SOME REMARKS ON THE PHOTOSYNTHESIS OF GREEN PLANTS

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I

Of recent years there have been considerable contributions to the literature of the photosynthesis in plants. One must mention here the papers of Franck (1) and Stoll (2), who developed the theory proposed by Willstätter (3), the interesting considerations of van Niel and Müller (4), and the detailed discussions by Gaffron and Wohl (5) and Emerson (6).

The most important of recent experimental discoveries in this field is the "photosynthetic unit" of Emerson and Arnold (7). These authors have shown that only a small fraction, about 1/2000, of the total amount of chlorophyll present takes part directly in the assimilation process. It seems that the chlorophyll is entering into photosynthesis only in certain photosynthetic units containing a few hundred molecules. Gaffron and Wohl (5) also concluded from the previous assimilation experiments of Willstätter and Stoll (8) that about 1000 chlorophyll molecules must cooperate to bring about the reduction of one molecule of CO₂ during the experimentally determined time of the "Blackman period."

Another important point is the rôle of iron in the assimilation of green plants. Although the need for iron in the photosynthesis of green plants has been known for a long time and the presence of iron in the chloroplasts was established by Moore (9) yet the rôle of iron has not been considered in the modern theories. It is not only that the plants grown in the absence of iron remain pale or colorless (chlorosis) and therefore show a low photosynthetic activity due to their
low chlorophyll content, but also, as Willstätter and Stoll (8) have shown, in these chlorotic plants some other important part of the photosynthetic mechanism must be imperfectly developed (10). It is conceivable that iron salts catalyze the oxidation of the colorless protochlorophyll, from which, according to Noack and Kiessling (11), chlorophyll is formed. Noack (12) has recently found a relatively large amount of ionic iron in the chloroplasts of different green plants. This suggests that iron salts may take a direct part in photosynthesis. It has been shown experimentally (13) that it is possible to reduce different fluorescent substances (e.g. chlorophyll) in the presence of ferrous salts by the action of light, which is absorbed by these substances in the visible region of the spectrum. Similarly the ferrous salts may reduce the CO$_2$ in the plants under the action of sunlight, a point which will be discussed more fully later.

It is well known that the assimilation process of the green plants can be represented by the following stoichiometric equation:

$$\text{CO}_2 + \text{H}_2\text{O} \rightarrow 4\text{CaH}_{12}\text{O}_6 + \text{O}_2 - 112 \text{ K cal.}$$

Warburg (14) has shown experimentally that 4 quanta must be absorbed for the reduction of one molecule of CO$_2$. In the case of red light ($\lambda \sim 660 \text{ m}\mu$) this corresponds to about 168 K cal. (per mol); i.e., with the small reserve energy of about 50 K cal. the plant is able to provide for the activation energies of all the processes in the hydrogenation of the CO$_2$. Every individual process involved must possess a high quantum efficiency and a small heat of activation, and the intermediate products must have a long lifetime. The last two conditions will be fulfilled with radicals as intermediate products and the high quantum efficiency is also compatible with this assumption, if the light reaction is followed by a sufficiently fast thermal reaction.

Willstätter and Stoll (8) have shown experimentally that a complex between chlorophyll and CO$_2$ is easily formed in the presence of water. It is possible that the Fe$^{++}$ ions present are able to reduce a chlorophyll-CO$_2$-complex in the plant under the influence of sunlight.

The decomposition of water into H and OH in the assimilation
process has previously been assumed by van Niel and others (4). However, the direct splitting of H$_2$O with a red quantum of energy ($\sim 50 \text{ K cal./mol}$) is impossible because of the high binding energy ($115 \text{ K cal./mol}$) of the H--OH bond. This can only be accomplished with the help of Fe$^{++}$ (or possibly another reducing agent) by sensitization with a substance which absorbs in the red region of the spectrum, as has been proved experimentally (16). The hydrogen formed hydrogenates the sensitizing substance. (The direct photochemical decomposition under the influence of Fe$^{++}$ with the accompanying formation of hydrogen gas requires an energy of at least 90 K cal. ($\lambda \sim 2900 \text{ Å}$) (17)).

In this connection the behavior of the green sulfur bacteria is of particular interest. According to van Niel and Müller (4) no catalase and most probably no iron are present in these bacteria. Consequently they should not be able to reduce (assimilate) CO$_2$ with H$_2$O alone. Actually they are only enabled to assimilate in the presence of H$_2$S which, in the course of the process is oxidized to elementary sulfur. In view of the much smaller binding energy of the S--H bond ($\sim 88 \text{ K cal.}$) one realizes how these bacteria can produce H atoms from H$_2$S (even in the near infrared), by using the quanta absorbed by the sensitizing bacterio-chlorophyll present. These conclusions are in good agreement with recent investigations on the photochemistry of SH-compounds in solution (18).

III

It seems at first very difficult to understand how the plant accumulates the 4 quanta necessary to reduce one molecule of CO$_2$ with practically no loss of energy. It is easy to show by a simple statistical calculation that if only those molecules of chlorophyll, which under the given conditions have absorbed at least 4 quanta of light, can take an active part in the assimilation, the quantum efficiency would be very small indeed. The quantum efficiency is, however, a relatively high one (14).

An explanation of this complete utilization of the absorbed energy is to be found in the peculiar structure of the chloroplasts and in the state of the chlorophyll in the living plastids. According to the investigations of Liebaldt (19) and Mencke (20) the chloroplast, which
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is optically anisotropic, consists of a lipoid phase, in which the chlorophyll is dissolved, which is itself dispersed in an aqueous "hydroid phase."

It seems that the chlorophyll has to fulfill two different functions, depending on its situation in the chloroplasts. The chlorophyll molecules on the surface of the lipoid phase (in contact with an aqueous phase containing Fe++) combine with CO₂ to form a light absorbing chlorophyll-CO₂-complex and in this way take part in the reduction of the CO₂.

The greater part of the chlorophyll molecules is dissolved in the interior of the lipoid phase and absorbs the energy of the light which is then stored in the form of electronic excitation energy. It is well known that electronic excitation energy is practically never directly transferred into kinetic energy (heat) (21). Therefore the quanta absorbed in the interior of the lipoid phase will be handed over from one chlorophyll molecule to another by a sort of resonance effect and eventually reach the chlorophyll molecules on the surface. In this way all the energy absorbed in the interior can ultimately be used for the assimilation process on the surface. This process implies that the chlorophyll molecules in the lipoid phase are in a state of strong mutual interaction. The observed shift of the absorption maximum of chlorophyll in the living plastids by 150–200 Å towards the red region as compared with chlorophyll in solution or in the colloidal state (22) may be due to interaction forces of this kind.

The above considerations require that the lifetime of the excited chlorophyll molecule in the lipoid phase shall be of the same order of magnitude as the average time of reaction necessary for the complete reduction of one CO₂ molecule (the so called Blackman period). The lifetime of the excited chlorophyll molecule in solution is about 0.01 second at 25°C. (Kautsky (23)) and the Blackman period was found to be 0.02 second at 25°C. (Emerson (29)). Both the lifetime of excited chlorophyll and the Blackman period increase considerably with falling temperature. It is also possible to explain Kautsky's observation that strongly assimilating leaves show a considerably weaker fluorescence than in the normal state (24). In the case of non-assimilating leaves the energy coming from the interior of the lipoid phase is not captured on the surface and is eventually given up
as fluorescent light. On the basis of these arguments the photosynthetic unit of Emerson and Arnold is determined by the ratio surface/volume of the lipoid globules in the plastid:

\[
\frac{\text{(Active) chlorophyll in the surface of the lipoid phase}}{\text{Chlorophyll dissolved in the interior of the lipoid phase}}
\]

The obvious implication is that for every chlorophyll molecule on the surface (actively reducing \(\text{CO}_2\)) there are about 500 molecules in the interior which provide it with the necessary 4 quanta.

This assumption does not seem to be in contradiction to the recent considerations of Kohn (25), which on the other hand are unable to explain the accumulation of energy.

With regard to the size of such a structure, there seems to be no particular restriction, as may be clear from the following remarks.

Assuming, for example that the globules (in the chloroplast) are small spheres (radius \(r\)), the chlorophyll molecule can be regarded (to a first approximation) as a cylinder, constituted by the porphine ring as the base of about 100Å (radius \(\rho\)) and the phytol-chain lying on top of it, the height of the cylinder being about \(h \sim 3\)Å. These dimensions are taken from measurements on surface films by Gorter (26) and A. Hughes (27).

We then have for the ratio:

\[
\frac{\text{Surface of the sphere}}{\text{Volume of the sphere}} \times \frac{\alpha_s}{\alpha_v} = \frac{\text{(porphine) surface of one chlorophyll molecule}}{\text{volume of 500 chlorophyll molecules}}
\]

where \(\alpha_s\) denotes the fraction of the (total) surface occupied by the chlorophyll and \(\alpha_v\) denotes the fraction of the (total) volume occupied by the chlorophyll.

From the above equation we obtain:

\[
\frac{4\pi r^2 \times \alpha_s}{\frac{4}{3}\pi r^3 \times \alpha_v} = \frac{\rho^2 \pi}{500 \rho^2 \pi h}
\]

\[
r = 3.500.3.10^{-4} \frac{\alpha_s}{\alpha_v} \text{ cm.} \sim 4.5.10^{-6} \frac{\alpha_v}{\alpha_s} \text{ cm.}
\]

We do not know the value of the ratio \(\frac{\alpha_s}{\alpha_v}\), but even assuming this to be about unity, we obtain for the radius of the little sphere repre-
SENTING the structural unit $r \sim 0, 4\mu$, which is of the order of 1/10 to 1/20 of the radius of a plastid. It is possible that the dark globules shown by photographs of chloroplasts in red light (28) represent this photosynthetic unit; the size of these globules being of the same order of magnitude as suggested above.

IV

The processes discussed above can be represented by a system of simple reactions showing the step-wise reduction of $CO_2$ to the $CH_2O$ stage. (The asterisks in the following equations denote excited forms of molecules.)

(Complex formation)

(a) Chlorophyll (surface) + $CO_2$ + $H_2O$ → Chl→$CO_2$-complex

(Light absorption)

(b) Chl→$CO_2$ + $hv$ → Chl→$CO_2$* (b') Chl→$CO_2$* → Chl→$CO_2$ + $hv$

1. (Reduction step) Chl→$CO_2$* + $Fe^{++}$ → Chl→$CO_2$- + $Fe^{+++}$; Chl→$CO_2$- + $H^+$ → Chl→$CO_2$OH 2$CO_2$OH → $H_2C_2O_4$ oxalic acid (by product)

(Light absorption)

(c) Chlorophyll (lipoid phase) + $hv$ → Chl* (c') Chl* → Chl + $hv$

(d) (Energy transfer) Chl→$CO_2$OH + Chl* → Chl→$CO_2$* + Chl

2. (Reduction step) Chl→$CO_2$OH* + $H_2O$ → Chl→$HCOOH$ + $OH$

(e) (Energy transfer) Chl→$HCOOH$ + Chl* → Chl→$HCOOH$* + Chl

3. (Reduction step) Chl→$HCOOH$ + $Fe^{++}$ → Chl→$HCOOH$- + $Fe^{+++}$; Chl→$H$. $COOH^-$ + $H^+$ → Chl.HC = O + $H_2O$

(f) (Energy transfer) Chl→$HCOOH$ + Chl* → Chl→$HCOOH$* + Chl

4. (Reduction step) Chl→$HCOOH$* + $H_2O$ → Chl→$H$.$CHO$ + $OH$

Reactions analogous to (1) and (3) have been observed experimentally, therefore the energy relations need not be considered in detail here. For reactions (2) and (4) the total energy, $Q$, is given by $Q = Nhv + E_{C-H} - D_{H_2O}$ where $D_{H_2O}$ (115 K cal.) is the energy (per mol) required to split $H_2O$ into $H$ and $OH$, $Nhv$ ($\sim 42$ K cal.) the excitation energy of chlorophyll and $E_{C-H}$ ($\sim 94$ K cal.) the energy of the C→H bond. We therefore have $Q \sim 25$ K cal., so that both reactions (2) and (4) can be regarded as sufficiently exothermal.

This scheme is but one of several alternative representations of the theory. In this case all processes, except reaction (1), are taking place during the Blackman period. The formation of oxygen is considered to take place with the help of catalase from hydrogen peroxide formed according to $2OH = H_2O_2$. Two OH radicals are formed.
directly in reaction (2) and (4), whereas two additional OH radicals
must be formed in the reduction of Fe$^{+++}$ formed in reaction (1) and
(3). $2\text{H}_2\text{O}_3$ yield finally 1 mol $\text{O}_2$. One might assume that the
Fe$^{+++}$ ions formed in the assimilation process are reduced to Fe$^{++}$
in the course of the respiration process and that the augmented res-
piration is thus connected with assimilation (30). It is possible that
the carotenoids of the chloroplast also enter into this process. For
the stationary state and constant illumination one can derive some
conclusions with regard to the kinetics which are in good agreement
with experiment (31).

(a) For small light intensities, when the rate of formation of the
Chl—CO$_2$-complex (reaction a) is fast with respect to the subsequent
reactions, the rate of assimilation is determined solely by the amount
of light absorbed per second ($I_{abs}$) and therefore the temperature
coefficient in this region must be unity. For complete absorption
(rate $\sim I_{abs} \sim I_a$) the rate varies linearly with the intensity of the
incident light ($I_a$). For small absorption (rate $\sim I_{abs} \sim I_a$ [Chl]
[CO$_2$]) the rate is also a linear function of the [Chl] and [CO$_2$] concen-
trations in the stationary state.

(b) For high light intensities the formation of the complex (Reac-
tion a) is the time determining factor. In the case of sufficiently
small CO$_2$ concentrations this can also occur at smaller light intensi-
ties. The rate is then given by: rate $\sim$ const. $e^{-Q/RT}$ [Chl] [CO$_2$],
where $Q$ is the heat of activation of reaction (a). In this case the rate
is independent of the light intensity, in agreement with the experi-
ments of Blackman (34), Warburg (14), and others. The dependence
on temperature is given by: log rate $\sim -\frac{Q}{RT}$ in agreement with the
experiments of Emerson (33). Using intermittent light the kinetics
are in some respects more complicated, because we cannot assume
stationary conditions. In fact, the time required to establish a
stationary state is of the same order as the light period.

The above discussion could readily be extended to provide some
other conclusions which, however, for the present seem to be of little
interest.
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SUMMARY

1. It is suggested that in the assimilation process of green plants the reduction of the CO₂ takes place with the help of Fe²⁺ ions (present in the chloroplast) under the influence of light, which is absorbed by a sensitizing chlorophyll-CO₂-complex.

2. It seems that the chlorophyll has to fulfill two different functions depending on its situation in the chloroplast. The chlorophyll molecules on the surface of the lipoid phase (in contact with an aqueous phase containing Fe²⁺) combine with CO₂ to form a light absorbing chlorophyll-CO₂-complex and in this way take part in the reduction of the CO₂.

   The light energy is also absorbed by the greater portion of the chlorophyll, which is dissolved in the interior of the lipoid phase, and eventually handed over to the chlorophyll molecules on the surface.

3. The photosynthetic unit of Emerson and Arnold may be determined by the ratio:

\[
\frac{\text{(Active) chlorophyll in the surface of the lipoid phase}}{\text{Chlorophyll dissolved in the interior of the lipoid phase}}
\]

so that for every chlorophyll molecule on the surface there are about 500 molecules in the interior, which provide it with the necessary quanta.

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