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THE MECHANISM OF THE INITIAL STEPS OF REACTIONS IN PHOTOSYNTHESIS

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ABSTRACT

The yield of oxygen production per light flash in photosynthesizing algae subjected to intermittent light has been studied as a function of the duration of both the light flashes and the dark periods between flashes. From these studies, information regarding the mechanism of the initial photochemical steps of photosynthesis has been obtained. There were found to be two reservoirs of chemical energy that stored energy absorbed during the flash for use during the subsequent dark period. The first of these was saturated during the shortest flashes employed; the second one was never saturated during the conditions employed and was filled at a rate that was approximately linear with respect to the duration of the light flash.

THE MECHANISM OF  
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## INTRODUCTION

Many experiments have been carried out with intermittent light in the hope of elucidating the mechanism of the initial steps of reactions in photosynthesis. But most previous studies have been concerned with the change of the photosynthetic yield per flash of a fixed period with different dark periods. From the results, various theories were proposed, including the theory of a "photosynthetic unit."<sup>1, 2</sup> Tamiya and Chiba<sup>3</sup> found that the maximum yield becomes temperature-dependent when the light intensity is very high, and in place of the "photosynthetic unit" theory they proposed a different mechanism in which the sensitizer S (probably chlorophyll) is converted to the excited sensitizer ( $S^*$ ), after which  $S^*$  reacts with other chemicals to bring about photosynthesis or is deactivated by a second-order temperature-dependent reaction. Despite differences in these theories, there is similarity in one respect--in the assumption that there is only one compound excited or one reservoir to be filled during the course of a flash, with other steps in photosynthesis occurring during a subsequent dark period.

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<sup>†</sup> The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

<sup>1</sup> H. Gaffron and K. Wohl, *Die Naturwissenschaften* 24, 81, 103 (1936).

<sup>2</sup> K. Wohl, *Z. Physik. Chem. B.* 37, 105, 122, 169, 186, 209 (1937); *New Phytologist* 39, 33 (1940); 40, 34 (1941).

<sup>3</sup> H. Tamiya and Y. Chiba, *Studies of the Tokugawa Institute* 6, No. 4 (1949).

The preliminary experiment reported here was carried out to study the effect of the flashing-light period on the maximum yield per flash when a sufficiently long dark period and a sufficiently high intensity are provided to saturate photosynthesis even with the flashing light. As will be shown later, it appears that at least two reservoirs are filled, or partially filled, in the course of light periods used in these experiments.

### Methods

The system used in this experiment to measure photosynthetic rate was the same as the system for our quantum-requirement experiments,<sup>4</sup> although the total volume was changed so as to increase the sensitivity, and the surface area of the cell containing the algal suspension was reduced so as to obtain more light per unit area of the suspension of algae. The photosynthetic rate was measured by observing the change of oxygen content in the closed system, by means of an analyzer measuring the paramagnetic property of oxygen. At the same time, the change of CO<sub>2</sub> was observed as a reference by an infrared CO<sub>2</sub> analyzer. To saturate the photosynthetic yield, two 1-kw projection lamps and a 400-w photospot illuminated the suspension of algae through three sheets of infrared-absorbing glass with a water cooling system. In front of the cell, which contained 25 ml of algal suspension, a sectored disc was rotated. Six kinds of discs were used; they have 1, 2, 3, 4, 6, and 9 open sectors of the same size in a disc that is 40 cm in diameter. The size of opening was made so as to obtain equal dark and light periods with a 9-hole disc. Therefore, dark periods relative to the light period in these discs are 17, 8, 5, 3.5, 2, and 1 respectively. If the rotating rate is fixed, the light period ( $t_e$ ) is the same for all discs, but the dark periods ( $t_d$ ) can be changed by changing the discs. From a set of data with a single rotation rate, one can obtain the change of yield per flash as a function of dark period for a fixed light period. The light period was changed by changing the rotation rate. The light periods were 1/360, 1/180, 1/90, and 1/45 sec.

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<sup>4</sup> J. A. Bassham, K. Shibata and M. Calvin, *Biochem. Biophys. Acta*, 17, 332 (1955).

An important requirement in this experiment is that the photosynthetic rate be saturated with respect to light intensity under the conditions used. The saturation was tested with the longest dark periods (one-hole disc) for two light periods,  $1/360$  sec and  $1/45$  sec. One of the results, where  $t_e = 1/360$  sec and  $t_d = 17/360$  sec, is shown in Fig. 1, where relative photosynthetic rate per flash ( $P_f$ ) is plotted against the relative light intensity ( $I_{rel}$ ). The arrow in the figure indicates the light intensity at which all other experiments reported here were carried out. With this light intensity, the yield is well saturated even with the one-hole disc. The concentration of the suspension of Scenedesmus was 0.2% in packed cell volume units.

### Results

The results are shown in Fig. 2, where the relative photosynthetic rate per flash ( $P_f$ ) is plotted against the dark period ( $t_d$ ). Each curve represents the change of photosynthetic rate for a particular light period ( $t_e$ ) as a function of the dark period. The points for zero dark period were calculated from the continuous-light rate. The relative unit of  $P_f$  was chosen so as to make  $P_f$  equal to unity for  $t_e = 1/360$  sec and  $t_d = 0$  sec; hence the values for the intercepts for the curves at  $t_e = 1/360$ ,  $1/180$ ,  $1/90$ , and  $1/45$  sec are 1, 2, 4, and 8, respectively. As can be seen in this figure,  $P_f$  increases linearly at short dark times and levels off to a maximum value. The behavior of this change of  $P_f$  as a function of the dark period agrees with the results that have been obtained by other investigators.

For the purpose of discussion the effect of the variation in light period,  $\Delta P'_e$ , was defined as the difference between the  $P_f$  value for zero dark period and the maximum  $P_f$  value for each curve. The values of  $\Delta P'_e$  and the corresponding absolute values of  $\Delta P_e$  are listed in Table I.

$\Delta P'_e$  is the measure of oxygen generation per flash in the dark in excess of the oxygen generation in the light. We postulate that in the course of a light period a first reservoir is filled by the energy of light, and must be full by the end of the light period because the photosynthetic rate is saturated against light intensity. Presumably, this initial step of photosynthesis would be a photochemical reaction, and some purely chemical reaction may follow it, with the additional possibility of some deactivation process. But, if only one reservoir is filled during the light flash and is responsible for all further reactions of photosynthesis,  $\Delta P'_e$  should be



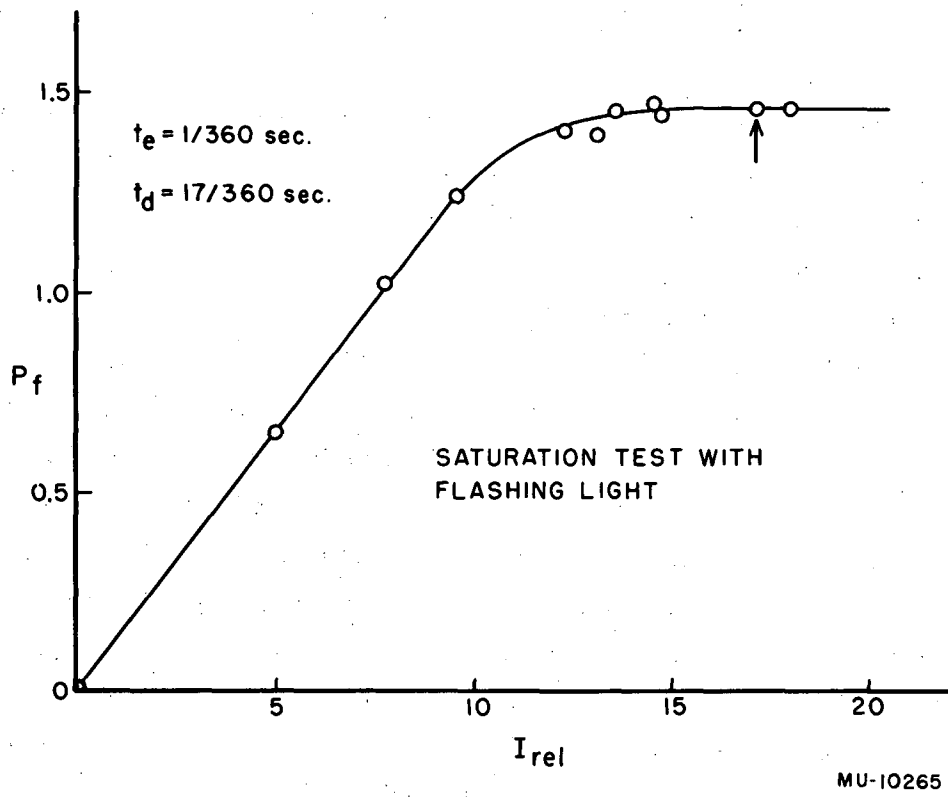


Fig. 1. Saturation test with flashing light.

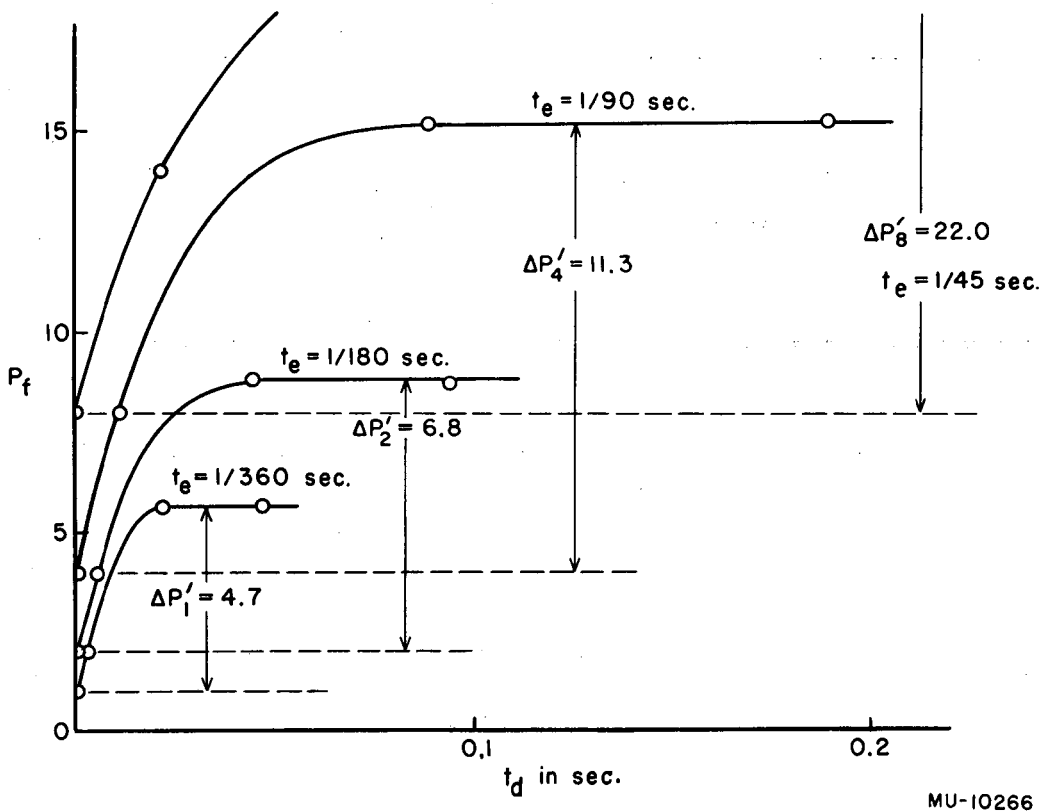


Fig. 2. Change of oxygen generation per flash with changing dark periods.

Table I

Extra yield of oxygen per flash in photosynthesis  
as a function of flash time

Ch (total chlorophyll-a content) =  
 $117 \times 10^{-8}$  mole per 25 ml of suspension

$t_e$	$\Delta P'_e$	$\Delta P_e$ ( $10^{-8}$ mole of $O_2$ per 25 ml)	$\Delta P_e / \text{Ch}$ $\Delta_e$ (%)
1/360	4.69	3.80	0.325
1/180	6.75	5.62	0.480
1/90	11.23	9.12	0.782
1/45	22.00	17.8	1.517
0	3	2.43	0.204

constant and independent of light period, because the light intensity is strong enough to saturate the reservoir in any light period used. This would be true no matter what the mechanism of reactions relating to this particular reservoir might be.

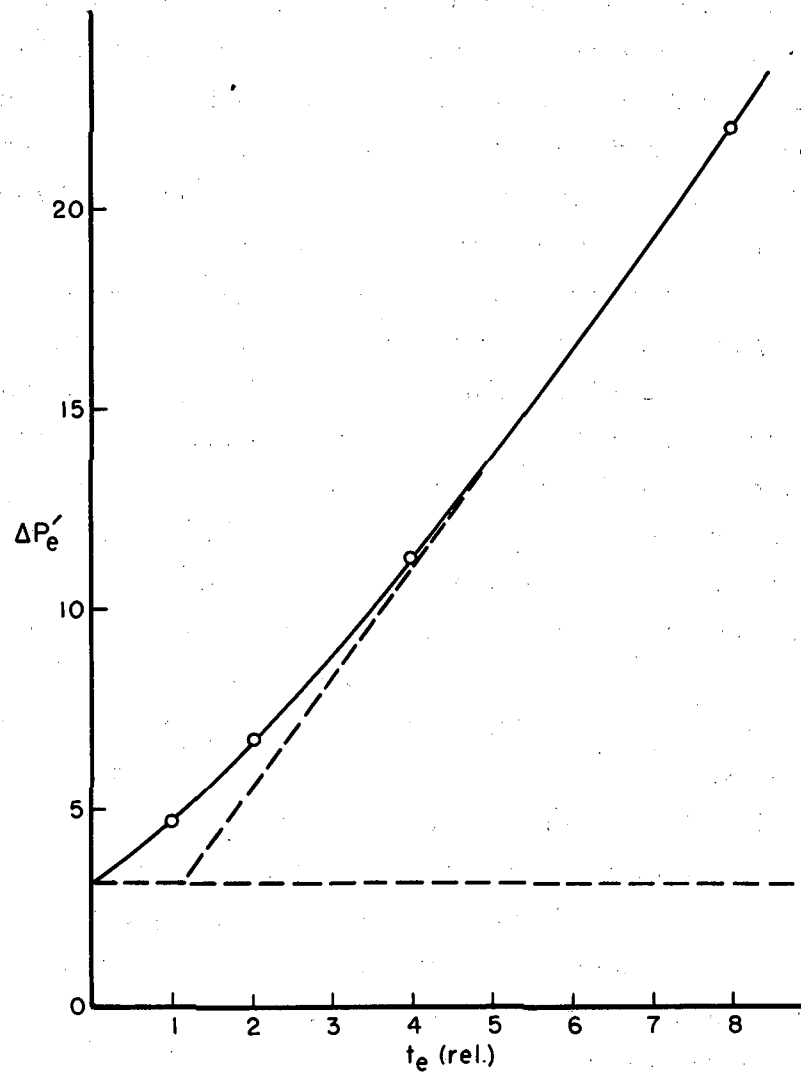
In Fig. 3  $\Delta P'_e$  is plotted against relative light period. As can be seen,  $\Delta P'_e$  increases with increasing light period instead of remaining constant, as would be the case that is indicated by the dotted horizontal line. The rate of increase becomes almost linear at longer light periods. This result indicates that there is a second reservoir, (which is partially filled) even in these rather short light periods. Almost from the beginning the second reservoir begins to be filled, as can be seen from the fact that the straight part of the curve can be extrapolated to pass through the origin. The ratio of  $\Delta P_e$  (absolute value) to total chlorophyll content of the sample of algae is listed in Table I. The ratio extrapolated to zero light period is 0.204%, the reciprocal of which is about 481 molecules of chlorophyll for each molecule of oxygen evolved. This number is much smaller than the previously reported photosynthetic unit of 2500. It may be that the value of 2500 resulted from weak light intensities employed in earlier experiments.

Another fact we might point out is that the first reservoir is almost filled at about 3 milliseconds. This time is calculated from the intersection of the horizontal straight line and the extrapolated straight part of the curve in Fig. 3. This time corresponds to the first fast decay period of chemiluminescence, which was observed by Strehler et al.<sup>5</sup>

The straightness of the curve in Fig. 3 at longer light periods indicates that the second reservoir is filled by a zero-order reaction, which is quite reasonable as an enzymatic reaction. If the light period is further increased, the curve in Fig. 3 should level off when the second reservoir is saturated.

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<sup>5</sup> B. Strehler, Arch. Biochem. Biophys. 34, 239 (1951); B. Strehler and W. Arnold, J. Gen. Physiol. 34, 809 (1951); W. Arnold and J. B. Davidson, J. Gen. Physiol. 37, 677 (1954); B. Strehler, reported at Gatlinburg Meeting on Photosynthesis, 1955; W. Arnold, reported at Gatlinburg Meeting on Photosynthesis, 1955.



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Fig. 3. Oxygen generation per flash with change of light period.