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ENERGY TRANSFER IN ORDERED AND UNORDERED PHOTOCHEMICAL SYSTEMS

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Gordon Tollin, Power B. Sogo, and Melvin Calvin

October 29, 1957

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ABSTRACT

A review is presented of the various mechanisms by which light energy absorbed by one molecule may be transformed or transferred to other molecules. It comprises examination of the processes occurring in fluid nonordered systems, followed by characterization of the changes necessary to carry over the basic concepts so introduced to a condensed and ordered system. Examples of model systems are presented. Finally, these concepts are applied to biological materials, especially particulate material derived from green plants.

Photoinduced electron spin resonance signals have been observed in isolated chloroplasts and other green plant materials; their growth time is not affected by reducing the temperature to -140°C. The luminescence of these materials has also been investigated under a variety of conditions. The results of these studies have been shown to be consistent with a mechanism involving the recombination of electrons and holes trapped in a quasi-crystalline lattice. Some details of such a mechanism have been proposed that suggest the mode of entry of the light energy into the photosynthetic pathway.

ENERGY TRANSFER IN ORDERED AND UNORDERED PHOTOCHEMICAL SYSTMES*†

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October 29, 1957

I. Introduction

The phenomenon of energy transfer has been receiving an ever-increasing amount of attention from physicists, physical chemists, and biochemists alike since the pioneering work of Franck[1] and Vavilov.[2] This concept has proved to be of fundamental importance for an understanding of many of the photoinduced phenomena of molecules, both in solution and in the solid state, and is proving to be of increasing significance to biology.

Our concern here is mainly with a qualitative discussion of the theoretical aspects of energy migration, with some of the experimental criteria of this phenomenon, and, finally, with its possible role in the primary quantum-conversion act in photosynthesis.

II. General Considerations of Energy Transfer in Unordered Systems

A. Spectroscopic Properties of Molecules in Solution

Some of the main qualitative features of the effects of visible and uv radiation on molecules in solution may be understood by a consideration of the diagram in Fig. 1. Process "a" represents the absorption of a quantum of light by the molecule, resulting in a change in its electronic state. Molecules in the lowest excited singlet state may then undergo one of four processes: they may emit a quantum of light as fluorsecence (process "b"); the electronic excitation energy may be degraded into heat (process "c"); a small portion of the electronic energy may be degraded into heat, concomitant with an unpairing of electron spins, resulting in an intersystem crossing into the lowest excited triplet state (process "d"); the quantum of excitation energy may be transferred to another molecule. Molecules in the lowest excited triplet state may, similarly, undergo one of three processes: phosphorescence (process "f"), thermal degradation (process "e"), or energy transfer to another molecule.

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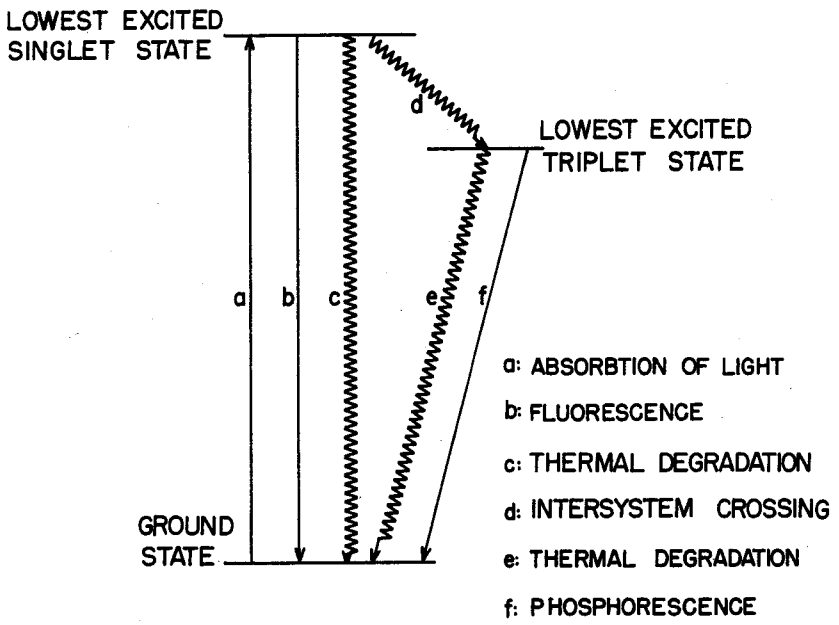
§National Science Foundation Postdoctoral Follow, 1956-1957.



LOWEST ELECTRONIC ENERGY LEVELS OF AN ISOLATED MOLECULE. STRAIGHT LINES REPRESENT RADIATIVE PROCESSES; ZIG-ZAG LINES REPRESENT RADIATIONLESS (THERMAL) PROCESSES.

MU-14255

Fig. 1. Lowest electronic energy levels of an isolated molecule. Straight lines represent radiative processes; zigzag lines represent radiationless (thermal) processes.

B. Theoretical Aspects of Energy Transfer

There are three mechanisms by which electronic excitation energy may be transferred from one molecule to another in unordered systems. These are:

(1) The emission of a quantum of radiation by the excited molecule followed by the reabsorption of this quantum by an unexcited molecule. This may be repeated many times. The probability of this process is determined simply by the Boer-Lambert law and by the geometry of the system. In general, the lifetime of the excited state of a particular molecule remains the same, but the lifetime of the emission in a finite system may be increased by the "imprisonment of radiation."[3] This mechanism has been shown to be of relatively minor importance in energy transfer in solution.[4, 5]

(2) The transfer of electronic excitation energy through close collisions between excited and unexcited molecules. The energy levels of the molecules are, in general, significantly perturbed by such collisions, and thus the absorption and emission spectra of the components may be changed. If, on occasion, only a small amount of the excitation energy is removed (and is transformed into vibrational energy of the acceptor molecule), the excited molecule may be brought into the triplet state.[6] Such a process may occur with particularly high probability if the acceptor molecule contains an atom of high atomic number or is paramagnetic.[7, 8] The close collision mechanism is believed to be the most important one in the fluorsecence of liquid organic solutions induced by high-energy radiation.[5] It has been shown to be unimportant in some uv-induced energy-transfer phenomena.[9]

(3) The transfer of electronic excitation energy through collisions over a distance of several molecular diameter (resonance transfer).[10-13] The main quantitative theory of resonance transfer is due to Forster[13] and is based on a calculation of a mutually induced dipole interaction between donor and acceptor molecules, both of which are capable of being excited to the same energy level. The theory predicts that the probability of transfer is proportional to the extent of the overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor, and also to the intensity of these transitions. This phenomenon may be thought of as analagous to the property of resonance in organic molecules, inasmuch as, during the actual collision, the interaction between the molecules makes it impossible to consider the excitation energy as belonging to only one of the partners; it must rather be thought of as belonging to both of them simultaneously. Thus, on subsequent separation of the colliding molecules, the energy has a definite calculable probability of being found in the previously unexcited molecule. Forster estimated that, for typical dye molecules (i.e., molecules with intense transitions in the visible region), the probability of energy transfer during an excited-state lifetime of 10^{-8} second becomes equal to the probability of fluorsecence when the colliding molecules come within about 100 A of each other, i.e., about 10 times their ordinary kinetic-collision diameter. The probability of transfer, and thus the number of molecules over which transfer occurs, is also inversely proportional to the sixth power of the distance between the molecules and thus directly proportional to the square of the concentration. In general, the absorption and emission spectra of the components are not changed by resonance transfer. This mechanism is generally considered to be the most important one in phonomena of energy transfer induced by visible or uv light.

C. Experimental Aspects of Energy Transfer

Efficient energy transfer has been demonstrated thus far only from aromatic compounds, with one exception, 1, 4-dioxane.[4] Energy transfer from many other types of organic molecules, both saturated and unsaturated, has been observed, but it was only with lower efficiency. This is probably because the excited-state lifetimes of these molecules are much shorter than those of the aromatic solvents. Thus, a higher concentration of acceptor molecules is necessary. With such poor solvents it is possible to increase the yield of energy transfer by adding a small amount, say 10%, of an efficient energy acceptor. This acceptor then mediates the transfer of energy from solvent to solute.

The general types of observable phenomena that can be interpreted in terms of energy transfer are as follows:

(1) Sensitized emission: in such measurements one dissolves a small amount of an emitting substance in a solvent that absorbs energy at somewhat shorter wavelengths (i.e., higher energy) than does the solute. Upon excitation of such a system with radiation absorbed only by the solvent, the resulting emitted light is that characteristic of the solute. The solvent emission is normally almost completely absent.

(2) Concentration depolarization: the fluorescence emitted by dilute solutions of organic molecules in viscous solvents is normally partially polarized, owing to a limited amount of orientation of the molecules. As the concentration is increased, however, the extent of polarization decreases. This is due to a transfer of excitation energy between molecules in different orientations.

(3) Self-quenching of fluorsecence: the quantum yield of fluorsecence of solutions of organic molecules generally decreases with increasing concentration. This phenomenon is made somewhat more complicated by the possible occurrence of other effects such as the equilibrium formation of relatively stable nonfluorsecent dimers.[14] In many cases, the nature of the concentration dependence and also the effects of temperature and viscosity enable one to decide between the mechanisms. If resonance transfer is to lead to self-quenching, some of the molecules must be in a nonfluorescent state and, furthermore, the lifetime of such a state must be comparable to or longer than the average time that the excitation energy spends in any one molecule. Forster suggests that such a nonfluorsecent energy sink is a statistical dimer.[13]

(4) Quenching of fluorsecence by solutes: this phenomenon is essentially similar to self-quenching in that the final energy acceptor must be nonfluorsecent, the electronic excitation energy eventually being degraded into the thermal energy of the solvent. Interpretations in terms of resonance transfer may be complicated by the occurrence of intermolecular spin-orbital perturbations[8] leading to an increased probability of an intersystem crossing into the triplet state.

It would be impossible here to review all the many systems in which the above phenomena have been observed. However, we will mention a few of the more significant examples. Forster, in a study of the concentration

dependence of the quenching of the fluorsecence of solutions of tryptaflavine by rhodamine B, demonstrated that nonradiative energy transfer occurs efficiently at distances as great as 70 A, in agreement with the predictions of his theory.[15] Similarly, Lavorel, in a study of alkaline fluorescein solutions, was able to calculate that a resonance transfer of energy occurred over an average of about 300 molecules.[16] Of particular biological significance is the demonstration by Watson and Livingston[17] and by Duysens[18] of the sensitization of chlorophyll a fluorsecence in methanol solution by chlorophyll b. In addition, there have been a number of studies of energy transfer in protein-dye conjugates[19] whereby energy absorbed in the protein portion of the conjugate (e.g., lysozyme, bovine plasma albumin, chymotrypsinogen and ribonuclease) excited the fluorescence of the dye (e.g., 1-dimethylaminonaphthalene-5-sulfonyl chloride).

A series of very interesting experiments by Terenin and Ermolaev has demonstrated sensitized phosphorescence in rigid solutions at -180°C.[20-22] These workers studied various combinations of naphthalene, benzaldehyde, biphenyl, and benzophenome. The donor molecule was selected to have its lowest excited singlet state below that of the acceptor and its lowest triplet above that of the acceptor. When such mixtures were illuminated with light absorbed only by the donor, the phosphorescence of the acceptor was sensitized and that of the donor quenched. They interpreted these results in terms of an energy transfer between the triplet states of the molecules involved.

Whereas the application of the concepts outlined above to biology must at the present time be reserved mainly for the future, there are a number of examples that one might cite in which energy transfer is of importance. The first, of somewhat trivial significance, is scintillation counting,[23] in which solutions of hydrocarbons are used to detect and measure high-energy radiations in tracer work. The second is the well-known demonstration of the transfer of energy from various plant pigments such as phycocyanin and phycoerythrin to chlorophyll in plant material.[24, 25] Finally, we might mention the experiments of Arnold and Meek, who have demonstrated the transfer of energy among chlorophyll molecules in the grana through a study of the polarization of the fluorsecence.[26] Rabinowitch has suggested that this energy migration is a result of resonance transfer and has estimated that, for excited-state lifetimes of about 10^{-9} second and intermolecular distances between chlorophyll molecules of about 10 A, energy-transfer chains of the order of 100 or 1000 molecules could easily occur.[27]

III. General Considerations of Energy Transfer in Ordered Systems

A. Theoretical Aspects

There are three mechanisms by which energy transfer may proceed in ordered systems. These are:

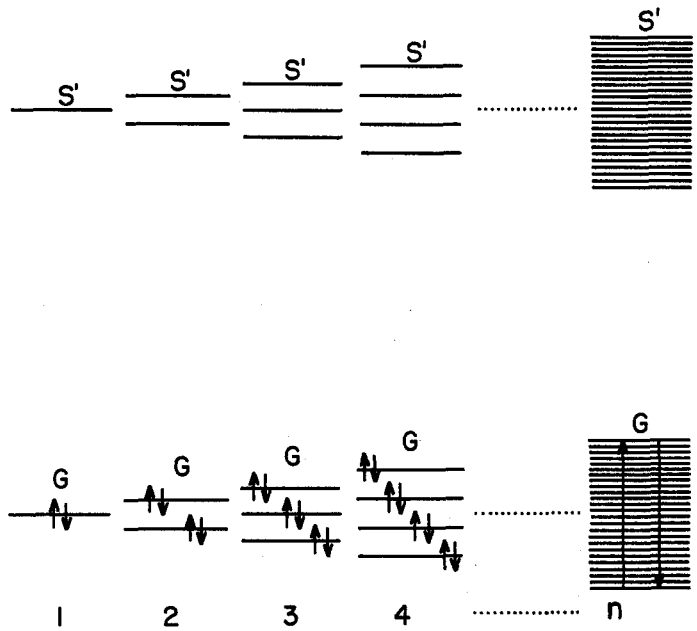
(1) the emission of a quantum of radiation followed by its reabsorption by an unexcited molecule. This is exactly analogous to the mechanism proposed for fluid solutions. The main proponent of this theory has been Birks.[23] There is some disagreement as to the importance of this mechanism,[28, 29] and it has been shown in a number of instances to be of little significance.[30, 31]

(2) resonance transfer analogous to unordered systems.[32, 33] According to this theory, the energy is transferred by an overlap of the electronic systems of the excited donor and the unexcited acceptor molecules. In this case, the interaction between molecules is small enough to permit them to be considered as individual electronic systems. The probability of transfer increases with the magnitude of the interaction.

(3) the migration of excitons throughout the crystal.[34-40] The main features of this theory may be understood by a consideration of the diagram in Fig. 2. The interactions between the π-orbitals of the n molecules in the crystal are so large as to lead to a splitting of the energy levels, resulting in the formation of n closely spaced levels. In general, the lower band of levels are completely occupied by electrons, whereas the upper bands are vacant. Absorption of a quantum of light raises an electron from the lowest (ground-state) band into the upper, (singlet-state) band. Inasmuch as the levels in this upper band are very closely spaced, at most temperatures the thermal energy is sufficient to allow the electron to move from any one level in the band to any other level. Thus, the excited state cannot be considered as belonging to any one molecule in the crystal, but rather must be considered as belonging to the crystal as a whole. We thus arrive at a concept of an excited state free to migrate throughout an ordered array of molecules. Such an excited state can be visualized as consisting of a negatively charged electron in the upper, or excited, band and a positively charged hole (the vacancy left by the electron when it is raised into the upper band) in the lower, or valence, band. This is shown schematically in Fig. 3. The components of electron-hole pair attract each other through ordinary Coulomb forces and migrate as a unit throughout the crystal. Such a state of the crystal is called an exciton, inasmuch as it is formally equivalent to a neutral, massless particle with spin zero traveling through the crystal. It is apparent that it is possible to have triplet excitons as well as singlet excitons.

In an ideal crystal, such an exciton would migrate throughout the crystal until either it recombined with the emission of a quantum of radiation or its energy became degraded into the lattice vibrations of the crystal. However, all real crystals contain imperfections in the lattice structure resulting from dislocations, vacancies, impurities, etc. Such imperfections cause some of the excitons to be ionized, i.e., the electron and the hole are no longer constrained to migrate as a unit, but rather each is capable of moving independently of the other. Furthermore, the crystal imperfections give rise to trapping centers that are capable of immobilizing the electrons and (or) holes. These traps may be considered as energy levels lying somewhat below the lowest level of the conduction band. Ultimately, the electrons and holes recombine with each other, but it is quite possible for them to have very different histories before recombination, spending various amounts of time trapped in impurities and imperfections in different parts of the crystal. In general, the most mobile entities in organic crystals are the holes.[41]

There is some controversy in the literature on whether exciton migration or resonance transfer is the most significant mechanism for energy transfer in ordered systems.[23] The objections of Franck and Livingston[32] and



SCHEMATIC REPRESENTATION OF THE FORMATION OF ENERGY BANDS IN CRYSTALS THROUGH THE INTERACTION OF n MOLECULES. THE PAIRS OF ARROWS REPRESENT ELECTRONS WITH ANTIPARALLEL SPINS. G = GROUND STATE, S' = LOWEST EXCITED SINGLET STATE.

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Fig. 2. Schematic representation of the formation of energy bands in crystals through the interaction of n molecules. The pairs of arrows represent electrons with antiparallel spins. G = ground state; S' = lowest excited singlet state.

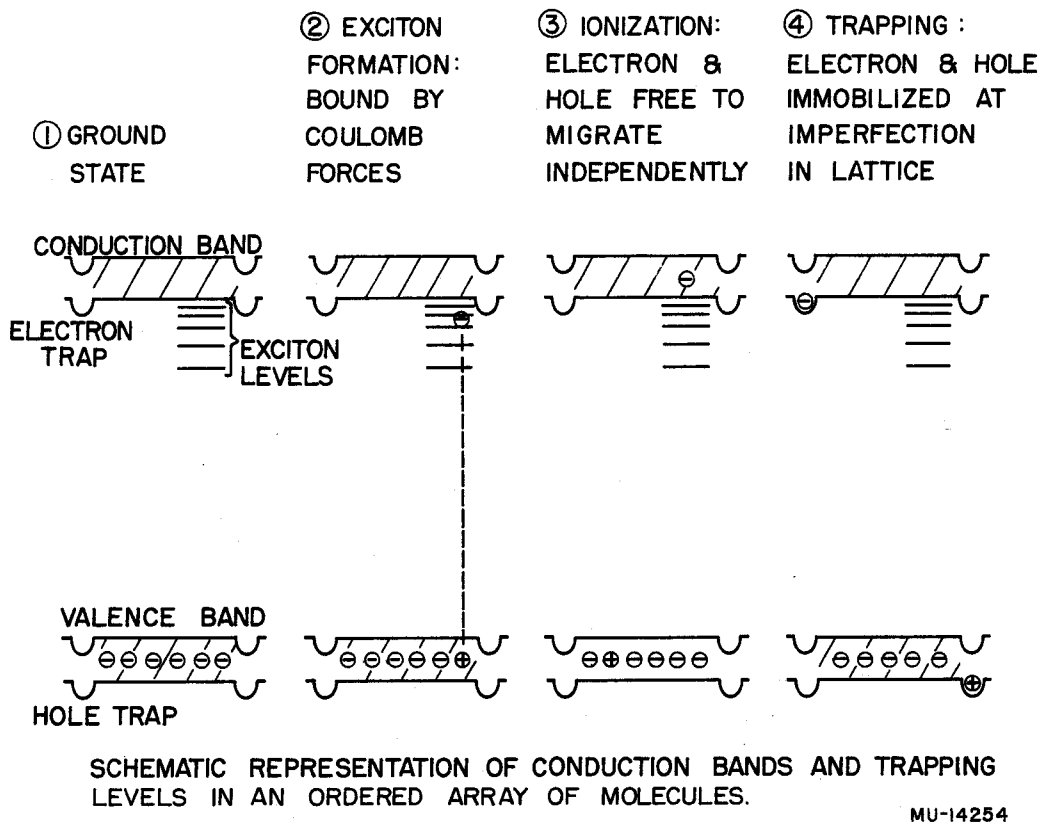


Fig. 3. Schematic representation of conduction bands and trapping levels in an ordered array of molecules.

of Livingston[33] to the exciton theory, in the case of anthracene crystals, are primarily that one cannot account for the absorption and emission spectra of the pure crystals in terms of it. However, it must be pointed out that an adequate quantum-mechanical interpretation of the absorption spectrum of crystalline anthracene has been given by Davydov[37] and by Craig and Hobbins[42] on the basis of exciton theory. Furthermore, recent work on the photoconductivity (see below) of anthracene crystals and on the sensitized fluorescene of impurities in anthracene crystals[43] suggests that both these phenomena represent alternative pathways for the degradation of an exciton, which probably takes place at a dislocation in the crystal.

B. Experimental Aspects

The above discussion, while greatly oversimplified, enables one to achieve a good deal of insight into many of the electronic properties of organic crystals, some of which include:

(1) photoconductivity: the electrons and holes formed as a result of the absorption of light, being free to migrate throughout the crystal, endow the crystal with the property of conducting an electric current. The exciton itself, being neutral, does not contribute to the photoconductivity.

(2) semiconductivity: at room temperature, the thermal energy is normally insufficient to achieve a very large dark population of the conduction band in most crystals. However, as the temperature is raised, more and more electrons are excited into the conduction band in accordance with Boltzmann's law. Thus, many crystals that are insulators at low temperatures exhibit an increasing conductivity as a function of temperature.

(3) luminescence: there are, in general, four main mechanisms of luminescence in organic crystalline semiconductors, not all of which need be operative simultaneously. These are:
(a) direct decay of the exciton (fluorsecence or phosphorescence).
(b) recombination and radiative decay of the electron and hole subsequent to ionization but prior to trapping.
(c) excitation of the trapped electron and (or) hole into the conduction band, followed by recombination and radiative decay.
(d) transfer of the excitation energy to a fluorescent impurity in the crystalline lattice (sensitized fluorescence).

Luminescence processes "a", "b", and "c" all lead to emissions of the same wavelength but with different time constants and temperature dependencies. Process "a" is relatively temperature-independent; process "b" may or may not exhibit a temperature coefficient, depending upon the actual mechanism of ionization; process "c" has a very definite temperature dependence as a function of the depth of the traps.

(4) thermoluminescence: if the trap depths are such that, at a given temperature, the excitation of the trapped electron or hole into the conduction band does not proceed at a measurable rate, irradiation followed by an increase in temperature leads to luminescence. Under such conditions, the luminescence-vs-temperature curve of the crystal exhibits peaks corresponding to the various trap depths.

A typical example of an energy-transfer process in molecular crystals is given by the study by Bowen and co-workers on anthracene crystals.[44] They found that the presence of 0.1% of naphthacene in anthracene almost completely quenches the blue-violet fluorescence of anthracene and replaces it with the yellow-green emission of naphthacene. The quantum yield of this process is only slightly less than that of the fluorescence of pure anthracene. Similarly, traces of anthracene in naphthalene replaces the uv fluorsecence of the naphthalene with the blue-violet anthracene fluorescence. The semiconductivity and photoconductivity of anthracene have been quite extensively studied,[43] and--of somewhat more biological interest-similar studies have been carried out on the phthalocyanines.[45]

A very interesting series of experiments on the germination of seed by Hendricks and co-workers and by Evanari and co-workers has shown that red light (5250 A-7000 A) stimulates such germination whereas infrared radiation (7000 A-8200 A) reverses this effect.[46-49] These effects show a marked resemblance to phenomena exhibited by many crystalline phosphors and, in fact, the suggestion has been made that these phenomena are the result of the formation of trapped electrons in a semiconducting system by the action of the red light and of the detrapping of the electrons by the infrared light. It will be interesting to see if further experimentation supports this viewpoint.

IV. Energy Transfer in Green Plant Materials

Katz,[50] in 1949, and, independently, Bradley and Calvin,[51] in 1955, suggested that aggregates of chlorophyll molecules in the chloroplasts might give rise to conduction bands in which photoproduced electrons and holes could migrate. Such a system would have the advantage of providing for a separation of the oxidizing and reducing entities known to be necessary for photosynthesis.

This concept has remained purely speculative until, quite recently, a number of researches have been published which suggest that something of this nature may indeed take place within chloroplasts. In 1956, Commoner and co-workers published evidence for the presence of a light-induced electron spin resonance (ESR) in spinach chloroplasts due to the photoproduction of unpaired electrons.[52] Again, in 1957, these workers have shown the presence of two kinds of unpaired spins, one of which is transformed into the other.[53] In 1957, Arnold and Sherwood studied dried chloroplast films and found them to exhibit semiconductivity and thermoluminescence.[54] In addition some studies by Strehler and co-workers have demonstrated the existence of temperature-dependent long-lived luminescences in algae and in chloroplasts.[55-58]

Our own experiments in this area began in 1956 with the demonstration by Sogo of a light-induced ESR signal in dried eucalyptus leaves. Inasmuch as these results were rather poorly reproducible, it was decided to study isolated chloroplasts.[59] Furthermore, when it became apparent that the spin resonance signals decayed fairly rapidly when the light was turned off, the possibility that at least part of the energy associated with these unpaired spins might appear as luminescence led us to a study of the light-emission properties of the chloroplasts.[60]

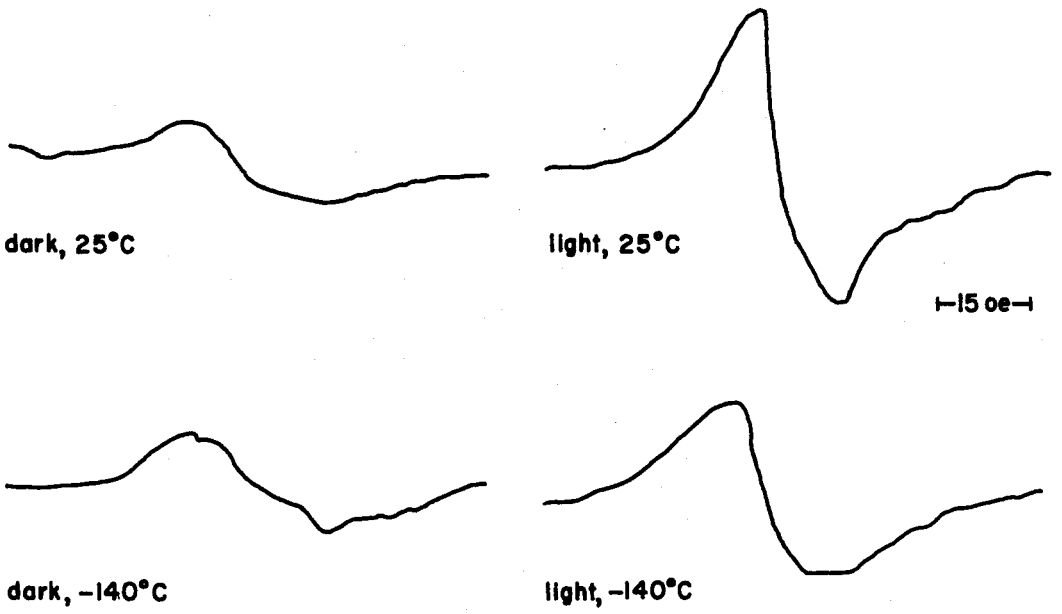
The chloroplasts are prepared by grinding spinach leaves in a blendor and carrying out a series of differential centrifugations.[59] These enable us to obtain what we shall call intact chloroplasts and large and small chloroplast fragments.

Some typical ESR curves for wet large chloroplast fragments are shown in Fig. 4. These curves are essentially plots of microwave power absorbed in the sample vs magnetic field strength. It is seen that there is an increase in the number of unpaired spins when the light is turned on, both at room temperature and at -140°C. These signals represent approximately 10^{16} unpaired spins. The wave-lengths of light effective in exciting these signals are between 3500 A and 4500 A and between 6000 A and 7000 A, indicating absorption by chlorophyll. A rough quantum-yield measurement indicates a value lying between 0.1 and 1.

Figure 5 shows some results of growth- and decay-time measurements on the same samples. In this case, the curves represent power absorption vs time at constant magnetic field strength. The half-time for the decay at 25°C is of the order of 30 seconds. At low light intensities (about 10^{15} quanta per second) the rise time is about 30 seconds and at higher light intensities (about 10^{16} quanta per second) the rise time is about 6 seconds. There is good reason to believe that even the 6-second figure is light-limited. At -140°C essentially the same rise times are observed, but the decay time is of the order of hours. This effect of cooling is completely reversible. With dried chloroplasts at 25°C, the rise times are similar but the decay times are of the order of hours. However, at 60°C the decay time of the dried material is of the order of seconds. These figures are summarized in Table I.

Some of the luminescence decay curves for wet whole spinach chloroplasts are shown in Fig. 6. The apparatus is designed so that we are able to observe continuously the light emitted from the chloroplasts approximately 0.1 second after excitation by a flash of light.[60] An analysis of these curves and those for intermediate temperatures demonstrates that the room-temperature emission consists of at least three components having different temperature dependencies and having half-lives of 0.15, 2, and 15 seconds, respectively. Approximately 6% of the total integrated light intensity up to about 7 seconds after the flash is due to the 0.15-second emission. When the chloroplasts are cooled, the slower components diminish in intensity and vanish at about -35°C. At this temperature, the decay curve is the same as that obtained by subtracting the slower components from the room-temperature curve. When the chloroplasts are cooled still further, the 0.15-second component diminishes in intensity, its decay constant remaining approximately the same, and is gone at about -100°C. At about -90°C, a fourth emission begins to grow in and gradually increases in intensity down to liquid nitrogen temperature. This emission has a half-life of about 0.3 second. These cooling effects are completely reversible. Both large and small spinach chloroplast fragments behave similarly.

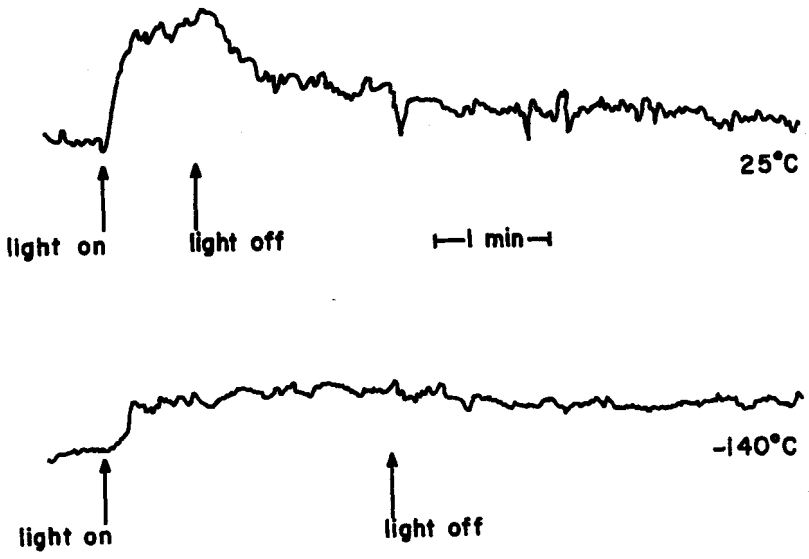
The excitation and emission spectra of the luminescence were measured, with Corning glass filters between the flash and the sample and between the sample and the detector. Such experiments demonstrate that



Spin Resonance Spectra from Wet, Large Chlorophyll Samples

MU-12842

Fig. 4. Typical spin resonance spectra from wet large chloroplast fragments.



Signal Growth and Decay Time Curves

MU-12873

Fig. 5. Some results of growth- and decay-time measurements on wet large chloroplast fragments.

Table I

Comparison of ESR and luminescence observations on chloroplasts

Material	t (°C)	Rise time		Decay time	
		ESR[a]	700-900 mμ luminescence[a]	ESR[a]	700-900mμ luminescence[a]
Wet chloroplasts	25	~sec (light-limited)	< 0.1 sec	~30 sec	0.15 sec (6%) 2 sec 15 sec (94%)
	-140	~sec (light-limited)	no signal	~hr	no signal
Dried chloroplasts	25	~min	no signal	~hr	no signal
	60	~sec	?	~sec	~sec

[a]Excited by wavelengths between 350 and 450 mμ or 600 and 700 mμ.

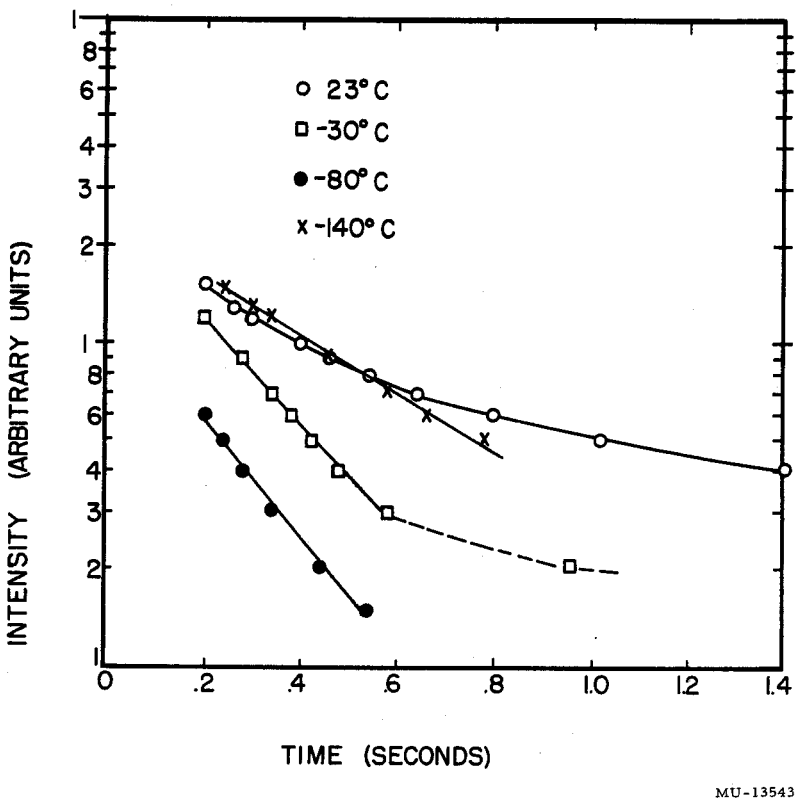


Fig. 6. Luminescence decay curves for wet whole spinach chloroplasts at four temperatures. Log intensity is plotted against time.

the room-temperature and -40°C emissions are excited by the same bands of wavelengths as induce the electron spin resonance, thus again indicating absorption by chlorophyll. These emissions consist of wavelengths lying between 7000 A and 9000 A. The crude measurements indicate that at least 90% of the emitted light is of longer wavelengths than 7000 A. The in vivo fluorsecence from the chlorophyll singlet lies mainly between 6500 A and 7200 A. This suggests that we are observing the lowest triplet state of chlorophyll rather than the lowest singlet. However, better spectra are needed to clarify this point and these are in the process of being measured in our laboratory. Similar experiments demonstrate that the low-temperature 0.3-second emission is excited only by wavelengths between 3500 A and 4500 A (light between 6000 A and 7000A has no effect) and that this emission consists of wavelengths between 10, 000 A and 12, 000 A.

Figure 7 shows the effects of allowing freshly prepared chloroplasts to stand in the dark at 23°C. Up to 8 hours, the luminescence gradually increases in intensity, and reaches a maximum intensity 2.7 times that of freshly prepared material. This larger signal exhibits the same decay curve, wavelength properties, and temperature behavior as does the original signal. Allowing the chloroplasts to stand still longer decreases the luminescence intensity and causes changes in the decay curve. After about 72 hours the luminescence has disappeared entirely, and the chloroplasts exhibit thermoluminescence similar to that observed by Arnold and Sherwood for quick-dried chloroplasts.[54]

While it is not possible to quantitatively compare the ESR results with the luminescence results at this time, there are a number of qualitative similarities that are significant (these are summarized in Table I):

(a) Both phenomena are excited by the same bands of wavelengths and both are due to absorption by chlorophyll.

(b) The 25°C decay times for wet chloroplasts are of the same order of magnitude for both phenomena. Inasmuch as the ESR spectrometer had a time constant of 2 seconds, the ESR decay corresponding to the shorter luminescence decay times could not have been detected.

(c) At -140°C the ESR decay times are of the order of hours and no luminescence could be detected (a luminescence with a decay time of the order of hours would be undetectable with the apparatus used in the studies reported here).

(d) At 25°C the decay time of the ESR for dried chloroplasts is of the order of hours and under similar conditions the chloroplasts did not luminesce.

(e) At 60°C the ESR of the dried chloroplasts had a decay time of the order of seconds. At this same temperature, we have observed a peak in the thermoluminescence of the dried chloroplasts.

The above similarities strongly suggest that the 7000-9000-A light emission of chloroplasts is at least in part the result of the decay of the unpaired spins detected by the ESR experiments. A quantitative comparison of the quantum yields, action spectra, and kinetic constants of these two

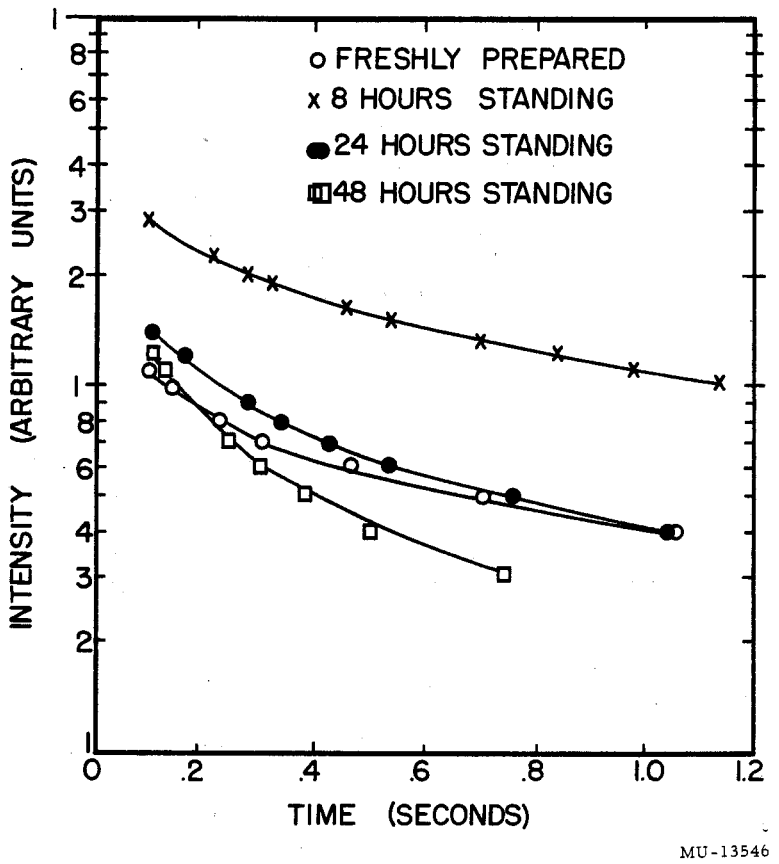


Fig. 7. Effects of allowing freshly prepared wet whole spinach chloroplasts to stand in the dark at 23° C.

phenomena is now being carried out. This should lead to a more definitive assessment of the relationships between them.

There are four possible mechanisms for the production of either ESR or delayed light emission in systems of the type we are concerned with here. These are:

(1) the production of radicals by the direct photodissociation of a single bond, followed by their recombination in the dark;

(2) the excitation and decay of a triplet state;

(3) the reversible photosensitization of chemical or enzymatic processes leading to the production of free radicals;

(4) production of trapped electrons in a quasi-ordered lattice.

Mechanism (1) is incompatible with the following considerations. No known stable naturally occurring chemical bond can be dissociated by 6000–7000-A light. Furthermore, decay times of the order of many seconds are not in the range to be expected for radical recombinations at relatively high temperatures. Finally, it is difficult to reconcile such a mechanism with the existence of three separate emissions of the same wavelength.

The excitation and decay of a long-lived triplet state, as in Mechanism (2), is incompatible with the observed definite temperature dependence of the chloroplast luminescence, i.e., it is very unlikely that lowering the temperature to -100°C would increase the triplet lifetime to the order of hours. Furthermore, such a mechanism cannot result in three separate emission acts having different time constants but of the same wavelength.

If enzymatic processes were involved here, as in Mechanism (3), cooling to -140°C should decrease the rates of these processes to essentially zero. This is not in accord with the fact that the rise time and the concentration of unpaired spins are about the same at this temperature as at 25°C. Similarly, the presence of the 0.15-second emission down to as low a temperature as -100°C rules out the participation of enzymatic processes in either the forward or reverse transformations in this case. If, then, only the 2- and 15-second emissions represent chemical processes, one would expect that cooling, by preventing the reaction leading to radical formation from taking place, would result in appearance of a greater amount of energy in the form of the 0.15-second decay. In fact, the emission at -80°C is less than it is at room temperature. Such a viewpoint is supported by the aging experiments mentioned earlier. Thus, if one assumes that the aging process involves the inactivation of enzymes, then the creation of centers (or radicals) for the 2- and 15-second emission processes by enzymatic means should be reduced. This reduction of competitive processes should then lead to an increase in the intensity of the 0.15-second emission together with a concomitant decrease in the intensities of the 2- and 15-second emissions. In fact, for aging periods up to 8 hours, all three emission intensities are increased by the same amount.

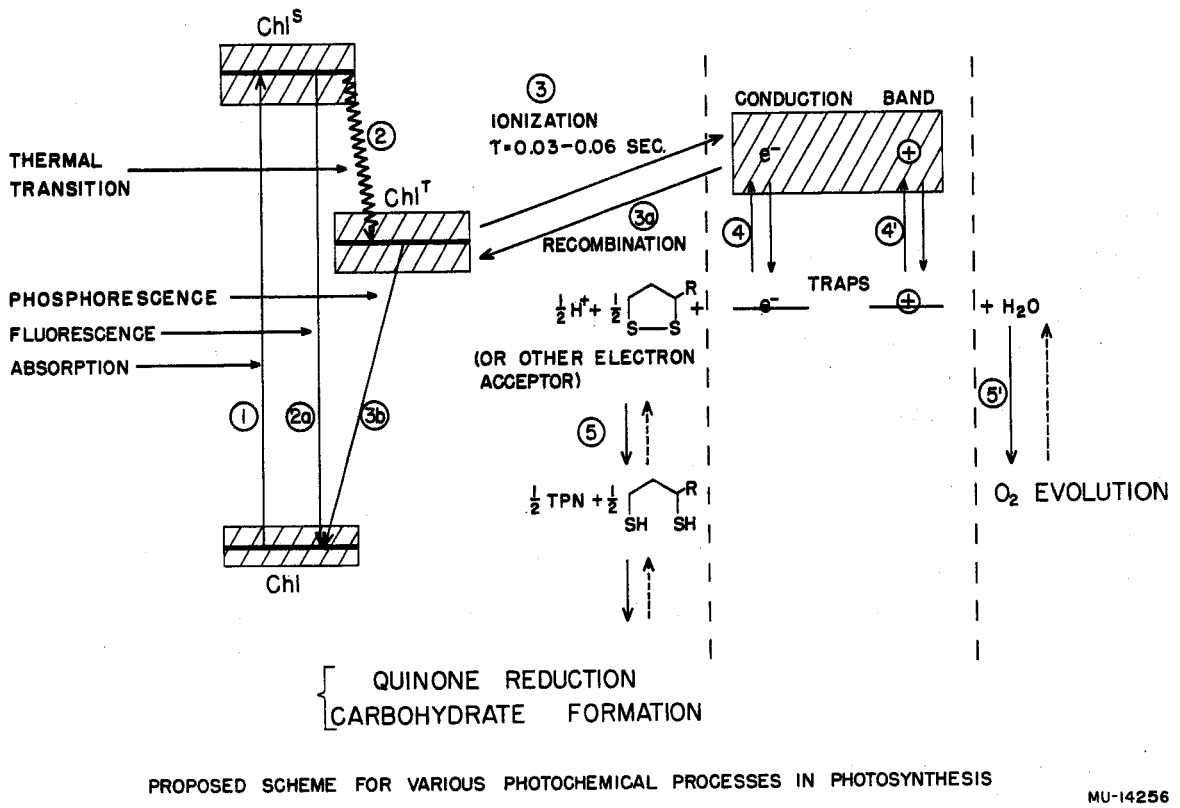
We are thus left with Mechanism (4) as the most likely explanation for the phenomena we are reporting here. We shall next see how such a scheme fits the data. Figure 8 is a schematic representation of the electronic energy bands in chloroplasts. Light is absorbed to produce the transition from the ground-state band of an aggregate of chlorophyll molecules to the first excited singlet state band (Process 1). From the first excited singlet, the energy is split between conversion to an exciton in the first excited triplet band (Process 2) and fluorescence (Process 2a). All these processes are well known in ordinary molecular systems and all will have time constants of the order of 10^{-8} second or less.

According to this picture, the triplet-state exciton undergo ionization, resulting in electrons and holes in the conduction band (Process 3). At the instant the exciting light is turned off, then, a certain fraction of these electrons and holes are in the traps (Processes 4 and 4'). The number of these traps in the chloroplast is probably very small, perhaps of the order of one per several thousand chlorophyll molecules. Thus, this scheme leads directly to the idea of a "photosynthetic unit."[61] A small proportion of the remaining electrons and holes are near enough to each other to recombine (Process 3a) and return to the ground state via Process 3b. We identify this recombination process with the rate-limiting step of the 0.15-second emission. The decay constant of such a process should be relatively temperature-independent, and the experimental results are in accord with this. The fact that the intensity of this emission decreases with the temperature suggests the existence of a process whose rate increases with decreasing temperature and which is competitive with the recombination. Whether this is the actual trapping of the electron or hole or is some side process is not known.

Arthur and Strehler have recently observed a temperature-independent emission in chloroplasts with a half-life of about 0.01 second.[57] It is possible that this emission represents the direct decay of the exciton via Process 3b.

The electrons and holes that are trapped give rise to a spin resonance signal. The traps are thermally depopulated and the resultant electrons and holes in the conduction band recombine and a temperature-dependent luminescence results. The 2- and 15-second-lifetime emission may then be identified with the depopulation of traps of different depts. At low temperatures, the thermal energy is insufficient to excite the electrons and holes out of the traps. This results in the disappearance of the luminescence and the appearance of a long-lived ESR signal. According to this picture, the thermoluminescence referred to earlier is the result of a deepening of the traps due to drying.

The electrons and holes in the traps may also be used up by enzymatic processes (Processes 5 and 5'). Any reversibility in these enzymatic processes would then lead to a long-lived luminescence which could be classified as a chemiluminescence. It is likely that some of the longer-lived emissions reported by Strehler and co-workers[55, 56] are of this nature, and perhaps also the 15-second emission reported here. The fact that almost



PROPOSED SCHEME FOR VARIOUS PHOTOCHEMICAL PROCESSES IN PHOTOSYNTHESIS

Fig. 8. Proposed scheme for various photochemical processes in photosynthesis.

three times as much energy is emitted as light in aged chloroplasts as in fresh chloroplasts suggest that these enzymes are easily inactiviated and that this enzymatic utilization represents the normal pathway for most of the electrons and holes in the living cell. In this way the light energy could be made available to the photosynthetic mechanism.

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