Title

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Professor Melvin Calvin

April 11, 1960

SOME PHOTOCHEMICAL AND PHOTOPHYSICAL REACTIONS OF CHLOROPHYLL AND ITS RELATIVES*

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ABSTRACT

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The solution photochemistry of chlorophyll and chlorophyll analogs is described. Many cases of electron transfer to or from the porphyrin macrocycle have been found, but in no case has any very large degree of energy storage been achieved. Because of the very rapid back-reaction for products with a ΔF of approximately -30 kcal, some solid state models in which such an energy storage might be achieved are described and their possible relation to the natural photosynthetic apparatus is given.

We can see that while the solid state model (phthalocyanine) allows an approach from a somewhat different point of view, the net result is the same as what was sought, but so far not found, when we looked at the solution chemistry of chlorophyll (and chlorophyll model substances), namely, the transfer of an electron, or hydrogen atom, from the excited porphyrin to an electron acceptor at a high reduction level which can be used to reduce the ultimate carbon dioxide reducers, followed by the donation of an electron ultimately from water to the remaining radical ion, or lattice, which produces the net results of the transfer of the hydrogen from water to carbon dioxide.

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How much of the solid state picture will be required to fully understand this separation of oxidant and reductant, I think is yet to be determined. However, I believe it is quite clear that we are coming to the same kind of conclusion from both ends, that is, from both the pure solution chemistry which involves electron transfer from donor to acceptor and from the solid state experiments which involve the same kind of electron transfer from donors to acceptors. The difference lies in the types of lattices involved. The back-reaction in the solid state experiments is demonstrably slower than one can visualize for the solution electron transfer reaction in which no provision is made for the rapid, relatively temperature-independent separation of the products, electron (reducing agent), and hole (oxidizing agent).

SOME PHOTOCHEMICAL AND PHOTOPHYSICAL REACTIONS OF CHLOROPHYLL AND ITS RELATIVES

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INTRODUCTION

This is a discussion of some of the photochemistry and photophysics of porphyrins which has accumulated in the course of the last fifteen to twenty years and which has some bearing on the problem in which we are primarily engaged, namely, the conversion of electromagnetic into chemical energy as it occurs in photosynthetic organisms. The history of the photochemical and similar properties of chlorophyll and its related materials is an old one. From the very beginning of the recognition of chlorophyll as a prime light-absorber and converter in green plants (26), there has been a steady flow of model experiments with chlorophyll, and related materials, trying to discover in simplified systems the types of transformations which conceivably might be taking place in the living organism in its operations for the conversion of electromagnetic into chemical energy.

From very early times, even before the chemical structure of chlorophyll and its relatives were known (32,76), a number of photochemical properties of the chlorophyll molecules in solution had been observed. These can be most easily described in two classifications. The first is a photosensitized oxidation reaction with molecular oxygen in which the chlorophyll is the photosensitizer, that is, the chlorophyll absorbs the light and causes, in some way, the oxidation of some other substrate with molecular oxygen; some of the pigment may be destroyed, depending on the conditions of the reaction, but

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conditions can be found in which the chlorophyll itself is relatively stable and acts as a photosensitizing dyestuff which will cause the oxidation of a good many substrates. Recent examples of this type of reaction are the studies of G. Schenck in Germany[57] in which he has studied the photosensitized oxidation of a whole variety of materials. One of the very early and more quantitative studies was that of Gaffron[29] in which he used chlorophyll as a photosensitizer for the oxidation of allylthiourea and, in fact, studied it thoroughly enough so that it could be used as an actinometer -- that is, as a means of measuring actual light intensity in a beam, particularly in the red. The quantum yield for this reaction, that is, the number of allylthiourea molecules oxidized per quantum absorbed by chlorophyll, is approximately one.

The other type of photochemical reaction in solution which chlorophyll is known to sensitize is a hydrogen transfer from some reducing agent to some oxidized substance. The classic example is the reduction of an azo dye, such as methyl red, by a reducing agent (hydrogen donor) such as ascorbic acid, and chlorophyll has long been known to sensitize the transfer of hydrogen from the reducing agent to the acceptor.

Both these types of cases, for the most part, are photosensitized reactions in which the thermodynamics favors the reaction itself, and the light largely serves the function of overcoming activation energy for the reaction. In general, then, there is not, in any of these reactions, a conversion of electromagnetic into chemical energy.

PHOTOCHEMISTRY OF CHLOROPHYLL MODELS

Relationships between Chlorophyll, Bacteriochlorophyll and Protochlorophyll

In 1937, I first became acquainted with the chemistry of porphyrins and recognized the relationship of porphin to chlorin. In order to see the type

of reasoning involved, I think it is best to look at the structural formulas of the principal energy-capturing molecules in the photosynthesizing organism. Figure 1 shows the structural formula of chlorophyll as we now believe it to be, and you will notice that it is a porphyrin with an isocyclic ring and an 'extra' pair of hydrogens on one of the pyrrole rings, making the chlorin a dihydroporphyrin. In the last few years, Linstead and his co-workers have proved that these two hydrogesn are trans to each other.(50)

It is interesting to see the relationship of the chlorophyll molecule to two others, one of which is bacteriochlorophyll (the light-capturing pigment in the photosynthetic bacteria), and that relationship can also be seen in Figure 1. There are two more 'extra' hydrogens in bacteriochlorophyll, presumably on a pyrrole ring on a diagonal from the hydrogen-bearing one in chlorophyll, and on the No. 1 pyrrole ring the vinyl group has been transformed into an acetyl group. The protochlorophyll (Figure 1) which is the material formed in plants when they are grown in the absence of light, can be recognized as the dehydrochlorophyll (a porphin). The No. 4 pyrrole ring in protochlorophyll has a double bond in it, and it has been shown by a number of workers (62) that the first thing that happens in etiolated plants (which have no chlorophyll in them) when the light is turned on, is the addition of the two hydrogens to the double bond in ring No. 4 to generate chlorophyll.

An examination of these three formulas shows this relationship of chlorophyll to protochlorophyll and bacteriochlorophyll very neatly. It shows that the macrocycle of chlorophyll lies midway in the oxidation level between that of protochlorophyll and that of bacteriochlorophyll. With the diffusion of the idea of Van Niel that the primary photochemical reaction of green plants involved the fission, or splitting, of the water molecule to give hydrogen and oxygen, or to give a reducing agent and an oxidizing agent, and that this reducing agent was used to reduce carbon dioxide and the

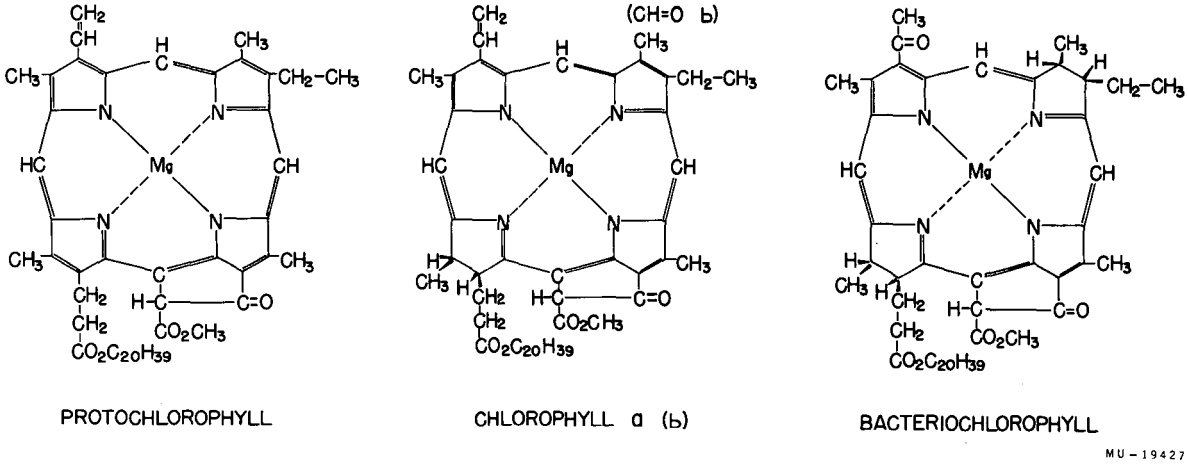

Fig. 1. Structural formulas of chlorophyll, bacteriochlorophyll and protochlorophyll.

oxidizing agent actually generated oxygen, it occurred to me that the function of the chlorophyll might be as a hydrogen carrier from the water toward the ultimate reducing agent which is used to reduce carbon dioxide. Today we believe one of these ultimate reducing agents to be pyridine nucleotide. It seemed likely that the chlorophyll might be functioning between the stage of chlorophyll and protochlorophyll, that is, between the stage of dihydroporphyrin and porphyrin, as a transferring agent of the hydrogen from water to something else.

This was a very early notion, and the earliest experiments which were devised to test it, such as doing photosynthesis in deuterated water to see if the two hydrogens that were picked up on the chlorophyll macorcycle were deuterium, failed of positive results. The first experiment of this kind was done by Ruben back before the war (53) and a second one was done in our own laboratory (6), using tritium in the hope we could detect smaller amounts of photosensitized exchange; this also failed to show a tritium incorporation into chlorophyll very much greater than that of new synthesis of the entire molecule.

These unsuccessful results then led to the next notion, namely, that the chlorophyll might be functioning not between the level of protochlorophyll and chlorophyll (between the level of porphyrin and dihydroporphyrin) but between the level of dihydroporphyrin and tetrahydroporphyrin, as represented by bacteriochlorophyll. In that case, any study of deuterium exchange in chlorophyll would fail. If the hydrogen transfer involved first the photochemical reduction of the chlorophyll (dihydroporphyrin) to tetrahydroporphyrin, and if this, then, was transferring its hydrogen to the acceptor which ultimately reduced CO_2, and we analyzed only for the dihydroporphyrin, we would not, of course, find any isotope in the dihydroform. It would have been passed on to the ultimate reducing agent.

This would require that in the green plant there should be traces of dihydrochlorophyll (tetrahydroporphyrin), although the steady state, or equilibrium, amount of dihydrochlorophyll might be minutely small and hard to discover. We have yet to perform an experiment in which we seek to find dihydrochlorophyll (or something close to it) in the green plant and to determine whether or not it undergoes a photosensitized isotope exchange. A similar experiment might very well be done in photosynthetic bacteria in which, presumably, the steady state, or equilibrium, amount of tetrahydroporphyrin (or dihydrochlorin) is large and the dihydroporphyrin (or chlorin) is small. This should show, if this type of transformation is the way in which the reaction is proceeding, a large and easily detectable photosensitized deuterium, or tritium, exchange. As far as I know, this experiment has not yet been done.

There is, however, one type of photosensitized deuterium exchange experiment which has been successful, and this is the experiment of Vishniac in which he has shown what appears to be the exchange of a labile proton, presumably on chlorophyll, which is photocatalyzed. He believed it to be the relatively labile isocyclic hydrogen which is exchangeable, since it is enolizable. Vishniac also believes to have shown a photosensitized, or photoaccelerated, exchange of some proton, on other compounds not identical with chlorophyll. Perhaps some of this could possibly be the dihydrochlorophyll mentioned earlier. Some of it might also be in the form of the next hydrogen carriers (see later). It remains to be seen what the exact nature of this exchange reaction is and whether it has any connection with the photosynthetic process.

Photochemical Hydrogen Transfer -- Model Systems

With this background it seems worthwhile to examine some model systems for photochemical hydrogen transfer. The model systems chosen (long before

the aforementioned exchange experiments with chlorophyll were done) were those which did not have the side chains on them which made the compound considerably more labile with respect to incidental transformations. Chlorophyll itself was relatively difficult to obtain in completely pure form, so we undertook to synthesize a model substance which would not be subject to the above-mentioned difficulties and which would have only the porphyrin nucleus and the dihydro- and tetrahydroporphyrin possibilities. Such a molecule is the simple tetraphenylporphyrin whose structure is shown in Figure 2 in which the four phenyl groups are on the bridging carbon atom and which contains a simple porphyrin nucleus. This material is relatively easy to synthesize. It is made simply by heating benzaldehyde with pyrrole in the presence of zinc ion to obtain a 10-15% yield of the zinc porphyrin with traces of zinc chlorin in which one of the double bonds is reduced. These substances can be separated by chromatography and fractional crystallization, their properties determined independently and unequivocally and their photochemistry studied. Figure 2 shows also the structure of the zinc chlorin in which one of the pyrrole rings is in the dehydro form, and Figure 3 shows the absorption spectra of these two forms. It is very easy to distinguish between the dihydroporphyrin, that is, the chlorin, and the porphyrin and the spectral difference between the two substances has been used to study the kinetics of the photochemical transformation of one to the other.(21)

The first of these transformations (and the easiest to study) was the photoinduced conversion of zinc dihydroporphyrin (chlorin) into zinc porphyrin using some hydrogen acceptor. A whole series of hydrogen acceptors were used, most of them being quinones or molecular oxygen.(33) It was easy to demonstrate a very clean photochemical conversion of dihydroporphyrin

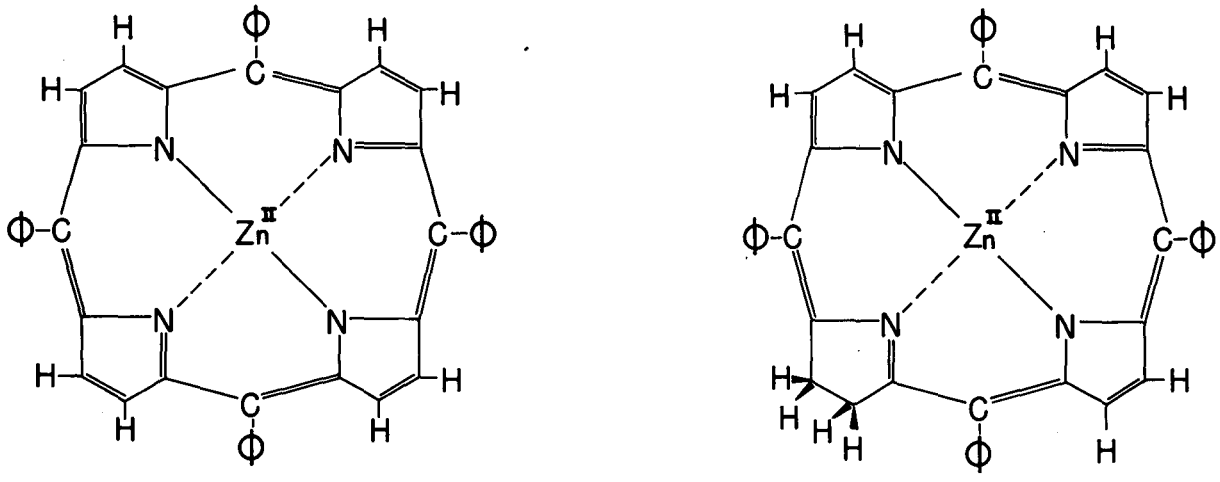

Fig. 2. Structural formula of zinc tetraphenylporphin and zinc tetraphenylchlorin.

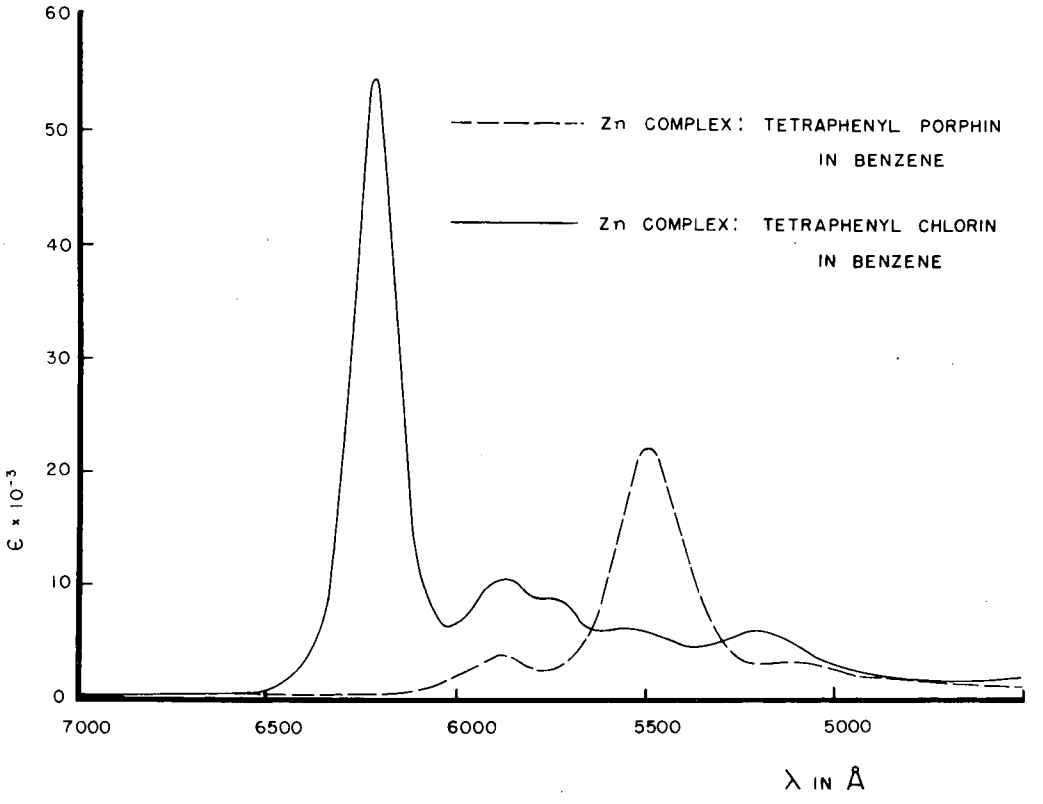

Fig. 3. Absorption spectra of zinc tetraphenylporphin and zinc tetraphenylchlorin.

into porphyrin and nothing else. Figure 4 shows the relative rates (quantum yields) of the transformations, and the relationship is clear between the rate of hydrogen transfer from chlorin to quinone and the ability of the quinone to hold the hydrogen, that is, the oxidation potential of the quinone. The greater the oxidation potential of the quinone, the faster is the transfer. Two series of experiments were done, on with para- and one with ortho-quinone; oxygen behaves like an ortho-quinone in the transformation and there is a very nice relation between the potential and the photochemical yield .

Unfortunately, none of these transfers of hydrogen from chlorin to these oxidation agents (hydrogen acceptors) involved the storage of chemical energy. In every case, the thermodynamics is such as to favor the system porphyrin + hydroquinone (over chlorin + quinone) and the light is simply overcoming the activation energy. The kinetics of these reactions were studied in some detail (Dorough and Calvin, [21]) and it was easy to demonstrate that a long-lived excited state of the chlorin was involved, since the rate of the reaction did not depend upon the concentration of the hydrogen acceptor at all, down to very low concentrations. This led to the suggestion that the excited state was the triplet state of the chlorin which has been found in a whole variety of chlorins, including chlorophyll a. [7, 21]

Figure 5 shows the kinetic analysis of this experiment. You can see that the quantum yield is dependent upon the ratio of k_3 to $k_3 + k_5$, and what we suggest is that the ratio depends upon the quinone. You will notice that the rate law does not contain a factor for the concentration of quinone, but the quantum yield does contain a factor which is dependent upon which quinone you use. In this way we have accounted for the kinetic results. In this case the hydrogen acceptor is a very good oxidizing agent and the transfer does not entail any energy storage. If the transfer of hydrogen could

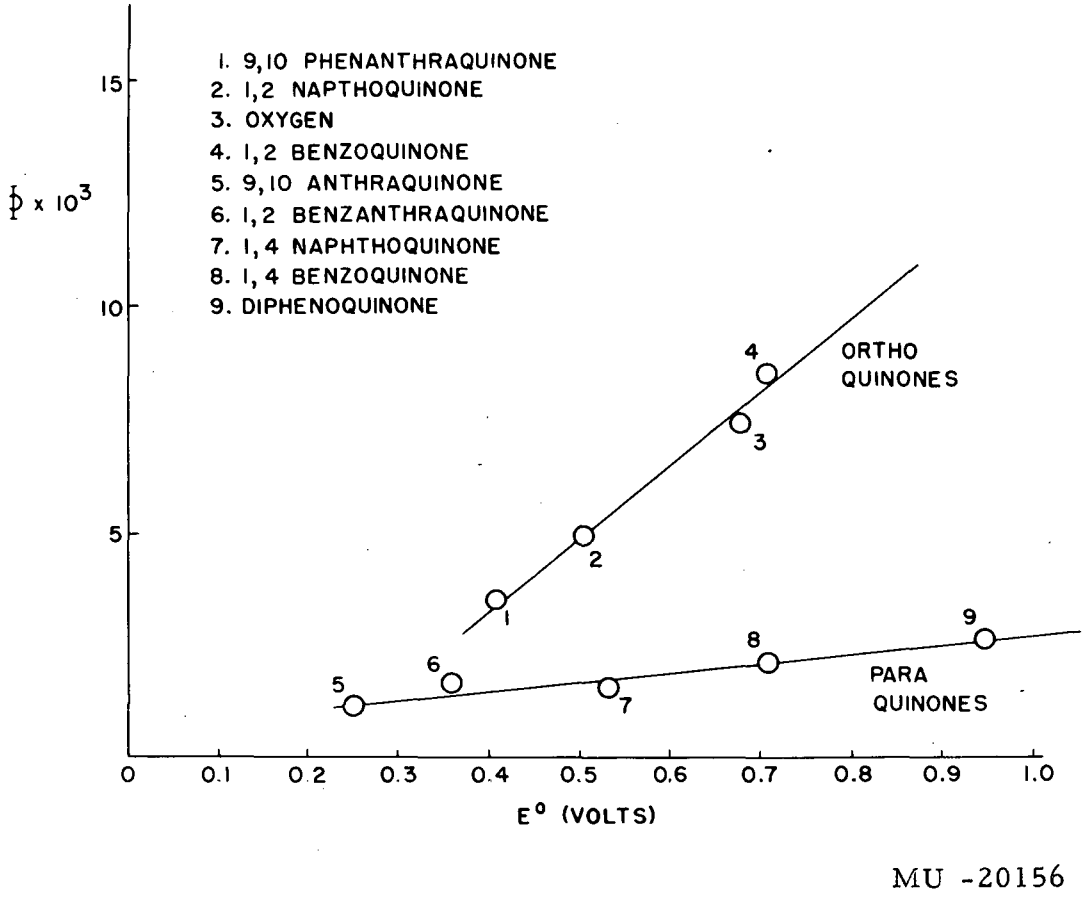

Fig. 4. Relation of quantum yield in photooxidation of zinc tetraphenylchlorin to the oxidation potential of hydrogen acceptor.

Excitation: $C_{Zn} + h\nu \xrightarrow[k_1]{} C^{S'}_{Zn}$

Deactivation of singlet:

$$\begin{cases} C^{S'}_{Zn} \xrightarrow[k_4]{} C_{Zn} \quad (h\nu \text{ or } kT) \\ C^{S'}_{Zn} \xrightarrow[k_2]{} C^{T}_{Zn} \end{cases}$$

Deactivation of triplet

$$\begin{cases} C^{T}_{Zn} + Q \xrightarrow[k_3]{} P_{Zn} + H_2Q \\ C^{T}_{Zn} + Q \xrightarrow[k_3]{} C_{Zn} + Q + kT \end{cases}$$

Rate law resulting from these steps:

$$(C_{Zn}) + \frac{1}{\Sigma d} \log \left(1 - 10^{-\Sigma (C_{Zn})\, d}\right) = \gamma\, Ka\, I_0\, t + \pi'$$

Where quantum yield, γ, equals: $\dfrac{k_2}{k_2 + k_4} \cdot \dfrac{k_3}{k_3 + k_5}$

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Fig. 5. Kinetic analysis of photooxidation of zinc tetraphenylchlorin.

be demonstrated from such a dihydroporphyrin to a more powerful reducing agent, that is, a molecule (i.e., pyridine nucleotide) which in its reduced form was more nearly like molecular hydrogen, perhaps something of more direct interest to photosynthesis could be shown.

The next question to be answered involved the possibility of doing the reverse reaction, i.e., the transfer of hydrogen from something which clearly was not as good a reducing agent as the dihydroporphyrin to the porphyrin to make the dihydroporphyrin; this actually would involve a storage of energy (58) Figure 6 shows the results of such an experiment. Here zinc tetraphenyl-porphyrin is being reduced by benzoin, which is an ene-diol resembling ascorbic acid in some respect. The solid line in Figure 6 is the spectrum of the porphyrin, and after 7 minutes of illumination the porphyrin is dropping and the chlorin is coming in. After one and one-half hours of illumination, most of the dye is in the form of the chlorin and most of the porphyrin is gone. The quantum yield of this reaction was extremely small, much smaller than that for the transformation in the other direction, but it involved, I believe, a storage of chemical energy.

The reaction does not generally stop at the dihydro- stage but goes on into the tetrahydro- and hexahydroporphyrin stages. The various relationships of these porphyrins are shown in Figure 7 which illustrates the hydrogen transfer reactions that have been demonstrated for this particular porphyrin. The first of these reactions was the transformation of chlorin by light into porphyrin using quinones as the acceptor. The reverse reaction, that is, the transformation of porphyrin into chlorin using ene-diols, is a variable one, depending on the ene-diol and also on the conditions of the reaction. The chlorin can then go further, into a tetrahydroporphyrin, which is a very good reaction compared to the first one. This result was in keeping with the notion that perhaps chlorophyll in the green plant was functioning not between

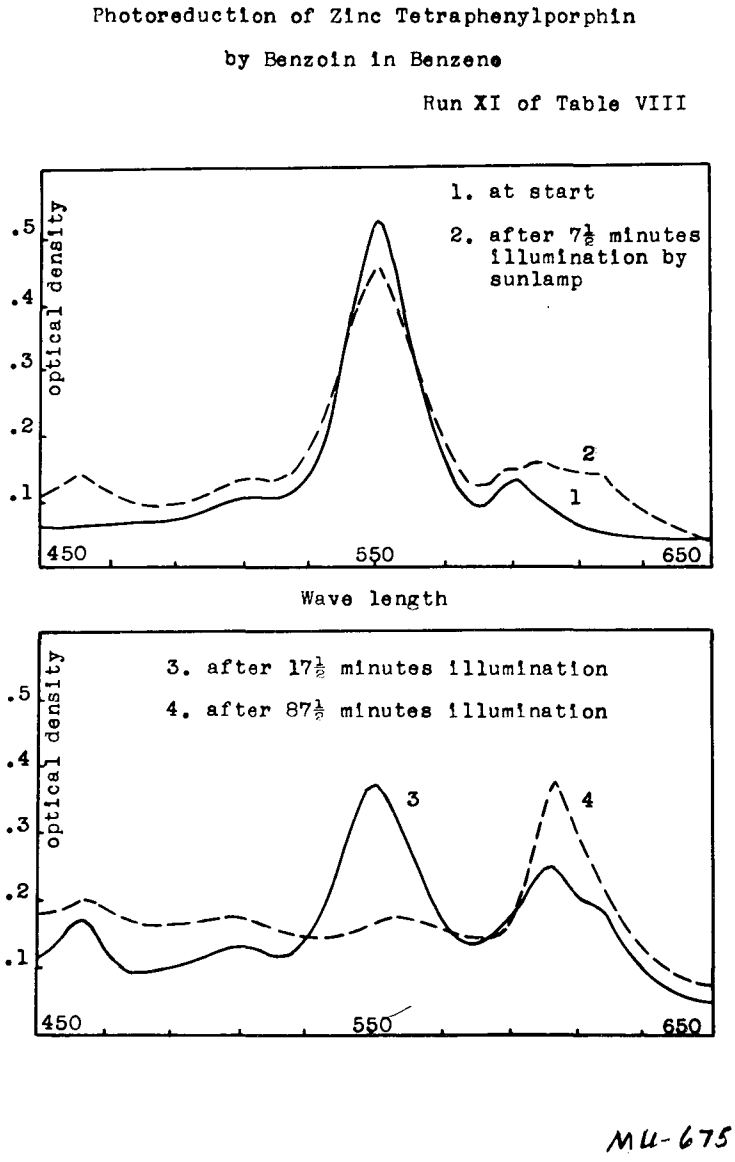

Fig. 6. Spectra showing photoreduction of zinc tetraphenylporphin by benzoin.

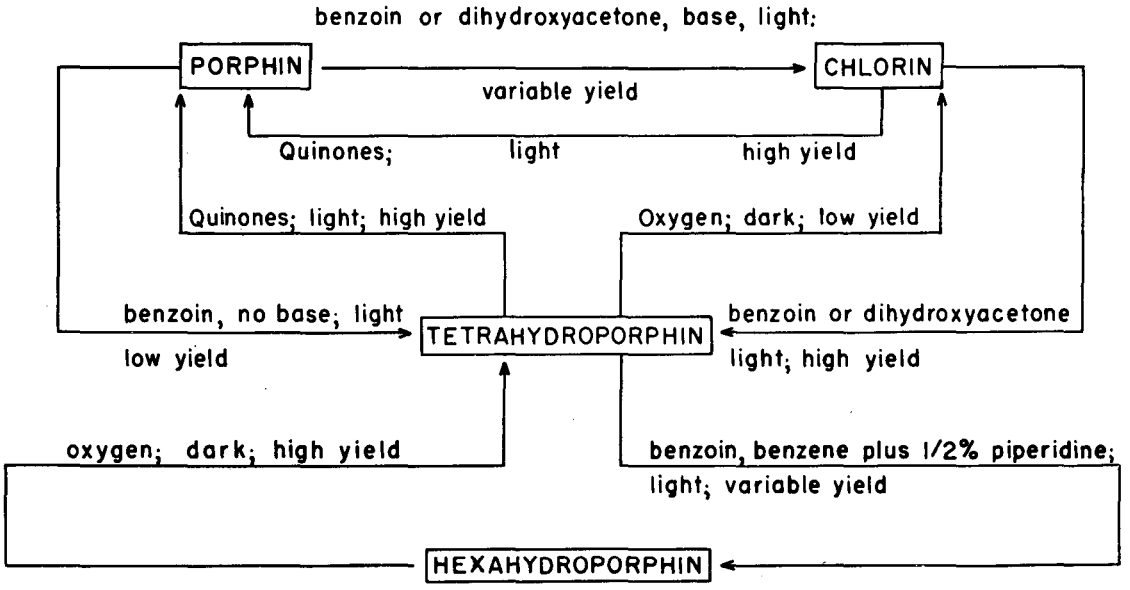

Fig. 7. Redox relations among the zinc tetraphenylporphyrins.

chlorophyll and protochlorophyll but between the chlorophyll and dihydro- or bacteriochlorophyll. The latter reaction does not stop at the tetrahydroporphyrin but under vigorous conditions it can be pushed to the hexahydroporphyrin.

The reverse reaction, namely, tetrahydroporphyrin and quinone, goes directly to porphin. The dihydro-form is not observed in between. The reaction of chlorin is much faster so that the accumulation of chlorin is not seen. The hexahydro-form will auto-oxidize, even in the dark, with oxygen to give the tetrahydroporphyrin very readily.

Thermodynamic Relationships

The question arises as to what indeed are the various energy levels of these porphyrins with respect to water, pyridine nucleotide, oxygen, etc., the various molecules which are involved in the process of photosynthesis itself. There is no direct and unequivocal information about the energy of these various transformations, primarily because the energy of hydrogenation of porphyrin to chlorin, or chlorin to dihydrochlorin, is not known. Only indirect information is available about this, and one must deduce, by indirection, what these energies might be. It is interesting to note what is evolved if one makes the best estimates one can about the energies involved in these transformations.

In the conversion of a porphyrin to a chlorin, a double bond is hydrogenated to give the dihydro compound. In doing so, the conjugated macrocycle is not destroyed. One such double bond can be removed without destroying the conjugated macrocycle, and in bacteriochlorophyll, two such double bonds can be removed and a conjugated macrocycle still exists. Therefore, as a first approximation, I would suggest that we use, in attempting the thermodynamic estimate, a ΔH for this reaction of about -30 kcal, which is

approximately that of a substituted olefin.*

$$\text{porphin} + H_2 \longrightarrow \text{chlorin} \qquad \Delta H \simeq -30 \text{ kcals} \qquad (1)$$

In order to estimate the energy for the hydrogenation of the pyridine nucleotide, one must remember that the free energy of this reaction is very nearly zero, that is, the reduction potential of TPN is very nearly the same as that of molecular hydrogen. If the free energy of this reaction is near zero, the heat will be equal to the entropy loss, which is 9 kcal.

$$\text{Pyridine nucleotide} + H_2 \longrightarrow PNH_2 \qquad \Delta H \simeq -9 \text{ kcals} \qquad (2)$$

The reason for this figure being so small is that a very large aromatic resonance is destroyed and that is why there is 20 kcal less energy evolved in the hydrogenation of this material (TPN) than in the hydrogenation of an ordinary olefin.

Combining these two reactions, one can write for the hydrogen transfer from chlorin to pyridine nucleotide to give reduced pyridine nucleotide:

$$\begin{matrix}\text{chlorin} \\ \text{or} \\ \text{(dehydrochlorin)}\end{matrix} + TPN \longrightarrow \begin{matrix}\text{Porphin} \\ \text{or} \\ \text{(chlorin)}\end{matrix} + TPNH_2 \qquad \Delta H \simeq 20 \text{ kcals} \qquad (3)$$

This is an 'uphill'reaction of the order of 20 kcal. To complete the cycle, the dihydroporphyrin has to be recovered. What is available now is a porphyrin (protochlorophyll, if the reaction is running between chlorophyll and protochlorophyll) which has to be returned to the chlorin stage in order for the reaction to continue. The hydrogen for this return must ultimately come from water, knowing what we do about the stoichiometry of the photosynthetic

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* The relation of protochlorophyll to chlorophyll might very well be materially different due to the steric requirements of the isocyclic (C_5) ring. Here one might expect somewhat less steric interference in the chlorophyll and thus the ΔH might be more negative.

reaction. The energy of getting hydrogen from water is +56 kcal. This reaction can now be combined with the hydrogenation of the porphyrin to the dihydroporphyrin:

$$H_2O \longrightarrow H_2 + 1/2\ O_2 \qquad \Delta H \simeq +57 \text{ kcals} \qquad (4)$$

$$\text{Porphin} + H_2O \rightarrow \text{chlorin} + 1/2\ O_2 \qquad \Delta H \simeq +26 \text{ kcals} \qquad (5)$$

The photolysis of water has now been divided into two steps, (5) and (3), one of which is the transfer of hydrogen to the porphyrin, if the reaction is running between porphyrin and chlorin (5). The calculations for the two reactions between chlorophyll and bacteriochlorophyll would be exactly the same as far as our precision is concerned. The total energy required for the reaction of two electrons -- the generation of one-half mole of oxygen -- has now been divided into two approximately equal parts, with one reaction 'uphill' approximately 20 kcal and the second one approximately 25 kcal.

In actual fact, this half mole of oxygen probably does not come off directly as molecular oxygen but goes through some oxygen acceptor species which then goes on to molecular oxygen. Reaction (5) would thus be broken into several steps, the size of which would depend on the nature of the unknown oxygen carrier.

Our model reactions have indeed shown that hydrogen can be transferred from dihydroporphyrin to an acceptor. Unfortunately, this particular hydrogen acceptor -- quinone -- is a very good one (not as poor a hydrogen acceptor as pyridine nucleotide), so it does not really correspond to such a transformation as (3) with a $+\Delta H$. The fact remains, however, that light will produce an excited state which induces the transfer of these hydrogen atoms to an

acceptor. The reduction of the double bond has been achieved only with much better hydrogen donors than water. They have always been relatively good reducing agents, such as ascorbic acid and a variety of other ene-diols or hydrazines which are the common materials used for this kind of transformation.

In fact, the photochemistry of chlorophyll models, and chlorophyll itself for that matter, has not yet produced in solution a model reaction in which such reactions as those described above, which involve relatively large energy storage acts, have been accomplished.

CHLOROPHYLL PHOTOSENSITIZED TRANSFORMATIONS

Hydrogen Transfer Reactions Catalyzed by Chlorophyll

Let us return to the sensitized hydrogen transfer reaction which is what one of the overall reactions of photosynthesis is presumed to be (hydrogen transfer from water to pyridine nucleotide) and determine which ones may be catalyzed by chlorophyll. The classic examples are the hydrogen transfers from materials such as ascorbic acid and hydrazine to dyestuff-acceptors such as azo dyes (methyl red). These reactions have been known for some time, and in the last 15 years they have been studied a great deal, particularly in the Soviet Union. One such reaction is called the Krasnovskii reaction after the man who has spent a great deal of time studying it.[42,43,44,46,47] Krasnovskii used chlorophyll and porphyrin model substances as sensitizers to transfer hydrogen from a variety of donors (ascorbic acid, particularly) to methyl red and other azo dyes. He did it under such conditions that he was able to show two steps as separate events, that is, the transfer of hydrogen from the hydrogen donor to chlorophyll to give some intermediate, followed by the transfer of hydrogen from this intermediate to the hydrogen acceptor,

giving back again the initial chlorophyll. By cooling the reaction mixture, and performing the experiment in a basic solvent such as pyridine, Krasnovskii was able to show that chlorophyll plus ascorbic acid, without the addition of a hydrogen acceptor, would go from a green color to a pink color. This pink color was presumed to be some intermediate, not necessarily bacteriochlorophyll, since the spectrum did not correspond. The reaction reverses in the dark, and the 'pink' intermediate is not a radical.[49]

The general result of all of these studies is shown in Figure 8[27,42,43,44,46,47] in which the whole series of transformations is systematized. The chlorophyll absorbs the light, the excited chlorophyll (probably in a triplet state, as the kinetics indicate that such is the case) removes either a hydrogen atom, or an electron from the donor (AH_2) to give what Krasnovskii believes to be a radical, or a radical ion (Ch), (at low temperatures, he believes he has caught this radical ion [45]) leaving behind a positive radical (AH_2^+) which then dissociates to give a proton. The free radical or ion (AH or AH_2^+) can then go ahead and reduce another chlorophyll, and the free radical ion of chlorophyll (Ch^-) can hand on the hydrogen atom, or electron (or both) to the dyestuff (D_y) to give back the chlorophyll starting material and the partly reduced semiquinone of the dye. This, then, finishes its reduction, either by combination with a radical or by taking a proton directly off the hydrogen donor itself, to give the colorless dyestuff and the dehydroascorbic acid or other dehydro compound. This is a generalized scheme which appears to apply for a whole variety of hydrogen donors, hydrogen acceptors and sensitizing dyes. The reaction will work, for example, with acridine orange as the sensitizing dye, for allylthiourea as the hydrogen donor, and for oxygen as the hydrogen acceptor.

$$Ch + h\nu \longrightarrow Ch^*$$

$$Ch^* + AH_2 \longrightarrow \dot{Ch}^- + \dot{A}H_2^+ \longrightarrow \dot{A}H + H^+$$

$$\dot{Ch}^- + \underset{\text{(RED)}}{D_y} \longrightarrow Ch + \dot{D}_y^-$$

$$\dot{D}_y^- + \underset{\text{(AH) etc.}}{AH_2} \longrightarrow \underset{\text{(COLORLESS)}}{D_yH_2} + A$$

CHLOROPHYLL SENSITIZED REDUCTION OF DYE BY ASCORBIC ACID (Krasnovskii, Evstigneev)

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Fig. 8.

In general, these reactions do not involve the storage of any energy, that is, the reaction hydrogen donor + dyestuff $\longrightarrow$ reduced dye + dehydro hydrogen donor (ascorbic acid, etc.) is thermodynamically positive; the energy is 'downhill' in that direction. However, there are a few cases in which the reaction seems to be reversible, that is, when the light is turned off there follows the reappearance of oxidized dyestuif. Whether this indeed represents a small degree of energy storage (a few kcal) or whether it represents a trace of oxygen in the reaction mixture which, in general, will oxidize most of these reduced materials, remains to be demonstrated. In any case, there is no great storage of free energy in this system; most frequently these reactions are 'downhill' thermodynamically and will not go backwards. When they do go backwards, it seems as though these dihydro dyestuffs are auto-oxidizable and traces of oxygen in the reaction mixture may carry them back.

There are a number of photochemical electron transfer reactions involving dyestuffs unrelated to chlorophyll which apparently do constitute some energy storage. One of these is the reaction (1,10,54) Here also the energy storage

$$\text{thionine} + Fe^{++} \underset{\text{dark}}{\overset{h\nu}{\rightleftarrows}} \text{leucothionine} + Fe^{+++}$$

is small ($\sim$ 5 kcals per quantum) at best.

PHOTOPHYSICAL EFFECTS IN MODEL SYSTEMS

Energy Transfer in Solid Systems

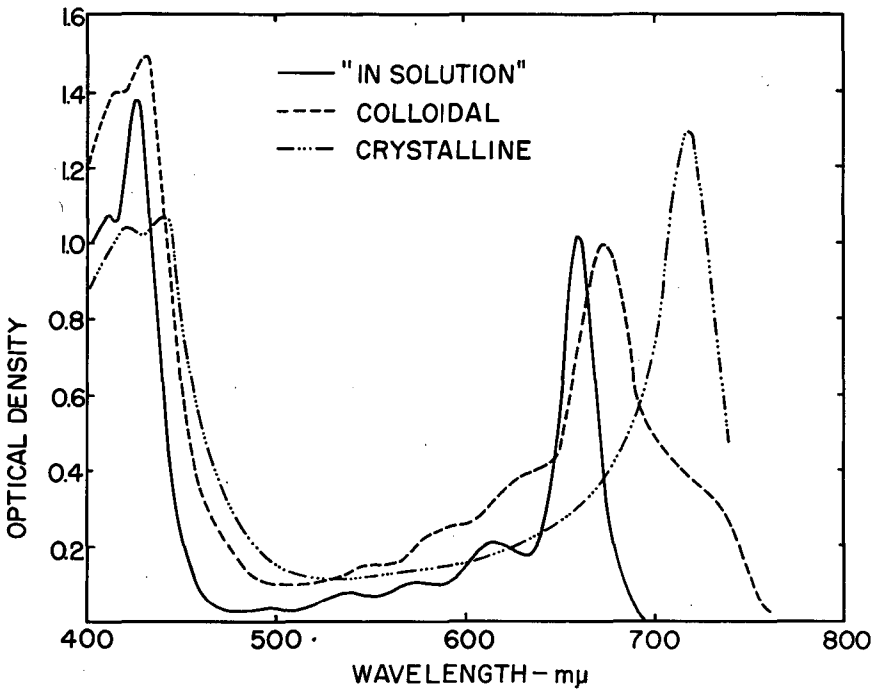
The failure of all of these various types of solution model reactions to provide a case where energy of the order of 20 to 40 kcal per quantum is being stored is in itself significant. I think it may be demonstrating that this is not the direction in which to look for the energy-storing reaction in photosynthesis. Some years back as the structure of the chloroplast became somewhat clearer to us (primarily through electron microscopy) [28, 66] and as our knowledge of the photochemical or photophysical behavior of ordered systems developed in the form of a body of theory and information on the photoresponse of atomic crystals [31, 70], the notion that the photosynthetic apparatus might not be functioning as ordinary molecules in solution but rather as something approaching molecular crystal behavior became popular. [13, 67]

We undertook to seek possible models for such systems in the laboratory. In addition to the electron microscopy on the chloroplasts, there was, of course, the very well known fact that the absorption spectrum of chlorophyll in the living organism is not identical with the absorption spectrum of chlorophyll in solution. This, together with the ordered structure that was seen in the electron microscope, suggested that the chlorophyll in the living organism might be in a physical form quite different from a true solution. The difference in the spectra is quite obvious. The solution spectrum of chlorophyll has a peak at about 6600 A and the living organism chlorophyll has its peak somewhere near 6800 A. This longer wavelength shift from 6600 to 6800 A is exactly the kind of shift observed

in the spectra of all sorts of pi-molecules when they are packed in crystals. When the pi-clouds of large, conjugated systems are brought close together there is an interaction which shifts the energy of the excited state (or the difference between the ground and the excited state). This is quite common in all pi-molecules and in chlorophyll it has been examined by Rabinowitch and by Trurnit. (35, 71) Figure 9 shows the so-called amorphous solid layers of chlorophyll have absorption in the 6800 A region, and when the chlorophyll layers are allowed to 'crystallize' the absorption spectrum moves out to almost 7200 A. Intermediate spectra of chlorophyll can be obtained, depending on the nature of the monolayers, in which the peaks lie between the 6600 A of the true chlorophyll solution peak and the 7200 A peak of the crystalline chlorophyll.

Phthalocyanine as a Model for Chlorophyll Energy Transfer

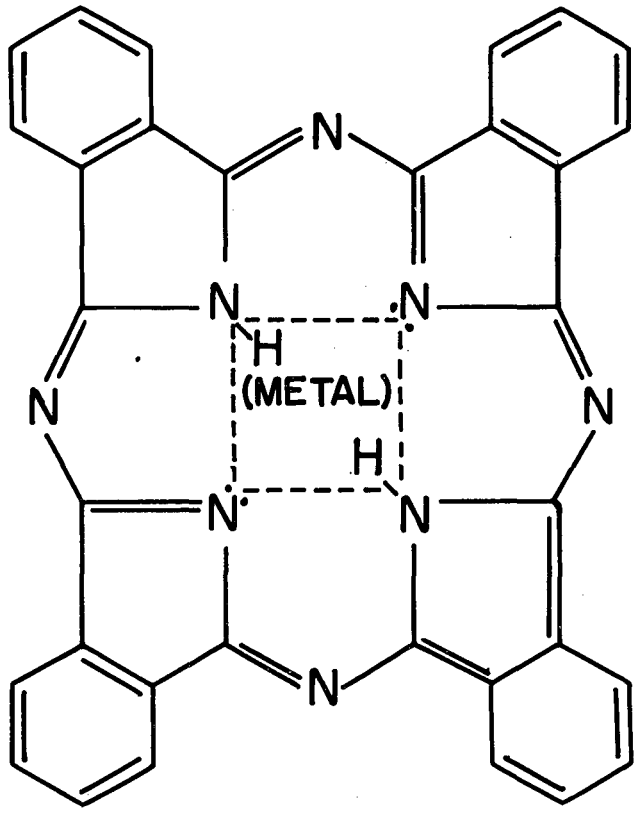
The ordered structure of the chloroplasts, as observed in the electron microscope, together with the difference in the chlorophyll spectra in solution and in the crystalline state, were some of the things which induced us to think in terms of solid lattices as a possible way in which the energy, which is absorbed in the chlorophyll molecule, might be handled in the chloroplast and in which the oxidizing and reducing power might be separated. Again, we sought models of various kinds so we could experimentally develop some concepts for such a separation, and here we turned to the experiments which were begun in the Soviet Union in 1949 by Vartanyan (73,74) and which were extended by Eley in England (22, 23, 24). Eley examined the electronic properties of crystals of a very stable molecule related to chlorophyll namely, phthalocyanine, the structure of which is shown in Figure 10. One can again recognize the tetrapyrrolic structure of the pigment and this molecule has



ABSORPTION SPECTRA OF CHLOROPHYLL IN VARIOUS STATES
(RABINOWITCH)

MU – 19447

Fig. 9.



PHTHALOCYANINE

MU – 19405

Fig. 10. Structural formula of phthalocyanine.

some resemblance to the porphyrin structure previously discussed. There are major differences, however, which must be kept in mind: the bridging atoms in phthalocyanine are nitrogen atoms instead of carbon atoms, and fused onto each of the pyrrole rings is a benzene ring which, of course, changes the nature of the compound considerably. It happens that I became familiar with this molecule in 1937, shortly after its discovery by Linstead, and participated in the demonstration of some of its catalytic abilities at the same time that Eley was working with it. (16,17) Eley has gone on to examine the electrical properties of phthalocyanine, and in recent years we turned to this also.

Phthalocyanine is a very stable substance, easy to prepare and not easy to destroy (compared to chlorophyll) and we used it as a model in our photophysical measurements. The first experiments undertaken were to demonstrate the effect of electron acceptors, or electron donors, added to crystalline phthalocyanine, on its conductivity and its photoinduced conductivity. These experiments were one step beyond what Vartanyan and Eley had done. They studied primarily the behavior of what they believed to be the pure phthalocyanine.

We made layers of phthalocyanine on a conductivity cell and then added electron donors or acceptor to it to see what effect these would have on the electrical conductivity in the dark and on the photoinduced conductivity. (38,39,40) Figure 11 shows such a conductivity cell with the electrodes, on top of which is placed a layer of phthalocyanine. On top of that is laid the layer of electron acceptor. The results of experiments using such a conductivity cell are shown in Figure 12 where the solid line gives the effect of added electron acceptor on the dark current. The dark current conductivity of such a sample, if electron acceptor is added, goes

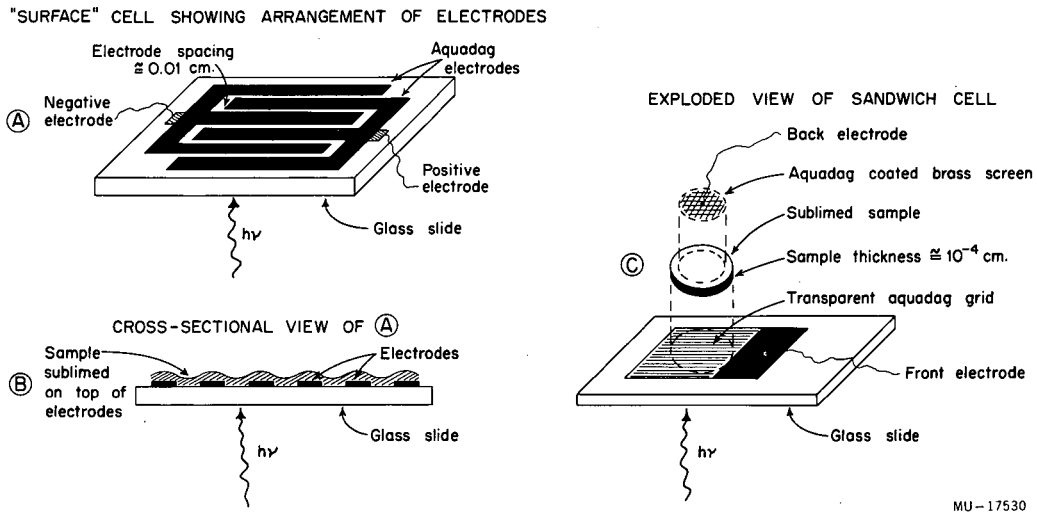


Fig. 11. Diagram of sample cells (conductivity).

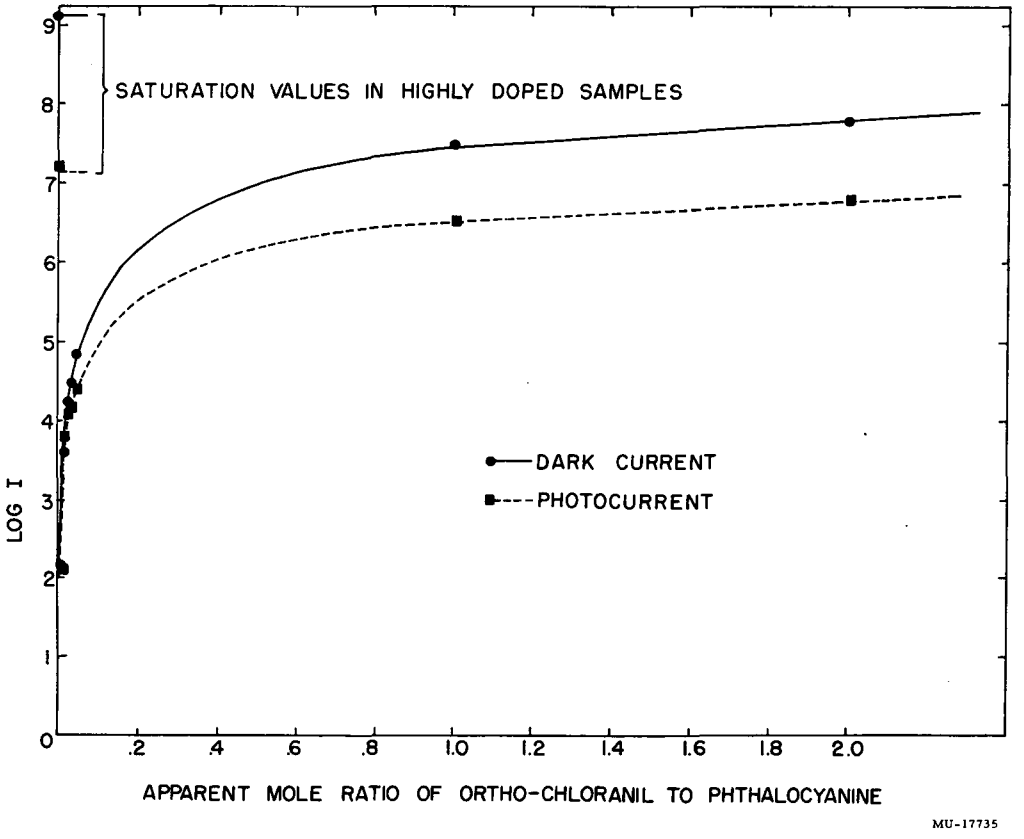


Fig. 12. Variation of dark conductivity and photoconductivity of phthalocyanine with amount of o-chloranil added.

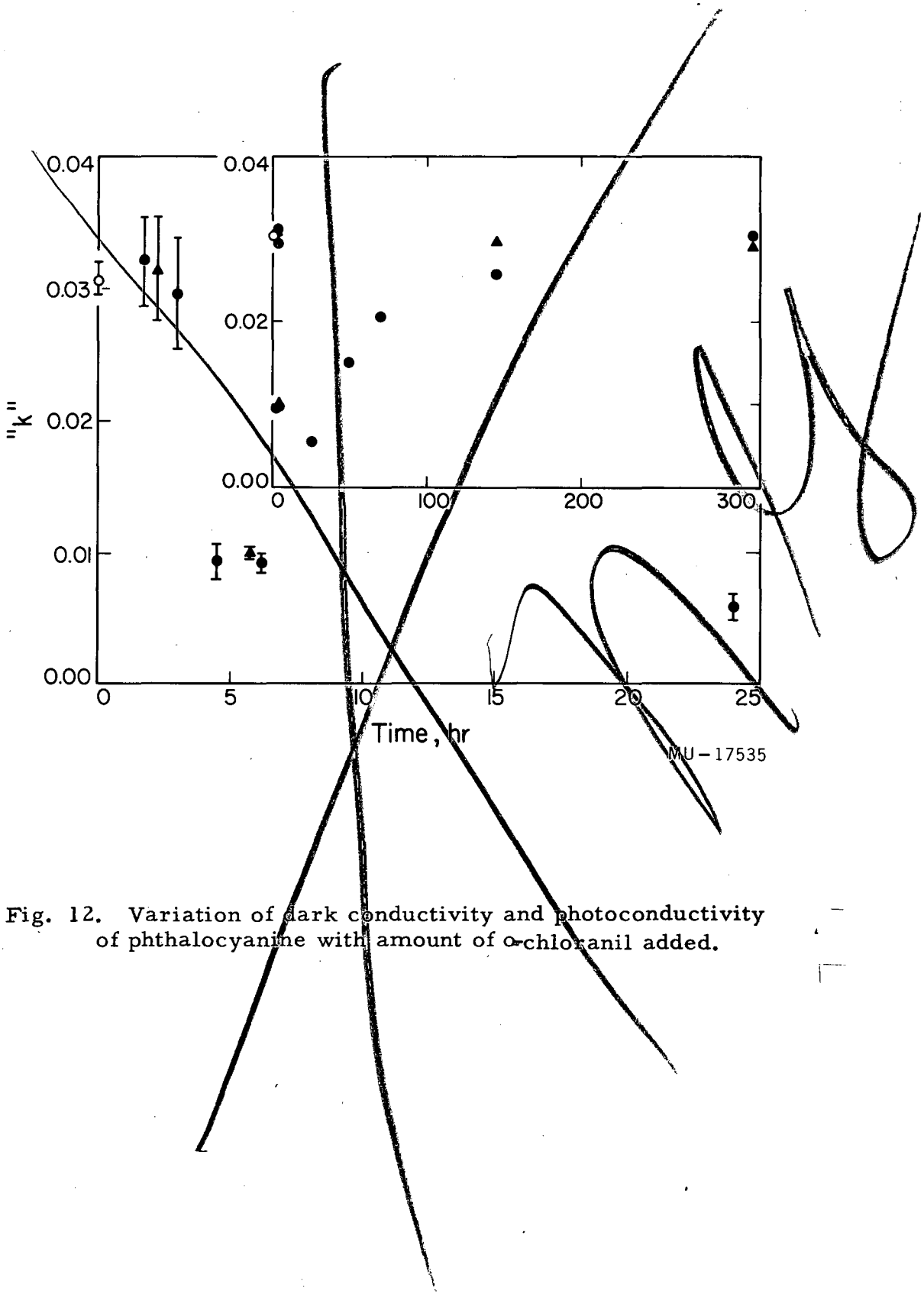


Fig. 12. Variation of dark conductivity and photoconductivity of phthalocyanine with amount of o-chloranil added.

up as much as seven powers of ten. The same type of thing is true of the photoinduced conductivity which goes up by as much as five powers of ten as electron acceptor is added on top of the layer of the phthalocyanine. I shall not try to review all of the kinetic and spectral studies which have been performed on this phthalocyanine system, but I shall show only a few of the highlights and then present to you what we believe to be the behavior of this molecular crystal in electronic terms.

When these electron acceptors were added to the phthalocyanine layer, it was found that electron transfer took place, from the phthalocyanine to the electron acceptor, even in the dark, as evidenced by the presence of free radical like signals, determined by electron spin resonance, in such a 'doped' or treated phthalocyanine sample. ([40]) This is shown in Figure 13, and the interesting fact is that by treating (doping) the phthalocyanine with electron accpetor (o-chloranil) we increase the dark current and also increase the light-induced conductivity. When the light is turned on such a sample as this, the number of unpaired electrons is decreased as indicated by the electron spin signal. Figure 14 shows how this system behaves. The conductivity, of course, goes in the reverse direction; when the light is turned on, the conductivity increases and when the light is turned off, the conductivity decreases, with rates corresponding to these spin signal changes.

Figure 15 shows the relationship between the kinetics of the spin signal behavior and the several other associated phenomena in the same lattice, such as conductivity, etc. Figure 16 shows the interpretation of these phenomena. They are interpreted in terms of electron transfer from phthalocyanine molecules, in the lattice, to the o-chloranil to give positive ion radicals of phthalocyanine which are in a crystal lattice, and these are

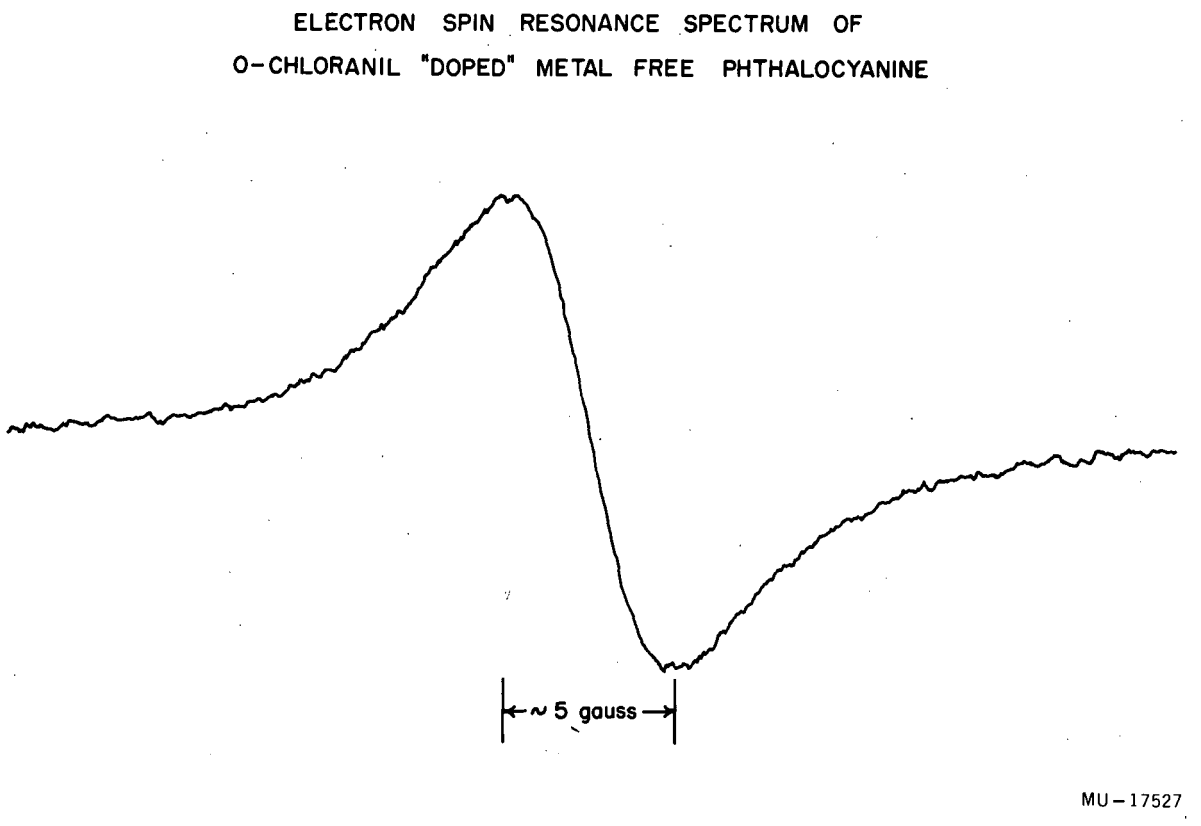


Fig. 13. Electron spin resonance (ESR) spectrum of o-chloranil-doped phthalocyanine. The curve represents the first derivative of the absorption.

EFFECT OF ILLUMINATION ON THE ELECTRON SPIN RESONANCE SIGNAL OF O-CHLORANIL "DOPED" METAL FREE PHTHALOCYANINE

CURVE REPRESENTS UNPAIRED SPIN CONCENTRATION VS. TIME

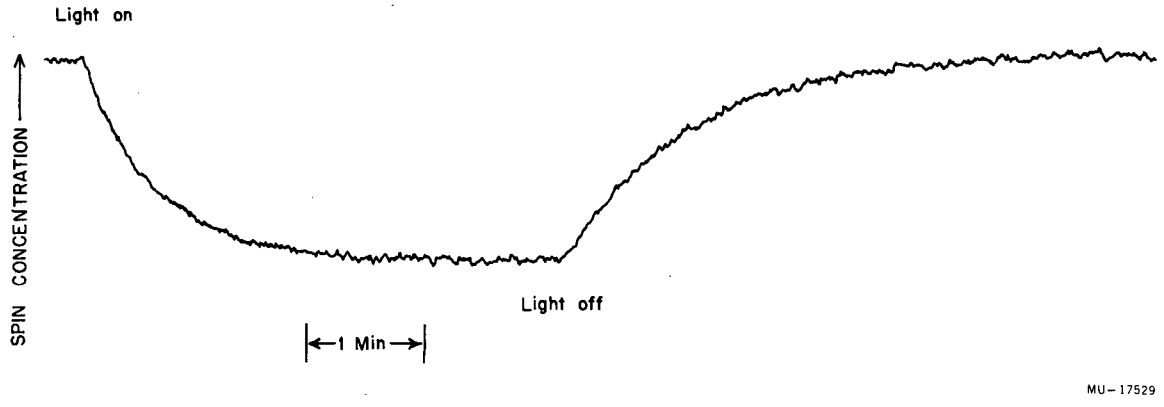


Fig. 14.

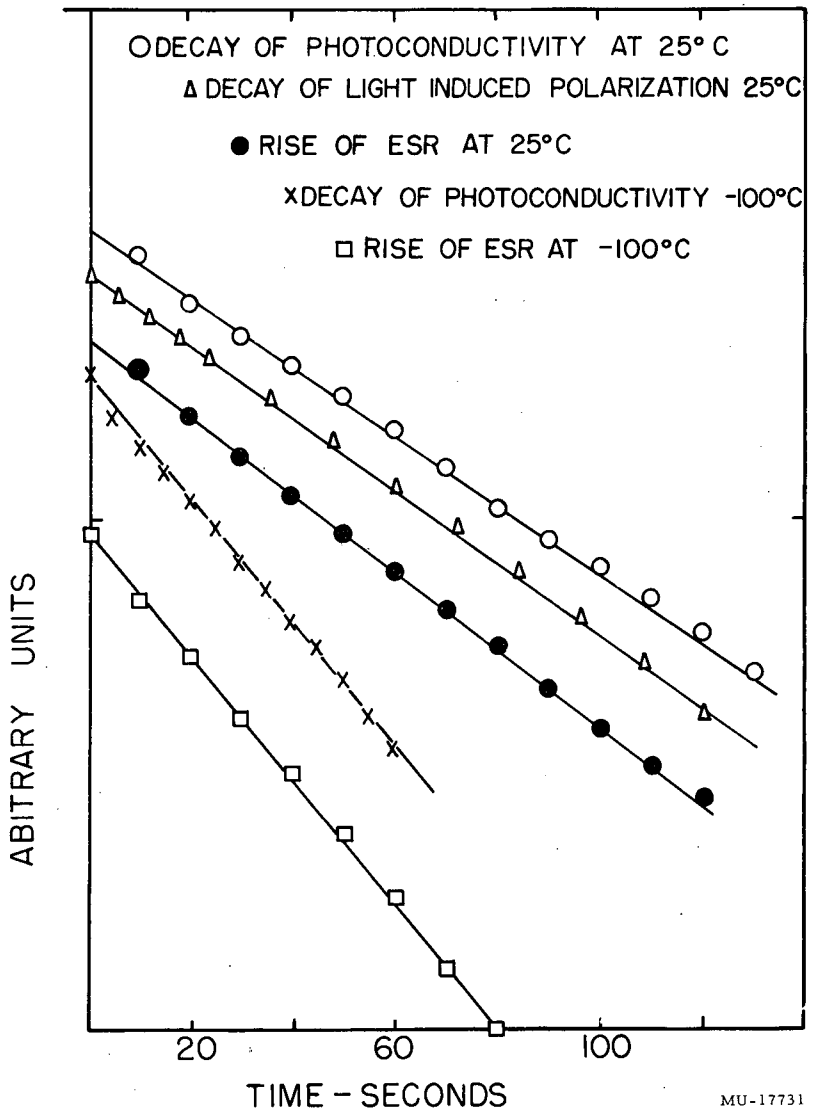


Fig. 15. Semilog plot of time dependence of photoconductivity , light-induced ESR and light-induced polarization in doped phthalocyanine.

1. $Pc + \underline{o}\text{-}Q \xrightarrow{DARK} \overset{\equiv}{Pc}^{+} + \underline{o}\text{-}\dot{Q}^{-}$

2. $Pc + \underline{o}\text{-}\dot{Q}^{-} \underset{DARK}{\overset{h\nu\ (7000\ \text{Å})}{\rightleftharpoons}} \overset{\equiv}{Pc}^{+} + \underline{o}\text{-}Q^{=}$

3. $Pc + \underline{o}\text{-}\dot{Q}^{-} \underset{DARK}{\overset{h\nu\ (4000\ \text{Å})}{\rightleftharpoons}} \overset{\equiv}{Pc}^{-} + \underline{o}\text{-}Q$

4. $\overset{\equiv}{Pc}^{-} + \overset{\equiv}{Pc}^{+} \xrightarrow{DARK} 2\ Pc$

REACTIONS OF A SOLID MATRIX OF Pc WITH A FILM OF o-Q.

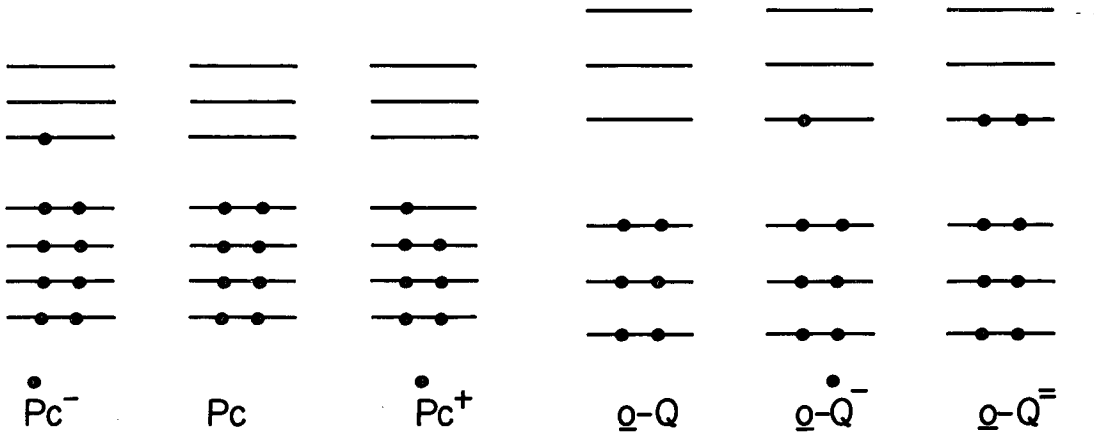
MU-19404

Fig. 16.

responsible for the conductivity (Figure 16-1). The electrons on the o-chloranil negative ion radical are not mobile and presumably they are the things which the ESR equipment sees. When the light is turned on, it is absorbed by the phthalocyanine, and the exciton can migrate around in the phthalocyanine until it is ionized (Figure 16-2). This ionization may take place either at some unknown center, or the exciton may come directly in contact with the o-chloranil negative ion, transferring a second electron to the o-chloranil negative ion, thus reducing the number of unpaired spins but increasing the conductivity. In the dark the reverse reaction occurs. When light at 4000 A is used, the reverse effect is observed, that is, there is a transfer of the electrons from the o-chloranil negative ion radical into an unoccupied orbital of the phthalocyanine crystal. (Figure 16-3) This is also an easily movable electron rather than a trapped one, and it will immediately and rapidly recombine with the conducting holes (positive ion centers) originally present in the lattice, thus resulting in a decrease in conductivity but an increase in electron spin signal (Figure 16-4).

Figure 17 shows a representation of the phthalocyanine negative ion, phthalocyanine itself, phthalocyanine positive ion radical, o-quinone, o-quinone negative ion radical and o-quinone double negative ion, in molecular orbital terms, to call to mind the way in which we have been thinking about the pi-energy levels of these conjugated molecules.

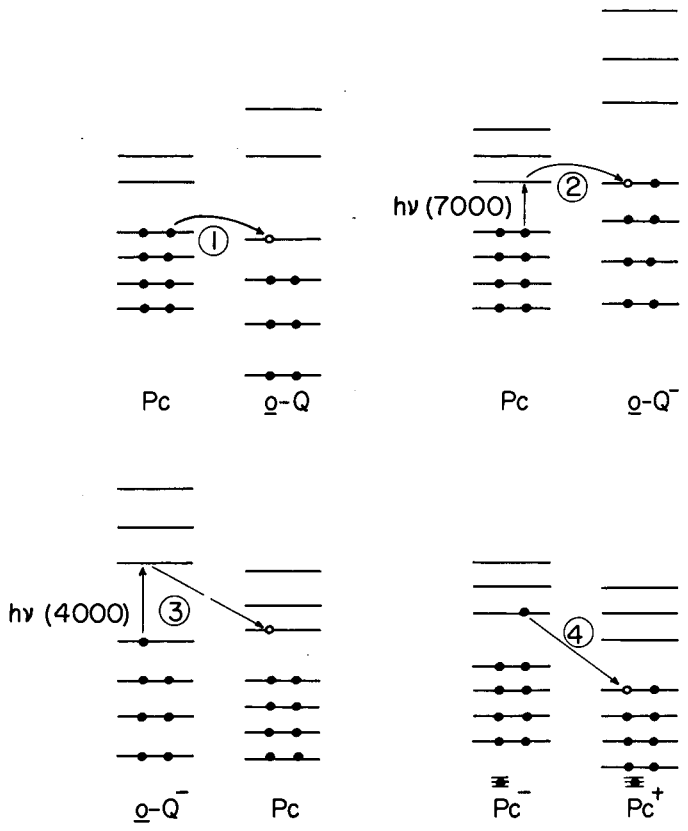
The actual processes which were seen in Figure 16 can now be illustrated in terms of molecular energy levels, and Figure 18 shows that interpretation. Reaction (1) is a transfer, in the dark, of an electron from the highest occupied phthalocyanine orbital to the lowest unoccupied o-quinone orbital, leading to the formation of phthalocyanine positive ions. This



SCHEMATIC REPRESENTATION OF THE ELECTRONIC ENERGY LEVELS OF VARIOUS Pc AND o-Q SPECIES (THE VARIOUS SPECIES SHOULD NOT BE COMPARED)

MU-19417

Fig. 17.



DIAGRAMMATIC MOLECULAR ORBITAL REPRESENTATION OF A SOLID MATRIX OF PHTHALOCYANINE WITH o-Q

MU-19402

Fig. 18.

occurs in the crystal lattice so these are conductivity holes, and the trapped electrons are in the o-quinone negative ion. The lowest unoccupied quinone level is shown below the highest phthalocyanine occupied level, and this reaction takes place quite spontaneously in the dark. The photochemical transformation (Reaction (2)) involves, first the excitation of the phthalocyanine itself, which could be represented by the raising of an electron from the highest occupied pi orbital(or from a N-n orbital) to the lowest unoccupied pi orbital which must lie very nearly at the same level as the singly-unoccupied orbital of the o-quinone negative ion. The reason for this shift of relative levels is that,whereas in the first instance transfer is occurring from one neutral molecule to another, here the transfer is from a neutral molecule to a negatively-charged already singly-occupied orbital.

The third process, in Figure 18, the one represented by the decrease in photoconductivity by illumination at 4000 A, involves the excitation of the electrons in the o-quinone negative ion up to an excited orbital which can then be transferred into the lowest unoccupied orbital of the phthalocyanine crystal (a conduction orbital). This, then, is a relatively mobile electron which can rapidly find and neutralize the conductivity holes in the phthalocyanine lattice, leading to a decrease in conductivity. These represent the principle processes shown in Figure 16.

Figure 19 shows the actual separation of charge that can be accomplished in this model system if it is constructed properly. Here is shown a matrix of phthalocyanine. on the surface of which lies an o-quinone layer. There will be some negative charge trapped in the o-quinone (acceptor) layer, and the positive charge will remain in the phthalocyanine (donor) layer. This will induce a polarization in the pair of electrodes

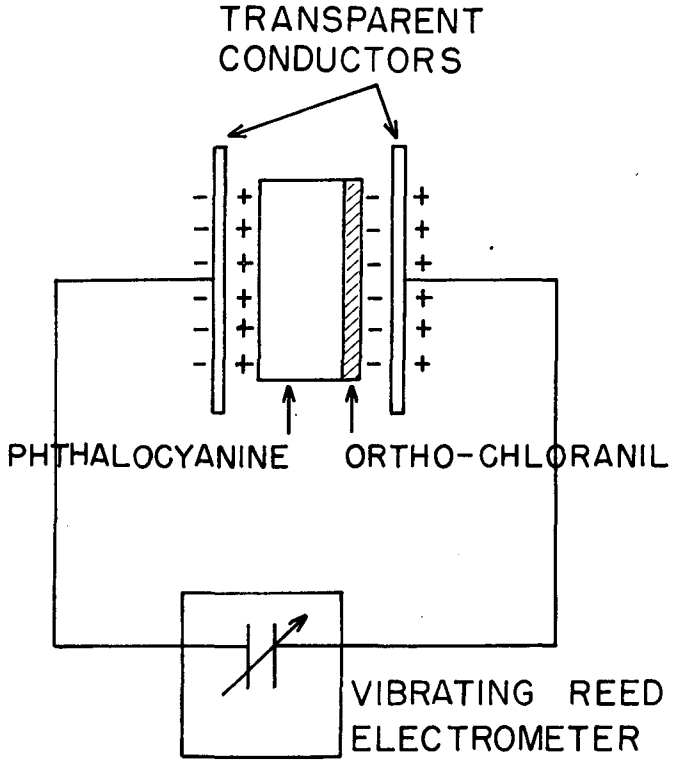


Fig. 19. Schematic diagram of polarization apparatus.

between which the double layer is placed. The polarization will be increased by shining light absorbed by phthalocyanine on the double layer, in which case there will be an additional accumulation of negative charge in the quinone and an additional accumulation pf positive charge in the phthalocyanine. This is exactly what happened (40), and it is a photochemically-induced separation of oxidizing power (positive holes) and reducing power (quinone double negative ions). Presumbly, this sort of thing could occur in the individual layers which can actually be seen in the chloroplasts.

THE RELATION TO THE PHOTOSYNTHETIC APPARATUS

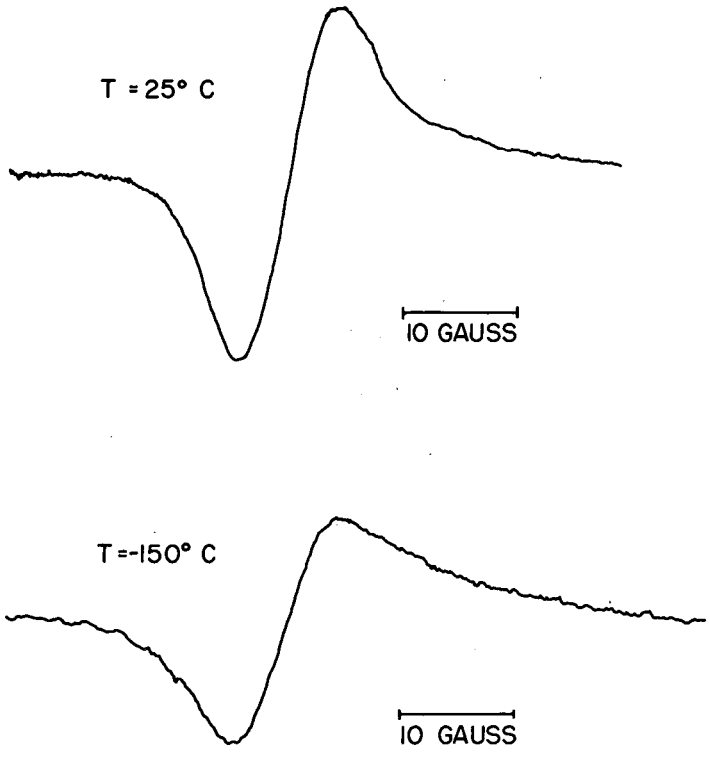
What bearing does this information have on the photosynthetic apparatus itself? The obvious relationship is that the phthalocyanine might be considered as a model for the chlorophyll layer itself. The electron acceptor, here listed as o-chloranil, might indeed be some electron acceptor in the chloroplasts such as Coenzyme Q_{255} (Plastoquinone), (8, 20, 41, 48, 60) which conceivably could have a function similar to the function that the o-chloranil has in the model system, but with certain differences.

Electron Spin Resonance in Chloroplast Materials

If this is a model for the actual chloroplast behavior, then we should see some of these electronic properties in the chloroplast itself. First of all, the change of absorption spectrum in the chloroplasts from that of a true solution has been mentioned earlier. Unfortunately, we cannot

place electrodes on either side of the lamellar layers of the chloroplasts as we have been ble to do in the phthalocyanine system, but there are a number of properties which cnn be observed. One of these is the generation and disappearance of the unshared electrons which we have seen manipulated in the model system. Figure 20 shows the photoproduction of unshared electron pairs in whole spinach chloroplasts at room temperature and at -150°C (64). The fact that unshared pairs of electrons can be produced by red light is in itself some indication that rather profound changes are occurring in the chloroplast. These cannot be due to triplet states because the interaction of the two electrons in a single triplet molecule in randomly-arranged chloroplasts would broaden the signal so as to make it unobservable. (34) The direct photochemical fission of a chemical bond (by some sort of predissociation process) seems entirely unlikely by a quantum supplying no more than 40 kcals of energy at most.

As in the model systems these signals must be due to the generation of unpaired electrons somewhere in the system. The fact that they can be induced by red light, and induced just as rapidly at -150°C as they are at +25°C, suggests that this is not due to an ordinary chemical reaction which requires any kind of activation energy. If that were the case, the formation reactions at -150° should have very different rates than the reactions at +25°, and they do not. In Figure 21 you can see the growth and decay of these signals, as far as they have been measured. At room temperature the signal rises just as fast as the instrument can measure it and part of the signal falls very rapidly when the light is turned off, thus indicating the presence of at least two different kinds of unpaired electrons. At -150° the rise of the signal is just as fast as the instrument can follow



LIGHT SIGNALS FROM WHOLE SPINACH CHLOROPLASTS

MU-14534

Fig. 20

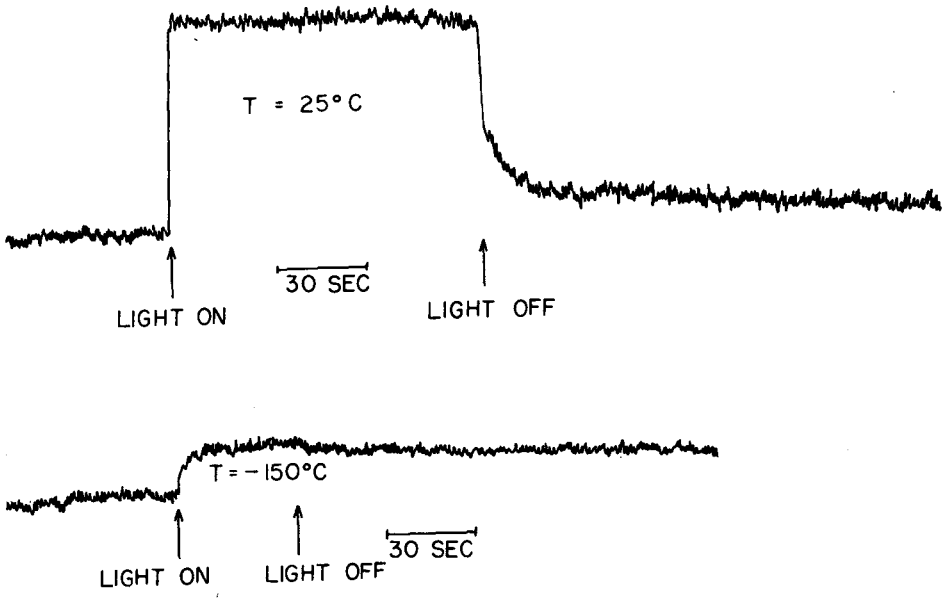

T = 25° C
30 SEC
LIGHT ON
LIGHT OFF
T = −150°C
LIGHT ON
LIGHT OFF
30 SEC
WHOLE SPINACH CHLOROPLASTS
MU-14535

Fig. 21

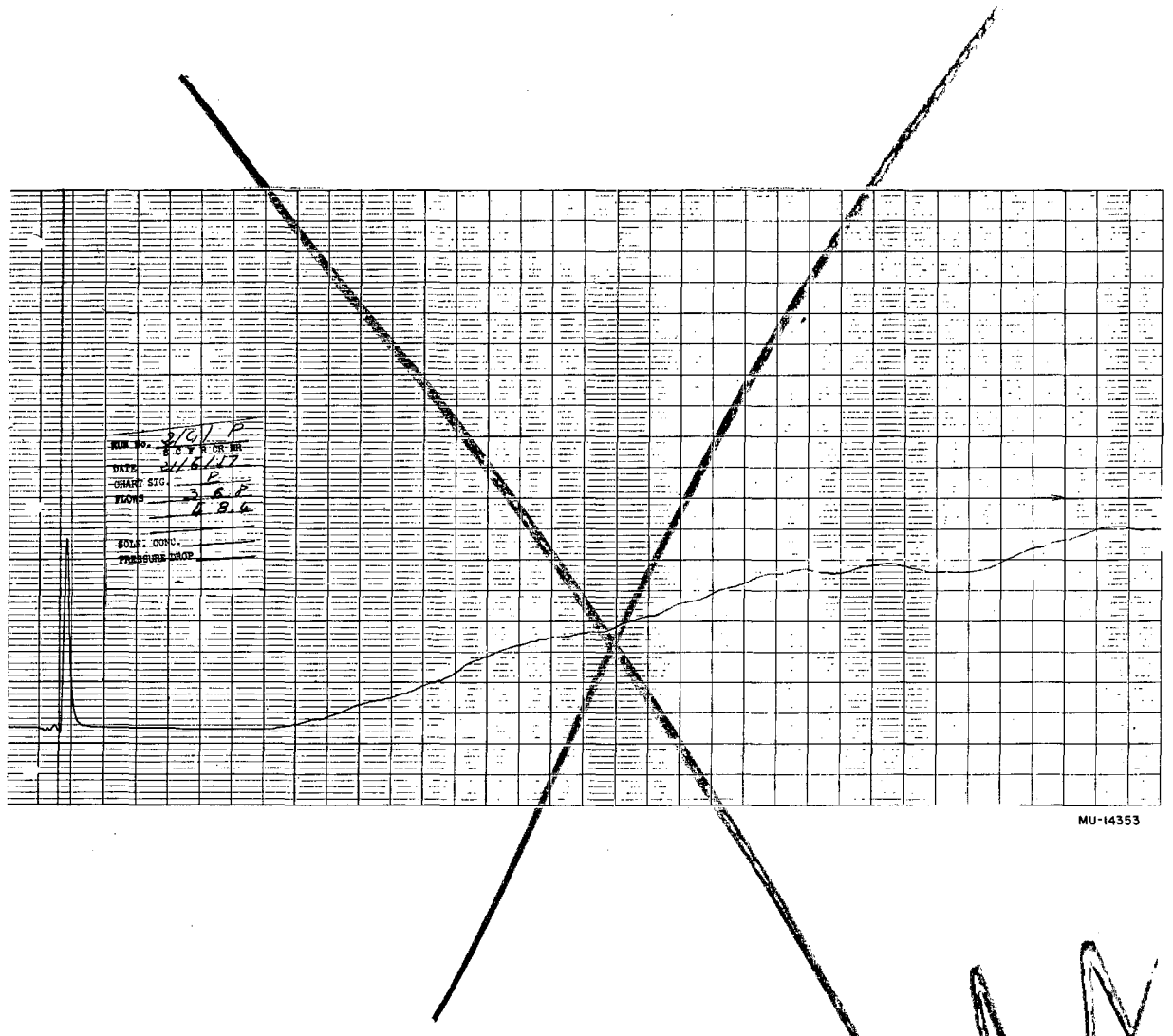

RUN NO. 2/GT P
DATE 4/6/17
CHART SIG. P
FLOWS 3 6 P
4 8 G
SOLN. CONC.
PRESSURE DROP
MU-14353

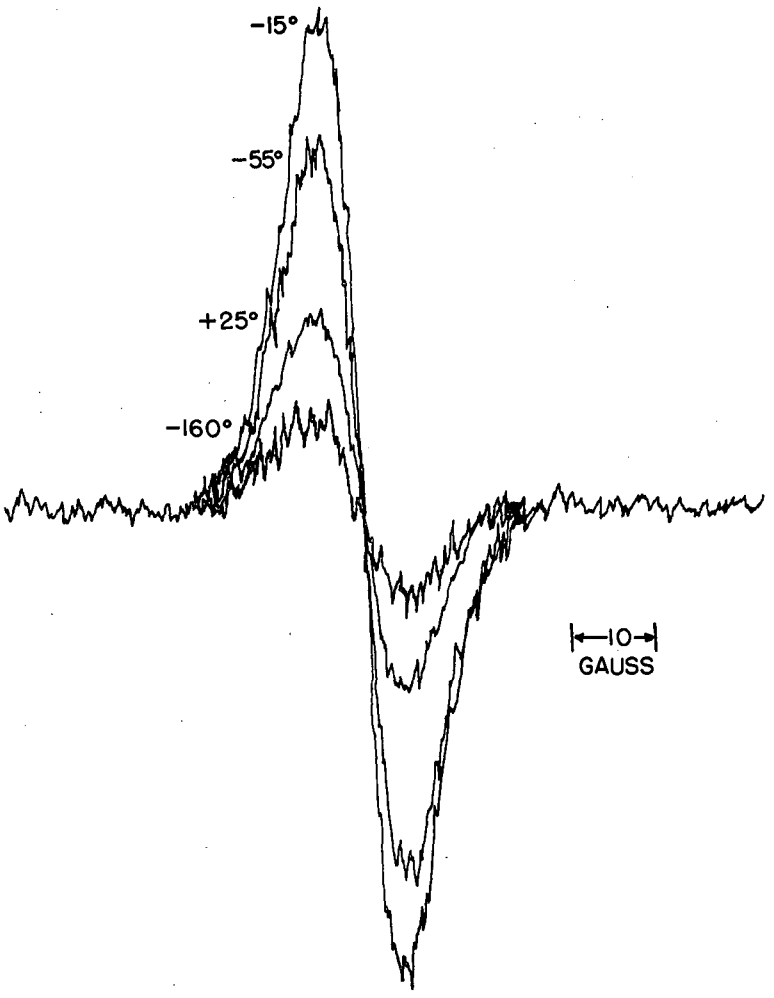
Fig. 21.

it, but in the case of the chloroplasts it does not fall at all at low temperatures, indicating that the back-reactions in this case do indeed have a temperature coefficient.

In the case of Rhodosprillum rubrum the reaction is also complex; some of it has a temperature coefficient and some of it does not. Figure 22 shows the Rhodosprillum signal growth and decay (14). At both +25° and -150° the growthand decay were as fast as the instrument could follow. In the intermediate temperature regions, some fraction of the decay was slow, indicating that this is a complex signal made up of several different kinds of unpaired electrons, probably formed in sequence. At low temperatures there are no slowly-formed unpaired electrons; they are formed extremely rapidly and they decay extremely rapidly.

The Structural Requirement

One could ask the question: Is the chloroplast needed to produce such unpaired electrons? Could not such unpaired electrons be produced photochemically, just using the chlorophyll and its associated pigments? (12,55) This experiment has been performed by several workers (61), including some in our own laboratory (2). Figure 23 shows the production of such unpaired electrons by the pigments which are extracted by methanol from chloroplasts. The signals so produced at 25°C are quite different from the signals produced in the whole chloroplasts, either at room temperature or at very low temperatures (Figure 23). The signals in the chloroplasts were 10 to 20 gauss wide and the signals in the chloroplast extracts are only 3 gauss wide. Furthermore, their decay at room temperature is slow compared to the decay of signals in the chloroplasts. It is clearly possible, therefore, to produce such signals in methanolic extracts, and if this methanolic extract is thoroughly dried, there is no signal. Furthermore, if the methanolic



ESR SIGNALS FROM *RHODOSPIRILLUM RUBRUM*
5 MINUTES CONTINUOUS ILLUMINATION

MU-15137

Fig. 22.

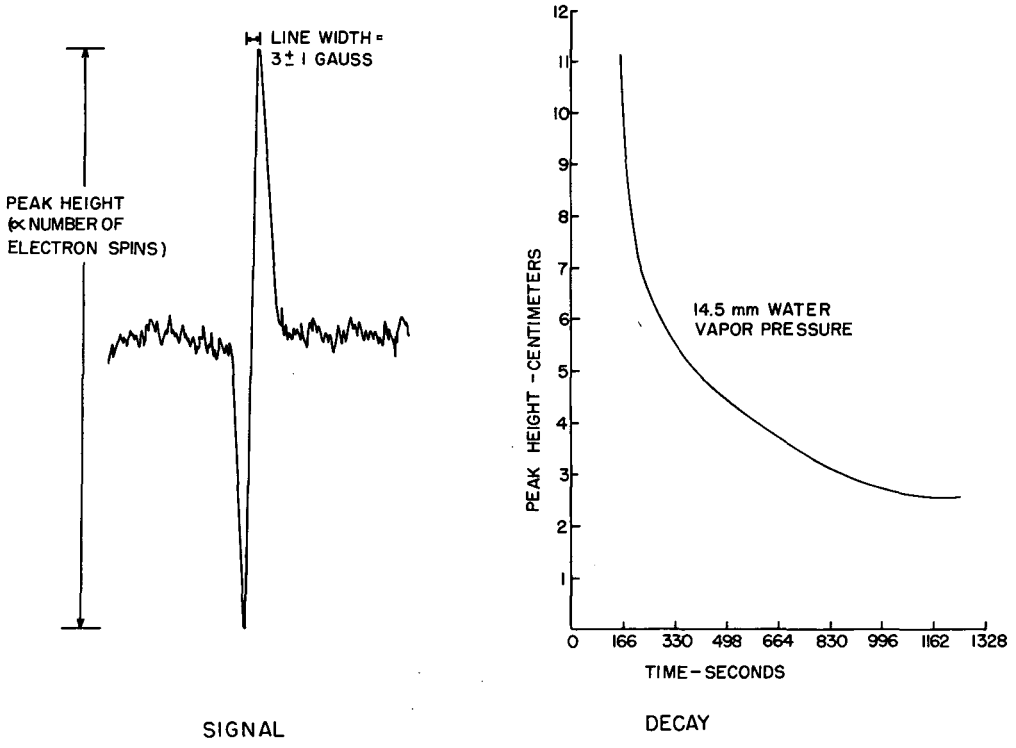


PHOTO INDUCED ESR OF METHANOL EXTRACT OF *CHLORELLA*

MU-19450

Fig. 23.

extracts are fractionated by petroleum ether so a cleaner chlorophyll is obtained, then the signals that are produced are broader and smaller.

Carotenoid Requirement

There is one dher type of experiment which has been performed in an attempt to determine the point at which these signals originate in the chloroplast and the factors which determine this point, and that is the experiment done with Rhodopseudomonas, of which we have two types: the wild type which contains carotenoid and the mutant (65) which does not contain the conjugated carotenoid. An attempt was made to see if the conjugated carotenoid was involved in the photoproduction of the spin signal (4,5). Very nearly the same characteristics in the signal are obtained (Figure 24) (3) whether the wild type or the mutant type (which does not contain the carotenoid) of Rhodopseudomonas is used. The signal is characterized by various physical methods such as the signal growth rate, decay rate, band width, etc.

Apparent Spectral Efficiency

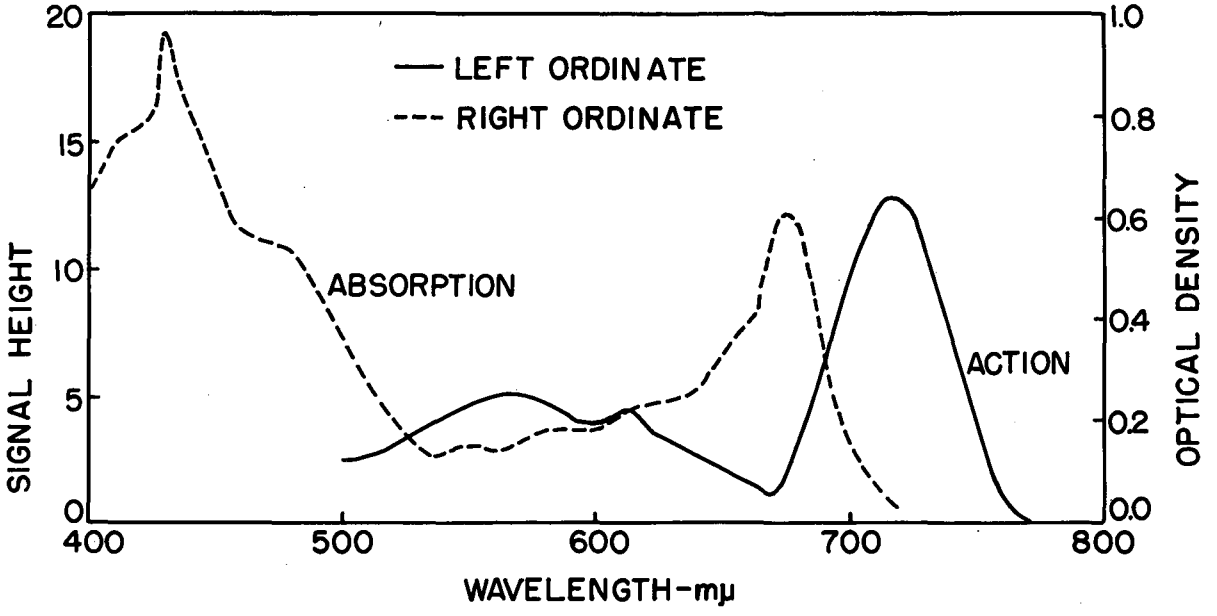
Finally, an apparent action spectrum for the production of these signals (63) both in the green plant material (Chloroplasts) (Figure 25) and in the red bacteria (Figure 26) are shown. This apparent action spectrum is a very curious one and has a peak at about 7200 A, with a minimum approximately where the peak of absorption of the chloroplyll in the living organism is, namely, at 6800 A. (59) A similar behavior for the apparent action spectrum of the signal production in the Rhodospirillum appears, namely, one in which the maximum for the production of the signal is at somewhat longer wavelength (9100 A) than the maximum for the pigment absorption (8800 A) (59).

PROPERTIES OF THE PHOTO-SPIN SIGNALS IN PURPLE BACTERIA

	RHODOPSEUDOMONAS SPHEROIDES (WILD)	*RHODOPSEUDOMONAS SPHEROIDES* (MUTANT)	*RHODOSPRILLUM RUBRUM*
g	<2 BY ~1%	<2 BY ~1%	2
LINE WIDTH	20 g	20 g	10 g
CHLOROPHYLL CONTENT	0.4 mg/100 ml sol.	0.2 mg/100 ml sol	—
SIGNAL AMPLITUDE	A_0 (SMALL)	A_0	$\gtrsim 5A_0$
INITIAL DECAY	SECONDS TEMPERATURE INDEPENDENT	SECONDS TEMPERATURE INDEPENDENT	< SECONDS TEMPERATURE INDEPENDENT
FINAL DECAY	HOURS TEMPERATURE DEPENDENT	HOURS TEMPERATURE DEPENDENT	MINUTES TEMPERATURE DEPENDENT

MU-19448

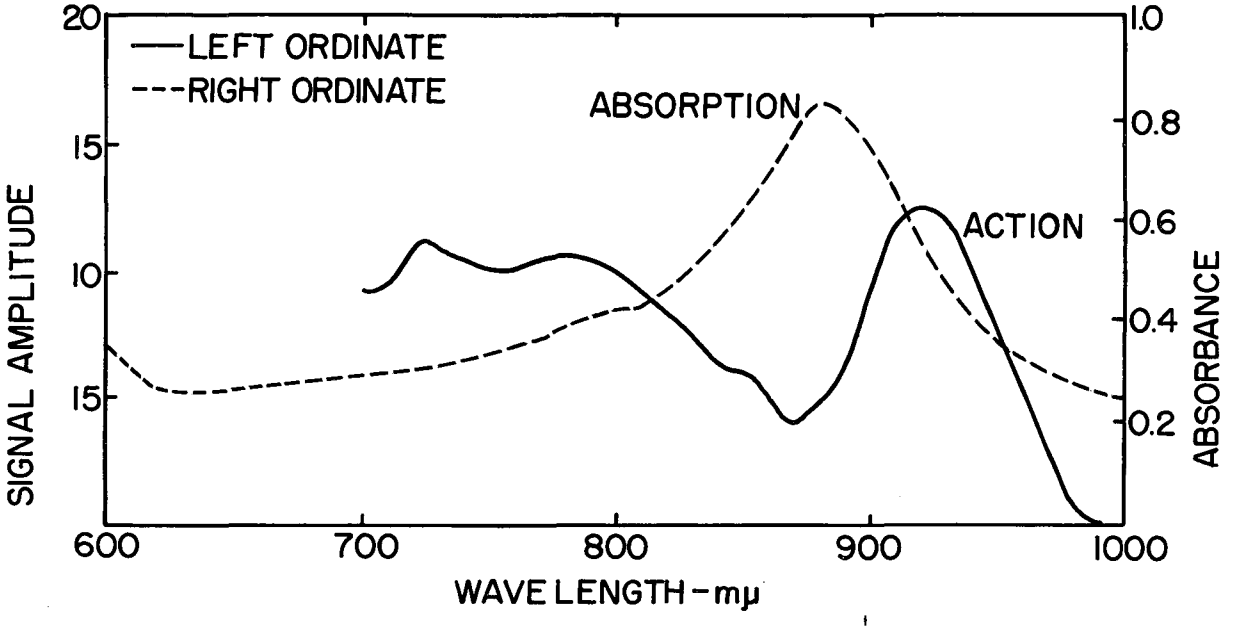
Fig. 24.



ABSORPTION AND ACTION SPECTRA OF CHLOROPLASTS (*SOGO*)
$I_0 = 10^{15}$ QUANTA/SEC. BAND WIDTH = 100 Å

MU-19446

Fig. 25.



ABSORPTION SPECTRUM (*SHIBATA, BENSON*) & ACTION SPECTRUM (*SOGO*) OF *RHODOSPRILLUM RUBRUM*. $I_0 = 5 \times 10^{14}$ QUANTA/SEC. BAND WIDTH = 66 Å

MU - 19449

Fig. 26.

Part of this shift of the wavelength for most efficient spin signal production is certainly due to the way in which the experiment was performed. The samples were totally-absorbing and relatively thick ($\sim$ 0.1 mm). This resulted in the total absorption of the maximally-absorbed light (6800 A for the chloroplasts and 8800 A for the Rhodospirillum) in a very thin layer near the surface of the sample. This situation resulted in the production of unpaired spins at a very much higher real concentration than would be the case if the light were absorbed throughout the sample. This latter situation would be approached by light of wavelengths not so strongly absorbing, such as wavelengths on either side of the absorption maximum in both cases (6800 A for the chloroplasts and 8800 A for the Rhodospirillum).

Since there is an indication that the decay rate of the spin signals is greater the higher their concentration, it is easy to see that any attempt to measure the number of spin signals produced for a constant incident number of quanta will be in error on the side of too few electrons per quantum absorbed, the greater is the concentration of the unpaired spins produced. This would depress the apparent number of spin signals produced at the very point of maximum light absorption, as indeed is the case. This, however, is not enough to account for the fact that the efficiency of spin signal production is actually higher on the longer side of the maximum light absorption than it is on the short side of the maximum. In order to account for this, another process must be invoked.

Something else besides the simple absorption of light by the chlorophyll into its ordinary excited state at 6800 A is involved in the production of the spin signal. Presumably there is another state, or another substance, which leads to the maximum at longer wavelengths. In

the crystal spectra of the monolayers of chlorophyll (Figure 9) there was indeed in the crystalline layers a peak at 7180 A. This, together with the fact that our spin signal occurred at ~7200 A, prompted us to seek some evidence for another excited state in the living organism, somewhere around 7200 A.

In looking back over our earlier studies on the luminescence of living organisms (68, 69) we did indeed find emission at 7200 A. In Figure 27 is shown the emission spectrum of Chlorella as it has been observed for long-lived emission, and you will notice that there is a peak at 6800 A but there is a very prominent shoulder at 7200 A. This was originally interpreted as due to self-abosprtion of the ordinary fluorescence. However, the fact that this is such an asymmetric curve now seems to suggest that there may be another emission band somewhere beyond 7000 A.

A better experiment was performed by Brody (11) in which he found exactly that: a very pronounced emission with a peak at 7180 A shown in Figure 28. The ordinary fluorescence spectrum of Chlorella has a peak at around 6900 A, but if one cools the Chlorella to -190°C a strong emission at 7180 A appears. This cannot be a self-absorption effect, because if it were, there would not be a minimum between the two absorption peaks. This means that there is a new state emitting, quite a different one from the one producing fluorescence emission at 6900 A. The triplet emission is out still further at around 7600 A and this has been observed in pure chlorophyll samples. Brody has seen the 7180 A peak both in whole Chlorella as well as in concentrated chlorophyll solutions, and he believes this to be the emission of a state of aggregated chlorophyll and quite different from the triplet emission but corresponding to something which exists in

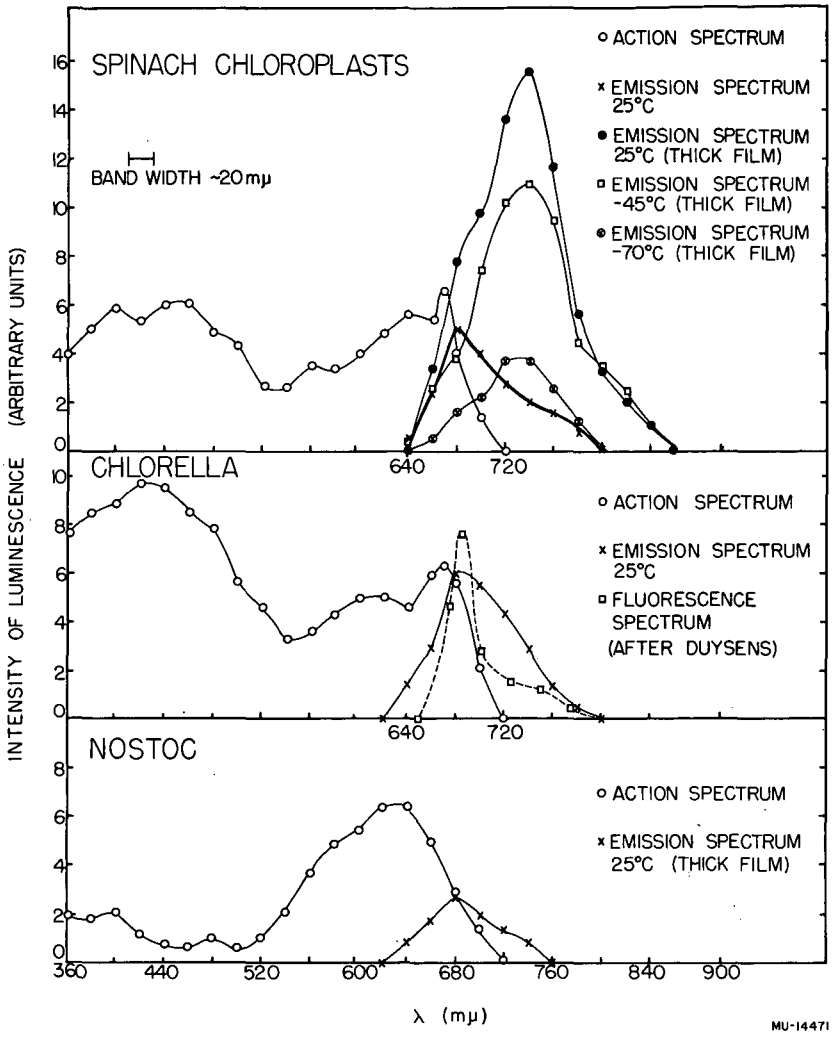
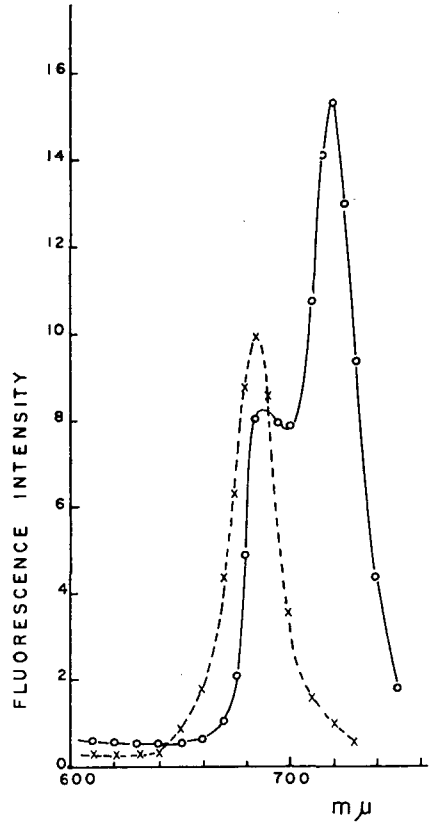


Fig. 27. Growth and decay curves of whole spinach chloroplasts.



Fluorescence spectra of *Chlorella* at room temperature (crosses) and -193°C (open circles). The fluoresence intensities indicated are the same for both curves. The decrease in fluorescence yield at 690 mμ is probably due to the increased scattering of the exciting and fluorescent light.

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Fig. 28.

the living organism. Another possibility is that it may be the lowest $n\pi^*$ state as distinct from the more readily reached $\pi\pi^*$ state.

One can presume, now, that excitation to such a state as this is required in order to produce the unpaired electron, and that this may occur either by resonance transfer of exciton energy amongst the chlorophyll molecules until it comes to molecules so situated that this state may be excited, or by direct excitation of this state by absorption, as presumably we have done when we examined the action spectrum for the production of unpaired spins. Failure to observe a distinct absorption peak at this point would have to be accounted for. What the nature of this emitting state is remains to be seen. It could be, of course, that this state is one from which an electron transfer to a certain low-lying acceptor occurs. Energy absorbed in the 6800 A state might be degraded to this emitting state to produce the same electron transfer, or might be used directly from the higher energy state to transfer an electron to a somewhat higher-lying acceptor.

It should be noted that this would, in fact, correspond to two different types of primary quantum conversion processes. Such an idea has already appeared in the work of Emerson (25) which has since been explored further by French and Myers (9,57). Emerson had observed that the apparent long wave limit for photosynthesis was shifted to still longer wavelengths (somewhat beyond 7000 A) if light of shorter wavelength (around 6500 A) was also present. A further examination of this effect by Myers and French seemed to confirm the suggestion that the quantum yield of an increment of 7000 A light is greater when light of shorter wavelength (around 6500 A) is also impinging than when it is not. In addition to this, Myers, following Blinks (9), observed a number of transients in changing from

one wavelength of light to another which are best interpreted in terms of the requirement for the collaboration of two different products resulting from two different quantum conversions, one in the region of 6500 A and another in the region of 7000 A.

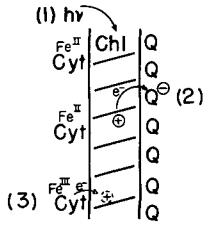
It is tempting to suggest, following the analogy of the phthalocyanine model experiments described earlier, that corresponding to the two model pigments, we have present in the chloroplast both chlorophyll and plastoquinone (8,20,48 The plastoquinone in this case would not have a sufficiently low-lying orbital to act as acceptor to the chlorophyll in its ground state, but could accept an electron from chlorophyll brought to an excited state by illumination, corresponding to the 7200 A emission. The transfer of a second electron to the quinone negative ion radical thus produced would require the excitation of the chlorophyll to a somewhat higher state which could result from absorption at 6800 A or shorter wavelength. The quinone double negative ion thus produced would then be a sufficiently powerful reducing agent in its lipid medium to reduce such enzymatic cofactors as lipoic acid or pyridine nucleotide (18.

The remaining positive ion in the chlorophyll matrix would have to find its way to some donor, ultimately accepting electrons from water. These donors might very well be other metal ions such as iron which is very common in the chloroplast and which is associated with chlorophyll. In fact, a low temperature ($70^{\circ}K$) light-induced electron abstraction from a ferrocytochrome in a bacterium has been reported (19). Room temperature photo-oxidation of the ferrocytochromes of photosynthetic bacteria has been known for some time (36,37).

The system might structurally then bear some resemblance to the model (Figure 19) which we have used, the chlorophyll layer having associated

with it on one side the electron acceptor, quinone, in a lipid environment and on the other side electron donor materials, such as the cytochromes, in an aqueous environment. Following the absorption of a quantum in chlorophyll (Figure 29, equation 1) it will migrate by resonance transfer to a suitable site near the quinone, at which point electron transfer to the quinone will take place (Figure 29, equation 2). The resulting vacancy, or chlorophyll positive ion, can then migrate by hole diffusion, that is, electron transfer from normal chlorophyll, into the vacant orbital of the neighboring chlorophyll positive ion. This process is the one in the entire sequence which most nearly resembles the properties of a semiconductor and permits the oxidizing point (the chlorophyll positive ion) to separate from the reducing point (the electrons in the quinone orbitals) by a process which is very nearly temperature-independent. The oxidizing point will make itself apparent as a chemical change, finally, when it captures an electron from a suitable reducing agent, in this case shown as a ferrocytochrome, thus producing a ferricytochrome and regenerating normal chlorophyll (Figure 29, equation 3).

It is conceivable that in order for the reduction of pyridine nucleotide to occur, possibly through lipoic acid (51,56), the quinone must be in the form of a di-anion, in which case a second electron transfer from an excited chlorophyll to a quinone negative ion radical, produced in equation 2 (Figure 29) will take place. This clearly will require somewhat greater energy than the first reaction, if only to overcome the electrostatic repulsion of the pre-existing negative charge. It is interesting to view these two steps as a possible means of understanding the collaborational requirement of light of two wavelengths (7000 A and 6500 A) which was mentioned earlier (52).



Cyt – CYTOCHROME AND/OR OTHER ELECTRON DONOR SYSTEMS (AQUEOUS PHASE)

Q – PLASTOQUINONE AND/OR OTHER ELECTRON ACCEPTOR SYSTEMS (TPN, LIPOIC ACID, ETC.) LIPID PHASE

Chl – CHLOROPHYLL

1. $Chl + h\nu \longrightarrow Chl^{*}$

2. $Chl^{*} + Q \longrightarrow \overset{\bullet}{Q}^{-} + \overset{\rightleftharpoons}{Chl}^{+}$

3. $\overset{\rightleftharpoons}{Chl}^{+} + Fe^{II} \longrightarrow Fe^{III} + Chl$

SCHEMATIC ARRANGEMENT OF CHLOROPHYLL AND POSSIBLE DONOR AND ACCEPTOR MOLECULES IN THE CHLOROPLAST

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Fig. 29. Schematic arrangement of chlorophyll and possible donor and acceptor molecules in the chloroplast.

Another alternative would involve the transfer of the electron from the donor (ferrocytochrome) to the excited chlorophyll as the first act. This would lead to a chlorophyll negative ion radical in which an electron has been placed in the lowest pi-orbital of the chlorophyll. The migration would then have to occur in this form until the acceptor site (quinone) is arrived at.

We prefer the first formulation described above, since every effort we have made to find either dark- or photoinduced electron transfer from a donor to the neutral phthalocyanine in our phthalocyanine model has failed. Beyond this, in practically every case in which it has been determined, the charge migration in an organic molecular crystal takes place via hole migration rather than via electron migration (30).

CONCLUSION

In summary, then, we can see that while the solid state model (phthalocyanine) allows an approach from a somewhat different point of view, the net result is the same as what was sought, but so far not found, when we looked at the solution chemistry of chlorophyll (and chlorophyll model substances), namely, the transfer of an electron, or hydrogen atom, from the excited porphyrin to an electron acceptor at a high reduction level which can be used to reduce the ultimate carbon dioxide reducers, followed by the donation of an electron, ultimately from water, to the remaining radical ion, or lattice, which produces the net result of the transfer of hydrogen from water to carbon dioxide.

How much of the solid state picture will be required to fully understand this separation of oxidant and reductant I think is yet to be determined. However, I believe it is quite clear that we are coming to the same

kind of conclusion from both ends, that is, from both the pure solution chemistry which involves electron transfer from donor to acceptor and from the solid state experiments which involve the same kind of electron transfer from donors to acceptors. The difference lies in the types of lattices involved. The back-reaction in the solid state experiments is demonstrably slower than one can visualize for the solution electron transfer reaction in which no provision is made for the rapid, relatively temperature-independent separation of the products, electron (reducing agent) and hole (oxidizing agent).

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