AN EXPERIMENTAL SEPARATION OF OXYGEN LIBERATION FROM CARBON DIOXIDE FIXATION IN PHOTOSYNTHESIS BY CHLORELLA*

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In the process of photosynthesis in Chlorella it is now known that the oxygen liberated during CO2 fixation and reduction originates from water (Ruben et al., 1941). It is further known that in some algae (not Chlorella) one can obtain carbon dioxide fixation and reduction without the release of oxygen (by adaptation to hydrogen, Gaffron, 1940, 1942 a, 1942 b, Gaffron and Rubin, 1942) and that in isolated chloroplasts one can obtain oxygen (by the reduction of ferric salts, Hill, 1940, Hill and Scarisbrick, 1940 a, 1940 b) without a simultaneous reduction of carbon dioxide. It therefore appears that, since the reactions of CO2 fixation and O2 liberation are only "loosely connected" (Franck and Gaffron, 1941), it should be possible by the choice of an appropriate technique, to cause oxygen liberation in intact cells without the simultaneous reduction of carbon dioxide; i.e., to separate the reactions liberating oxygen from those concerned with carbon dioxide fixation. The results reported in this paper are based upon the concept that if such is indeed the case, it should be possible to obtain oxygen from illuminated Chlorella cells by supplying reducible materials other than carbon dioxide.

Preliminary Studies

The technique we have employed is illustrated by the data given in Fig. 1. The algal strain used was Chlorella pyrenoidosa, previously described, grown in the light with CO2 as the sole carbon source in the medium and under the same conditions as previously recorded (Manning et al., 1938). 1 ml. of a washed suspension (washed and suspended in saline) obtained after 10 days growth and containing 96 mg. dry weight of algal cells per ml. was placed in each of three Warburg flasks. 1 ml. M/30 phosphate buffer (pH 7.0) was added, 0.2 ml. 20 per cent KOH placed in the center well, and 0.5 ml. of water, 0.5 ml. of M/10 acetaldehyde, or 0.5 ml. M/10 of benzaldehyde was supplied to the side arms. These were placed in the water bath at 28°C. Oxygen uptake was determined in the dark at 5 minute intervals for 20 minutes. The shaking rate employed throughout was 115 two cm. strokes per minute; well above that necessary for instantaneous gas exchange. The flasks were then illu-

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minated with approximately 900 foot candles of light (determined with a Weston photometer) obtained from two photoflood bulbs suspended 1 foot above the bath. After illumination of the flasks containing water or acetaldehyde in the side arm (Fig. 1) there is a release of gas for the first 10 minutes (20 to 30 minutes) after which there is a slow uptake. This gas is presumed to be oxygen from traces of CO₂ in solution, from the reduction of intermediates between CO₂ and the final product of photosynthesis, or perhaps resulting from the "CO₂ gush" reactions described by Blinks and Skow (1938) and Emerson and Lewis (1941). As shown in Fig. 1, this gas release tended to vary from flask to flask. However in a relatively short time

![Graph showing oxygen production](image)

**Fig. 1.** The production of oxygen from acetaldehyde and benzaldehyde. 1 ml. algal cell suspension (96 mg. dry weight), 1 ml. M/30 phosphate buffer, pH 7.0; 0.2 ml. 20 per cent KOH in center well 0.5 ml. of acetaldehyde (M/10), benzaldehyde (M/10) or water in side arm. 28°C. Illumination, 900 f. c.

(10 minutes or less) the curves became constant. Illumination was continued. Since the light intensity was above the compensation point this latter period served to remove traces of carbon dioxide in the algal suspension, which had not been completely removed by absorption in the KOH present. The side arm, containing the agent to be studied, was then tipped in and illumination continued for a period. The addition of water caused no change in the oxygen uptake observed in the light. The addition of acetaldehyde or benzaldehyde caused the production of a gas. This gas was not CO₂ (since the KOH would have reabsorbed it). If benzaldehyde is added to an illuminated algal suspension in an atmosphere of nitrogen (under conditions comparable to those of Fig. 1), a gas is evolved. If replicate flasks containing either alkaline pyrogallol or yellow phosphorus in the side arm are employed, no pressure change is found. We therefore conclude that the gas evolved in the first instance is oxygen since it appears to be absorbed by alkaline pyrogallol or yellow
phosphorus. Also, the gas evolved can be detected and measured by the dropping mercury electrode used under the conditions described by Petering et al. (1939) and since this is specific for oxygen (under the conditions employed) there seems to be no need to include data on this point here.

Using this method (as in Fig. 1) we were able to obtain appreciable quantities of oxygen (50–100 μl.) from ferric phosphate and other ferric salts, acetaldehyde, benzaldehyde, parabanic acid, nitro-urea, and sodium carbonate (the latter being a source of CO₂). We were unable to obtain oxygen from the following materials: potassium nitrate, potassium dichromate, p-dimethylaminobenzaldehyde, formaldehyde, butyraldehyde, dimethylglyoxime, cystine, alizarin, quinalizarin, methylene blue, urea, methyl urea, cyuranic acid, allantoin, uracil, xanthine, alloxan, succinate, citrate, fumarate, acetate, lactate, malate, isocitrate, pyruvate, glucose, xylose, arabinose, hexose diphosphate, hexose monophosphate, or phosphogluconic acid. It is of some interest that while parabanic acid and nitro-urea were able to cause oxygen liberation, urea and methyl urea were not. This is probably a reflection of the ring structure of crystalline urea (Werner, 1923) and of the fact that the methyl group of methyl urea is attached to the oxygen rather than to the nitrogens. Parabanic acid and nitro-urea both have free carbonyl groups. It is also evident that the reactions with which we are dealing are not the result of a simple reducing action of illuminated tissue since only a relatively small number of materials are capable of causing oxygen production and a slight change in their structure alters this reaction.

With intact *Chlorella* cells we were unable to use the technique of Hill and Scarisbrick (1940 b) (whereby the ferrous salt formed during the reduction is prevented from rapid reoxidation in the cells by combination with ferricyanide) in order to study the quantitative relationships between the oxygen produced and the ferric salts supplied. The ferricyanide is itself reduced by the algal suspension in light and the resulting complex of reactions so far defies precise analysis. We therefore chose benzaldehyde as an agent for the study of the oxygen-liberating process since it seemed to act in much the same manner as acetaldehyde but was not as volatile. It could also probably be excluded as either a normal intermediate in photosynthesis or a convenient source of carbon dioxide.

Quantitative Relationships between Benzaldehyde Supplied and Oxygen Liberated

The amount of oxygen liberated from a given quantity of benzaldehyde was found to depend upon three primary factors: (1) the length of time the benzaldehyde had been in contact with the algal suspension before the illumination period; (2) the length of the illumination period before the addition of benzaldehyde; (3) the “physiological state” of the algal suspension. (Three “states” could be distinguished as described below.)
The first two factors are illustrated in Fig. 2. A series of replicate flasks were prepared with each containing 1 ml. washed algal suspension (45 mg. dry weight), 1 ml.

**TABLE I**

*Oxygen Production from Benzaldehyde*

<table>
<thead>
<tr>
<th>Flask</th>
<th>Benzaldehyde additions</th>
<th>O₂ produced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time from start</td>
<td>Time in dark</td>
</tr>
<tr>
<td>A</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>B</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>110</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>130</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>190</td>
<td>120</td>
</tr>
</tbody>
</table>

Each flask contains 1 ml. washed algal cell suspension (45 mg. dry weight), 1 ml. M/20 phosphate buffer, pH 7.0; 0.2 ml. 20 per cent KOH in center well, and 0.5 ml. M/50 benzaldehyde in the side arm. At 10 minutes the benzaldehyde was added in flask A, at 40 minutes the benzaldehyde was added in flask B, at 70 minutes the entire series was illuminated, at 100 minutes flask C was tipped, etc., as indicated in the figure. The amount of oxygen produced in each case is given in Table I. It may be
first noted that with replicate aliquots of the same algal suspension it is possible to alter the amount of oxygen obtained from a given quantity of benzaldehyde. First, less oxygen is produced on illumination the longer the time benzaldehyde has been in contact with the algal cells in the dark; e.g., \( Q_{\text{benzaldehyde}} \) in flask A = 1.9, B = 2.2. Second, when benzaldehyde is added in the light the oxygen produced is dependent upon the length of the previous period of illumination; the longer that period, the less oxygen is produced (cf. flasks C, D, E). No oxygen is produced if the benzaldehyde is added in the dark after a long period of illumination (cf. flask F).

Turning to the first effect, it is evident that the decreased oxygen production is not due to the oxidation of benzaldehyde since there is no alteration in the rate of oxygen uptake upon its addition in the dark (cf. flasks A and B). Yet the benzaldehyde has been apparently removed in the dark. A reaction fulfilling such conditions would be a dismutation of the benzaldehyde into benzoic acid and benzyl alcohol. We therefore have studied the possibility of a dismutation of the benzaldehyde as expressed by equation 1.

\[
2\text{C}_6\text{H}_5\text{CHO} \rightarrow \text{C}_6\text{H}_5\text{CH}_2\text{OH} + \text{C}_6\text{H}_5\text{COOH}
\]  

That such a reaction exists in \textit{Chlorella} is evident from Fig. 3. If benzaldehyde be added in the dark to algal cells suspended in bicarbonate, an “extra carbon dioxide” production due to the formation of acid can be observed (Fig. 3B). This cannot be due to the production of carbon dioxide from the benzaldehyde itself since the latter added in the absence of bicarbonate causes no carbon dioxide formation (cf. Fig. 3A). In neither case does the addition of benzaldehyde alter the rate of oxygen uptake, although these rates are not identical in the phosphate or bicarbonate buffers. This, of course, is a reflection of the influence of ionic balance upon the rate of respiration of \textit{Chlorella}. Genevois (1927) has reported that \textit{Chlorella} respire on acetaldehyde with an \( Q \) of 1.3; i.e., excessive carbon dioxide is produced from the acetaldehyde. One can obtain data entirely comparable to those of Fig. 3 using acetaldehyde in place of benzaldehyde. Since Genevois employed bicarbonate buffers throughout his work we feel that the acid production of this type of dismutation was the source of his “extra” carbon dioxide. We have, however, been unable to confirm his observations of a marked increase in oxygen uptake due to the addition of acetaldehyde.

It is evident from Fig. 3B that the amount of carbon dioxide released from the bicarbonate is not stoichiometrically related to the benzaldehyde supplied; i.e., the benzoic acid found is less than that expected from the amount of benzaldehyde added. There must, therefore, be other reactions in addition to the dismutation which remove the benzaldehyde.

The conditions imposed, i.e. that benzaldehyde does not cause an increased respiration when added in the dark, rather severely limit the other types of reactions which can occur. One which has seemed to us probable arises from
an explanation of the second factor observed; i.e., that the amount of oxygen found is dependent upon the length of the previous illumination period. It seemed probable to us that illumination in the absence of carbon dioxide would tend to build up reducing materials in the algal cells (and liberate some oxygen). These reducible materials might be sufficiently stable and sufficiently active to reduce some of the benzaldehyde to benzyl alcohol. This would account for the effects observed in Figs. 2 and 3; but further proof that this type of reaction occurs is not available. We have called it an "internal reduction." However, there is no doubt that reactions exist in Chlorella leading to the disappearance of added benzaldehyde without either increased
respiration in the dark or oxygen production in the light. The lack, then, of
exact stoichiometric relation between benzaldehyde added and oxygen produced
(when it exists, see below) is due to chemical reactions which cause the disap-
pearance of the benzaldehyde from the reacting mixture before it can be used
in oxygen production.

The third factor involved, that of the "physiological state" of the algal cells
is more difficult to control. Using the same species of alga, cultivated on the
same medium under the same conditions and handled throughout in a rather
closely similar manner, we have obtained suspensions having three different
"physiological states." In general, but not invariably, the younger suspen-
sions tend to follow case I while older suspensions tend to follow cases II or III.
Suspensions stored in the refrigerator are almost invariably case III. The
"physiological states" are described below.

Case I.—In these suspensions all of the benzaldehyde added appears as
oxygen in the proportions expressed in equation 2. In these suspensions the

\[
2\text{C}_{6}\text{H}_{5}\text{CHO} + 2\text{H}_{2}\text{O} \rightarrow 2\text{C}_{6}\text{H}_{5}\text{CHOH} + \text{O}_{2}
\]

"internal reduction" or the dismutation reactions described above appear to
be negligible. Upon the addition of benzaldehyde in the dark in the presence
of bicarbonate no appreciable "extra CO\text{2}" is formed. The action of this type
of suspension is illustrated in Fig. 4, which also gives a comparison between
the rate of oxygen production from benzaldehyde and from equivalent quanti-
ties of Na₂CO₃ (this will be discussed later in the section on Rates of oxygen production). In this case the addition of 0.5 ml. of M/50 benzaldehyde (224 μl.) caused the production of 119 μl. of oxygen (112 μl. theoretical, cf. equation 2). Upon illumination for relatively long periods of time before the addition of benzaldehyde the amount of oxygen produced is less ("internal reduction") and storage of such suspensions in the refrigerator for 1 to 2 days will convert them into Case II or Case III types.

Case II.—In certain other suspensions one is able to account for all of the benzaldehyde disappearing by means of the simultaneous action of reactions expressed in equations 1 and 2.

For example, 224 μl. of benzaldehyde (0.5 ml. M/50) when added in the light to such a suspension produced 81.6 μl. of oxygen over a 20 minute period, after which the curve leveled off. When added in the dark in the presence of bicarbonate and 5 per cent CO₂, the same amount of benzaldehyde produced 28.2 μl. of CO₂ in 20 minutes and continued at that rate for almost 60 minutes. From equation 2, the 81.6 μl. of oxygen produced would be equivalent to 163.2 μl. of benzaldehyde added. From equation 1, the 28.2 μl. of CO₂ (= 28.2 μl. of benzoic acid) would be equivalent to 56.4 μl. of the benzaldehyde added.

We have therefore accounted for 219.6 (98 per cent) of the 224 μl. of benzaldehyde originally added. It is of interest that the oxygen produced is proportional, not to the benzoic acid formed, but to that part of the benzaldehyde which did not form benzoic acid.

We have also found that the dismutation reaction (equation 1) is very sensitive to the presence of iodoacetic acid while the oxygen-liberating reactions are not. In the same experiment as described above, in the presence of M/1800 iodoacetate, the addition of 224 μl. of benzaldehyde caused the production of 114 μl. of oxygen (equivalent to 228 μl. of benzaldehyde). All (102 per cent) of the benzaldehyde follows the pathway described by equation 2 in the presence of M/1800 iodoacetate.

Case III.—In still other suspensions (usually older, stored, or excessively illuminated) one is unable to account for all of the benzaldehyde supplied in terms of the reactions expressed in equations 1 and 2. In these cases the addition of iodoacetate (M/600 to M/1200) increases oxygen production about in proportion to that which can be attributed to dismutation but does not enable one to reach stoichiometric quantities. Apparently in these suspensions the benzaldehyde disappears because of the occurrence of both the dismutation and the "internal reduction" reactions.

The conclusion which arises from the experiments outlined above is that the lack of a direct stoichiometric relationship between the oxygen produced and the benzaldehyde added when it occurs is due to the presence of reactions other than that expressed by oxygen production (equation 2) which convert the benzaldehyde into other materials.
Evidence That Carbon Dioxide Is not an Intermediate in the Production of Oxygen from Benzaldehyde

The reaction involving benzaldehyde which is of most interest to us is the one concerned with the production of oxygen (equation 2) rather than the dismutation or the postulated "internal reduction." The fact that oxygen can be produced (sometimes in stoichiometric proportions) when benzaldehyde is added to illuminated Chlorella cells may be interpreted in either of two ways: (1) that the benzaldehyde is reduced by the action of light, carbon dioxide playing no part in this reduction, or (2) that the benzaldehyde serves as a source of carbon dioxide and that what really is being observed is a normal photosynthesis (fixation and reduction of carbon dioxide and oxygen liberation from water). It is of interest that the data of Warburg and Negelein (1920) showed that carbon dioxide was an important intermediate in the production of oxygen from nitric acid.

This is a difficult problem to approach particularly in view of the physiological variation encountered, and the apparent complexity of the pathways by which benzaldehyde reacts without producing oxygen. However, it seemed to us that if carbon dioxide were an intermediate in the production of oxygen from benzaldehyde; if somehow, the latter served as a source of carbon dioxide, it ought to be possible to demonstrate the carbon dioxide.

Of course, the addition of benzaldehyde in the dark did not cause the formation of carbon dioxide (Fig. 3 A). Nevertheless, it might do so in the light. If carbon dioxide were produced from the benzaldehyde, and if conditions were so adjusted that carbon dioxide could escape into the air, then, in the presence of KOH some of this carbon dioxide could be absorbed. Hence one could determine whether the benzaldehyde served as a source of carbon dioxide by determining, quantitatively, the amount of oxygen produced from a given quantity of benzaldehyde in the presence or absence of KOH. In order to work in the absence of KOH, however, one would need to employ a rather long preliminary light period to be certain of consuming all of the carbon dioxide in the system by normal photosynthesis before the benzaldehyde was supplied. Moreover, if carbon dioxide is formed from the benzaldehyde in the light, one would expect less oxygen to be formed in the flasks which contain KOH. The data of Fig. 5 are the result of a study of this possibility. The point which concerns us here is not how much oxygen was liberated but if there was any difference between the amount of oxygen produced from the same quantity of benzaldehyde in the presence or absence of KOH. Obviously at pH 7 in phosphate buffer (parts A and B) there is no essential difference due to the presence of KOH. In citrate (C) at pH 5.8 there is also no difference attributable to the KOH. In all of these cases oxygen was being produced before the addition of benzaldehyde; more oxygen in the presence than in the absence of KOH. In saline (part D) before the addition of benzaldehyde, this situation is reversed.
The origin of the oxygen so produced is not definitely known but presumably it arises from the reduction of reducible materials within the cell. However, in

![Graph](image)

Fig. 5. Oxygen production in the presence and in the absence of KOH. Part A, 1 ml. algal cell suspension (equivalent to 0.06 ml. packed wet cells, suspension 20), 1 ml. M/100 phosphate buffer, pH 7.0, 0.5 ml. M/50 benzaldehyde in side arm; 0.2 ml. 20 per cent KOH in center well. Parts B, C, and D, 1 ml. algal cell suspension (equivalent to 0.06 ml. packed wet cells, suspension 21), 1 ml. 0.6 per cent NaCl, 1 ml. M/100 citrate buffer, pH 5.8, or 1 ml. M/100 phosphate buffer, pH 7.0; otherwise as for Part A. 28°C. Illumination, continued for 90 minutes before the readings recorded in the figure, 600 f. c.

all cases, the addition of benzaldehyde results in no appreciable difference attributable to the KOH.

It might be argued that the previous experiments (Fig. 5) are dependent upon the assumption that the conditions were so adjusted that carbon dioxide, if formed, could escape and that this assumption is not correct. Aside from the fact that at pH 5.5-5.8 the carbon dioxide is all merely in solution and not
in a bound form, and that thus it is in equilibrium with gaseous carbon dioxide (and hence can escape) the data of Table II show that the conditions are such that if carbon dioxide were formed it actually would escape in the sense that added carbonate (whose carbon dioxide pressure is actually zero) has partially escaped into the air at pH 7 (more at pH 5.5) inasmuch as less oxygen is produced from it in the presence than in the absence of KOH (the latter producing the theoretical amount). It should be emphasized that the level of carbon dioxide supplied is such that it is entirely comparable to that presumed to be produced from the benzaldehyde and hence in the latter case there should have been some measurable difference between the presence of KOH and its absence in the form of more oxygen production in the latter case. The only explanation for the results observed (Table II) with the benzaldehyde which is consistent with these facts is that the benzaldehyde did not cause the production of carbon dioxide.

Obviously a stricter test of this technique would be to employ a material which would liberate its carbon dioxide rather slowly. We have, however, been unable to find such a material and attempts to use organic acids (in the hope that they might be decarboxylated) were not successful, in that no oxygen was produced (see preliminary experiments).

We are able to conceive of another possible objection. One could presume that the benzaldehyde liberated carbon dioxide within the cell (only in the light) and that this carbon dioxide did not have time to diffuse out before being used in normal photosynthesis. It is, of course, difficult ever to be sure of the exact nature of reactions which occur in intact cells. One can, therefore, take the position that in this work carbon dioxide has been produced in the inside of the cell (only in the light) upon the addition of benzaldehyde and that its

### Table II

*Influence of the Presence of KOH upon the Production of Oxygen from Benzaldehyde and Carbonate*

<table>
<thead>
<tr>
<th>Benzaldehyde</th>
<th>Carbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ produced from 10 µM benzaldehyde</td>
<td>O₂ produced from 10 µM Na₂CO₃</td>
</tr>
<tr>
<td>pH</td>
<td>+ KOH</td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
</tr>
<tr>
<td>6.8</td>
<td>57.8</td>
</tr>
<tr>
<td>5.8</td>
<td>58.5</td>
</tr>
<tr>
<td>4.6</td>
<td>49.5</td>
</tr>
</tbody>
</table>

2 ml algal cell suspension (0.06 ml packed wet cells) in m/100 phosphate buffer, pH adjusted with HCl by means of Beckman glass electrode; shaking rate, 115 2-cm. strokes/min. 28°C. Illumination, 600 f. c. for 120 minutes before addition of benzaldehyde or carbonate. Figure recorded is microliters of O₂ produced after addition. KOH when present was 0.2 ml 10 per cent. Benzaldehyde: suspension 167, theory, 112 µl. O₂; reaction complete in 20 minutes. Carbonate: suspension 186, theory, 224 µl. O₂ reaction complete in 15 minutes.
use in photosynthesis was such that none of it escaped. We, however, have discarded this possibility on the following grounds. First, there is no positive evidence of such internal carbon dioxide production. Second, in view of the extremely rapid transfer of carbon dioxide between the cell and the medium, the concept of an internal carbon dioxide production leads to the idea that the postulated enzymes liberating carbon dioxide from benzaldehyde and those fixing carbon dioxide must be so arranged that the carbon dioxide does not diffuse in the cell from one to the other, since, from purely kinetic considerations, if there were diffusion inside the cell some of the carbon dioxide should escape and hence be detected in experiments such as those recorded in Table II and in Fig. 5. Third, since the benzaldehyde which follows the light reaction (oxygen liberation) does so in accordance with equation 2, this would mean the production of 1 mole of carbon dioxide per 2 moles of benzaldehyde. We consider it exceedingly improbable that *Chlorella* cells are equipped to carry out what must be a rather complex reaction, particularly with a system operating only in the light which also must be closely adjacent to the carbon dioxide fixation system within the cell that the carbon dioxide is not required to diffuse for any distance through the cell interior. Obviously one cannot experimentally approach this problem since so long as the results are negative (i.e., no evidence of carbon dioxide production), it could be argued that the methods were not sufficient to demonstrate the internal production of carbon dioxide. Positive results must be obtained which, so far, have not been observed. But it seems obvious to us that this argument of internal carbon dioxide production from benzaldehyde in the light but not in the dark necessitates such a questionable group of assumptions that it can be discarded on this ground. This situation appears to us to be a justifiable application of Occam's razor and we therefore take the position that these data represent a direct reduction of benzaldehyde and that they do not mean that benzaldehyde serves as a source of carbon dioxide.

It should also be noted that the proof that carbon dioxide is not produced by the addition of benzaldehyde is independent of any assumptions regarding the carbon dioxide content of the solutions at the time of adding the benzaldehyde. We feel that after 10 to 20 minutes' illumination with KOH (and perhaps slightly longer in its absence) carbon dioxide is absent from the solutions. This is, however, a difficult matter to prove since the respiratory system of *Chlorella* in addition to being insensitive to HCN is not sensitive to high concentrations of acid (pH 1–2) and if acid is added in the light in an effort to release any “bound carbon dioxide,” an oxygen uptake continues for some time thereafter. There is no gas released upon the addition of acid, but the continuing oxygen uptake obscures small amounts that might be released. It is highly improbable that “bound carbon dioxide” would exist after the treatments employed. The acid, of course, would have very little effect upon dissolved carbon dioxide. We believe the system to be free of carbon dioxide,
since there is no carbon dioxide in the gas phase (KOH present) and since the cells have been illuminated for long periods at light intensities far above the compensation point, which should have served to use up traces of carbon dioxide which had escaped absorption by the KOH.

We are therefore forced to conclude that carbon dioxide is not formed by the addition of benzaldehyde in the light, and that the oxygen formed on such addition results from a more or less direct reduction of the benzaldehyde itself. Since oxygen is here produced, and since carbon dioxide is not the material being reduced, this constitutes a liberation of oxygen without carbon dioxide reduction. This, to our minds, constitutes an experimental separation of the reactions liberating oxygen from those concerned with carbon dioxide fixation.

We are, of course, willing to grant that one or more carrier systems may transport the hydrogen released from water to the reducing enzyme acting on the benzaldehyde. We do not regard benzaldehyde as a "normal" intermediate in photosynthesis (i.e., the production of oxygen by carbon dioxide fixation and reduction) but merely as a rather stable carbonyl group which is able to act with enzymes whose normal substrate is some other compound whose nature can only be surmised.

Rate of Oxygen Production from Benzaldehyde

If one examines the rate at which benzaldehyde causes the liberation of oxygen, it will be noted that the highest values obtained approach a $Q_o$ of $+10$ whereas it is possible to obtain $Q_o$'s for oxygen production from carbon dioxide in excess of $+100$. This might lead to the position that since the rate of oxygen production was "low" and since there were admittedly unknown dark reactions in operation, it would be hard to concede that a photoreduction of the benzaldehyde indeed occurred. This position, however, results from the neglect of a very fundamental consideration. $Q_o$'s greater than $+100$ occur at relatively high light intensities, and, which is more important, when the cell is bathed in excess carbon dioxide; i.e., when all of its enzyme systems are saturated with carbon dioxide and its products. Actually a comparison of the rates obtained under these conditions with the rates obtained here is not possible; the conditions of operation are entirely different. The only comparisons of value would be between systems saturated with carbon dioxide ($Q_o = > + 100$) and those saturated with benzaldehyde ($Q_o$, unknown) or between the rate of oxygen production from Na$_2$CO$_3$ (as a source of carbon dioxide) and from benzaldehyde under the same conditions. These latter comparisons (as shown in Fig. 4) show that the benzaldehyde produces oxygen at roughly half the rate of oxygen production from Na$_2$CO$_3$. In this case (Fig. 4) over a period of 15 minutes (maximum rate for carbonate) the benzaldehyde released 48 $\mu$l. of oxygen; the Na$_2$CO$_3$ released 85 $\mu$l. of oxygen; i.e., the rate of oxygen liberation from benzaldehyde was 56 per cent of that liberated from Na$_2$CO$_3$. The values for several experiments range from 45 to 60 per cent.
It has also been suggested that the rate of oxygen production from benzaldehyde should be at least as great as the rate of oxygen production from carbon dioxide. Obviously, however, this is not a necessary condition. The rate of oxygen production depends upon the rate at which the hydrogen produced from water can reach the reducible material. With an “unnatural” material such as benzaldehyde this rate may be any value whatsoever (conditioned by the dissociation constant of the benzaldehyde and the reducing enzyme and the diffusion to that enzyme) and need bear no relation to the rate of oxygen production from carbon dioxide. We wish to emphasize that the effects observed are not small nor is their rate extremely low.

SUMMARY

Using intact cells of Chlorella pyrenoidosa it is possible to obtain oxygen by the reduction of certain reducible materials other than carbon dioxide. Of these, benzaldehyde was studied in some detail. This reduction does not involve the production of carbon dioxide from the benzaldehyde. Stoichiometrical relationships as expressed by the following equation:

\[ 2 \text{C}_2\text{H}_4\text{CHO} + 2\text{H}_2\text{O} \rightarrow 2\text{C}_2\text{H}_5\text{CH}_2\text{OH} + \text{O}_2 \]

are somewhat difficult to obtain because the benzaldehyde can disappear from the reaction mixtures by dark reactions. The technique is now available which permits detailed studies of the oxygen-liberating mechanisms in photosynthesis.

LITERATURE CITED