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EFFECTS OF LIGHT ON RESPIRATION AND DEVELOPMENT OF PHOTOSYNTHETIC CELLS

Renewal Application

and

Progress Report

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ABSTRACT

The oxyhydrogen reaction in the presence and absence of CO_2 was studied in H_2- adapted Scenedesmus obliquus by monitoring the initial rates of H_2, O_2 and $^{14}CO_2$ uptake and the effect of inhibitors on these rates with gas sensing electrodes and isotopic techniques. Glucose and acetate respiration was competitive with H_2uptake. KCN inhibited equally respiration and the oxyhydrogen reaction in the presence and absence of CO_2. It was concluded that the oxhydrogen reaction both in the absence and presence of CO_2 has properties in common with components of respiration and photosynthesis. Participation of these two processes in the oxyhydrogen reaction would require a closely linked shuttle between mitochondrion and chloroplast. In this grant renewal, we will be concerned with the isolation of protoplasts and chloroplasts from a H_2 - adapted alga in order to elucidate the cooperation between the two organelles.

Acetate has been shown to stimulate H_2 photoproduction in H_2-adapted algae even more so than an uncoupler of electron transport. In some algae, glucose functions similarly. We plan to evaluate the role of these compounds either in terms of the glyoxylate cycle or electron acceptors resulting in formation of alcohols.

In our last report, we proposed the term "chloroplast respiration" to account for the breakdown of polyglucan within the chloroplast. A means of reoxidizing reduced pyridine nucleotide was required to complete the cycle. A new enzyme ascorbic acid reduced pyridine nucleotide peroxidase has been isolated from the chloroplast. We propose to continue with the characterization of this enzyme.

PROGRESS REPORT

I. Oxyhydrogen Reaction

A. Absence of CO_2

The oxyhydrogen reaction in green algae, a process first noted and studied in depth by Gaffron (1), involves a simultaneous uptake of H_2 and O_2 under darkness. Using Warburg manometric techniques, he usually observed a quotient of H_2 and O_2 equal to one. In the presence of CO_2, uptake of CO_2 was observed and the quotient of H_2 : O_2 changed to two. Horwitz (2) used a mass spectrometer to monitor O_2 and CO_2 levels continously and observed that both cellular respiration and the oxyhydrogen reaction coupled to CO_2 reduction had the same dependence on O_2 tension and that the rates of O_2 uptake for the two reactions fell within the same range. The conclusion was reached that the oxyhydrogen reaction had some properties in common with respiration.

Interestingly, during the reduction of N_2 to ammonia, a considerable fraction of the electron flow through the nitrogenase complex is utilized in the reduction of protons, resulting in the evolution of H_2 (3). The result of several surveys shows H_2 evolution representing a mean loss of 29% of the total electron flow to the nitrogenase system (4). The discovery (5,6) of a H_2 - oxidizing system in legume nodules and other N_2 - fixing organisms (7,8) has created interest in the H_2 - recycling process. Peterson and Burris (9) and Bothe et al (10) have reported that H_2 oxidation supported ATP formation and provided respiratory protection for nitrogenase in blue green algae.

Similarly, the oxyhydrogen reaction has been envisaged as a protective mechanism against the aerobic inactivation of hydrogenase. In contrast to nodule bacteroids and to the blue-green algae, the energy generated in the oxyhydrogen reaction can be coupled to CO_2 reduction via the reductive pentose phosphate cycle (11).

In our study, the initial velocities of H_2, O_2, and CO_2 uptake in H_2 - adapted Scenedesmus obliquus and the effect of inhibitor, glucose and accetate on these uptake rates were monitored. The rates of H_2 and O_2 uptake were monitored continuously during the first few minutes of the oxyhydrogen reaction using gas sensing electrodes while

the assimilation of CO_2 was followed by isotopic methodology.

As seen in Figure 1. the addition of 1 atm of H_2 increased the rate of O_2 uptake by only 30%. By contrast, the rate of H_2 uptake was highly dependent on the presence of O_2. Since H_2 uptake increased at a rate much greater than O_2 uptake with increasing pH_2, it appears that H_2 may compete successfully with endogenous electron souces in the mitochondrial reduction of O_2.

The next experiments were designed to determine if H_2- uptake was affected by exogenous substrates such as glucose and acetate. H_2 uptake was inhibited by glucose and acetate (Fig. 2). In bacteroids, Ruiz-Argueso et al. (12) reported that succinate, acetate and formate also inhibited H_2 uptake. It would appear that the pathways of substrate-stimulated respiration and H_2 oxidation share a common electron transport chain component(s).

The effects of KCN and the uncoupler, carbonylcyanide-p-trifluoro-methoxyphenyl-hydrazone (FCCP) were compared on both respiration and the oxyhydrogen reaction (Eig.3) Hydrogen and oxygen uptake were nearly equally affected by KCN and is consistent with the results of Bothe et al. (10) and Peterson and Burris (9) both of whom reported that cyanide inhibited the oxyhydrogen reaction in blue-green algae.

FCCP accelerated the rate of O_2 uptake in respiration indicating that energy conservation rather than a supply of endogenous electron donors limited mitochondrial electron transport. The rates of H_2 and O_2 uptake in the oxyhydrogen reaction followed the same pattern as that of cellular respiration, namely, an increasing rate up to 5 micromolar FCCP. In sharp contrast, uncouplers inhibited H_2 - uptake but accelerated O_2 uptake in blue-green algae (7) and in nodule bacteroids (13). The similar response of respiration and the oxyhydrogen reaction to cyanide may indicate that the primary terminal oxidase in both processes was cytochrome oxidase. The finding with the uncoupler suggests that the oxyhydrogen reaction is apparently limited by energy conservation. Clearly further work is needed to clarify the pathway of electron transport from H_2 to O_2.

B. Presence of CO_2

The chemosynthetic reduction of CO_2 coupled to the oxyhydrogen reaction did not affect the H_2 : O_2 ratio indicating it made little demand upon that reaction. On the other hand, all inhibitors of the oxyhydrogen reaction such as KCN(Fig.4) caused a strong inhibition of CO_2 reduction. Dark CO_2 reduction in the absence of H_2 was little disturbed by cyanide.

Thus, the oxyhydrogen reaction has properties in common with components from both respiration and photosynthesis. This would require a closely linked relationship between mitochondrion and chloroplast.

II H_2 Photoevolution. Acetate and uncouplers.

Acetate stimulates H_2 photoevolution in Chlamydomonas reinhardi (Table I). The effect was roughly two-fold but most importantly even higher than that caused by FCCP, the uncoupler. The optimal concentration was high being in the order of 5mM (Fig.5)

III Chloroplast Respiration

This term was proposed in the last progress report to account for the oxidation of starch within the chloroplast recognizing the fact that ATP and pyridine nucleotide do not cross the chloroplast membrane. The oxidation of glyceraldehyde -3-phosphate to glycerate-3-phosphate is coupled to substrate ATP formation and the formation of NAD (P) H. NADPH can be reoxidized by O_2 through the ferredoxin-NADP reductase enzyme. A missing key enzyme is a means of reoxidizing NADH. We can now report the isolation of pyridine-nucleotide ascorbate peroxidase in leaves of Sedum praealtum, Pisum sativum, Spinacia oleracea and in cells of Euglena gracilis. Partially purified spinach enzyme had substrate K_m's of 5uM for NADH, 50uM for H_2 $O_2$and 300uM for ascorbate at a pH optimum of 6.8 The spinach enzyme is inhibited by cyanide and alpha-alpha dipyridyl indicating Fe^{2+} involvement. About 20% of the total cellular activity was found in the chloroplast. The enzyme may have a dual purpose: (a) reoxidization of NADH: (b) detoxification of chloroplast generated H_2 O_2. Light treatment of spinach chloroplasts resulted

in a decreased activity, a property eliminated by the presence of 10 uM DCMU or subsequent darkness. The pH optimum of 6.8 is consistent with a stromal pH of 7.0 in the dark.

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Figure Legends

Fig. 1 Effect of pH_2 on O_2 and H_2 uptake rates.

(●): O_2 uptake and (▲) uptake under 0.02 atm O_2.

(■): H_2 uptake in the absence of O_2.

Fig.2 Effect of acetate and glucose on O_2 and H_2 uptake rates. The gases (0.02 atm O_2, 0.10 atm H_2) present are indicated in the figure. Acetate and glucose concentrations were 10 mM.

Fig.3 Effect of FCCP on O_2 and H_2 uptake rates. (●): O_2 uptake measured under 0.02 atm O_2 but in the absence of H_2. (▲): O_2 uptake and (■) H_2 uptake measured in the presence of 0.02 atm O_2 and 0.10 atm H_2.

Fig.4 Effect of KCN on the oxyhydrogen reaction in the presence of CO_2. Where used, the KCN concentration was 200 uM, [^{14}C[bicarbonate (60 uCi/umol) was 10 uM, pO_2 was 0.02 atm, and pH_2 was 0.10 atm.

Fig. 1

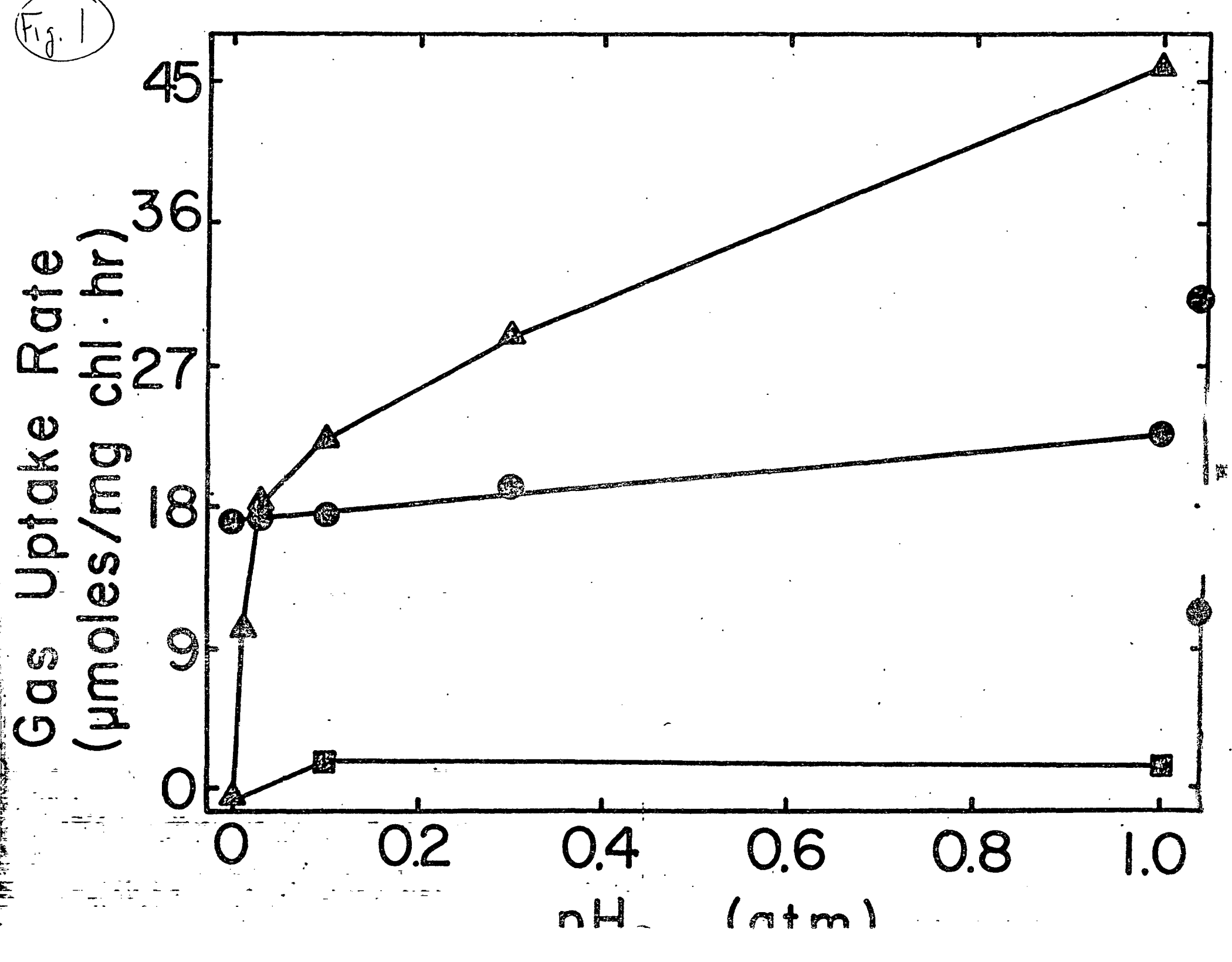
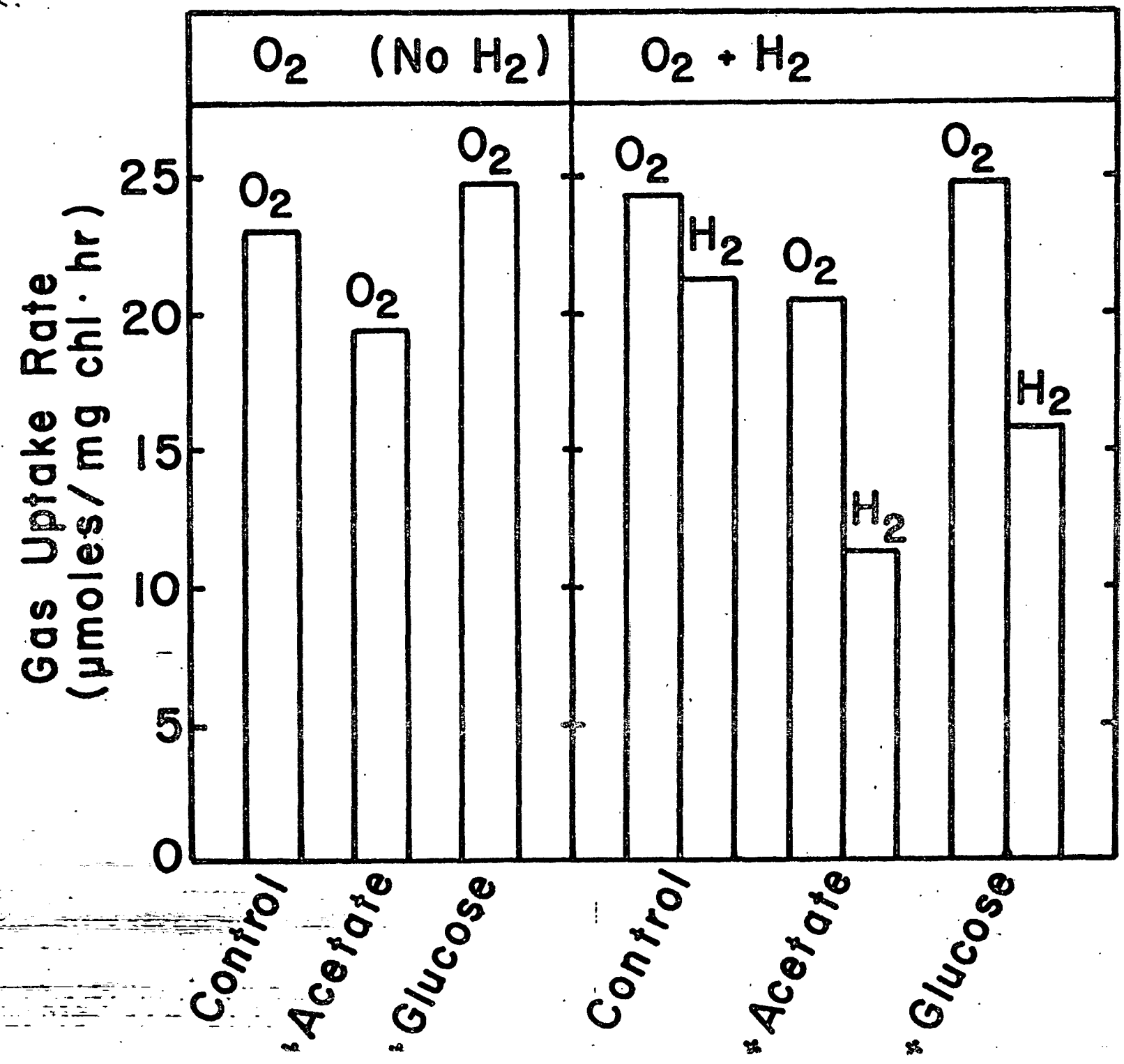
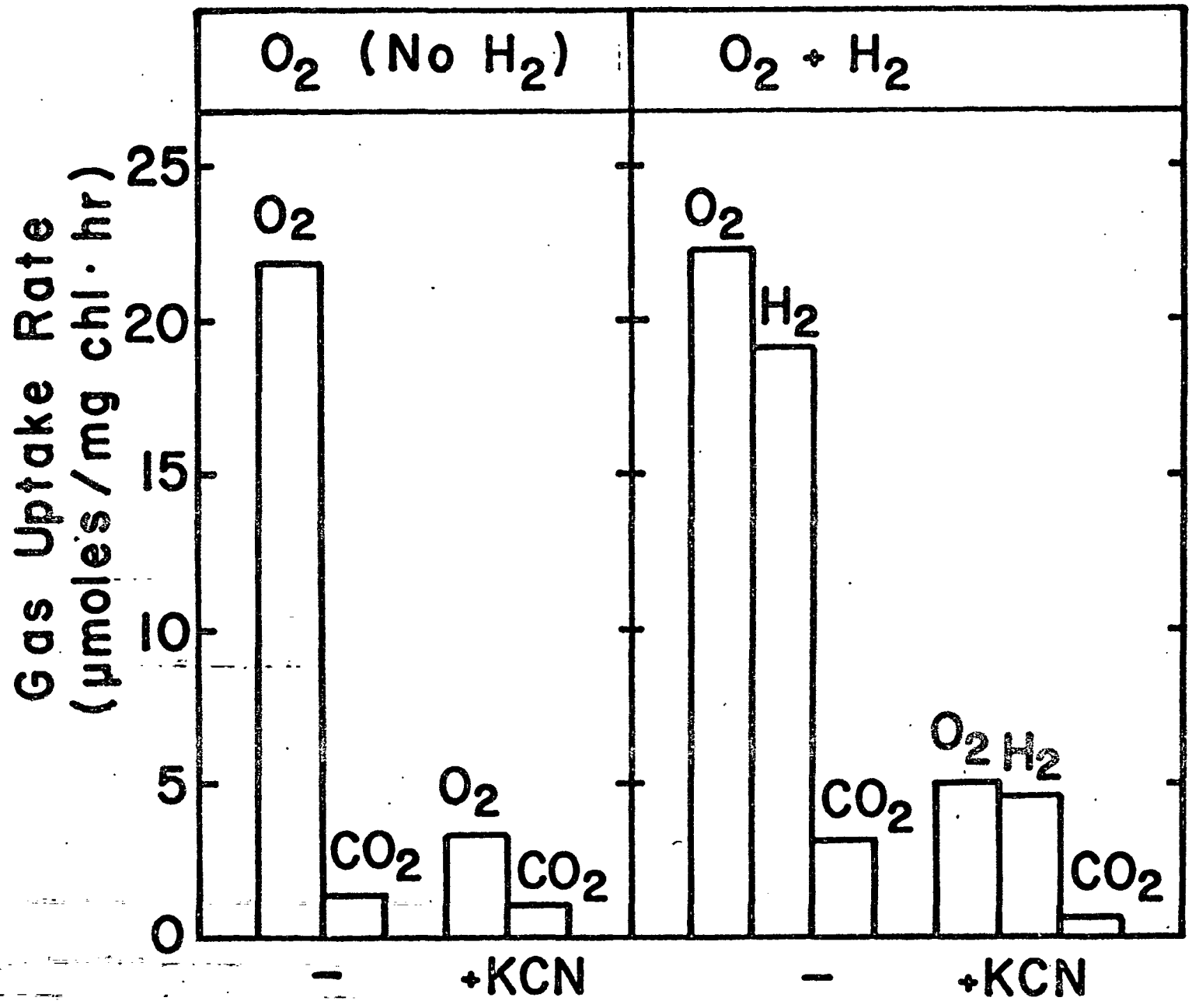

Gas Uptake Rate
(μmoles/mg chl · hr)
45
36
27
18
9
0
0
0.2
0.4
0.6
0.8
1.0
pH (atm)

Fig 2.


O2 (No H2)
O2 + H2
Gas Uptake Rate
(μmoles/mg chl · hr)
0
5
10
15
20
25
O2
O2
O2
O2
H2
O2
H2
O2
H2
Control
+Acetate
+Glucose
Control
+Acetate
+Glucose

Gas Uptake Rate (μmoles/mg chl·hr)
36
32
28
24
20
2
0
0
2
4
6
8
10
[FCCP] (μM)

Fig 4


O2 (No H2)
O2 + H2
Gas Uptake Rate
(μmoles/mg chl·hr)
25
20
15
10
5
0
O2
CO2
O2
CO2
O2
H2
CO2
O2 H2
CO2
–
+KCN
–
+KCN

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1962-64; 71-74	Consultant to NSF: Metabolic Biology Panel
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