REDUCTION OF CARBON DIOXIDE COUPLED WITH THE OXYHYDROGEN REACTION IN ALGAE

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Several species of algae have the capacity to include molecular hydrogen in their metabolism. This capacity becomes apparent only after anaerobic incubation and is lost again in contact with oxygen. While traces of oxygen are sufficient to prevent the appearance of the reactions with hydrogen, measurable amounts of oxygen are tolerated *after* adaptation to the hydrogen metabolism. This tolerance is due to the fact that the oxygen is rapidly reduced to water. The present investigation of this reduction, the oxyhydrogen reaction, in algae was undertaken when it was found that carbon dioxide is reduced in the dark simultaneously with the formation of water from oxygen. Though here observed for the first time in green plants this coupled oxido-reduction of carbon dioxide is known to occur in several strains of bacteria. The surprisingly high yield of carbon reduced per molecule of oxygen in so called *Knallgas* bacteria has been pointed out by those who first discovered it (34) and gave rise to a treatise on thermodynamics in living cells (35). Since that time the problem has certainly not become less interesting. The question of how the formation of water is coupled with the reduction of carbon dioxide is still unsolved.

Now, at a time when experiments with radioactively labeled carbon have shown that carbon dioxide can reenter along many ways the carbon cycle in living cells, this type of reaction deserves renewed attention. The dark reduction with hydrogen lies on the border line between autotrophic and heterotrophic carbon assimilation. Free hydrogen is the simplest of all reducing agents. Both autotrophic and heterotrophic bacteria are known in which molecular hydrogen can replace either inorganic or organic hydrogen donors. In the present case the dark reduction of carbon dioxide acquires a particular importance since it occurs in the same cell that carries on photoreduction and photosynthesis. Observations concerning the dark reduction could supplement our knowledge of the mechanism of photosynthesis. The investigations reported below have, indeed, revealed many striking similarities between light and dark reactions, especially a certain parallelism in the effect of specific poisons.

Summarizing these results one can hardly avoid the conclusion that with the exception of the typical light absorption by chlorophyll both photoreduction and dark reduction of carbon dioxide in green algae proceed along the same

pathways. It would be premature, of course, to say that the bacterial dark reduction of carbon dioxide with hydrogen requires a similar mechanism.

Material and Method

(*a*) *General Remarks.*—Unicellular algae of the genera *Scenedesmus*, *Rhapidium*, *Ankistrodesmus*, and perhaps many more, which contain or develop a hydrogenase system, can reduce oxygen with hydrogen under the proper anaerobic conditions. Several species of *Scenedesmus* have been used in most of the experiments, because they are easier to cultivate than the other algae named above. For culture methods see a recent review (36). The anaerobic treatment is the same as described for photoreduction (Papers I and II).

Warburg's manometric method has been used to measure the gas exchange of the algae, as in the previous investigations. The measurements of the oxyhydrogen reaction are in some respects simpler, in other respects more difficult than the experiments on photoreduction. They are simpler because there is no doubt that the complete reduction of oxygen with hydrogen yields water. They are more difficult because the gas exchange does not include two, but three gases: O_2, H_2, and CO_2. While the nature of the reactions could be established beyond doubt, the determination of the number of molecules of carbon dioxide reduced per molecule of oxygen required the comparison of a large number of experiments since the result of a single measurement is liable to large fluctuations. The observed deviations, however, are not due solely to the method. Under anaerobic conditions in nitrogen, the algae ferment, producing carbon dioxide, organic acids, and hydrogen. If the fermentation is carried on under hydrogen, as was the case in the present experiments, the algae not only ferment but continue to absorb various amounts of hydrogen. (Compare Table XI in Paper II.) It is the underlying basal metabolism which often introduces an error up to 20 per cent in the individual measurement. This error lies always in the same direction. More hydrogen is absorbed during the oxyhydrogen reaction than is due solely to the latter. A proper correction is easily applied by deducting metabolic rates observed in control experiments or during the periods before and after the reaction in question.

(*b*) *Details of Method.*—To prove the reduction of carbon dioxide in the course of the oxyhydrogen reaction the difference in the amount of total CO_2 before and after the reaction has been determined. The initial amount was known either because an analyzed gas mixture was used to fill the manometer vessel, or because carbon dioxide was released previous to the measurement inside the vessel from a carbonate solution and could be measured manometrically. After the oxyhydrogen reaction had run to completion the remaining carbon dioxide was absorbed completely by a potassium hydroxide solution. Flasks serving a similar purpose, the so called Dixon-Keilin vessels, are described in the literature (37). The latter have a rather massive stopcock at the bottom of the flask which prevents their use in photochemical experiments. We have employed two other ways to absorb carbon dioxide. In the first method the side arm of the manometer vessel has a wide opening which is closed by a ground-in stopper protruding into the side arm in form of a paddle. By turning the stopper the paddle breaks a delicate glass bulb containing the potassium hydroxide solution. The solution, spreading over a piece of filter paper, now rapidly absorbs all carbon dioxide

(see Fig. 14 *b*). No gas bubbles are allowed in the glass bulb. If it is important to know the gas volume and the amount of liquid in the vessel, the glass bulb is weighed before and after filling. For filling it is simpler to boil some water in the bulb and dip the capillary end into the potassium hydroxide solution, than to use vacuum in a desiccator. After breaking off most of the capillary the opening is sealed with a little paraffin or wax. A drawback of the method is that it depends so much on the skill of blowing the glass bulbs. A bulb which does not break when the paddle is turned means the loss of an experiment which has already been running for a day or two.

The second method depends on a type of vessel we have been using for quite some time. The side arm is connected with the main part of the vessel through a large hole in the ground joint where vessel and manometer fit together. The side arm can be closed and opened by turning the vessel around its vertical axis (Fig. 14 *a*). The supporting springs are attached to a concentric ring turning freely around the capillary. It is unnecessary to remove the vessels from the thermostat or to touch them at all

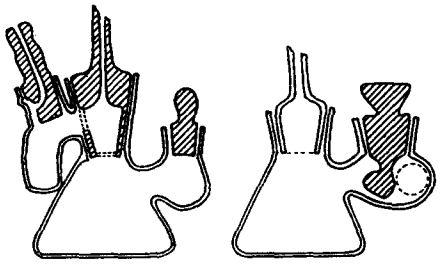

FIG. 14. For explanation see text.

with the fingers. Thermal pressure changes are thereby prevented. The great advantage of using these vessels is that the side arm can be closed and opened several times in the course of an experiment. A disadvantage is the existence of a separate gas space above the potassium hydroxide solution. While the side arm remains closed the pressure changes in the main part of the vessel, due to the algal metabolism plus the change in barometric pressure, have no effect on the isolated gas space. When the side arm is opened again there will be a sudden equalization of pressure between the main and the side compartment. Two ways are open to take care of the difficulty. One way is to reset the manometer reading and to re-establish the pressure prevailing at the moment of the closing just before the side arm is reopened. The other requires the computation of the expected change in manometer reading. In any case it is recommendable to open the side arm first for a moment only to the point where a pressure exchange, but no appreciable gas exchange, occurs. This allows checking both methods as to the absence of any pressure jumps or as to the amount of the computed correction.

When a certain gas exchange occurs while the side arm is open the manometric reading will be different (smaller) than when the side arm is closed. In other words, we have two vessel constants, K_1 and K_2, for the smaller and for the larger gas volume.

A certain gas exchange of x cubic millimeters then is given by:

$x = P_1K_1 = P_2K_2$, where P_1 or P_2 are manometric pressure changes in millimeters of Brodie solution (1,000 mm. = 1 atmosphere = 762 mm. Hg). When the gas exchange has taken place with the side arm closed and afterwards the arm is opened the reading P_1 will in this moment change to P_2, just as if the gas exchange had occurred in the greater volume. The difference, $\Delta P = P_1 - P_2$ is the correction which has to be applied after all the carbon dioxide has been absorbed. Since a continuous gas absorption begins once the side arm has been opened, ΔP cannot be measured accurately. The difference can be computed according to

$$\Delta P = P_1 - P_1 \frac{K_1}{K_2} = P_1 \cdot \left(1 - \frac{K_1}{K_2}\right) \tag{1}$$

K_1 and K_2, are the vessel constants according to Warburg's well known equations. (See Dixon (37).)

The solubilities of oxygen and hydrogen in water are small and approximately equa

$$\alpha_{O_2}^{25^\circ} = 0.03; \qquad \alpha_{H_2}^{25^\circ} = 0.02$$

We can use, therefore, the same constants for computations involving the exchange of these two gases. Furthermore, since the vessel constants for oxygen and hydrogen are equal a plot of experimental data in millimeters of manometric readings will give an undistorted picture of the event as far as the oxyhydrogen reaction is concerned. The question arises whether it is allowed to proceed in this manner, if carbon dioxide is reduced together with oxygen. The following calculation shows that the error introduced by plotting all types of experiments in millimeters of gas pressure changes is slight. Let us assume that one-half molecule of carbon dioxide is reduced to the level of carbohydrate together with 1 molecule of oxygen. The balance of the reaction can be written

(*a*) $$2O_2 + 1CO_2 + 6H_2 \rightarrow \underset{\text{(carbohydrate)}}{1(CH_2O)} + 5H_2O$$

Only one-ninth of the disappearing gas volume is carbon dioxide. On account of its greater solubility in water the vessel constant for CO_2 may be 1.6, for instance, instead of 1.3 as for the other gases.

In letting the reaction proceed with 50 c.mm. of oxygen we expect according to equation (*a*) at best a total gas uptake of 225 c.mm. This would correspond to a manometric pressure change of $\frac{200}{1.3} + \frac{25}{1.6} = 170$ mm. Neglecting the different vessel constants for carbon dioxide and multiplying 170 with 1.3 we obtain 221 c.mm. instead of 225 c.mm. This is an error of 2 per cent.

It is very easy to introduce into several manometer vessels approximately the same amount of oxygen and to measure the amount introduced into each vessel very accurately, whereas one would need an elaborate apparatus to introduce exactly the same volume of oxygen into every one of the vessels. In sets of several parallel measurements the procedure was as follows: All manometers are set so that the pressure within the vessel is 300 mm. below atmospheric pressure (1 atmosphere =

10,000 mm. Brodie solution). A vent stopper leading to the air is turned once very quickly. Some air is drawn into the vessel and no gas within the vessel can escape by diffusion against the air current. The manometer vessels are left standing for 3 to 5 minutes. The air introduced diffuses throughout the vessel and attains the temperature of the thermostat. Now a reading is taken and the vessels are set in motion. With the beginning of shaking the oxygen diffuses rapidly into the cell suspension and the reaction starts. A pressure difference of about 200 mm. before and after the introduction of air can be measured accurately. Since 0.207 is the percentage of oxygen in air, 200 mm. of air are equivalent to 41.4 mm. of O_2. The correct zero point for the beginning of the oxyhydrogen reaction is not the second reading taken while the vessels were still standing, because the air has not reached the equilibrium distribution yet between the gaseous and the liquid phase. Consequently a little more gas than is due to the metabolic reaction will be absorbed by the cell suspension during the first minutes of shaking. Correction for this may be made by taking the solubility of air in the suspension medium into account. When several vessels are treated in the same way the amounts of oxygen introduced into the vessels differ from a few per cent up to 20 per cent.

The shape of the reaction curve is independent of the partial pressure of oxygen, the rates are proportional to it. Consequently for comparison all curves can be superimposed. The data of one experiment are multiplied with a factor given by the ratio of the two different oxygen partial pressures. In Table XX the data of several measurements have been computed on the assumption that exactly the same amount of oxygen, equal to 0.5 volume per cent, had been introduced into the vessel. The obvious coincidence justifies the procedure outlined. All comparative measurements have been plotted in a like manner.

Influence of Carbon Dioxide on the Oxyhydrogen Reaction

The simple reduction of oxygen with hydrogen to water by living cells should require an uptake of the respective gases in the ratio of 1 O_2 to 2 H_2. If one introduces into a vessel containing a suspension of adapted algae an amount of oxygen corresponding to a rise of 50 mm. in partial pressure, one can expect the oxygen to disappear together with twice the volume of hydrogen. At the end of the reaction the manometric pressure should be 100 mm. lower than at the beginning of the experiment, before the addition of oxygen. Actually this is observed only under special conditions as we shall see below. An experiment was performed with hydrogen containing carbon dioxide of the same partial pressure as used in cultivating the algae. Another vessel contained pure hydrogen and potassium hydroxide solution in the side arm in order to absorb carbon dioxide which might be formed if some of the oxygen would not react with hydrogen but oxidize organic substances. Curves *a* and *b* in Fig. 15 are the observed results in presence or absence of carbon dioxide. Table XX contains similar experiments in more detail. Much more than the theoretical amount of gas is taken up by the algae in presence of carbon dioxide and less in its absence. The incomplete reduction of oxygen

TABLE XX

Oxyhydrogen Reaction in Presence and Absence of Carbon Dioxide

0.02 cc. of cells of *Scenedesmus obliquus* species Pringsheim in 2 cc. of 0.033 M phosphate buffer. pH 5.4. (*a*) 0.2 cc. of 0.1 M K_2CO_3 added from a side arm after incubation. Vessel contains now 450 c.mm. of CO_2, 60 per cent of which are retained in the solution. (*b*) 0.2 cc. of 8 per cent NaOH in side arm to absorb all CO_2. Temperature: 25°. Incubation: 12 hours in H_2.

	(*a*) CO_2 present						(*b*) CO_2 absent					
Volume of vessels, *cc.*	14.9		16.0		15.0		15.4		15.2		15.4	
O_2 introduced, *mm.*	57		46		60		41		48		44	
Time	Mm. of gas absorbed, (1) observed, (2) calculated for 50 mm. of O_2											
	1	2	1	2	1	2	1	2	1	2	1	2
min.												
60	76	67	66	72	87	72	33	40	36	37	33	37
120	144	126	119	129	162	135	59	72	67	70	61	69
150	166	146	137	149	185	154	68	83	77	80	70	80
180	180	158	148	161	199	166	71	86	83	86	75	85
240	203	178	165	179	221	184	81	99	93	97	85	97
300	214	187	172	187	230	192	85	104	97	101	88	100
360	219	193	176	191	235	196	87	106	100	104	91	103
420	224	196	179	195	239	199	90	110	102	106	93	106
580	226	198	182	198	243	203	91	111	107	111	95	108

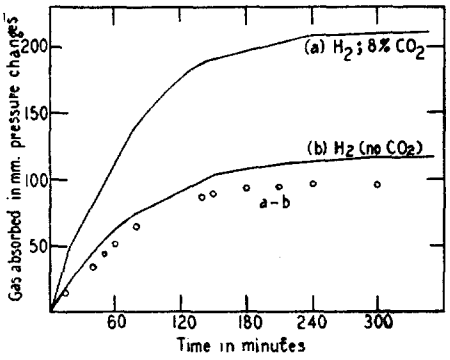

FIG. 15. Oxyhydrogen reaction in presence and absence of carbon dioxide.

is easily explainable by side reactions. The oxygen might partly be used up by hydrogen donors other than hydrogen or even by normal respiration. The excess absorption of hydrogen, however, indicates the simultaneous reduction of some substance other than O_2. Measurements of the total amount of

carbon dioxide before and after the completion of the oxyhydrogen reaction revealed that considerable amounts of carbon dioxide had disappeared together with the excess hydrogen. Table XXI contains some of the data. We see that the hydrogen taken up in excess over the amount necessary for

TABLE XXI

Disappearance of Carbon Dioxide in the Course of the Oxyhydrogen Reaction in the Algae Scenedesmus and Rhaphidium

Ca. 0.03 cc. of cells in 3 cc. of various media. Gas phase: H_2 with 0.5 to 1.7 vol. per cent CO_2. O_2 introduced measured directly. CO_2 disappeared measured as difference between the initial and the final carbon dioxide content. H_2 absorbed measured as difference between the total pressure change and the pressure change due to the absorption of oxygen and of carbon dioxide.

Algae and medium	Duration of experiment	Gas exchange in experiment with oxygen			Gas exchange in fermenting control	
		O_2 introduced	CO_2 disappeared	H_2 absorbed	CO_2	H_2
	hrs.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
Scenedesmus D_3 (old culture) in M/15 phosphate buffer, pH 6.5	44	278	90	609	—	—
Scenedesmus D_3 in M/20 phosphate buffer, pH 6.0	47	258	71	596	+2.5	+4
Scenedesmus D_3 in culture medium	18	298	82	962	+34	−49
	12	267	75	761	+55	−105
	27	470*	130	820	+11	−8
Rhaphidium polymorphium in culture medium, pH 5.7	23	196	81	560	±0	−50
Scenedesmus D_3 in culture medium	19	420	128	1,210	+11	−18
Scenedesmus D_3 in M/20 KH_2PO_4	17	206	65	567	+5	−1

* Not all oxygen absorbed. Algae turned to aerobic conditions, oxyhydrogen reaction stopped.

the formation of water is not always sufficient to bring the carbon of the missing carbonic acid to the carbohydrate level. It can hardly be expected, however, that the ratio $\frac{\text{Additional } H_2 \text{ absorbed}}{CO_2 \text{ disappeared}}$ will be exactly 2 in each experiment. The often apparently incomplete reduction of oxygen and the fermentation processes (see Paper II) are factors which must cause appreciable deviations. Since in photoreduction and photosynthesis the reduction of carbon dioxide proceeds to the level of carbohydrate, it is

very probable that also here carbohydrate is formed. As long as a chemical analysis of the substances produced under these conditions is wanting this remains, however, an assumption.

The Ratio of Carbon Reduced Per Molecule of Oxygen Absorbed

In continuation of the experiments it was soon found that the course of the oxyhydrogen reaction, and with it the yield of reduced carbon, varied much more with experimental conditions than did photosynthesis in the same set of algae. A statistical survey of the results of 129 measurements gives a clear answer as to the maximum amount of gas absorbed per unit of oxygen.

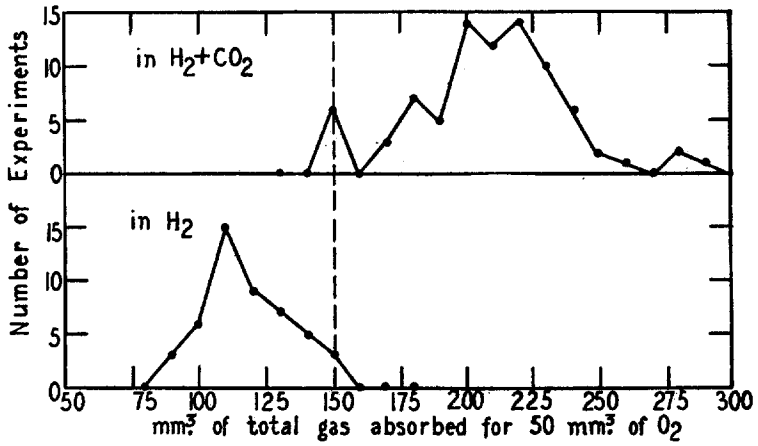

FIG. 16. Total amount of gas absorbed upon addition of 50 c.mm. of oxygen in presence and absence of carbon dioxide.

In Fig. 16, the individual results have been arranged into columns 10 c.mm. apart. Data between the numbers 96 and 105, for instance, have been counted as 100; between 106 and 115 as 110, etc. These data fall into two groups, one in which carbon dioxide was known to be present and in another in which it was known or supposed to be absent.

In the group without carbon dioxide, the majority of experiments point to the absorption of only two volumes of gas (1 O_2 + 1 H_2), the rest indicate an approach to a maximum of three volumes, (1 O_2 + 2 H_2). In the other group, three volumes constitute the minimum yield found only in a fraction of all the experiments, while the majority shows an absorption of four to four and one-half volumes, that is, 200 to 225 c.mm. of gas for 50 c.mm. of oxygen. There is no doubt that the formation of water, *i.e.* the absorption of one part

of oxygen with two parts of hydrogen, is the exception. The oxyhydrogen reaction either stops before or surpasses it by including the reduction of some carbon dioxide. Therefore it is the presence of carbon dioxide which decides which course will be taken. We can state that as soon as carbon dioxide is present, the oxyhydrogen reaction goes to completion. Water is formed always, simultaneously carbon dioxide is reduced in varying amounts.

If we consider first the group of experiments where carbon dioxide was absent, we may be allowed to disregard those experiments in which water has

TABLE XXII

Absence of Respiration during Oxyhydrogen Reaction

0.03 cc. of cells of *Scenedesmus* D_3, washed with 0.03 M Na_2HPO_4 then suspended in 3 cc. or 4.5 cc. of medium. Gas phase: H_2. Temperature: 25°. Adaptation time: 6 hours. Final CO_2 content measured by acidifying with H_2SO_4. Duration of experiment: 2 days.

	0.03 cc. of cells in 3 cc. 0.06 M Na_2HPO_4				0.045 cc. of cells in 4.5 cc. of phosphate buffer		
	pH 9.0				pH 7.9		
Volume of vessels, *cc.*	16.7	18.2	18.4	18.5	15.0	16.0	15.2
Total amount of O_2, introduced in several portions of about 50 mm. each *mm.*	0	374	321	327	160	172	185
Total amount of gas absorbed ($O_2 + H_2$) *mm.*	(+7)	−651	−748	−776	−405	−464	−482
Difference of total CO_2 content of suspension before and after experiment *mm.*	+40	+24	+20	+25	±0	−7	+14

apparently been formed and which overlap with similar results in the carbon dioxide group. Because fermentation continually supplies the cell with small amounts of carbon dioxide, it is not surprising that part of the experiments in absence of known amounts of carbon dioxide should yield results as if this gas were present. Significant is the fact that in the absence of carbon dioxide the oxyhydrogen reaction fails to go to completion in most of the experiments. In these experiments the ratio of hydrogen to oxygen is about 1:1. We learn from these data that the two molecules of hydrogen which reduce oxygen to water are not equivalent in their mode of action. First an intermediate compound is formed from oxygen and one molecule of hydrogen, and it depends

on the presence of carbon dioxide whether the reaction will utilize more hydrogen to form water or continue in unknown directions. Carbon dioxide is not merely the hydrogen acceptor in a coupled reaction, but occupies in addition a key position directing the course of the oxyhydrogen reaction. (Compare literature in reference 38.)

As to the fate of the intermediate compound in absence of carbon dioxide, it is unlikely that the substance accumulates. The incomplete reaction with oxygen can take place several times in succession without apparent damage to the algal cells (compare Table XXII). If such an intermediate (probably a kind of peroxide) would not disappear by a reaction with internal hydrogen donors, the first thing to happen would be the inactivation of the hydrogenase system. In fact, the latter invariably becomes inactivated, if the rate of oxygen uptake (determined by the partial pressure of oxygen) is too high or if the reaction is inhibited by cyanide (see below).

Turning now to the group of experiments in the presence of carbon dioxide in which the oxygen is completely reduced by hydrogen, we were interested to know how much gas would be absorbed in addition to the two volumes of hydrogen needed for the formation of water. From Fig. 16, it is apparent that up to three additional volumes of gas absorbed all intermediate values may be found. Fig. 16 shows that, after reaching a peak at about 225 c.mm. total gas absorbed for each 50 c.mm. of O_2, the number of experiments giving greater values drop off rapidly. No case is recorded where the total uptake surpasses 300 c.mm. of gas. An examination of the highest values above 250 c.mm. has shown invariably that they belong to experiments of very long duration or of large and questionable corrections.

50 c.mm. (or mm.) of O_2 is our "standard" volume. We conclude that not more than 1.5 volumes of additional gas are absorbed by the plant for 2 volumes of hydrogen utilized in the oxyhydrogen reaction. It has been shown (Table XXI) that carbon dioxide is taken up together with an excess of hydrogen. If in this reaction carbohydrate is produced, the ratio of carbon dioxide to hydrogen should be 1:2. With the understanding that the coupling between the formation of water and the reduction of carbon dioxide is stoichiometric, we can expect for each volume of oxygen absorbed the following maximal amounts of "extra" gas.

(1) $\frac{1}{2}\ H_2 + \frac{1}{4}\ CO_2 = \frac{3}{4}$ volume
(2) $1\ H_2 + 0.5\ CO_2 = 1.5$ volumes
(3) $1.5\ H_2 + 0.75\ CO_2 = 2.25$ volumes
(4) $2\ H_2 + 1\ CO_2 = 3$ volumes

Of all possibilities only the second fits the great majority of observations and is further supported by other experiments reported below. There is no doubt that it is possible to obtain results which apparently agree with the third or

even fourth case, but only if the very end of the absorption process continuing for many hours is ascribed to the oxyhydrogen reaction and not to a secondary absorption of hydrogen connected with fermentation; and if no correction is applied for this continuing metabolism as well as for the gas exchange observable prior to the experiment. The observed yield of 0.5 molecule CO_2 for 1 molecule O_2 is the highest well established value in the literature on the dark reduction of carbon dioxide coupled with the oxyhydrogen reaction. Ruhland's (34) best results gave 0.3 molecule CO_2.

The Part Played by Respiration during the Oxyhydrogen Reaction

In all cases where the oxyhydrogen reaction proceeds with the absorption of two or more equivalents of hydrogen for each molecule of oxygen, there is little doubt that practically none of the oxygen absorbed by the cells is used in normal respiration. In discussing the fact that the incomplete reaction in absence of carbon dioxide, which leads to the uptake of one molecule of hydrogen only, is not harmful to the cell, it was concluded that the first intermediate must react further and disappear by way of an internal reduction. In connection with this unknown step, a formation of carbon dioxide appeared possible. Table XXII shows the results of several experiments made with the purpose of establishing any such production of carbon dioxide. The algae were suspended in alkaline phosphate solution which retains any carbon dioxide released from the cells as carbonate or bicarbonate. The partial pressure of free carbon dioxide was so low that the reaction remained incomplete, as explained in the preceding paragraph. Only about one molecule of hydrogen was taken up for each molecule of oxygen absorbed. The quantities of oxygen used were much greater than in most of the other experiments. To make this possible, small and tolerable amounts of oxygen were introduced repeatedly. The difference determined by acid titration between the carbonate content of the solution before and after the experiment was always less than one-tenth of the corresponding amount of oxygen. This small production of carbon dioxide can easily be accounted for by fermentation. The amount of carbon dioxide formed during the same time in a fermenting control was even larger. Since we can hardly assume that the first step in the oxyhydrogen reaction leads to a very stable intermediate, it follows that the internal hydrogen donor reacting in place of the second molecule of hydrogen does not yield carbon dioxide in this process. Certainly normal respiration is suppressed completely under the circumstances favoring the oxyhydrogen reaction. This is remarkable because less than 1 per cent of oxygen can still support respiration in these algae when the low partial pressure is applied in direct exchange for air. On the other hand, once a return to aerobic conditions has been enforced by a small "excess" of oxygen the oxygen uptake now continues through respiration.

Partial Reactions

In the preceding paragraph it has been shown that the course of the oxyhydrogen reaction involves two or more steps which differ in importance for the induced reduction of carbon dioxide. A study of these partial reactions is encouraged by the fact that they are clearly demonstrated when the course of the reaction is plotted in curves showing either the total amount of gas absorbed, or the rate of absorption, *versus* time. The same breaks or sudden changes in slope which can be seen in Figs. 15, 19, 21, and 22 have been verified in 75 per cent of more than a hundred single experiments. Small deviations in the readings are sufficient to obliterate this peculiarity. No such well defined breaks are obtained if the curves are drawn through observation points which are few and far apart or if the data of several parallel experiments are averaged and plotted.

Our interpretation of the broken curves is that the very first partial reaction is faster than some of those which follow. In general the curves have three to four distinct breaks and hence consist of as many straight lines of different slope. In several cases these straight lines, when traced back to the ordinate, intersect it at points having the same number of millimeters or multiples thereof as the initial partial pressure of oxygen. This observation is general and therefore cannot be ascribed to chance. The obvious interpretation is that the absorption of oxygen, of hydrogen, and of carbon dioxide starts nearly simultaneously, each of these reactions proceeding at a different rate. Probably the rates of absorption for each one of the three hydrogen equivalents are also different. Whenever a partial reaction has come to completion, its rate drops to zero, and consequently the overall rate diminishes. The initial absorption of oxygen is artificially maintained at a slow rate. Since it sets the pace, any ensuing partial reaction faster than this one remains unnoticed. Two problems present themselves: (1) Which partial reaction, if its rate becomes too fast, leads to inactivation? and (2) Which partial reaction is coupled with the reduction of carbon dioxide? In answer to the first question, we can state that the rate of oxygen absorption rises in proportion to the partial pressure of O_2. (Compare Table XX.) Depending on individual conditions of the algal cultures, up to two volumes per cent of oxygen may be tolerated. Inactivation is observed usually above one volume per cent of O_2. The maximum rate obtainable without inactivation is approximately equal to the maximum rate of photoreduction in the same cells. (Compare Table IV in reference 22.) With a small "excess" of oxygen inactivation is a comparatively slow process. Large amounts of oxygen (ten to twenty volumes per cent) produce momentarily a very high rate of oxygen uptake followed by quick inactivation. The very first uptake of oxygen, then, seems to be a fast reaction leading to the accumulation of an intermediate which, if it is not removed, brings about inactivation. This agrees with the observation that

more oxygen, than would be tolerated initially, may be added to the cell suspension after the first break in the velocity curve has been passed. It is therefore possible to maintain the reaction for an indefinite period of time at the initial or even at a higher rate. Consequently oxygen will be consumed by the cells in large quantities. The experiments of Table XXI, proving the disappearance of carbon dioxide, as well as those of Table XXII, were made in this manner.

As to the coupling with the carbon dioxide reduction it is significant that from the start of the oxygen absorption the rates in the presence or absence of this gas differ. If the reduction of carbon dioxide were a slow reaction the experimental observation presumably would have been a prolonged uptake of hydrogen at a more or less steady rate in excess of the amount necessary to form water. Fig. 15 shows that this is not the case. The initial rate in presence of CO_2 is approximately doubled. Plots of the difference of the curves with and without carbon dioxide demonstrate that the induced reduction is completed in about half the time required for the total process (Figs. 20 and 21).

Influence of Cyanide

The inhibition of adaptation to the hydrogen metabolism by cyanide has been described in Paper I. Here we shall discuss the manner in which cyanide influences the oxyhydrogen reaction after adaptation has been completed. Table XXIII shows that relatively small concentrations of cyanide inhibit the oxyhydrogen reaction definitely. If larger amounts of cyanide are added in advance, hardly any hydrogen is absorbed, the oxygen disappears slowly, and the algae lose the ability of performing any kind of metabolism. Hence the effect of very high concentrations of cyanide hardly permits one to draw conclusions as to the mode of action of the poison (contrary to the experiments with high concentrations of hydroxylamine). The experiment shown in Fig. 17 was made with a cyanide concentration which inhibited photoreduction about 50 per cent. The coupled reduction of carbon dioxide is stopped whenever HCN is added. The reduction of oxygen continues. A comparison of the action of cyanide in the presence (Fig. 17) or absence (Fig. 18) of carbon dioxide clearly demonstrates that it is the induced reduction of carbon dioxide which is most sensitive to cyanide. The first part of the oxyhydrogen reaction leading to a "peroxide" takes place. (The word "peroxide" here is used only as a short expression for the fact that oxygen and hydrogen are taken up in equal volumes.) From then on a further reduction of the "peroxide" is inhibited. If "peroxide" is formed and accumulates it would not be surprising to observe that the hydrogenase system becomes inactivated whenever the oxyhydrogen reaction occurs in the presence of cyanide. The percentage of inactivation is related to the time at which the poison is added after the start of the oxyhydrogen reaction. (Compare Table XXIV.) These findings

agree well with the observation that cyanide enhances the return of photoreduction to normal photosynthesis and that it prevents the adaptation even at concentrations which, added after adaptation, permit photoreduction to proceed at a measurable rate. In the experiment of Table XXIII, for instance, where during the oxyhydrogen reaction the hydrogenase system becomes inactivated in the presence of cyanide, we find only 13 per cent inhibition of photoreduction before and a normal rate of photosynthesis after the turnback.

TABLE XXIII

Inactivation of the Hydrogenase System by Oxygen in Presence of Cyanide

0.048 cc. of cells of *Scenedesmus* D_3 in 4 cc. of 0.05 M phosphate buffer. pH 6.5. Gas phase H_2; 8 per cent CO_2. Temperature 26°. Poison added anaerobically 2 hours before experiment.

		1	2
Volume of vessels, *cc.*		16.0	15.4
Cyanide concentration		—	2×10^{-4} M HCN
	Observation time	Rate of gas exchange in $\frac{\text{mm.}}{10 \text{ min.}}$	
(*a*) Light. 2000 lux. Photoreduction	17 min.	−52	−45
(*b*) Dark. Oxyhydrogen reaction with 50 mm. of O_2	After 10 min.	−22	−8
	After 60 min.	−15	−5
	After 120 min.	−10	−1
(*c*) Light. 2000 lux. Photoreduction in (1). Photosynthesis in (2)	20 min.	−66	+9

Influence of Hydroxylamine

With hydroxylamine the effects appear to be very complex. Firstly: This poison, contrary to cyanide, may be added (after adaptation of the algae) in large concentrations before or during the oxyhydrogen reaction with hardly any effect upon its course. One obtains results similar to those shown in Fig. 19, which should be compared with Fig. 17. If, however, the presence of poison is tested by irradiation of the plant, one observes that photoreduction is inhibited, the turn more difficult, and any possibility for oxygen production completely blocked. The poison must have penetrated into the cell and into the chloroplast. See Table XXV, No. 1, *d-h*.

Secondly: Hydroxylamine, if added anaerobically as in the first case, is often found to inhibit strongly the coupled reduction of carbon dioxide. This is demonstrated in Figs. 20 *a* and *b* showing the course of the oxyhydrogen

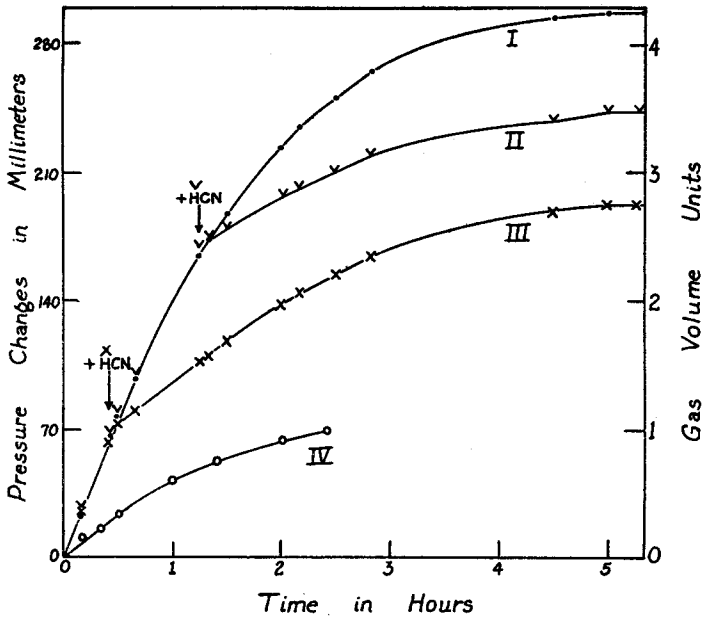

FIG. 17. Inhibition of the dark reduction of carbon dioxide by 2×10^{-4} M cyanide. Poison added at different times in the course of the oxyhydrogen reaction. The gas phase in this experiment was H_2 with 4 per cent CO_2.

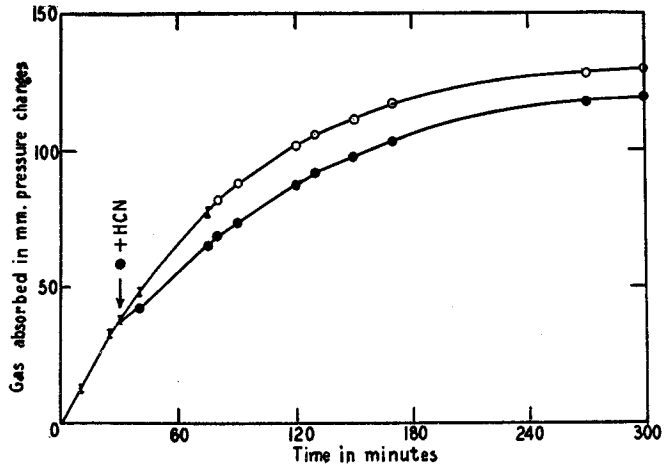

FIG. 18. Effect of 2×10^{-4} M cyanide upon the oxyhydrogen reaction in absence of carbon dioxide.

reaction with and without poison. The absorption of oxygen continues in the poisoned algae together with either one or two equivalents of hydrogen.

Curve *c*, the difference between curve *a* and *b*, demonstrates the time course of the partial reaction concerned with the reduction of carbon dioxide. In order to stop the dark reduction of carbon dioxide, the amount of hydroxylamine has to be at least as great as required for a measurable inhibition of photoreduction, that is much larger than needed for a complete inhibition of photosynthesis. When hydroxylamine is added, however, aerobically before adaptation, the same reaction occurs that was described for photoreduction: The

TABLE XXIV

Inactivation of the Hydrogen Metabolism by Small Amounts of Oxygen in Presence of Cyanide

0.05 cc. of cells of *S. obliquus* in 4 cc. of 0.02 M KH_2PO_4. Temperature: 25°. Gas phase: H_2; 4 per cent CO_2. Preceding dark period: 12 hours. Cyanide added at successive time intervals in different vessels during the course of the oxyhydrogen reaction. Final concentration of poison: 2×10^{-4} M. Remaining activity tested by photoreduction after completion of the dark reaction.

Condition	Time	1	2	3	4
		Rate of pressure changes in $\frac{\text{mm.}}{10 \text{ min.}}$			
	min.				
(*a*) Light. 2600 lux	After 10	−56	−62	−54	−66
(*b*) Dark. Reaction with 70 mm. O_2	After 30	−19	−21	−22	No O_2 added
		+ HCN		−17	
	75	−7.5	−20		
			+ HCN		
	150	−5	−4.5	−7.5	
	300	−1	−1	−1	
				+ HCN	
(*c*) Light. 2600 lux	After 5	−10	−24	−34	−66
	10	+1	0	−28	−73
	15	+6	+6	−4	—

inhibition is either complete for lack of adaptation, or it is partial and then the oxyhydrogen reaction proceeds only to the intermediate "peroxide" level (Fig. 21). See also Table XXV, Nos. 2 and 3.

Thirdly: In some cases where the oxyhydrogen reaction yielded only water and for no apparent reason was not coupled with the reduction of carbon dioxide, addition of hydroxylamine restored the coupling. In other words, more hydrogen was absorbed in presence of the poison than without it. The experiment could be repeated several times and it is certainly not due to an error. See Fig. 22.

Summarizing the experiments with hydroxylamine, one can say that the poison has no effect on the course of the oxyhydrogen reaction unless its con-

centration is unusually high, and that, if it takes effect, it will inhibit the reduction of carbon dioxide, whereas the reaction between hydrogen and oxygen may continue to the peroxide level or to the formation of water.

The much greater inhibitions found when the hydroxylamine is added aerobically before the adaptation are a problem which does not concern the oxyhydrogen reactions as such. Photoreduction as well as the oxyhydrogen reaction can be used as tests for this peculiar type of inhibition.

We know now that the turnback from photoreduction to normal photosynthesis and to aerobic conditions under the influence of excess light is

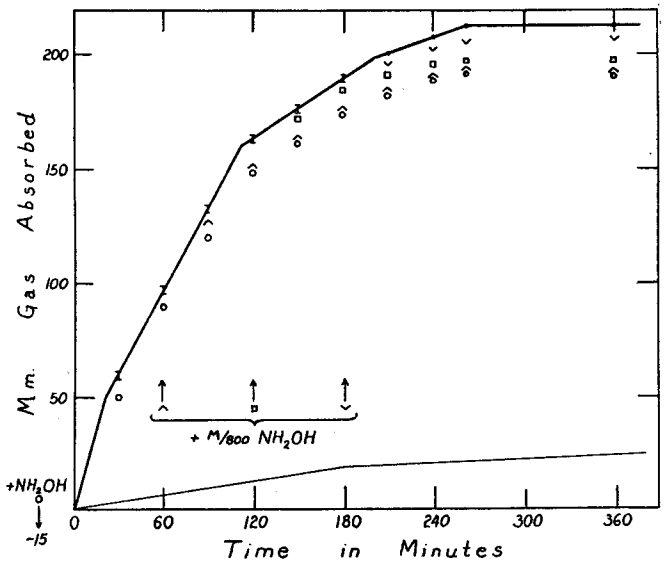

FIG. 19. Effect of M/800 hydroxylamine on the oxyhydrogen reaction (CO_2 present). Poison added after adaptation.

prevented or retarded by hydroxylamine (Paper I). No such "protection" by hydroxylamine exists if the turn is enforced by an excess of oxygen. This is the one point where photoreduction and oxyhydrogen reaction do not give corresponding results. On the other hand, hydroxylamine does not enhance the inactivation as does cyanide.

One might think that the peculiar differences found in the effect of hydroxylamine were caused by the use of different strains of algae or of algal samples grown under unequal conditions. Table XXV proves that this is not the case. With the same culture distributed equally in four vessels, most of the reactions described were observed. In vessel 2, sufficient hydroxylamine was added aerobically to inhibit the rate of normal photosynthesis by 60 per cent. The intensity of illumination is so low that there is still propor-

tionality between rate and intensity. The inhibition remains the same however, when the intensity is cut in half. This conforms to the observation of Weller and Franck (6) that hydroxylamine inhibits some photochemical

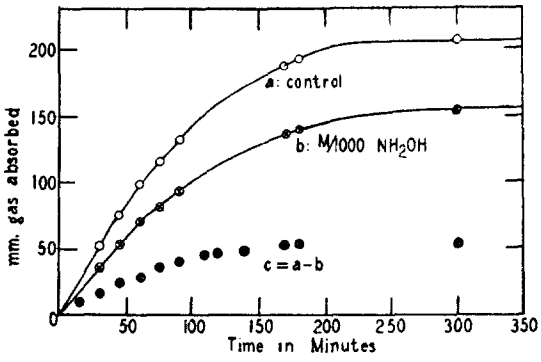

FIG. 20*a*

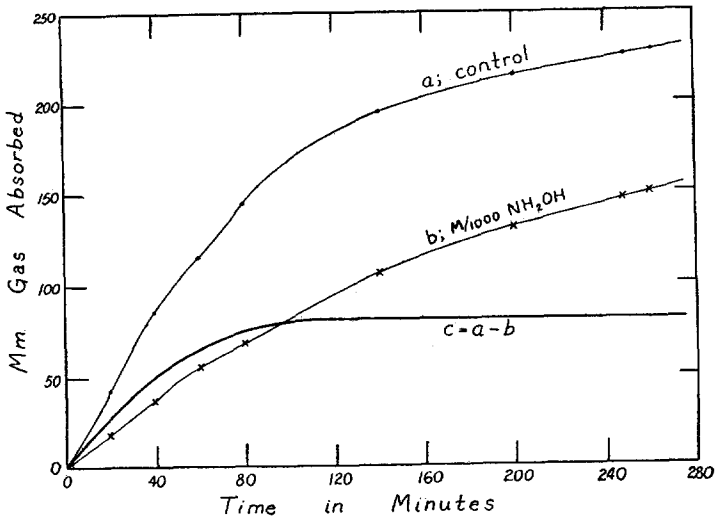

FIG. 20*b*

FIGS 20*a* and 20*b*. Inhibition of the dark reduction of carbon dioxide by hydroxylamine. Poison added after adaptation.

reactions in photosynthesis, in spite of the fact that it seems to inhibit specifically the production of oxygen (Paper I). During adaptation to hydrogen, the gas exchange of the poisoned algae is affected strongly, yet after 6 hours we find typical photoreduction at about 50 per cent of that of the unpoisoned algae. This inhibition lasts for a dark period of about 20 hours. During this time some oxygen has been added twice and the oxyhydrogen reaction proceeds

TABLE XXV

Inhibition of Photoreduction and Dark Reduction by Hydroxylamine

0.034 cc. of cells of *Scenedesmus* D_3 in 4 cc. of 0.1 M bicarbonate solution (equal parts of $NaHCO_3$ and $KHCO_3$). Gas phase: H_2; 4 per cent CO_2. Temperature: 25°. Alternate dark and light periods. Light reactions measured as rates of gas exchange in mm./10 minutes. Dark reaction measured as millimeters of total gas absorbed for 50 mm. of O_2.

No. of experiment		1		2		3		4	
Volume of vessels, *cc.*		15.2		15.0		15.4		14.9	
Poison added		—		M/5000 NH_2OH		—		—	
Condition	Time	Photosynthesis rate in mm./10 min.		Photosynthesis rate in mm./10 min.		Photosynthesis rate in mm./10 min.			
Not adapted									
(*a*) Light. 3200 lux	5 min.	+34		+14		+34		—	
1700 lux	20 min.	+20		+8		+19		—	
(*b*) Dark, refilled with H_2/CO_2. Adaptation..	300 min.	−2.7		−1 → 0.5		−2.7		−3	
		Photoreduction in mm./10 min.	Oxyreduction in total mm.	Photoreduction in mm./10 min.	Oxyreduction in total mm.	+M/1000 NH_2OH: Photoreduction in mm./10 min.	+M/1000 NH_2OH: Oxyreduction in total mm.	Photoreduction in mm./10 min.	Oxyreduction in total mm.
(*c*) Light. 800 lux	10 min.	−42		−24		−16		—	
(*d*) Dark + 50 mm. O_2.....	14 hrs.		250		195		151		—
Dark + 50 mm. O_2.....	6 hrs.		207		188		109		—
(*e*) Light. 800 lux	30 min.	−32		−24		−4		New control	
(*f*) Dark.....	30 min.	+ M/1000 NH_2OH		+ M/5000 NH_2OH		——			——
(*g*) Light. 800 lux	15 min.	−36		−37		−7		−42	
Light. 3200 lux	5 min.	−60		−78		−4		−78	
Light. 3200 lux	10 min.	−70		−102		−5		+4(!)	
(*h*) Dark.....	2 hrs.	——		——		——			——
Dark + 50 mm. O_2.....	5 hrs.		220		230		150		258
Dark + 50 mm. O_2.....	16 hrs.		209		224		241		248
(*i*) Light. 800 lux	30 min.	−27		−32		−30		−39	
Light. 3200 lux	20 min.	−47		−94		−78		−72 → +22(!)	
(*j*) Dark + 50 mm. O_2.....	3.5 hrs.		211		225		208		209
(*k*) Light. 3200 lux	10 min.	−40		−108		−102		−88 (turns)	
1700 lux	40 min.	−36		−66		−66		+8	

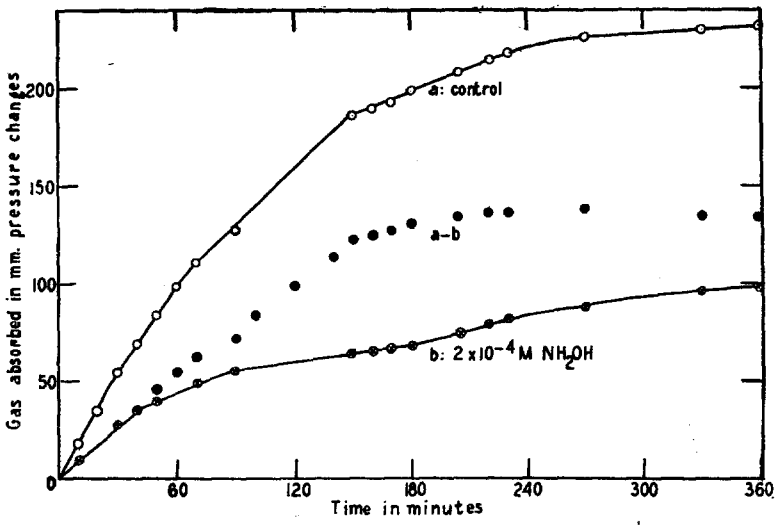

FIG. 21. Inhibition of the dark reduction of CO_2 by hydroxylamine. Poison added in air before adaptation.

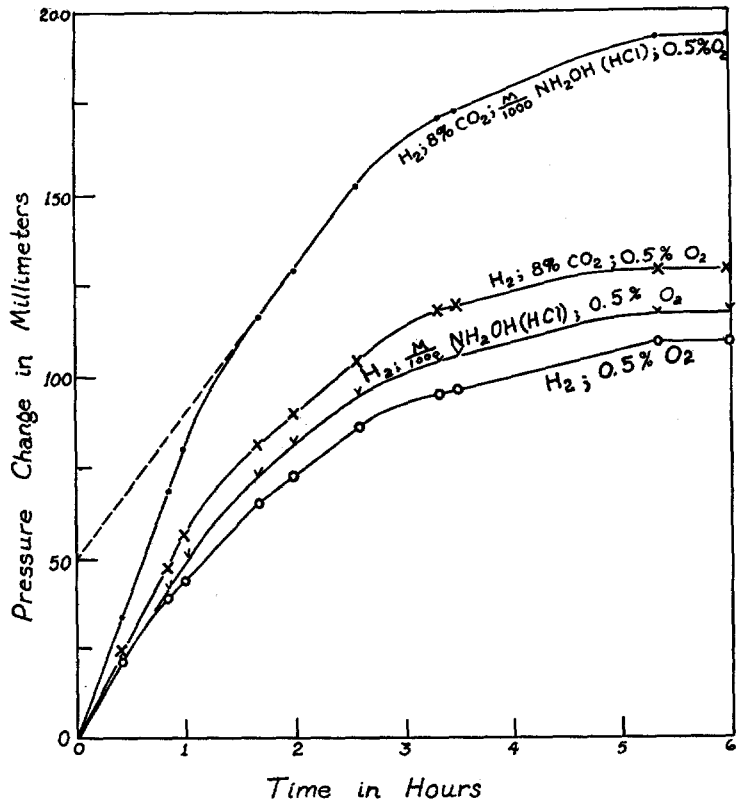

FIG. 22. Effect of hydroxylamine upon the incomplete dark reduction of carbon dioxide.

in a partially inhibited form. Adding now the same amount of poison which the algae had received aerobically the day before, not only produces no further inhibition, but it enhances suddenly the rate of photoreduction from 24 to 37 mm./10 min. and the value for the oxyhydrogen reaction attains the theoretical maximum. The presence of the poison is evident from the protection it provides against the turn to aerobic photosynthesis under the influence of the fourfold intensity. Compare vessel 4.

In vessel 3, hydroxylamine is added after 6 hours of adaptation. A concentration of 10^{-3}M instantaneously produces a strong inhibition of photoreduction (60 per cent) and of the dark reduction. Both effects increased

TABLE XXVI

Effect of Dinitrophenol on the Coupled Dark Reduction of Carbon Dioxide Compared with the Inhibition of Photoreduction

0.027 cc. of cells (*a*) in 4 cc. of 0.03 M KH_2PO_4, (*b*) in 0.05 M phosphate buffer. pH 6.0. Gas phase: H_2; 4 per cent CO_2.

	(*a*)			(*b*)	
	Dinitrophenol added after 15 hrs. of adaptation Temperature 25°			Dinitrophenol added aerobically before adaptation Temperature 38°	
Final concentration of dinitrophenol.....	0	2×10^{-6} M	1×10^{-4} M	0	2×10^{-5} M
Relative rates of photosynthesis at 3300 lux.....	100	100	50	100	55
Relative rates of photoreduction at 1700 lux.....	100	85	60	100	60
Oxyreduction. Total gas absorbed in mm. for 50 mm. of O_2.....	210	218	204	212	154

first with time and then vanish completely on the 3rd day. In the case of the dark reduction, the changes are not gradual but in clear-cut steps corresponding to the formation of water, then "peroxide," again water, and finally uninhibited coupled reduction.

The same amount of poison if added not after 6 but after 26 hours of anaerobic conditions leaves the yield of the oxyhydrogen reaction unchanged and inhibits photoreduction measurably only at higher light intensities.

The parallelism between the dark and the photoreduction of carbon dioxide with respect to the influence of hydroxylamine is striking. In general photoreduction remains more sensitive. No quantitative determinations have been made on the disappearance of the hydroxylamine from the algal suspension, but it is certain that it decomposes slowly by way of side reactions in the course of an experiment lasting several days. Similarly a disappearance of the effect upon the oxyhydrogen reaction is accompanied by a fading of the

inhibition of photoreduction. Nearly always enough poison remains in the solution to prevent normal photosynthesis under aerobic conditions.

The Influence of Dinitrophenol

Photosynthesis and photoreduction with hydrogen are inhibited strongly by dinitrophenol (Paper I). The oxyhydrogen reaction appears to be less sensi-

TABLE XXVII

Inhibition of Hydrogen Absorption by Glucose

0.034 cc. of cells of *Scenedesmus* D_3 in 4 cc. 0.1 M bicarbonate. Temperature: 25°. Gas phase: H_2; 4 per cent CO_2. Cells 4 days in H_2.

	1	2	3
Volume of vessels, *cc.*	15.4	15.0	14.9
Oxyhydrogen reaction. Mm. of total gas absorbed for 50 mm. of O_2	211	190	217
Poison added in (3). 20 hrs. dark	—	—	$+10^{-3}$ M NH_2OH
Rate of photoreduction (mm./5 min.)	37	38	32
Glucose added; 30 min. dark	—	+0.5 per cent glucose	+0.5 per cent glucose
Rate of photoreduction (mm./5 min.)			
First 5 min.	28	15	6
After 15 min.	39	34	22
Oxyhydrogen reaction.* Mm. of total gas (corrected) absorbed for 50 mm. O_2 during 8 hrs.	221	96	75
Rate of photoreduction (mm./5 min.)			
First 5 min.	24	12	8
Second 5 min.	36	30	20
After 14 hrs. dark and 20 min. light	35	37	16

* Compare Fig. 23.

tive to this poison, as shown in Table XXVI. If an inhibition occurs, then it is again the coupled reduction of carbon dioxide, not the formation of water, which is inhibited.

The Influence of Glucose

The photochemical utilization of molecular hydrogen is looked upon as the simplest case of a photoreduction which might as well proceed with the aid of other more complicated hydrogen donors (1, 22). One of the main points supporting this view is the fact that yeast extracts containing carbohydrates

or glucose greatly inhibit the uptake of hydrogen in photoreduction (1) and enhance the photochemical production of hydrogen in the absence of reducible carbon dioxide (Paper II). Glucose (in dilute solution) certainly has no inhibitory effect on respiration, fermentation, or photosynthesis in the same algae. A few experiments have been made to show that also with glucose there is parallelism between the induced dark reduction and photoreduction. In a medium containing glucose (0.05–1 per cent) the algae absorb less hydrogen in the course of the oxyhydrogen reaction than in the glucose-free control.

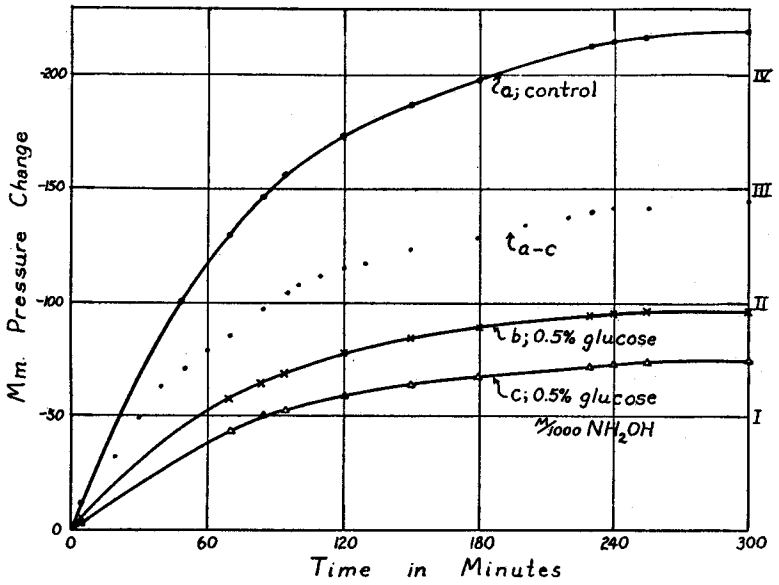

FIG. 23. Inhibition of the hydrogen uptake during the oxyhydrogen reaction in presence of glucose.

It is not surprising that the differences between the results obtained with and without glucose vary widely from experiment to experiment, since the activities of the competing enzyme systems need to change only little in order to produce greatly divergent results. The important problem is how much the uptake of hydrogen can be suppressed by the presence of glucose.

In the experiment shown in Table XXVII 0.5 per cent glucose did not greatly influence photoreduction before or after the reaction with hydrogen except during the first 5 minutes of illumination. The amount of gas absorbed in the course of the oxyhydrogen reaction, however, is less than one-half of the normal. In a second experiment a similar batch of algae in the same medium was used but it contained about 0.001 M hydroxylamine which had been added anaerobically a few hours earlier. These algae could be expected to absorb at least one or two equivalents of hydrogen. The curves in Fig. 23

demonstrate the suppression of hydrogen absorption. In presence of glucose only little more gas disappears than corresponds to the amount of oxygen introduced. The "excess" gas might be carbon dioxide in this particular case. Since the supply of oxygen is small compared with that of glucose it is probable that the latter is oxidized only partly and without a release of CO_2. Further experiments are necessary to prove definitely a reduction of carbon dioxide coupled with the partial oxidation of glucose. It has been mentioned before that the algae absorb considerable amounts of hydrogen during the first hours of anaerobic incubation. It appears likely that such partially oxidized carbohydrates play a part as acceptors for hydrogen.

DISCUSSION AND CONCLUSIONS

Lately many papers have been published concerning dark reactions in living cells which involve carbon dioxide as a reactant. (For a recent review on the subject see reference 38.) In comparing different results it is important to distinguish clearly what is meant by "carbon dioxide reduction." In the literature we find reduction, fixation, assimilation, etc., of carbon dioxide treated as if these words were synonyms. For instance, Ruben, Kamen, and Hassid (4), using the radioactive tracer method, have shown that, contrary to what has been found under the conditions described in this paper, carbon dioxide is not reduced in the dark under normal aerobic conditions. What they found is a reversible fixation of carbon dioxide in the plant which is a reaction postulated as an important preliminary step in photosynthesis since the times of Willstätter and Stoll. If we write this fixation, following the authors, as a carboxylation it is evident where the difference between the first equation and the following ones lies.

$$\begin{aligned} &(1)\ RH + CO_2 \rightleftarrows RCO\cdot OH \text{ (Fixation)} \\ &(2)\ RCO\,OH + H_2 \rightarrow RCHO + H_2O \\ &(3)\ RCHO + H_2 \rightarrow RCH_2OH \\ &(4)\ RCH_2OH + H_2 \rightarrow R\,C\,H_3 + H_2O \end{aligned} \Big\} \text{(Reduction)}$$

In the methane bacteria (25, 39) the carbon is completely reduced, but the methane is liberated from the cell, hence it cannot be assimilated. In liver cells (40) carbon dioxide is fixed and assimilated, but perhaps not reduced. In this and similar cases, it is not known whether after fixation in the form of a carboxyl group the carbon becomes reduced, and little attention has been paid thus far to the different possibilities. A clear terminology may help in clarifying the problems.

The data presented in this paper prove that in *Scenedesmus* and similar algae carbon dioxide is reduced by molecular hydrogen in the dark with the aid of the oxyhydrogen reaction. The reduction proceeds to the level of carbohydrate. The yield is one-half molecule of carbon dioxide reduced for one molecule of absorbed oxygen.

The problem confronting us now is to devise a mechanism which will explain how this yield is possible. We find in a paper of Yamagata and Nakamura a plausible scheme (41) for a pure oxyhydrogen reaction. A hydrogenase and an oxygen-transferring system will readily yield water, but nothing else.

In order to link the oxyhydrogen reaction with a reduction of carbon dioxide, we have to make use of the following experimental facts: (1) The presence of carbon dioxide is necessary for the undisturbed formation of water, otherwise the reaction proceeds only to the "peroxide" level. In other words, CO_2 must be not only an acceptor for hydrogen but an essential part of an intermediate substance. (2) Photoreduction and dark reduction appear to have a great similarity as shown by the effect of poisons.

Elsewhere we have said that the photochemical processes in a green plant can be described as forcing the constituents of a molecule of water, H and OH, into a complex $Q \cdot R \cdot (CO_2)$, whereby oxidized and reduced substances, QOH and $R(H) \cdot (CO_2)$ are formed. On paper it is possible to do the same not photochemically but by oxidation and reduction; then we obtain:

(1) $$2Q \cdot R \cdot (CO_2) + O_2 + 2HDo \rightarrow \underset{|}{2R}(CO_2) + 2QOH + 2Do$$

and

(2) $$\underset{|}{RCO_2} + HDo \rightarrow R(H)(CO_2) + Do$$

HDo means any hydrogen donor, in our case free hydrogen. Once the same products are formed as in photoreduction, there is no difficulty in assuming that they react further along identical lines. In this way the induced dark reduction of carbon dioxide can be described as a partial reversal of photoreduction that leads us automatically to the required maximum yield of half a molecule of carbon dioxide reduced per molecule of oxygen absorbed. A detailed discussion of the principle mentioned is only possible in close connection with a discussion of the facts observed in photoreduction and photosynthesis and therefore reserved for another communication.

SUMMARY

1. Unicellular algae possessing a hydrogenase system (*Scenedesmus* and other species), and having been adapted by anaerobic incubation to the hydrogen metabolism, reduce oxygen to water according to the equation $O_2 + 2H_2 \rightarrow 2H_2O$.

2. The oxyhydrogen reaction proceeds undisturbed only in the presence of carbon dioxide, which simultaneously is reduced according to the equation $CO_2 + 2H_2 \rightarrow H_2O + (CH_2O) =$ (carbohydrate).

3. The maximum yield of the induced reduction is one-half molecule of carbon dioxide reduced for each molecule of oxygen absorbed.

4. Partial reactions are recognizable in the course of the formation of water

and it is with the absorption of the second equivalent of hydrogen that the carbon dioxide reduction appears to be coupled.

5. The velocity of the reaction increases in proportion to the partial pressure of oxygen, but only up to a certain point where any excess of oxygen causes the inactivation of the hydrogenase system. The reaction then ends prematurely.

6. During the oxyhydrogen reaction little or no oxygen is consumed for normal respiratory processes.

7. Small concentrations of cyanide, affecting neither photosynthesis nor photoreduction in the same cells, first inhibit the induced reduction of carbon dioxide and then lead to a complete inactivation of the hydrogenase system.

8. Hydroxylamine, added after adaptation, has either no inhibitory effect at all, or prevents solely the induced reduction of carbon dioxide without inactivating the hydrogenase system.

9. Dinitrophenol prevents the dark reduction of carbon dioxide while the reduction of oxygen continues to the formation of water.

10. Glucose diminishes the absorption of hydrogen, probably in its capacity as a competing hydrogen donor.

11. The induced reduction of carbon dioxide can be described as an oxido-reduction similar to that produced photochemically in the same cells.

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