{O2}	−0.15**	5.7	0.3**
P_{CO2}	1.2	0.4	1.4
U_{O2}	0.6	3.1	1.3
U_{CO2}	0	2.8	0.2
ΔP_{CO2}		0.9	
ΔU_{CO2}		2.1	
$\Delta P_{CO2} + \Delta U_{O2} + U_{CO2}$		5.8	

* Expt. 2107. 5.3 μl cells. 26.0° C. Gas phase, CO_2 : O_2 : He (3 : 4 : 493). Constants (cf. equations 7 and 8) : j_{O2}, 0.932; F_{O2}, 36×10^{-6} per chart div.; f_{O2}, 6.36×10^{-6} ml (N.T.P.) O_2 sec^{-1} per chart div. min^{-1}; k_{O2}, 0.13 ml sec^{-1}; j_{CO2}, 1.121; F_{CO2}, 22.4×10^{-6} per chart div.; f_{CO2}, 5.17×10^{-6} ml (N.T.P.) CO_2 sec^{-1} per chart div. min^{-1}; k_{CO2}, 0.072 ml sec^{-1}. Units: M, chart div.; R, chart div. min^{-1}; all others, μml sec^{-1} μl^{-1}.

** Oxygen production may be assumed not to occur in darkness; departure of tabular values from zero indicates experimental error.

mus in phosphate buffer, it may have been fortuitous. Other experimental material or different media may influence interfacial properties in such a way that k becomes a significant function of cell concentration. We found this to be the case with another organism (Ochromonas). Complications of this kind also were reported by Habermann for chloroplast suspensions (11), and were noted by Dr. Robert Emerson for Chlorella suspensions (personal communication).

For a given kind of gas we were concerned in these experiments with 2 isotopic species; these must be considered separately. A pair of M_s values were calculated from measurements of M and R for the respective isotopic masses. Thus a pair of isotope concentrations—consequently a ratio—in the liquid phase was determined. Ratios calculated in this way, being much better approximations of the ratios existing at the site of gas consumption, were used in preference to the gas phase ratios as measured. From these considerations it is possible to improve on equations 2 and 3, which were first approximations involving only gas phase quantities, by substitution of corresponding expressions based on liquid phase quantities obtained by taking into account the diffusion barrier at the gas-liquid interface. Corrections of this sort also were applied to mass spectrometer data by Horwitz and Allen (12).

Values of M and M* and of their rates of change were measured in the gas phase in arbitrary units of pen excursion on the recorder chart. For this and for other reasons several conversion factors must be introduced in order that results may be expressed in the desired units. The overall calculations are indicated by the following equations which are revisions of equations 2 and 3:

$$P = (Rfj) - (R^*fj)\left[\frac{a\,MF - Rf\left(\frac{1}{k}\right)}{a\,M^*F - R^*f\left(\frac{1}{k}\right)}\right] \quad (7)$$

$$U = R^*fj\left[1 + \frac{a\,MF - Rf\left(\frac{1}{k}\right)}{a\,M^*F - R^*f\left(\frac{1}{k}\right)}\right] \quad (8)$$

for which the following symbols are defined (for the case where O_2 is the gas considered):

P and U = production and consumption rates for). Units, ml per sec per μl cells $\times 10^{-6}$.

M and M* = concentrations of the 2 isotopic forms of O_2 measured on the recorder chart as mass spectrometer peak heights for the respective masses. Units, arbitrary scale divisions.

R and R* = rates of change of M and M* respectively. Units, scale divisions per minute.

$j = \left[\frac{a\,v + V}{Vv}\right]\left[\frac{273}{T}\right]$, where v and V are liquid and gas volumes (ml) and T is the absolute temperature.

F = C/S, where C is the oxygen fraction of the gas used for calibration and S is the mass spectrometer peak height (in scale div) for mass 32 when measuring the calibration gas at 1 atmosphere.

$f = F \cdot V/60$.

k = interface transfer constant as previously defined. For analogous measurements on CO_2, masses 44 and 45, a set of similar equations with comparable constants was applied. The units of P_{O2}, U_{O2}, P_{CO2}, and U_{CO2} are ml sec^{-1}. The final values are expressed on a unit cell volume basis.

Results and Discussion

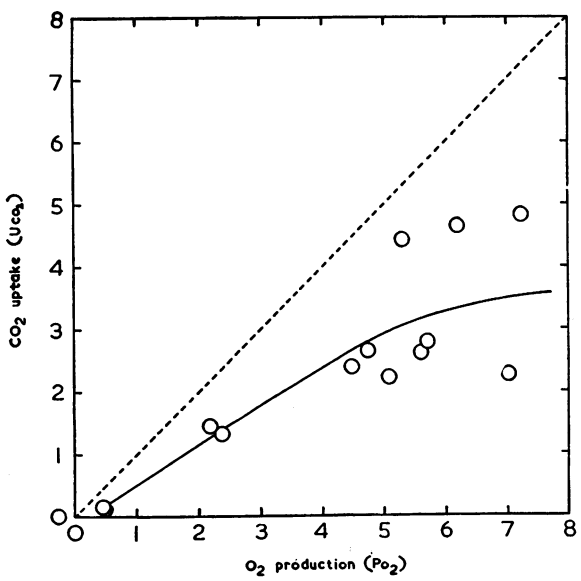
Figure 4 shows an example of mass spectrometer data for the metabolism of 2 isotopic forms of O_2 (masses 32 and 34) and 2 of CO_2 (masses 44 and 45). The essential measurements on a chart record such as figure 4 are recorded in table I along with results of computations leading to the true production and consumption rates of both O_2 and CO_2. The table also shows the final result of light-dark comparisons. Note that the O_2 production rate in the light period, Po_2 is nearly the same as the sum of: deficit in CO_2 production, enhanced O_2 consumption, and CO_2 uptake as shown by the last entry in the table. This agreement is predicted by equation 1. Data on 13 light intervals, such as that on which table I was based, with dark periods before and after for comparison, were obtained over a range of light intensities. Values of U_{CO2}, ΔP_{CO2}, and ΔU_{O2} calculated for each illumination period, were plotted against corresponding values of P_{O2} in figures 5 to 7.

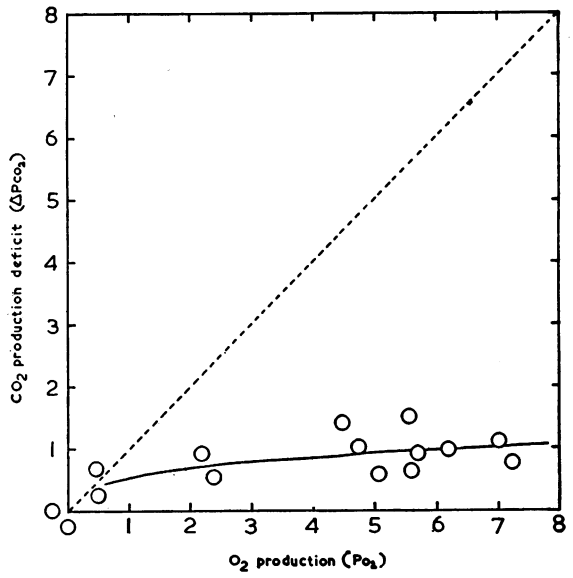
Figure 5 shows the relation between rate of CO_2 utilization and O_2 production. If the photosynthetic quotient were actually unity, the points would all lie along the diagonal. This was not the case as the uptake of CO_2 fell short of being equivalent to O_2 production especially at higher rates of photosynthesis.

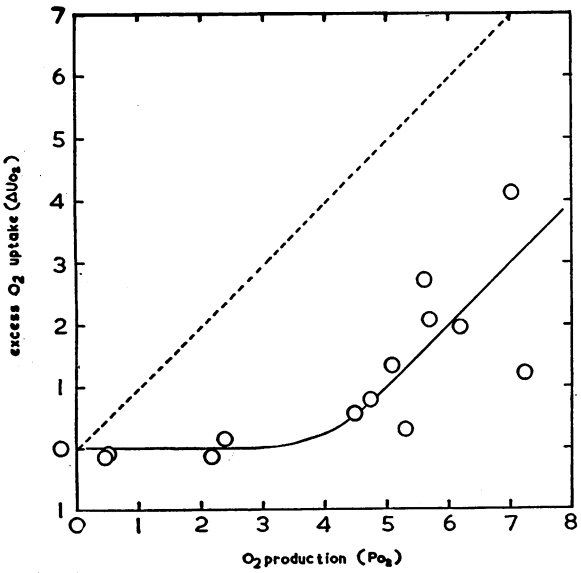
Figure 6 shows the light induced deficit in respiratory CO_2 production. Compared with the dark rate CO_2 production was reduced to about one half by light, an effect which varied little with photosynthetic rate.

Figure 7 shows the photostimulated fraction of the O_2 consumption which was negligible at low light intensities but which became large at higher rates of O_2 evolution. It may be noted that, at low light intensities, neither stimulation nor inhibition of respiratory O_2 uptake occurred. Under these conditions reliable measurements of photosynthetic O_2 production could have been made on such Ankistrodesmus suspensions by ordinary methods not involving the use of tracers. However, in the case of CO_2 metabolism, results were less reassuring. Because of the suppression of CO_2 production in light of even low intensity, measurements without tracer of net CO_2 exchange are not readily interpreted.

Each point in figure 8 is the absolute sum of ordinate values in figures 5 through 7 plotted against O_2 production rate. These points lie along the diagonal indicating that, over the range of light conditions employed, the rate of production of photosynthetic reductant (corresponding to the rate of oxidant production and measured as O_2 evolution) was equivalent to the sum of the CO_2 consumption rate, the deficit in the rate of CO_2 production, and the increase in the rate of O_2 absorption. This is the condition imposed by equation 1. Thus, figure 8 demonstrates that the data were generally in agreement with the equation over a relatively wide range of light intensities (up to about 6 times compensation). The data are consistent with the assumption of an effect of light on respiratory metabolism mediated by a reductant rather







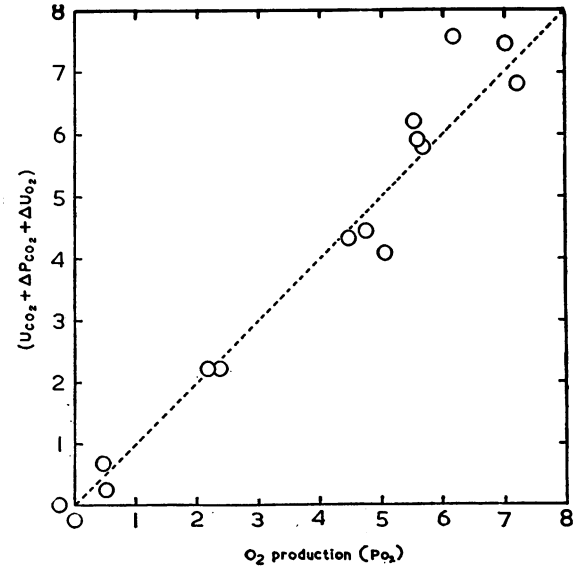


FIG. 5 (*top, left*). Relation between rates of CO_2 uptake and O_2 production in the light. If the photosynthetic quotient actually were unity, all points would lie on the diagonal.

FIG. 6 (*top, right*). Relation between deficit in CO_2 production rate caused by light and the rate of O_2 production.

FIG. 7 (*below, left*). Relation between the enhancement by light of the rate of O_2 consumption and the O_2 production rate.

FIG. 8 (*below, right*). Comparison of light induced deficit of CO_2 evolution rate, increment in O_2 consumption rate, and CO_2 uptake rate with the rate of O_2 production. Prediction according to equation 1 requires that the points should lie along the diagonal.

than by an oxidant. At low light intensities—low rates of reductant generation—only CO_2 evolution is affected. At higher light intensities enhancement of O_2 consumption occurs as well.

From kinetic data of this type it is of course not possible to decide where in the oxidative process the photosynthetic reductant interacts with respiratory metabolites, coenzymes, or electron carriers. It may be noted also that the enhanced O_2 consumption observed at the higher light intensities is not readily dismissed as photooxidation not directly related to photosynthesis and respiration, since the increment in O_2 consumption was matched by increasing O_2 production. If the usual kind of photooxidation were superimposed on photosynthetic and respiratory gas exchange, results would not agree stoichiometrically with equation 1.

During some of the experiments described above further data were taken while the cell suspensions were allowed to photosynthesize until the CO_2 was depleted to the level of CO_2-compensation. As this condition was approached, the CO_2 isotope ratio, M_{44}/M_{45}, became so large that calculations of production and consumption rates were subject to large error as the net rate of CO_2 uptake approached zero. Oxygen computations were as reliable as in other experiments. Figure 9 illustrates the changes which were observed in 2 representative experiments. After CO_2-compensation was attained, the rate of O_2 consumption usually was higher and O_2 production invariably lower than was the case for normal photosynthesis with an adequate concentration of CO_2. Evolution and uptake of O_2 were then steady and essentially in balance. Just before the condition of compensation was reached, a transient stimulation of O_2 exchange sometimes was observed (as in experiment 1707 of fig 9). When such experiments were performed on suspensions without supplementary glucose, this transient usually was especially pronounced.

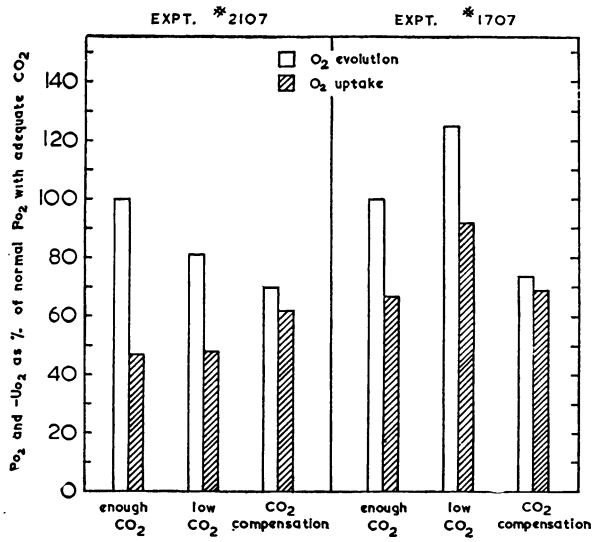


FIG. 9. Comparison of rates of O_2 production and consumption under conditions of CO_2 depletion. Two separate experiments are shown.

It is reasonable to suggest that results such as these, obtained as the cells run out of CO_2, demonstrate a change in fate of the photochemical reductant which, when deprived of its normal oxidant, reduces O_2 instead. The steady state which finally obtains is one in which O_2 consumption is elevated sufficiently to match—or nearly match—O_2 evolution; i.e., net gas exchange approaches zero and an O_2 exchange in ratio 1 : 1 prevails. The rates of O_2 consumption observed under such compensation conditions, while higher than normal for the light intensity used, were about equivalent to—but did not exceed—the maximal rate of extra O_2 uptake observed in any light period where sufficient CO_2 was present. This maximal rate was observed only at a significantly higher light intensity in the case of normal photosynthesis. From the fact that O_2 production rates were not maintained under CO_2 deficient conditions, as illustrated in figure 9, we may deduce that O_2 reduction by photochemical reductant either is less efficient than CO_2 reduction or, more probably, when CO_2 compensation prevails, secondary changes may inhibit energy transfer from chlorophyll, increase the probability of back reactions, and thus lower the O_2 production rate as suggested by Franck and coworkers (*7*, 8, 9, 17).

SUMMARY

The gaseous metabolism of the alga, *Ankistrodesmus braunii*, was measured in darkness and in light of various intensities. Cells were suspended in phosphate buffer containing glucose and the gas phase, enriched with O_2 and CO_2 isotopes, was monitored with a recording mass spectrometer. In contrast to earlier results with other experimental material, isotope exchange involving CO_2 was not prohibitively rapid so that with this gas as well as with O_2 it was possible to calculate simultaneous production and consumption during the light. These calculations depended on knowledge of the isotope ratio prevailing at the site of gas consumption and, since the ratio (especially in the case of O_2) generally was not the same in liquid and gas phases, corrections were introduced which took into account the effect of the diffusion barrier at the gas-liquid interface; calculations therefore were based on isotope ratios prevailing in the liquid phase which were considered close approximations to those at the intracellular reaction site. It was found that light induced an inhibition of CO_2 evolution which was nearly independent of light intensity whereas O_2 consumption in the light was unaffected at low intensity but enhanced at high intensities. The results were quantitatively in agreement with interaction between a photosynthetic reductant and the respiratory mechanism.

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