ON THE INTERRELATION OF RESPIRATION AND PHOTOSYNTHESIS IN GREEN PLANTS

by

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INTRODUCTION

During a previous investigation we found anomalies in the light intensity assimilation curves measured in *Chlorella* suspensions. The linear relation between the rate of photosynthesis and the quantity of light absorbed by the cells did not hold for low intensities.

From the phenomenon, that per molecule of O_2 evolved the same number of light quanta was not always used, we concluded that more than one photochemically induced process occurs in the green plant. Fig. 1 shows a typical experiment in which the rate of dark respiration (R) and a number of assimilation rates (P) at medium light intensities have been measured. The perfectly straight line $P_1 - P_4$ cuts the ordinate at H between O and R. From these experiments we concluded that the ratio OH = HR amounted to exactly 1:1.

We interpreted this observation in the simplest way, *viz.*, by accepting the occurrence of two photochemically induced processes. The first one ($P_1 - P_4$), being normal photosynthesis, is characterized by a quantum number q (quanta absorbed per mol O_2 evolved), whereas the quantum number of the second process amounts to ½ q.

This interpretation led to some theoretical considerations regarding both processes and we felt obliged to collect more experimental material to gain more insight into the following questions:

1. Averaging about 50 experiments we found OH = HR (Fig. 1). It seemed useful to inquire how accurately this relation holds in separate experiments. To this purpose it was necessary to use an apparatus with which measurements could be performed with a still greater accuracy.

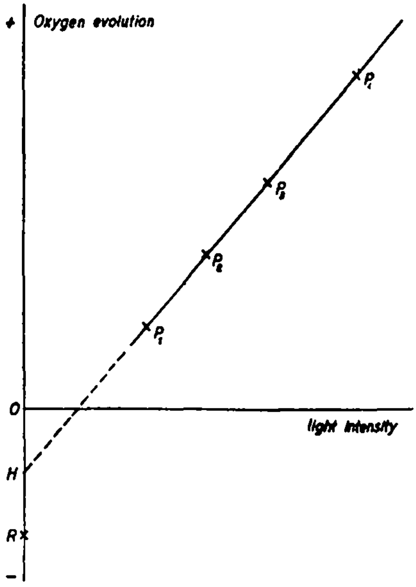

Fig. 1. Relation between light intensity and oxygen evolution according to earlier experiments

2. For the greater part our algal material was cultivated via a medium containing glucose. It was interesting to examine whether the same phenomenon is shown by inorganically grown algae as well as by other green plants.

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3. The assumption of only one extra photochemically induced process beside normal photosynthesis was the simplest interpretation but not the only possible one. Therefore we wished to obtain more information concerning the exact shape of the curve in the range R–P_1 (Fig. 1).

TECHNIQUE OF MEASUREMENT

A special type of differential volumeter (KOK AND VELTKAMP[6]) was constructed. 10–20 μl Algae were suspended per ml Warburg buffer no. 9.

The vessels used were filled in such a way that per cm^2 irradiated bottom surface 3–6 μl cells were present.

In this way part of the incident light was abosorbed (35–50%).

The light source used was a Philips HP 300 high pressure mercury lamp fed by an a.c. stabilizer. Only the green and yellow mercury lines were used. As light of these wave-lengths is not strongly absorbed by chlorophyll, relatively thick suspensions — giving higher respiration rates and still giving good linearity of the assimilation curves — could be used.

Different light intensities, measured in relative units with the aid of a thermopile-galvanometer system, were obtained by placing Scott NG filters between lamp and vessel. The various light intensities were applied one after the other. Before each measurement of the rate of pressure change (during 5 to 15 minutes) the algae were pre-illuminated during 10 to 15 minutes.

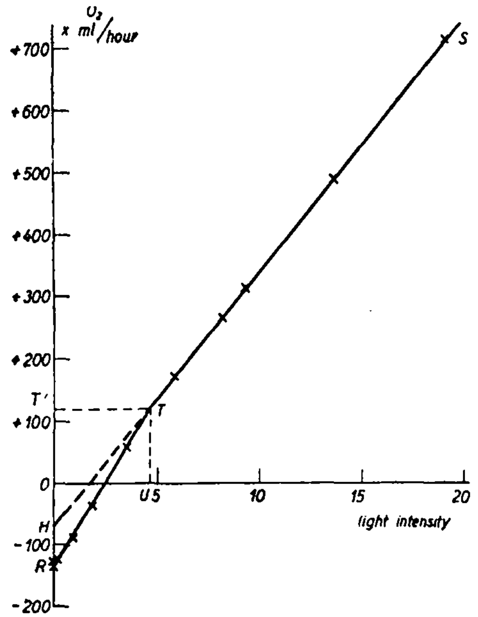

Fig. 2. *Chlorella*, grown in Knop-solution, during 4 days. Exp. 9-4-48

Dark respiration was measured at the start, middle and end of a series of light intensities.

Before each experiment we exposed the algal suspension to strong light during about half an hour; following this procedure the respiration rate was found to be more constant (Fig. 5A.)

In order to get high respiration rates most experiments were performed at 29–30° C. Measurements on leaves were made with small pieces floating in the buffer medium.

EXPERIMENTS

In Fig. 2 a typical curve found with inorganically grown algae is represented. It shows two strictly linear parts, with a sharp bend at T. The production of part ST cuts the ordinate at H, OH being exactly HR (OR represents the magnitude of the dark respiration). OT^1 is about equal to OR, the ratio of the slopes of RT and TS being about 1.3. The ratio OT^1 : OR was found to be fairly constant in various measurements performed with inorganically grown algae of the strain used by us.

Quite other curves were found for algae grown in a medium containing glucose. An example is given in Fig. 3.

In this case we also found two straight lines, but the point of intersection (T) was now situated below the abscissa and point H shifted towards R. It is remarkable in this case that $T^1H = HR$ and so the slope of RT is exactly twice the slope of TS.

When we interpret TS as photosynthesis (the q quanta process) RT represents really a ½ q quanta process.

As contrasted with inorganically grown algae the ratio OT^1: OR varied largely in these experiments.

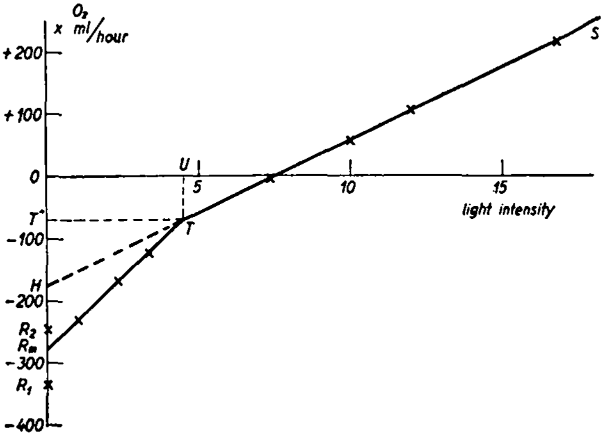

Fig. 3. *Chlorella*, grown in Knop solution + 100 mg glucose per L, during 3 days. Exp. 26-2-48.

Experiments were made with algae cultured in media containing diverse amounts of glucose (between 0.01–1.5 %), and showing largely varying respiration rates. A distinct correlation could not be detected. Mostly the ratio OT^1: T^1R varied between 1:1 and 3:1, in some experiments T was found very near to the abscissa or somewhat above it, in one experiment only, no bend was found at all.

In these experiments (more so than it was the case with inorganically grown algae) the decreasing value of the strong dark respiration during the time of measurement proved to be inconvenient.

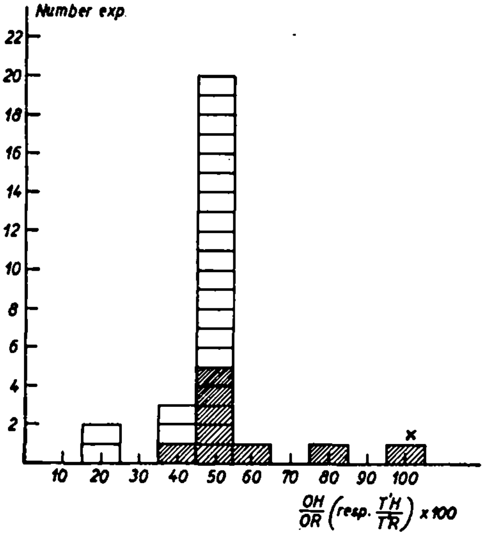

Fig. 4. Classification of results

We see that the position of point T may vary depending upon the conditions. Still more interesting is the question whether such a variability holds for point H too:

In total 28 experiments were made with *Chlorella*.

We estimated for each curve the point of intersection of P_1–P_4 with the ordinate (H Fig. 1) and we computed the value $\frac{OH}{OR}$ × 100. If curves were found as represented in Fig. 3, we computed $\frac{T^1H}{T^1R}$ × 100 (shaded squares in Fig. 4). The argumentation for these different ways of computation will be given on page 629.

The results, classified in groups increasing by 10%, are given in Fig. 4. From the distribution found we may conclude that experimental errors only are responsible for the finding of other values than 50%. (The experiment marked X, showing no bend at all, was already mentioned above).

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This means that the ratio $\frac{OH}{HR}$ or $\frac{T^1H}{HR}$ equals 1 under all circumstances.

Finally we made some experiments with two other green plants. With the alga *Haematocococcus fluviatilis* (*Volvocales*), grown in an inorganic medium (Knop solution), we found curves of the type represented in Fig. 5. Here we found T^1H greater than HR.

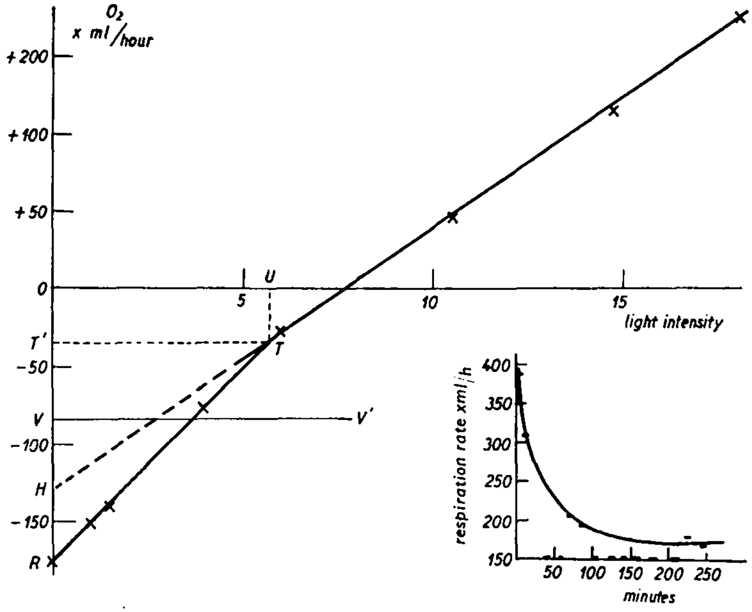

Fig. 5. *Haematococcus*, grown in Knop solution, during 14 days. Exp. 23-3-48. Fig. 5A indicates the decrease of respiration rate with time.

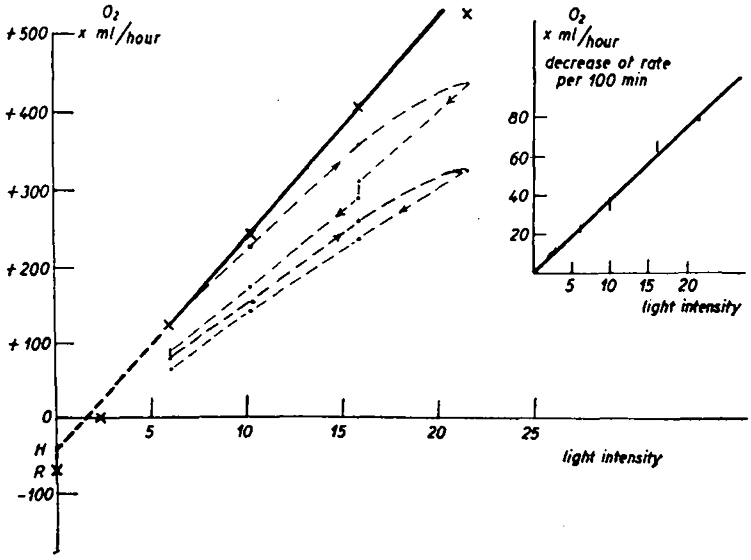

Fig. 6. *Cabomba* leaves . . . .>. . . Rates measured one after the other. Decrease fairly proportional with time and with light intensity cf. Fig. 6A. ×——× Assimilation curve as computed.

Fig. 5A shows the strong decrease of the respiration rate with time. By way of interpolation for each light intensity a correction was computed.

Leaves of the submersed aquatic plant *Cabomba*, floating in Warburg buffer showed a decreasing assimilation rate during our experiments (due to the high p_H?), As this decrease was fairly proportional with time, it was possible to eliminate this effect to a large extent by measuring with each sample of leaves an assimilation curve several times.

Respiration rates were found to be fairly constant in this case. The light intensity assimilation-curves distinctly showed the effect as represented in Fig. 6, OH being somewhat greater than HR.

No accurate information was obtained regarding the range RT (Fig. 2), although we received the impression that point T was situated as shown in Fig. 2 or at still higher light intensities.

DISCUSSION

As we found a perfectly straight line between R and T in the case of *Chlorella* (Fig. 2), and a sharp bend at T, the existence of only one extra process characterized by a different quantum number, seems to be highly probable.

As we invariably found OH (resp. T^1H) equal to HR, exactly half the amount of light quanta per oxygen molecule evolved (or not respired) is used in this process as compared to photosynthesis. A further indication in this direction is given by the fact that under special circumstances (Fig. 3) the slope of RT was indeed twice the slope of TS, and a higher ratio never occurred.

The position of the transition point T was found to be variable with external conditions and plant material. This is easily explained as a variability of the ratio in which the absorbed quanta are distributed over the two processes running simultaneously at low light intensities.

As RT is a straight line, we must assume that this ratio is exactly constant in the range of light intensities OU (Fig. 2). At U a transition point T is reached and the $\frac{1}{2}$ q quantum process is saturated. The light absorbed over that at U is used by the q quanta process only, until at very high intensities this latter process also attains saturation.

As can be derived from the slope of the curve represented in Fig. 2, in the range RT $^2/_3$ of the light absorbed is used here by the q quanta process and $^1/_3$ by the $\frac{1}{2}$ q quanta process.

In the case represented in Fig. 3 in range RT all light absorbed is used by the $\frac{1}{2}$ q quanta process, as we will discuss below.

In our previous paper we interpreted the $\frac{1}{2}$ q quanta process as "light respiration": a direct supply of energy from light quanta to the cell metabolism. (The favourable energetic yield resulting from the omission of the carbohydrate stage occurring in the cycle photosynthesis-respiration.)

We introduced the supposition that simultaneously with the substitution of its function a decrease of the rate of dark respiration occurs. In such a way that the total energy supply of the cell — the sum of the products of both processes is always constant (at least during an experiment). In this picture at point T (Fig. 2) total suppression of dark respiration is reached and the $\frac{1}{2}$ q quanta process is saturated, providing the cell with the same amount of energy as the respiration process furnished in the dark.

If we apply this trend of thought to Fig. 3, we have to assume that now part of the dark oxygen uptake (OT[1]) is *not* suppressed by the ½ q quanta process, but goes on undisturbed in the light.

It follows from our experiments that deviations from linearity in the assimilation curves at low light intensities are not restricted to *Chlorella*. In leaves of higher plants where next to green cells colourless tissues occur, the latter tissues can be expected to interfere, since in them respiration can hardly be affected by photochemical products in the green cells. So a priori we would expect to find here also a phenomenon as given in Fig. 3, where point H is shifted towards R cf. Fig. 6.

The curve found with *Haematococcus* needs further discussion: Firstly we find a similar shift of point H as with organically grown *Chlorella* cells.

In these cases we have not to deal with different types of cells (coloured or colourless) and we can imagine two possibilities: either there are two types of oxygen consuming processes running in the dark, only one of both being suppressed by light, or the influence of photochemical metabolites is restricted to the neighbourhood of the chloroplast.

Secondly the slope of RT in Fig. 5 indicates a simultaneous occurrence of the two photochemically induced processes; it seems probable that the part of the dark oxygen consumption not influenced by light amounts to OV (HV = HR). This computation is suggested by the analogy with the other curves: considering VV_1 as the abscissa we obtain a similar curve as given in Fig. 2.

According to these views we have to accept in this organism the simultaneous occurrence at low light intensities of 4 processes:

1. unsubstitutable dark respiration; 2. substitutable dark respiration; 3. light respiration (½ q quanta process); 4. photosynthesis (q quanta process).

We may accept a localization of the photochemical process (in the chloroplasts), but a distribution of respiration over the whole cell. Then it is indicated by the various curves obtained that the photochemically formed intermediate, base for the ½ q quanta process, must have a rather long lifetime.

Finally we may point here to investigations of several authors who determined light intensity assimilation curves with leaves as well as with algae. In many cases the relation found at medium intensities was interpreted as a smoothly bent curve, though a straight line, not crossing the origin, fits much better with the measurements plotted (WASSINK *et al.*[2], KOPP[3], GABRIELSEN[4, 5]). We like to interpret all these curves in the same way as we did our own ones. The measurements of GABRIELSEN point to the occurrence of the phenomenon described in leaves of higher plants.

SUMMARY

The relation between light intensity and rate of photosynthesis was studied at low and medium intensities with suspensions of *Chlorella, Haematococcus,* and leaves of *Cabomba*. Various types of curves were found, all showing two strictly linear parts with a sharp bend between.

In the discussion of the various curves we started from the more or less complete suppression of dark respiration by the photochemical reaction.

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RÉSUMÉ

La vitesse de la photosynthèse a été mesurée relativement aux intensités lumineuses faibles et moyennes avec des suspensions de *Chlorella*, *Haematococcus* et des feuilles de *Cabomba*.

Des courbes d'un caractère divers ont été trouvées, chacune présentant deux portions rectilignes séparées par un coude accentué. Pour la discussion des diverses courbes, nous avons pris pour point de départ la suppression plus on moins totale de la respiration due à la réaction photochimique.

ZUSAMMENFASSUNG

Das Verhältnis zwischen Lichtintensität und Geschwindigkeit der Photosynthese wurde bei schwachen und mittleren Intensitäten an Suspensionen von *Chlorella*, *Haematococcus* und an Blättern von *Cabomba* gemessen. Es wurden verschiedenartige Kurven gefunden, die aber alle aus zwei geradlinigen Stücken bestehen. Bei der Besprechung der verschiedenen Kurven gingen wir von der mehr oder weniger vollständigen Ausschaltung der Dunkelatmung durch die photochemische Reaktion aus.

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