Effects of DCMU and Antimycin A on Photoassimilation of Glucose in Chlorella[1, 2]

W. Tanner, L. Dächsel, and O. Kandler

Institut f. Angewandte Botanik der Technischen Hochschule München

Two basically different systems have been described by which chloroplast preparations are able to synthesize ATP in the light: cyclic and noncyclic photophosphorylation (2, 3). Other systems producing ATP in the light, e.g. pseudocyclic (or aerobic) and oxidative photophosphorylations might only be variations of the 2 basic types (26, 27).

A main feature of true cyclic photophosphorylation in chloroplasts is its resistance to DCMU[3] and CMU (16), inhibitors of photosynthetic O_2 production (5, 29). According to the concept of 2 light reactions in photosynthesis (8, 11, 15, 32) this DCMU resistance means that cyclic photophosphorylation should be driven only by the first light reaction and not depend on the second one, which is responsible for the splitting of water. Such evidence has been obtained by Tagawa et al. (22) for ferredoxin-catalyzed cyclic photophosphorylation in chloroplast preparations.

In spite of a vast literature on photophosphorylation in vitro, much less is known about photophosphorylation in vivo. The best evidence for photophosphorylation in vivo has been obtained by following glucose assimilation of *Chlorella* (13, 14) and of leaf discs (18), acetate assimilation of *Chlamydobotrys* (19, 31), inhibition of photoreduction by glucose in *Ankistrodesmus* (6), and light-enhanced P^{32} incorporation in *Helodea densa* (20). In the following paper the question was investigated whether cyclic photophosphorylation of the type observed in chloroplasts also occurs in vivo. The influence of DCMU and antimycin A on light dependent glucose assimilation in *Chlorella* was studied in the absence of CO_2 and O_2.

Materials and Methods

The same strain of *Chlorella pyrenoidosa* was used as previously (12). The culture medium contained in 1 liter of destilled water: 0.4 g KNO_3, 0.1 g $Ca(NO_3)_2 \cdot 4\ H_2O$, 0.1 g $MgSO_4 \cdot 7\ H_2O$, 0.1 g KCl, 0.1 g $KH_2PO_4 \cdot H_2O$, 2 mg $FeCl_3$, 5 ml saturated EDTA solution (free acid), and 1 ml Hoagland's A-Z solution. The algae were grown in 1-liter flasks at a light intensity of 3000 lux. An air-CO_2 mixture (ca. 1–2 % CO_2) was bubbled through the cultures. The algae were harvested within 3- to 5-day intervals and subsequently starved for 24 hours at 18° in 0.02 M potassium phosphate buffer pH 7.1.

All experiments were carried out in 15-ml rectangular Warburg vessels. In the light these were surrounded by a tin mantle in a way that the light could enter the vessel only from the bottom. The light intensities given were those measured at the bottom of the vessels; they were varied by neutral screens. Philips Attralux tungsten lamps served as a light source. Photosynthesis was measured manometrically in 0.1 M carbonate buffer ($NaHCO_3$: Na_2CO_3=9:1). $C^{14}O_2$ fixations were carried out in a small lollipop. An air-$C^{14}O_2$ mixture (0.44 % CO_2) was bubbled through 2.8 ml algal suspension in 0.04 M Tris-HCl pH 8.9. The suspension (0.2 ml) was killed by adding it to 2-ml cold absolute ethanol containing 5 % acetic acid. The total of 2.2 ml was then plated on 2 aluminum planchets and counted with a windowless methane flow counter Frieseke a. Hoepfner 407A. All experiments were carried out at 27°.

Incubation Conditions for the Measurement of Glucose Uptake in the Light (+ N_2) and Dark (+ air). The algae were suspended in 0.04 M Tris-HCl buffer pH 8.9, with 5 mg glucose in a total volume of 2.8 ml. This suspension had a chlorophyll content of 200 to 220 γ/ml as determined by the method of Arnon (1). Alkaline pyrogallol (0.2 ml) for the light and 20 % KOH (0.2 ml) for the dark experiments were present in the side arm. For the light-dependent uptake the samples were flushed for 10 minutes with purified N_2 which was passed through an alkaline pyrogallol solution. O_2 uptake was followed during determination of the oxidative glucose assimilation. Glucose was determined according to the method of Folin and Wu (9, 10).

The different amounts of antimycin A were added in 0.05 ml methanol. The same amount of methanol was added to all the controls. DCMU was dissolved in methanol giving a concentration of 10^{-2} M. A dilution series with water was then prepared to give the final concentrations wanted.

Results

Effects of DCMU on Respiration and Oxidative Glucose Assimilation. In order to determine if DCMU was affecting physiological processes in ad-

[1] Received May 1, 1965.

[2] Supported by Deutsche Forschungsgemeinschaft.

[3] Abbreviations used; DCMU=3-(3,4-dichlorophenyl)-1,1-dimethylurea; CMU=3-(4-chlorophenyl)-1,1-dimethylurea.

dition to photosynthesis, its influence on respiration and oxidative phosphorylation was studied. The latter was followed by the oxidative glucose assimilation (13, 14). Glucose was added to the algae-buffer suspension before the algae were distributed into the Warburg vessels. The vessels already contained the various amounts of DCMU. After all the additions the vessels were equilibrated for 5 minutes. A zero time control was centrifuged at 0° immediately after closing the manometers.

DCMU concentrations higher than 10^{-6} M slightly increased glucose respiration (fig 1) and with 5×10^{-5} M a stimulation of 10 % was obtained. Also, the endogenous respiration was increased to the same extent. In contrast the glucose uptake was slightly inhibited at concentrations of 5×10^{-6} M and higher. With 5×10^{-5} M DCMU an inhibition of 16 % was

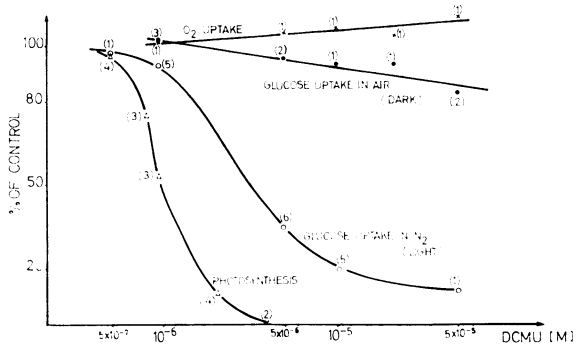

FIG. 1. Effect of DCMU on O_2 uptake in the presence of glucose (glucose respiration), oxidative- and photoassimilation of glucose, and photosynthetic O_2 evolution. Light intensity 34,000 lux. Average values of (n) experiments.

observed. These rather high concentrations of DCMU thus exert a weak uncoupling effect on the respiratory metabolism of *Chlorella*. The ratio of moles of O_2 consumed to moles of glucose assimilated increases by about 40 % (table I).

Effects of DCMU on Photosynthesis and Photoassimilation of Glucose. The glucose uptake in the light was followed under anaerobic conditions. The experiment was started as described above. One vessel was covered with black cloth and carried through the experiment under otherwise identical conditions. All values of light-dependent glucose assimilation given in the paper are based on the dark/N_2 sample as control (see line 4, table II). An example of an experiment is given in table II. For comparison the

Table II. *Glucose Assimilation in* Chlorella

Experimental conditions: see Methods. Incubation time was 3 hours 15 minutes.

Conditions	Amount of glucose determined (γ)	Calculated glucose uptake (γ)
0 Time control	5200	...
Dark/N_2	5020	180
Light/N_2	1926	3274
Light/N_2-Dark/N_2	...	3094
Dark/O_2	1570	3630

uptake under aerobic conditions, which on an average was 10 to 20 % higher than in light + N_2, is included in the table. The uptake under anaerobic conditions in the dark was generally smaller than 5 % of the amount taken up in the light.

In the presence of DCMU glucose uptake in light is considerably inhibited (fig 1). A concentration of 4×10^{-6} M resulted in an inhibition of 55 %. However, at the same concentration photosynthesis, measured as O_2 production of the algae in carbonate buffer, was completely inhibited. The 2 curves do not run parallel and even with the highest DCMU concentration tested it was not possible to inhibit the glucose uptake completely. Trebst and Eck (25) found that cyclic photophosphorylation with some cofactors (e.g. vitamin K) is only DCMU-resistant if the cofactor is in its reduced form at the time of DCMU addition. This was achieved by preilluminating the chloroplasts before the addition of poison. To check if a similar effect can be observed in vivo, algae were illuminated for 30 minutes under anaerobic conditions and subsequently a solution containing DCMU + glucose was tipped from the sidearm into the algae suspension. This treatment, however, did not release the inhibiting effect of DCMU.

The different susceptibility of photosynthesis and light-dependent glucose uptake to DCMU could have been a result of the difference in light saturation of these 2 processes. It has been noticed earlier (13) that the light-dependent glucose uptake is saturated at much lower light intensities than photosynthesis. Figure 2 shows this under the experimental conditions used here. The rate of photosynthesis was linear up to 6000 lux and even increased up to 14,500 lux. The light-dependent glucose uptake, however, was already saturated at 1200 lux. Since the experiments of figure 1 were carried out at 34,000 lux it was possible that this extreme oversaturation was causing the relatively smaller susceptibility of the glucose uptake.

Table I. *Uncoupling Effect of DCMU and Antimycin A in* Chlorella *Expressed as Amount of O_2 to Amount of Glucose Taken Up*

DCMU	Conc (M)	0	4.5×10^{-6}	1×10^{-5}	4.5×10^{-5}
	Mole O_2/mole glucose	0.96	0.99	1.24	1.33
Antimycin A	Conc (γ/2.8 ml)	0	40	100	200
	Mole O_2/mole glucose	0.85	1.41	1.56	1.82

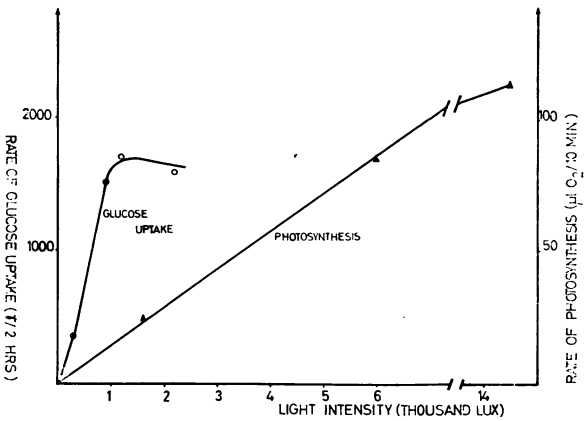

FIG. 2. Rate of photoassimilation of glucose and rate of photosynthetic O_2 evolution in relation to light intensity. Experimental conditions: see Methods.

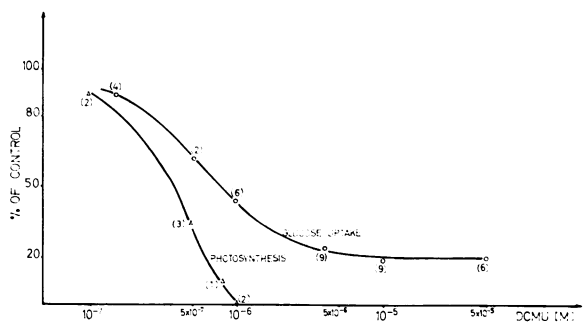

FIG. 3. Effect of DCMU on photoassimilation of glucose and photosynthetic O_2 evolution. Light intensity 1200 lux. Average values of (n) experiments.

To check this possibility glucose uptake experiments were conducted at 1200 lux. As shown in figure 3 at low light intensity glucose uptake was indeed more sensitive to DCMU; thus 10^{-6} M DCMU showed a 55 % inhibition whereas it was only 7 % at 34,000 lux. However, photosynthesis also is more sensitive to DCMU at low light intensities. Therefore, the different response of photosynthetic O_2 evolution and photoassimilation of glucose to DCMU in the end remained the same at both light intensities. It should be pointed out, however, that it was not possible to inhibit glucose uptake completely even with rather high DCMU concentrations. Thus, a plateau is reached between DCMU concentrations of 5×10^{-6} and 5×10^{-5} M. Tagawa et al. (21) have shown that antimycin A inhibits a ferredoxin-catalyzed cyclic phosphorylation in chloroplasts which is insensitive to DCMU. It was of interest, therefore, to see if the light-dependent glucose uptake in *Chlorella* can be inhibited by antimycin A. For comparison the effect of antimycin A on respiration and oxidative phosphorylation was also investigated.

Effects of Antimycin A on Respiration and Oxidative Phosphorylation. Antimycin A inhibits the oxidative glucose uptake to maximally 60 % but increases the respiration up to 20 % (fig 4). These effects are stronger than the corresponding ones with DCMU and, therefore, lead to a greater uncoupling. This is reflected in the higher O_2/glucose ratios (table I). In contrast to DCMU the endogenous respiration is considerably increased (400 γ antimycin A more than double endogenous O_2 uptake).

Effects of Antimycin A on Photoassimilation and on Photosynthesis. The experiments in figure 4 were carried out at light intensities of 1200 and 14,500 lux. The addition of antimycin A resulted in a strong inhibition of glucose uptake at the low light intensity, but it was considerably released at 14,500 lux. Antimycin A at a concentration of 200 γ/2.8 ml (1.3×10^{-4} M) inhibited glucose uptake

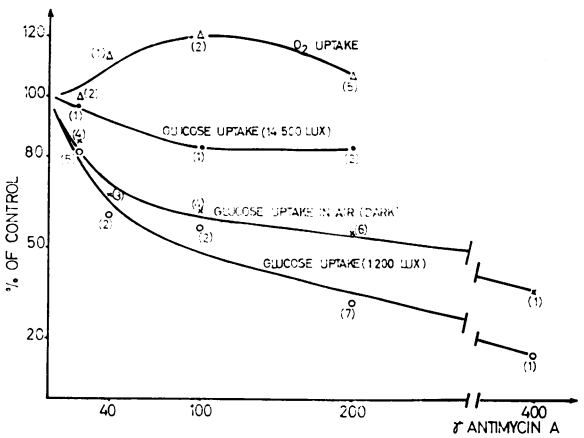

FIG. 4. Effect of antimycin A on glucose respiration, oxidative- and photoassimilation of glucose. Light intensities as indicated. Average values of (n) experiments.

Table III. *Effect of Antimycin A on Photosynthesis in* Chlorella

Method used	Light intensity	Amount of antimycin added (γ)	
	Lux	100	200
		% Inhibition	
Manometry	34,000	16	21
	14,500	18	24
$C^{14}O_2$ Fixation	14,500	...	23
	1200	...	30

at 1200 lux by more than 60 % and the maximal inhibition observed was 85 % with 400 γ antimycin. Photosynthesis was less inhibited. The results are summarized in table III showing the effect of different light intensities and of 100 and 200 γ antimycin on O_2 evolution and $C^{14}O_2$ fixation, respectively. The $C^{14}O_2$ method had to be used at low light intensities, since antimycin increases endogenous respi-

Table IV. *Additive Effect of DCMU and Antimycin A on the Photoassimilation of Glucose*

Expt	Inhibition of glucose uptake in % DCMU (2×10^{-5} M)	DCMU (2×10^{-5} M) + 200 γ Antimycin	Antimycin (200 γ)
1	81.2	88.7	
2	72.4	72.2	
3	71.4	78.7	
4	75.6	81.8	
5	77.2	81.8	
6	80.9	85.5	
Avg	76.4	81.4	65.0*

* Average value of 7 individual experiments (see fig 4). These experiments were carried out separately.

ration considerably (> 100 %) and, therefore, it becomes difficult to evaluate the actual photosynthesis with manometric techniques. Photosynthesis was inhibited up to 30 %. High light intensities, however, released the inhibition to a much smaller extent than that of glucose uptake. When *Chlorella* was not starved before the experiment, photosynthesis was even less affected by antimycin A, thereby increasing the difference in susceptibility of glucose uptake and photosynthesis (23). It was surprising that DCMU and antimycin A yield approximately the same inhibition of photoassimilation of glucose in weak light.

In view of the results of Urbach and Simonis (28) it was of interest to study the simultaneous action of the 2 poisons on the light-dependent glucose uptake. In table IV the inhibitions observed with 2×10^{-5} M DCMU and 200 γ antimycin alone are compared to those of both poisons together. Each value represents the average of duplicate samples. In the presence of 2×10^{-5} M DCMU glucose uptake was inhibited by 76.4 % (total average of 6 experiments) whereas the additional presence of 200 γ antimycin A increased the inhibition to 81.4 %. This difference is rather small and the results are far from showing additivity, i.e. a 100 % inhibition.

Discussion

At first we would like to compare our results with those of other authors. Butt and Peel (7) also observed inhibition of light-dependent glucose uptake of *Chlorella* by 5×10^{-6} M DCMU. Marré et al. (17) have investigated the effect of CMU on the light-enhanced glucose-C^{14} incorporation into leaves at a concentration where O_2 evolution is completely inhibited. The synthetic events (starch and cellulose synthesis) were inhibited by 47 % with 5×10^{-5} M CMU and the total light-enhanced glucose incorporation by 28 %.

The light-dependent P^{32} incorporation into *Ankistrodesmus* studied by Urbach and Simonis (28) most likely is based on the same mechanism as photoassimilation of glucose. This anaerobic P^{32} incorporation is also strongly inhibited by DCMU, however, only slightly by antimycin A alone. The latter result can be explained by the observation that a high light intensity, as it was applied by Urbach and Simonis, releases antimycin inhibition of glucose uptake to a considerable extent (fig 4). In contrast to our results (Urbach and Simonis) obtained, however, an additive effect if DCMU and antimycin A were added together.

Wiessner and Gaffron (30) studying anaerobic photoassimilation of acetate in *Chlamydobotrys* also observed a DCMU inhibition in the absence of CO_2. However, this DCMU sensitivity could be released if some O_2 was introduced into their system. Since in the presence of O_2 a strong oxidative glucose assimilation takes place in *Chlorella*, it is not possible to check this O_2 effect in this organism. In addition there seems to be a difference between these 2 systems since in *Chlorella* the aerobic photoassimilation of glucose is only about 50 % higher than the anaerobic (13) whereas the photoassimilation of acetate in *Chlamydobotrys* is more than 4 times higher in the presence of O_2 (30).

Summarizing the comparison of the results of other authors with ours we can say there is a general agreement concerning the main effects, but there are certain deviations which might be caused by differences of the objects chosen as well as the processes used as indicator for photophosphorylation.

It should be pointed out that the phosphorylation which is responsible for the anaerobic photoassimilation of glucose must be a cyclic process. A noncyclic phosphorylation cannot take place in the absence of CO_2, the natural hydrogen acceptor, whereas a pseudocyclic or aerobic phosphorylation (free O_2 would have to participate) is unlikely under the anaerobic conditions used (N_2-flushing; alkaline pyrogallol present during the whole experiment).

The rather high inhibition of glucose uptake by DCMU seems to indicate at first sight a different mechanism for cyclic photophosphorylation in vivo and in vitro. However, at DCMU concentrations where photosynthesis is completely inhibited glucose uptake is inhibited only by 55 % (fig 1, 3). In addition recent experiments have shown that the inhibition of glucose uptake by DCMU can be separated into 2 effects: A) the inhibition of an induction phase lasting 30 to 60 minutes from the time the glucose is added and B) an inhibition during steady state photoassimilation of glucose (24). If only the latter effect is taken into consideration the difference in susceptibility of photosynthesis and glucose uptake is even greater. At concentrations inhibiting photosynthesis completely, photoassimilation still proceeds at a rate 70 to 80 % of the control rate. The plateau at the higher DCMU concentrations does not exceed 60 % inhibition (23, 24). Two sites for DCMU inhibition have to be postulated, therefore: A) the splitting of water [light reaction II (8)], and B) at higher concentrations an inhibition somewhere in the system of light reaction I. Two sites for DCMU

inhibition have already been suggested from in vitro results by Asahi and Jagendorf (4).

Antimycin A also shows a high inhibition of photoassimilation of glucose. There is, however, no difference if antimycin A is added in the beginning or 1 hour after glucose addition, i.e. during steady state glucose uptake. Since photosynthesis is much less affected by antimycin A, it seems likely that cytochrome b_6 is only involved in cyclic photophosphorylation as has been suggested by Tagawa et al. (21).

The observation that DCMU together with antimycin A does not show additive inhibition is also in agreement with the interpretation that in our system antimycin A as well as DCMU at high concentrations inhibit cyclic photophosphorylation.

Summary

The effects of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and antimycin A on respiration, photosynthesis, oxidative glucose assimilation, and photoassimilation of glucose in the absence of oxygen and carbon dioxide in *Chlorella pyrenoidosa* were investigated.

DCMU at concentrations more than 10^{-6} M has only a minor effect on oxygen uptake and on oxidative glucose assimilation. Antimycin A acts like a rather strong uncoupling agent since the uptake of oxygen is increased up to 20 % and glucose assimilation inhibited to about 50 %.

The degree of inhibition of photosynthesis and photoassimilation of glucose by DCMU and antimycin A is strongly influenced by the light intensities used; higher light intensities result in less inhibition. Photosynthesis is more sensitive to DCMU than photoassimilation of glucose. At concentrations which inhibit photosynthesis completely photoassimilation of glucose is inhibited by 55 %. At these concentrations the inhibition amounts to only 20 to 30 % if DCMU is added 1 hour after the glucose is supplied, i.e. at a time when photoassimilation of glucose has reached a steady state rate. It is postulated that DCMU, besides inhibiting light reaction II, at high concentrations also inhibits cyclic photophosphorylation dependent only on light reaction I.

In contrast to DCMU antimycin A inhibits photoassimilation more than photosynthesis. An inhibition of glucose uptake up to 70 % was observed. This suggests the participation of cytochrome b_6 in cyclic photophosphorylation. The addition of both poisons together did not result in an additive inhibition.

Literature Cited

1. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 1–15.
2. Arnon, D. I., M. B. Allen, and F. R. Whatley. 1954. Photosynthesis by isolated chloroplasts. Nature 174: 394–96.
3. Arnon, D. I., F. R. Whatley, and M. B. Allen. 1958. Assimilatory power in photosynthesis. Photosynthetic phosphorylation by isolated chloroplasts is coupled with TPN reduction. Science 127: 1026–34.
4. Asahi, T. and A. T. Jagendorf. 1963. A spinach enzyme functioning to reverse the inhibition of cyclic electron flow by *p*-chlorophenyl-1, 1-dimethylurea at high concentrations. Arch. Biochem. Biophys. 100: 531–41.
5. Bishop, N. I. 1958. The influence of the herbicide, DCMU, on the oxygen-evolving system of photosynthesis. Biochim. Biophys. Acta 27: 205–06.
6. Bishop, N. I. 1961. The photometabolism of glucose by hydrogen-adapted alga. Biochim. Biophys. Acta 51: 323–32.
7. Butt, V. S. and M. Peel. 1963. The participation of glycollate oxidase in glucose uptake by illuminated *Chlorella* suspensions. Biochem. J. 88: 31 p.
8. Duysens, L. N. M., J. Amesz, and B. M. Kamp. 1961. Two photochemical systems in photosynthesis. Nature 190: 510–11.
9. Folin, O. and H. Wu. 1920. A system of blood analysis. Supplement I. A simplified and improved method for determination of sugar. J. Biol. Chem. 41: 367–74.
10. Folin, O. and H. Wu. 1929. Two revised copper methods for blood sugar determination. J. Biol. Chem. 82: 83–93.
11. Hill, R. and F. Bendall. 1960. Function of the two cytochrome components in chloroplasts: a working hypothesis. Nature 186: 136–37.
12. Kandler, O. 1950. Über die Beziehungen zwischen Phosphathaushalt und Photosynthese I. Phosphatspiegelschwankungen bei *Chlorella pyrenoidoso* als Folge des Licht-Dunkel-Wechsels. Z. Naturforsch. 5b: 423–37.
13. Kandler, O. 1954. Über die Beziehungen zwischen Phosphathaushalt und Photosynthese II. Gesteigerter Glucoseeinbau im Licht als Indikator einer lichtabhängigen Phosphorylierung. Z. Naturforsch. 9b: 625–44.
14. Kandler, O. 1955. Über die Beziehungen zwischen Phosphathaushalt und Photosynthese III. Hemmungsanalyse der lichtabhängigen Phosphorylierung. Z. Naturforsch. 10b: 38–46.
15. Kok, B. 1961. Partial purification and determination of oxidation reduction potential of the photosynthetic chlorophyll complex absorbing at 700 mμ. Biochim. Biophys. Acta 48: 527–33.
16 Losada, M. and D. I. Arnon. 1963. Selective inhibitors of photosynthesis. In: Metabolic Inhibitors. R. M. Hochster and J. H. Quastel, eds. Academic Press, New York.
17. Marré, E., G. Forti, R. Bianchetti, and B. Parisi. 1963. Utilization of photosynthetic chemical energy for metabolic processes different from CO_2 fixation. In: La Photosynthèse. Ed. Centre Natl. Rech. Sci., Paris.
18. Maclachlan, G. A. and H. K. Porter. 1959. Replacement of oxidation by light as the energy source for glucose metabolism in tobacco leaf. Proc. Royal Soc. 150: 460–73.
19. Pringsheim, E. G. and W. Wiessner. 1960. Photoassimilation of acetate by green organisms. Nature 188: 919–21.

20. SIMONIS, W. AND K. H. GRUBE. 1953. Weitere Untersuchungen über Phosphathaushalt und Photosynthese. Z. Naturforsch. 8b: 312–17.
21. TAGAWA, K., H. Y. TSUJIMOTO, AND D. I. ARNON. 1963. Role of chloroplast ferredoxin in the energy conversion process of photosynthesis. Proc. Natl. Acad. Sci. U. S. 49: 567–72.
22. TAGAWA, K., H. Y. TSUJIMOTO, AND D. I. ARNON. 1963. Analysis of photosynthetic reactions by the use of monochromatic light. Nature 199: 1247–52.
23. TANNER, W., E. LOOS, AND O. KANDLER. 1965. Photoassimilation of glucose by *Chlorella* in monochromatic light of 658 and 711 mμ. In: Currents in Photosynthesis. J. B. Thomas and J. C. Goedheer, eds. Ad. Donker Rotterdam.
24. TANNER, W. AND O. KANDLER. Z. Pflanzenphys. In preparation.
25. TREBST, A. AND H. ECK. 1961. Untersuchungen über die Beteiligung des Sauerstoffs in photosynthetischen Reaktionen mit Hilfe von Hemmstoffen. Z. Naturforsch. 16b: 455–61.
26. TREBST, A., H. ECK, AND S. WAGNER. 1963. Effects of quinones and oxygen in the electron transport system of chloroplasts. In: Photosynthetic Mechanisms of Green Plants. Natl. Acad. Sci., Natl Res. Council, Washington.
27. TREBST, A. 1964. Neuere Vorstellungen über den Mechanismus der Photosynthese. Ber. Deut. Botan. Ges. 77: 123–42.
28. URBACH, W. AND W. SIMONIS. 1964. Inhibitor studies on the photophosphorylation in vivo by unicellular algae (*Ankistrodesmus*) with antimycin A, HOQNO, salicylaldoxime and DCMU. Biochem. Biophys. Res. Commun. 17: 39–45.
29. WESSELS, J. S. C. AND R. VAN DER VEEN. 1956. The action of some derivatives of phenylurethan and of 3-phenyl-1,1-dimethylurea on the Hill reaction. Biochim. Biophys. Acta 19: 548–49.
30. WIESSNER, W. AND H. GAFFRON. 1964. Role of photosynthesis in the light-induced assimilation of acetate by *Chlamydobotrys*. Nature 201: 725–26.
31. WIESSNER, W. 1965. Quantum requirement for acetate assimilation and its significance for quantum measurements in photophosphorylation. Nature 205: 56–57.
32. WITT, H. T., A. MÜLLER, AND B. RUMBERG. 1961. Experimental evidence for the mechanism of photosynthesis. Nature 191: 194–95.