An Attempted Theory of Photosynthesis

J. Franck and K. F. Herzfeld

very hygroscopic, discrepancy between observed and calculated values is probably due mostly to water.

$\Delta\nu = 372$ (2) $(\pm e)$; 455 (2) $(k, i, \pm e)$; 752 (6) $(k, i, f, \pm e)$; 955 (6) (k, i, e); 1047 (1) (k, i, e); 1173 (3) (k, i, e); 1289 (3) (k, e); 1418 (1) (k); 1455 (5) (k, i, e); 2374 $(\frac{1}{2})$ (k); 2440 (1) (k); 2598 (1) (k); 2828 (4) (k, e); 2930 (5) (k, i, e); 2967 (3) (k); 2991 (3) (k, i, e); 3037 (8) (k, i, e).

14. *Trimethyl amineoxide hydrochloride*, $(CH_3)_3N^+(OH)$ Cl^- (25 percent aqueous solution, $+0.2N$ HCl). Prepared from trimethyl amine and hydrogen peroxide, with subsequent addition of HCl. Four times recryst. from ethyl or methyl alcohol and ether. M.pt. 216–217° (decomp.).

$\Delta\nu = 382$ (3) (e); 500 $(2vb)$ (k, e); 754 (7) (k, f, e); 947 (7) (k, e); 1132 (2) (k, i, e); 1263 $(2b)$ (k, e); 1412 (2) (k, e); 1449 (7) (k, e); 1658 $(1vb)$ (e) (water); 2104 $(1vb)$ (k); 2186 $(1vb)$ (k); 2426 (0) (k); 2597 (2) (k); 2674 $(\frac{1}{2})$ (k); 2811 (3) (k); 2886 (3) (k); 2974 $(10vb)$ (k, i, e); 3043 $(10vb)$ (k, i, e).

15. *Sodium glycinate*, $H_2N \cdot CH_2COO^- \cdot Na^+$ [from Hoffman-LaRoche C.P. glycine twice recrystallized, $+0.97$ equivalent of C.P. NaOH] in water, solution containing 27 percent of glycine.

$\Delta\nu = 518$ (3) (k, e); 904 (5) (k, e); 1113 (2) (k, e); 1354 (2) (k, e); 1409 (4) (k, e); 1444 (1) (k, e); 1602 (0) (e) (water ?); 2953 (2) (k, e); 3332 (2) (k).

16. *Sodium dl-alaninate*, $H_2N \cdot CH(CH_3) \cdot COO^- Na^+$, [from Eastman dl-alanine, three times recrystallized, and 0.98 equivalent C.P. NaOH]. Aqueous solution containing 33 percent of dl-alanine.

$\Delta\nu = 543$ $(3b)$ (f, e); 853 (4) (k, f, e); 926 $(2vb)$ (k, e); 1034 $(\frac{1}{2})$ (e); 1079 (2) (e); 1162 (0) (e); 1305 $(\frac{1}{2})$ (e); 1362 (2) (k, e); 1414 (4) (k, e); 1463 (3) (k, e); 2893 (2) (k); 2946 (4) (k); 2988 (2) (k); 3313 (3) (k).

Both Na glycinate and Na alaninate show indications of a frequency near $K - 3380$, obscured by heavy continuous background.

17. *Glycine*, $^+H_3N \cdot CH_2 \cdot COO^-$ [23 percent solution in water]. (See reference 8.) A new study of the spectrum of this substance revealed the Raman shift 1448 cm^{-1} (1), excited by both the K and E mercury lines. The finding of this line confirms the observations of Wright and Lee, Nature, **136**, 300 (1935).

The presence of the doubtful line at 1491 in glycine, which we had previously reported, could not be confirmed.

TABLE V. *Polarization of lines in the Raman spectra studied.*

"*P*" indicates that a given line in question is strongly polarized (ρ definitely less than 6/7). "*D*" indicates that the line is nearly depolarized ($\rho = 6/7$ or not much less.)

1. *Methyl amine hydrochloride*: 995(P), 1466(D), 1625 $\pm 50(D)$, 2975(P), 3032(D).

2. *Dimethyl amine hydrochloride*: 412(P), 895(P), 1030 (D), 1106(D), 1249(P ?), 1482(D), 2978(P), 3040(D).

3. *Trimethyl amine hydrochloride*: 406(D), 468(P), 821(P), 987(D), 1067(?), 1248(D), 1464(D), 2894(P), 2969(P), 3030(D).

4. *Tetramethyl ammonium chloride*: 372(D), 455(D), 752(P), 955(D), 1455(D), 2930(P), 2967(P), 2991(P), 3037(D).

The line at 752 cm^{-1} in tetramethyl ammonium chloride is very highly polarized (ρ approaching zero). The lines near 2980 cm^{-1} in all four compounds also are very strongly polarized.

An Attempted Theory of Photosynthesis

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An attempt is made to explain quantitatively many observations described in the literature on the photosynthetic production of oxygen in its dependence on light intensity, time of irradiation, etc. Four photochemical steps and two dark reactions are assumed, in which among others, a peracid, formic acid and a peraldehyde occur. These are the same intermediate compounds as in auto-oxydation processes, so that the similarity between these two inverse processes is striking. Light saturation is explained by back chain reactions initiated by photolytical decomposition of the per-compounds. The agreement between observations and calculations is good. The picture gained for the photosynthesis of CO_2 can be applied in the same way for that of plant acids but the plant acids can also be photooxidized in a reaction sensitized by chlorophyll.

ABOUT a year ago one of the authors[1] published an attempt to bring the theory of photosynthesis of Willstätter and Stoll[2] into agreement with the results of modern atomic physics, especially in the field of fluorescence and spectroscopy. It was shown that this was possible in principle. One preparatory photochemical reaction, followed by the four main photochemical reactions of photosynthesis, was assumed.

Since the publication of this paper, however, it has been shown by several authors that the application of these equations presents difficul-

[1] J. Franck. Chem. Rev. **17**, 443 (1935).
[2] R. Willstätter, Naturwiss. **21**, 252 (1933); A. Stoll, ibid **20**, 955 (1932); R. Willstätter and A. Stoll, *Chlorophyllbuch* (Bayr. Akad).

ties. Gaffron[3] has proved in particular that there is no necessity for the assumption of a preparatory reaction, namely the dehydrogenation of chlorophyll under the influence of oxygen and light, before photosynthesis can start. He could explain the necessity for the presence of oxygen in a much more plausible way. This point of view was therefore accepted in a paper on the fluorescence of chlorophyll by Franck and R. W. Wood.[4]

The main criticism which Gaffron and Wohl[5] level against the four proposed photochemical reactions is that the absorption of four quanta of red light supplies an energy which is ~ 20 kilocalories smaller than the heat of reaction necessary to form one molecule of formaldehyde and two of hydrogen peroxide from one of carbon dioxide and three of water. But even if one accepts these figures, they do not prove the impossibility of the original assumptions. According to the present authors,[6] one can gain sufficient energy from thermal vibrations to compensate the difference in the case of photochemical processes in polyatomic molecules, provided they show fluorescence, as is the case here. Nevertheless, it remains doubtful whether the intermediate products would be stable enough to avoid considerable back reaction, particularly for weak radiation.

Therefore, it seemed worthwhile to change the assumptions. One might question whether one should try to write specific photochemical reactions in view of our present relatively small knowledge of photosynthesis, but firstly such discussions lead to new experiments, and secondly it seems important to see whether it is possible to account for this biological problem without deviating from the usual laws of physics and chemistry.

In photosynthesis, most of the recent investigators[7] have concluded this to be impossible. They agree with Arnold, that the experiments in plants make the assumption of a photosynthetic unit necessary, in which a large number of molecules cooperate in a way not encountered in vitro. The present authors intend to show here that the assumption of such units is unnecessary and that a picture for photosynthesis can be given, into which all experiments hitherto published fit and which makes use only of the experience gained in other photochemical reactions. To do this, we shall discuss first the experiments which seem to make the "photosynthetic unit" necessary.

The name unit is given to a group of about 1000 chlorophyll molecules connected with one carbon dioxide molecule. The chlorophyll molecules of the unit are supposed to absorb light practically independently of each other, but the energy so absorbed by the whole unit is supposed to be available for the reduction of the one carbon dioxide molecule connected with it. The hypothesis was introduced to explain three groups of experiments.

Firstly, no induction period for the production of oxygen is observed for small light intensities.[8] If at the start of the illumination every individual chlorophyll molecule were connected with one carbon dioxide molecule and if four quanta would be necessary before the liberation of oxygen, the length of the induction period would be equal to the average time it takes for a chlorophyll molecule to absorb four quanta under the conditions of the experiment. This time is so long that the induction period would have been found. If on the other hand, it is only necessary for the whole unit of 1000 chlorophyll molecules to absorb four quanta, this time becomes 1000 times shorter and would be difficult to detect.

Secondly the unit explains the fact that the so-called light saturation occurs, in the presence of much CO_2, with a light intensity[9] much smaller than expected. The saturation has hitherto been explained by the occurrence of a dark reaction (Blackman reaction) in addition to the photochemical reactions in the course of photosynthesis. With intensity sufficiently high, the dark reaction would become the slowest

[3] H. Gaffron, Naturwiss. **23**, 528 (1935).
[4] J. Franck and R. W. Wood, J. Chem. Phys. **4**, 551 (1936).
[5] H. Gaffron und K. Wohl, Naturwiss. **24**, 81 (1936).
[6] J. Franck and K. F. Herzfeld, J. Phys. Chem. **41**, 97 (1927).
[7] R. Emerson and W. Arnold, J. Gen. Physiol. **16**, 191 (1932); **15**, 391 (1931); W. Arnold and H. Kohn, ibid., **18**, 109 (1934); H. Gaffron and K. Wohl, reference 5; H. J. Kohn, Nature **137**, 706 (1936); J. Weiss, Nature **137**, 997 (1936) and **138**, 80 (1936).

[8] O. Warburg, *Über die katalyt. Wirkungen der leb. Substanz.* (Berlin, 1928).
[9] R. Emerson and W. Arnold, reference 7. More literature, see in Emerson's review, "Ergebnisse der Enzymforschung" **5**, 305 (1936).

reaction in the sequence and therefore limit the whole sequence to its own speed. The intensity at which half the saturation yield is produced would correspond to the condition that the dark reaction and the light reaction have the same speed. As the velocity of the dark reaction is known (see next paragraph) to be about 50 sec.$^{-1}$, the velocity constant of the light reaction can be calculated. It turns out so large that it again necessitates the absorption of four quanta by a unit of 1000 chlorophyll molecules for the reduction of one carbon dioxide molecule.

The third argument for the unit is independent of the assumption that four quanta have to be absorbed by any particular chlorophyll molecule to reduce one CO_2 molecule. It is based on Emerson and Arnold's[7] investigations of photosynthesis with light flashes of very short duration. The dark pauses between flashes, if long enough, allow the completion of the dark reaction. By changing these intervals, Emerson and Arnold measured the duration of the dark reaction. Changing the intensity of the light flashes, they observed a saturation for the individual flashes. The total energy absorbed per second, is, under these conditions, much smaller than the one necessary to produce saturation for continuous illumination. Arnold explains this by the assumption that all the carbon dioxide which is, at the beginning of the flash, combined with chlorophyll, is reduced by one flash and the subsequent dark reaction. The comparison between the amount of oxygen developed per flash and the amount of chlorophyll gives again about 1000 times as many chlorophyll molecules as carbon dioxide.

The agreement in the size of the unit, arrived at in the three ways (the numbers vary from 500 to 2600) has convinced most authors of the necessity of the hypothesis. The main objections are the following: Chlorophyll solutions emit fluorescent light if the chlorophyll is in true solution. Polymerization of the chlorophyll into colloidal particles made up entirely of chlorophyll destroys the fluorescence. In the plant, fluorescence occurs just as in true solutions, therefore the chlorophyll in the plant must be in molecular dispersion. We assume, however, that it is adsorbed on the surface of colloidal particles of a different material (chloroplasts). This is in agree-

ment with the usual opinion that the chlorophyll in the plant has a colloidal distribution. The observations on fluorescence show that the individual chlorophyll molecules are separated.

In all organic (true) solutions of chlorophyll, the yield of the fluorescence is rather small.[10] This must be interpreted by the high probability of a chemical reaction of the excited chlorophyll molecule, either a dissociation of the molecule itself or some reaction with the organic solvent. Therefore, if 1000 chlorophyll molecules are coupled closely in a unit and one is excited, the major part of the absorbed energy would not be used for the reduction of CO_2, but for reactions between the chlorophyll molecules themselves. It might be added that under suitable illumination organic solutions of chlorophyll containing oxygen show the same type of change with time in the intensity of fluorescence as was found by Kautsky for leaves. Only the time scale is different.[11] This also indicates the same kind of state, i.e., molecular dispersion.

Therefore, we prefer to explain the experiments in a different way. We will assume that the saturation is due to back reactions induced by light and proceeding in chains.

Warburg's experiments show that four quanta of red light reduce carbonic acid to the state of reduction of an aldehyde and that probably two peroxide molecules are formed, which, under the action of an enzyme, split off oxygen. The energy relations mentioned above make it improbable that H_2O_2 is the peroxide. The same is true of formic peracid[12] and formic peraldehyde, since it is known that at least the former will react with water to give formic acid and hydrogen peroxide with a negligible heat of reaction. Therefore formic peracid needs about the same energy as hydrogen peroxide.

If a peroxide is formed which needs 5–10 kcal. less than hydrogen peroxide, energy considerations present no difficulties.

The lack of an induction period at low light intensities shows from our point of view that the peroxide is formed in the plant upon the absorption of the first quantum. Therefore, the energy

[10] J. A. Prins, Nature **134**, 457 (1934).
[11] J. Franck and H. Levi, Zeits. f. physik. Chemie **B27**, 409 (1935).
[12] See for instance, K. Wohl, Zeits. f. physik. Chemie **B31**, 152 (1935).

to transform carbonic acid into this peroxide cannot be larger than ~43 kcal. These considerations lead us to the following picture. We assume that in the plant the chlorophyll carbonic acid complex is attached to an organic molecule ROH present in abundance, in which the strength of the OH bond to R has the usual value of ~70 kcal. The ROH may for instance be a protein which forms the main body of the chloroplasts, on the surface of which the chlorophyll is adsorbed. The chlorophyll molecules will then be able to move along the surface as a two-dimensional gas (Vollmer). Using the data for the formation of performic acid from carbonic acid and the usual values for the C—H bonds and C—R bonds, one gets the energy necessary for

the formation of the peracid
$$\begin{array}{cc} HO & R \\ \backslash & / \\ & C—O—OH \\ / \\ HO \end{array}$$
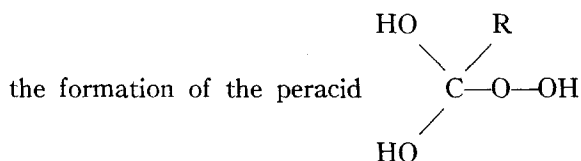
from
$$\begin{array}{c} HO \\ \backslash \\ C=O \\ / \\ HO \end{array}$$
and ROH. This turns out to be

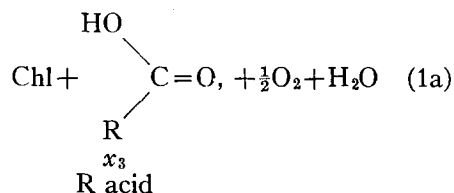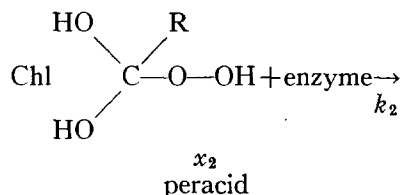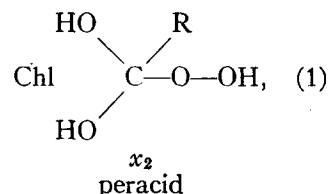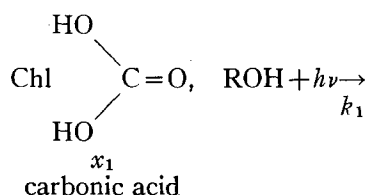a little smaller than the energy available through the first absorption act.[13] In close analogy to the first photochemical reaction, the third one is then the transformation of formic acid into the

peraldehyde
$$\begin{array}{cc} HO & R \\ \backslash & / \\ & C—O—OH \\ / \\ H \end{array}$$
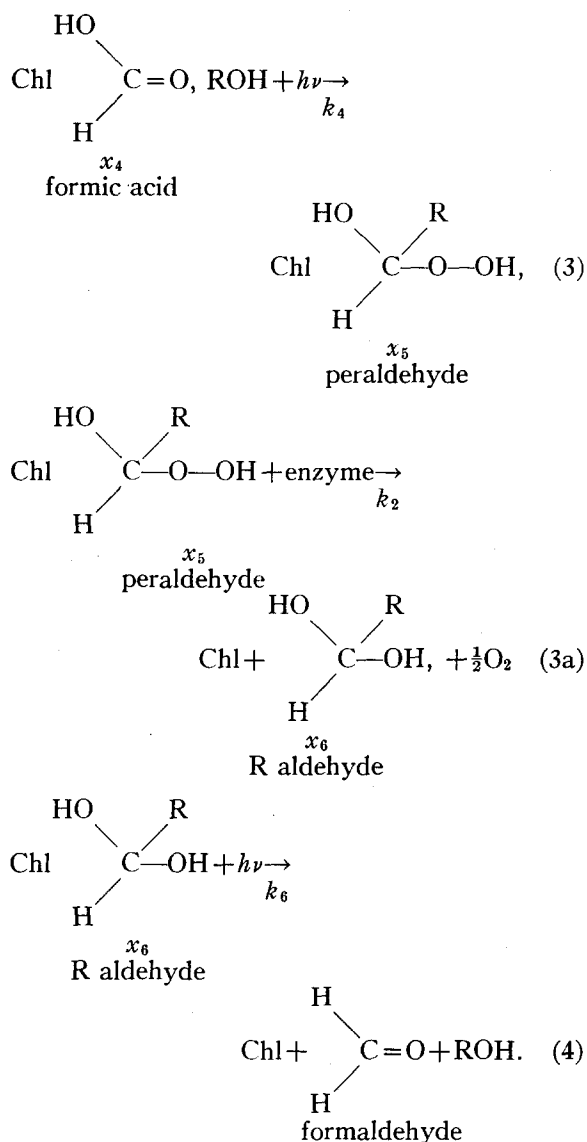Reactions one
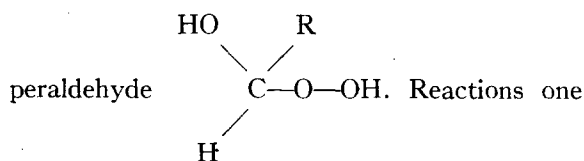
and three are followed by dark reactions in which the peracid or the peraldehyde is reduced under the influence of enzymes to the acid and the aldehyde. Reactions two and four transform

[13] We take $h\nu = 6700A \sim 43$ kcal. as the energy available for one absorption act, because $\lambda 6700A$ is the long wavelength limit of strong absorption in the chlorophyll. Ranging from 6700A toward greater wave-lengths the absorption band has a weak tail caused by the presence in the thermal distribution of a small proportion of molecules with vibration energies up to a few tenths of a volt. Absorption of these greater wave-lengths leads to the same excited state because of the contribution from thermal energy. Consequently this extra amount of thermal energy is used in addition to light energy when photosynthesis is performed with light $\lambda > 6700A$, but the absorption is this spectral region will not become appreciable unless very thick layers of chlorophyll are used.

the acid and the aldehyde into formic acid and formaldehyde and restore the catalyst ROH. So far as our knowledge of the heats of reactions which occur in these equations goes, no difficulties are encountered. At least in the first two photochemical steps, there are even available small amounts of excess energy, so that some activation energy to prevent back reaction might be present. In the following equations, concentrations x, names and constants of reaction velocities k have been written under the corresponding chemical formulas. The symbol Chl is an abbreviation for chlorophyll.

$$\begin{array}{c} HO \\ \backslash \\ Chl \quad C=O, \quad ROH+h\nu \rightarrow \\ / \qquad\qquad\qquad k_1 \\ HO \\ x_1 \\ \text{carbonic acid} \end{array}$$

$$\begin{array}{c} HO \quad R \\ \backslash \; / \\ Chl \quad C—O—OH, \quad (1) \\ / \\ HO \\ x_2 \\ \text{peracid} \end{array}$$

$$\begin{array}{c} HO \quad R \\ \backslash \; / \\ Chl \quad C—O—OH+\text{enzyme}\rightarrow \\ / \qqu\qquad\qquad\qquad k_2 \\ HO \\ x_2 \\ \text{peracid} \end{array}$$

$$\begin{array}{c} HO \\ \backslash \\ Chl+ \quad C=O, \; +\frac{1}{2}O_2+H_2O \quad (1a) \\ / \\ R \\ x_3 \\ \text{R acid} \end{array}$$

$$\begin{array}{c} HO \\ \backslash \\ Chl \quad C=O+H_2O+h\nu\rightarrow \\ / \qquad\qquad\qquad k_3 \\ R \\ x_3 \\ \text{R acid} \end{array}$$

$$\begin{array}{c} HO \\ \backslash \\ Chl \quad C=O, \; ROH, \quad (2) \\ / \\ H \\ x_4 \\ \text{formic acid} \end{array}$$

$$\underset{\substack{x_4 \\ \text{formic acid}}}{\overset{\text{HO}}{\underset{\text{H}}{\diagup}}\text{Chl}\diagdown\text{C}=\text{O}}, \text{ROH}+h\nu\underset{k_4}{\rightarrow}$$

$$\underset{\substack{x_5 \\ \text{peraldehyde}}}{\text{Chl}\overset{\text{HO}}{\underset{\text{H}}{\diagup}}\text{C}\diagdown\overset{R}{\diagup}\text{O}\text{—O—OH}}, \quad (3)$$

$$\underset{\substack{x_5 \\ \text{peraldehyde}}}{\text{Chl}\overset{\text{HO}}{\underset{\text{H}}{\diagup}}\text{C}\diagdown\overset{R}{\diagup}\text{—O—OH}}+\text{enzyme}\underset{k_2}{\rightarrow}$$

$$\underset{\substack{x_6 \\ R\text{ aldehyde}}}{\text{Chl}+\overset{\text{HO}}{\underset{\text{H}}{\diagup}}\text{C}\diagdown\overset{R}{\diagup}\text{—OH}}, +\tfrac{1}{2}\text{O}_2 \quad (3a)$$

$$\underset{\substack{x_6 \\ R\text{ aldehyde}}}{\text{Chl}\overset{\text{HO}}{\underset{\text{H}}{\diagup}}\text{C}\diagdown\overset{R}{\diagup}\text{—OH}}+h\nu\underset{k_6}{\rightarrow}$$

$$\underset{\text{formaldehyde}}{\text{Chl}+\overset{\text{H}}{\underset{\text{H}}{\diagup}}\text{C}=\text{O}+\text{ROH}.} \quad (4)$$

One could change Eq. (4) so that it would give glycolaldehyde instead of formaldehyde as end product, but a discussion of this question does not seem justified at the present stage. We do not suppose that the preceding equations represent any final solution, but they explain so many facts that they are at least a good working hypothesis;[14]

[14] Following the ideas of Weiss, reference 7, one might try to replace the catalyst ROH used in the preceding equations by Ferri ions. But not enough iron is present in the plant to provide each chlorophyll molecule with an Fe ion. Moreover, Warburg[8] has shown that while the Blackman reaction (and photosynthesis at high light intensities) is influenced by cyanide in a way typical for a Fe catalysis, photosynthesis at low intensities is not affected. He concludes therefore that Fe catalysis does not play any role in the purely photochemical part of photosynthesis.

the assumption that the peroxides[15] mentioned above are intermediate products of photosynthesis makes the photoreduction of carbonic acid and formic acid entirely analogous to the reverse processes of autoxydation of aldehydes to formic acid and carbonic acid, as observed in vitro. The photochemical equations thus gained for photosynthesis can be applied in the same way to the photosynthesis of plant acids formed as intermediate states of respiration. The only difference between these higher acids on the one hand, and carbonic acid on the other, is that the latter will only undergo photosynthesis, while the former will also be photoxidized under suitable conditions. This photooxidation provides for the explanation of the Kautsky[16] curves of fluorescence and the induction period,[8] both observed only with strong illumination in the presence of oxygen.
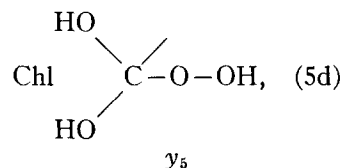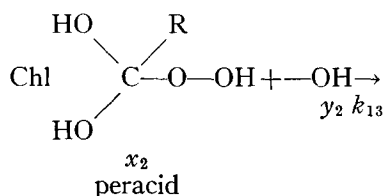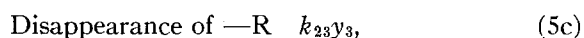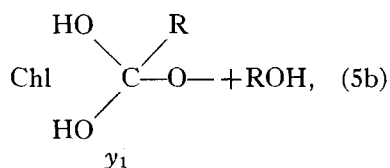
It is particularly important that the binding energy of —OH to —O— in both the peroxides used above is so weak (<40 kcal. according to calculations from the heat of formation), that the radical —OH will be split off whenever the complex Chl peracid or Chl peraldehyde absorbs a quantum of light. This fact gives an explanation for the shape and the constants of the saturation curves, both for continuous and flashing light. The probability of such an absorption is negligible with weak illumination because the average time between two absorption acts of a chlorophyll molecule is much greater than the time within which the enzyme reduces the permolecule. But if the light intensity is increased, this probability becomes greater. It is about 1/10 for an intensity that gives almost saturation in normal leaves. (This value is calculated from the velocity of the Blackman reaction, the number of light quanta impinging per second, and the number of chlorophyll mo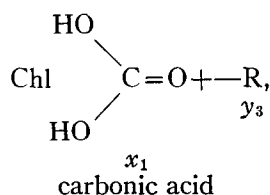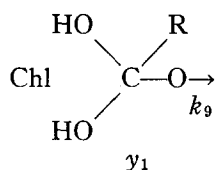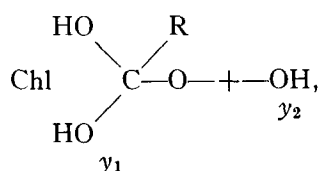lecules in the leaf.) The photodecomposition of the permolecule into two radicals will then start chain reactions in the usual way. In our case, these *chain reactions are back reactions* of the peracid to carbonic acid and ROH, and of

[15] Compare R. Willstätter and A. Stoll, reference 2.
[16] H. Kautski and co-workers, Naturwiss 19, 964 (1931); 19, 1043 (1931), and 24, 317 (1936). Ber. d. Dtsch. Chem. Ges. 65, 1762 (1932); 66, 1588 (1933) and 68, 152 (1935). Biochem. Zeits. 274, 423 (1934); 278, 373 (1935) and 284, 412 (1936).

the peraldehyde to formic acid and ROH. The following equations describe tentatively these chains.

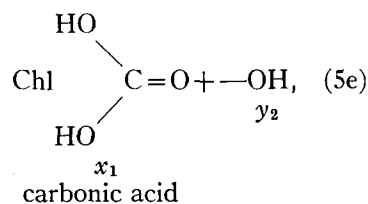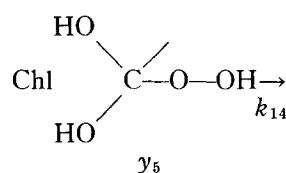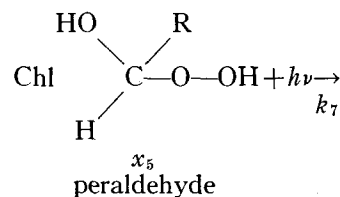There are two photochemical reactions (5) and (6) both splitting off the weakly bound OH, one for the peracid, one for the peraldehyde. In each case there are two possibilities for chains, one with —R(Eqs. (a) and (b)), one with —OH (Eqs. (d) and (e)) and there are two reactions terminating the chain ((c) and (f)). We write again concentrations (y for the radicals) and velocity constants below the chemical equations
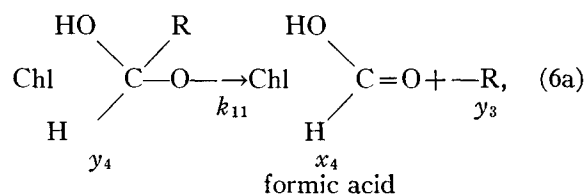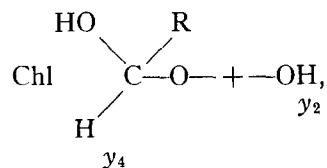
$$
\begin{array}{c}
\text{HO} \qquad \text{R} \\
\diagdown \quad \diagup \\
\text{Chl} \quad \text{C—O—OH} + h\nu \rightarrow \\
\diagup \qquad\qquad k_7 \\
\text{HO} \\
x_2 \\
\text{peracid}
\end{array}
\tag{5}
$$

$$
\begin{array}{c}
\text{HO} \qquad \text{R} \\
\diagdown \quad \diagup \\
\text{Chl} \quad \text{C—O—+—OH,} \\
\diagup \qquad\qquad y_2 \\
\text{HO} \\
y_1
\end{array}
$$

$$
\begin{array}{c}
\text{HO} \qquad \text{R} \\
\diagdown \quad \diagup \\
\text{Chl} \quad \text{C—O} \rightarrow \\
\diagup \qquad k_9 \\
\text{HO} \\
y_1
\end{array}
\tag{5a}
$$

$$
\begin{array}{c}
\text{HO} \\
\diagdown \\
\text{Chl} \quad \text{C=O+—R,} \\
\diagup \qquad\quad y_3 \\
\text{HO} \\
x_1 \\
\text{carbonic acid}
\end{array}
$$

$$
\begin{array}{c}
\text{HO} \qquad \text{R} \\
\diagdown \quad \diagup \\
\text{Chl} \quad \text{C—O—OH} + \text{—R} \rightarrow \\
\diagup \qquad\qquad y_3\, k_{10} \\
\text{HO} \\
x_2 \\
\text{peracid}
\end{array}
$$

$$
\begin{array}{c}
\text{HO} \qquad \text{R} \\
\diagdown \quad \diagup \\
\text{Chl} \quad \text{C—O—+ROH,} \\
\diagup \\
\text{HO} \\
y_1
\end{array}
\tag{5b}
$$

Disappearance of —R $k_{23}y_3$, $\qquad$ (5c)

$$
\begin{array}{c}
\text{HO} \qquad \text{R} \\
\diagdown \quad \diagup \\
\text{Chl} \quad \text{C—O—OH} + \text{—OH} \rightarrow \\
\diagup \qquad\qquad y_2\, k_{13} \\
\text{HO} \\
x_2 \\
\text{peracid}
\end{array}
$$

$$
\begin{array}{c}
\text{HO} \\
\diagdown \quad \diagup \\
\text{Chl} \quad \text{C—O—OH,} \\
\diagup \\
\text{HO} \\
y_5
\end{array}
\tag{5d}
$$

$$
\begin{array}{c}
\text{HO} \\
\diagdown \quad \diagup \\
\text{Chl} \quad \text{C—O—OH} \rightarrow \\
\diagup \qquad k_{14} \\
\text{HO} \\
y_5
\end{array}
$$

$$
\begin{array}{c}
\text{HO} \\
\diagdown \\
\text{Chl} \quad \text{C=O+—OH,} \\
\diagup \qquad\quad y_2 \\
\text{HO} \\
x_1 \\
\text{carbonic acid}
\end{array}
\tag{5e}
$$

Disappearance of —OH $k_{22}y_2$, $\qquad$ (5f)

$$
\begin{array}{c}
\text{HO} \qquad \text{R} \\
\diagdown \quad \diagup \\
\text{Chl} \quad \text{C—O—OH} + h\nu \rightarrow \\
\diagup \qquad\qquad k_7 \\
\text{H} \\
x_5 \\
\text{peraldehyde}
\end{array}
\tag{6}
$$

$$
\begin{array}{c}
\text{HO} \qquad \text{R} \\
\diagdown \quad \diagup \\
\text{Chl} \quad \text{C—O—+—OH,} \\
\diagup \qquad\qquad y_2 \\
\text{H} \\
y_4
\end{array}
$$

$$
\begin{array}{cc}
\text{HO} \quad \text{R} & \text{HO} \\
\diagdown \ \diagup & \diagdown \\
\text{Chl} \ \text{C—O—} \rightarrow \text{Chl} & \text{C=O+—R,} \\
\diagup \qquad k_{11} & \diagup \qquad\quad y_3 \\
\text{H} & \text{H} \\
y_4 & x_4 \\
& \text{formic acid}
\end{array}
\tag{6a}
$$

$$\begin{array}{c} \text{HO} \quad \text{R} \\ \backslash \quad / \\ \text{Chl} \quad \text{C—O—OH} + \text{—R} \rightarrow \\ / \qquad\qquad\qquad y_3 \, k_{12} \\ \text{H} \\ x_5 \end{array}$$

peraldehyde

$$\begin{array}{c} \text{HO} \quad \text{R} \\ \backslash \quad / \\ \text{Chl} \quad \text{C—O—} + \text{ROH}, \quad (6b) \\ / \\ \text{H} \\ y_4 \end{array}$$

$$(6c) = (5c)$$

$$\begin{array}{c} \text{HO} \quad \text{R} \\ \backslash \quad / \\ \text{Chl} \quad \text{C—O—OH} + \text{—OH} \rightarrow \\ / \qquad\qquad\qquad y_2 \, k_{15} \\ \text{H} \\ x_5 \end{array}$$

peraldehyde

$$\begin{array}{c} \text{HO} \\ \backslash \quad / \\ \text{Chl} \quad \text{C—O—OH} + \text{ROH}, \quad (6d) \\ / \\ \text{HO} \\ y_6 \end{array}$$

$$\begin{array}{c} \text{HO} \\ \backslash \quad / \\ \text{Chl} \quad \text{C—O—OH} \rightarrow \\ / \qquad\qquad k_{16} \\ \text{HO} \\ y_6 \end{array}$$

$$\begin{array}{c} \text{HO} \\ \backslash \\ \text{Chl} \quad \text{C} = \text{O} + \text{—OH}, \quad (6e) \\ / \qquad\qquad\quad y_2 \\ \text{H} \\ x_4 \end{array}$$
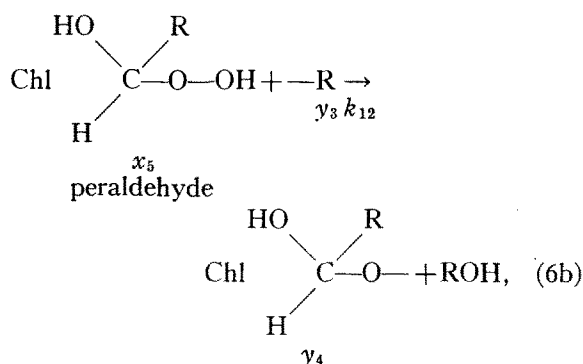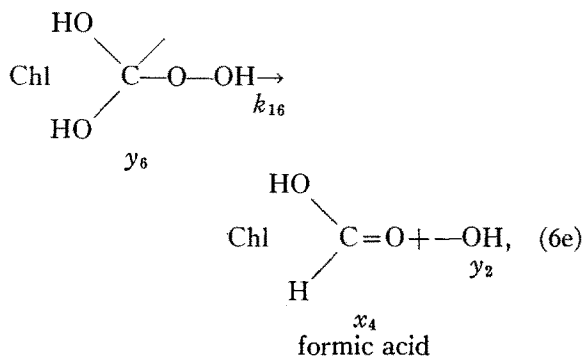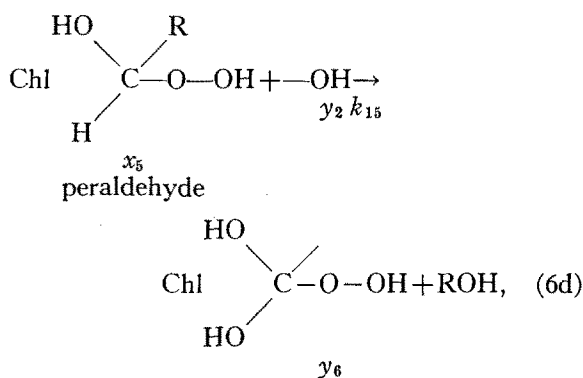
formic acid

$$(6f) = (5f)$$

Differential Equations for the Reactions

We write the equations for the different compounds which occur in the course of photosynthesis (concentrations x_2 to x_6) and of the radicals occurring in the chains (concentrations y_1 to y_6). It should be remembered that x_2 and x_5 are the concentrations of the peracid and peraldehyde respectively, and that k_1, k_3, k_4, k_6, k_7 are proportional to the light intensity I and as the absorption occurs in the chlorophyll, all equal, namely equal to the rate of light absorption in chlorophyll.

$$dx_2/dt = k_1 x_1 - k_2 x_2 - k_7 x_2 - k_{10} x_2 y_3 - k_{13} x_2 y_2, \quad (7)$$

$$dx_3/dt = k_2 x_2 - k_3 x_3, \quad (8)$$

$$dx_4/dt = k_3 x_3 - k_4 x_4 + k_{11} y_4 + k_{16} y_6, \quad (9)$$

$$dx_5/dt = k_4 x_4 - k_2 x_5 - k_7 x_5 - k_{12} y_3 x_5 - k_{15} y_2 x_5, \quad (10)$$

$$dx_6/dt = k_2 x_5 - k_6 x_6, \quad (11)$$

$$dy_1/dt = k_7 x_2 - k_9 y_1 + k_{10} x_2 y_3, \quad (12)$$

$$dy_2/dt = k_7(x_2 + x_5) - k_{13} y_2 x_2 + k_{14} y_5 \\ - k_{15} y_2 x_5 + k_{16} y_6 - k_{22} y_2, \quad (13)$$

$$dy_3/dt = k_9 y_1 - k_{10} x_2 y_3 + k_{11} y_4 \\ - k_{12} y_3 x_5 - k_{23} y_3, \quad (14)$$

$$dy_4/dt = k_7 x_5 - k_{11} y_4 + k_{12} x_5 y_3, \quad (15)$$

$$dy_5/dt = k_{13} x_2 y_2 - k_{14} y_5, \quad (16)$$

$$dy_6/dt = k_{15} y_2 x_5 - k_{16} y_6. \quad (17)$$

In these equations we have assumed the chains to be terminated by the first order disappearance of the radicals OH and R, $-k_{22} y_2$ and $-k_{23} y_3$. It will be shown afterwards that a first order reaction is necessary to give saturation. That y_2 and y_3 alone have been chosen is due to the fact that the other radicals y_1, y_5, y_4, y_6 are very unstable so that the amount of R— and —OH is much greater than that of the large radicals and so the overwhelming number of chains is terminated by reactions taking R and OH out of the chain.

Adding, on the one hand (12), (14) and (15), on the other (13), (16) and (17), finally (8), (9), (10), (15) and (17) gives

$$(d/dt)(y_1 + y_3 + y_4) = k_7(x_2 + x_5) - k_{23} y_3, \quad (18)$$

$$(d/dt)(y_2 + y_5 + y_6) = k_7(x_2 + x_5) - k_{22} y_2, \quad (19)$$

$$(d/dt)(x_3 + x_4 + x_5 + y_4 + y_6) = k_2(x_2 - x_5). \quad (20)$$

Furthermore, if N is the total number of chlorophyll molecules taking part in photosynthesis, we have

$$N = x_1 + x_2 + x_3 + x_4 + x_5 + x_6 \quad (21)$$

the number of radicals being too small to count.

The Chain Equations

With stationary illumination, all the left sides of Eqs. (7) to (20) are zero. Therefore, (18) and

(19) determine the concentration of the R— and —OH radicals. Eqs. (18) and (19) say, of course, that the stationary state for the concentrations of the radicals is reached if for every percompound dissociated by light one R— and one —OH are destroyed by the chain-ending reactions. If we put these results into Eqs. (12), (15), (16), (17) equated to zero, we find the following equations for the radicals

$$y_2 = (k_7/k_{22})(x_2+x_5), \tag{22}$$

$$y_3 = (k_7/k_{23})(x_2+x_5), \tag{23}$$

$$y_5 = \frac{k_{13}}{k_{14}}x_2 y_2 = \frac{k_{13}}{k_{14}}\frac{k_7}{k_{22}}x_2(x_2+x_5), \tag{24}$$

$$y_1 = \frac{1}{k_9}(k_7 x_2 + k_{10}x_2 y_3)$$

$$= \frac{k_7}{k_9}x_2\left\{1+\frac{k_{10}}{k_{23}}(x_2+x_5)\right\}, \tag{25}$$

$$y_4 = \frac{1}{k_{11}}(k_7 x_5 + k_{12}x_5 y_3)$$

$$= \frac{k_7}{k_{11}}x_5\left\{1+\frac{k_{12}}{k_{23}}(x_2+x_5)\right\}, \tag{26}$$

$$y_6 = \frac{k_{15}}{k_{16}}y_2 x_5 = \frac{k_7}{k_{22}}\frac{k_{15}}{k_{16}}x_5(x_2+x_5). \tag{27}$$

We abbreviate

$$Z = k_{10}/k_{23} + k_{13}/k_{22}, \tag{28}$$

$$Z' = k_{15}/k_{22} + k_{12}/k_{23}, \tag{28'}$$

We now substitute the values found for the radical concentrations back into (7) to (11)

$$dx_2/dt = k_1 x_1 - x_2\{k_2+k_7+k_7 Z(x_2+x_5)\}, \tag{7'}$$

$$dx_3/dt = k_2 x_2 - k_3 x_3, \tag{8'}$$

$$dx_4/dt = k_3 x_3 - k_4 x_4 + k_7 x_5\{1+Z'(x_2+x_5)\}, \tag{9'}$$

$$dx_5/dt = k_4 x_4 - x_5\{k_2+k_7+k_7 Z'(x_2+x_5)\}, \tag{10'}$$

$$dx_6/dt = k_2 x_5 - k_6 x_6. \tag{11'}$$

We name now two assumptions, which are in no way necessary to our physicochemical considerations, but lighten the mathematical task so far as the flash illumination is concerned.

Firstly, we assume

$$Z = Z', \tag{28''}$$

which means that the speed of reaction of the peracid with the radicals R and OH is the same as that of the peraldehyde with the same radicals, which seems plausible.

Secondly, we assume that the duration of the chains is short even compared with the duration of a flash (10^{-5} sec.) so that the concentrations of the other molecules have not changed appreciably during the time between the starting and the ending of one chain. In this case, formulas (22) to (27) for the concentrations of the radicals, which have been calculated for constant concentration, can still be used with concentrations of the molecules x_2, x_5 varying with time, the variation during the life of one chain being assumed small.

Under these assumptions, one can talk uniformly about both continuous and flash illumination.

As the number of chains starting per second is given by $k_7 x_2$ and $k_7 x_5$, and the total number of chain reactions $k_7 Z(x_2+x_5)x_2$ and $k_7 Z(x_2+x_5)x_5$ (see 7', 9', 10'), it follows that the chain length is $Z(x_2+x_5)$.

CONTINUOUS ILLUMINATION

Then, in the steady state, the left side of Eqs. (7') to (11') and (20) are zero. Taking into account the equality of all the light reaction constants, one finds

$$x_2 = x_5, \quad x_3 = x_6 = k_2 x_2/k_1, \quad x_4 = x_1, \tag{29}$$

$$k_1 x_1 = x_2\{k_2+k_1+2Z k_1 x_2\}, \tag{30}$$

$$N = 2x_1 + 2x_2 + 2(k_2 x_2/k_1), \tag{21'}$$

or $$(N/4)k_1 = x_2\{k_2+k_1+Z k_1 x_2\}. \tag{31}$$

If we call the number of oxygen molecules developed per second V, we have

$$V = \tfrac{1}{2}k_2(x_2+x_5) = k_2 x_2 \tag{32}$$

or $$\frac{N}{4}k_1 = V + Z\frac{k_1}{k_2^2}V^2 \tag{31'}$$

k_1 can always be neglected[17] next to either k_2 or

---

[17] If the intensity is weak, $k_1 < k_2$. If the light is strong $k_1 < k_2 Z(x_2+x_5)$.

Zk_1x_2 and has been left out. The equation shows clearly the behavior of the system. If the light intensity is small, the second member on the right side is small compared with the first, i.e., the action of the enzyme can catch up with the production of the percompounds and nothing is lost by back photoreaction. The yield is $\frac{1}{4}$:

$$V = (N/4)k_1. \qquad (31'')$$

From (30) follows $k_2x_2 \sim k_1x_1$; (29) gives then

$$x_1 = x_4 = x_3 = x_6.$$

The chlorophyll is occupied one-fourth each by $CO_2(x_1)$, R acid (x_3), formic acid (x_4) and R aldehyde (x_6), the percompounds being decomposed too rapidly to count. With increasing intensity, more and more of the percompounds are consumed by the chains before the enzyme has time to act, until saturation is reached, given by

$$V^2_{max} = (N/4)(k_2{}^2/Z),$$
$$V_{max} = Nk_2(1/4NZ)^{\frac{1}{2}}. \qquad (33)$$

The quantity $(4/NZ)^{\frac{1}{2}}$ is the factor by which the yield is smaller than it would be if the Blackman reaction were alone (i.e., in the absence of chain back reactions) responsible for saturation ($V = (N/4)k_2$); it should therefore be 1/500 to 1/2500, or $NZ \sim 10^6$ (10^6 corresponds to the first, $25 \cdot 10^6$ to the second number) which is not unreasonable for the chain length for unit concentration of the percompound (the chain length in photosynthesis at saturation is only $\sim 10^3$, as $x_2 \sim N \cdot 10^{-3}$). At saturation, CO_2 and formic acid occupy each almost half of the chlorophyll, the R acid and R aldehyde small fractions, the percompounds still less (since k_2 still $> k_1$).

The equation for the dependency of V on the light intensity I can be written, from (31') and (33)

$$KI = V/(1 - V^2/V^2_{max}), \qquad (34)$$

with $K = (N/4)(k_1/I)$. This is plotted in Figs. 1 and 2 and shows good agreement with the measurements, which are discussed by Smith[18] and other observers, although the form is different from the one suggested by Smith. It explains also the dependency of photosynthesis on chlorophyll, as only the amount of light absorbed counts and N enters in the same way as I.

[18] E. Smith, Nat. Acad. of Sciences America **22**, 504 (1936).

Therefore, photosynthesis has the same dependence on the amount of chlorophyll as on light intensity. If the amount of CO_2 adsorbed is proportional to the external CO_2 pressure, the same relation should hold for the dependency on CO_2 pressure. In fact, Smith represents the dependency of photosynthesis on CO_2 by the same curve as the dependency on light intensity.

FLASH SATURATION

We consider the stationary state, when the flashing has gone on for some time and the effects of subsequent flashes are all equal. We call the beginning of a flash time 0, its end time τ, the duration of the dark interval T. In the stationary state, everything must be the same at $t = T + \tau$, when the next flash starts, as it was at $t = 0$.

During the dark period, we have the following equations

$$dx_2/dt = -k_2x_2, \qquad (7'')$$

$$dx_3/dt = k_2x_2, \qquad (8'')$$

$$dx_4/dt = 0, \qquad (9'')$$

$$dx_5/dt = -k_2x_5, \qquad (10'')$$

$$dx_6/dt = k_2x_5. \qquad (11'')$$

That gives

$$\left.\begin{matrix} x_2 = {}_\tau x_2(1 - e^{-k_2 t}) \\ x_5 = {}_\tau x_5(1 - e^{-k_2 t}) \end{matrix}\right\} t \text{ counted from } \tau. \qquad (35)$$

Integrate then (20) from $t = 0$ to $t = \tau + T$. We have then as the left side the difference of $x_3 + x_4 + x_5 + y_4 + y_6$ at these two moments, which difference is zero. On the right, there is no light reaction, and we can therefore neglect the integration over the very short flash. We find, therefore,

$${}_\tau x_2(1 - e^{-k_2 T}) = {}_\tau x_5(1 - e^{-k_2 T}),$$

or ${}_\tau x_2 = {}_\tau x_5$, and, therefore, x_2 and x_5 are equal during the dark period.

Now on comparing (10') with (7'), one sees that this is possible if $x_1 = x_4$, because then the equations for x_2 and x_5 become identical, and if these quantities are equal at one moment, they will remain so.

Even for the largest available intensities, only a small fraction of the chlorophyll molecules absorb a quantum during one flash (according to
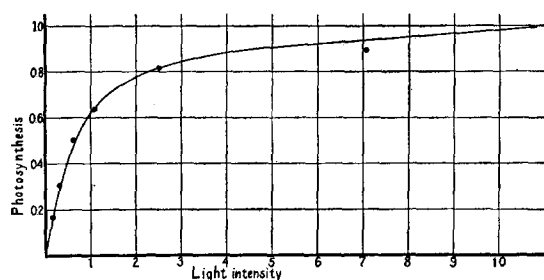
FIG. 1. Degree of saturation for continuous illumination as function of the light intensity. Abscissa: light intensity. The units are such that intensity 1 means that four times as many light quanta are absorbed as oxygen molecules are developed at saturation. The curve is calculated according to Eqs. (31'), (34). The measurements are from Warburg.[8]

Kohn's[7] data, ~ 0.01). Therefore, the relative change that the number of molecules of any kind which are not reacting during the dark (i.e., all except the percompounds) undergoes during one flash is small, and we can substitute the average value. We find therefore from (8'), (8'')

$$k_3 \bar{x}_3 \tau = \int_\tau^T k_2 x_2 dt = {}_\tau x_2(1 - e^{-k_2 T}), \qquad (36)$$

which says that during the flash just as much R acid is transformed into formic acid as is supplied in the dark interval by decomposition of peracid. Similarly from (11'), (11'')

$$k_6 \bar{x}_6 \tau = \int k_2 x_5 dt = {}_\tau x_2(1 - e^{-k_2 T}). \qquad (36')$$

We call v the amount of oxygen developed per flash (not per second), which is

$$v = \tfrac{1}{2} \int k_2(x_2 + x_5) dt = {}_\tau x_2(1 - e^{-k_2 T}). \qquad (37)$$

Neglecting during the flash k_2, we get by integrating the equation (see 7')

$$dx_2/dt = k_1 \bar{x}_1 - 2k_1 Z x_2^2 \qquad (7''')$$

the following result

$$\frac{(k_1 x_1)^{\frac{1}{2}} - (2k_1 Z)^{\frac{1}{2}} x_2}{(k_1 x_1)^{\frac{1}{2}} + (2k_1 Z)^{\frac{1}{2}} x_2} = \frac{(k_1 x_1)^{\frac{1}{2}} - (2k_1 Z)^{\frac{1}{2}} {}_0 x_2}{(k_1 x_1)^{\frac{1}{2}} + (2k_1 Z)^{\frac{1}{2}} {}_0 x_2}$$

$$\times \exp. \left(-2k_1 x_1 \left(\frac{2Z}{x_1} \right)^{\frac{1}{2}} t \right) \qquad (38)$$

in which x_1 has been written instead of $\bar{x}_1$ but

$$_0 x_2 = {}_T x_2 = {}_\tau x_2 e^{-k_2 T}.$$

One has, in addition, from (21'), (36)

$$N/2 = x_1 + {}_\tau x_2 \{ 1 + (1/k_1 \tau)(1 - e^{-k_2 T}) \},$$

or, neglecting $k_1 \tau$ compared to 1 and assuming $k_2 T > k_1 \tau$

$$(N/2)k_1 \tau - {}_\tau x_2(1 - e^{-k_2 T}) = k_1 \tau x_1. \qquad (21'')$$

Writing now simply x instead of ${}_\tau x_2$, one gets as equation for the unknown x as function of $k_1 \tau$ and T

$$\frac{[(N/2)k_1 \tau - x(1 - e^{-k_2 T})]^{\frac{1}{2}} - (2k_1 \tau Z)^{\frac{1}{2}} x}{[(N/2)k_1 \tau - x(1 - e^{-k_2 T})]^{\frac{1}{2}} + (2k_1 \tau Z)^{\frac{1}{2}} x}$$

$$= \frac{[(N/2)k_1 \tau - x(1 - e^{-k_2 T})]^{\frac{1}{2}} - (2k_1 \tau Z)^{\frac{1}{2}} x e^{-k_2 T}}{[(N/2)k_1 \tau - x(1 - e^{-k_2 T})]^{\frac{1}{2}} + (2k_1 \tau Z)^{\frac{1}{2}} x e^{-k_2 T}}$$

$$\times \exp. \left\{ -2(2k_1 \tau Z)^{\frac{1}{2}} \left[\frac{N}{2} k_1 \tau - x(1 - e^{-k_2 T}) \right]^{\frac{1}{2}} \right\}. \qquad (39)$$

This formula is too complicated to be discussed for all possible cases. We see, however, that if the light is very weak ${}_\tau x_2 = k_1 x_1 \tau + {}_0 x_2$ {from (7')}. (No chain back reactions, all percompounds formed are still present at the end of the flash,
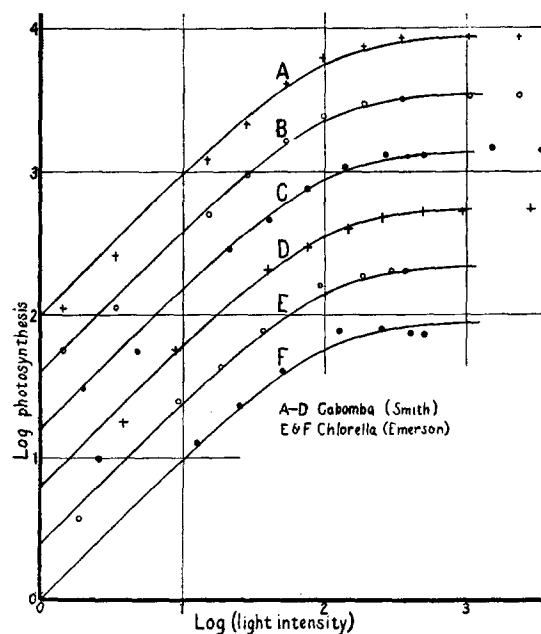

FIG. 2. Logarithms of photosynthesis against logarithms of light intensities. The curves are calculated according to Eqs. (31'), (34), but the absolute values are not given. The points mark experimental data. The measurements are taken from E. Smith.[18]

time τ.) Then

$$x_1 = N/4$$

and as with weak continuous light, the chlorophyll is equally divided between CO_2, R acid, formic acid and R aldehyde.

$$v = {}_\tau x_2 (1 - e^{-k_2 T}) = {}_\tau x_2 - {}_0 x_2 = k_1 x_1 \tau = k_1 (N/4)\tau.$$

The amount of oxygen developed per flash is proportional to the light energy, with quantum yield $\frac{1}{4}$, independent of the interval between flashes.

If, on the other hand, the light intensity is high enough to give flash saturation, the exponent on the right side of (39) is very large and therefore,

$$(N/2)k_1\tau - x(1 - e^{-k_2 T}) = 2k_1\tau Z x^2,$$

but the second member on the left is also small and we have

$$x^2 = N/4Z. \tag{39'}$$

That means simply that the stationary concentration of the percompounds is established during the duration of the flash, independently of whether at the beginning of a flash any remnants from the preceding flash are still present. The back reaction chains are working so fast, that these remnants are consumed, and the concentrations of the percompounds is the same as in continuous light (where it would, however, take much longer to establish the steady state, due to the smaller intensity of the light used).

During a dark interval $T < 1/k_2$ however, not all of the percompounds can be decomposed enzymatically before the chains of the next flash destroy them, and so we find

$$v_{\text{sat}} = (N/4Z)^{\frac{1}{2}}(1 - e^{-k_2 T}), \tag{39}$$

giving the correct experimental dependance on T. If T is $\sim 1/2k_2$ the production of O_2 *per second* is about 80 percent of that produced at saturation by continuous illumination, at $T = 1/k_2$, about 63 percent.

The variation of v with light intensity will be discussed only for long dark intervals $(k_2 T \gg 1)$, so that the equation takes the following form

$$\frac{((N/2)k_1\tau - v)^{\frac{1}{2}} - (2k_1\tau Z)^{\frac{1}{2}}v}{((N/2)k_1\tau - v)^{\frac{1}{2}} + (2k_1\tau Z)^{\frac{1}{2}}v}$$

$$= \exp.\{-2(2k_1\tau Z)^{\frac{1}{2}}((N/2)k_1\tau - v)^{\frac{1}{2}}\}. \tag{40}$$
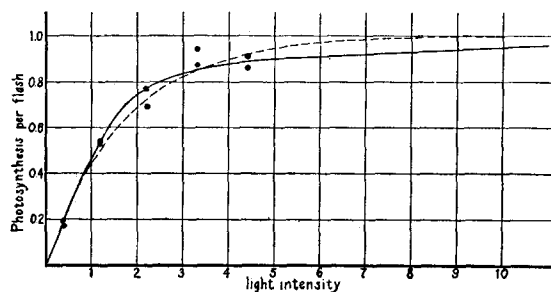

FIG. 3. Photosynthesis per flash in intermittent light. Ordinate photosynthesis as fraction of saturation value. Abscissa light intensity. The full curve is calculated from Eq. (40'). Units so that light intensity 1 means: Per flash there are twice as many quanta absorbed as molecules of oxygen are produced at saturation. The dotted curve is calculated from the Eq. $(1) - v/V_{\text{max}} = \exp. -0.575$ I which has the form suggested by Kohn.[7] The points are experimental results by R. Emerson and W. Arnold.[7]

Call again $\qquad V_{\text{max}} = (N/4Z)^{\frac{1}{2}}, \tag{41}$

so that $\qquad N/V_{\text{max}} = (4NZ)^{\frac{1}{2}} \tag{41'}$

is the ratio of chlorophyll molecules present to oxygen molecules produced per flash at saturation.

Call $\qquad v' = (N/2)k_1\tau \tag{41''}$

half of the numbers of quanta absorbed per flash. Then

$$\frac{(1 - v/v')^{\frac{1}{2}} - v/V_{\text{max}}}{(1 - v/v')^{\frac{1}{2}} + v/V_{\text{max}}}$$

$$= \exp.\left\{-2\frac{v'}{V_{\text{max}}}\left(1 - \frac{v}{v'}\right)^{\frac{1}{2}}\right\}. \tag{40'}$$

The equation was solved numerically,[19] the result is plotted in Fig. 3. Kohn has made plausible the form

$$1 - v/V_{\text{max}} = \exp. \ (-kI).$$

The main reason why our curve deviates from this is the shift in the amount of carbonic and formic acid from $\frac{1}{4}$ of the chlorophyll each at low intensities to $\frac{1}{2}$ each at saturation. However,

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[19] This was done by writing (40') in the form

$$\frac{1 - v/v'}{v/v'} = u\frac{1 + e^{-2u}}{1 - e^{-2u}} \text{ with } u = \frac{v'}{V_{\text{max}}}\left(1 - \frac{v}{v'}\right)^{\frac{1}{2}},$$

calculating the left side for a set of values of u and then from u and the so found

$$\frac{1 - v/v'}{v/v'} = \frac{v'}{v} - 1, \quad \text{finding} \quad \frac{v'}{V_{\text{max}}} \quad \text{and} \quad \frac{v}{V_{\text{max}}}.$$

Kohn's curve deviates appreciably from ours only quite close to saturation, as Fig. 3 shows. The numerical values of Kohn would lead to

$$(4NZ)^{\frac{1}{3}} \sim 1000$$

(1 mm^3 of oxygen $= 2.7 \cdot 10^{16}$ molecules in 3 minutes $= 3 \times 60 \times 15 = 2700$ flashes, i.e., 10^{13} molecules of oxygen per flash, $\sim 10^{16}$ molecules of chlorophyll) in fair agreement with the values for continuous illumination. But his light intensity

$$k_1 \tau = 0.01, \quad \text{i.e.,} \quad v' = \tfrac{1}{2} \cdot 10^{14}, \quad v'/V_{max} = 5$$

would lead to 90 percent saturation instead of the 99 percent he claims.

DISCUSSION OF THE ASSUMPTIONS

The explanation of saturation by dark back reaction is out of the question. If it were monomolecular, it would affect the yield in weak light just as much as in strong light; if bimolecular, it would give a yield proportional to the square root of light intensity instead of saturation.

Therefore, the back reaction must be contingent on light. The first question to decide is whether the final product, oxygen, is consumed or the percompounds, peracid and peraldehyde. This question can be decided from a consideration of flash saturation. Oxygen is produced by the action of enzyme on the percompound, which is so slow (50 sec.$^{-1}$) that only a negligible amount is produced during the flash itself (duration $< 10^{-4}$ sec.). Therefore, if flash saturation is to be explained by O_2 consumption, we would have to assume a persistent oxygen level. If this came from the outside (equilibrium with atmosphere) one would not get saturation, as one would have to subtract from a production of O_2 proportional to I (light intensity) a consumption proportional to I or I^2, leaving either something $\sim I$ or something falling off to zero after a maximum. If the level is determined by production and consumption of oxygen—which means assuming the escape slow compared with the production—then one would have this level independent of the distance between flashes and increasing oxygen development per flash with increase of the interval between flashes, i.e., the time available for oxygen escape, contrary to experience. Therefore, for flash illumination the

back reaction must affect the percompounds only.

If in continuous illumination both percompound and oxygen would be consumed, one would not get saturation; because a mechanism giving saturation in flash illumination would make the percompounds independent of light intensity as in flash saturation, therefore the amount of oxygen produced would be independent of the intensity and an additional consumption of oxygen would make the amount escaping decrease with increasing high intensity.

Of course, this does not mean that the radicals do not react with oxygen at all. This would be contrary to the experience with the oxidation of aldehydes by oxygen. It only means that the radicals react much faster with the peroxides than with oxygen, probably because the heat of activation for a reaction between radical and oxygen is considerably higher than for a reaction between the radical and peroxide. That is quite plausible in view of the stability of the oxygen molecule and the instability of the peroxides.

Now the peracid is produced proportional to the first power of I. To get saturation for continuous light, the consumption must (at high light intensity) also be proportional to I and some powers of the concentration x_2 of the peracid, as is seen from

$$\text{production} \sim I = \text{consumption } I x_2^3.$$

That only first powers in I occur follows also from the experimental result of Emerson and Arnold,[7] that photosynthesis depends only on the product of light intensity and duration of the flash. During the flash everything but photoprocesses can be ignored. One divides then the kinetic equations by I. On the left, Idt appears in the denominator, and no other I should appear in the equations.

This being recognized, the back reaction (consumption of the percompound) must depend on the absorption of light by the percompound. But according to Kohn's data only a small fraction of the peroxide produced during one flash can have occasion to absorb a light quantum. Nevertheless, most of it must be consumed by the back reaction before it can be decomposed by enzyme, as the oxygen developed is only a fraction of the peroxide produced (i.e., of the

number of light quanta absorbed). Therefore the few optically excited permolecules must start long chains which consume most of the permolecules. But this consumption, as said before, must be proportional to the light intensity, i.e., to the number of chains that have been started. That is only possible if the ending of the chain occurs in a reaction containing the chain carrier in the first order, as in (5c) and (5f). Had we assumed an ending of the chain by a reunion of two radicals, the rate of production of oxygen would be proportional to the square root of the number of chains started, i.e., to the square root of the light intensity, and there would be no saturation. A first-order ending of the chains might be explained by the destruction of the radicals in a heterogeneous reaction, which is quite plausible. The power in which the concentration of the peroxide enters the equation does not affect the fact of saturation (provided it is positive), but the way in which saturation is reached. Our equations lead to a chain length proportional to the concentration of the percompound. This is responsible for the good agreement with the experimental curves. Had we taken also the ending of the chain to be proportional to the percompound, $k_{22} \sim k_{22}'x_2$, the chain length would have been constant, and the formula for continuous illumination would have been the usual one

$$KI = V/(1 - V/V_{max})$$

which does not agree so well with Smith's results.

Time of Approach to Steady State

If we start out with a plant that has been in the dark for a long time in the presence of a great excess of CO_2, we can assume that practically all the chlorophyll molecules are connected with carbonic acid.

We want to calculate the order of magnitude of the time it takes to reach the stationary state with high light intensity.

The intermediate substance which is present in the stationary state in the greatest amount is formic acid, namely in an amount equal to that of CO_2, or about half that of chlorophyll. Now the reaction that limits the production of formic acid is essentially the enzyme decomposition of the peracid, the next stage—the R-acid—being
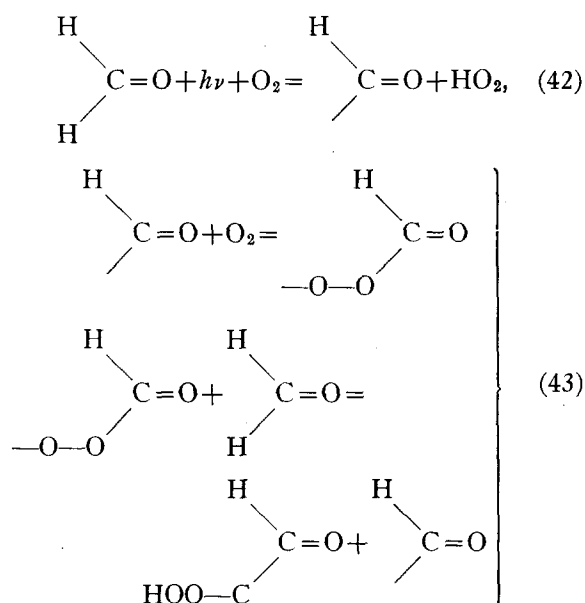
present in such a small quantity that its stationary state is reached much more quickly.

In saturation for continuous illumination, Emerson and Arnold find a production of about $2 \cdot 10^{-10}$ mole of O_2 per second for $0.5 \cdot 10^{-8}$ mole of chlorophyll, or a production of 1/25 molecule of peracid per second per molecule of chlorophyll. Therefore, the order of magnitude of the time necessary to reach the stationary state will be somewhat larger than 25 seconds, or about 1 minute. For flash saturation, the same authors get $2 \cdot 10^{-12}$ mole O_2 per flash under the same conditions, or 1/2500 molecules of peracid per molecule of chlorophyll per flash, which agrees well with Kohn. Therefore, one would need somewhat more than 2500 flashes, or about 3 minutes in Kohn's experiment.

Oxidation Processes

As was mentioned above, the intermediate products of the four photochemical steps of photosynthesis are very similar to the intermediate compounds that are generally assumed to occur in autoxidation processes in vitro, both in those induced by light and in those which occur at high temperatures.[20] Of course, the oxidation processes, being strongly exothermic, proceed by chains, which is not the case in photosynthesis.

We may use as an example the photochemical oxidation of formaldehyde by molecular oxygen:

$$\begin{matrix} H \\ \diagdown \\ C=O+h\nu+O_2= \\ \diagup \\ H \end{matrix} \quad \begin{matrix} H \\ \diagdown \\ C=O+HO_2, \\ \diagup \end{matrix} \quad (42)$$

$$(43)$$

The peraldehyde then reacts with aldehyde to give formic acid.

In plants, the intermediate products of respiration will be particularly affected by photo-oxidation. There are different opinions about the mechanism of the oxidation which goes on under the influence of the respiration ferment, but doubtlessly acids of the same level of oxidation as formic and carbonic acid occur as intermediate products. As higher carbohydrates are oxidized, these intermediate products are more complicated than H_2CO_3 or H_2CO_2, but we see no reason why these plant acids should be photosynthesized in a way different from that for H_2CO_3, as Warburg has concluded. His experiments can also be interpreted in a different manner and his opinion seems to us to be in contradiction with some experiments of Emerson and Arnold.

In the plant acids, the photooxidation by oxygen molecules—possible only if the acids are adsorbed to chlorophyll—can take place in all the side branches, and is therefore much more probable under the same conditions than in formic acid.

If the plant has been in the dark for some time, a considerable amount of those plant acids will have accumulated by respiration. The stationary concentration is much higher in the dark than in light, since the acids are transformed photochemically into aldehydes (reduction) and carbonic acid (photooxidation). Even in the presence of much CO_2, a part of the chlorophyll will be occupied by adsorbed plant acids after a certain time in the dark.

If we now start an illumination, every quantum absorbed by a chlorophyll with an attached CO_2, and a majority absorbed by chlorophyll with plant acids attached, will start photosynthesis, producing directly peracids or peraldehydes in the first step. Some quanta, however, will be absorbed by chlorophyll molecules which have plant acids absorbed, at a moment when an O_2 molecule is in contact with the latter. In this case, a radical will originate from this complex (42), starting a chain which uses molecular oxygen to build up a considerable number of peracid molecules (43). Because of the many side chains of the plant acids, we cannot neglect the chains with oxygen here, as we did

with CO_2 and H_2CO_2. Therefore, in this stage, peracid or peraldehyde is formed, partly with a consumption of oxygen.

There exists in consequence an induction period[8] with a diminished oxygen production and diminished CO_2 consumption which lasts until the concentration of the plant acids is reduced to the value corresponding to the stationary state for illumination. Therefore for strong light this induction period should be shorter than for weak light.

On the other hand, the total underproduction of oxygen during the induction period will be much larger for strong light,[21] as the peracids will be able to form radicals by absorption of light before they are decomposed by enzyme. These radicals start chains partially compensating photosynthesis, as has been shown in (5) and (6). These chains are rapid compared with the chains involved in photooxidation. Due to the photooxidation of the plant acids, there are now more chains starting back reaction in comparison with the quantity of peracid involved in photosynthesis than would be the case without the plant acids.

The slight dependence of the yield of photosynthesis under strong illumination on the external pressure of oxygen, which Warburg found, can also be explained. Even in the stationary state with strong illumination, there will be produced a certain amount of plant acids by respiration, which will then be partly photosynthesized, partly photooxidized. The photooxidation constitutes a small steady drain on the oxygen developed. The division between photosynthesis and photooxidation is shifted toward photooxidation with an increasing amount of O_2 present, until the limiting state observed by Warburg is reached.

If one strongly illuminates leaves in the presence of oxygen, they emit fluorescent light with an intensity which changes with time of irradiation. The intensity increases rapidly in the first second of the illumination, reaches a maximum and falls off slowly to a stationary

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[20] See, for instance, the theory of H. L. T. Baeckström, Zeits. f. physik. Chemie **B25**, 99 (1934), and the discussion in the Bunsengesellschaft in July, 1936; Zeits. f. Elektrochemie **42**, 439–498 (1936).

[21] The effect has in fact not yet been found for weak light.

value. This behavior and the existence of the induction period can be explained in the same way. The explanation for the form of the dependency of the fluorescent light on time has been given by Franck and Wood. There are no reasons to change the general conclusions, but it is now possible to come to a more definite picture in respect to the chemical substances involved. After a dark pause part of the chlorophyll in the leaf has adsorbed plant acids which will use the absorbed light for photosynthesis and for photooxidation. Therefore the fluorescence is weak in the first moment of illumination. The chain reaction of photooxidation produces radicals in the side branches of the plant acids. These radicals have a chance to react with ROH instead with oxygen. Such a reaction breaks the chain and renders the complexes unable to proceed with photosynthesis until ROH is restored. Thus short lived complexes are formed which are unable to use the light absorbed for photochemical purposes and are thus strong reemitters of light. The production of these complexes gives the rise of the curve. The decay following the maximum is due to the consumption of the plant acids.

BIOLOGICAL ASPECT

Of a great importance for the role of chlorophyll in the living plant is its specificity. It has, besides the absorption in the red, also regions of absorption in the blue and in the ultraviolet, so that it can make use of a much wider range in the spectrum. But if these much larger quanta could be used directly for photochemical reactions, such reactions, involving much higher energies, could be destructive for the plant. Therefore a mechanism must be present by which only an energy corresponding to $\lambda = 660\mu$m is used for photochemical reactions, even if such larger

quanta are absorbed. Recently Teller[22] has shown how that is possible for polyatomic molecules. If a molecule is brought into a highly excited state, it may drop back quickly into the lowest excited state without radiation, the difference of energy going over into vibrational energy and being conducted away as heat. From the lowest excited state, it can either go back into the normal state under the emission of fluorescence or react photochemically.

While we do not know why chlorophyll has this property, it is shown that it does have it, by the fact that the color of its fluorescence is always red, even if excited by yellow-green or blue light.

The proposed chemical mechanism permits us to understand how the plant is protected in another manner. The back reactions which we discussed in the chapter on photosynthesis protect the plant from too large a rate of photosynthesis if the light intensity becomes too high; otherwise too much aldehyde would be produced (overfeeding).

Similarly, if there is too little CO_2 present, the products of respiration (the plant acids) can be photosynthesized and this play between the products of photosynthesis and photo- and catalytic oxidation prevents an attack on the main structure of the plant. Only a long intense illumination in the absence of CO_2 would lead to a photooxidation of the main material of the plant, just as only with long starvation of an animal the vital parts are attacked.

In conclusion, we wish to express our thanks for the many helpful criticisms and suggestions we have received in the course of discussions. For these we are indebted to many scientists among them especially to Dr. Dean Burk of the Fixed Nitrogen laboratory, and to Dr. A. Corwin, chemistry department of the Johns Hopkins University.

[22] E. Teller, J. Phys. Chem. **41**, 109 (1937).