PHYSICOCHEMICAL ASPECTS OF SOME RECENT WORK ON PHOTOSYNTHESIS

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Much of our present knowledge of the photochemistry of chlorophyll has been acquired during the last ten years. No other substance has had its photochemical properties studied so broadly and intensively.[1] In spite of the effort which has gone into these studies, there are obvious gaps in our factual knowledge of its spectroscopic and photochemical properties and the interpretation of these facts is in part arbitrary and uncertain. Interpretation is rendered difficult by the complexity of the problem. Chlorophyll appears to exhibit every known type of radiative and non-radiative transition between its several electronic states and to undergo or sensitise practically all of the various types of photochemical reactions which have been observed for all other polyatomic molecules. From the viewpoint of photochemistry, chlorophyll is an extraordinarily versatile compound. Its photochemistry is interesting, not only intrinsically, but also because of its relationship to the primary act of photosynthesis. The same amount of study scattered amongst dozens of compounds would have contributed much less to the progress of the photochemistry of polyatomic molecules than has the intensive investigation of this one substance.

Changes in the environment of chlorophyll molecules profoundly affect their spectroscopic properties. Measurements have been made with chlorophyll in dilute and in concentrated solutions, with microcrystals, with unimolecular films, and when adsorbed on solid surfaces. Even in dilute solutions, the spectroscopic properties of chlorophyll are strongly influenced by the nature of the solvent and by the temperature. In more concentrated solutions, new phenomena appear, presumably, owing to the intermolecular transfer of energy of excitation and to the reversible formation of dimers. Microcrystalline chlorophyll is non-fluorescent and exhibits photoconductivity and related effects.

There is much more information available about dilute solutions of chlorophyll than about its other states of aggregation. In the plant cell, the pigment is present in a highly concentrated and partially ordered condition. However, it is not unreasonable to treat chlorophyll present under these conditions as having properties which are modifications of those observed in dilute solution. For these several reasons, emphasis in this discussion will be placed upon the studies of dilute solutions. Material which was published before 1956 has been exhaustively reviewed by E. Rabinowitch[1] in his three-volume monograph on photosynthesis.

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[1] Rabinowitch, "Photosynthesis," Vol. I (1945), Vol. II, Part I (1951), Vol. II, Part II (1956), Interscience Publ., New York.

In the present article, we shall attempt to discuss in detail the results of studies which have appeared in the last five years and to outline those aspects of older studies which are essential to an understanding of the general problem.

Molecular Structure

Chlorophyll is a substituted, magnesium-complexed, dihydroporphin. This pigment occurs in Nature either as chlorophyll *a*, whose structural formula[2] is given in Fig. 1, or as chlorophyll *b* in which a carbonyl group is substituted for the methyl group marked with an asterisk. In acid solutions, chlorophyll is readily hydrolysed; the magnesium being replaced by two hydrogen atoms. This compound is called phæophytin. The spectroscopic and photochemical properties of chlorophyll are markedly influenced by the presence of the magnesium atom and of the cyclopentenone ring (v). In homogeneous solutions, the phytol group, $C_{20}H_{39}$, has little or no effect upon the properties of the molecule, although it undoubtedly plays an important role in determining the spatial arrangement of the pigment molecules in the chloroplast.

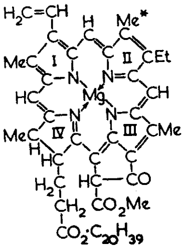

FIG. 1. *The structural formula of chlorophyll* a.

There are two other naturally occurring chlorophyll-like pigments whose photochemical properties have been extensively studied. One of these, protochlorophyll, is the precursor of chlorophyll in green plants. Chemically, it is similar to chlorophyll *a*, except that it contains two less hydrogen atoms, none of the pyrrole rings being reduced. The other, bacteriochlorophyll, occurs in certain photosynthetic bacteria. It is a tetrahydroporphin, ring II, as well as ring IV, being reduced. In place of the red (660 mμ) absorption of chlorophyll *a*, its ethereal solutions absorb strongly in the near infrared (770 mμ).

In addition to these four magnesium-containing compounds and their phæophytins, photochemical studies have been made of some porphyrins, of tetraphenylporphin, and of phthalocyanine. Both the metal-free compounds and their complexes with various metals have been studied. While there are important individual differences, the photochemical properties of these several compounds are in general similar.

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[2] Ref. 1, pp. 438—448.

Absorption Spectra

Detailed knowledge of the absorption spectra of chlorophyll has been of value in the determination of the nature of its several electronic states. Spectrophotometric measurements are used in a routine way to measure the concentration of solutions and to estimate the purity of samples of chlorophyll and its derivatives. The first definitive spectra of dilute solutions of chlorophylls *a* and *b*, in the visible and near ultraviolet, were published by Zscheile and Comar in 1941.[3] Subsequent measurements,[4] using highly purified samples, have necessitated only minor modifications of these spectra.

Attempts have been made to calculate the energy levels of porphin and dihydro- and tetrahydro-porphins by means of the free-electron model[5] and the LCAO-MO approximation.[6] Although the agreement between observed and calculated energy levels is moderately good, the calculations scarcely permit an unambiguous interpretation of the nature of the several excited states.

The infrared spectra of the chlorophylls and of their derivatives and analogues have been measured.[4b,7] Holt[7c] has demonstrated that these spectra are particularly useful in identifying the changes in the substituent groups which occur when chlorophyll is allomerised, etc. The corresponding changes in the electronic spectra are more difficult to interpret and in some cases are scarcely detectable. For example, the visible and ultraviolet absorption spectra of chlorophyll *a* and of the corresponding chlorophyllide (in which the phytol "tail", $C_{20}H_{39}$, is replaced by an ethyl radical) are practically identical.[8]

The metal-complexed porphyrins and chlorins (including chlorophylls *a* and *b*) form stable monosolvates with nucleophilic reagents (*i.e.*, Brönsted bases) such as water, alcohols, amines, ketones, and ethers.[9] The ordinary absorption spectra of the chlorophylls are those of the solvates. The principal absorption bands of the unsolvated compounds appear broader,[10] owing to the superposition of new bands shifted toward the red.[11] For example, in dry benzene the normal maximum of chlorophyll *b* at 6450 is accompanied by a second peak at 6650 Å. Freed and Sancier have proposed[11a] an interesting interpretation of their results; however,

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[3] Zscheile and Comar, *Bot. Gaz.*, 1941, **102**, 463.

[4] (*a*) Smith and Benitez, *Carnegie Inst. Year Book*, 1954, **53**, 168; (*b*) Stoll and Wiedemann, *Helv. Chim. Acta*, 1959, **42**, 679.

[5] Ref. 1, pp. 1793—1798.

[6] (*a*) Platt, in "Radiation Biology", Vol. III, McGraw-Hill, New York, 1956, pp. 94—114; (*b*) Matlow, *J. Chem. Phys.*, 1955, **23**, 673.

[7] (*a*) Ref. 1, pp. 1811—1815; (*b*) Holt and Jacobs, *Plant Physiol.*, 1953, **30**, 553; (*c*) Holt, *Canad. J. Biochem. Physiol.*, 1958, **36**, 439.

[8] Holt and Jacobs, *Amer. J. Bot.*, 1954, **41**, 710.

[9] Livingston and Weil, *Nature*, 1952, **170**, 750.

[10] Livingston, Watson, and McArdle, *J. Amer. Chem. Soc.*, 1949, **71**, 1542.

[11] (*a*) Freed and Sancier, *J. Amer. Chem. Soc.*, 1954, **76**, 198; (*b*) Freed, *Science*, 1957, **125** 1248.

no reasonable explanation has been suggested which is compatible with all of the reported observations.

In 1952 Jacobs and Holt[12] published an outline of a procedure for the preparation of crystals of chlorophylls *a* and *b*. Subsequent experiments[4b,13] have confirmed the validity of the first report. Relative to the solution spectra, the principal absorption maxima of suspensions of microcrystals are shifted strongly to the red, the shift being about 80 mμ for the red band of chlorophyll *a*. This is much greater than any known solvent effect. The absorption spectra of colloidal chlorophyll and of chlorophyll *in vivo* resemble more nearly the spectra of solutions than those of the crystalline chlorophyll. Jacobs has attempted to explain the marked effect of crystallisation upon the wavelengths of the maxima in terms of Heller and Marcus's theory[14] of the excitation of virtual dipoles in an infinite isotropic lattice. Unimolecular films of chlorophyll have been prepared[13b] which exhibit the absorption spectra of either amorphous or crystalline chlorophyll, depending upon their surface concentration.

The Electronically Excited States of the Monosolvates

The photochemical reactions of polyatomic molecules are determined largely by the properties of their electronically excited states. The more important of these properties are the available energy, the intrinsic mean life, the maximum yield of fluorescence (or phosphorescence), and the probabilities of chemical reactions of the molecule in each of its excited states. These characteristic properties are influenced by the temperature and by the nature of the solvent.

The absorption of visible light by chlorophyll corresponds to a transition from the ground to the first (or second) excited singlet (*Q*) state. Molecules in their first excited (*i.e.*, fluorescent) states have a relatively large probability of undergoing radiationless transitions to their ground triplet state, which is known to play an important role in photochemistry. In addition to these π–π (or *Q*) states, *n*–π (or *U* or *W*) states[6a] are probably important in determining the spectroscopic and photochemical properties of chlorophyll and related compounds.

The formation of a monosolvate[10] has a marked effect upon the spectroscopic properties of chlorophyll, probably by changing the energy of its *n*–π states, relative to the corresponding π–π states.[6a] Chlorophyll dissolved in ordinarily pure, but not-especially dried solvents exists principally as a monosolvate. Since the great majority of measurements have been made with such "wet" solutions, it will be convenient to discuss them separately. The differences between the properties of the solvated and non-solvated molecules are described and interpreted in a later section of this Review.

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[12] Jacobs and Holt, *J. Chem. Phys.*, 1952, **20**, 1326.

[13] (*a*) Jacobs, Vatter, and Holt, *Arch. Biochem. Biophys.*, 1954, **53**, 228; (*b*) Zill, Colmano, and Trurnit, *Science*, 1958, **128**, 478.

[14] Heller and Marcus, *Phys. Rev.*, 1951, **84**, 809.

The Fluorescent State.—For chlorophyll, as for practically all polyatomic molecules, the maximum intensity of fluorescence is situated at the long-wavelength side of the first absorption peak. The overlap between the absorption and emission bands is greater for chlorophyll than it is for most dyes and pigments. The red absorption of chlorophyll *a* has a maximum at 6600 Å and extends to 7000 Å. The corresponding fluorescence peak is at 6645 Å, and fluorescence emission is detectable at wave lengths as short as 6300 Å. Except for thin layers of very dilute solutions, reabsorption greatly distorts the appearance of the fluorescence spectrum.[15]

The energy corresponding to the zero–zero transition from the ground to the fluorescent state can be estimated by comparing the absorption and emission spectra. It is approximately 43 kcal./mole.

The quantum yield of fluorescence, ϕ_{fl}, of a solution is the ratio of the number of quanta emitted to the number absorbed. Except for very dilute solutions, the quantum yield is reduced by reabsorption and self-quenching. The most recent and probably the most reliable measurements of the maximum values for the quantum yields of fluorescence were published by Weber and Teale[16] in 1957. Some of their results are listed in Table 1.

TABLE 1. *Maximum quantum yields of fluorescence*

Solvent	λ(mμ) of exciting light	Fluorescent yield, ϕ_{fl}: chlorophyll *a*	chlorophyll *b*
Benzene*	Hg 366	0·33	0·11
Benzene	Cd 644	0·32	0·12
Ethyl ether	Cd 644	0·32	0·12
Acetone	Cd 644	0·30	0·09
Ethanol	Cd 644	0·23	0·10
Methanol	Cd 644	0·23	0·10
Cyclohexanol	Cd 644	0·30	

* Presumably "wet" benzene was used, since chlorophyll is not detectably fluorescent in carefully dried hydrocarbons.[10]

With the exception of the values for methanolic (and probably ethanolic) solutions, these data are consistent with the earlier *corrected* values of Forster and Livingston[17] and the *uncorrected* values of Latimer, Bannister, and Rabinowitch.[18] The correction referred to above was proposed by Shepp[19] to take care of the reflection of the fluorescent light at the boundary of the samples. Although some such correction should certainly be applied to Forster and Livingston's data, and presumably to those of Latimer *et al.*, the 1·20 factor proposed by Shepp may be too large. In

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[15] Ref. 1, pp. 603—609, 740—747.

[16] Weber and Teale, *Trans. Faraday Soc.*, 1957, **53**, 646.

[17] Forster and Livingston, *J. Chem. Phys.*, 1952, **20**, 1315.

[18] Latimer, Bannister, and Rabinowitch, *Science*, 1956, **124**, 585.

[19] Shepp, *J. Chem. Phys.*, 1956, **25**, 579.

contrast to the results of Weber and Teale, both of the earlier sets of measurements indicate that the fluorescence yield of chlorophyll *b* is about two-fold less in methanol than in ether but the yields for chlorophyll *a* are identical in these two solvents. Tumerman,[20] who used a mixture of chlorophylls *a* and *b* (probably chiefly component *a*), reports quantum yields 5% greater for ethanol than for either acetone or ether. The cause of this disagreement is not known. Apart from this discrepancy, the authors' estimate[16] of an uncertainty of ±10% for the quantum yields appears reasonable. For phæophytin *a* in benzene, the quantum yield is 0·18 ± 0·02.

The intrinsic mean life time, τ^0, of an excited state is the value which the mean life would have if the excited molecule could lose its energy only by emitting a photon of fluorescent light. The actual mean life, τ, of the state is equal to the product of the intrinsic life and the quantum yield: $\tau = \phi_{fl}\tau^0$. τ and ϕ_{fl} vary with the temperature and the nature of the solvent but their ratio, τ^0, is little influenced by these factors. τ^0 can be calculated from the integrated extinction coefficients, corresponding to the transition from the ground to the fluorescent state, by means of the modified Ladenburg equation.[21] The actual mean life can be determined directly by measuring[22] the decreasing intensity of fluorescence as a function of time, following excitation by a very brief pulse of light. When the decay of the excited state follows a first-order law, it is more convenient to measure τ by the phase-shift method.[23] It can also be evaluated indirectly from measurements of the degree of polarisation of fluorescence as a function of the viscosity of a series of solvents.[24] The mean life of the fluorescent state of chlorophyll has been evaluated by all of these methods and results representative of the several types of measurements are presented in Table 2.

TABLE 2. *Actual mean life time of the fluorescent state*

Pigment	Solvent	$\tau \times 10^9$ (sec.)	Method	Ref.
Chlorophyll *a*	Ethyl ether	5·1	Direct	22
Chlorophyll *a*	Ethyl ether	4·5—6·3*	Landenburg	22
Chlorophyll *a*	Ethyl ether	5·1	Phase shift	25
Chlorophyll *a*	Toluene (wet?)	4·4	Phase shift	25

* These values were obtained from the calculated values of τ^0 by using $\phi_{fl} = 0{\cdot}30$ for chlorophyll *a* and $\phi_{fl} = 0{\cdot}13$ for chlorophyll *b*. The indicated spread of values is the result of the uncertainty as to what range of frequencies corresponds to this electronic transition.

[20] Tumerman, *Doklady Akad. Nauk S.S.S.R.*, 1957, **117**, 605; *Soviet Physics*, 1957, **2**, 525.

[21] Förster, "Fluoreszenz organischer Verbindungen," Vandenhoeck u. Ruprecht, Göttingen, 1951, pp. 156—159.

[22] Brody and Rabinowitch, *Science*, 1957, **125**, 555.

[23] Rollefson and Bailey, *J. Chem. Phys.*, 1953, **21**, 1315.

[24] Ref. 21, pp. 168—172.

[25] Dmitrievsky, Ermolaev, and Terenin, *Doklady Akad. Nauk S.S.S.R.*, 1957, **114**, 751.

TABLE 2—*continued*

Pigment	Solvent	$\tau \times 10^9$ (sec.)	Method	Ref.
Chlorophyll *a*	Benzene (wet?)	7·8	Direct	22
Chlorophyll *a*	Cyclohexanol	6·5	Polarisation	26
Chlorophyll *a*	Pyridine	5·3	Phase shift	25
Chlorophyll *a*	Ethanol	5·0	Phase shift	25
Chlorophyll *b*	Ethyl ether	3·9	Direct	22
Chlorophyll *b*	Ethyl ether	3·0—4·2*	Ladenburg	22
Chlorophyll *b*	Ethyl ether	3·0	Phase shift	25
Chlorophyll *b*	Toluene (wet?)	4·5	Phase shift	25
Chlorophyll *b*	Benzene (wet?)	6·3	Direct	22
Chlorophyll *b*	Pyridine	4·7	Phase shift	25
Chlorophyll *b*	Ethanol	3·4	Phase shift	25
Chlorophyll *a* + *b*	Ethanol	5·3	Phase shift	20
Chlorophyllide *a*	Ethyl ether	5·1	Phase shift	25
Phæophytin *a*	Ethyl ether	5·3	Phase shift	25
Phæophytin *a*	Toluene (wet?)	5·6	Phase shift	25

The Triplet State.—The existence of a long-lived energetic form of chlorophyll has been unequivocally established from photochemical kinetic evidence and from a comparison of the quantum yields of fluorescence with the yields of certain autoxidations, photosensitised by chlorophyll. Presumably this form of chlorophyll is the long-lived species whose properties can be studied by means of the photoflash technique and which is responsible for the weak phosphorescence exhibited by chlorophyll *b* in rigid solvents at low temperatures. It is commonly assumed that this species is the lowest triplet state. Although this identification has not been proved for chlorophyll, in the remaining discussion we shall assume that it is correct.

At low temperatures in glassy solvents, most aromatic compounds are strongly phosphorescent. Chlorophyll is an exception to this rule. Earlier reports of phosphorescence of chlorophyll *a* were in error: no phosphorescence of dilute solutions of this compound has been detected, although it has been sought for by several investigators. Chlorophyll *b* phosphoresces weakly[27] in ether–pentane–ethanol mixtures at liquid-nitrogen temperatures. The emission has its maximum intensity at about 8650 Å; its mean life is approximately 0·03 sec., and its quantum yield is less than 0·01. It is noteworthy that Cu-phæophorbide *a*, which is non-fluorescent, phosphoresces strongly in low-temperature, rigid solvents.

The absorption spectrum of the triplet and the kinetics of its decay can be measured (at ordinary temperatures in fluid solvents) by the use of

[26] Weil, Doctoral Diss., University of Minnesota, 1952.

[27] Becker and Kasha. *J. Amer. Chem. Soc.*, 1955, 77, 3669

flash-photolytic apparatus. The photochemical formation of the triplet state of chlorophyll is an efficient process. For example, over 90% of the chlorophyll molecules present in 15 ml. of a 2×10^{-6}M-solution, in pyridine, are raised to their triplet state by a single 50-Joule flash of a few microseconds' duration. Absorption spectra of such solutions have been obtained both by the photographic[28-30] and by the photoelectric[31] modifications of the photolytic-flash technique. The triplet spectrum (Fig. 2) was obtained by Linschitz and Sarkanen,[31] using the photoelectric method. The other available data,[28-30] while probably less reliable, are in satisfactory agreement with these curves. Unlike the corresponding spectra of the aromatic hydrocarbons, the absorption spectra of the triplet states of chlorophyll and related compounds[29,32] are broad and relatively

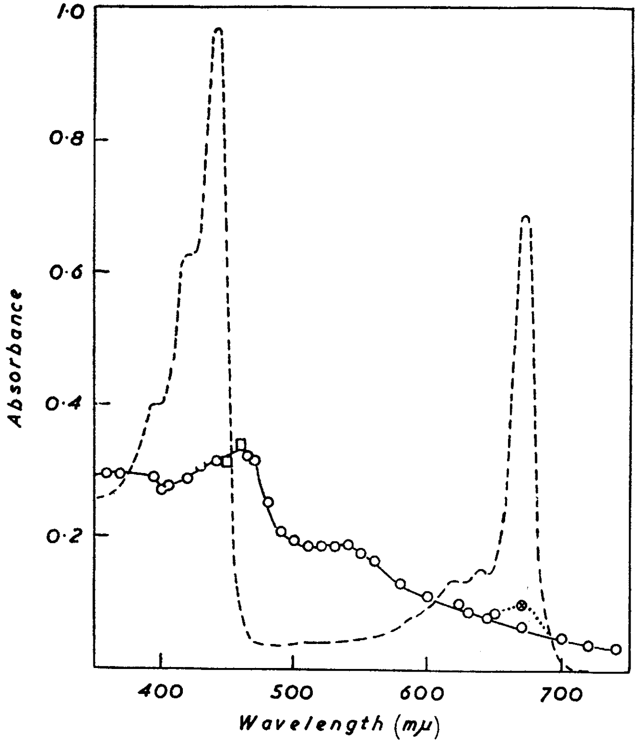

FIG. 2. *The absorption spectra of chlorophyll* a *in its triplet and in its ground state.* (Reproduced, by permission, from *J. Amer. Chem. Soc.*, 1958, **80**, 4826.)

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[28] Livingston, *J. Amer. Chem. Soc.*, 1955, 77, 2179.

[29] Livingston and Fujimori, *J. Amer. Chem. Soc.*, 1958, **80**, 5610.

[30] Claesson, Lindquist, and Halmström, *Nature*, 1959, **183**, 661.

[31] Linschitz and Sarkanen, *J. Amer. Chem. Soc.*, 1958, **80**, 4826.

[32] Linschitz and Pekkarinen, *J. Amer. Chem. Soc.*, in the press.

structureless, extending into the near infrared. Their principal maxima occur at the long-wavelength edge of the Soret bands (of the singlet state).

The disappearance of the triplet state of chlorophyll *a* or *b*, in dilute fluid solutions, conforms to the following equation. It is not a simple first order process:

$$-\mathrm{d}[\mathrm{GH'}]/\mathrm{d}t = k_\mathrm{A}[\mathrm{GH'}] + k_\mathrm{B}[\mathrm{GH'}]^2 + k_\mathrm{C}[\mathrm{GH}][\mathrm{GH'}]$$

Table 3 summarises the available data.

TABLE 3. *Rate constants for the decay of the triplet state of chlorophyll*

Pigment	Solvent	$k_\mathrm{A} \times 10^2$ (sec.$^{-1}$)	$k_\mathrm{B} \times 10^9$ (l. mole^{-1} sec.$^{-1}$)	$k_\mathrm{C} \times 10^7$ (l. mole^{-1} sec.$^{-1}$)	Ref.
Chlorophyll *a*	Pyridine	7·3	1·3	2	33
Chlorophyll *a*	Pyridine	6·7	1·5	2	31
Chlorophyll *a*	Benzene	4·4	2·1	5	31
Chlorophyll *a*	Cyclohexanol	7·7	0·02	~0·2	33
Chlorophyll *b*	Pyridine	3·1	1·6	2	31
Chlorophyll *b*	Benzene	3·3	2·2	9	31
Chlorophyll *b*	Benzene (wet)	4·0	2·4	4	30

When chlorophyll in dilute solutions (less than 10^{-5}M) is illuminated with light of ordinary intensity, the mean life of the chlorophyll triplet is equal to k_A^{-1}, approx. 2×10^{-3} sec. The self quenching process, $\mathrm{GH} + \mathrm{GH'} \rightarrow 2\mathrm{GH}$, becomes dominant at high concentrations (greater than

TABLE 4. *Summary of some properties of the excited states**

State	ΔE (kcal./mole)	$\phi_{(\mathrm{max.})}$	$\tau_{(\mathrm{max.})}$ (sec.)
Chlorophyll *a*			
First excited singlet	43 ee	0·32 ee	$5{\cdot}3 \times 10^{-9}$ py
Lowest triplet	—	$\leqslant 0{\cdot}01$	$1{\cdot}4 \times 10^{-3}$ py
Chlorophyll *b*			
First excited singlet	44 ee	0·12 ee	$4{\cdot}7 \times 10^{-9}$ py
Lowest triplet	33 epa	$< 0{\cdot}01$ epa	$3{\cdot}1 \times 10^{-3}$ py
Phæophytin *a*			
First excited singlet	42 ee	0·18 b	$5{\cdot}3 \times 10^{-9}$ ee (*a* + *b*)
Lowest triplet	—	$\leqslant 0{\cdot}01$	—

* *Solvents:* b—Benzene; ee—ethyl ether; epa—ether–pentane–alcohol at −180°C; py—pyridine. (*a* + *b*) denotes that a mixture of chlorophylls *a* and *b* was used.

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[33] Fujimori and Livingston, *Nature*, 1957, **180**, 1036.

5×10^{-5}M), where the lifetime of the triplet state is inversely proportional to the concentration of chlorophyll. The second-order process, $2GH' \rightarrow 2GH$ (or $GH + GH'$), can be safely neglected for all intensities normally used in photochemical experimentation but strongly reduces the mean life observed in flash photolytic studies.

Within the limits of uncertainty, the value of k_B is the same for chlorophylls *a* and *b*. While the data are scanty, k_B appears to be inversely proportional to the viscosity of the solvent and to be not much less than the value of the rate constant, k_D, for a bimolecular reaction whose rate is determined by the number of encounters. Approximately, $k_D = 8\boldsymbol{R}T/30\eta$, where η is the viscosity coefficient in centipoises.

The Quenching of the Fluorescent and Triplet States by Added Substances

Bimolecular interactions of excited pigment molecules with foreign molecules (either added or present as impurities) can reduce the intensity of the fluorescence of the pigment. This process is known as the diffusional quenching of fluorescence. Foreign substances can also quench fluorescence by forming thermally-stable, non-fluorescent addition compounds with the pigment. Diffusional quenching shortens the mean life of the excited state and its efficiency is low in viscous solutions. The second, or static type of quenching does not affect the mean life of the fluorescent state and its efficiency is not directly related to the viscosity of the solvent. There is some evidence indicating that fluorescence of chlorophyll is subject to both diffusional and static quenching. However, diffusional quenching appears to be the dominant type for the quenchers which have been investigated, and to simplify the discussion we shall assume that it is the only type operative.

Properly speaking, quenching refers to an effect upon the emission of light by the solution, not upon the properties of the excited state. However, it is convenient, and common practice, to say that the fluorescent state is quenched when its mean life is shortened by the addition of a foreign substance. An obvious extension of this usage is to say that the triplet state is quenched when its mean life is shortened by the presence of a foreign compound. This terminology will be employed in the present discussion.

The following reaction steps are essential to an analysis of the diffusional quenching process. In some of them, the solvent may play a direct role, although this is not indicated as they are written. Unimportant steps have been omitted. For example, both kinetic and spectroscopic evidence indicate that for chlorophyll the radiationless transition from the first excited to the ground singlet state ($GH^* \rightarrow GH$) is negligible compared to competitive processes. Since the present section is limited to a discussion of dilute solution, the self-quenching of fluorescence has also been omitted.

1	$h\nu + \mathrm{GH} \rightarrow \mathrm{GH}^*$	Excitation
2	$\mathrm{GH}^* \rightarrow \mathrm{GH} + h\nu_f$	Emission of fluorescence
3	$\mathrm{GH}^* \rightarrow \mathrm{GH}'$	Formation of the triplet
4	$\mathrm{Q} + \mathrm{GH}^* \rightarrow \mathrm{GH} + \mathrm{Q}$ (or products)	Diffusional quenching of fluorescence
5	$\mathrm{GH}' \rightarrow \mathrm{GH}$	Spontaneous decay of triplet
6	$\mathrm{GH} + \mathrm{GH}' \rightarrow 2\mathrm{GH}$	Self-quenching of triplet
7	$2\mathrm{GH}' \rightarrow 2\mathrm{GH}$ (or $\mathrm{GH} + \mathrm{GH}'$)	Second-order decay of triplet
8	$\mathrm{Q} + \mathrm{GH}' \rightarrow \mathrm{GH} + \mathrm{Q}$ (or products)	Diffusional quenching of triplet

If no irreversible photochemical reactions occur,

$$\mathrm{d}[\mathrm{GH}^*]/\mathrm{d}t = 0 \text{ and } \mathrm{d}[\mathrm{GH}']/\mathrm{d}t = 0$$

under conditions of steady illumination. It follows that

$$I_{\mathrm{abs}} = (k_2 + k_3 + k_4\,[\mathrm{Q}])\,[\mathrm{GH}^*]$$
$$I_{\mathrm{fl}} = k_2\,[\mathrm{GH}^*]$$

By definition, $\phi_{\mathrm{fl}} = I_{\mathrm{fl}}/I_{\mathrm{abs}}$

and, therefore, $\phi_{\mathrm{fl}} = k_2/(k_2 + k_3 + k_4\,[\mathrm{Q}])$

The (modified) Stern–Vollmer relation follows directly:

$$\phi_{\mathrm{fl,(max.)}}/\phi_{\mathrm{fl}} = 1 + k_4[\mathrm{Q}]/(k_2 + k_3) = 1 + k_4\tau'[\mathrm{Q}]$$

The Stern–Vollmer quenching constant is $K_{\mathrm{Q}} = k_4\tau'$, where τ' is the actual mean life in the absence of quenchers.

The bimolecular quenching constant, k_8, can be obtained from flash-photolytic measurements of the rates of disappearance of the triplet in the presence and absence of quenchers:

$$-\,\mathrm{d}[\mathrm{GH}']/\mathrm{d}t = (k_5 + k_6[\mathrm{GH}] + k_7[\mathrm{GH}'] + k_8[\mathrm{Q}])\,[\mathrm{GH}']$$

If the total concentration of chlorophyll is represented by M, $[\mathrm{GH}] = \mathrm{M} - [\mathrm{GH}']$, we may write

$$\begin{aligned} -\,\mathrm{d}[\mathrm{GH}']/\mathrm{d}t &= (k_5 + k_6\mathrm{M} + k_8[\mathrm{Q}])\,[\mathrm{GH}'] + (k_7 - k_6)\,[\mathrm{GH}']^2 \\ &= k_{\mathrm{I}}[\mathrm{GH}'] + k_{\mathrm{II}}[\mathrm{GH}']^2 \end{aligned}$$

Values for k_{I} and k_{II} may be obtained from the experimental data, by either graphical or analytical calculation; k_8 is equal to the difference between the values of k_{I}, corresponding to the presence and absence of a quencher, divided by its concentration.

In 1950, Livingston and Ke[34] demonstrated that the fluorescence of chlorophyll is strongly quenched by quinones, aryl nitro-compounds, some nitroso-compounds and azo-dyes, in addition to oxygen and nitric oxide. A few amines (notably *p*-aminophenol, phenylhydrazine, and dimethylaniline) quench weakly. Many active reducing agents (such as aniline,

[34] Livingston and Ke, *J. Amer. Chem. Soc.*, 1950, **72**, 909.

quinol, and thiourea) are practically without effect upon the intensity of fluorescence. Qualitatively similar results were obtained by Evstigneev, Gavrilova, and Krasnovskii.[35] The quenching of fluorescence by oxygen, benzoquinone, and *m*-dinitrobenzene (and presumably other reagents) is not accompanied by an appreciable chemical change. The fluorescence of porphyrins[36] and of Mg-phthalocyanine[35] is quenched by the same types of reagent. It is undeniable, for the relatively large number of compounds which were investigated,[34,35] that the effective quenchers are oxidizing agents and that reducing agents are either non-quenchers or quench only weakly. It should not be concluded from these observations that quenching is necessarily the result of an electron-transfer process. One possible alternative[34] is that the quencher induces a transition of the pigment from its fluorescent to its lowest triplet state and concurrently the quencher molecule goes from its ground to its lowest triplet state. Whether this mechanism is energetically feasible in all cases studied is unknown; however, it remains a possible explanation.

TABLE 5. *Bimolecular constants, k_8, for the quenching of the triplet state of chlorophyll* a

Quencher	Solvent	$k_8 \times 10^{-9}$ (l. mole^{-1}sec.$^{-1}$)	Ref.
p-Benzoquinone	Benzene[a]	2·4 ± 0·5	37
Oxygen	Benzene	1·1 ± 0·3	37
m-Dinitrobenzene	Toluene	1·2 ± 0·4[b]	39
β-Carotene	Benzene	1·3 ± 0·3	37
β-Carotene	Toluene	0·7 ± 0·2	39
β-Carotene	Benzene	1·5 ± 0·3[c]	37
α-Carotene	Benzene	1·6 ± 0·3	37
Luteol	Benzene	0·6 ± 0·3	37
t-Retinene	Benzene	0·3 ± 0·1	39
Cyclo-octatetraene	Methanol	0·0012 ± 0·0002	37

[a] Benzene used in the first series of experiments[37] was (intentionally) wet, but had been otherwise purified. The benzene and toluene used in the other experiments[39] did not contain added water but had not been exhaustively dried.

[b] This result was obtained by Ichimura and contradicts the earlier negative observation,[37] which was of a preliminary nature and was probably in error.

[c] Chlorophyll *b* was used in these experiments.

The relatively scanty quantitative information regarding the quenching of the triplet state is summarised in Table 5. In addition to the compounds listed therein, Fujimori[37] investigated the quenching action of phenylhydrazine, ascorbic acid, allylthiourea, and ethyl *N*-phenyl carbamate. None of them showed quenching action at concentrations as high as 0·010M; or,

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[35] Evstigneev, Gavrilova, and Krasnovskii, *Doklady Akad. Nauk S.S.S.R.*, 1950, **74**, 315.

[36] Livingston, Ramarao, and Thompson, *J. Amer. Chem. Soc.*, 1952, **74**, 1073.

[37] Fujimori and Livingston, *Nature*, 1957, **180**, 1036.

for phenylhydrazine, ascorbic acid, and allylthiourea, at concentrations of 0·10M. Ascorbic acid and allylthiourea were tested in dry pyridine; the others in methanol. Recently, Pugh[38] demonstrated that, if pyridine containing 2% or more of water is used as the solvent, ascorbic acid is a moderately effective quencher. He also demonstrated[38] that, unlike retinene, vitamin A is not an efficient quencher.

With the exception of oxygen, the list of efficient quenchers for the triplet state could not have been predicted upon any simple basis. However, some of the results are in good agreement with predictions based upon classical photochemical studies. For example, Schenck[40] has interpreted his extensive studies of pigment-sensitised photochemical reactions as indicating that the triplet states of pigments are quenched efficiently by benzoquinone and moderately by cyclo-octatetraene. These predictions were confirmed[37] by direct measurement, at least for the case of chlorophyll. The effects of the carotenoids and retinene were unexpected and remain unexplained.

TABLE 6. *Comparison of bimolecular quenching constants with* $k_D = 8RT/30\eta$.*

Quenching of the fluorescent state

Quencher	Solvent	K_Q(l. mole^{-1})	τ(sec.)	$k_4 = K_Q/\tau$ (l. mole^{-1} sec.$^{-1}$)	k_4/k_D
p-Benzoquinone	Methanol	104 (34)	7×10^{-9} (28)	15×10^9	1·3
p-Benzoquinone	Methanol	143 (41)	7×10^{-9} (28)	21×10^9	1·8
Dinitrobenzene	Methanol	86 (34)	7×10^{-9} (28)	12×10^9	1·0
Oxygen	Ethanol	44 (34)	5×10^{-9} (31)	9×10^9	1·5

Quenching of the triplet state

Quencher	Solvent	k_8 (l. mole^{-1}sec.$^{-1}$)	k_8/k_D
p-Benzoquinone	Benzene	$2{\cdot}4 \times 10^9$ (37)	0·22
Dinitrobenzene	Toluene	$1{\cdot}2 \times 10^9$ (39)	0·10
Oxygen	Benzene	$1{\cdot}1 \times 10^9$ (37)	0·10

* References in parentheses.

In terms of the diffusional mechanism, the maximum efficiency of quenching is limited (approximately) by the rate of encounters between molecules of the excited pigment and the reagent. Table 6 presents a comparison between the bimolecular quenching constants and the limiting rate constant, $k_D = 8RT/30\eta$(l. mole^{-1}sec.$^{-1}$). For fluorescence quenching, the agreement between k_4 and k_D is surprisingly close. The bimolecular

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[38] Pugh, unpublished work at University of Minnesota, 1959.
[39] Livingston and Pugh, *Discuss. Faraday Soc.*, 1959, **27**, 144.
[40] Schenck and Ritter, *Naturwiss.*, 1954, **41**, 334.
[41] Goedheer, *Biochim. Biophys. Acta*, 1958, **27**, 478.

constants for the quenching of the triplet state are about ten-fold smaller. This difference in efficiency is not peculiar to the case of chlorophyll; a similar difference was observed[42] for the quenching of the triplet and fluorescent states of anthracene by oxygen.

Effects of Environment upon the Electronic States

In this discussion, we have chosen to regard as the normal state of chlorophyll, the monosolvated compound in dilute solution at ordinary temperatures. Chlorophyll will be considered to be in a perturbed state when it is absorbed on solids, present as crystals, in concentrated solutions, in solution at low temperatures, or dissolved in dry hydrocarbons.

Effect of Solvation.—The most striking of the environmental effects upon chlorophyll are those which accompany exhaustive drying of non-basic solvents, such as hydrocarbons, halogenated hydrocarbons, etc. It has been definitely established[9–11,43] that the chlorophylls (and other metal-complexed chlorins and porphins) form stable 1:1 addition compounds with Brönsted bases (*i.e.*, nucleophilic reagents). These bases include ethers and ketones, in addition to hydroxylic solvents and amines. Phæophytins and metal-free porphyrins do not form such addition compounds at ordinary temperatures.[9,43] For either oxygen bases or nitrogen bases considered separately, the stabilities of the compounds are symbatic to the basicities of the addenda. However, compared to amines, oxygen bases form abnormally stable addition compounds;[9] *e.g.*, the stability constant of the methanol complex is about 200-fold greater than that of the aniline complex.

When the solvent is a dry hydrocarbon, the absorption bands of chlorophyll appear unusually broad. This apparent broadening is due to the occurrence of shoulders on the long-wavelength side of the principal bands.[10,11,44] Exhaustive drying increases the relative height of the secondary peak, but in no case is the normal band eliminated. The double maxima appear to be characteristic of the unsolvated chlorophylls.[11] Lowering the temperature favours the normal band,[11] corresponding to standard molar enthalpies, of the addition reaction, in the range from −1500 (aniline) to −6000 cal. (heptylamine).

Solvation changes the absorption spectra of all of the metal-complexed chlorins and porphins which have been studied, but chlorophyll is unique in being strongly fluorescent when solvated and non-fluorescent in the unsolvated state.[9,10,43] The equilibrium constants for compound formation can be calculated from either spectrophotometric or fluorescence-intensity measurements. In a few cases, both methods were used[9] and identical values of the equilibrium constants were obtained. This demonstrates that equilibrium is attained in the dark and that either the equilibrium constant

[42] Livingston and Subba Rao, *J. Phys. Chem.*, 1959, **63**, 794.
[43] Evstigneev, Gavrilova, and Krasnovskii. *Doklady Akad. Nauk S.S.S.R.*, 1950, **70**, 261.
[44] Fernandez and Becker, *J. Chem. Phys.*, 1959, **31**, 467.

is the same for chlorophyll in its ground and fluorescent states or else the mean life of the addition compound is long compared to the mean duration of fluorescence.

In 1949, Livingston *et al.*[10] postulated that the addition resulted from the formation of a hydrogen bond between the labile hydrogens of the activator and the keto-oxygen of ring v of chlorophyll. This postulate was not compatible with the observed activation of fluorescence by simple ethers, but the authors dismissed this discrepancy as due to the presence of hydroxylic impurities in the ethers. Very shortly afterwards, Evstigneev *et al.*[43] pointed out that the change in fluorescence intensity did not occur if phæophytin was substituted for chlorophyll. They advanced the alternative explanation that the activator is bonded to the central metal atom of the pigment. Although some of the evidence which they cited[43] was later shown to be in error,[9] there can be no reasonable doubt of the validity of their postulate. The addition compounds are formed by metal-complexed pigments, regardless of the presence or absence of ring v, but are not formed by metal-free pigments.

Since chlorophyll dissolved in dry hydrocarbons is not detectably fluorescent ($\phi_{\text{fl}}<0{\cdot}01$), the mean life of the first excited singlet state must be very short, certainly less than 10^{-10} sec. A plausible interpretation is that the non-radiative transition from the first-excited singlet to the lowest triplet state is much faster in unsolvated than in monosolvated chlorophyll. If this were true, the yield per flash of the triplet should be higher in dry than in wet solvents. Preliminary experiments[29] indicated that this is not the case. Recently, Pugh[38] has made a careful, flash-photolytic study of dilute solutions of chlorophyll *a* in very dry and in water-saturated toluene. He found that the yield is four- or five-fold less in dry than in wet toluene and that the rate of decay (more exactly, the values of k_A, k_B, and k_C) is the same in wet and in dry solutions. The triplet–triplet absorption spectrum appears to be the same in dry as it is in wet solutions.

The situation is further complicated by the observation of Fernandez and Becker[44] that dry, non-fluorescent solutions of chlorophyll *a* exhibit, at liquid-nitrogen temperatures, a moderately long-lived luminescence with its intensity maximum at λ7550 Å. The authors report that the intensity of luminescence of these rigid, low-temperature solutions is comparable to that of the fluorescence of ordinary (wet) chlorophyll solutions. They also state that the mean duration of the luminescence at liquid-nitrogen temperatures is equal to or greater than 5×10^{-4} sec. The same solutions at room temperature, likewise, luminesce in the near infrared but with an intensity about one-twentieth of the value at −180°c Although, in the concentrated solutions which these authors used an appreciable fraction of the chlorophyll must be present as dimers or higher aggregates,[45] it is improbable that the state of aggregation was solely

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[45] (*a*) Lavorel, *J. Phys. Chem.*, 1957, **61**, 1600; *J. Chim. phys.*, 1958, **55**, 905; (*b*) Weber and Teale, *Trans. Faraday Soc.*, 1958, **54**, 640.

responsible for all of their observations. [The strange results which they report for chlorophyll *b* were very probably due to the high concentration (5×10^{-5}M) and the methylpentane, used as a solvent. It is well known[46] that chlorophyll *b* is only slightly soluble in aliphatic hydrocarbons of low molecular weight, even at room temperature. At low temperature, it is probable that the pigment existed chiefly as a colloidal suspension.]

These several observations may be interpreted in terms of a modified form[44] of the scheme originally proposed by Kasha[47] and by Platt.[6a] Fig. 3 is an energy-level diagram corresponding to this modified scheme.

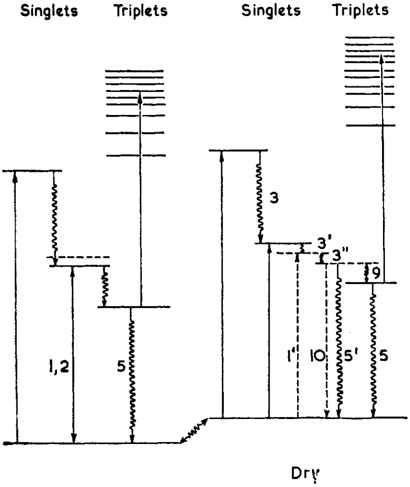

FIG. 3. *A tentative energy-level diagram for monosolvated and unsolvated chlorophyll.*

The left half of this diagram represents the energy levels and transitions of the monosolvate; the right half, the corresponding levels and transition of unsolvated chlorophyll *a*. Non-radiative transitions are indicated by zig-zag vertical lines; radiative transitions by straight vertical lines. A broken line indicates that the transition is observable but weak. Energy levels corresponding to π–π states are represented by solid horizontal lines; those corresponding to n–π states by dotted lines. For simplicity, the overlapping oscillational levels which accompany electronic states have been omitted and only 0–0 transitions are indicated on the diagram.

The vertical lines on the extreme left of each half of the diagram represent the absorption of a photon in the region of the Soret band. Since no corresponding fluorescence is observable, the arrow is single headed. The next pair of (zig-zag) lines represents the highly probable, non-radiative transitions from the second to the first excited singlet states. The

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[46] Willstätter and Stoll, "Untersuchungen über Chlorophyll," Springer, Berlin, 1913, pp. 157—166.

[47] Kasha, *Discuss. Faraday Soc.*, 1950, **9**, 14.

normal absorption and emission of red light is indicated by the arrows labelled 1, 2 (and 1′). The straight, solid, vertical lines on the right of each half of the diagram represent the triplet–triplet absorption which is observed in flash-photolytic experiments. The upper triplet level is indicated by a series of horizontal lines to suggest the unusually broad, structureless nature of the triplet–triplet absorption.

The energy of n–π levels is shifted by solvation.[6,47] We shall assume that for a monosolvate it lies above the π–π singlet and, therefore, does not interfere with the emission of fluorescence. Absorption corresponding to this n–π level would lie within the normal red band and, being weak, would be completely masked by the stronger π–π absorption. Molecules which reach the first excited singlet state must either fluoresce or pass, by an act of internal conversion, to the lowest triplet state; thence they go non-radiatively to the ground state.

In dry hydrocarbons, we assume that the n–π singlet levels are slightly below the corresponding π–π levels. The non-radiative transition, 3′, is so fast that it eliminates the competing radiative transition, 2, and no fluorescence is observed. The absorption process, 1′, is responsible for the long-wavelength shoulder of the red band, which is observed in dry solutions. To explain the results of Fernandez and Becker[44] we shall postulate that a relatively fast, radiationless transition, 3″, leads to the n–π triplet state. From this state, the molecule may undergo non-radiative transitions to either the ground state, 5′, or the π–π triplet state, 9. Alternatively, the molecule may emit, 10, a photon of near infrared (λ 7550 Å) light. At room temperatures, the radiationless processes dominate, but the radiative process becomes comparable at low temperatures. To fit the scheme to Pugh's flash-photolytic data, we must further assume that, at ordinary temperatures, process 5′ is four or five times faster than process 9, and that the mean life of the π–π triplet is at least 20-fold greater than that of the n–π triplet. Under these conditions, the *observed* mean life and absorption spectrum of the triplet state would be the same in wet and dry solvents but the yield of triplet would be four- or five-fold smaller in dry solutions.

Properties of Concentrated Solutions.—Intermolecular transfer of energy of excitation occurs efficiently, by inductive resonance,[21] in moderately concentrated solutions of chlorophyll monosolvates, as is indicated by the self-quenching[26,48] and concentration depolarisation[26,42] of fluorescence and by the sensitisation of the fluorescence of chlorophyll *a* by chlorophyll *b*.

The self-quenching of the fluorescence of chlorophyll is appreciable in solutions whose concentration is 2 × 10^{-4}M or greater. It is as efficient in a viscous solvent, cyclohexanol, as in acetone or ether.[26,48] These data conform to the simple empirical relation, $\phi_{\text{fl}}{}^{\circ}/\phi_{\text{fl}} = 1 + 6270\text{M}^2$, but are also consistent with Wavilow's theoretical equation.[49]

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[48] Watson and Livingston, *J. Chem. Phys.*, 1950, **18**, 802.
[49] Wavilow, *J. Phys. U.S.S.R.*, 1943, **7**, 141.

The concentration depolarisation of fluorescence of chlorophyll was studied by Weil[26] in cyclohexanol and by Goedheer[50] in ricinus oil. Weil's data are represented by the empirical equation $P^\circ/P = 1 + 177\text{M}$, where P represents the degree of polarisation. Goedheer reports the following values for the molar concentrations at which the degree of polarisation is reduced to half of its maximum value: chlorophyll *a*, $6 \times 10^{-4}\text{M}$ and chlorophyll *b*, $3{\cdot}5 \times 10^{-3}\text{M}$. These results are consistent with Förster's inductive-resonance explanation of concentration depolarisation.[21]

The sensitisation of the fluorescence of chlorophyll *a* by chlorophyll *b* was observed independently by Watson and Livingston[48] and by Duysens.[51] These authors agree that the efficiency of transfer of energy from chlorophyll *b* to chlorophyll *a* (in an equimolar mixture) reaches a maximum of about one half at a concentration of 10^{-3}M. At higher concentrations of the donor, self-quenching reduces the efficiency of transfer. These results agree surprisingly well with Förster's prediction.[21]

In the intact plant cell, the fluorescence of chlorophyll *a* is sensitised by carotenoids. The corresponding sensitisation *in vitro* was recently observed by Teale,[52] using equimolar mixtures of carotenoids and chlorophyll dispersed in micelles. The local concentration of each of the pigments was about 0·1M. Under these conditions, when chlorophyll *a* was the acceptor and *β*-carotene, lutein, or fucoxanthol was the donor, the probabilities of transfer were 0·1, 0·6, and 1·00, respectively. When chlorophyll *b* was substituted for chlorophyll *a*, the probabilities of transfer were reduced five-fold or more. When phæophytin was the acceptor, there was no detectable sensitisation of its fluorescence. These marked individual differences suggest that the transfer is not simply the result of inductive resonance but is chiefly due to the formation of addition compounds between the donors and acceptors. In these micelles and in chlorophyll-free micelles, the long-wavelength absorption limit of the carotenoids is shifted strongly toward the red. The chlorophyll absorption spectra are about the same in the micelles as they are in dilute solutions.

In general, the quantum yield of fluorescence does not depend upon the wavelength of the exciting light. However, it has been observed that the yield for solutions of dyes and pigments is less than normal when the wavelength of the exciting light is on the red side of the first absorption band. In the earlier literature, this behaviour was accepted as characteristic of such solutions and several *ad hoc* theories were proposed to explain it. Lavorel[45a] and, independently, Weber and Teale[45b] have demonstrated that this decrease in the fluorescence yield occurs only when the concentration of the pigment is relatively high. It appears to be the consequence of the formation of non-fluorescent dimers. In the absorption spectrum of the dimers the single bands of the monomer are split. As a result of partial dimerisation, the quantum yield exhibits a minor minimum

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[50] Goedheer, Doctoral Diss., Utrecht, 1957.

[51] Duysens, *Nature*, 1951, **168**, 548.

[52] Teale, *Nature*, 1958, **181**, 415.

when the wavelength of the exciting light is on the blue side and a sharp decline when it is on the red side of the (monomer) absorption band. In the antistokes region where the total absorption is small, relatively low concentrations of dimer can produce a marked decrease in the quantum yield.

It has been suggested[53] that dimerisation, which reduces the efficiency of fluorescence, should increase the yield of phosphorescence. Recent observations by Brody[54] are probably an example of this behaviour. He noted an intense luminescence, with a maximum at $\lambda 715$ mμ, from concentrated ethanolic solutions of chlorophyll *a* at -193°C. Dilute solutions (10^{-6}M) exhibit neither the luminescence at 715 mμ nor the concurrent weakening of the normal fluorescence, whose maximum is at 670 mμ. Analogous results were obtained with chlorophyll *b*. The author concludes that the appearance of the long-wavelength luminescence and the weakening of the normal fluorescence are the result of dimerisation which is favoured by low temperatures.

Properties of Solid Films.—After five years of intensive investigation in a number of laboratories and much enthusiastic and repetitive discussion, it must be admitted that the case for or against the "biological solar battery" is not proved. It was suggested about ten years ago[55] that the ordered aggregates of pigment and protein molecules which constitute the chloroplasts might act as an organic analogue to the "solar battery" and so produce an electrochemical "splitting of water", leading to the reduction of carbon dioxide and the evolution of oxygen. This mechanism necessarily involves the following components: a light-induced charge separation; photoconduction of holes and electrons; something analogous to two spatially separated, reversible electrodes; and a return path for electricity, presumably through a continuous ionic solution. This concept has had a strong intuitive appeal to many biologists; however, detailed analyses of the problem[56] indicate that it involves serious (although, in principle, not insurmountable) difficulties.

Interesting, positive evidence for the existence of some of the components of the biological solar battery have been reported. Electron spin resonance measurements[57] have proved that free electrons are produced by the illumination of chloroplasts and of some other chlorophyll-containing material. Films of chlorophyll and of similar pigments are photoconductive, the mobile carrier being the positive hole rather than the

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[53] McRae and Kasha, *J. Chem. Phys.*, 1958, **28**, 721.

[54] Brody, *Science*, 1958, **128**, 838.

[55] Katz, in "Photosynthesis in Plants", Iowa State College Press, 1949, pp. 287—292.

[56] (*a*) Brugger and Franck, *Arch. Biochem. Biophys.*, 1958, **75**, 465; (*b*) Rabinowitch, *Discuss. Faraday Soc.*, 1959, **27**, 161.

[57] (*a*) Commoner, Heise, and Townsend, *Proc. Nat. Acad. Sci.*, 1956, **42**, 710; (*b*) Sogo, Pon, and Calvin, *ibid.*, 1957, **43**, 387.

electron.[58–62] Photochemical charge separation, at the boundaries between films of organic compounds, has been reported.[60,63] Several types of long-lived, faint luminescence of chloroplasts and intact cells have been observed and sometimes interpreted as the result of the recombination of trapped electrons and holes. This interpretation, although possible, has in no sense been established.

One of the weakest links in this chain of evidence is the lack of reliable determinations of the quantum yields of free electrons, etc. While most of the available semi-quantitative information suggests that these yields are very small, Tollen *et al.*[64] state that their electron spin resonance measurements indicate a quantum yield of free electrons greater than 0·1. No details have been published in support of this statement, which, if confirmed, would be of primary importance. The photoconductivity of organic films appears to be a complex phenomenon[58] and its analogy to the photoconductivity of inorganic semiconductors is probably more formal than physically real.[65]

There can be no reasonable doubt of the real importance of the spatial arrangement of the pigment, enzymes, and other substances present in the chloroplasts. This arrangement must play an essential role in the process of photosynthesis. However, the "solar battery" hypothesis, as originally proposed and as still supported by some biologists, appears to be as naïve and unreasonable as the view of the opposing extremists, *viz.*, that efficient photosynthesis could occur in a homogeneous fluid system.

Reversible Photochemical Reactions

Reversible Photobleaching or Phototropy.—Under certain specific conditions, chlorophyll undergoes reversible photochemical reactions. In 1937 Porret and Rabinowitch[66] discovered that air-free methanolic solutions of chlorophyll are reversibly bleached when strongly illuminated. The effect is small; the radiation from a 2000-watt carbon arc produces only a 1% change in the absorption of red light by a 2×10^{-5}M solution of chlorophyll. The absorption spectrum of the reduced form has not been determined reliably, but as compared with normal chlorophyll both of the major bands are decreased and the absorption in the intermediate region is increased. The extent of the bleaching is proportional to the square-root of the intensity of the absorbed light. The back reaction is too fast to be measured by ordinary, classical methods and only the steady-state changes

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[58] Weigl, *J. Mol. Spectroscopy*, 1957, **1**, 216.

[59] Nelson, *J. Chem. Phys.*, 1957, **27**, 864.

[60] Arnold and Maclay, *Brookhaven Symposia in Biology*, 1958, **11**, 1.

[61] Terenin and Putzeiko, *J. Chim. phys.*, 1958, **55**, 681.

[62] Terenin, Putzeiko, and Akrimov, *Discuss. Faraday Soc.*, 1959, **27**, 83.

[63] Tollin, *Brookhaven Symposia in Biology* (*Bioenergetics*), in the press.

[64] Tollin, Sogo, and Calvin, *J. Chim. phys.*, 1959, **55**, 919.

[65] (*a*) Kasha, *Rev. Mod. Phys.*, 1959, **31**, 162; (*b*) Garrett, *Brookhaven Symposia ni Biology* (*Bioenergetics*), in the press.

[66] Porret and Rabinowitch, *Nature*, 1937, **140**, 321.

have been studied. The effect has been observed in methanol, ethanol, and acetone but not in dry or wet benzene. It requires the addition of 1% of methanol to benzene to restore the steady-state change to half of its normal value. The effect is inhibited by oxygen; 5×10^{-6}M-oxygen in methanol largely eliminates the reversible change. Although there is some irreversible bleaching in the presence of oxygen, the action of oxygen is not merely to convert reversible into irreversible bleaching. In an air-saturated solution in methanol, the quantum yield of the irreversible reaction is about 4×10^{-5} but the corresponding *lower limit* for the yield of the bleached form, in an anaerobic solution, is 3×10^{-3}. The extent of bleaching is not appreciably affected by moderately high concentrations of allylthiourea or isopentylamine. Certain oxidising agents (notably iodine and Methyl Red) greatly increase the bleaching, probably by changing the nature of the reaction. These phenomena were studied intensively by Livingston and his co-workers and their results have been adequately reviewed by Rabinowitch.[67]

The measurements were rather poorly reproducible, probably owing to undetected impurities in the solvents or pigments. Although no detailed explanation of the results has been established, certain general conclusion may be safely stated. The dependence upon the square-root of the light intensity shows that the reverse, thermal reaction is bimolecular in respect to the photo-products. This, as well as the magnitude of the effect and the influence of solvents, demonstrates that the steady-state bleached form is not the triplet state. Presumably the bleached species is a pair of radicals or ions which are formed by the reaction of an excited chlorophyll molecule with the solvent or with an adventitious (oxidising?) impurity. The high efficiency of the inhibition by oxygen suggests that the active excited species is the triplet state. These assumptions being made, the generalised mechanism may be written as follows:

$$
\begin{array}{rcll}
\mathrm{GH} + h\nu & \twoheadrightarrow & \mathrm{GH}' & \phi_{\mathrm{T}} I_{\mathrm{abs.}} \\
\mathrm{GH}' & \longrightarrow & \mathrm{GH} & k_{\mathrm{a}}\,[\mathrm{GH}'] \\
\mathrm{O_2} + \mathrm{GH}' & \twoheadrightarrow & \mathrm{GH} + \mathrm{O_2} & k_{\mathrm{b}}\,[\mathrm{O_2}]\,[\mathrm{GH}'] \\
\mathrm{S} + \mathrm{GH}' & \longrightarrow & \mathrm{g} + \mathrm{s} & k_{\mathrm{c}}\,[\mathrm{S}]\,[\mathrm{GH}'] \\
\mathrm{g} + \mathrm{s} & \longrightarrow & \mathrm{GH} + \mathrm{S} & k_{\mathrm{d}}\,[\mathrm{g}]^2
\end{array}
$$

where S stands for the solvent or impurity and g + s for the pair of radicals or ions. At the steady state, the change in the concentration of chlorophyll should be

$$\Delta[\mathrm{GH}] = [\mathrm{g}] = \left[\frac{(k_{\mathrm{c}}/k_{\mathrm{d}})\ [\mathrm{S}]\phi_{\mathrm{T}}}{k_{\mathrm{a}} + k_{\mathrm{b}}[\mathrm{O_2}] + k_{\mathrm{c}}[\mathrm{S}]}\right]^{\frac{1}{2}} I_{\mathrm{abs.}}^{\frac{1}{2}}$$

The values of k_{a} and k_{b} must be consistent with k_5 and k_8, as determined from flash-photolytic measurements, and $\phi_{\mathrm{T}} = 1 - \phi_{\mathrm{fl}}$. Within the limits

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[67] Ref. 1, pp. 483—494, 1487—1501.

of uncertainty of the available data, this equation is consistent with the experimental results.

If radicals are formed by the illumination of an air-free chlorophyll solution, containing a suitable monomer such as methyl methacrylate, they should induce readily detectable polymerisation. Two such studies have been performed,[68] and a slow polymerisation was observed in ethanol or pyridine but not acetone or benzene. The polymerisation is greatly accelerated by the presence of reducing agents but is inhibited by oxygen. One author[68a] reports that the yield, in the absence of added reducing agents, was "considerably below unity" and that the average molecular weight of the polymer was approximately 10^5. This corresponds to a very inefficient initiation process, $\phi_i < 10^{-5}$. These results indicate that either the products, g + s, are not radicals or if they are radicals they do not initiate polymerisation efficiently. Rüppel and Witt[69] demonstrated that chlorophyll, dissolved in hexane, is not detectably photoionised. However, this is not surprising, since chlorophyll is not reversibly bleached in such solvents.

Reversible Photo-oxidation.—In the same year that Porret and Rabinowitch discovered the reversible photobleaching of chlorophyll, Rabinowitch and Weiss[70] observed a rapid, reversible reaction between chlorophyll and ferric salts. In anhydrous alcohol, chlorophyll can be quantitatively regenerated, from the yellowish product of the reaction between ferric salts and chlorophyll, by the immediate addition of an excess of ferrous salt or other suitable reducing agent. However, if the bleached solution is allowed to stand before the reductant is added, or if the solvent contains water, allomerised rather than native chlorophyll is formed.[71] In dry alcoholic solutions containing comparable amounts of ferric and ferrous salts an equilibrium between normal and oxidised chlorophyll is attained fairly rapidly. Illumination with red light displaces the equilibrium, favouring the bleached form of chlorophyll. It appears probable, though it has not been proved, that the yellowish product is a reactive, oxidised derivative of chlorophyll. A similar, but transitory, pale yellow solution is formed during the allomerisation of chlorophyll with dilute solutions of iodine or bromine.[71] The alternative interpretation of these observations, which was advanced by Ashkinazi *et al.*,[72] is not justified by the evidence which they cite.

Linschitz *et al.*[73] studied the reversible photochemical reaction between chlorophyll and benzoquinone. This, like the ferric reaction, appears to be

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[68] (*a*) Uri, *J. Amer. Chem. Soc.*, 1952, **74**, 5508; (*b*) Krasnovskii and Umrikhina, *Doklady Akad. Nauk S.S.S.R.*, 1955, **104**, 882.

[69] Rüppel and Witt, *Z. phys. Chem. (Frankfurt)*, 1958, **15**, 21.

[70] Ref., 1, pp. 464—466, 488, 489, 1499, 1500.

[71] Watson, *J. Amer. Chem. Soc.*, 1953, **75**, 2522.

[72] Ashkinazi *et al.*, *Doklady Akad. Nauk S.S.S.R.*, 1950, **74**, 315; 1951, **80**, 385; 1954, **102**, 767.

[73] (*a*) Linschitz and Rennert, *Nature*, 1952, **169**, 193; (*b*) Korn, Doctoral Diss. Syracuse University, 1955.

a reversible photochemical oxidation of chlorophyll. If a mixture of chlorophyll and an excess of benzoquinone is illuminated in EPA (a mixture of ethyl ether, pentane, and ethanol) at −170°C the chlorophyll is almost completely bleached. At this, or lower, temperatures the reaction products are stable and the chlorophyll derivative has a spectrum practically identical with that of the Molisch brown phase.[74] At −75°C or above, no appreciable colour change is produced by steady illumination, presumably owing to the rapid rate of the reverse reaction. At a still lower temperature, −183°C, the photochemical reaction is inhibited. Either the photochemical reaction has an intrinsic energy of activation or it cannot occur in a rigid medium. No reaction was observed when chlorophyll was replaced by phæophytin or tetraphenylchlorin.

In view of the important role which the reversible oxidation of chlorophyll may play in photosynthesis and in oxidation–reduction reactions sensitised by chlorophyll *in vitro*, it is most unfortunate that neither of these reactions has been studied intensively or quantitatively.

Reversible Photoreduction.—The reversible photoreduction of chlorophyll was observed by Krasnovskii in 1948.[75] Only a limited number of reducing agents (including ascorbic acid, dihydroxymaleic acid, cysteine, hydrogen sulphide, and phenylhydrazine) react in this way. The basic reagent, phenylhydrazine, reacts as efficiently in toluene as in pyridine,[76] but the other compounds reduce chlorophyll only in basic solvents. The first experiments were performed in (supposedly) dry pyridine and the reduced form of chlorophyll observed was a pink pigment having an absorption maximum at about 5200 Å. The regeneration of the green colour of the solution occurs rapidly if air is admitted to the solution or other oxidising agents are added; otherwise, the green colour returns slowly. Chlorophyll is not completely regenerated; part of it is converted into phæophytin. The details of these photochemical reactions and of the photoreduction of analogous pigments have been described in a series of papers by Krasnovskii, Evstigneev, and their co-workers, which have been reviewed critically by Rabinowitch.[77]

In the photoreduction of chlorophyll an excited molecule of this substance must react with one of the reductant. If the reacting molecule were in its first excited singlet state, the reaction should quench the fluorescence of chlorophyll. Evstigneev *et al.*[35] have shown that no such quenching occurs. It has also been demonstrated[37] that, in dry pyridine, high concentrations of ascorbic acid have no effect upon the half-life of the triplet state of chlorophyll. Recent work by Bannister[78] seems to render compatible these apparently inconsistent observations. He demonstrated that the

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[74] Weller, *J. Amer. Chem. Soc.*, 1954, **76**, 5819.

[75] Krasnovskii, *Doklady Akad. Nauk S.S.S.R.*, 1948, **60**, 421.

[76] Evstigneev and Gavrilova, *Doklady Akad. Nauk S.S.S.R.*, 1953, **91**, 899.

[77] Ref. 1, pp. 1501—1507; see also, Rackow and König, *Z. Electrochem.*, 1958, **62**, 482.

[78] Bannister, *Plant Physiol.*, 1959, **34**, 246.

photoreduction of chlorophyll by ascorbic acid does not occur detectably in dry pyridine but requires the presence of a moderate concentration of water. In pyridine containing 20 or 30% of water and 0·10M-ascorbic acid, the pink pigment does not accumulate and reoxidation produces phæophytin rather than the original chlorophyll. In such solutions, the quantum yield of the reduction of chlorophyll is in the range 0·05—0·10. At low concentrations of water (2 or 3%) the yield of the photoreduction product is small but the process is much more nearly reversible. Presumably the "dry pyridine" used by Krasnovskii in his original experiments[75] contained several units % of water.

The publication of Bannister's results led Pugh[38] to re-examine the effect of ascorbic acid upon the mean life of the triplet state of chlorophyll. His results may be summarised as follows: In dry pyridine, ascorbic acid (even in concentrations as high as 0·10M) has no detectable effect upon the decay of the chlorophyll triplet. In pyridine containing 20% of water, ascorbic acid noticeably shortens the mean life of the triplet, and the corresponding value of the quenching constant, k_8, is about 5×10^5 l.mole^{-1} sec.$^{-1}$. Quenching is also observable in pyridine containing 2% of water, but under these conditions the quenching constant is about 1×10^5 l.mole^{-1} sec.$^{-1}$. It should be noted that these rate constants are approximately 10,000-fold smaller than the corresponding constants for quenching by oxygen, benzoquinone, etc. The flash-photolytic traces[29–31] indicate that a second labile species, having a longer life than the triplet state, is also formed in wet pyridine containing ascorbic acid. Its mean life is about 0·01 sec. and it absorbs more strongly at 4800 than at 5520 Å. These properties demonstrate that this labile substance is not Krasnovskii's pink pigment but some forerunner of it.

It was demonstrated in 1953 by Evstigneev and Gavrilova[79] that the pink pigment is not the primary product of photoreduction. The illumination of suitable solutions of chlorophyll and ascorbic acid, containing a reference and an inert electrode, changes the potential of the electrode much more rapidly than it produces changes in the absorption spectrum. In the dark, the original potential of the cell is restored in 2 or 3 minutes but the reappearance of the green colour is much slower. These differences are especially marked at low temperatures. The same authors observed[80] photo-conductivity when they illuminated a mixture of chlorophyll and phenylhydrazine in air-free, dry pyridine. These and other related measurements[77] demonstrate that the photoreduction of chlorophyll produces at least two labile intermediates in addition to the pink pigment.

The flow sheet shown in Fig. 4 is consistent with the principal observations of Bannister, Evstigneev, Krasnovskii, and Pugh on the photoreduction of chlorophyll and phæophytin. Its details are by no means

[79] Evstigneev and Gavrilova, *Doklady Akad. Nauk S.S.S.R.*, 1953, **92**, 381; 1954, **95**, 381; see also, Hendrich, *Roczniki Chem.*, 1958, **32**, 107.

[80] Evstigneev and Gavrilova, *Doklady Akad. Nauk S.S.S.R.*, 1955, **103**, 97.

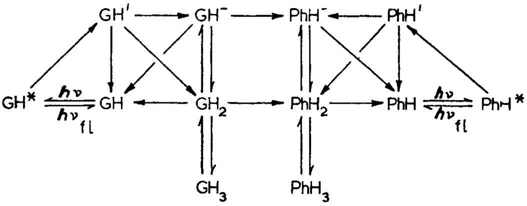

FIG. 4. *Intermediates in the reversible photoreduction of chlorophyll and phæophytin.*

established, and the process may be more complicated than the diagram indicates. The symbol PhH represents phæophytin. The "pink pigments" are indicated by GH_3 and PhH_3; and the Evstigneev intermediate radicals and radical ions by GH_2, GH^-, PhH_2, and PhH^-.

Photochemical Reactions sensitised by Chlorophyll

The facts presented in the preceeding discussion place severe restrictions upon the mechanisms which otherwise might be advanced in explanation of the role of chlorophyll in sensitised reactions. The results of kinetic measurements of naturally occurring photosynthesis are so complicated that not even the general nature of the primary act of this process can be established. However, the accumulated information about the properties of electronically excited chlorophyll has proved useful[81] in the elimination of mechanisms which previously seemed plausible.

For chlorophyll-sensitised reactions occurring in homogeneous solutions, the problem is much simpler and it probably is not unduly optimistic to hope that the mechanisms of some of these reactions will be determined with reasonable certainty within the next few years. Chlorophyll efficiently sensitises the photochemical auto-oxidation of a wide variety of reducing agents. In contrast, relatively few oxidation–reduction reactions (not involving oxygen) are sensitised, and these only in hydroxylic solvents. A large number of such reactions have been qualitatively investigated and a few have been studied intensively and quantitatively. The results of these studies and their possible interpretations have been summarised recently.[82,83] While some of the published interpretations are now outdated, it is doubtful if a complete re-examination of the problem is at present justifiable.

The observation that certain photobiological processes occur efficiently at liquid-nitrogen temperatures has been cited as evidence that they are not simple chemical reactions but must involve some special mechanism,

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[81] (*a*) Brugger and Franck, *Arch. Biochem. Biophys.*, 1958, **75**, 465; (*b*) Franck, *Proc. Nat. Acad. Sci.*, 1958, **44**, 941; (*c*) Franck, in "Handbuch der Pflanzenphysiologie", Vol. V, Springer, Heidelberg, in the press.

[82] Rabinowitch, ref. 1, pp. 507—521, 545—547, 1507—1528.

[83] Livingston, in "Handbuch der Pflanzenphysiologie", Vol. V, Springer, Heidelberg, in the press.

such as the "solar battery". In view of the fact that the Rose Bengal-sensitised auto-oxidation of terpene has a quantum yield of about 0·25 at −156°c in a mixture of organic solvent,[84] this interpretation of the biochemical reactions must be regarded with some suspicion.

It was reported recently[85] that chlorophyll in dilute solution sensitises the photoisomerisation of poly-*cis*-carotenes. It does not detectably sensitise[38] the photoisomerisation of *trans*-retinene. These reactions are worthy of extensive, quantitative study. Photoisomerisations sensitised by stable polyatomic molecules have not been previously reported and may be of importance in relation to photobiological processes.

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[84] Schenck, Kinkel, and Koch, *Naturwiss.*, 1954, **41**, 425; see also Evstigneev and Gavrilova, *Doklady Akad. Nauk S.S.S.R.*, 1954, **98**, 1017.

[85] Claes and Nakayama, *Nature*, 1959, **183**, 1053.