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ENERGY CONVERSION IN PHOTOSYNTHETIC PROCESSES

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ABSTRACT

The concepts of solid-state photophysics are applied to biological materials, especially particulate matter 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.

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The chemical pathway by which carbon is transformed from its low-energy form, carbon dioxide, into its high-energy form, principally carbohydrate, has been fairly completely mapped by use of tracer carbon, and our present knowledge of it is exhibited in Fig. 1. The photochemical apparatus that provides the driving force for running the carbon cycle from Co_2 toward polysaccharide, designated by [E] in Fig. 1, is contained in the chloroplasts of green plants and in the chromatophores of the simpler organisms such as photosynthetic bacteria and blue-green algae. The microstructure of this apparatus has been investigated down to the level that may be reached with an electron microscope, and such an electron micrograph is shown in Fig. 2. The universal appearance of these ordered structures in all photosynthetic equipment suggests that the primary quantum conversion occurs in a quasi-crystalline phase. One might be justified, therefore, in speaking of the primary quantum conversion in photosynthesis as a photophysical operation rather than a photochemical one.

Katz[1] in 1949, and, independently, Bradley and Calvin[2] 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.[3] Again, in 1957, these workers have shown the presence of two kinds of unpaired spins, one of which is transformed into the other.[4] In 1957, Arnold and Sherwood studied dried chloroplast films and found them to exhibit semi-conductivity and thermoluminescence.[5] In addition, some studies by Strehler and co-workers have demonstrated the existence of temperature-dependent, long-lived luminescences in algae and in chloroplasts.[6-9] Finally, the photo-conductivity of chlorophyll films has been observed.[10]

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.[11] 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.

*The work described in this paper was sponsored by the U.S. Atomic Energy Commission.

The chloroplasts are prepared by grinding spinach leaves in a blender and carrying out a series of differential centrifugations.[11] These enable us to obtain what we shall call "intact chloroplasts" and "large and small chloroplast fragments."

Some typical ESR curves for wet whole spinach chloroplasts are shown in Fig. 3. These curves are essentially derivative plots of microwave power absorbed in the sample vs magnetic field strength. These signals represent approximately 10^{16} unpaired spins. The wave lengths of light effective in exciting these signals are between 3500 Å and 4500 Å and between 6000 Å and 7000 Å, indicating absorption by chlorophyll. A rough quantum-yield measurement indicates a value lying between 0.1 and 1.

Figure 4 shows some results of growth- and decay-time measurements on the samples. In this case, the curves represent power absorption vs time at constant magnetic field strength. An analysis of the 25° decay curve (Fig. 5) produced the two decay times of 1 second and 10 seconds. The room-temperature rise time for the light-produced signal is less than the 0.2-second response time of the detection apparatus. At -150°C essentially the same rise time is observed, but the decay time is on 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 on the order of hours. However, at 60°C the decay time of the dried material is on 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.[12] 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 -40°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 apparently 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 by use of a Bausch and Lomb grating monochromator between the flash and the sample and between the sample and the detector.[13] The curves are shown in Fig. 7. The action spectra for Chlorella° and for spinach chloroplasts are quite similar to the absorption spectra of these materials. The action spectrum for Nostoc, on the other hand, shows a relatively low activity for chlorophyll and carotenoids and a high activity for phycocyanin. Thus, electronic excitation energy may be transferred, with some degree of efficiency, from carotenoid to chlorophyll in Chlorella and in spinach chloroplasts, but such transfer occurs only rather poorly in Nostoc.

Table I

Comparison of ESR and luminescence observations on chloroplasts

Temperature (°C)	Relative ESR light signal	Rise time		Decay time	
		ESR[a]	Luminescence at 600—800 mμ	ESR[a]	Luminescence at 600—800 mμ
Wet Fresh Chloroplasts					
25	3	<0.2 sec[b]	<0.1 sec[b]	< 1 sec[b] (60%) 10 sec 40%	0.15 sec (6%) 2 sec 15 sec
-35	9	< 1 sec[b] (75%) 12 sec (25%)	<0.1 sec[b]	< 1 sec[b] (33%) 10 sec (33%) 3 min (33%)	0.15 sec (33%) 2 sec [illegible]
-140	4	< 1 sec	no signal	~hr	no signal
Dried Chloroplasts					
25	...	~min	no signal	~hr	no signal
60	...	~sec	...	~sec	~sec

[a]Instrument limited

[b]Excited by wave lengths between 350 and 450 mμ or 600 and 700 mμ.

The room-temperature emission spectra of thin films of Chlorella[14] and of chloroplasts demonstrates that the luminescence is the result of a transition between the first excited singlet state and the ground state of chlorophyll. The somewhat broadened shape of the luminescence spectra in comparison with a typical fluorescence spectrum is the result of the relatively large monochromator slit widths necessitated by the low intensity of the luminescence. Filter experiments indicate that the 0.15-, 2- and 15-second emissions all have the same spectral distribution.

In an earlier publication it was tentatively suggested, on the basis of measurements with filters on thick films of material, that the luminescence of spinach chloroplasts was the result of a triplet-state—to—ground-state emission of chlorophyll.[12] This is now recognized as having been due largely to the self-absorption distortion of the emission spectrum.

Emission spectra for thick films of spinach chlorophasts at three temperatures are also plotted in Fig. 7. Thick films were used in order to compensate for the low intensity of the low-temperature emissions (see above). The markedly different shape of these spectra, in comparison with the thin-film spectra, is due to self-absorption. However, it is apparent that the curves at all three temperatures are identical in shape, thus suggesting that they are the result of the same electronic transition. This temperature independence of the spectrum of emission indicates that the triplet state of chlorophyll is not involved at all in the delayed light emission of spinach chloroplasts.

The luminescence of Nostoc is very much weaker than that of either Chlorella or spinach chloroplasts, therefore necessitating the use of thick films of material. In view of this, it would be expected that if the emission were due solely to chlorophyll, the spectrum would be similar to those obtained with thick films of chloroplasts. However, it is not similar. This suggests that a significant portion of the emitted light originates in phycocyanin(which has a fluorescence peak at about 660 mμ) and is self-absorbed in the thick layer.

Similar experiments using Corning glass filters in place of the monochromator demonstrate that the low-temperature 0.3-second emission of chloroplasts is excited only by wave lengths between 3500 Å and 4500 Å (light between 6000 Å and 7000 Å has no effect), and that this emission consists of wave lengths between 10,000 and 12,000 Å.

Figure 8 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, wave-length properties, and temperature behavior as does the original signal. Allowing the chloroplasts to stand even 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.[5]

Although 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 wave lengths 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.

(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 6000—8000-Å light emission of chloroplasts is at least in part the result of the decay of some of the unpaired spins detected by the ESR experiments. That some of the radicals decay by nonluminescent processes is indicated by the fact that the light emission at -35°C is smaller than at room temperature. A quantitative comparison of the quantum yields, action spectra, and kinetic constant of these two 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, involving migration of the fragments, 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-Å 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 wave length.

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 -140°C would

increase the triplet lifetime to the order of hours, as required by the spin-resonance studies as well as by the fact that no triplet emission can be observed. Furthermore, such a mechanism cannot result in three separate emission acts having different times constants but of the same wave length.

Cooling to -140°C should decrease the rates of any chemical or enzymatic processes occurring here, as in Mechanism (3), to essentially zero. Thus, the unpaired spins that are produced at this temperature cannot be considered as having arisen in this manner. On the other hand, the ESR results at -35°C, that is, the appearance of a 12-second rise time, suggest that part of the radicals formed at that temperature and at room temperature are due to chemical transformations. The larger spin signal at -40°C may be accounted for by assumption of a greater temperature coefficient for the decay of these chemically produced radicals than for their formation.

Some insight into which of the decays are associated with the chemical reactions and which with the purely physical mechanism may be gotten from the following considerations. 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-second emission represents a chemical process, 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-second emission process 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 intensity of the 2-second emission. In fact, for aging periods up to 8 hours, both emission intensities are increased by the same amount.

We are thus left with Mechanism (4) as the most likely explanation for the primary process of the phenomena we are reporting here. We shall next see how such a scheme fits the data.

Figure 9 is a schematic representation of the electronic energy bands in chloroplasts. Inasmuch as the band width is proportional to the square of the transition probability for the transition from ground state to excited state,[15] the excited singlet state is much broader than the corresponding triplet state. Thus, there may be a good deal of overlap between the energy levels of these two states. It is necessary to postulate such overlap in order to provide a relatively temperature-independent pathway between the states to account for the inability to observe triplet-state emission, even at -70°C.

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. Singlet-state excitons may then undergo one of three competing processes:

(1) They may decay to the ground state via fluorescence emission ($\tau \cong 10^{-9}$ sec

(2) They may ionize with the formation of electrons and holes in conduction bands ($\tau \sim 10^{-9}$ sec). Calculations have shown that such a lifetime would permit the exciton to migrate over from 100 to 1000 molecules.[16]

(3) They may cross over in a radiationless transition into the triplet state in times as short as 10^{-12} sec.

If the triplet-state conversion is important in chloroplasts, ionization into the conduction bands may occur from this state. The electrons and holes in the conduction band migrate and ultimately are trapped at suitable points in the lattice. Characteristic lifetimes of 0.01 to 0.1 second have been observed in many types of experiments on photosynthetic materials.[2, 3, 11, 12] According to the present hypothesis, this would represent the time required for the population of the traps. If ionization occurs from the singlet state, this time constant may be identified with the lifetime of one of the charge carriers in the conduction band. Such an hypothesis has some support from the fact that, for germanium, the intrinsic minority carrier lifetime is calculated to be 0.75 second.[17] Experimentally, lifetimes on inorganic semiconductors may range from 10^{-18} second to several seconds.[18] No corresponding measurements have been made for organic semiconductors. If, on the other hand, ionization occurs from the triplet state, the 0.01- to 0.1-second time constant may represent either the ionization time constant or a carrier lifetime.

It is not possible, at present, to decide which of the two mechanisms, direct ionization from the singlet state or ionization from the triplet state, is operative in chloroplasts. Indeed, it may be that both processes occur simultaneously.

The number of 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."[19] 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. Such recombination may occur directly into the singlet state or into the singlet state via the triplet state. The 2- and 15-second-lifetime emissions can be identified with the depopulation of traps of different depths. The 0.15-second decay may represent either the depopulation of a shallow trap or the lifetime of one of the charge carriers in the conduction band. Further experimentation is in progress to determine the nature of the 0.15-second decay as well as the 0.01-second decay reported by Arthur and Strehler.[8]

At low temperature, the thermal energy is insufficient to excite the electrons and holes out of the traps, and enzymatic production and decay of radicals no longer occurs. This results in the disappearance of the luminescence and the appearance of a long-lived ESR signal. The thermoluminescence referred to earlier may be the result of a deepening of the trapping levels due to drying.

The electrons and holes in the traps may also be used up by enzymatic processes. 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[6] are of this nature, and perhaps also the 15-second emission reported here. If these enzymatic processes involve free radicals, similar decay times will occur in the spin-resonance analysis. The fact that almost three times as much energy is emitted as light in aged chloroplasts as in fresh chloroplasts suggests that these enzymes are easily inactivated. A similar increase in the number of light-induced

radicals in aged chloroplasts is found in spin resonance experiments. These observations suggest that 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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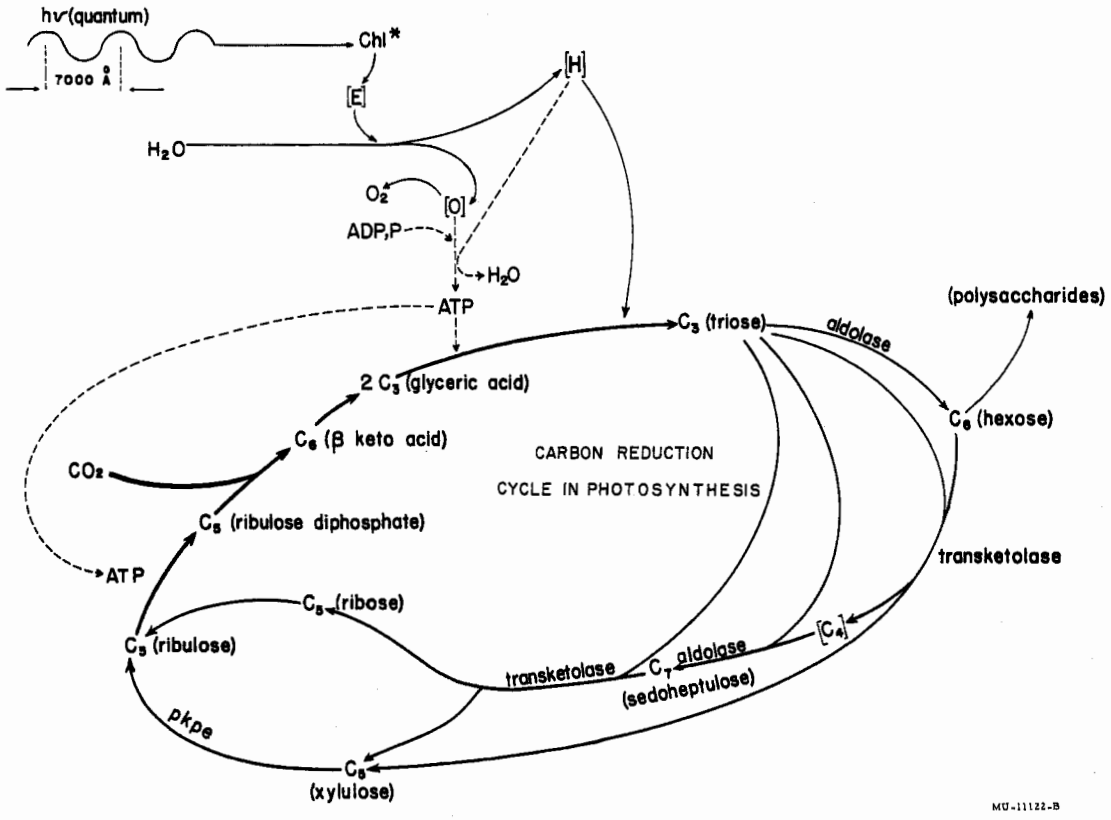

Fig. 1. Proposed cycle for carbon reduction in photosynthesis.

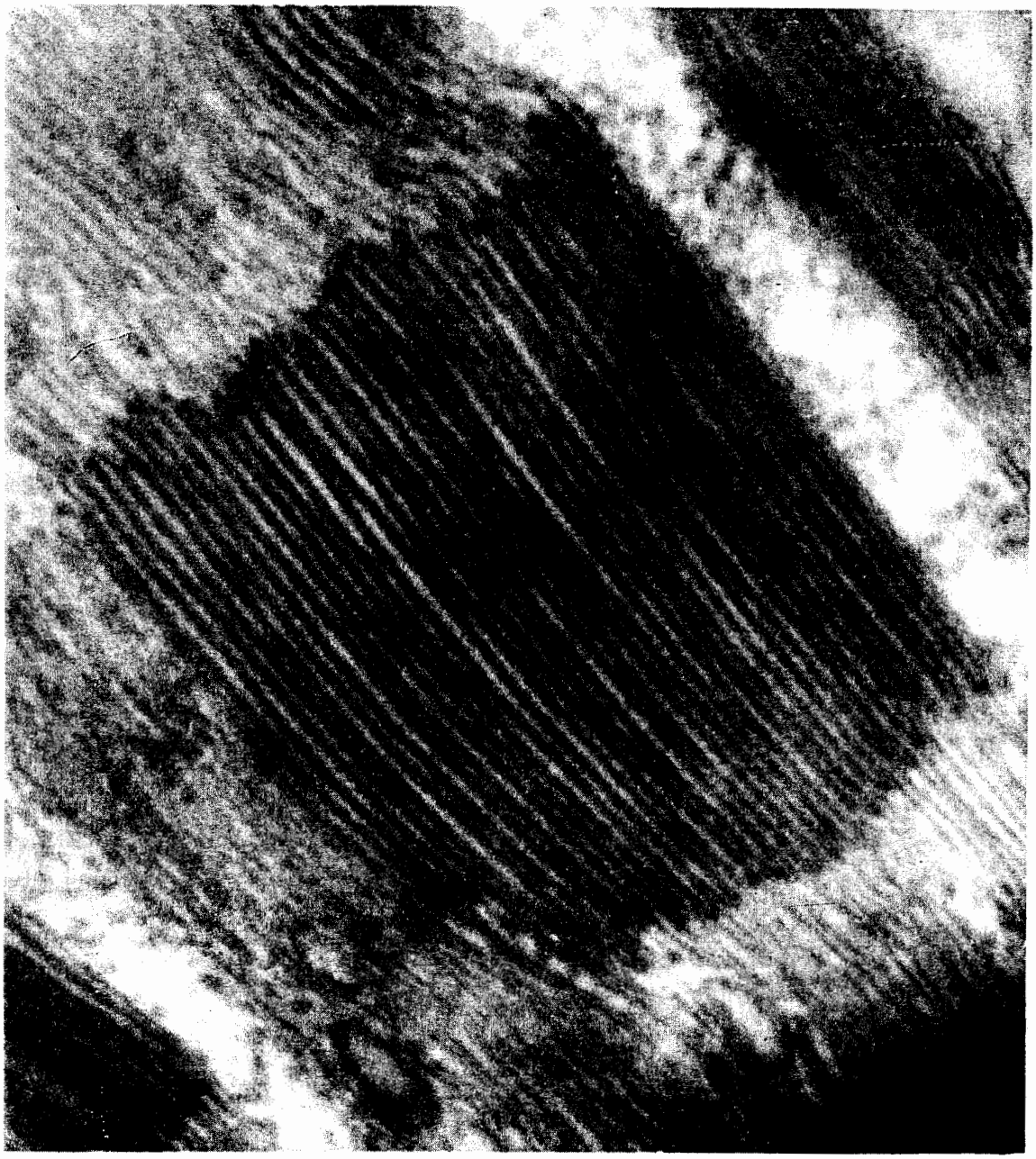

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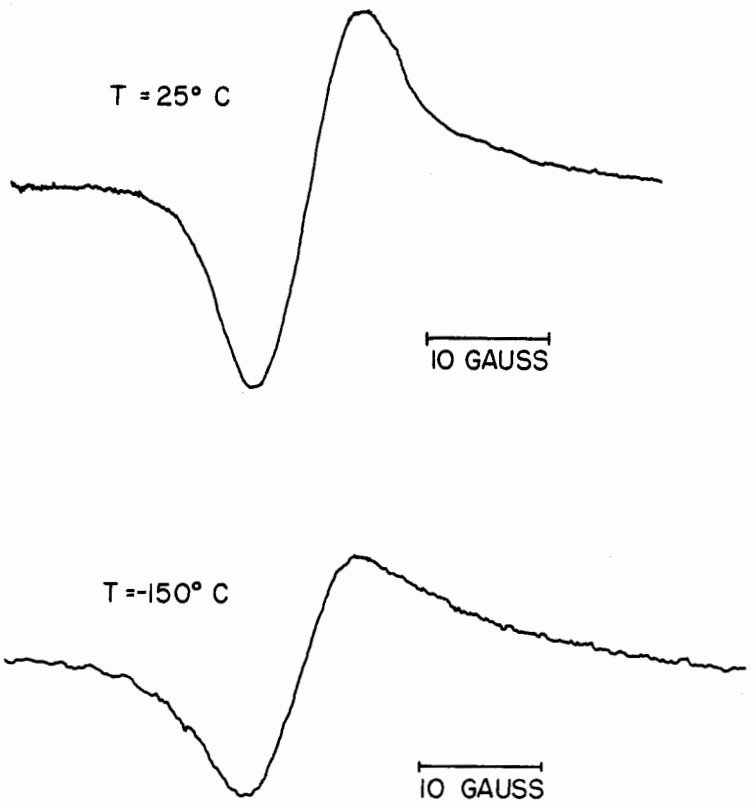
Fig. 2. Ultrastructure of chloroplasts.



LIGHT SIGNALS FROM WHOLE SPINACH CHLOROPLASTS

MU-14534

Fig. 3. Light signals from whole spinach chloroplasts.

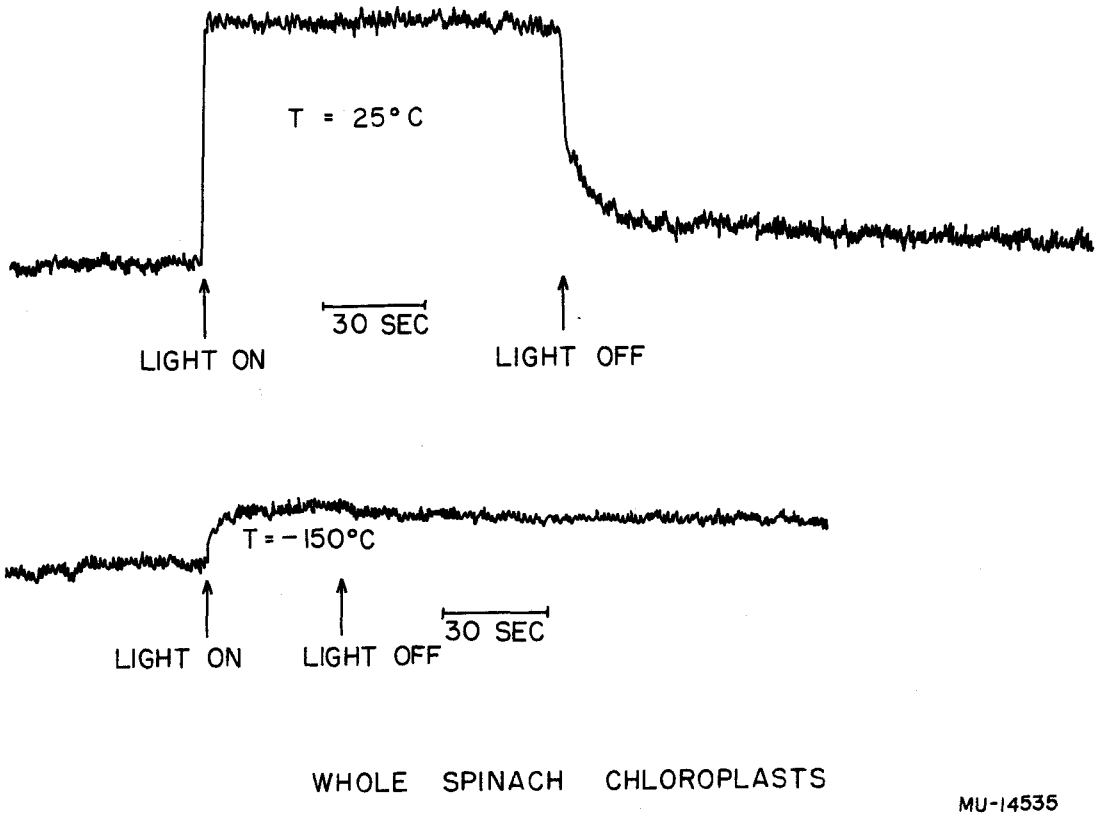


Fig. 4. Growth and decay curves of whole spinach chloroplasts at T = 25°C.

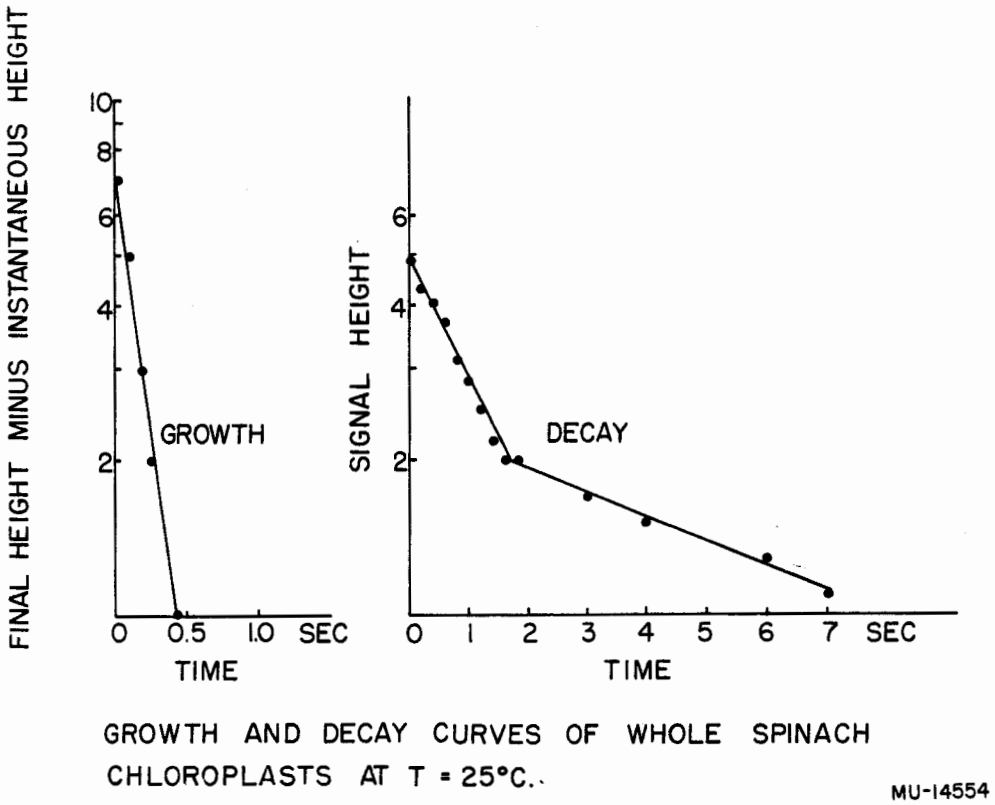


Fig. 5. Analysis of growth and decay curves of whole spinach chloroplasts at T = 25°C.

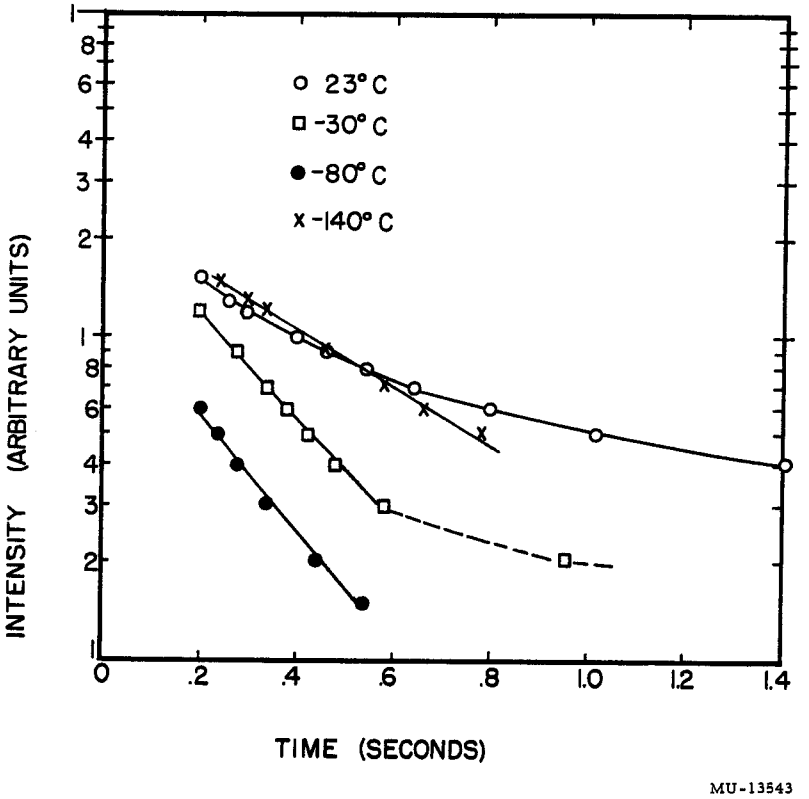


Fig. 6. Luminescence decay curves for wet whole spinach chloroplasts at four temperatures. Log intensity is plotted against time.

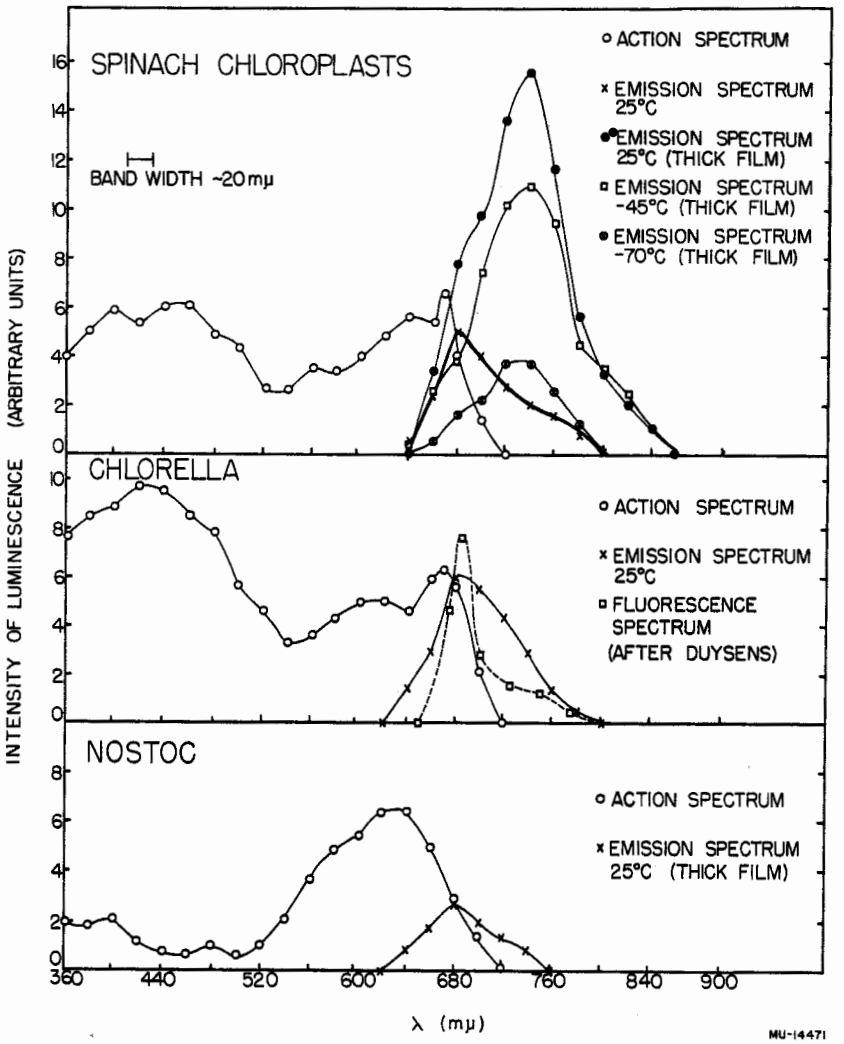


Fig. 7. Excitation and emission spectra for Chlorella, spinach chloroplasts, and Nostoc.

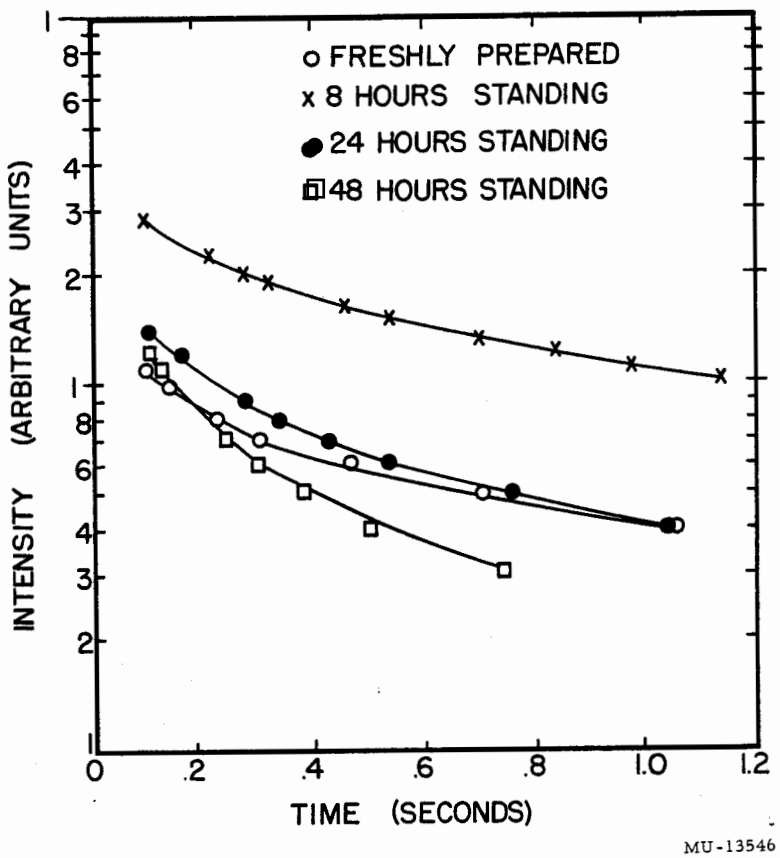


Fig. 8. Effects of allowing freshly prepared wet whole spinach chloroplasts to stand in the dark at 23°C.

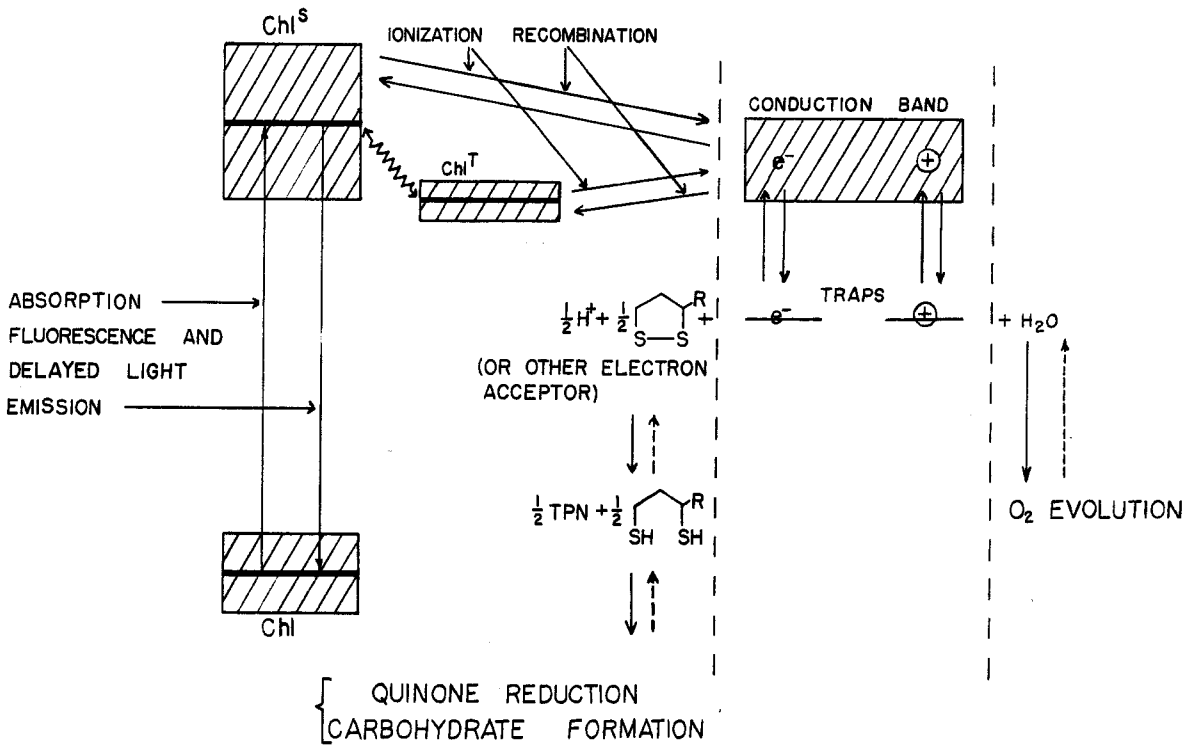


Fig. 9. Propsed scheme for various photochemical processes in photosynthesis.