REMARKS ON PHOTOSYNTHESIS

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In this paper the author wishes to show that every theory of photosynthesis must conform to several principles well established in molecular physics; using these as a basis in conjunction with chemical knowledge, it is possible to write down a set of photochemical processes which, while they do not constitute a complete theory, are plausible and in agreement with the results of atomic physics. The author has recently published a short article (4) on carbon dioxide assimilation, which he presented at the Symposium on Photochemistry held at the San Francisco Meeting of the American Chemical Society; the present paper contains the material of the original article with the addition, at the end of the paper, of some considerations which take into account papers subsequently published and the discussion at the symposium.

It is known that in the process of photosynthesis four light quanta (9), which are absorbed by chlorophyll and transferred to the carbon dioxide, are used to reduce one carbon dioxide molecule to formaldehyde and to set one oxygen molecule free. This result is in good agreement with thermochemical relations; the energy required to reduce carbon dioxide and water to formaldehyde and hydrogen peroxide is equal to the energy of $3\frac{1}{2}$ light quanta of red light which promotes photosynthesis. As an intermediate state between carbon dioxide and formaldehyde the formation of formic acid is assumed. Willstätter (10) and Stoll (8) offered strong evidence that chlorophyll not only acts as a sensitizer, but that it enters into the course of the chemical reactions. Chlorophyll, having two especially loosely bound hydrogen atoms (3, 8), is assumed to give off these atoms in reducing carbon dioxide and to regain the hydrogen by dissociating water (10). The steps of the reactions, assumed by Willstätter, seem not to be in conformance with the amount of energy available when red light, which is known to promote photosynthesis, is absorbed. According to results of Willstätter and Stoll (11), the presence of oxygen is necessary to start photosynthesis. Willstätter supposes, therefore, that monodehydrochlorophyll and not the fully hydrogenated chlorophyll is the substance which enters into the reactions of photosynthesis. A new method

of attacking the problem was presented by Kautsky (7), who studied the fluorescence of living leaves under different conditions and especially the influence of oxygen on the light emission. Since reëmitted light can not be used for photochemical processes, researches on fluorescence and its quenching allow conclusions to be drawn about the photochemical yield. Kautsky found that the intensity of fluorescence was not constant but was a complicated function of the time of irradiation, and he explained his results with assumptions which seem not to be in accordance with other experience in the field of fluorescence of liquids. The author therefore made an attempt to discuss the photochemical part of photosynthesis in a way which avoids these difficulties and offers possible explanations for the features of photosynthesis mentioned above.

For the sake of simplicity we will use in the following equations the symbols: HH Chph for fully hydrogenated chlorophyll; H Chph for monodehydrochlorophyll; Chph for dehydrogenated chlorophyll; OH Chph for dehydrogenated chlorophyll to which is bound one OH radical.

The binding force of one H atom in HH Chph can be calculated (4) from the color of the afterglow of irradiated chlorophyll observed by Kautsky in chlorophyll solutions in the absence of oxygen, if one assumes that the afterglow is excited by the recombination process between H and H Chph. This value allows one then to calculate a lower limit for the binding force of the second loosely bound H atom from the stability of HH Chph, which does not disintegrate spontaneously into H_2 and Chph. An upper limit can be derived by the assumption that HOH Chph is unstable and will break up into H_2O and Chph. This instability of HOH Chph would make it impossible that HH Chph can be used for the process of photosynthesis. Before photosynthesis starts a so-called induction period takes place. It is assumed that in this time H Chph is formed. This process is described by the following equations:

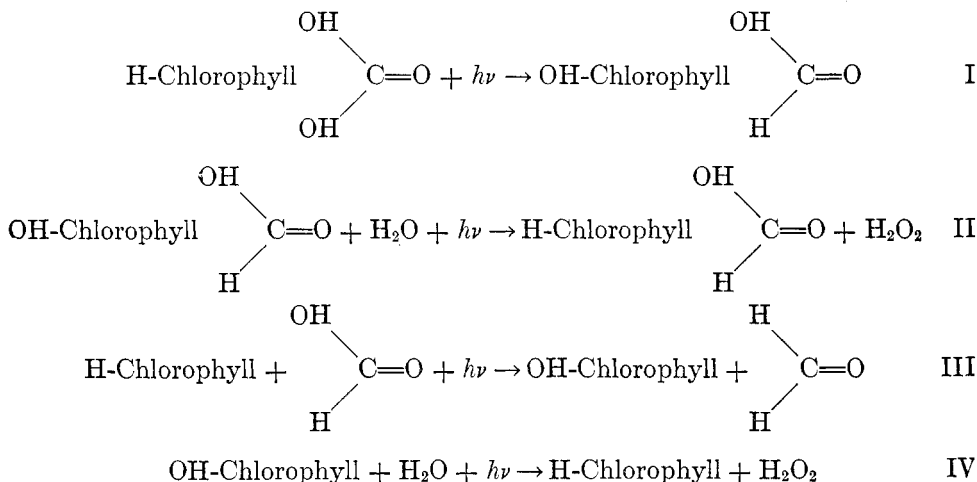$$HH\ Chph + h\nu \rightarrow H\ Chph + H \tag{1}$$
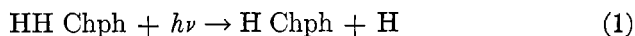
The yield of equation 1 is very low, because (5) two radicals formed in solution recombine to a large extent. If oxygen is not present, a somewhat slower back reaction will practically entirely restore HH Chph and give an afterglow by chemiluminescence. If oxygen is present, reaction 1 is followed by:

$$H + O_2 \rightarrow HO_2 \tag{2}$$
$$HO_2 + HO_2 \rightarrow H_2O_2 + O_2 \tag{3}$$

Equation 1 having a low quantum yield, the process of converting HH Chph to H Chph in the presence of oxygen takes a time from several
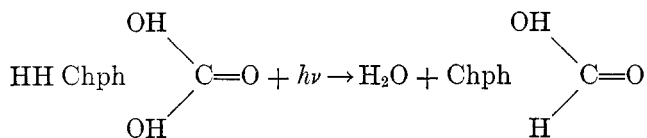
seconds to a minute, being of course a function of the intensity of the irradiating light.

The main processes are given by the following equations:

$$\text{H-Chlorophyll} \quad \overset{\displaystyle \text{OH}}{\underset{\displaystyle \text{OH}}{\diagdown\!\!\diagup}}\!\!C\!=\!O + h\nu \rightarrow \text{OH-Chlorophyll} \quad \overset{\displaystyle \text{OH}}{\underset{\displaystyle \text{H}}{\diagdown\!\!\diagup}}\!\!C\!=\!O \qquad \text{I}$$

$$\text{OH-Chlorophyll} \quad \overset{\displaystyle \text{OH}}{\underset{\displaystyle \text{H}}{\diagdown\!\!\diagup}}\!\!C\!=\!O + H_2O + h\nu \rightarrow \text{H-Chlorophyll} \quad \overset{\displaystyle \text{OH}}{\underset{\displaystyle \text{H}}{\diagdown\!\!\diagup}}\!\!C\!=\!O + H_2O_2 \quad \text{II}$$

$$\text{H-Chlorophyll} + \quad \overset{\displaystyle \text{OH}}{\underset{\displaystyle \text{H}}{\diagdown\!\!\diagup}}\!\!C\!=\!O + h\nu \rightarrow \text{OH-Chlorophyll} + \quad \overset{\displaystyle \text{H}}{\underset{\displaystyle \text{H}}{\diagdown\!\!\diagup}}\!\!C\!=\!O \qquad \text{III}$$

$$\text{OH-Chlorophyll} + H_2O + h\nu \rightarrow \text{H-Chlorophyll} + H_2O_2 \qquad \text{IV}$$

The process induced by the light is in all these four equations an exchange between an H atom and an OH radical. The binding energies of H and OH in carbon dioxide and in formic acid are practically the same, as can be shown by thermochemical results. Also if we assume for OH Chph a very weak binding force, reaction I should be slightly exothermic. Under the same assumption about OH Chph, reactions II and IV should easily proceed in the same direction as the arrow.

If radiation is stopped the H Chph can take up a second hydrogen atom in the dark, in a slow reaction with formic acid or formaldehyde, destroying a small part of the substances gained by photosynthesis. This interprets Kautsky's results, which indicate that the same intensity–time relationship after exposure to light is reproducible only after a lapse of the order of fifteen minutes in the dark. In the absence of oxygen the reaction which takes place should be

$$\text{HH Chph} \quad \overset{\displaystyle \text{OH}}{\underset{\displaystyle \text{OH}}{\diagdown\!\!\diagup}}\!\!C\!=\!O + h\nu \rightarrow H_2O + \text{Chph} \quad \overset{\displaystyle \text{OH}}{\underset{\displaystyle \text{H}}{\diagdown\!\!\diagup}}\!\!C\!=\!O$$

followed by a slow back reaction in the reverse direction. Dehydrogenated chlorophyll will thus reëmit light, not being able to start one of the photochemical reactions mentioned above. The shape and intensity of Kaut-

sky's intensity–time curves can be interpreted on the basis of the assumptions made above. Those assumptions are suggested as working hypotheses. It is of course possible to use the four main equations but replace in them H Chph by HH Chph and OH Chph by HOH Chph. But this would have sense only if one abandons the hypothesis that HOH Chph is unstable.

The main points of the interpretation given above are as follows:
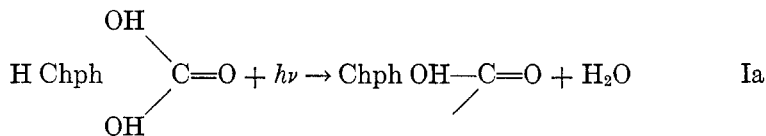
1. If four quanta are necessary to reduce one carbon dioxide molecule, four different photochemical reactions have to be considered, since assumptions about storing up energy in the form of excitation energy of molecules are impossible. Also hypotheses about metastable states with a long life-time cannot be assumed because the reactions take place in a condensed system.

2. Each photochemical partial reaction has to correspond to the energy amount of one quantum of red light, which gives photosynthesis.

3. Each individual photochemical step has to take place with the yield 1, in accordance with the total quantum yield. Therefore, only those photochemical partial reactions may be considered in which at least one of the products is not a radical, so that back reactions would not take place.
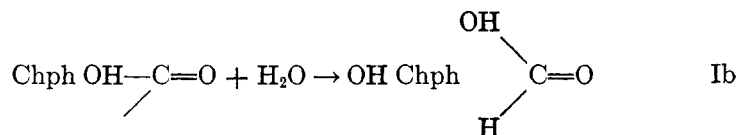
REMARKS ADDED AFTER THE DISCUSSION

A difficulty in the system of reactions presented above is the assumption in equations I and III that an exchange of places between an H atom at the H Chph and an OH radical at the carbon dioxide shall take place in one elementary act.[1] It is possible to avoid this difficulty in assuming two steps for these reactions. If the binding force between H and Chph is not larger than ~ 64 kg-cal. (which may be plausible for loosely bound hydrogen compared with the normal C—H binding force ~ 90 kg-cal.), this can be done. It will be enough to discuss only equations Ia and Ib. The replacement of equation III by two steps has to take place in a corresponding way.

$$\text{H Chph}\begin{array}{c}\text{OH}\\ \diagdown\\ \diagup\\ \text{OH}\end{array}\hspace{-0.3em}\text{C}\!=\!\text{O} + h\nu \rightarrow \text{Chph OH}\!-\!\begin{array}{c}\text{C}\!=\!\text{O}\\ \diagup\end{array} + \text{H}_2\text{O} \qquad\qquad \text{Ia}$$

followed by a dark reaction

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[1] See, for instance, the discussion remark of Bates, but I may use the opportunity to mention also letters received from F. O. Rice of Baltimore and Schoenberg of Edinburgh, which point out the same difficulty.

$$\text{Chph OH}\!-\!\overset{\diagup}{\underset{\diagup}{\text{C}}}\!=\!\text{O} + H_2O \rightarrow \text{OH Chph} \quad \overset{\text{OH}\diagdown}{\underset{\text{H}\diagup}{\text{C}}}\!=\!\text{O} \qquad \text{Ib}$$

The condition that equation Ia can take place with the yield 1 is that the quanta are not big enough to split off the hydrogen atom from the chlorophyll. Quanta of ultra-violet light which could do so should then give a much smaller quantum yield if they are absorbed at all by the chlorophyll. The dissociation process should be followed in most cases by a back reaction instead of the water formation. For equation Ib, one should expect a heat of activation so that the reaction velocity is dependent on the temperature. Reaction Ib seems to fulfill all the conditions which are necessary to understand the results published in very interesting papers by Emerson and Arnold (2) and Arnold and Kohn (1). Also Kautsky's intensity–time relations may be interpreted in this way, but more experimental proof is necessary here.

The exchange of places between H and OH according to equations II and IV will not offer such difficulties, as is pointed out by Pauling in the discussion (see below).

The assumption that H Chph and not HH Chph plays the important rôle for photosynthesis was founded on Willstätter and Stoll's results that oxygen is necessary to start photosynthesis, and on Kautsky's observation concerning the afterglow of the fluorescence. Gaffron (6) has published in the meantime a paper in which he states that photosynthesis starts also without oxygen. He explains Willstätter and Stoll's results by the presence of products of fermentation, which poison photosynthesis and have to be removed, for instance, by longer irradiation. This result is not contrary to the assumptions put forward above. It is reasonable to assume other acceptors for hydrogen atoms in the plant if oxygen is not present. Also carbon dioxide may act as acceptor, but the other acceptors will of course not be so effective as oxygen. The result of a lack of oxygen would then be that the induction period is lengthened. But we have also to consider the possibility that the hypothesis is wrong and that HH Chph is the important substance in photosynthesis. As was mentioned above, the four main equations can then be used in the same way, substituting always HH Chph for H Chph and HOH Chph for OH Chph. In this case it would be necessary to assume that the first hydrogen atom is bound more strongly to the H Chph than was calculated from the afterglow (~ 64 kg-cal. instead of ~ 40 kg-cal.). The basis for this calculation was that the afterglow also occurs with red light. Kautsky supposes so, but his observations are made with the whole light of an arc, so that this point

needs more experimental evidence.   It may be also that HH Chph and H Chph are equally important, the first for photosynthesis with longer wave lengths, the second for shorter wave lengths.   Kautsky's observations on the fluorescence in living leaves are made with blue light and even shorter wave lengths.

REFERENCES

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(2) EMERSON, R., AND ARNOLD, W.: J. Gen. Physiol. **15**, 391 (1932); **16**, 191 (1932).
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(5) FRANCK AND RABINOWITSCH: Trans. Faraday Soc. **30**, 152 (1934).
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(7) KAUTSKY, H., HIRSCH, H., AND DAVIDSHÖFFER, F.: Ber. **65**, 1762 (1932).
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(11) WILLSTÄTTER AND STOLL: Chlorophyllbuch. Bayr. Akad.

DISCUSSION

PROFESSOR BATES: Professor Franck, in discussing the energetics of the various steps in his mechanism, mentioned only the change in heat content.   Is it not possible that there might be large activation energies involved which would change the whole complexion of things?   For example, step 4 is of the type that in the gas phase usually requires considerable activation energy, being a "double displacement."   In the mechanism for the hydrogen atom–oxygen reaction we find evidence that the reaction corresponding to 4,

$$HO_2 + H_2 \rightarrow H_2O + OH$$

requires enough activation energy to make the reaction occur either through

$$HO_2 + H_2 \rightarrow H_2O_2 + H$$

or

$$HO_2 + HO_2 \rightarrow H_2O_2 + O_2$$
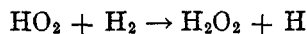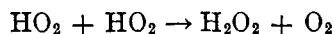
PROFESSOR LINUS PAULING (California Institute of Technology): Professor Franck has postulated that in the active state much of the chlorophyll is present in the form of H Chph or OH Chph.   Each of these molecules contains an odd number of electrons, and has a magnetic moment of 1.73 Bohr magnetons.   Hence their presence could be detected by measurements of the magnetic susceptibility of the illuminated substance.

Dr. Bates has remarked that the activation energies for the reactions postulated by Professor Franck may be very large. It may be that this is avoided because of the special properties of the odd molecules; for example, a water molecule may be attached by the free valence of OH Chph in such a way that the hydrogen peroxide-producing reaction follows immediately the formation of a single bond between the two oxygen atoms, for which the activation energy may be small.

PROFESSOR FRANCK: I agree entirely with the remarks of Dr. Pauling, but for the main equations 1 and 3, difficulties similar to those mentioned by Dr. Bates remain (see the improvement made in the last section of the paper "Remarks on Photosynthesis").

DR. LARS ONSAGER (Yale University): According to the scheme suggested by Dr. Franck, it appears that hydrogen peroxide in sufficient concentration ought to react with H-chlorophyll with the emission of light. Has this reaction been investigated? Might I also ask if Dr. Franck considers it possible to carry on photosynthesis in vitro?

PROFESSOR FRANCK: So far as I am aware, research regarding chemiluminescence in this special case has not been carried out. It would be difficult to separate this light emission from other processes which give light, for instance, fluorescence. Moreover, I would expect if at all a very weak effect, because the reaction should be endothermic and the concentration of H Chph is very small. Photosynthesis in vitro has never succeeded up to this time. Still I hope that it would be possible to find the right conditions. The difficulty seems to be that Chph in water gives, not a real solution, but a colloid in which one Chph molecule which absorbs light attacks another one. It would be necessary to imitate the chloroplasts. That will mean that it would be necessary to have Chph molecules scattered on the surface of a stable lipoid colloidal substance in water.

DR. JAMES H. C. SMITH (Carnegie Institution of Washington, Stanford): The interpretation which Professor Franck has given to the experimental observations of Kautsky on the fluorescent effects in leaves (Kautsky, Hans, Hirsch, A., and Flesch, W.: Ber. **68,** 152 (1935) and on the afterglow of chlorophyll in solution (Kautsky, Hans, Hirsch, A., and Davidshöffer, F.: Ber. **65,** 1762 (1932)) is very interesting. The chief significance of this proposal lies in its reasonable correlation of some of the photochemical phenomena occurring in photosynthetic organs with observations made on simpler photochemical systems. These correlations will undoubtedly aid in the interpretation of the photosynthetic process.

The quenching of the fluorescence and afterglow of chlorophyll by oxygen has been interpreted by Professor Franck to be a reaction between oxygen and the hydrogen dissociated from chlorophyll by the action of

light, which reaction forms monodehydrochlorophyll. This reaction is assumed to be necessary to prepare the chlorophyll for the subsequent reduction of carbon dioxide. In this scheme oxygen is necessary for the initiation of the photosynthetic reactions. Recent experiments by Gaffron (Naturwissenchaften **23**, 528 (1935)) have indicated that molecular oxygen is not necessary for carbon assimilation, and on the basis of these experiments it appears probable that molecular oxygen is not involved in the first step in photosynthesis.

Gaffron has also shown that immediately following illumination carbon dioxide was absorbed more quickly than oxygen was evolved. This is significant in that it indicates that carbon dioxide absorption and oxygen evolution may be distinct processes. This agrees with Professor Franck's proposed reactions, in that the evolution of oxygen from the hydrogen peroxide formed by the reduction of carbon dioxide would be a step separate from the absorption of the carbon dioxide.

In a discussion such as this, one should probably limit oneself to the details of the paper presented, but in this instance it seems advisable to point out that photosynthesis is a complicated biological process in which many factors are operative, the disentanglement of which and the evaluation of their individual contributions will require considerable experimentation.

While it is desirable to reduce the photosynthetic process to simple terms, experiments on plants indicate that the process is very complex. Harder's experiments (Planta **20**, 699 (1933)) have shown that the effects caused by light of different intensities are complicated. The plant's behavior is conditioned by its previous history. The rate of photosynthesis is a function of the ratio of the light used in cultivation and in measurement. When the ratio of intensity of light of cultivation to intensity of light used in measurement is small, there is a rapid acceleration in the rate of photosynthetic activity with time. When the ratio is large there is a rapid decrease in the photosynthetic rate. These effects persist for a considerable period of time. Other factors, such as length of periods of illumination, nutrition, and temperature, cause complicated effects. In view of these observations it scarcely seems probable that the photosynthetic reactions can be circumscribed by as simplified a process as has been proposed by Professor Franck.

One other relation should also be mentioned in connection with the proposed mechanism. In all photosynthetic organisms which have been investigated thus far, whether the purple bacteria or the higher plants, the *green* pigments are always accompanied by the *yellow* pigments. The individual pigments in the purple bacteria differ from those in the higher plants, but the same type of complex apparently exists (van Niel, C. B.,

and Smith, James H. C.: Arch. Microbiologie **6,** 219 (1935)). Although it is dangerous to conclude that because the *yellow* pigments always accompany the *green* pigments in the photosynthetic organs they must be involved in the photosynthetic reactions, it seems highly probable, because of this close association, that they may play a rôle in the process and ultimately may have to be included in the formulation of the reactions involved.

PROFESSOR FRANCK: I had not seen Gaffron's paper until now, but so far as I understand these results, they are not in contradiction to my assumptions because oxygen can be replaced by other less effective acceptors of hydrogen atoms in the plant (see the remarks added to the paper).

With the general remarks of Dr. Smith I agree, in so far as I do not claim to have the real solution of the whole problem. I wish only to show that it is possible to sketch a picture with a few simple assumptions. The only point which is in favor of my picture seems to be that I use only principles which are in accordance with the results of atomic physics.

As to the last remarks, I may mention that most of the experiments made with plants and also the interesting experiments with flashlights (Emerson and Arnold) are carried out under conditions in which an overdosage of light is used. This gives, of course, very interesting and important results, but of course not very simple ones, which only can be expected if the amount of light, the amount of Chph and CO_2, etc., are carefully balanced against each other, as was done in Warburg's experiments with the quantum yield (see the remark added to the paper).

DRS. O. L. INMAN AND PAUL ROTHEMUND (Kettering Foundation, Antioch College) (communicated): We are yet unwilling to accept Dr. Franck's statement that "it is known that in the process of photosynthesis four light quanta which are absorbed from chlorophyll and transferred to the carbon dioxide are used to reduce one carbon dioxide molecule to formaldehyde and to make one oxygen molecule free." It seems to us necessary that Warburg's work should be carefully substantiated before we accept these as established facts in the photosynthetic mechanism. The statement that oxygen is necessary to initiate photosynthesis is not by any means a settled question. E. N. Harvey (Plant Physiol. **3,** 85 (1928)) concluded that several marine algae and one flowering plant (*Zostera*) could carry out the first steps of photosynthesis in the absence of oxygen even to a pressure which corresponds to about 10^{-53} atmospheres. Harvey's work has been successfully repeated by us with a large variety of land plants. Such hypotheses as Dr. Franck's have value, since they tend to focus attention on certain phases of the supposed mechanism which need more experimental support.

DR. VERNON M. ALBERS (Kettering Foundation, Antioch College)

(communicated): Dr. Knorr and I have studied the fluorescence spectra of the chlorophylls and some of their derivatives, during photodecomposition, in acetone solution, under atmospheres of air, oxygen, carbon dioxide, and nitrogen, using the total radiation from Pyrex mercury arcs. The time required to bleach a solution of a given substance varied with the different atmospheres, in general being greatest under oxygen and least under nitrogen, but the intensity of the fluorescence was not noticeably different. In some cases the rate of bleaching was as much as twenty times as great under nitrogen as under oxygen. This would indicate that in these solutions of the chlorophylls and chlorophyll derivatives the yield of fluorescence is not inversely proportional to the photochemical yield, but is independent of the photochemical yield.