HIGH INITIAL RATES OF GAS-EXCHANGE IN RESPIRATION AND PHOTOSYNTHESIS OF *CHLORELLA**

by

B. KOK AND C. J. P. SPRUIT

*T.N.O. Solar Energy Research Group, Laboratory of Plant Physiological Research**,*
Agricultural University, Wageningen (Netherlands)

We recently described an apparatus for measuring photosynthesis, based on gas exchange phenomena, allowing rapid changes to be followed with a high degree of sensitivity and accuracy[8].

We have now used this apparatus in studies of gas exchange during transitory phases from light to darkness and *vice versa*. At the same time we corroborated these measurements by polarographic and potentiometric measurements of oxygen and carbon dioxide respectively.

A study of this type appeared still more desirable because BURK AND WARBURG[1-7] recently published extensive experiments in which, during the first minute of illumination, *Chlorella* cells showed a gas evolution about 4 times greater than that occurring after attainment of the steady state. Similarly, an increased gas uptake was found immediately after the light was switched off or decreased in intensity. They suggested that there is a primary photosynthetic step in which, for each light quantum absorbed, one molecule of O_2 is evolved and one molecule of CO_2 reduced ($\gamma = -1.0$). In a subsequent combustion stage three quarters of these products formed by the light were supposed to react back to yield the additional chemical energy required for the further reduction of the remaining quarter.

The "one quantum" slopes during transitions from light to dark or *vice versa* could be observed manometrically in acid suspension media only and at sufficiently high partial CO_2 pressures (5–50%, depending upon the type of cells used[4,6]).

From measurements made by the two vessel manometric method it was computed that at all times γ (CO_2/O_2) was constant and close to minus unity. It is evident, that this latter observation was the main basis for the hypothesis developed by WARBURG *et al.*, and that any large transitory deviation of γ from unity indicating "gush" or "induction" phenomena should be excluded.

In view of the fact that such phenomena have been occasionally described in the literature (*cf. e.g.*[12,16]) we have given special attention to their occurrence under the conditions of our experiments.

EXPERIMENTAL METHODS

In a separate paper[8] one of us described a volumetric apparatus of high relative and absolute sensitivity. A small fraction of the suspension itself serves as pressure index fluid, and the amount

---

* This work was aided by a grant from the Ch. F. Kettering Foundation.

** 121st Comm. of this Laboratory, 40th Comm. on Photosynthesis.

of liquid to be used or the intensity of shaking is not limited by the hazard of splashing into the manometer capillary. Equilibration between gas and fluid phases therefore can be very fast. Since, moreover, the changes in volume are automatically recorded, this apparatus appeared well suited for the study of transitory gas exchange phenomena.

The recording system of this instrument was used in conjunction with three different types of reaction vessel, *viz.*:

a. microvessels as described in[8];

b. manometer vessels of standard dimensions and with ordinary shaking arrangement;

c. a special vessel which simultaneously allowed volumetry, polarography and potentiometry.

Method a:

The total volume of the reaction vessel was 290 μl; for the experiments to be described, it was filled with either 230, 100 or 50 μl of *Chlorella* suspension.

TABLE I

	$V_f(\mu l)$	V_f/Vg	kO_2	kCO_2	KO_2	KO_2/kO_2	kCO_2/kO_2
a:	230	3.8	1.58	6.58	2.1	1.3	4.16
b:	100	0.77	4.53	6.63	14.7	3.3	1.46
c:	50	0.21	5.63	6.70	40	7.2	1.2

The "vessel constants" (the amount of gas in 10^{-3} μl corresponding to one division of the delivery screw) for these conditions (19° C) are given in Table I. KO_2 was computed by taking $\gamma = -1.0$.

It will be apparent that an 18 fold variation of V_f/Vg yields a variation of kCO_2 of less than 2 %. kO_2 varies 3.5 fold and KO_2 even 20 fold. Most noteworthy is the favourable vessel constant and the small difference between kO_2 and KO_2 when the maximum amount of liquid was used ($k/K = 0.79$). In this case, therefore, the readings represent mainly oxygen exchange, if either carbonate-bicarbonate buffer mixture or acid medium is used. In the latter medium only 25 % of the CO_2 exchange contributes to the deflection of the meniscus.

Method b:

To be able to use the recording system for studying the influence of shaking rates, and to make measurements with the two-vessel method, the set-up illustrated in Fig. 1 was used. A flexible stainless steel capillary (a 1 mm injection needle) connected the index capillary with the liquid in the reaction vessel. The latter was mounted in such a way that it could be shaken in a horizontal plane with an amplitude of two cm and at any speed desired.

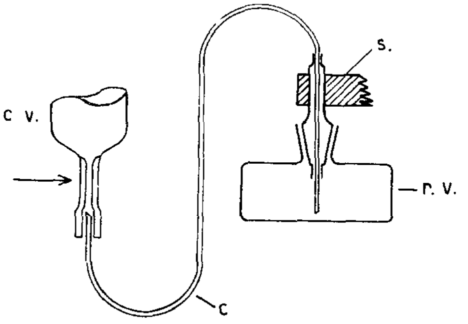

Fig. 1. Large-size reaction vessels (*r.v.*) and method of attachment to compensation vessel (*c.v.*), via stainless steel capillary (*c*). *s*: arm of shaking arrangement. The arrow indicates the index meniscus. Vessels were either cylindrical, with volumes of 14.6 and 21.5 ml, or rectangular, with inside dimensions 17 × 30 × 28 mm.

Method c:

In this series of experiments, oxygen was determined by a polarographic procedure, which was a modification of the one described by DAMASCHKE *et al.*[9]. In the same polarography vessel, carbon dioxide exchange could be observed by measuring the pH-changes with a glass electrode. It was also possible to measure changes in gas pressure in this vessel by using it as the reaction vessel of the recording volumeter instead of the one shown in Fig. 1 (*cf.* Fig. 2).

To a certain extent the combination of the three methods, *viz.* volumetry, polarography and potentiometry, necessitated a compromise as to the sensitivity of these methods. Oxygen determination by the polarograph is most accurate at low total oxygen concentration, preferably with no gas phase present. Under the latter condition, measurement of gas exchange is impossible. In some experiments therefore we first filled the vessel partially with algal suspension. After a number of volumetric determinations, more suspension medium was added so as to fill the vessel completely and polarographic measurements were started. By the use of a gas phase with a low oxygen content, it was found possible to make a simultaneous record of the changes in volume and of the polarographic current.

Potentiometric pH measurement, with the glass electrode could be combined with either method and carried out in a vessel with or without a gas phase. High carbon dioxide concentrations, often required in this investigation, result in a relatively low sensitivity. This necessitated very sensitive E.M.F.-measurements (full scale 2.5 mV on a Brown recorder). In this way, pH-changes as small as 0.001 pH could be observed.

Calibration of the polarograph and potentiometer in terms of oxygen and CO_2-concentration could be carried out with the aid of microburettes attached to the vessel and filled with solutions of these gases. The response to such conditions was complete within a few seconds. For the problems studied, the instrumental lag was therefore negligible. Further details of the apparatus will be given in a forthcoming paper[10].

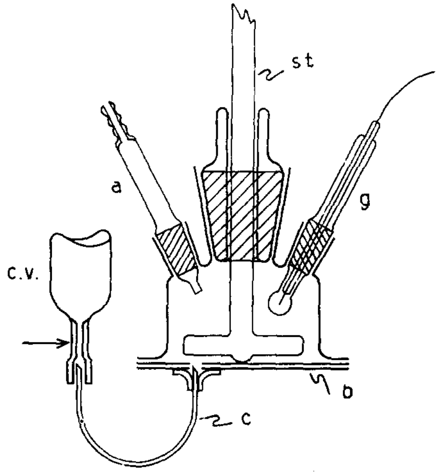

Fig. 2. Vessel used for the simultaneous measurement of oxygen by polarography and carbon dioxide by pH-measurement. For measurement of gas exchange, the vessel was also connected to the compensation vessel, see Fig. 1. *St.*: stirrer ("KPG-Rührwelle", Jenaer glass) with four blades. *a*: Agar bridge, *g*: glass electrode. The electrodes for polarography and the microburettes were attached in the same way (not shown). *c*: stainless steel capillary, *b*: removable bottom, *c.v.*: compensation vessel.

OBSERVATIONS WITH THE MICRO VOLUMETER

High transitory rates of gas exchange were observed (irrespective of the cellular material used) if certain experimental conditions were fulfilled. Such rates were small if the cells were suspended in an alkaline buffer medium. They were also small if the cells were suspended in an acid medium with a small gas space in the reaction vessel (Table I, *a*). But if a suspension in acid medium in combination with a large gas space (Table I, *b* and *c*) was studied, high transitory rates were always observed. This is illustrated by the transitions from darkness to light in the three recordings of Fig. 3, made with about the same CO_2-concentration. The experiment of Fig. 4 was done with 10% CO_2. This figure plots the first derivative of the volumes taken from the original record shown in the inset and represents the rate of volume change. Upon illumination, a high positive gas exchange occurs, lasting for a few seconds. The rate then decreases, may change its sign and there is an indication of a second maximum before the steady state is attained. Upon darkening, the transition is more or less a mirror image of the dark-light transition. The negative rate of volume change sets in just as abruptly, but it does not reach quite as high a level, and declines more slowly. In this case, too, a second "wave" can sometimes be noticed before the steady state is reached. The areas under the D–L and the L–D transition peaks are about equal. The steepest slope observed upon illumination may surpass 10–20 fold the steady state slope.

It is evident that the photosynthetic "yield", computed by subtracting the gas exchange in dark from that in light will vary greatly, depending upon which corresponding parts or periods of the respective curves are used for the computation.

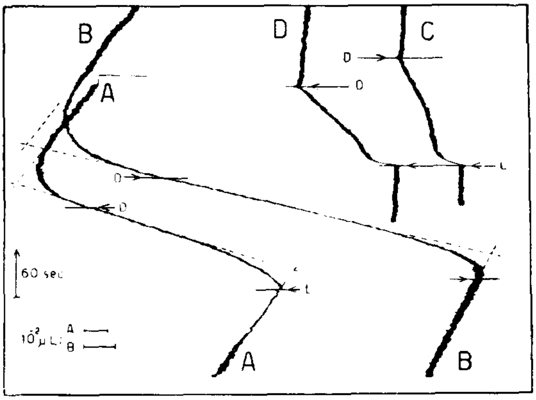

Fig. 3. Volumeter recordings of parallel aliquots of cells suspended in: A. 230 μl acid medium, B. in 230 μl carbonate buffer (pH 8.8, 2 % CO_2), C. and D. in 100 μl acid medium. In expts. A, B. and C, the light intensities were the same. In expt. D, a higher intensity was used.

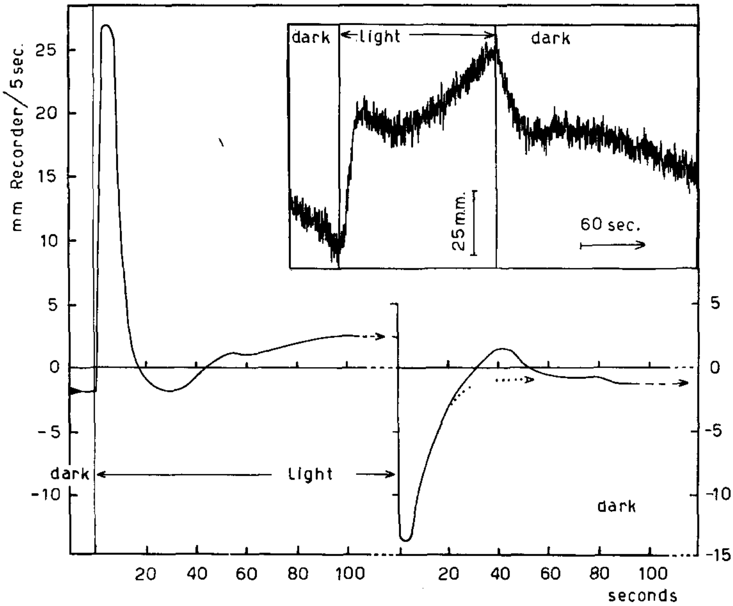

Fig. 4. Differentiation of the recording shown in the inset. Cells suspended in 100 μl acid medium, 10 % CO_2.

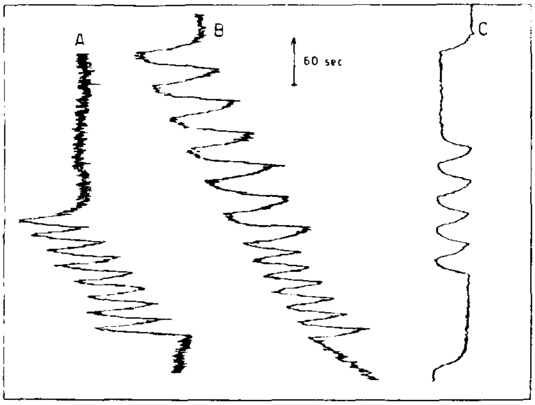

Fig. 5. Cells suspended in 100 μl culture medium, pH 5.0. Intermittent illumination with a given intensity (A and B) and with a 3 × lower intensity (C). A. 10 % CO_2, B. and C. 50 % CO_2. As compared to A and B, in recording C movement was 3 × slower and sensitivity 1.5 × decreased. Pen moved to the left for positive, to the right for negative exchanges. Respiration was often a little irregular and decreased after such exposures (*cf.* A).

The shorter the light and dark periods are chosen, *e.g.* down to 10 sec, the higher are the yields obtained.

As shown in Fig. 5 the transition phenomena can be repeated for any number of light-dark cycles. The first outburst and pick-up are usually the largest, but the cyclic changes upon illumination and darkening soon become fairly constant. Yields computed for the individual cycles may surpass factorfold the steady state photosynthetic yields.

The "net gain", *i.e.* the yield over a period of intermittent illumination with equal light and dark periods, was usually a little less than the gain observed in continuous light of half the intensity.

The results described so far are typical for a number of experiments with four different strains of *Chlorella* grown under various conditions of temperature, light intensity and light quality, and used in various suspension densities and at temperatures between 15° C and 30° C.

The transition effects were found in *all* samples tried, be it with differences in magnitude, light saturation value or, in particular, sensitivity to the CO_2-concentration.

The experiments described so far in no way give an exhaustive description of the effects which can be observed under various conditions. A long dark period may cause an enhanced outburst upon illumination. The pick-up after darkening may appear much smaller than this burst. This does not necessarily imply, however, that the total amount of gas taken up is actually smaller: a slow uptake may be obscured by the simultaneous adaptation of the respiratory gas exchange. After darkening and completion of the pick-up, respiration often appears anomalous, and the rate may be decreased even to zero for a while.

Influence of CO_2-concentration

The CO_2 pressure used was found to play an important role; as is illustrated in the experiment shown in Fig. 6, the transitory phenomena are relatively small at low partial CO_2 pressure; they continue to increase in magnitude when higher concentrations (until virtually 100%) are used. The dark "pick-up" usually required a higher CO_2 tension to appear to its full extent than did the "gush" upon illumination (*cf.* expt. Fig. 3 made at 3% CO_2). Fig. 6 also shows that the "net gain" decreases with increasing CO_2 pressure. This resembles WARBURG's "*Aufspaltung ohne Gewinn*" (ref.[3], pag. 419–420).

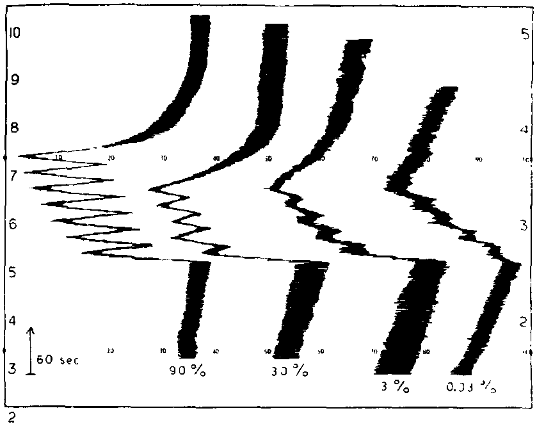

Fig. 6. Influence of CO_2 pressure upon the magnitude of transitory rates. *Chlorella* cells suspended in 0.1 ml culture medium 23° C. The vessel was first filled with air. After a series of measurements it was evacuated and refilled with air containing 3% CO_2. In the same way 10, 30 and 50% CO_2 in air and finally 90% CO_2 + 10% O_2 were successively studied. Transition effects increased with increasing (CO_2).

Correlation with photosynthesis

Several observations indicate that the transitory effects are only remotely correlated with the photosynthetic process as such. Both the initial rate, and the total volume change involved in outburst and pick-up increased with increasing light intensity. However, the transitory effects attained a maximum rate at a light intensity much lower than that required for saturating steady-state photosynthesis. Differences of more than 10 fold were observed. Moreover, both gush and pick-up require far higher concentrations of CO_2 than photosynthesis. Though the steady state rates may eventually be depressed, the magnitude of the transitory effects may continue to increase with increasing CO_2 concentration (*cf.* Fig. 6).

High transitory rates are only due to CO_2 exchange

Since gush and pick-up are found most clearly in acid media, the role played by each of the two gases O_2 and CO_2 should first be determined.

In the experiments of Fig. 3, made at 2–3% CO_2, the net photosynthetic rates computed from the steady states were practically identical, *viz.* 0.070 in A, 0.075 in B and 0.066 in C (μl per min, accepting $\gamma = -1$). There appears to be only a slight difference between the curves A and B. In both cases CO_2 exchange influenced the readings to a small extent: in acid medium to about 25%, and in buffer to about 10%. Curve C, however, closely resembled curves as illustrated in Fig. 4. We conclude that the anomalies are only found if CO_2 exchange contributes appreciably to the readings, *i.e.* in acid media and with a relatively large gas space.

EXPERIMENTS WITH STANDARD SIZE MANOMETER VESSELS

In Fig. 7, an experiment made in an open manometer (left) is compared with a similar one (right), taken from WARBURG's publications (ref.[3], Fig. 1, Protokoll 1). The time course of the rates of gas evolution is very similar in the two cases, as well as the quantum yields if computed taking $\gamma = -1$. We have failed to obtain this value of γ in our experiments however, in contrast to the findings reported by WARBURG *et al.*

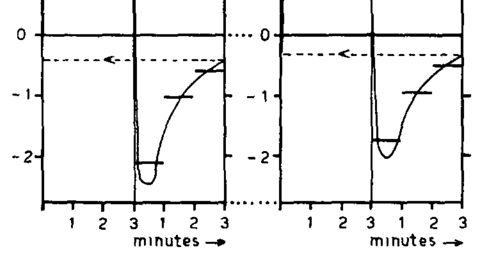

Fig. 7. Left: Transitory rates as observed in an open manometer. 23° C, 15% CO_2, $V_f = 5$ ml, $V_g = 20$ ml. Totally absorbed red light covered the entire bottom area of the vessel. The vessel contained a sample of the same *Chlorella* suspension as used in the recording volumeter for expt. Fig. 6 (performed simultaneously with this experiment). Light-dark periods of 3 minutes each are given. Manometers were read at 30 min interval and plotted by 1 min intervals. Rates are averages of 4 cycles of 6 minutes. Right: Rate curve as given by WARBURG *et al.*[3] protocol I. The rate of pressure change corresponding to $\Phi = 0.1$ (for $\gamma = -1.0$) is indicated in both figures, a tenfold higher rate thus would correspond to $\Phi = 1.0$.

In most of our experiments, we used two vessels of 14.6 and 21.5 ml volume respectively. The small vessel was filled with 7 ml algal suspension. The larger one was filled with the same amount of cells suspended in either 7 or 14 ml liquid. This gave three sets of data for computing O_2 and CO_2 exchange.

If the first few minutes of illumination were disregarded, the three computations yielded closely agreeing results. At high CO_2 concentrations low steady state values of γ (*e.g.* —0.3) were observed. In most experiments we failed to obtain consistent γ values from the initial rates. We are inclined to attribute this failure only partly to the variability of the transitory effect as such. In our apparatus the three measurements could not be made simultaneously. Special care was taken, however, to pretreat the samples as uniformly as possible. The values of γ appeared to depend upon whether the initial slopes, the average rate during the first half-minute of illumination or the total amount of gas involved in the outburst was used for computation. The only source of systematic error we have been able to find is a difference in the speed of equilibration of the vessels even if shaken at exactly the same speed. Indeed we found that the observed initial rate of pressure change (in contrast to the steady state rate) depends strongly upon the shaking speed and also upon the shape and filling of the vessel. Fig. 8 shows the influence of the shaking rate upon the initial slope as observed in a number of reaction vessels.

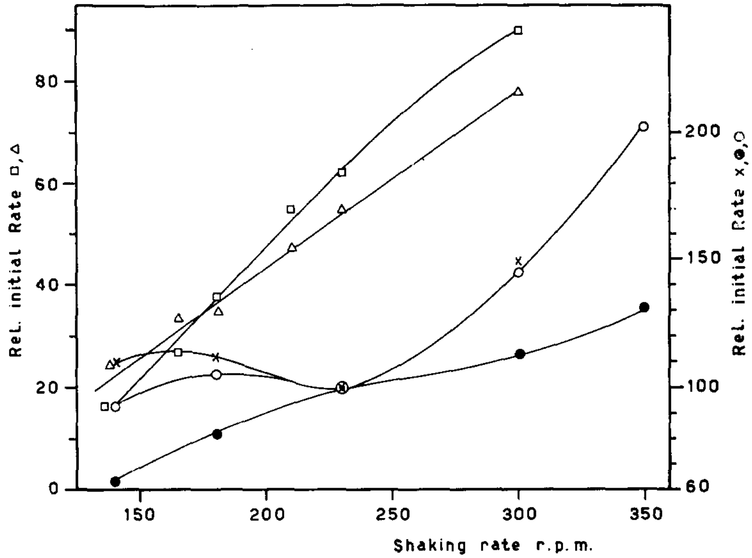

Fig. 8. Correlation between initial slope and shaking rate. Cells suspended in acid medium (15 % CO_2). □: transition from light to darkness, rectangular vessel, △: transition from dark to light (same vessel), ○, ● and ×: experiments with the two cylindrical vessels (see Fig. 1), ○: small vessel with 7 ml liquid, ×: large vessel with 7 ml liquid, ●: large vessel with 14 ml liquid. For the experiments in cylindrical vessels, the initial rates at 230 r.p.m. were arbitrarily taken as 100.

We made a few experiments to determine the rate of equilibration for oxygen and carbon dioxide separately, in the rectangular vessel used for the experiment of Fig. 8, and filled with either 6 or 9 ml liquid. Oxygen equilibration was measured either with *Chlorella* cells suspended in bicarbonate buffer (oxygen evolution) or by using the actinometric fluid of WARBURG AND SCHOCKEN[11] (oxygen consumption). CO_2 equilibration was studied by using the photochemical CO_2 evolution from mercuric chloride and oxalate (EDER reaction).

This reaction had the disadvantage that the calomel formed tended to clog the volumeter capillary.

Gas exchange was recorded during transition from darkness to light or *vice versa.* The value of τ in: $v_t = v_m(1 - e^{t/\tau})$ in which v_t represents the rate at the moment t and v_m the final rate, was computed from the records.

Fig. 9 shows a few results of such experiments. It appears that over a certain range of shaking rates τ varies exponentially with this rate.

It is evident that the phenomena to which Figs. 8 and 9 refer make any computation of γ values during the transition phase extremely ambiguous; for this reason we have refrained from discussing γ values in any detail.

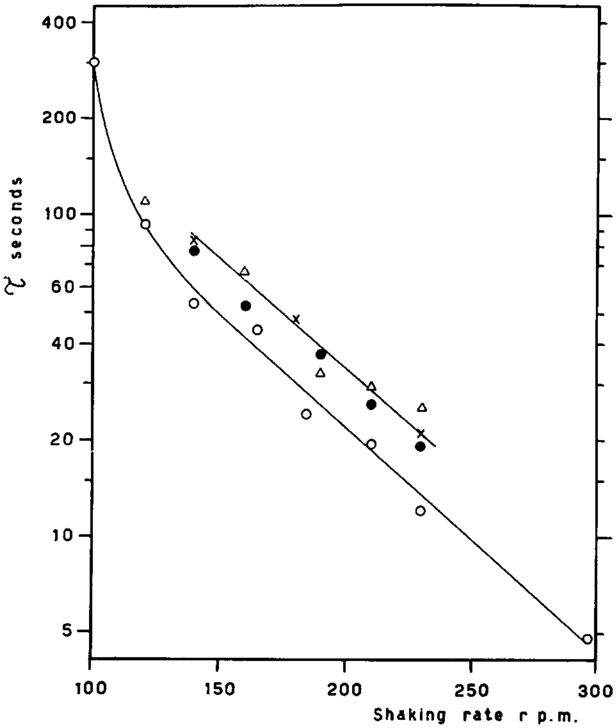

Fig. 9. Equilibration times for oxygen and carbon dioxide, as a function of shaking rate, in the rectangular vessel. × : *Chlorella* cells suspended in 9 ml carbonate buffer. ○: *Chlorella* cells in 6 ml carbonate buffer. ● : 6 ml WARBURG-SCHOCKEN actinometric fluid. △: 6 ml oxalate-mercuric chloride solution (Eder-solution).

POLAROGRAPHIC AND POTENTIOMETRIC EXPERIMENTS

An unambiguous assignment of any transitory effect to a particular gas is not possible with manometric and volumetric methods. According to method *c* (page 213) we have followed potentiometrically the time course of carbon dioxide uptake and polarographically that of oxygen exchange in addition to the volumetric measurements. In a completely filled vessel, such as used in the experiment of Fig. 10, there is no time delay due to equilibration between gas and liquid phase. Fig. 10 shows records obtained with a suspension of *Chlorella* in 0.02 *M* KCl solution in equilibrium with 20% CO_2. No increased rates of oxygen exchange are found during transition.

In several cases, however, the pH records show a production of carbon dioxide at the start of the illumination, followed by an uptake upon darkening.

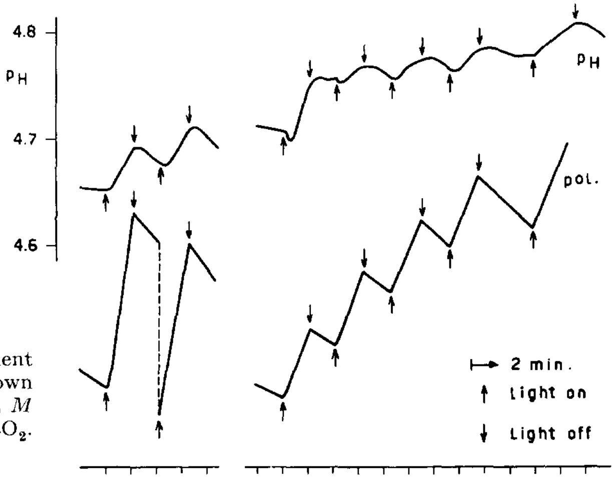

Fig. 10. Simultaneous measurement of O_2 and CO_2 in the vessel shown in Fig. 2. Cells suspended in 0.05 *M* KCl + 0.001 *M* $NaHCO_3$ + 20% CO_2.

In other experiments, the occurrence of such an outburst is not evident, but in all cases the response of the pH is delayed with respect to changes in light intensity. This also can very well be interpreted as production of CO_2 at the start, and an uptake of CO_2 after the end of an illumination period. It thus appears that the rate of CO_2 production as observed in darkness either remains the same immediately after the start of illumination, or increases temporarily. After darkening of the cells an increased rate of CO_2 uptake is not observed; CO_2 uptake merely "overshoots" temporarily. Fig. 11a and b show simultaneous recordings of volumeter and pH changes in a partly filled vessel. In Fig. 11a the pH record shows an outburst of CO_2 upon illumination and an overshoot upon darkening, and in Fig. 11b there is only overshoot in both cases lasting for about 30 seconds. As the volumeter vessel contained a relatively large fluid phase, the effect upon volume changes is not as pronounced as it would have been in a vessel with less liquid, but is still clearly visible. Finally, Fig. 11c gives polarograph recordings in the vessel completely filled with an algal suspension equilibrated with air +20% CO_2 (right). Later, part of the liquid was replaced by the same gas mixture and volumeter recordings were started (left). The polarographic measurements show that, apart from a slight induction,

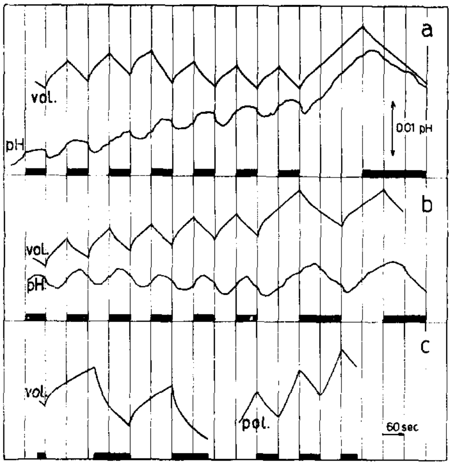

Fig. 11. Recordings of volumetric, potentiometric and polarographic response. Black bars indicate dark periods. Upward movement of the recorder pen represents pressure increase, uptake of CO_2 and production of oxygen. Cells suspended in 0.05 *M* KCl, a. and b. 50% CO_2, c. 20% CO_2.

oxygen exchange reacts immediately to illumination or darkening. These results confirm the conclusions drawn from experiments with the recording volumeter, that anomalous CO_2 exchange is the cause of the high transitory rates of gas exchange as measured. Additional observations with the polarographic and potentiometric method will be published shortly[10].

Even without the occurrence of large productions and intakes of CO_2 upon changes in light intensity, the delayed response of this gas may explain high transitory rates. In this simple case, the response of the volumeter can be easily predicted:

Suppose a light intensity I_1 gives rise to a steady state oxygen production 1 and a carbon dioxide production —1 ($\gamma = -1$). The volumeter index under these conditions will move with the rate:

$$\frac{1}{kO_2} - \frac{1}{kCO_2} \tag{1}$$

If the intensity is changed to I_2, resulting in a new rate of photosynthesis n, the index will move with the rate:

$$\frac{n}{kO_2} - \frac{n}{kCO_2} \tag{2}$$

If there is a time lag in the response of CO_2 exchange to changes in light intensity, the rate during the transition phase after going from I_1 to I_2 will be equal to:

$$\frac{n}{kO_2} - \frac{1}{kCO_2} \tag{3}$$

The ratio of transitory to steady state rate of the volumetric index is then given by the ratio (3) over (2):

$$N_1 = \frac{na - 1}{na - n}$$

in which $a = kCO_2/kO_2$.

In Fig. 12a, N_1 is plotted versus n for a few values of a. The value of N_1 is not the same for a decrease ($n < 1$) or an increase ($n > 1$) in the light intensity.

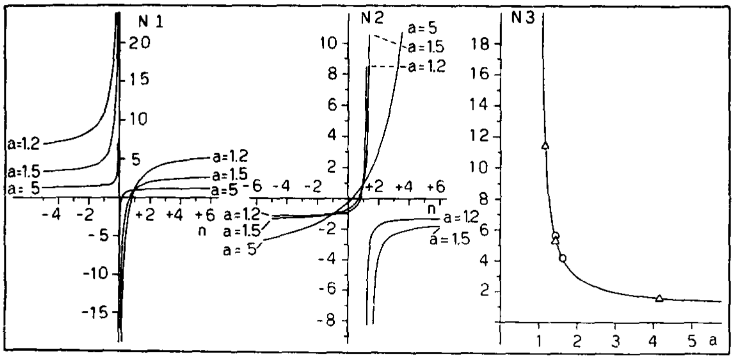

Fig. 12. See text.

The transitory rates are also different. The ratio N_2 of the initial rate at intensity I_2 over the initial rate at intensity I_1 is given by:

$$N_2 = \frac{na - 1}{a - n}$$

A plot of N_2 versus n is given in Fig. 12b for three values of a. For $n = \infty$, *i.e.* the intensity I_1 yields compensation, the ratio N_2 equals $-a$. Since in most standard manometer vessels, a is between 1 and 1.5, the ratio will not deviate far from -1, as long as I_1 is close to the compensation point.

If we are to compute quantum yields from transitory, rather than from steady state rates, we have to subtract the transitory slope during $I_2 \rightarrow I_1$ from the transitory slope $I_1 \rightarrow I_2$. This difference amounts to:

$$\frac{n}{k\mathrm{O}_2} - \frac{1}{k\mathrm{CO}_2} + \frac{n}{k\mathrm{CO}_2} - \frac{1}{k\mathrm{O}_2} \tag{4}$$

On the other hand, if we subtract the steady state rates, we obtain:

$$\frac{n}{k\mathrm{O}_2} - \frac{n}{k\mathrm{CO}_2} + \frac{1}{k\mathrm{CO}_2} - \frac{1}{k\mathrm{O}_2} \tag{5}$$

The ratio between the yields computed in these two ways represents the apparent increase in quantum yield during transition, N_3:

$$N_3 = \frac{a + 1}{a - 1}$$

In Fig. 12c N_3 is plotted against a. Values of a for a few vessels as used by WARBURG *et al.*[3] (circles) as well as by the present authors (triangles), are indicated in this figure. For the values of a, generally used, apparent increases in quantum yield during transition compared with steady state yields of 4 to 8 are obtained.

The occurrence of real outbursts and uptakes of CO_2 tends to further increase the value of N_3. Manometric equilibration, on the other hand may decrease N_3.

DISCUSSION

Observations as those reported above have also been made by several other authors. BRACKETT *et al.*[12], HILL *et al.*[13], BROWN[14] and WHITTINGHAM[15], using a variety of methods, failed to observe increased rates of oxygen exchange during transitions. Their results were in close agreement with those shown in Figs. 10 and 11.

On the other hand, anomalous transitory CO_2 exchange was observed with *Chlorella* cells as early as 1941 by EMERSON AND LEWIS[16] with the manometric method. Their description of the effect and of the factors which govern it is largely confirmed by our data, if we accept a difference in the rate of manometric equilibration. VAN DER VEEN[17] used the more specific hot-wire apparatus to study this phenomenon.

The question arises to what extent the effects studied by BURK AND WARBURG are identical with the phenomena presented in this paper. The principal difference is that we failed to obtain ratios of O_2 and CO_2 exchange during transitions consistently approaching a value of -1.

On the other hand, there are arguments strongly supporting the identity of the effects. Firstly, all of our samples of *Chlorella* cells showed the high transitory rates. Secondly, there are many other points of similarity. Like WARBURG *et al.* we found that high CO_2 concentrations are essential for obtaining high transitory rates, the light-dark transition effects requiring the highest pressure. Likewise, we found the net gain to decrease at excessive CO_2 pressures (*Aufspaltung ohne Gewinn*). The increase of apparent initial quantum yield (*cf.* Fig. 8) with increasing shaking rate was also observed by BURK[18]. With our methods, we could even observe apparent quantum yields far better than 1, if computations of gas exchange were based on the assumption that CO_2/O_2 would be equal to -1 (*cf.* also p. 215).

SUMMARY

With combined methods of volumetry, polarography and potentiometry we studied transitory phenomena as occurring in *Chlorella* suspensions upon changes in light intensity. High transitory rates as observed in the volumeter were shown to be caused by anomalous carbon dioxide

exchange only. During the transitory stages, the photosynthetic quotient may therefore deviate largely from minus unity. This fact must be considered if short exposures to light and darkness are used for the computation of photosynthetic quantum yields.

RÉSUMÉ

A l'aide d'une combinaison de méthodes volumétriques, polarographiques et potentiométriques, les auteurs ont étudiés les phénomènes transitoires qui se produisent dans des suspensions de *Chlorella* quand on modifie l'intensité lumineuse. Les vitesses transitoires très élevées observées volumétriquement se sont révélées être dues exclusivement à un échange anormal de gaz carbonique. Au cours des étapes transitoires, le quotient de photosynthèse peut donc dépasser largement l'unité. Ce fait doit être pris en considération lorsque des expositions courtes à la lumière et à l'obscurité sont utilisées à la détermination des rendements quantiques de photosynthèse.

ZUSAMMENFASSUNG

An Hand von kombinierten volumetrischen, polarographischen und potentiometrischen Methoden wurden bei Änderungen der Lichtintensität in *Chlorella*-Suspensionen eintretende Übergangsphenomene untersucht. Es wurde festgestellt, dass volumetrisch angezeigte hohe Übergangsgeschwindigkeiten nur durch einen anormalen Kohlendioxydaustausch verursacht werden. Deshalb kann der photosynthetische Quotient während der Übergangsstadien starke Abweichungen vom negativen Einheitswert aufweisen. Es muss mit dieser Tatsache gerechnet werden, wenn Berechnungen der photosynthetischen Quantenausbeute durch kurzes Exponieren in Licht und Dunkelheit angestellt werden sollen.

REFERENCES

[1] D. Burk and O. Warburg, *Naturwiss.*, 37 (1950) 560.
[2] D. Burk and O. Warburg, *Z. Naturforsch.*, 6b (1951) 12.
[3] O. Warburg, H. Geleick and K. Briese, *ibid.*, 6b (1951) 417.
[4] O. Warburg, *Z. Electrochem.*, 55 (1951) 447.
[5] O. Warburg, *Naturwiss.*, 39 (1952) 337.
[6] O. Warburg, G. Krippahl, W. Buchholz and W. Schröder, *Z. Naturforsch.*, 8b (1953) 675.
[7] O. Warburg, *Z. angew. Chem.*, 66 (1954) 493.
[8] B. Kok, *Biochim. Biophys. Acta*, 16 (1955) 35.
[9] K. Damaschke, P. Tödt, D. Burk and O. Warburg, *Biochim. Biophys. Acta*, 12 (1953) 347.
[10] C. J. P. Spruit and B. Kok, *Biochim. Biophys. Acta* (in the press).
[11] O. Warburg and V. Schocken, *Arch. Biochem.*, 21 (1949) 363.
[12] P. G. Brackett, R. A. Olson and R. G. Crickard, *J. Gen. Physiol.*, 36 (1953) 563.
[13] R. Hill and C. P. Wittingham, *New Phytologist*, 52 (1953) 134.
[14] A. H. Brown, *Am. J. Bot.*, 9 (1953) 719.
[15] C. P. Wittingham, *Plant Physiol.*, 29 (5954) 473.
[16] R. Emerson and C. M. Lewis, *Am. J. Botany*, 28 (1945) 789.
[17] R. v. d. Veen, *Physiol. Plantarium*, 3 (1950) 247.
[18] D. Burk, Paper read before Natl. Sci. Foundation Meetings on Photosynthesis, Gatlinburg (Tenn.), Oct., 1952.

Received May 2nd, 1955