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REVERSIBLE PHOTOCHEMICAL REDUCTION OF CHLOROPHYLL AND ITS ANALOGUES AND DERIVATIVES

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INTRODUCTION

One method of obtaining knowledge about the photochemical stage of photosynthesis is to study the changes undergone by plant pigments on exposure to light. In 1948 we succeeded in detecting the ability of chlorophyll and its analogues to undergo reversible photochemical reduction[1]. This reaction is accompanied by the formation of active photoproducts, which "store" the energy of the light quanta, reacting reversibly after the termination of the exposure to regenerate the original pigment molecule. Further research showed that reversible photoreduction forms the basis of the photosensitising action of chlorophyll in solution, and can be used to explain the mechanism of several photobiological processes. During the past decade much work in the USSR and abroad has been concerned with the photoreduction of chlorophyll and its analogues and derivatives, and it is timely to review this work now. Brief summaries have been given in previous papers by the present author[2].

The Laboratory of Photobiochemistry of the Institute of Biochemistry of the Academy of Sciences of the USSR has carried out a comparative study of the properties of chlorophyll and its analogues and derivatives, which has enabled us to determine the significance of individual molecular groupings in the pigments and of the nature of the central metal atom in the development of photochemical properties. Investigations have been carried out on pigments isolated from various organisms and purified by chromatography, and also on pigments in the form of natural proteins directly in biological structures (in suspensions of granules and chloroplasts) and the leaves of plants.

Studies have been made on chlorophylls *a* and *b* of green leaves, bacteriochlorophyll of photosynthesising bacteria, protochlorophyll of etiolated leaves and the seed capsules of the Cucurbitaceae, the corresponding phaeophytins, various

porphyrins, phycobilins, and on synthetic analogues of these pigments, the phthalocyanines. In the course of this work methods for the separation and chromatographic purification of these plant pigments were worked out and applied.

All the pigments studied have characteristic absorption and fluorescence spectra, which vary not only with chemical changes in the pigments, but also with change in their molecular condition, e.g. aggregation to form colloidal particles, formation of coordination compounds, etc. The investigations reported below were therefore based on spectrophotometric measurements made photoelectrically during the course of reaction. Spectrophotometry in vacuum tubes adapted for measurements using Beckman and SF-4 instruments was extensively employed in the work. The reaction was also studied in several cases by making measurements of fluorescence spectra, the photoelectric potential of an inert electrode, and the photoconductivity of the reaction mixture. The formation of free radicals during the reaction was measured by methods based on the initiation of photopolymerisation and on electron paramagnetic resonance.

The following methods were employed to study the active reaction intermediates and to disentangle the separate stages in the reaction: (1) use of media in which the intermediate reaction products are stabilised; organic bases were found to have such an effect; (2) use of low temperatures, which allowed the photochemical stage of electron transfer to be isolated from the subsequent dark stages of the reaction; and (3) use of deuterated hydrogen donors which, together with the experiments at low temperatures, enabled the proton-transfer stage to be studied.

All these methods were employed in combination with measurements which enabled the formation of photoproducts to be followed directly during illumination of the reaction system and the products which "survived" the period of exposure to be traced.

I. REVERSIBLE PHOTOREDUCTION

1. Spectrophotometric Measurements

Chlorophyll. Brief exposure to red light of pyridine solutions of chlorophyll containing a reducing agent (ascorbic acid or phenylhydrazine) without access of air leads to the formation of a "red" photoreduction product having an absorption maximum at 520–525 mμ. When the light is switched off, the reverse reaction occurs, with regeneration of the chlorophyll and restoration of the green colour of the solution; this happens more rapidly when oxidising agents, atmospheric oxygen, etc., are introduced[1,2].

Partial conversion to phaeophytin was observed as a side reaction, especially in the presence of excess of ascorbic acid and with longer periods of illumination. The reversibility of the reaction was the more complete witn chlorophyll *a*; with chlorophyll *b* reduction in pyridine also gave products which could not be reconverted[3].

Reaction could be observed also in alcoholic solutions containing organic bases — pyridine, nicotine, phenylhydrazine, hexamethylenetetramine — and in pyridine in the presence of up to ~ 50% of water[3,4].

Continuing these investigations, Evstigneev and Gavrilova[5] showed that in toluene solution chlorophyll is photoreduced by phenylhydrazine. Under these conditions it was possible to photoreduce chlorophyll *b* with good reversibility. When the temperature was lowered to -40°, the formation of the "red" photoreduced form of chlorophyll was extremely slow.

Holt and Rabinowitch[6], repeating our experiments, noted that the photoreduction and its reversal could be carried out over and over again with the formation of an ever increasing amount of irreversibly changed chlorophyll. Linshitz and Weissman[7] observed the formation of a "red" photoreduced form of chlorophyll in phenylhydrazine. Lynch and French[8] repeated the photoreduction of chlorophyll in pyridine, and noted that reaction can be observed on adding 50% of pyridine to an aqueous suspension of chloroplasts. Rackow and Konig[9], in a study of the photoreduction of chlorophyll, showed that when the cyclopentanone ring of the chlorophyll molecule was broken, they were unable to observe the reaction, which in their opinion shows that this group must be present in the molecule for the reaction to occur. This conclusion is evidently unjustified, since several other analogues and derivatives of chlorophyll which contain no cyclopentanone ring (porphyrins, magnesium phthalocyanine) are fully capable of photoreduction.

Bannister[10], in his thesis on the reversible photoreduction of chlorophyll, concluded that the reaction takes place only when alcohol or water is introduced into the pyridine, which favours the formation of the ascorbate ion the active electron-donor. The quantum efficiency for the formation of the "red" reduced form of chlorophyll reaches 0.05 with 40% of water in the pyridine; formation of phaeophytin was also observed during the photoreaction. In ethereal solution, free from oxygen, chlorophyll is photoreduced by phenylhydrazine to a form having absorption maxima at 410 and 520 mμ. In agreement with the work carried out in our laboratory, Bannister concludes that it is not a singlet but a triplet excited state of chlorophyll which takes part in the reaction.

Phaeophytin. We have observed the reversible photoreduction of phaeophytin with the formation of a "red" form having a similar spectrum to the corresponding form of chlorophyll[3].

Detailed comparative studies of chlorophyll and phaeophytin were made by Evstigneev and Gavrilova, who observed a more rapid reduction of phaeophytin[11]. They found that at -40° in pyridine solution phaeophytin forms a brown intermediate photoproduct, which passes into the red photoreduced form on heating[12]. This "primary" reduced form of phaeophytin and other pigments has been investigated in detail by Evstigneev[13].

Ethyl chlorophyllide. This behaves like chlorophyll, giving a "red" reduced form. Holt[14] noted the reversible photoreduction of the chlorophyllide in the presence of Versene (tetrasodium ethylene diaminetetraacetate).

Protochlorophyll and its derivatives. On photoreduction with ascorbic acid, protochlorophyll forms a brown photoproduct, with an absorption maximum at 470 mμ, which is reversibly reoxidised to form the green protochlorophyll[15]. It was not possible to observe the formation of chlorophyll, although prolonged illumination leads to the formation of a small amount of a product having an absorption maximum at 675 mμ. Protophaeophytin is also capable of reversible photoreduction. Destruction of the cyclopentanone ring of these compounds by means of piperidine or alcoholic alkali leads to the formation of porphins, which are photoreduced like other porphyrins

(see below). Evstigneev and Gavrilova measured at -40° the absorption spectra of the primary photoreduced form of protophaeophytin which has analogous properties to those of the primary photoreduced form of phaeophytin[16].

Bacteriochlorophyll and bacteriophaeophytin. The photoreduction of bacteriochlorophyll by ascorbic acid in pyridine results in the formation of photoproducts which possess no characteristic absorption spectrum in the visible region[17]. Reaction is more complete, and more reversible, in strongly basic media, i.e. in the presence of piperidine, ammonia, or phenylhydrazine as reducing agents. With sodium sulphide the reaction is readily reversible in air, and a green photoreduction product with a maximum at 660 mμ is formed.

Bacteriophaeophytin under these conditions forms a stable green photoreduction product[17].

Bacterioviridin. A study of the properties of the pigment of green sulphur bacteria, which are similar to those of chlorophyll, has shown that this pigment forms a "red" photoreduced form like the photoreduction product of chlorophyll *a*. The phaeophytin obtained from bacterioviridin also has similar properties to phaeophytin *a*, forming primary and secondary photoreduced forms. Bacterioviridin differs from chlorophyll in its greater rate of photooxidation in solution; in this it approaches bacteriochlorophyll, breaking down extremely rapidly on illumination.

Porphyrins. Protoporphyrin, haematoporphyrin, the porphyrin of photosynthesising bacteria, and the porphyrin obtained from protochlorophyll proved to be similar in behaviour; differences were observed only in the rates of the forward and reverse reactions[19-21].

The photoreduction of these compounds has been studied in organic solvents and in acid and alkaline aqueous solutions. In the bases (piperidine and pyridine) reaction with ascorbic acid usually leads to the formation of a green photoproduct, having an absorption maximum at 640–660 mμ, which is reversibly reconverted into a porphyrin in the presence of air. In water containing 10% of pyridine a photoproduct with maximum absorption at 740–750 mμ is formed[19]. The two compounds are linked by an acid–base equilibrium, passing from one into the other with change in the basicity of the medium[20]. These products evidently differ in the degree of dissociation of the carboxyl groups of the porphyrin.

In acid aqueous solutions the absorption spectrum of the porphyrins is markedly changed by the addition of protons to the nitrogen atoms of the pyrrole nuclei; an intermediate photoreduced product having maximum absorption at 520 mμ is formed[21] (see Fig. 2).

At -40° formation of a "primary" form of haematoporphyrin with a maximum at 450 mμ was observed; on heating this underwent partial conversion into a "green" photoreduced form with an absorption maximum at 650 mμ[16].

Metal complexes of chlorophyll and its analogues. We have been unable to photoreduce the copper complexes of phaeophytin, but the fluorescent zinc complex was capable of photoreduction in the experiments of Vostrilova and Dulova[22]. Rackow and Konig[9] pointed out the facile reversibility of the photoreduction of this compound.

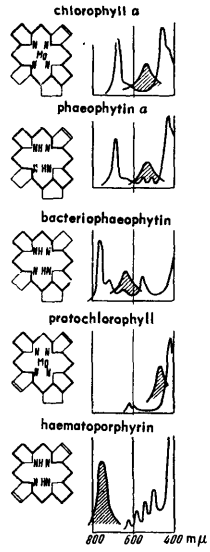


Fig. 1. Changes in the absorption spectra of chlorophyll and its analogues on photoreduction with ascorbic acid in pyridine[2] (skeletal structure of pigment molecules shown); the curves show the shape of the absorption spectrum of the pigment before reaction and after the reverse reaction; the absorption spectra of the intermediate photoreduced forms are shaded.

Dain and his collaborators[23,24] established and made a detailed investigation of the photoreduction of iron complexes of chlorophyll; they assumed that the reaction involves electron transfer with a complex of the iron–chlorophyll with hydroxide ions, water molecules, and

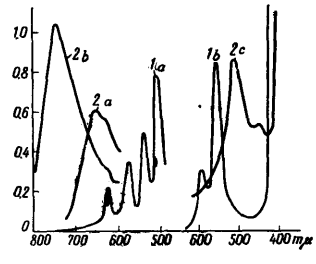


Fig. 2. Absorption spectra of photoreduced forms of haematoporphyrin in basic and acidic media[20-21]: 1) absorption spectrum of porphyrin before photoreduction and after the reverse reaction in the dark: *a*) in a pyridine–ethanol mixture; *b*) in *N* H_2SO_4; 2) after photoreduction with ascorbic acid: *a*) in pyridine; *b*) in a pyridine–ethanol mixture; *c*) in *N* H_2SO_4.

metal ions from the reducing agent. Dain and Ashkinazi[23,24] supposed that in the excited state the electronic system of these pigments is unstable, leading to subsequent (after absorption of light) electron transfer within the complex from the organic skeleton of the molecule to the central iron atom, which thus undergoes photoreduction. The resulting charge deficiency is made up by oxidation of the solvent. Since these electron transfers, following one upon the other, occur immediately after the actual absorption of light, the life of the excited state is considerably shortened. This is shown, in the opinion of these workers, by a slight broadening of the absorption bands, and the disappearance of fluorescence and the vibrational structure.

We have observed an extremely slow photoreduction of cytochrome *c* under the influence of visible light in anaerobic conditions, and the photooxidation of its reduced form in the presence of air[25,26].

Phthalocyanines. In the irradiation of a pyridine solution of magnesium-phthalocyanine containing ascorbic acid, the formation of irreversibly reduced products was accompanied by a photoreduction stage which could be rapidly reversed in the dark[27].

On carrying out the reaction at a low temperature, Evstigneev and Gavrilova observed a rapid formation of the "red" photoreduced form with a maximum at 550 mμ, which on admission of air was instantly converted back to the original phthalocyanine[28]. As in the case of chlorophyll, the spectrum of the "primary" photoreduced form of this compound could not be obtained. Copper-phthalocyanine does not undergo a reaction of this type.

Carotenoids. Attempts have been made in our laboratory to detect the photoreduction of β-carotene, xanthophyll, and the carotenoids of photosynthesising bacteria in various media with different reducing agents, but under no conditions was it possible to observe the formation of reversibly reduced products of these compounds[29]

Phycobilins. The phycoerythrin of red algae and phycocyan do not undergo this reaction nor have any photosensitising action[30]; attempts have been made to photoreduce erythrobilin, obtained by the acid hydrolysis of phycoerythrin, but this compound also proved unable to enter into a photoreaction.

2. Pigment Structure and Capacity for Photoreduction

On the basis of the above results we came to the conclusion that photoreduction can be observed with all derivatives and analogues of chlorophyll which possess a chlorin, bacteriochlorin, or porphyrin structure with a central atom of hydrogen, magnesium, or zinc.

The spectroscopic properties and the reactivities of the reduced forms of the various pigments are different in different media. Magnesium complexes usually form more active (more rapidly reconverted) reduced forms than do phaeophytins, containing no magnesium.

Comparison of the reactivity with the structure of the various pigments shows that the main criterion determining the reversible photoreduction of a pigment is the presence in its molecule of a cyclic system of conjugated double bonds linking the four pyrrole rings.

3. Comparison of the Properties of Intermediate Forms Produced by Photoreduction and by "Dark" Reduction with Zinc

Timiryazev was the first to discover the ability of chlorophyll to be reduced by metallic zinc and its subsequent regeneration by the action of air on the reduced compounds[31].

Kuhn and Winterstein demonstrated the identity of chlorophyll *a* before reduction with zinc and after reoxidation[32]. Rothemund, Albers, and Knorr[33] showed that the spectra of the pigment after regeneration and of the original chlorophyll are not identical.

In our laboratory[34] a study has been made of the reduction of chlorophyll and its analogues in pyridine solution with metallic zinc in the presence of acetic acid; under these conditions regeneration is followed by replacement of magnesium by zinc with formation of a zinc complex of the chlorophyll. The intermediate products include a compound having the spectrum of the "red" photoreduced form of chlorophyll. Evstigneev and Gavrilova[35] studied the effect of the amount of acid present on the zinc reduction, and found that with a minimum quantity of acid it is possible to obtain a reduction product of chlorophyll whose spectrum is identical with that of the "red" photoreduced form, from which chlorophyll, and not its zinc complex, is regenerated.

When a pyridine solution of chlorophyll is reduced with zinc amalgam in a vacuum, products having a different absorption spectrum from that of the red form are formed. Possibly, under these conditions partial formation occurs of products, of a one-electron reduction, corresponding to the primary photoreduced form of chlorophyll, whose spectrum has not yet been determined; on exposure to oxygen, the reverse reaction occurs instantaneously with formation of chlorophyll. Reduction of haematoporphyrin under these conditions leads to the formation of a product spectroscopically identical with the "primary" photoreduced form.

Thus it is possible, by varying the conditions of reduction of chlorophyll with zinc, to obtain intermediate compounds which are identical with photoreduction products.

4. Changes in Fluorescence during Photoreduction

All the porphyrin pigments studied possess a characteristic fluorescence, usually red in colour, only bacteriochlorophyll having a fluorescence maximum in the near infra-red region (780 mμ).

At the start of the investigation we observed that photoreduction is accompanied by a reversible diminution in the red fluorescence of chlorophyll and the appearance of the orange luminescence of the photoreduced form at 600–630 mμ. Evstigneev[5] observed, on cooling photoreduced chlorophyll to the temperature of liquid nitrogen, the appearance of a green fluorescence with maxima (in toluene solution) at 525–520 and 550–570 mμ. Gachkovskii[36] described the appearance of a series of fluorescence maxima, including an intense maximum at 730 mμ, during the photoreduction of chlorophyll and phaeophytin.

Krasnovskii and Erokhin[37] have undertaken systematic studies on the fluorescence spectra of pigments during their photoreduction with ascorbic acid in pyridine. With all the pigments investigated — chlorophyll *a* and *b*, protochlorophyll, bacterioviridin, and the corresponding phaeophytins

and porphyrins — fluorescence of the photoreduced forms was observed. Only in the case of the primary photoreduced forms of phaeophytins were well defined fluorescence maxima not found at low temperatures. We were also unable to observe the fluorescence maximum at 730 mμ of the photoreduced form of chlorophyll and phaeophytin described by Gachkovskii[36]. It is evident that in this work the fluorescence was recorded together with incident light scattered by the frozen material. A group of mercury lines near 733 mμ was used and was probably transmitted by the combination of light filters.

5. Change in Redox Potential E_0 during the Photoreaction

Krasnovskii and Brin[38] used a redox-indicator method to estimate the shift in E_0 for the system after reaction. The photoreaction of chlorophyll with ascorbic acid was carried out in a vacuum tube having a side outlet, after which solutions of compounds having different values of E_0 were run into the photoreduction products in the dark, and the rate of the reverse reaction was observed spectrophotometrically.

Before exposure, the chlorophyll–ascorbic acid system in pyridine can reduce compounds having E_0 about 0.05 V (methylene blue), but after exposure reduction of compounds with E_0 about -0.35 V (safranine T, pyridine nucleotides) was observed. These results were confirmed by measurements of the potential of an inert electrode in the reaction system. We observed a sharp change in the potential of such electrode on illuminating the chlorophyll–ascorbic acid system, as in the experiments of Rabinowitch[39] on the thionine–iron ions system.

Evstigneev and Gavrilova made a detailed study of the variation of the photoelectric potential of a platinum electrode on illumination of the reaction solution[40]. It had been shown in special experiments that the photopotential is determined not by the illumination of the electrode but by the formation of photoproducts in the bulk of the solution. Lowering the temperature was observed to increase the value of E_0 owing to the decrease in the rate of the reverse reactions and the increase in the steady-state concentration of the photoproduct. The change was observed at -40° (i.e. under conditions such that no formation of the "red" form of chlorophyll occurred). These observations led to the conclusion that the potential is determined by the formation of the primary, readily reconvertible "electrode-active" reduced form of the pigments, studied in detail by Evstigneev[13]. The intermediate formation of the "primary" reduced form was also supported by Bannister's work[10] on the quantum efficiency of the reaction as a function of the concentration of reducing agent. On illumination of solutions of chlorophyll and its analogues containing ascorbic acid the value of E_0 reached -0.35 V, in agreement with the experiments in which redox indicators[38] were used. Thus the "electrode-active" form and the "red" reduced form have similar values of E_0.

Hendrich[41] observed a change in the photoelectric potential on illumination of the chlorophyll–ascorbic acid system, and studied the effect of a series of inorganic ions on this process. He found that Cu^{2+} and Ni^{2+} ions, which can combine with ascorbic acid, prevent the development of a photoelectric potential.

On illuminating the chlorophyll–phenylhydrazine system in a vacuum, Evstigneev and Gavrilova observed an increase in electrical conductivity[42]; when the light was switched off, the conductivity returned to its previous value, which confirms the formation of ionic photoproducts. Comparison of the kinetics of the photoconductance and of the development of the photopotential at different temperatures indicates that it is the "electrode-active" products which are responsible for the photoconductivity. Thus at -40° the photoconductivity and the photopotential remain after the light has been switched off, whereas at +20° the effects are rapidly reversed in the dark.

The potential of a platinum electrode in the chlorophyll–ascorbic acid system in pyridine becomes 0.3–0.35 V more negative after illumination, indicating the formation of electrode-active products (the photoreduced form of the pigments), which give up their electron to the electrode in accordance with the following scheme for the primary photoprocess:

$$X + AH_2 \rightleftharpoons \cdot X^- + \cdot AH_2^+; \quad AH_2^+ \rightarrow \cdot AH + H^+;$$

further dissociation is possible in the presence of strong bases:

$$\cdot AH \rightarrow A^- + H^+.$$

The question arises as to why the negatively charged platinum electrode takes up the charge of the reduced form of the pigment, but does not give up an electron to the oxidised form of the hydrogen-donor.

Factors which play a part here are evidently the preferential adsorption of photoproducts of a given character on the platinum and the kinetics of the secondary changes undergone by the photoproducts. If, for example, the rate of dismutation of one of the pair of semiquinones ($\cdot X^-$ or $\cdot AH_2^+$) is greater than that of the other, the steady-state concentration of the latter compound will be greater than would appear from the electrode potential. In addition, the oxidised product (monodehydroascorbic acid) ·AH is probably able to act as a reducing agent, giving up an electron to the electrode with the formation of dehydroascorbic acid. It is thus possible that both photoproducts formed during the reaction will tend to give up an electron to the electrode. It is therefore desirable to investigate the electrode activity not only of the primary reduced form of the pigments but also of the "semi-oxidised" compound which is formed.

In this connection it is useful to compare what happens with a reducing agent which is chemically quite different in character, namely ferrous ions. Krasnovskii and Umrikhina[21] have studied the photopotential of a platinum electrode in aqueous acid solutions of a porphyrin–ascorbic acid system; here the photopotential became more negative, just as in the chlorophyll–ascorbate system in pyridine. However, with ferrous oxalate as electron-donor, the photopotential became more positive, evidently because the ferric ions formed by the elementary photoprocess

$$X + Fe^{2+} \rightleftharpoons \cdot X^- + Fe^{3+}$$

are more electrode-active. The kinetics of the photopotential variation of an electrode in these systems require more detailed study.

6. Reductant Molecules (Electron-Donors) which React with Chlorophyll and its Analogues

The reaction was observed in its purest form, yielding long-lived products in pyridine, using ascorbic acid and other dienols, phenylhydrazine, hydrogen sulphide, or cysteine[1-3]. To investigate the readily reversible "latent"

formation of primary photoproducts, it was necessary to use methods enabling such formation to be observed during the period of illumination. Sensitive methods were: measurement of the photopotential, sensitised photopolymerisation, and sensitised reduction of irreversibly reducible electron-acceptors.

A change in E_0 towards more negative values was produced by the reaction with chlorophyll of quinol, catechol, thiosinamine, and several other compounds[13]. Oster and Holt[43] used ethylenediaminetetraacetic acid in reactions of this type. The activity of ferrous compounds was shown by the polymerisation method[44,45]. The sensitised oxidation of cytochrome in the presence of chlorophyll, which we have observed[25,26], indicates the possibility of a reversible process here also. It appears from the experiments of Rackow and Konig[9] that haemochromogens could be used as electron-donors. Thus, an excited chlorophyll molecule can acquire an electron from systems with values of E_0 between -0.3 and +0.3 V.

It is probable that chlorophyll could react with cytochrome *b* detected in chloroplasts[46], for which the value of E_0 is close to that for ascorbic acid. Up till now it has remained obscure whether an excited pigment molecule can acquire an electron from a water molecule.

Use of deuterated donors. The use of deuterated ascorbic acid slows down the photoreduction of chlorophyll and the production of the "red" form[47]; the reverse dark reaction also takes place more slowly. Hill's reaction is retarded by increasing the quantity of D_2O in the water[47].

The D_2O, while retarding formation of the "red" form of phaeophytin, does not affect the rate of formation of the "primary" photoreduced form[48]; at low temperatures the rate of formation of the primary form of phaeophytin is the same whether the ascorbic acid used is deuterated or not[48]. These observations confirm the occurrence of electron transfer in the formation of the "primary" form and the participation of a proton in the formation of the more stable "red" form.

Reaction with solvent. Porret and Rabinowitch[49] were the first to observe the reversible decolorisation of chlorophyll in methanol. Livingston[50-52] studied the reversible spectral changes, occurring in chlorophyll solutions, by flash photolysis and found an increase in absorption at 525 mμ, corresponding to the maximum for the photoreduced form of chlorophyll. Linshitz and Rennert[53] detected reversible changes in the absorption spectrum of chlorophyll on illuminating it in vitreous media at low temperatures. Linshitz and Abrahamson[54], using the technique of flash photolysis with chlorophyll solutions in pyridine, noted a reversible lowering of the absorption maximum of chlorophyll in the red region of the spectrum and the appearance of a maximum in the green region at 525 mμ, together with a slight increase in absorption at 700 mμ. They noted that the life of the product absorbing at 525 mμ is longer in pyridine than in other solvents, and the reversibility of the process is better than in purified methanol.

Terenin and his collaborators[55] observed by means of a flash technique a reversible fall in the absorption maximum in solutions of magnesium-phthalocyanine, and the appearance of new maxima in haematoporphyrin[56].

In experiments of this type the solution is subjected to the action of an extremely powerful flash of light, lasting for about 10^{-5} sec, produced in a discharge tube by the discharge from a bank of condensers (50 joules); a "recording" light flash from another discharge tube is passed through the solution $10^{-4}-10^{-5}$ sec after the activating flash.

We have already suggested[57,58] that the effects observed in these experiments can be explained by the reversible photoreduction of chlorophyll, in which solvent molecules act as electron (hydrogen) donors. Actually, oxygen inhibited reversible effects in Livingston's experiments in conformity with the rapid reaction of oxidising agents with the photoreduced form of chlorophyll.

The fact that, in the flash photolysis experiments, formation of photoreduced forms of the pigment is mainly observed is supported, in our opinion, by comparatively recent experiments of Livingston and Fujimori[59]. They studied the effect of various compounds on the half-life $\tau_{1/2}$ of the metastable excited state of chlorophyll, and found that oxidant molecules shorten $\tau_{1/2}$, whereas reducing agents (ascorbic acid) have no effect on the value of $\tau_{1/2}$.

These results are readily understood on the assumption that a photoreduced form of chlorophyll is produced by the light flashes; clearly, this form will not react with a reducing agent (ascorbic acid) but does react with an oxidising agent (quinone). The effect of carotene in these experiments agrees with the effect of this compound on the photoreduction of chlorophyll observed in our own experiments[29,60].

The spectrum of the "triplet" state in the papers of Livingston and Linshitz is similar to that of the "primary" photoreduced form of phaeophytin observed by Evstigneev, and it contains also an absorption region corresponding to the "red" form of chlorophyll. In these experiments the main effect is evidently the absorption of an electron by an excited pigment molecule, whereas any formation of a shorter-lived metastable state is superimposed on the main effect and is difficult to distinguish with the recording technique employed (see also Terenin[56]).

7. Effect of the Acid–Base Properties of the Medium

Formation of relatively stable photoreduced forms of chlorophyll can be clearly observed in pyridine. We have already put forward the hypothesis that this stability is associated with acid–base equilibria, under which the proton is situated "between" the pigment molecule which has absorbed an electron and a molecule of the medium, a base:

$$\cdot X^- \,..\, H^+ \,..\, N\!<$$

The formation of these relatively stable compounds is possible only when the proton affinities of the pigment molecule and of the medium (the base) have certain values. The optimum basic strength of the medium for the formation of stable reduced forms differs with different pigments. It was to be expected that in a more basic medium than pyridine, formation of negative radical-ions $\cdot X^-$, readily undergoing the reverse reaction, would predominate. In more "acidic" media the formation of radicals $\cdot XH$ could be expected with the addition of a single proton, and of positively charged radical-ions $\cdot XH_2^+$ with the addition of two protons.

In fact, if a few drops of a strong base, piperidine or ammonia, are added to a pyridine solution of the "red" form, the reverse reaction occurs rapidly (owing to ionisation of the reduced form)[20]; in conformity with this, piperidine or ammonia, added to a pyridine solution of chlorophyll, hinder the formation of the "red" form during the photoreaction. These experiments must be carried

out quickly and preferably at low temperatures, since even 1% of piperidine in pyridine leads to a gradual breakdown of the cyclopentane ring of the chlorophyll molecule. If the reaction is carried out in pyridine which has been carefully dried with calcium hydride, formation of the "red" form takes place far more slowly than in ordinary reactive, moist pyridine, which is explained by the presence of insufficient "hydrogen ions" in the dry pyridine for the formation of the "red" form.

In the case of haematoporphyrin, a reduced form with an absorption maximum at 650 mμ is formed in strongly basic media (piperidine), but in less basic media (alcohol–pyridine mixtures) one with a maximum at 740 mμ is produced. By varying the basic strength of the medium, it is possible to change repeatedly and reversibly the position of the absorption maximum of the reduced forms[20].

Thus the conversion of the "650" into the "745" form is governed not by different redox levels, as was assumed earlier[19], but by acid–base equilibria. An optimum acidity is observed for the formation of the reduced forms of different pigments with zinc according to the method of Timiryazev[35].

Thus the spectral properties and reactivities of photoreduced forms of the pigments vary within wide limits depending on the basic strength of the solvent. More detailed study is required of the effect of the basic strength of the medium on the properties and behaviour of the electron-donor molecules which are "semi-oxidised" during the reaction; it must be assumed that monodehydroascorbic acid is also more stable in basic media, where it occurs in the form of a radical-ion, and it is evidently this form which is detected by measurements of electron paramagnetic resonance (see below).

8. Formation of Free Radicals during Photoreduction

Oxidant molecules (quinones, nitro compounds) quench the fluorescence of chlorophyll, but do not react with it photochemically, whereas reducing agents, which do react, exert no "physically" extinguishing effect on the fluorescence of the pigment[61]. These observations indicate that it is possibly the metastable pigment molecules which take part in the reaction. These are, according to Terenin, G.N.Lewis, and Kasha, and as is now universally accepted, in a triplet or biradical state. The reversal of electron spin on the formation of the long-lived metastable level determines the continued existence of a "hole", a vacant electronic level formed as a result of the excitation of a pigment molecule.

In the elementary step of photoreduction an electron is taken up by an excited chlorophyll molecule from a donor molecule, which should be accompanied by formation of a pair of radical-ions; subsequent transfer of a proton can lead to the formation of a pair of radicals in non-ionic form:

$$X + AH_2 \rightleftharpoons {\cdot}X^- + {\cdot}AH_2^+ \rightleftharpoons {\cdot}XH + {\cdot}AH.$$

Uri[62] has noted that chlorophyll in the presence of ascorbic acid initiates the polymerisation of methyl methacrylate.

A detailed investigation[44,45] of the initiation of polymerisation by the pigment–hydrogen-donor system in pyridine solution and in aqueous emulsion has shown that polymerisation is initiated by chlorophyll and all its analogues and derivatives. Phycobilins, which are not subject to photoreduction, had no effect on polymerisation. The most active electron-donors were dienols, cysteine, and ferrous compounds. (Incidentally, we were unable to detect reaction between Fe^{2+} and chlorophyll by other methods.) Polymerisation was inhibited by compounds (e.g. safranine, riboflavin) which react vigorously with the photoreduced forms of the pigments.

Under anaerobic conditions polymerisation is evidently initiated by the radical-ion reduced form of the pigment, and by products of the photoreduction of oxygen (OH, HO_2) when the latter is present. In support of Rosenberg's remark[63] about the possible effect of the triplet state of the pigment in these experiments, it must be pointed out that active polymerisation was observed only in the presence of electron-donor molecules. Linshitz and Weissman[7] found no paramagnetic resonance with the "red" reduced form of chlorophyll, and it is not yet clear whether this form is a radical or a non-radical dismutation product XA_2. The second possibility must be regarded as the more probable. We have pointed out[58] that, if measurements of paramagnetic resonance are made during illumination of the chlorophyll–ascorbic acid system, an effect due to formation of radical-ions will be detected with a sufficiently sensitive apparatus. Measurements carried out soon after this showed[45] that illumination of chlorophyll–ascorbic or phaeophytin–ascorbic acid vacuum systems in the resonance machine, by a quartz mercury lamp, gave a signal, which immediately disappeared when the light was switched off. The signal was strongest for illumination of the phaeophytin–ascorbic acid system at -40°, i.e. under conditions favouring the formation of the radical-ion reduced form. In the absence of a reducing agent it was not possible to observe the effect.

As we have already mentioned, in the elementary photoprocess of electron transfer a pair of radical-ions is formed — the radical reduced form of the pigment $\cdot\cdot X^-$ and the radical oxidised form of ascorbic acid $\cdot AH^+$, which in strong bases can be in the form of a negatively charged radical-ion.

Commoner[64] reported to the Fourth International Biochemical Congress that the structure of the electron-paramagnetic-resonance signal observed during the photoreduction of chlorophyll by ascorbic acid corresponds to the radical oxidised form of the latter. These conclusions are in agreement with data obtained in the Institutes of Chemical Physics and of Biochemistry of the Academy of Sciences of the USSR[65], where it has been shown that the signal has the same structure for the photoreduction by ascorbic acid of various pigments — chlorophyll, haematoporphyrin, phaeophytin, and magnesium-phthalocyanine (see Fig. 3). A

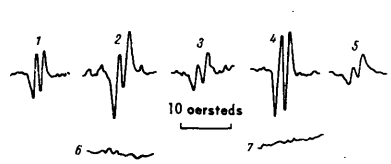


Fig. 3. Electron-paramagnetic-resonance spectrum produced during illumination of pigment–ascorbic acid system[65]: 1) chlorophyll *a*; 2) chlorophyll *a* + chlorophyll *b*; 3) phaeophytin *a* + phaeophytin *b*; 4) haematoporphyrin; 5) magnesium-phthalocyanine; 6) ascorbic acid without pigment; 7) chlorophyll without ascorbic acid.

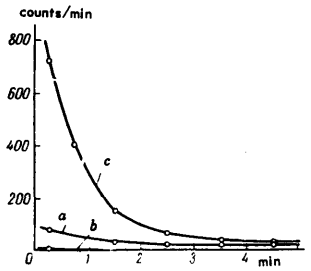


Fig. 4. Chemiluminescence produced during oxidation of photoreduced form of chlorophyll (measurements with photon counter)[69]: *a*) after illumination of the chlorophyll — ascorbic system in the absence of air; *b*) admission of air in the dark after photoreduction; *c*) admission of air and introduction of ammonia solution after photoreduction.

signal of the same structure is observed during the illumination of ascorbic acid in piperidine, yielding a coloured complex with this strong base. When other reducing agents (phenylhydrazine) are used, no signal can be observed, evidently because of the very small photostationary concentration of the radical reduced form of chlorophyll.

The oxidised form of ascorbic acid formed during photoreduction (monodehydroascorbic acid) is relatively stable, does not undergo rapid secondary reactions (dismutation, etc.) in basic media, and probably exists in the form of a radical-ion.

During this work an electron-paramagnetic-resonance signal of the same structure as in the photoreduction of chlorophyll was observed on illumination of the blades of certain grasses directly in the resonance machine.

9. Chemiluminescence during the Reverse Reactions of the Photoreduction Products of Chlorophyll

Strehler and Arnold[66] detected in green leaves and chloroplasts an extremely weak red afterglow, lasting for several minutes after the light had been switched off. Further investigation showed that the glow consists of several components of different duration. This feeble glow could be detected by means of a photon counter, a photomultiplier cooled in liquid nitrogen serving as collector.

To get an idea of the nature of the glow observed in leaves, it is desirable to investigate all types of chemiluminescence exhibited by chlorophyll. Thus a red glow accompanying the oxidation of chlorophyll and its analogues with tetralin peroxide has been known for a long time[67,68].

By means of the photon counter, chemiluminescence could be observed during the reversible oxidation of chlorophyll which had been photoreduced with ascorbic acid[69]. After illumination of a pyridine solution of chlorophyll containing ascorbic acid or phenylhydrazine, the reverse reaction (in the dark) is accompanied in the absence of air by an extremely feeble afterglow. Admission of air increases the effect by accelerating the reverse reaction. Introduction of ammonia, which greatly accelerates the reverse reaction of the "red" photoreduced form of chlorophyll[20], also leads to a very great intensification of the luminescence effect[69] (see Fig. 4).

In the absence of reducing agent no glow is observed under these experimental conditions. The mechanism of the process requires investigation; it may be that the dark reaction between the reduced form of chlorophyll and oxygen is accompanied by the formation of a biradical, a triplet state. At the expense of the heat content of the system, this passes into an excited singlet state which emits a glow spectrally similar to the fluorescence:

$$XH_2 + O_2 \rightarrow XH_2{\cdot}O_2 \rightarrow {\cdot}X{\cdot} + H_2O_2,$$

$${\cdot}X{\cdot} \rightarrow X^* \rightarrow X + h\nu.$$

Excited states can be formed also during the reverse reactions of the radical-ion photoproducts:

$${\cdot}XH + {\cdot}AH \rightarrow X^* + AH_2; \quad X^* \rightarrow X + h\nu.$$

Further work should show whether the long-lived glow in Strehler and Arnold's experiments can be attributed to the oxidation of chlorophyll or of its photoreduced form.

10. Formation of Association Complexes before Reaction

Contact between the reactant molecules is a condition for the occurrence of the primary photoprocess; the duration of this contact may vary. The life of the "active complex" at the moment of collision is very brief, but the formation of long-lived association complexes is also possible, these breaking down after the occurrence of electron transfer.

In our case three types of association with a hydrogen-donor molecule are conceivable, formed respectively with the pigment molecule in its ground state, with an excited molecule in a singlet state, or with a long-lived excited molecule in a triplet state.

The formation of long-lived complexes of chlorophyll with ascorbic acid in pyridine medium is unlikely, since the molecules of the medium (a base) themselves coordinate with the central magnesium atom of the chlorophyll molecule[70]. In fact, the introduction of ascorbic acid into a pyridine solution of chlorophyll does not affect its absorption spectrum. However, the formation of short-lived complexes, in which electron transfer occurs, is possible. On the other hand, Dain and his collaborators[28] observed a change in the absorption spectrum when chlorophyll reacts with metal ions, which, in the opinion of these workers, indicated the formation of long-lived complexes.

The formation of complexes with a molecule in an excited singlet state could probably be ascertained by measuring the half-life $\tau_{1/2}$ of the excited state of the pigment when an ascorbate or other hydrogen-donor is introduced; such experiments have evidently not been carried out.

Similar experiments could be made with pigments in the triplet state, the great chemical activity of which[71] is responsible for their tendency to form complexes.

The experiments of Evstigneev and Gavrilova[111] have shown that the rate of formation of the primary photoreduced form of phaeophytin is independent of temperature if the viscosity of the medium does not change greatly; if, however, the viscosity is increased by introducing glycerol, the rate of reaction falls. When the solution is frozen, no reaction takes place; these workers consider that kinetic

collisions are necessary for reaction to occur, and the existence of complexes long-lived is unlikely when not illuminated.

11. Mechanism of Photoreduction

Using Michaelis' hypothesis of a "one-electron" mechanism for oxidation–reduction reactions, we suggested[1] a scheme for the process consisting of the following stages:

1. Absorption of an electron by an excited (probably a metastable biradical) pigment molecule X from a hydrogen-donor molecule AH_2 with formation of a pair of radical-ions. We have pointed out the probability of the absorption of an electron by the cyclic system of conjugated double bonds of the pigment molecule (see scheme in Fig. 5):

$$X + AH_2 \rightleftharpoons {\cdot}X^- + {\cdot}AH_2^+; \quad {\cdot}AH_2^+ \rightleftharpoons {\cdot}AH + H^+.$$

2. Transfer of a proton combined with the molecules of the basic medium, with formation of a pair of radicals and subsequent formation of non-radical compounds:

$${\cdot}X^- + {\cdot}AH_2^+ \rightarrow {\cdot}XH + {\cdot}AH \rightarrow XH_2 + A.$$

The experimental results support the main features of this scheme.

A summary of the main experimental results (described in the preceding sections of the review), supporting this reaction mechanism, is given below.

Experimental results	Consequence
1. Reversible photoreduction is shown by all pigments having a porphin, chlorin, bacteriochlorin, or azaporphin structure (with H, Mg, or Zn at the centre of the molecule), independently of the nature of the side groups.	The existence in the pigment molecule of a cyclic system of conjugated double bonds linking the four pyrrole nuclei is an essential condition for reaction.
2. The basic strength of the medium in which reaction occurs strongly influences the spectral properties and the reactivity of the products of the photoreduction.	The various types of photoreduction products are linked by acid-base equilibria (proton transfer).
3. On illumination of a pigment–hydrogen-donor system the potential of an inert electrode becomes more negative and the electrical conductivity of the system increases.	Products which are ionic in character are formed, together with reactive photoproducts, which give up an electron to the electrode.
4. Illumination at low temperature inhibits the formation of stable photoreduction products; under these conditions the reversible primary process occurs rapidly, accompanied by the effects indicated in the previous paragraph.	The reaction includes a photochemical stage of electron transfer and a dark stage.
5. When deuterated hydrogen-donors are used, the dark stage of the reaction is slowed down.	The dark stage is associated with proton transfer.
6. On illumination, the pigment–hydrogen-donor system initiates the photopolymerisation of methyl methacrylate. During illumination of the pigment–hydrogen-donor system the appearance of an electron-paramagnetic-resonance spectrum is observed: this vanishes after the light has been switched off.	Reaction is accompanied by the formation of photoproducts which are of the nature of free radicals (or radical-ions).

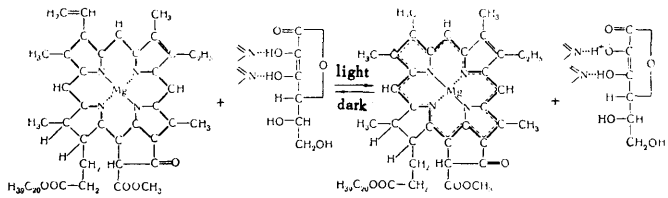


Fig. 5. Photoreduction of chlorophyll with ascorbic acid. The heavy line shows one of the possible cyclic paths of conjugation of the system of double bonds of the chlorophyll molecule. The dotted line shows the absorption of an electron by the system of conjugated bonds of an excited chlorophyll molecule; >N represents molecules of the basic medium, linked by a hydrogen bond to the reactant molecules.

When conditions are such that the dark stages of the reaction, associated with transfer of a proton (hydrogen), are inhibited, electron transfer is observed in its "pure" form. In media of different basic strength different protolytic equilibria are established. The dependence of the spectral properties of the photoproducts on the nature of the medium is especially clearly observed with the porphyrins, in which various stages of acid–base equilibrium are possible owing to the presence of acidic (carboxyl) and basic (pyrrole nitrogen) groups. The number of protons added to the molecule and the number of electrons transferred are not in general the same, and the photoproducts may be cationic or anionic in character, as can be seen from the marked differences in the properties of the photoreduced forms of haematoporphyrin in different media, from strong bases (piperidine) to strong acids (an aqueous solution of sulphuric acid).

Further studies of the structure of the paramagnetic-resonance bands of the photoproducts will probably make possible the location of the electron and the proton in the pigment molecule.

II. OXIDATION–REDUCTION REACTIONS PHOTOSENSITISED BY CHLOROPHYLL

When a chlorophyll–electron-donor–electron-acceptor system is illuminated with red light, a sensitised reduction of the acceptor molecules is observed, brought about by the light quanta absorbed by the chlorophyll[72]. This property is possessed by all the chlorophyll analogues studied (including magnesium-phthalocyanine) which can undergo photoreduction. With bacteriochlorophyll and bacteriophaeophytin the reactions are activated by near infra-red radiation[73,74].

The course of the reaction can be conveniently followed by means of spectrophotometric measurements in Tunberg vacuum tubes, either the change in optical density at the absorption maxima of the acceptors or the development of new maxima of the reduced compounds being observed.

Using ascorbic acid (E_0 = +0.05 V) as electron-donor, we observed the photoreduction of riboflavin (E_0 = -0.22 V), safranine T (E_0 = -0.3 V), and pyridine nucleotides (E_0 = = -0.32 V). When the light is switched off, the reverse reactions take place at a different rate, sometimes requiring the introduction of oxygen to regenerate the dye from the leuco form.

During these reactions the energy of the light is "stored", there being an increase in the free energy of the system, characterised by the value of ΔE_0 for the donor ($E_0 = +0.05$ V) and the acceptor ($E_0 = -0.32$ V) of the electron; a rough estimate of the stored free energy can be made: $\Delta F = 2 \times \times 23.06 \times 0.4 = 18.4$ kcal.

The comparative slowness of the reverse reactions in these processes is to be explained by the rapid dismutation of the semiquinones first formed to leuco compounds; during exposure the rate of dismutation is greater than the rate of the reverse reactions of the primary photoproducts.

We were able to observe the chlorophyll-sensitised oxidation–reduction of cytochrome *c* in aqueous medium [25,26]; in this reaction proteins (water ?) could serve as electron-donors. Photooxidation of the reduced cytochrome was carried out in the presence of atmospheric oxygen.

When azo dyes are employed as electron-acceptors, they undergo irreversible reduction; the azo dye methyl red has been used for a long time in reactions of this type [75,76]. However, reduction of methyl red by ascorbic acid and phenylhydrazine occurs slowly and in the dark, the reaction being very greatly accelerated by light.

In several sensitised reactions which occur under aerobic conditions, oxygen is the ultimate electron-acceptor. The chlorophyll-sensitised oxidation of several organic compounds has been known for a long time, but hitherto there has been no single view as to the mechanism of this process. The photooxidation of several hydrogen-donors, capable of the photoreduction of chlorophyll and phaeophytin, has been observed by means of Warburg's manometric method [77,78]. Probably in this case also, the dark stage of the reaction is in effect the regeneration of chlorophyll from the intermediate photoreduced form by oxidation with oxygen. The alternative mechanism of the primary photochemical formation of a peroxide of chlorophyll, which is reduced in the dark by the hydrogen-donor, is less probable, although we have detected labile photooxidation products of chlorophyll and bacteriochlorophyll, which slowly react with ascorbic acid to regenerate the pigment [17,79,80]. The photoreduction of chlorophyll by a hydrogen-donor is far more rapid than the photochemical reaction with oxygen.

That the sensitised reactions under investigation pass through a stage of reversible photoreduction of the sensitising pigment is suggested by the following facts:

1. Sensitised reduction is observed in a system containing hydrogen-donors which can take part in the photoreduction of chlorophyll.
2. Accumulation of the photoreduced forms of the pigments cannot be observed in a ternary system containing an electron-donor, a pigment, and an electron-acceptor, owing to the rapid reaction of the photoreduced forms with the acceptor molecules; only after the whole of the acceptor has undergone "sensitised" reduction does formation of the photoreduced form of the sensitising pigment begin. This is shown as an induction period in the sensitised reactions (cf. ref.21).
3. Finally, it is possible to carry out separately the photochemical stage of the reaction (formation of photoreduced forms of the pigment) and their dark reaction with the electron-acceptor molecules. In the case of chlorophyll this is illustrated by the experiments with the "red" photoreduced form [38], whereas the "primary" photoreduced forms react with acceptors far more rapidly, as was shown by Evstigneev's experiments [13]. Extremely significant facts are the effect of acceptors on the photopotential of an electrode in the chlorophyll–ascorbic acid system [28] and the "damping" effect of these compounds on the photoconductivity of this system [81], due to their rapid reaction with the photoreduced form of chlorophyll.

Thus experimental facts support the conclusion that the sensitised reactions occur via a stage of reversible photoreduction of chlorophyll and its analogues. The mechanism of sensitisation, established in experiments with chlorophyll, is similar to the scheme put forward by Weiss [82] more than twenty years ago for sensitising dyes which form leuco compounds.

In our laboratory a detailed investigation has been made of the part played by the photoreduced forms of pigments in sensitised reactions, but a more profound study is required of the role in these reactions of the "semi-oxidised" forms of electron-donor molecules of the type of monodehydroascorbic acid, the steady-state concentration of which is quite high, as is shown by electron-paramagnetic-resonance measurements.

These "semi-oxidised" compounds are evidently capable not only of the reverse reactions with the reduced forms of the pigment but also of further oxidation (to dehydroascorbic acid), by transferring their hydrogen to an acceptor — a molecule of oxygen, methyl red, etc. However, the limits of the reducing power of these compounds are obscure: they can hardly reduce systems having such a potential as high as that of safranine or the pyridine nucleotides.

Thus the mechanism of the transfer of hydrogen from a molecule AH_2 to a molecule B, sensitised by chlorophyll or its analogues, can be represented by the following scheme:

1. Photoreduction:

$$X + AH_2 \rightleftharpoons \cdot X^- + \cdot AH_2^+ \rightleftharpoons \cdot XH + \cdot AH.$$

2. Dark stages of the reaction with the hydrogen-acceptor (electron-acceptor) B; regeneration of chlorophyll from photoreduced forms:

$$\cdot X^- + B \rightarrow X + \cdot B^-; \quad \cdot B^- + H^+ \rightarrow \cdot BH.$$

$$\cdot XH + B \rightarrow X + \cdot BH; \text{ possibly also } \cdot AH + B \rightarrow A + \cdot BH.$$

3. Dismutation of semiquinone forms:

$$2 \cdot BH \rightarrow B + BH_2.$$

Overall process:

$$AH_2 + B + (X) \rightarrow A + BH_2 + (X).$$

Such a reaction mechanism has received direct experimental verification in solution, but with heterogeneous sensitisation on pigment crystals and in colloidal solutions it is extremely difficult to separate the individual stages of the reaction occurring at the phase boundary. It may be assumed that the elementary steps of electron transfer from acceptor to pigment molecule (hole") at the phase boundary and the compensating transfer of an electron to the acceptor molecule are retained here too. We have indicated the possibility of such a mechanism in studying the photocatalytic action of heterogeneous sensitisers [83,84]. The experiments of Evstigneev and Terenin on the photopotentials of solid films of pigments in aqueous media also suggest this [85].

III. PHOTOCHEMICAL REACTIONS OF COLLOIDAL SOLUTIONS OF CHLOROPHYLL

Reactions of the chlorophyll molecule in organic solvents have been described above; we shall now examine the properties of chlorophyll in aggregated forms, in aqueous colloidal solutions.

It has long been known that such colloids are formed by running solutions of chlorophyll (in alcohol, acetone, dioxan, pyridine) into an excess of water or of a buffer solution. We have found that colloidal solutions of chlorophyll are obtained also by the direct dissolution in water of a chromatographic band of chlorophyll adsorbed on sugar. The position of the absorption maximum and the particle size depend on the type of solvent and the pH of the aqueous medium; at pH 4–5 coarser particles are formed having maximum absorption at 690 mμ, and at pH 8–9 extremely stable colloids result with a maximum at 670–672 mμ [86]. In contrast to these systems, we have found that colloids obtained by running solutions of chlorophyll into aqueous solutions of detergents (anionic, cationic, and non-ionic) possess a bright fluorescence, an absorption maximum at 669–670 mμ, and photochemical properties similar to those of chlorophyll in solution [77,87]. This is probably due to the fact that the pigment molecules are arranged in the form of a monolayer on the detergent micelle, i.e. they are in a dispersed form.

Colloidal solutions of chlorophyll in water possess a photosensitising action, which is most marked if the ultimate electron-acceptor is reduced irreversibly, as occurs with oxygen and azo dyes (methyl red). In some experiments the polymerisation of acrylamide was observed on illuminating aqueous colloidal solutions of chlorophyll with red light and in a vacuum.

In contrast to the comparatively feeble activity of these systems, colloids obtained using detergents have a strong sensitising action, as great as that of chlorophyll in solution. Doubtless the fundamental mechanism of the process in solution is retained here. The photosensitising action of coagulated colloidal chlorophyll has been described in recent papers from our laboratory [88].

IV. PHOTOCHEMICAL PROPERTIES OF CHLOROPHYLL IN THE GRANULES OF CHLOROPLASTS

The properties of the chlorophyll in granules were studied in viscous aqueous glycerol media, which permitted accurate spectrophotometric measurements as a result of their transparency and their "colloidal" stability. Such systems permit work to be carried out also at low temperatures.

Such "green solutions" of leaves of the sugar beet containing an ascorbate showed on illumination small reversible changes (up to 3%) in the optical density in the red absorption maximum of chlorophyll, which are attributable to reversible photoreduction; changes to more negative values were also observed in the photopotential of an inert electrode. With a Beckman spectrophotometer we were unable to detect any definite changes in optical density at 520 mμ. In the absence of ascorbate, effects of smaller magnitude were observed. Such reversible photochemical changes in homogenates of green leaves are to be ascribed to the "monomeric" form of chlorophyll, which has closely similar properties to those of chlorophyll in solution [86,89,90].

It is well known that in an "oxidising" medium homogenates of leaves show Hill's reaction, i.e. evolution of oxygen coupled with reduction of the oxidising agents — Fe^{3+}, quinones, etc. In a "reducing" medium, in the presence of sodium ascorbate, homogenates of leaves are capable of reversible photosensitised reduction of safranine and other compounds and also of irreversible reduction of oxygen and methyl red [91].

Leaves of plants possessing a large amount of the rapidly decolorised monomeric form are more active in reactions of this type; e.g. leaves of the sugar beet are more active than leaves of the bean.

The biological role of the different forms of chlorophyll and the part they play in the reactions of photosynthesis is not yet clear. It has been estimated that the transfer of a single electron from water to carbon dioxide requires about two quanta of red light, and this may possibly require two types of photochemical reactions with a reserve of intermediate compounds between them. Different types of reactions are possibly associated with different forms of chlorophyll. In any case, the ability of a chlorophyll molecule to take up an electron when illuminated is retained in the "collective" of molecules in aggregated forms. The transfer of energy between the pigment molecules in such aggregates (in the granules of plastids) can probably occur in the form of a "hole" and an unpaired electron, and at the level of the triplet state, e.g. by the mechanism suggested by Terenin and Ermolaev [92,93].

V. REVERSIBLE PHOTOCHEMICAL CHANGES UNDERGONE BY CHLOROPHYLL IN THE LIVING PLANT LEAF

Special methods are required for recording the rapid reversible changes undergone by chlorophyll during the photochemical reactions occurring in the living leaf. Techniques used for this purpose at present are differential spectrophotometry, and flash photolysis, fluorescence, and electron paramagnetic resonance.

Coleman et al. [94] were able to observe reversible changes in the red absorption maximum of chlorophyll in a suspension of *Chlorella*, and also in the vicinity of 520 mμ, which can be attributed to reversible photoreduction. In a subsequent paper Coleman and Rabinowitch [95] pointed out the similarity in the differential spectra after illumination of a suspension of *Chlorella* and after the photoreduction of chlorophyll with ascorbic acid; arguments are given for the occurrence of the reversible photoreduction of chlorophyll in living organisms. The fact requires explanation, however, that in the photoreduction of chlorophyll the ratio of the fall in optical density in the red absorption maximum ($\Delta D_{670\ m\mu}$) to the increase in density in the maximum of the photoreduced form ($\Delta D_{520\ m\mu}$) has a value of 2–3, whereas in *Chlorella* cells this ratio hardly reaches unity. In Witt's experiments [96], in which a flash technique was employed, the fall in the optimum density in the red absorption maximum corresponded to the reversible fall in the red fluorescence of chlorophyll and the appearance of a maximum at 520 mμ.

The appearance of this maximum after living leaves have been illuminated was first discovered by Duysens [97], and subsequently studied by Witt [96], Bell [98], and other workers. The agreement between this maximum and the absorption of the "red" photoreduced form of chlorophyll is remarkable.

The experiments of Osipova and Timofeeva [99] have shown a high content of basic amino-acids in the proteins of the granules; on the basis of these results, Rackow and Konig [9] have pointed out the possibility of detecting the photoreduced form of chlorophyll in Witt's experiments. It is not clear,

however, whether the ratio between the fall in optical density in the red absorption of chlorophyll and in the maximum at 520 mμ, observed during photoreduction, is maintained in these experiments. Clearly, the conditions of acid–base equilibrium in chloroplasts may differ greatly from those in pyridine solutions, and reversible photoreduction of chlorophyll may occur also without the accumulation of the "red" form[91].

Although the nature of the absorption maximum appearing at 515–520 mμ in leaves cannot yet be regarded as cleared up, it is highly probable that the reversible change in the red absorption maximum of chlorophyll can be ascribed to photoreduction.

Use of fluorescence spectroscopy will probably be of great help in ascertaining the nature of the changes undergone by chlorophyll in leaves. Measurements by Litvin[100] have shown the presence in living green leaves of fluorescence maxima within the range of the photoreduced forms of chlorophyll, but it is still difficult to interpret these results. It is also necessary to determine whether the phenomenon of a separate stage of photoreduction of chlorophyll is connected with the appearance of the paramagnetic-resonance bands observed by Commoner[101] and later by Calvin and his collaborators[102].

The phenomenon of chemisynthetic luminescence, observed in the leaves of plants, suspensions of chloroplasts, and solutions of chlorophyll, indicates that similar photoproducts may be formed in all these cases.

VI. REVERSIBLE PHOTOREDUCTION AND POSSIBLE MECHANISM OF THE PARTICIPATION OF CHLOROPHYLL AND ITS ANALOGUES IN PHOTOBIOLOGICAL PROCESSES

The above account has shown that chlorophyll and porphyrin pigments in general have the characteristic ability to undergo reversible photochemical reduction, the reversible absorption of an electron (or hydrogen) from donor molecules. We have already put forward the hypothesis that this may play a part in separate stages of the participation of chlorophyll and its analogues in the reactions of photosynthesis and other photobiological phenomena.

1. Photosynthesis[112]

The work reported above has established that the photosynthesising action of chlorophyll in reactions involving the photochemical transfer of hydrogen (or an electron) is determined by its ability to undergo reversible photoreduction.

In the actual photochemical step an excited pigment molecule takes up an electron (hydrogen) from the donor molecule, and in the dark stage it transfers the electron to the acceptor molecule. These results make it possible to set up the working hypothesis that during photosynthesis chlorophyll also takes part in the reversible transfer of an electron (raising it above the level of the reduced pyridine nucleotides), in agreement with several investigations which have shown the possibility of the photoreduction of pyridine nucleotides in suspensions of chloroplasts and in the leaves of plants[103–105].

The suggestion that chlorophyll takes part in an electron-transfer process with the intermediate formation of free radicals accords with the results of measurements of electron-paramagnetic-resonance in chloroplasts and in leaves[101,102] and with observations on the presence of an extremely feeble long-lived afterglow (chemiluminescence) after leaves have been illuminated[66].

The experimental results which have now accumulated indicate that the operation of the cycle of enzymic reactions for the reduction of carbon dioxide necessitates the photochemical formation of reduced pyridine nucleotides and energy-rich phosphate esters.

The photochemical transfer of an electron (hydrogen) "upward" to the level of reduced pyridine nucleotides makes possible the operation of the carbon dioxide assimilation cycle according to Calvin's scheme[106]. The formation of the energy-rich phosphate esters required for the functioning of the carbon dioxide reduction cycle occurs, according to the work of Arnon[107], by anaerobic photosynthetic phosphorylation, in which adenosine triphosphate is formed from adenosine diphosphate and inorganic phosphate by oxidation–reduction processes — dark reactions activated by photoproducts, involving biocatalytic systems — cytochromes, naphthoquinone derivatives (vitamin K), etc. Thus the "downward" drop of the photochemically mobilised electron makes possible phosphorylation and the "storage" of energy in adenosine triphosphate.

2. Photodynamic Action of Porphyrins

The basis of this phenomenon is the dye-photosensitised photooxidation of metabolites or proteins of importance in vital processes, leading to the haemolysis of erythrocytes and a series of far reaching disturbances of the normal functioning of organisms[108]. The mechanism of the primary photochemical process underlying the photodynamic effect is not yet clear. The investigations of the mechanism of the photosensitising action of porphyrins discussed here provide a basis for the hypothesis that the primary stage in this process is photoreduction of the pigment. Thus our work has established the ability of porphyrins to undergo reversible photoreduction by dienols, cysteine, and several other organic compounds.

Oxidation of the photoreduced forms of porphyrins by oxygen or other oxidising agents leads to the reverse process of the regeneration of the original pigment molecule. In this process, as in the case of chlorophyll, the radical-ion forms of the pigment are the most active. It may be assumed that the mechanism of the separate steps in the photodynamic effect corresponds to the scheme of reactions photosensitised by porphyrins given above (see Part II); AH_2 represents a molecule of the metabolite undergoing oxidation (a protein amino-acid, etc.), and X a molecule of the porphyrin pigment.

3. Other Photobiological Reactions

Study of various photobiological processes — photoperiodism, germination of seeds under the influence of light, etc. — in the work of Bortwick, Hendricks, et al.[109] led to the discovery of a maximum in the activating spectrum at 650 mμ; the effect of red light was cancelled by subsequent illumination with an effective spectral maximum at 740 mμ. These observations led the authors to the hypothesis that a pigment system is present, which undergoes reversible photochemical changes with the formation of photoproducts possessing absorption maxima at 650 and 740 mμ.

It has not yet proved possible to find transitions of this type in pigments in plants. We have already pointed out above that the reversible photoreduction of porphyrins can be observed, leading to the formation of photoproducts having maximum absorption at 740 mμ; it is significant that infra-red irradiation accelerates the reverse reaction of these photoproducts[110].

It is possible that the reversible formation of photoreduction products of porphyrin pigments may play a part also in several photobiological processes of this type.

CONCLUSION

Chlorophyll and its analogues and derivatives undergo reversible photochemical reduction. During this reaction photoproducts which store the light energy are formed; in the dark stage of the process these photoproducts undergo the reverse reactions, leading to the formation of the original pigment molecule. The ability of chlorophyll and its analogues to undergo reversible photoreduction underlies the photosensitising action of these pigments in oxidation–reduction reactions.

The experimental results obtained provide a basis for the working hypothesis that the ability of chlorophyll and its analogues to undergo reactions involving photochemical electron (or hydrogen) transfer may be linked with the part played by these pigments in photobiological processes.

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PHYSICOCHEMICAL PROPERTIES OF CHLORINE TRIFLUORIDE

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INTRODUCTION

Elemental fluorine can now be made in large quantities and this has facilitated the study of the physical and chemical properties of a number of inorganic fluorides. Among these halogen fluorides are of great theoretical and practical interest. Emeléus and Woolf [1], have shown that the halogen fluorides are ionising solvents which dissociate according to the equation

$$2MF_n \rightleftharpoons MF_{n-1}^{+} + MF_{n+1}^{-}.$$

The physical and chemical properties of the halogen fluorides can be explained by considering them as self-ionising solvents. The great experimental difficulties associated with the handling of these highly reactive compounds did not permit reliable determinations of their physical constants until recently. Table 1 gives some physical properties of known halogen fluorides (figures for elemental fluorine are given for comparison) [2-41].

All the halogen fluorides are powerful fluorinating agents and it is interesting to consider their chemical properties from this point of view. The compounds can be arranged in the following order of reactivity: $ClF_3 > BrF_5 > IF_7 > ClF > BrF_3 > IF_5 > BrF$, although in a number of cases the characteristics of the halogen fluorides as fluorinating agents are known only qualitatively. Chlorine trifluoride is the most reactive of the known halogen fluorides.

The halogen fluorides are of practical importance as fluorinating agents in the preparation of a number of higher fluorides [21-24] and especially of uranium hexafluoride, used in the regeneration of nuclear fuel [25-28]. Complete combustion of the fuel is not achieved in atomic reactors. Consequently several combustion cycles with intermediate regeneration cycles are necessary for its complete utilisation. Fractional distillation is the most suitable method for regenerating nuclear fuel because the technology of this process has been well developed and uranium is regenerated