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Effect of Light on Respiration and Development of Photosynthetic Cells

Progress Report

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Abstract

1. Hydrogen photoevolution and photoreduction by algae. The distribution of H_2 photoevolution was surveyed in a variety of marine and fresh water algae. Of 30 strains including representatives of all major algal groups, sustained H_2-photoevolution was observed only in Scenedesmus obliquus and Chlamydomonas reinhardi. The multicellular, marine red algae produced H_2 but light was not a factor. The effect of the photosynthetic inhibitors dibromothymoquinone and disalicyldenepropanediamine were studied. The results indicate that H_2 may be evolved under the influence of light either from water or a reduced carbon compound.

2. Chloroplast shuttle. A new enzyme, non-reversible, D-glyceraldehyde-3-P dehydrogenase associated with TPN was recently characterized. This enzyme coupled to photosynthesis in an intact spinach chloroplast has been used to characterize the transportation of substrates across the double membrane. The factors affecting elimination of glyceraldehyde-3-P such as phosphate, concentration of CO_2, and rate of photosynthesis have been evaluated.

Regulation of Photosynthesis and Respiration by Light

1. Hydrogen Metabolism:

The distribution of light dependent H_2 evolution was surveyed in a variety of marine and fresh water algae (Table 1). In each case, H_2 evolution was detected only after a period of anaerobiosis (adaptation period). Of the 30 strains tested, sustained light-induced H_2 evolution was detected only in the two green unicellular forms, Scenedesmus and Chlamydomonas. The red algae yielded H_2 but light did not appear to influence the rate. Most of the other algae showed a light dependent burst of gas which is assumed to be that of H_2. The initial rate of photoevolution was 3 µl per mg dry wet per hour but, it declined within the next 20 min. in the light. Longer periods of anaerobiosis as long as 1-2 days under N_2 or H_2 or addition of organic substrates to the reaction mixture had no stimulatory effect. The lack of H_2 photoevolution in most of the algae may be related to inactivation of hydrogenase by O_2 released through the photooxidation of water.

Adaptation

Adaptation of hydrogenase is usually a slow but highly variable process. The time required varied with the strain of the alga and it ranges from minutes in Chlamydomonas to 20 hours in the red algae. In our study, we found that the nutritional status of the cell affected the adaptation period. Thus acetate grown hydrogenase containing algae required a shorter period of anaerobic adaptation.

Figure 1 and 2 illustrate that H_2 photoevolution catalyzed by Chlamydomonas or Scenedesmus was either unaffected or stimulated when

the uncoupler FCCP was added after anaerobic adaptation. Addition of FCCP before the cells were made anaerobic inhibited H_2 photoevolution by Chlamydomonas but stimulated it in Scenedesmus. Moreover, when FCCP was added prior to illumination H_2 photoevolution by Chlamydomonas was inhibited gradually during the following 5 hours (Figure 3). Since FCCP is not an inhibitor of the formation of adaptive enzymes, the process of adaptation in Chlamydomonas seems to involve a reductive activation of hydrogenase through the process of oxidative carbon metabolism.

To clarify further whether anaerobic adaptation provided for a more reducing atmosphere within the cells we followed fluorimetrically the steady state pool of cellular pyridine nucleotides under aerobic and anaerobic conditions. Figure 4 illustrates a typical dark anaerobic reduction of NAD(P) in Chlamydomonas. On bubbling air, there was an immediate drop in blue fluorescence until the initial value was restored. Table 2 summarizes the steady state pools of NAD(P)H detected fluorimetrically in several green algae. The amount of NAD(P)H in Chlamydomonas is far higher than in the other algae.

The formation of reduced pyridine nucleotides in the cell is correlated with hydrogenase associated metabolism (Table 3). Heterotrophically grown cells require shorter periods at anaerobic adaptation and exhibit higher rates of H_2 photoevolution. In studies with Scenedesmus, it was demonstrated that the adaptation period for hydrogenase was correlated with the adaptation period of NAD(P)H formation. However, the rate of H_2 photoevolution was independent of the amount of cellular (NAD(P)H. As was already suggested (1, 2), it may well be that Scenedesmus can utilize both stored reduced intermediates and water as sources for H_2 photoevolution depending on the appropriate conditions.

H_2 Photoevolution

Some characteristics of the mechanism of H_2 photoevolution by Chlamydomonas and Scenedesmus are illustrated in Table 4. Hydrogen photoevolution by Scenedesmus was characterized by sensitivity to DCMU and by stimulation of photoevolution by FCCP added before or after the adaptation period (Figure 2). In contrast, the pathway in Chlamydomonas was characterized by insensitivity to DCMU, stimulation by acetate or succinate and inhibition by FCCP added before the anaerobic period (Figure 1). Both pathways were inhibited by low concentrations of the antagonist of plastoquinone, DBMIB (3), (Figure 5) and by substrate levels of p-benzoquinone (Table 4). It was clear that plastoquinone is a necessary mediator for H_2 photoevolution from the water splitting reaction in Scenedesmus or from cellular reduced compounds in Chlamydomonas.

The questions of the role of ferredoxin in H_2 evolution was approached by use of the inhibitor, DSPD (4, 5). Figure 6 illustrates slight inhibition of H_2 photoevolution by DSPD. If it is presumed that DSPD can penetrate the cells and so inhibit ferredoxin reduction, then the site of H_2 photoevolution will be located between photosystem I and ferredoxin. Under these conditions a reduced product of photosystem I preceding ferredoxin can serve as an H_2 donor.

H_2 Photoreduction

Table 5 shows the requirements for assimilation of H_2 coupled to the reduction of CO_2 in the light of Scenedesmus and Chlamydomonas. As already shown (6), this process takes place in the presence of DCMU and does not involve the photolysis of H_2O. The reaction requires ATP and CO_2 for H_2 uptake. Addition of uncoupler (FCCP) abolishes H_2 uptake

and stimulated H_2 photoevolution. The presence of acetate in the reaction mixture inhibited H_2 photoreduction implying a competition for ATP.

Figure 5 and Table 4 illustrate that in the presence of appropriate amounts of DBMIB or p-benzoquninoe, H_2 photoreduction is severly inhibited.

These observations confirm the participation of plastoquinone in H_2 photoreduction. Figure 6 illustrates slight inhibition of H_2 photoreduction by DSPD. Under these conditions, H_2 via hydrogenase can donate electrons to another cofactor between photosystem I and ferredoxin. The kinetics of H_2 photoreduction is illustrated in Figure 7. Under our conditions, the cells were able to photoreduce at a rate of 5 μmoles H_2 per mg chlorophyll per hour.

2. Glyceraldehyde-3-P and Glycerate-3-P Shuttle:

Plant enzymes contain three enzymes for the oxidation of glyceraldehyde-3-P (G-3-P). Two catalyze the phosphate dependent, reversible oxidation of G-3-P to 1,3-diPGA; the third enzyme is independent of phosphate and is irreversible.

During photosynthesis, TPNH and ATP are produced. Photosynthetically generated ATP and TPNH are not directly available for extra-chloroplastic reactions due to the impermeability of the inner chloroplast membrane to these compounds (7, 8). A shuttle system involving G-3-P and PGA has been suggested involving two enzymes external to the chloroplast: DPN-linked reversible G-3-P dehydrogenase and PGA kinase and, therefore, is dependent upon the levels of ATP, ADP, inorganic phosphate, DPN and DPNH (9). Recently Kelly and Gibbs (10) have proposed an analogous shuttle system for the indirect transfer of TPNH from chloroplast to cytoplasm. Replacing the two enzymes of the previously described shuttle is the TPN-irreversible enzyme which does not require inorganic phosphate. Another

advantage of the Kelly-Gibbs system is the extremely low Km (3 μM) for TPN (11). In the past year we have evaluated this system which may not only be a means of exporting reducing power but also a means of controlling the oxidation pentose phosphate cycle.

Surprisingly, the shuttle system (G-3-P irreversible dehydrogenase) brought about a significant increase in CO_2 fixation, O_2 evolution and exogenous TPNH reduction in the presence of bicarbonate-^{14}C, PGA, ribose-5-P or fructose-1,6-diP (Table 6). The effect of the sugar phosphates on TPN reduction was difficult to evaluate because of high rates observed in the dark. Therefore, use of these compounds was omitted in other studies.

We investigated the effect of bicarbonate concentration on the rates of CO_2 fixation and the G-3-P leak. As seen in Figure 8, increasing the bicarbonate concentration resulted in a rise in CO_2 fixation concomitant with an increase in G-3-P leak. Interestly, between 0.5 and 5 mM bicarbonate, the ratio of TPNH/CO_2 remained fairly constant.

Figure 9, shows the effect of increasing concentrations of shuttle enzyme on CO_2 fixation and TPN reduction. Between zero and 3.4 enzyme units exogenous TPNH formation and CO_2 fixation increased from 2.0 to 7.1 and from 7.9 to 14.0 μmoles per mg chlorophyll per hour.

With these data, much speculation is in order. Thus we wondered if the shuttle enzyme is pulling G-3-P from the chloroplast or whether this leakage is independent of the shuttle enzyme. The ability of higher concentrations of enzyme to cause an increase in G-3-P leak indicates that the enzyme actively pulls G-3-P from inside the chloroplast and in doing so, enhances CO_2 fixation. To explain the stimulatory effect of G-3-P leakage on CO_2 fixation we propose a "pulling mechanism" exerted by the shuttle system. This would accelerate the flow of carbon substrate with-

in the cycle thus resulting in a faster regeneration of ribulose-1,5-diP acceptor and an increased rate of carboxylation.

Increased rates of CO_2 fixation from increasing bicarbonate and the addition of PGA, both in the presence of the shuttle enzyme, suggest that the rate of G-3-P leak is dependent on the rate of CO_2 via the carbon reduction cycle.

Table I

Distribution of H_2 Evolution among Algae

Phylum	H_2 evolution
Chlorophyta	
Chlorococcales	
Scenedesmus obliquus	+
Chlorella pyrenoidosa	-
Chlorella autotrophica	-
Volvocales	
Chlamydomonas reinhardii	+
Dunaliella parva	-
Dunaliella tertiolecta	-
Chlamydomonas sp. (marine)	-
Pyramimonas sp.	-
Platymonas sp.	-
Siphonales	
Codium sp.	+
Ulvales	
Ulva lactuca	-
Entermorpha linza	-
Dasycladales	
Acetabularia mediterranea	-
Cladophorales	
Cladophora gracilis	-
Phaeophyta	
Fucales	
Fucus vesiculosus	-
Chrysophyta	
Bacillariophyceae	
Cyclotella cryptics	-
Cyanophyta	
Oscillatorriales	
Oscillatoria sp.	-
Chroococcales	
Synechococcus sp.	-
Euglenophyta	
Euglena gracilis	-
Rhodophyta	
Gigartinales	
Chondrus crispus	+
Cryptonemiales	
Corallina officinalis	+
Ceramiales	
Ceramium fastigiatum	+
Callithamnion roseum	+
Champia parvula	?
Dasya pedicellata	?

Table II

Steady State Amounts of NAD(P)H in several Autotrophic and Heterotrophic Green Algae

Species	Growth Conditions				
	CO_2	Glucose		Acetate	
	Light	Light	Dark	Light	Dark
	nmoles NAD(P)H/mg dry weight				
Chlamydomonas reinhardii	5.2	No growth		13.6	9.7
Scenedesmus obliquus	0.0	2.4	4.3	1.3	11.8
Chlorella pyrenoidosa	0.0	0.0	1.3	2.6	2.6
Chlorogonium elongatum	--	No growth		2.0	--
Dunaliella parva	--	No growth		2.1	--

Table III

Rates of H_2 Photoevolution by several Autotrophic and Heterotrophic Green Algae

Species	Growth Conditions				
	CO_2	Glucose		Acetate	
	Light	Light	Dark	Light	Dark
	$\mu l H_2$ evolved/mg dry weight/hr				
Chlamydomonas reinhardii	2.0	No growth		6.4	0.6
Scenedesmus obliquus	1.2	2.5	2.5	5.0	4.5
Chlorella pyrenoidosa	0.0	0.0	0.0	0.0	0.0
Chlorogonium elongatum	0.0	No growth		0.0	0.0

Tabla IV

Effect of FCCP and Photosynthetic Electron Transport Inhibitors on H_2 Photoevolution by Scenedesmus and Chlamydomonas

Additions	Chlamydomonas reinhardii	Scenedesmus obliquus
	$\mu l H_2$ evolved/mg dry weight/hr	
Control	3.48	2.68
+ FCCP (10^{-5}M)	3.50	3.15
+ DBMIB (1μM)	0.92	0.74
+ BQ (10^{-4}M)	3.48	2.68
+ BQ (10^{-3}M)	0.13	0.54
+ DBMIB (1μM) + BQ (10^{-3}M)	0.13	0.74
+ BQ (10^{-3}M) + FCCP (10^{-5}M)	0.24	0.74
+ DCMU (10^{-5}M)	3.00	0.05

Table V

Effect of FCCP and Photosynthetic Electron Transports Inhibitors on H_2 Photoreduction by Chlamydomonas and Scenedesmus

Treatment	H_2 Photoreduction	
	Chlamydomonas reinhardii	Scenedesmus obliquus
	$\mu l H_2$ uptake/mg dry weight/hr	
Complete	13.0	6.6
System minus $NaHCO_3$	0.1	0.1
System + 10^{-5}M FCCP	+0.5	+0.4
System + 10^{-5}M DBMIB	0	0
System minus DCMU + 10^{-5}M DBMIB	0	0
System + 10^{-3}M p-Benzoquinone	0	0

Table VI

The Effect of NR-G3P-DH on the Rates of CO_2 Fixation, Oxygen Evolution and G3P Leak (NADP Reduction) Out of the Chloroplast.

In this experiment $^{14}CO_2$ fixation and NADP reduction were carried out simultaniously in the same test tubes placed in a 25°c water bath illuminated for 10 minutes with two Sylvania flood bulbs so that the total light intensity was 2000 ft-c. The 1 ml reaction mixtures were of the composition described in Material and Methods, except that the pH was set at 8.3, and the bicarbonate was either eliminated or set at the concentrations indicated. PGA, R5P and FDP were added to the reaction mixture as indicated. One enzyme unit was placed in the appropriate tubes. The chlorophyll concentrations was 40 µg/ml. CO_2 was determined as described under methods. NADPH was assayed enzymatically (after removal of the chloroplast by centrifugation) with L-glutamic dehydrogenase.

Oxygen evolution was analyzed in parallel reaction mixtures with the same composition and chloroplast preparation as for the $^{14}CO_2$ fixation and NADPH reduction determinations. The reaction mixtures were contained in a 1 ml compartment connected to a Clark-type oxygen electrode which had been previously calibrated. Oxygen evolution was recorded during 10 min. of photosynthesis with a light intensity of 2000 ft-c. Actinic light was provided from a 500 w projector lamp ~~and~~ filtered through a saturated $CuSO_4$ solution.

Table VI

COMPONENT ADDED	OXYGEN EVOLUTION*		CO_2 FIXATION*		NADP REDUCTION* (LIGHT)		NADP REDUCTION* (DARK)
	-Enzyme	+Enzyme**	-Enzyme	+Enzyme**	-Enzyme	+Enzyme**	+Enzyme**
1mM HCO_3^-	4	12	--	--	1	5	.6
1mM PGA	17	25	--	--	3	11	1
1mM R5P	17	35	--	--	2	22	12
1mM FDP	10	25	--	--	3	28	22
5mM HCO_3^-	15	22	17	22	1	3.2	1
5mM HCO_3^- + 1mM PGA	13	16	13	16	3	8	1
5mM HCO_3^- + 1mM R5P	15	18	15	21	2	18	12
5mM HCO_3^- + 1mM FDP	14	18	14	19	3	21	22

* μmoles x $mg \cdot chl^{-1}$ x hr^{-1}
**One unit of NR-G3P-DH/ml.

Fig 1

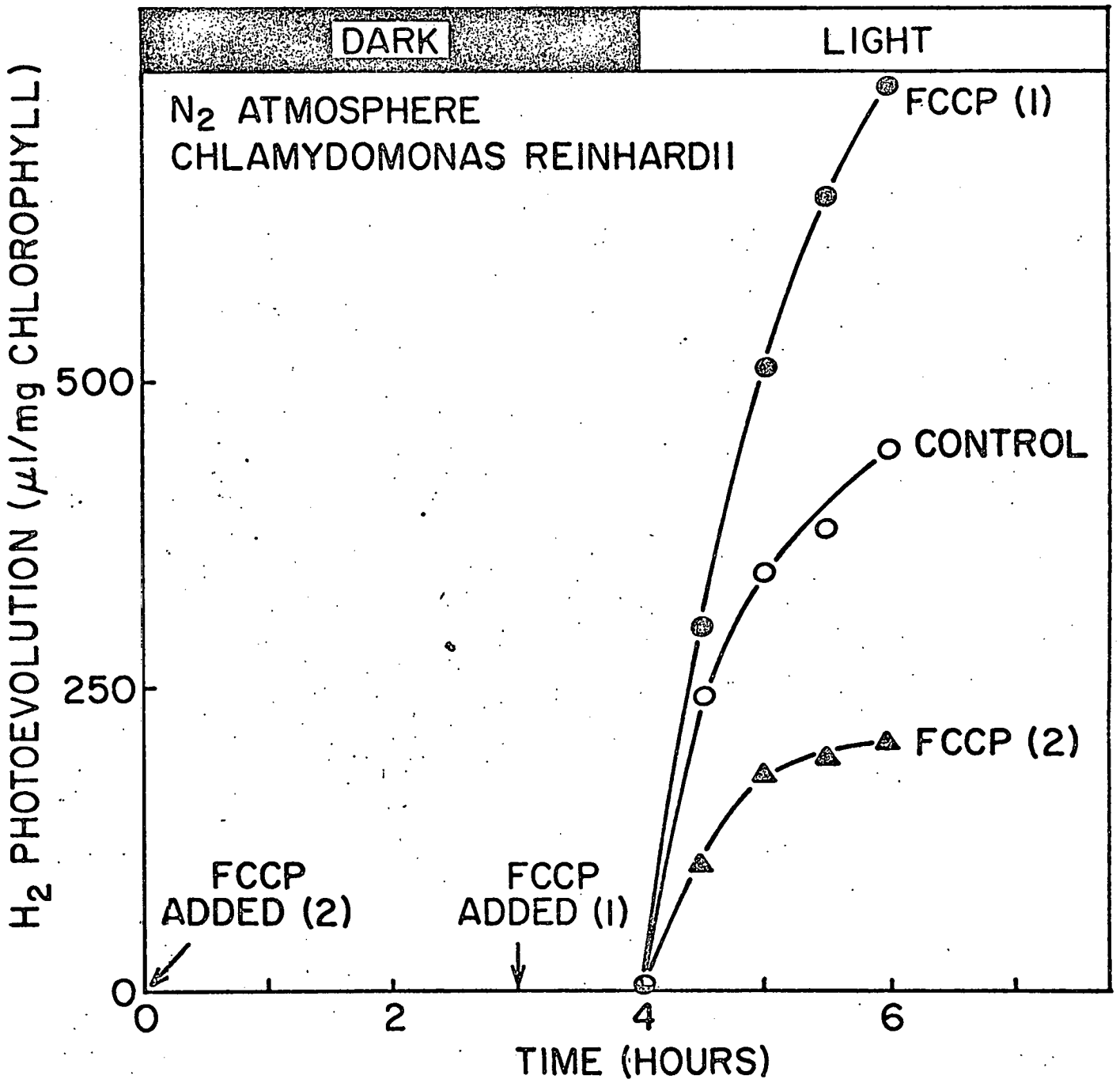

DARK
LIGHT
N_2 ATMOSPHERE
CHLAMYDOMONAS REINHARDII
FCCP (1)
CONTROL
FCCP (2)
FCCP ADDED (2)
FCCP ADDED (1)
H_2 PHOTOEVOLUTION (μl/mg CHLOROPHYLL)
0
250
500
0
2
4
6
TIME (HOURS)

Fig 2

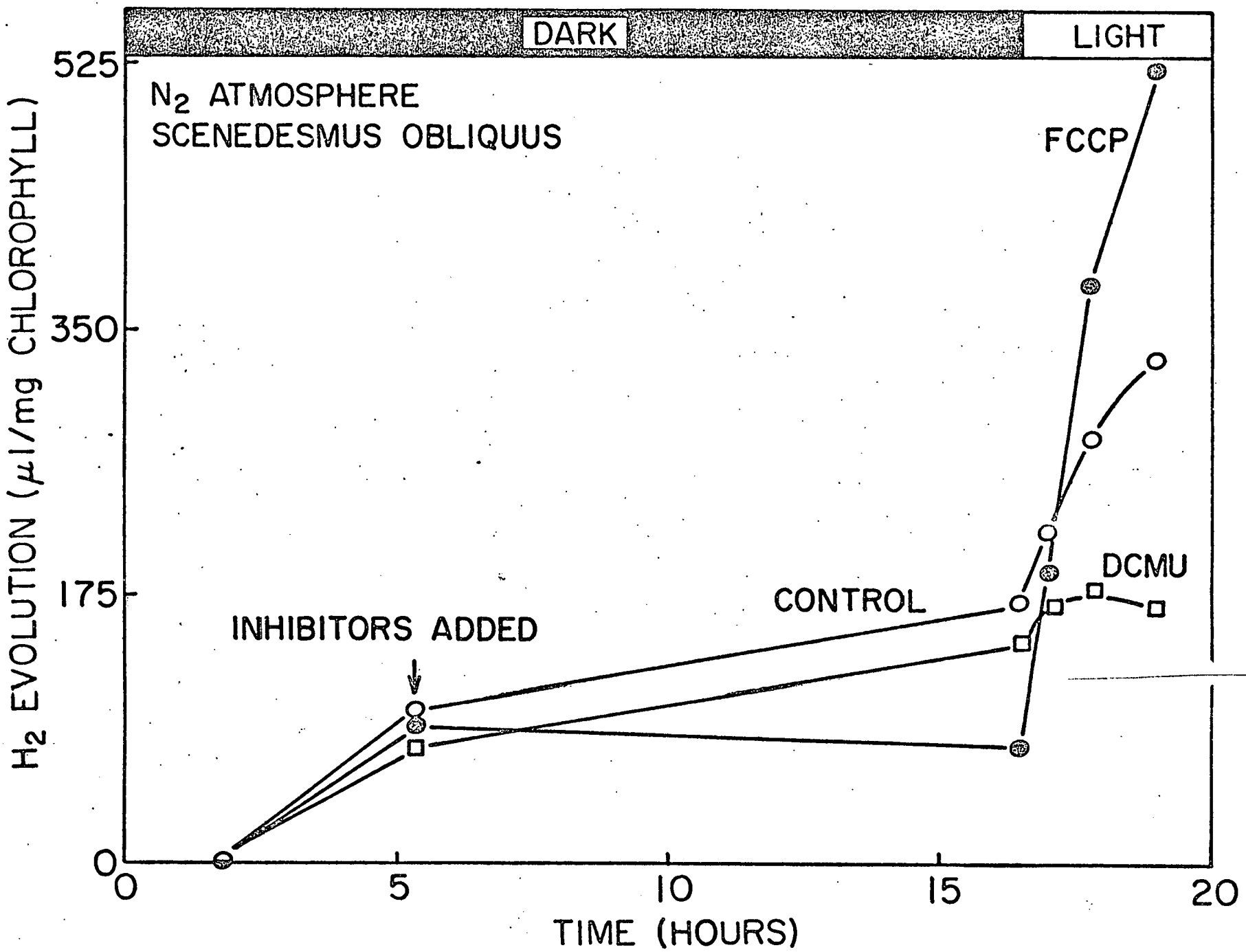

DARK
LIGHT
N_2 ATMOSPHERE
SCENEDESMUS OBLIQUUS
FCCP
DCMU
CONTROL
INHIBITORS ADDED
H_2 EVOLUTION (μl/mg CHLOROPHYLL)
525
350
175
0
0
5
10
15
20
TIME (HOURS)

Fig 3

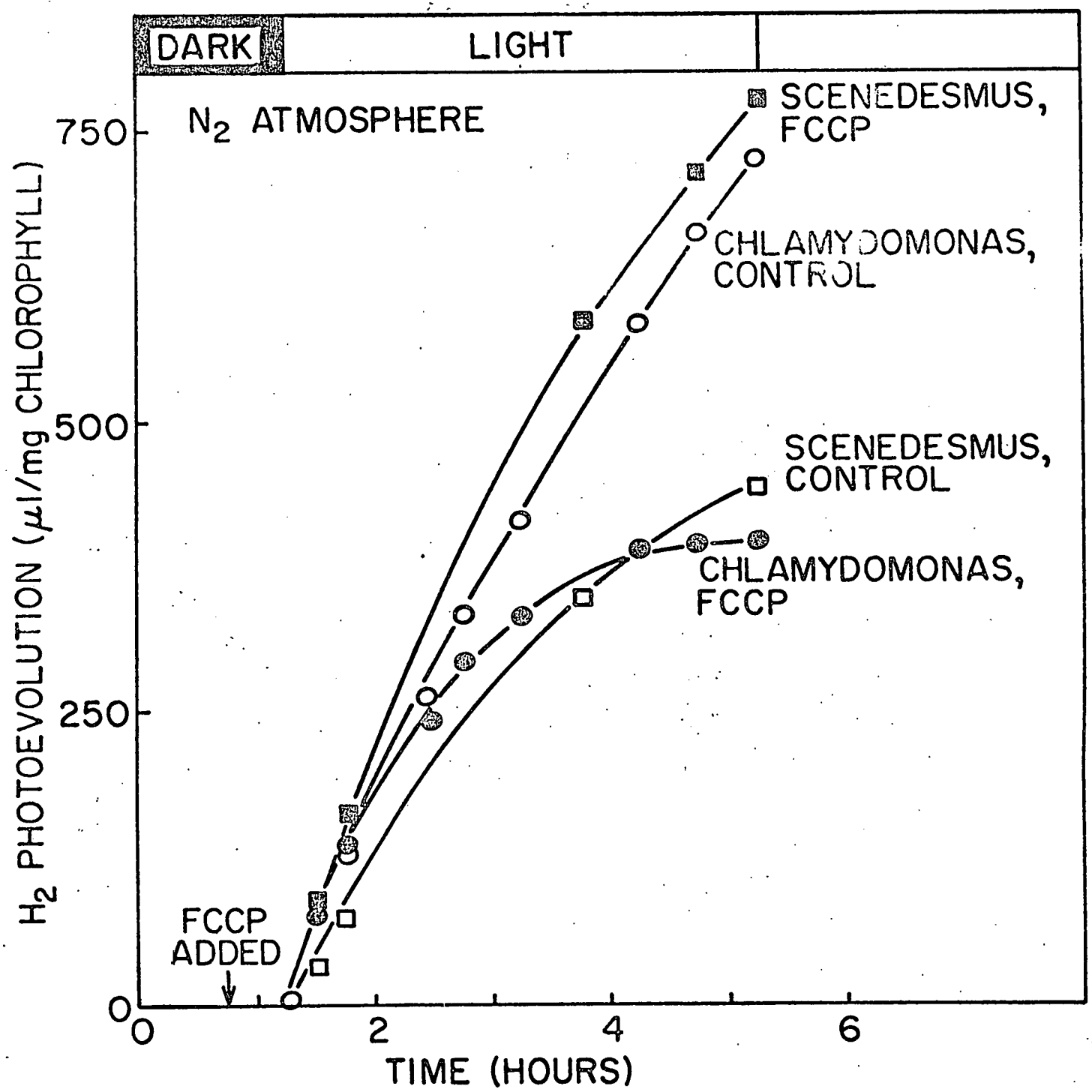

DARK
LIGHT
N2 ATMOSPHERE
SCENEDESMUS, FCCP
CHLAMYDOMONAS, CONTROL
SCENEDESMUS, CONTROL
CHLAMYDOMONAS, FCCP
FCCP ADDED
H2 PHOTOEVOLUTION (µl/mg CHLOROPHYLL)
750
500
250
0
0
2
4
6
TIME (HOURS)

Fig 4

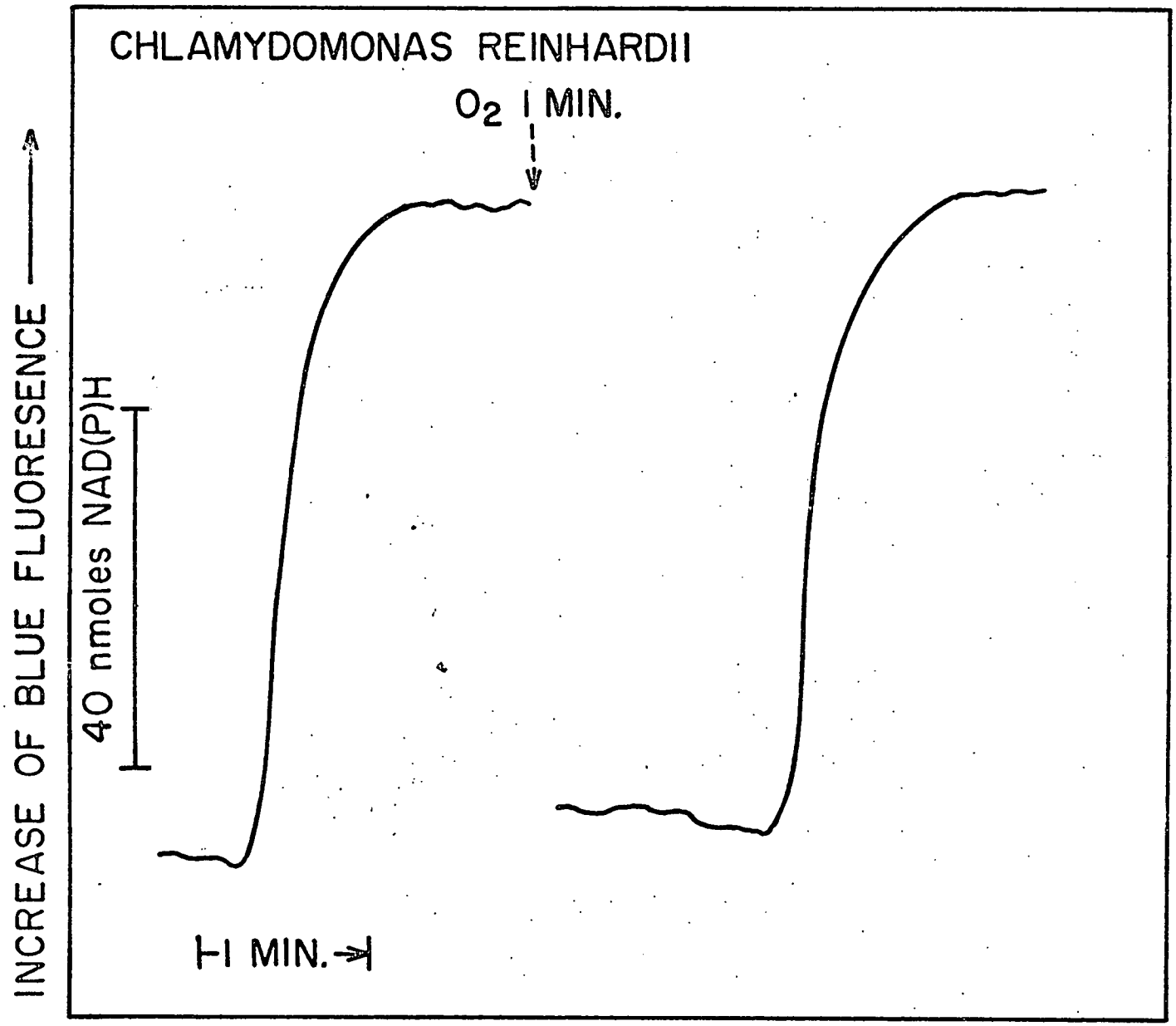

CHLAMYDOMONAS REINHARDII
O_2 1 MIN.
INCREASE OF BLUE FLUORESCENCE
40 nmoles NAD(P)H
1 MIN.

Fig 5

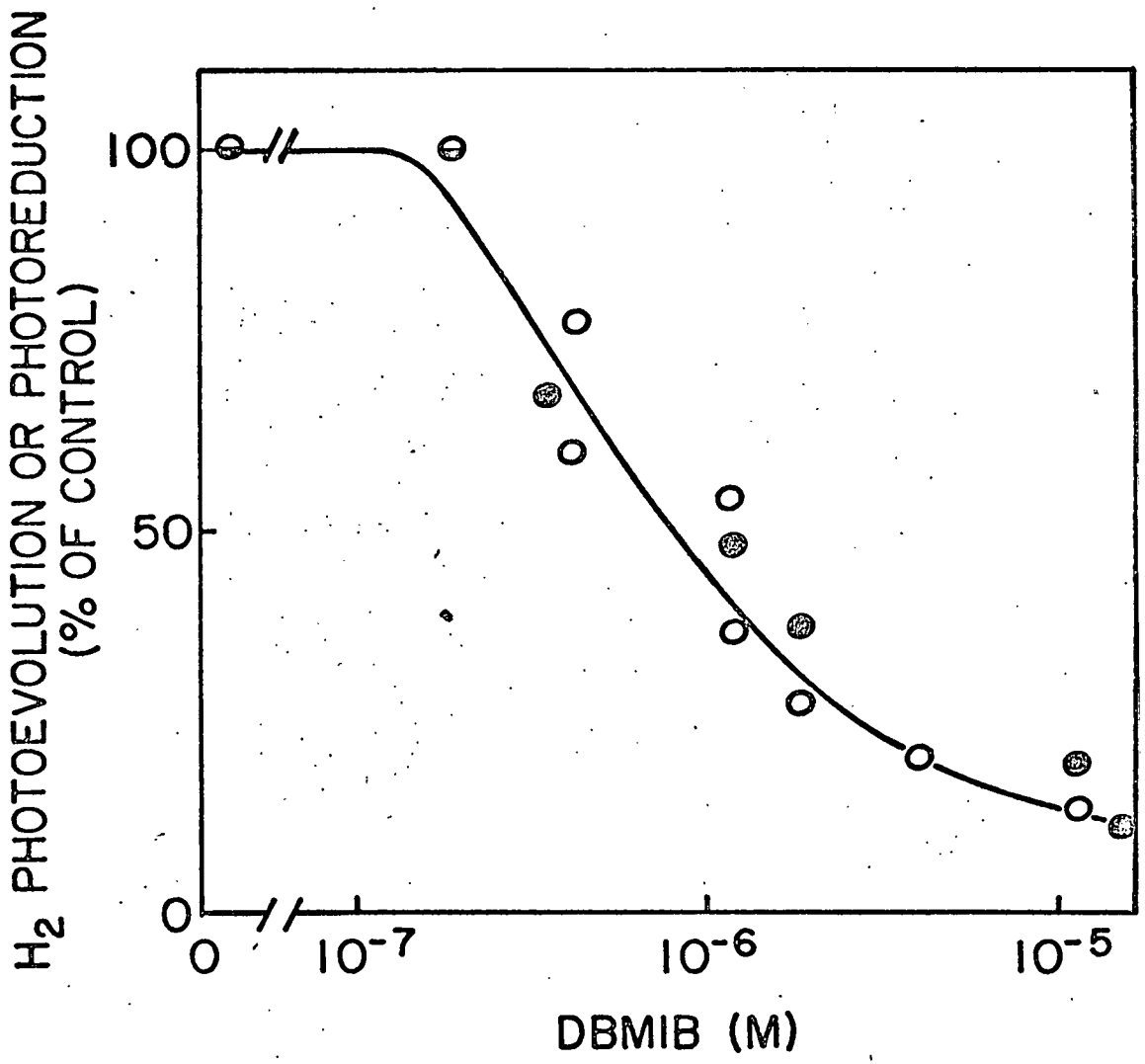

H_2 PHOTOEVOLUTION OR PHOTOREDUCTION
(% OF CONTROL)
100
50
0
0
10^{-7}
10^{-6}
10^{-5}
DBMIB (M)

Fig 6

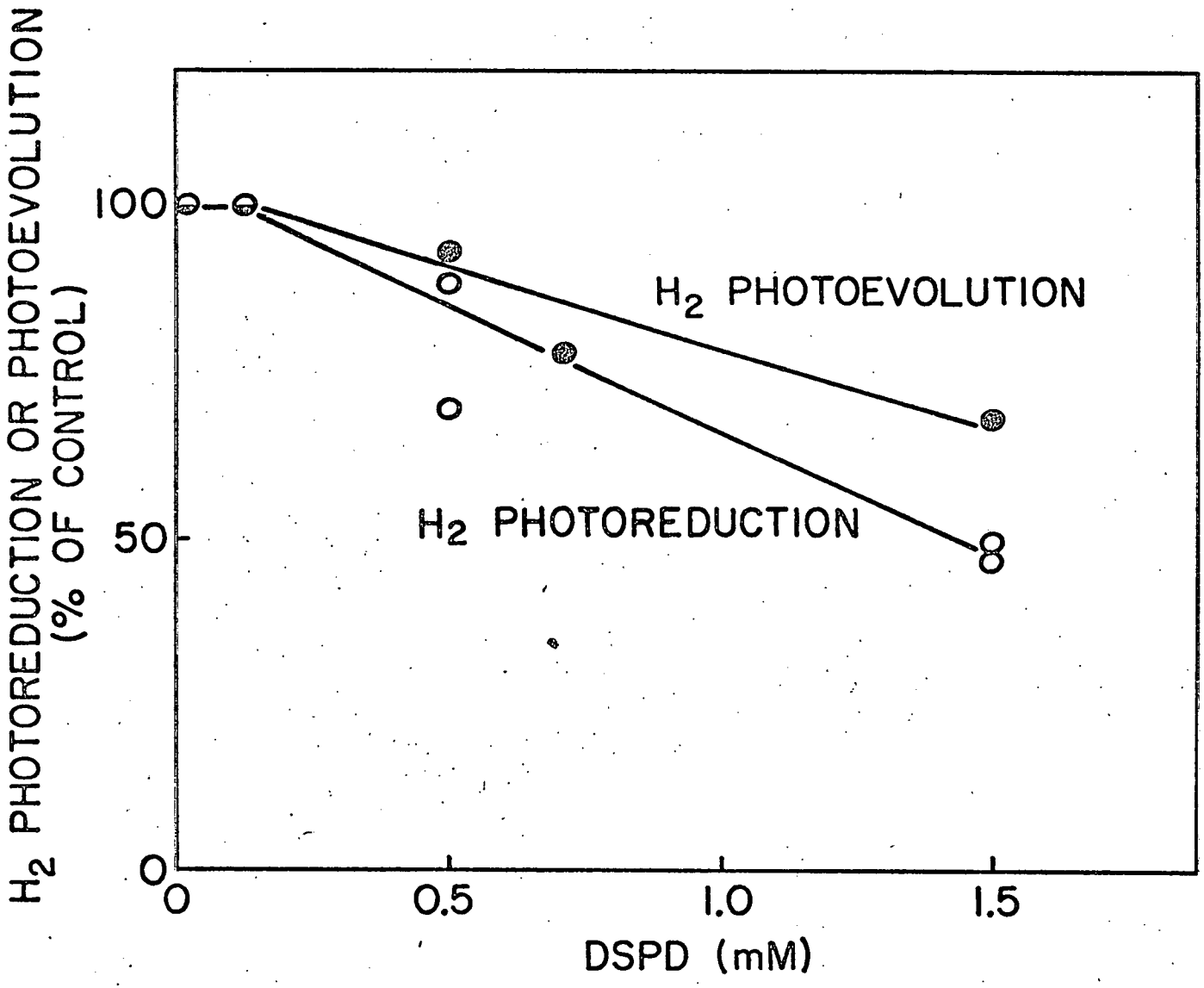
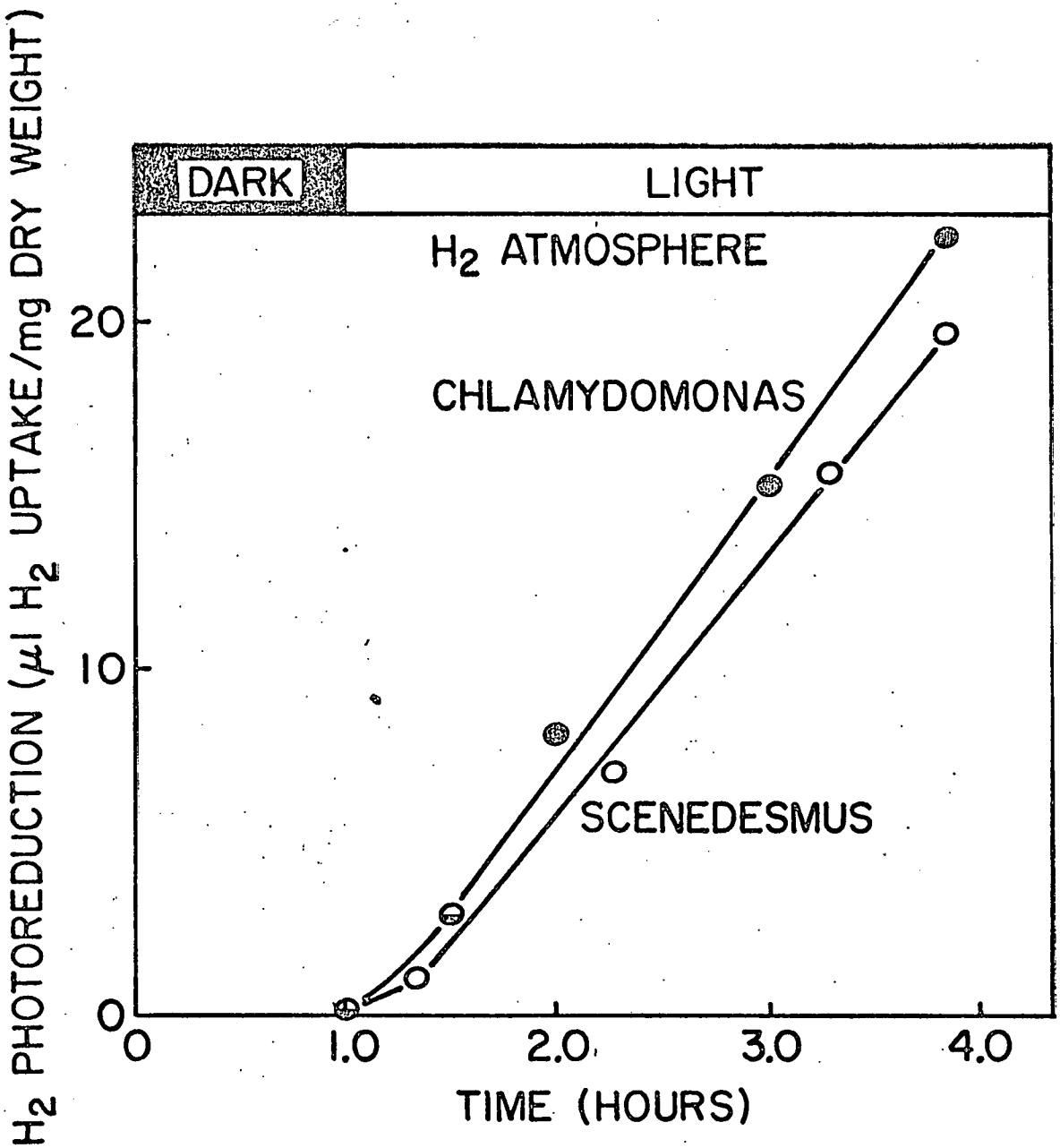

H2 PHOTOREDUCTION OR PHOTOEVOLUTION (% OF CONTROL)
100
50
0
0
0.5
1.0
1.5
DSPD (mM)
H2 PHOTOEVOLUTION
H2 PHOTOREDUCTION

Fig 7


DARK
LIGHT
H2 ATMOSPHERE
CHLAMYDOMONAS
SCENEDESMUS
H2 PHOTOREDUCTION (µl H2 UPTAKE/mg DRY WEIGHT)
0
10
20
0
1.0
2.0
3.0
4.0
TIME (HOURS)

THE EFFECT OF $NaHCO_3$ CONCENTRATION ON CO_2 FIXATION AND G3P LEAK IN THE PRESENCE OF NRG3

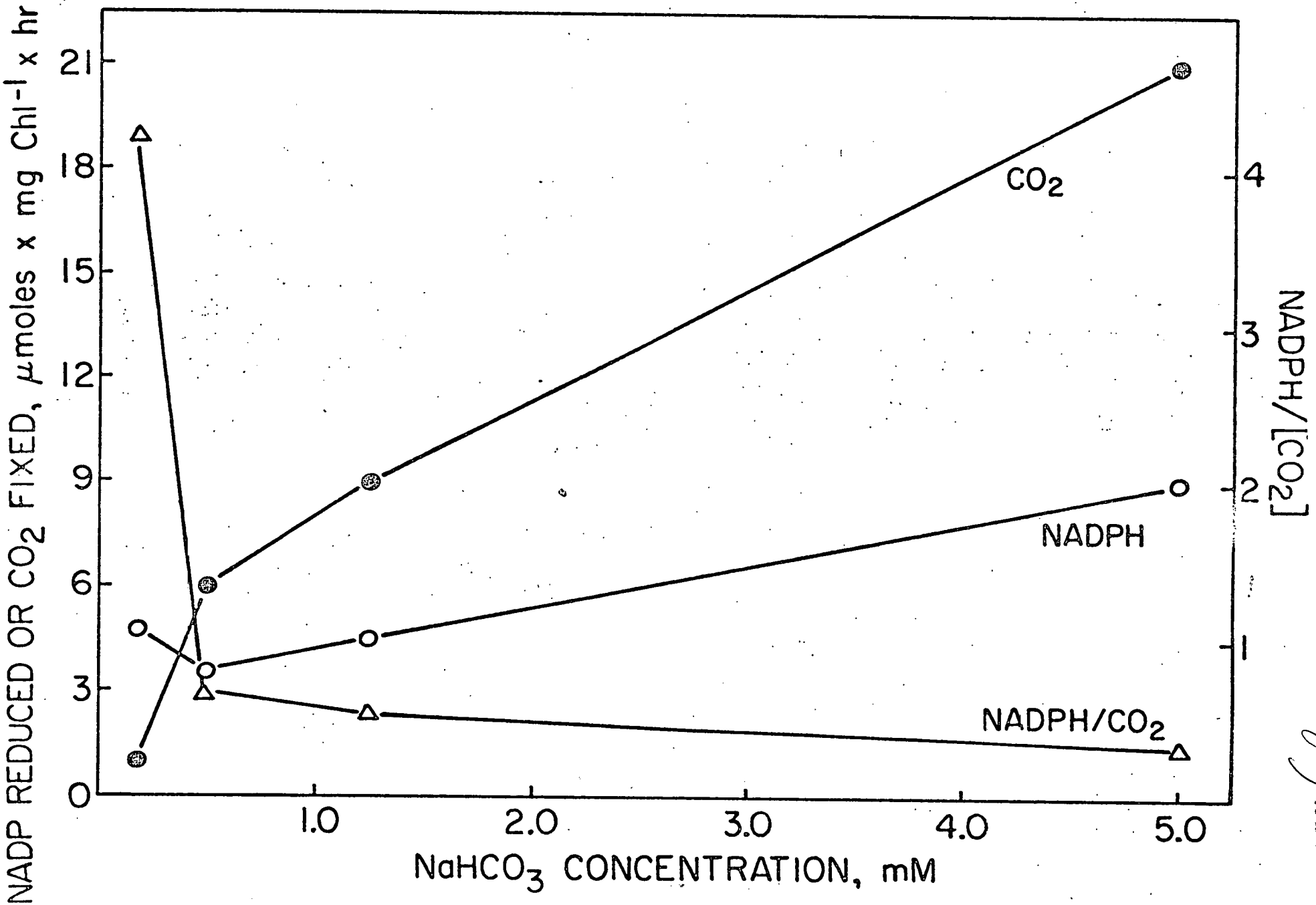


Fig 1

fig 9

THE EFFECT OF NRG3PDH ON CO_2 FIXATION AND THE LEAK OF G3P