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QUANTUM CONVERSION IN PHOTOSYNTHESIS

Melvin Calvin

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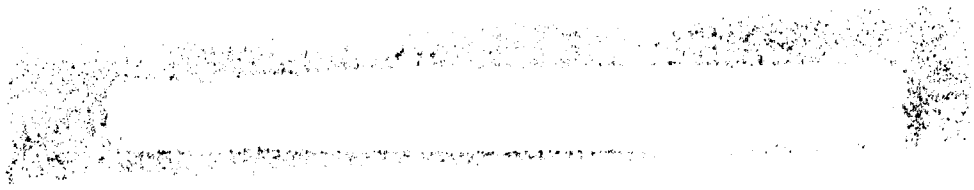

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QUANTUM CONVERSION IN PHOTOSYNTHESIS*

Melvin Calvin**

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ABSTRACT

A new suggestion is made based on model work associated with similar measurements on the biological material itself. The primary quantum conversion act is an ionization occurring in a charge transfer complex. This is what it amounts to in chemical terms. But this process cannot occur in isolated charge transfer molecules in solution because the products cannot escape from each other. The primary quantum conversion as it occurs in modern photosynthesis can only take place in a laminated structure where the electrons and holes can escape from each other by electron migration and not by atomic migrations. This is the essential feature introduced here which differs from all the previous notions of how quantum conversion occurs in chemistry or biology.

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INTRODUCTION

One can hardly begin a discussion of the problem of photosynthesis, or any specific aspect of it, without writing a small equation which will define and delimit the discussion. The overall reaction of photosynthesis, the reaction by which green plants convert electromagnetic into chemical energy, is usually written in this form:

$$CO_2 + H_2O \xrightarrow{h\nu} (CH_2O)_n + O_2$$

You will recognize that the substances on the left-hand side of the equation (CO_2 and H_2O) are the elements of carbon, oxygen and hydrogen in their lowest energy form, and the substances on the right-hand side of the equation (carbohydrate and oxygen) represent these same elements at a higher chemical potential. The carbohydrate and the oxygen normally, in the animal body and in the plant too, for that matter, can back react, producing carbon dioxide and water and, at the same time, liberate energy in one form or another -- energy for growth, energy for heat, energy for whatever purpose the organism might want it.

Certain aspects of this problem of energy conversion are not going to be the subject of this discussion, partly because they have been resolved and partly because we know little about them. These are the two aspects which I am going to eliminate. First to be restricted is the part that we know something about and which has been resolved: this is the part in which the carbon passes from carbon dioxide into carbohydrates. By the use of tracer carbon, we were able in the past fourteen years to draw a rather complete road map from carbon dioxide to the various chemical compounds which go to make up the plant (Bassham and Calvin, 1957; Bassham and Calvin, 1960; Bassham and Calvin, in press; Bassham, 1959) principally

carbohydrates. The other aspect of the energy storage problem, the conversion of the oxygen from water to molecular oxygen, is at the opposite end of the knowledge level, and we know nothing, really, about how the single oxygen atom in the water molecule finds another one and becomes an oxygen molecule -- in other words, how is the oxygen-oxygen bond created. We have some ideas about it, but very few in contrast to what we know about the construction (the actual building) of carbon compounds. But we know very little about how we put together an oxygen molecule (Dorough and Calvin, 1951; Anderson, Blass and Calvin, 1959; Sapoznikov, Eidelman, Bazhanova and Popova, 1959; Mason, 1957).

In between these two phases of our knowledge of the process of photosynthesis and energy conversion lies the area of the present discussion. It is the aspect in which the electromagnetic quantum -- the light quantum -- is absorbed by the chlorophyll to give an excited electronic state of chlorophyll, and then something happens to this excited electronic state, during which time it is converted into chemical potential -- definite molecular species which, upon back reaction, could liberate energy. That particular step is the primary concern of this paper.

To isolate, for consideration, that step from the equation as it is written, we may describe the events as follows:

See diagram on following page

The quantum is first absorbed by the chlorophyll molecule; then
(p for primary)
something happens/to the excited chlorophyll to produce two chemical species ([O] and [R], for example) which later can go on, one of them [O] to become molecular oxygen in some way, (1) and the other one [R] leading to the reduction of carbon dioxide to carbohydrate (2). Along these two routs various other energy-containing species may be created, such as phosphoric anhydride (ATP or $\sim$ P). A phosphoric anhydride species, represented by ATP, would, of course, be an energy storage product. These may be created on either, or both, sides. Further than that there may be even back reaction (3) between these intermediates -- oxidants and reductants -- which also could create various products of higher energy. The obvious one to use here is, of course, the

pyrophosphate linkage. The creation of a pyrophosphate linkage of this sort in a water milieu is storing energy.

PHOTOCHEMISTRY OF CHLOROPHYLL

We shall not try to describe the biochemical detail of any of the steps beyond (p). We shall be limited to the very first thing that happens to the quantum after it has been absorbed by the chlorophyll molecule to produce an excited state of the chlorophyll. What are the very first forms in which stable (definable) chemical species different from electronically-excited molecules (such as excited chlorophyll) appear? We will not be concerned with how the intermediate oxidant [O] becomes oxygen (1) or what other intermediate oxidants might be, nor will we consider what the hydrogen carriers might be which eventually reduce carbon dioxide to carbohydrate (2) or how, along the line (2) as they drop in potential, they might produce other high energy containing materials such as ATP. The recombination (3) oxidant and reductant which might also occur as succeeding chemical steps, will also lie outside our present concern. Our concern is the immediate fate of the excited chlorophyll and what could possibly be the very first of these species here called oxidants and reductants.

In order to try and get some idea of what could happen to the excited chlorophyll, we introduce two additional ideas. First of all, we shall examine the biological apparatus which performs this operation (insofar as we know what molecules that biological apparatus is made of and how it is constructed), and, secondly, we shall explore some model experiments which are based upon what we believe is the construction of this biological apparatus. This latter is almost exclusively

physical chemistry or physical-organic chemistry. Then I would like to go back and apply the concepts which are devised from the combination of the structural information and our model researches, to the biological material itself -- experimental observations on the biological material designed to simulate or reproduce the observations that were made on the model systems.

Photochemistry of Chlorophyll in Solution

Before going into the details of this, it seems worthwhile to introduce the point of view which dominates these discussions. From the very beginning of our knowledge of the structure of chlorophyll, beginning in 1911 when Willstätter and Stoll (1939) first had a pretty good idea of what the structure was, chemists and biologists and biochemists went to work trying to understand the photochemistry of chlorophyll itself. As they extracted chlorophyll from leaves of green plants and worked on the structure of it, they studied its photochemical behavior as well. The Fischer formula has since been confirmed completely (Woodward et al, 1960), and we can now go along with complete confidence in it.

From the very beginning the photochemists went to work to try and understand something about the energy conversion by an examination of the photochemistry of chlorophyll in solution. Over a period of some 40 years they did a wide variety of experiments in an attempt to see how the energy of a 40 kcal quantum (which is what is involved here) could be converted in a single act into chemical potential. An enormous literature (Gaffron, 1933; Schenck, 1957; Krasnovskii, 1960; Livingston, 1960) exists on the photochemistry of chlorophyll and models of it. A great many attempts have been made to find ways in which the energy of 40 kcal in an excited electronic state might be

used in a single act to create two chemical species which potentially could back-react with about 40 kcal -- in other words, to store almost all of that 40 kcal. Even if only 35 kcal were stored, that would be a lot to store in particles created at the same point. This search has not been successful, in spite of 40 years work, and the many men's lives involved in it. The attempt to find a chemical reaction, either sensitized by chlorophyll or by any of its analogs or by model substances representing it, in which the energy of 40 kcal would be converted into a pair of chemical species storing something of the order of 30-35 kcal (the efficiency of this process must be very high) has not succeeded.

In retrospect, it is not very surprising that it should have not yet succeeded. If this energy conversion process is going to take place in chlorophyll molecules which are simply in ordinary solution, randomly moving about and in contact with a variety of molecules with which they could react and to which they could give energy, it is necessary to create, in one operation, a pair of energy rich species A and B.* Then A + B by definition, in their back reaction have 35 kcal of energy to set free, and they have to be created in one act right on or near the chlorophyll molecule. You can see, therefore, that some rather tricky kinetics must be involved. Most chemical reactions do not have activation energies that high -- usually they are only around 20 kcal. If we have to store 35 kcal from the starting point (let us define A·B as the starting point -- and this could be a molecule or molecular system) the end product, A + B, has to be

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* These may be in different parts of the same molecule in which case the photoreaction might be called a rearrangement.

35 kcal above it. If this product is not to return immediately, there has to be a barrier between it and the starting point so that the system won't fall back immediately in the back reaction. This cannot be done; if we are going to store 35 kcal and we have only 40 kcal in the quantum with which to do it the barrier can't be more than 5 kcal high and the back reaction would be too fast. This is essentially what the problem is: To separate the products which are themselves of high potential energy for reaction before back reaction can take place. This is very hard to do in ordinary statistical chemical reactions. In fact, it has not yet been done.

There are a number of cases in which the photochemist has succeeded in storing energy in a straightforward photochemical reaction in solution, but, in general, those storages are very small -- a few kcal at most -- and 40-60 kcal quanta are used to accomplish this. The situation, therefore, is just the reverse of the natural reactions of chlorophyll. Instead of the product being 35 kcal above the starting point, it is only 5 kcal, with a 50 kcal quantum to help, and the barrier can be quite high (45 kcals by these numbers). You can succeed in that kind of a storage problem

The point of view that I am going to take is that this 35 kcal energy storage is <u>not</u> the result of ordinary statistical photochemistry in solution, but rather is the result of a <u>photophysical</u> process in an organized solid, or quasi-solid, matrix. How this is achieved in this case, in contrast to solution chemistry, is going to be the substance of this discussion. We did model work to show that this was possible in model systems. We then went on to ask if the phenomena we see in the model systems could be reproduced in the biological material itself.

PHOTOPHYSICAL EFFECTS IN MODEL SYSTEMS

Energy Transfer in Model Systems

One of the factors which contributed to the adoption of this viewpoint was the examination of the structure of the biological apparatus which accomplished the energy conversion (Steinmann and Sjostrand, 1953; Frey-Wyssling, 1957). Figure 1 shows the chloroplast of a green plant in which this energy transfer occurs. The green particles, called the chloroplasts, inside the cell contain the chlorophyll, and it is in these (a few microns in size) that the energy conversion process occurs. Figure 2 is an electron micrograph of a single chloroplast, at much higher magnification, which shows the internal structure of one of the chloroplasts shown in Figure 1. You can see that this is not just a 'bag of molecules.' There is a very high degree of organized structure to be seen inside the chloroplasts. The dark areas are the so-called lamellae which are present in all photosynthetic organisms. In this particular one (tobacco) these lamellae are arranged in stacks, and the term'granum' has been applied to a single one of these ellipsoidal packages which can be separated from the chloroplasts. There is, then, a high degree of order to be found inside the chloroplast. In fact, if one takes a smaller section of this granum at still higher magnification, one can see that these are made up of what look like little oval sacks pressed together. The darkest areas appear to be the contact areas between the two surfaces of completely enclosed oval, or ellipsoidal, sacks.

Figure 3 shows a diagram of our concept of what the layers of the chloroplast are composed of (Park and Pon, in press). Each of the dark areas represents a contact between the surface of two of the

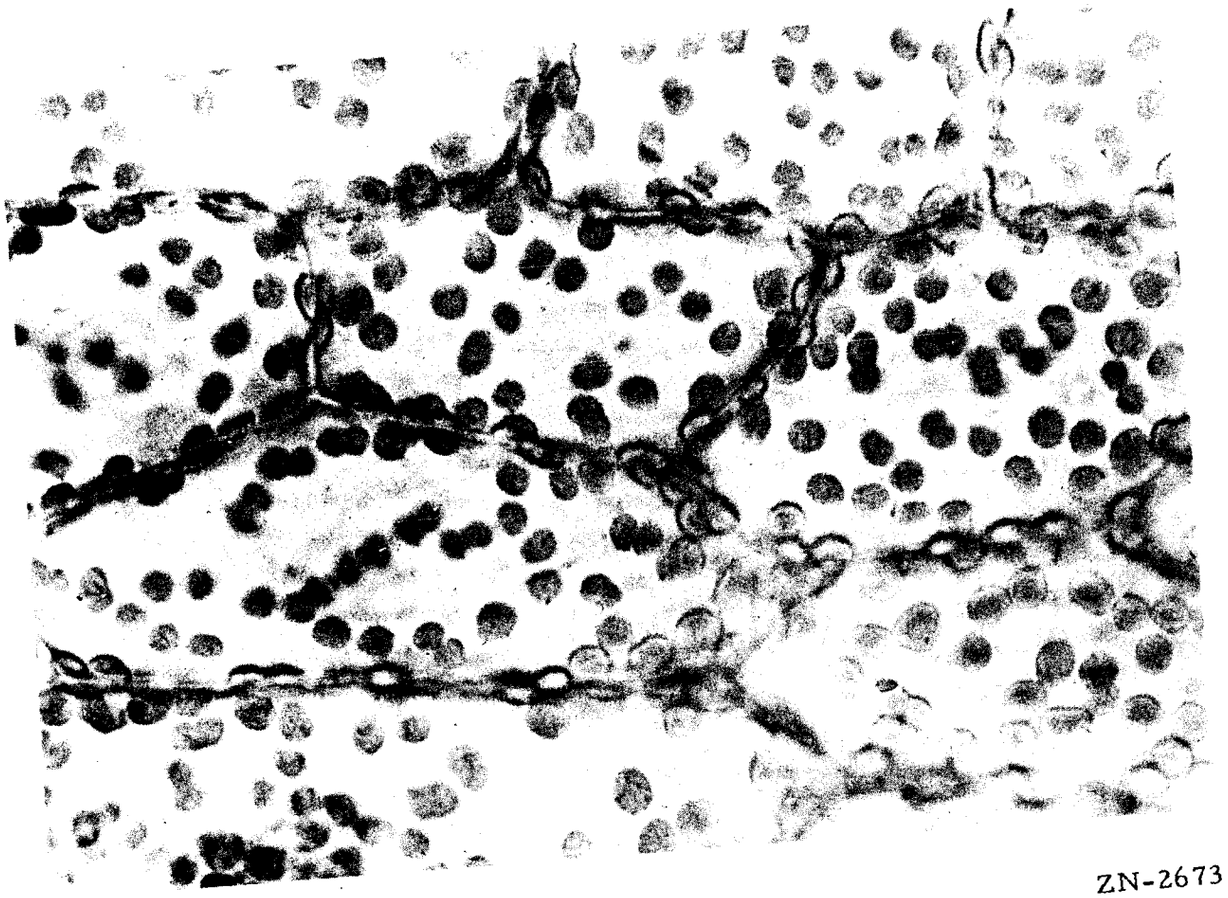

Fig. 1. Cells of liverwort showing chloroplasts.

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Fig. 2. Tobacco Chloroplasts. 24-36 hrs in dark before fixing with permanganate (Weier).

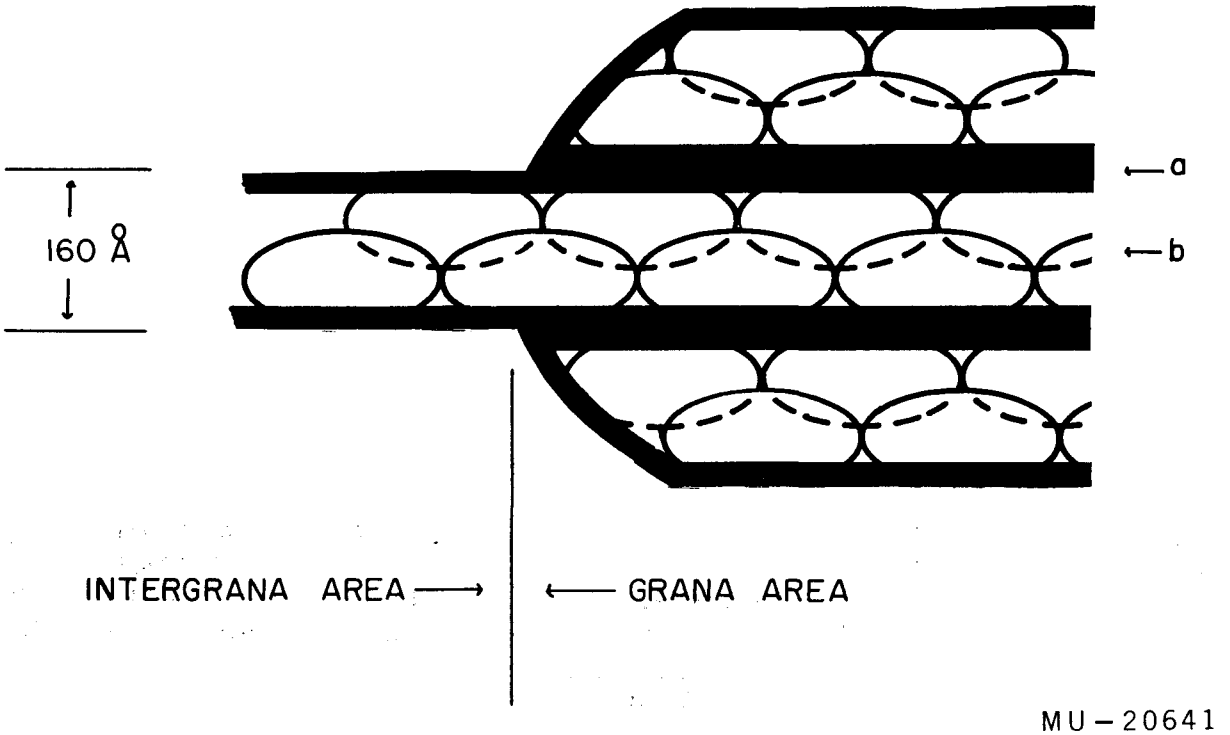

Fig. 3. Model for chloroplast lamellar structure (Park and Pon, in press).

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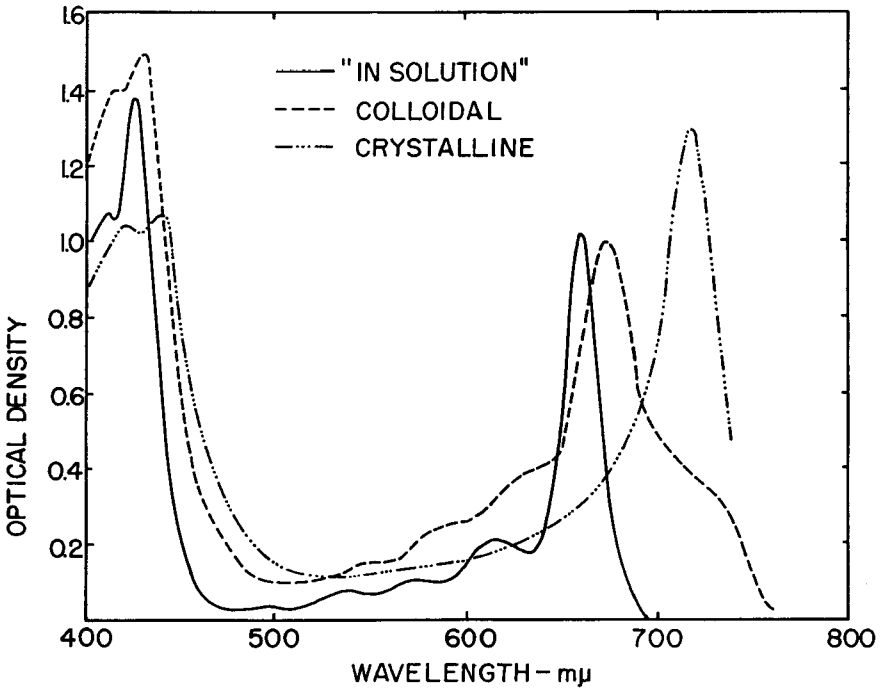


Fig. 4. Absorption spectra of chlorophyll in various states.

absorption spectrum of chlorophyll in the plant itself resembles the latter two more than the first one.

So you see the plant chlorophyll is not chlorophyll in solution; it is lipid, protein and chlorophyll (with other pigments) in a tight package; in a semicrystalline form. I am not emphasizing the spectrum itself as the only bit of evidence, but simply as one piece indicating the ordered array which the chlorophyll in the chloroplast itself is likely to turn out to have when we know it.

Relations between Chlorophyll, Protochlorophyll and Bacteriochlorophyll

What is the molecule we are talking about? Figure 5 shows three of the chlorophylls with which we are normally concerned. The middle structure shows chlorophylls a and b; chlorophyll a has a methyl group in the 3-position and chlorophyll b has a formyl group (formaldehyde) in that position. Bacteriochlorophyll is found in all the photosynthetic bacteria which do not make oxygen but which do reduce CO_2. The essential difference between plant chlorophyll and bacteriochlorophyll is the fact that the latter has two extra hydrogens on the opposite pyrrole ring (at positions 3 and 4) as compared to a double bond for the plant chlorophyll; the total redox level remains the same, since the 2-vinyl group is now oxidized to acetyl. The hydrogen atoms are just at a different place. In both the plant chlorophyll and bacteriochlorophyll, the macrocycle remains conjugated, but it is somewhat more limited in the bacteriochlorophyll.

Protochlorophyll belongs to the class of compounds known as porphyrins; it is dehydrogenated at positions 7 and 8 compared to chlorophyll and that is the only difference between them. The protochlorophyll appears in etiolated plants, that is, plants grown in the dark from

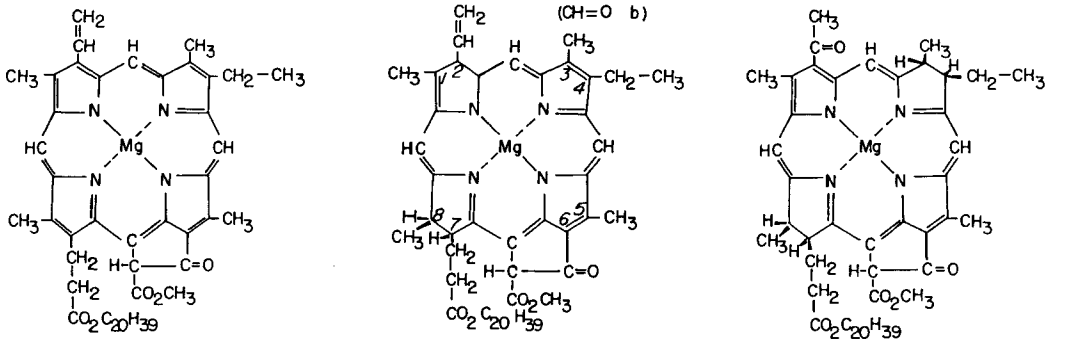


Fig. 5. Structures of protochlorophyll, chlorophyll a and b and bacteriochlorophyll.

seed and which have never seen the light. Protochlorophyll is converted into chlorophyll immediately upon illumination (Smith and Coomber, 1955). I might say that these 'extra' hydrogens have held a fascination for everyone -- the 7 and 8 pair and the 3 and 4 pair. These are the two points of the chlorophyll that people have focussed their attention on for the last 20 years in an attempt to try and do solution photochemistry. We did it, too, (Seely and Calvin, 1955). We thought that perhaps that one or the other of these pairs of hydrogen atoms were being transferred back and forth by the photochemical reaction, but now the evidence seems to indicate that this is not the case and the chlorophyll is not functioning in such a way.

The main feature of the chlorophyll structure is this big conjugate macrocycle, the so-called dihydroporphyrin ring (chlorin ring) which is the light-absorbing entity of the photosynthetic apparatus. This is the thing that makes plants green. The phytol side chain would seem to be part of the architecture which holds the molecule in place. I don't believe the phytol chain plays a part in the energy transmission directly, at least. The 6800 Å -40 kcal quantum is absorbed by the electronic system of this conjugated macrocycle with the magnesium in the center, and from there on we don't know what happens. This is what we are trying to discover and are speculating about.

Presumably, a very similar process goes on in the bacteria with the bacteriochlorophyll, the difference being that in the bacteria, oxygen is not liberated. The primary oxidant is instead reduced by some chemical reducing agent other than water.

So much, then, for what we know about the biological equipment that is going to perform this energy conversion job which we have

described earlier. I have not mentioned the accessory pigments, of which there are several and at least one of which is probably going to turn out to be as important as chlorophyll. People generally overlook this, although when you stop to think about it, it shouldn't really be overlooked. The fact is that wherever there is chlorophyll, wherever there is photosynthesis, there is also carotenoid. In general, people have tended to ignore this, or at least have not given enough weight to the fact that the carotenoid is also present in every case where there is photosynthesis, and somehow these two things must be very closely associated. The carotenoid is the long conjugated carbon chain (polyisoprene with 10 to 12 double bonds in it and some oxygen at each end) and a variety of functions have been proposed for it: oxygen carrier (Dorough and Calvin, 1951), electron carrier (Calvin, 1958; Platt, 1959), hydrogen carrier (Calvin, 1959a; Shlyk, Godnev, Rotfard and Lyakhovich, 1957), and probably one of them is right, but the trick is to know which one.

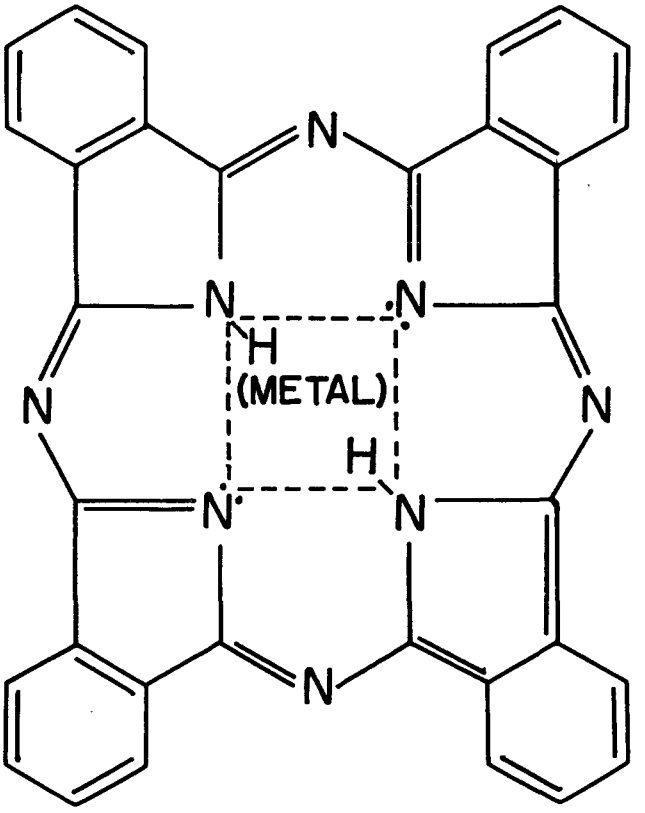
With this structural background on the photobiological apparatus, let us turn first to the question of generating an idea as to how it might work (other than ordinary solution photochemistry) in the solid state, i.e., the organized state which very certainly exists. Then we will describe some of the model experiments which have been done in an attempt to expand, or explore, the concepts which were generated by the combination of knowing the fact that there is such a fine structure; that the flat chlorophyll molecules tend to lay one upon the other; and that there is something different about the way the crystal, or pseudo-crystal, behaves from the way the molecules in solution behave.

Phthalocyanine as a Model for Chlorophyll Energy Transfer

About 1950 the developments in solid state physics finally reached the chemists (at least they reached me then). By this I mean the developments in our knowledge of the electrical and magnetic properties of atomic and ionic crystals had reached a stage,both of technical development and understanding, which allowed us to apply some of the notions which were common amongst the physicists developing this work to the kinds of molecules and the kinds of systems which we had in this biological apparatus, particularly these big, flat aromatic systems such as chlorophyll.

I had for some years been working with porphyrin analogs. The first of these, and the one that is still one of the most popular, I encountered in 1936, the year it was discovered in England, and this is the molecule of phthalocyanine. It is a synthetic compound which resembles, in some respects, the structure of the tetrapyrrole which you saw in chlorophyll. Phthalocyanine differs from chlorophyll in certain rather important aspects, but the most important difference was that it was easily made compared to chlorophyll, easily handled and very stable -- and none of these things was true of chlorophyll. This is the reason we selected phthalocyanine as a model of the porphyrin structure found in the chlorophyll in an attempt to find out how the solid array of molecules might differ in their physical and chemical properties and reaction to light from molecules in solution.

The structure of phthalocyanine was determined in 1935-36 by Linstead (Linstead, Eisner, Ficken and Johns, 1955) at the Imperial College. It is shown in Figure 6. It is made from phthalonitrile and metal; the ring closure occurs very readily. It has the elements of the tetrapyrrole in it, but/differs it



PHTHALOCYANINE

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Fig. 6. Structural formula of phthalocyanine.

from a true tetrapyrrole in that the bridging atom instead of being CH is nitrogen, so it is called a tetrazaporphyrin. It also has benzene rings fused onto the pyrrole rings. Phthalocyanine is a very stable substance and is widely used in various forms as a dyestuff.

With this as our starting point we sought to make systems which might resemble the laminated system which appeared to exist in the chloroplast. The idea that organic substances such as phthalocyanine might be electronic conductors under certain conditions was actually born, as far as I was concerned, in a discussion with Professor Michael Polanyi (University of Manchester) at the time we received the phthalocyanine from Linstead, back in 1936. We didn't do anything about it then except insofar as we used it as a catalyst for hydrogen activation, much like platinum. That was about the extent of my early activity with phthalocyanine as a possible electronic conductor. (Calvin, Cockbain and Polanyi, 1936; Calvin, Eley and Polanyi, 1936). One of my associates in the laboratory at Manchester, D. D. Eley, also working with phthalocyanine, went to work along the electronic lines,and some twelve years later he published the first paper, I think, on this subject, in which he demonstrated that phthalocyanine behaved as an organic semiconductor. (Eley, 1948).

This was enough to trigger us again, and now the basic idea was born that the energy conversion process in the chloroplast might be a process in which the excited chlorophyll molecule had some of the properties of an organic semiconductor. The transformation from an excited chlorophyll molecule into chemical potential was envisaged as <u>separation of charge rather than a separation of atoms</u>. We now had to devise the physical configuration of these molecules which might permit the demonstration that this phenomena could occur.

The structure of the actual photosynthetic apparatus is such as to suggest a laminated structure in which there were chlorophyll molecules arranged

in some order, perhaps with carotenoids and dher lipid-type of materials on one side. On one side of the chlorophyll layer there could be electron-accepting species and on the other side of the layer there could be electron-donating species. In this way one could visualize a laminated system resembling the donor-acceptor systems in the atomic and ionic lattices that the physicists had been describing, which did succeed in converting electromagnetic energy into charge separation in a fairly well understood manner.

We proceeded to explore this idea and develop it to see what the limitations of it were and what the requirements were for producing charge separation in an organic system using light. First, we had to show that the material was indeed a semiconductor. We performed the same experiments that Eley had done and came out with pretty much the same general results. The next step was taken when we started to construct laminated (layered) structures in which we added either electron donors or electron acceptors to the phthalocyanine (chlorophyll analogue) layer. (Kearns and Calvin, 1958; Kearns, 1960; Kearns, Tollin and Calvin, 1960). Our first measurements were purely of conductivity: Could these layers carry an electronic current in the dark? What would happen to the conductivity of such a system if one put donor or acceptor layers together in such a configuration?

Figure 7 shows the diagram of the apparatus which was used to perform these experiments. The electrode system shown here was actually an interleaving of two aquadag combs, and laying on top of it, by sublimation or evaporation, was the layer of the sample. We have performed the experiment with phthalocyanine and with about half a dozen other aromatic pi-electron containing systems. The lamination was achieved by putting on the back surface of the sublimed layer the donor or acceptor system, whichever it might be. Most of

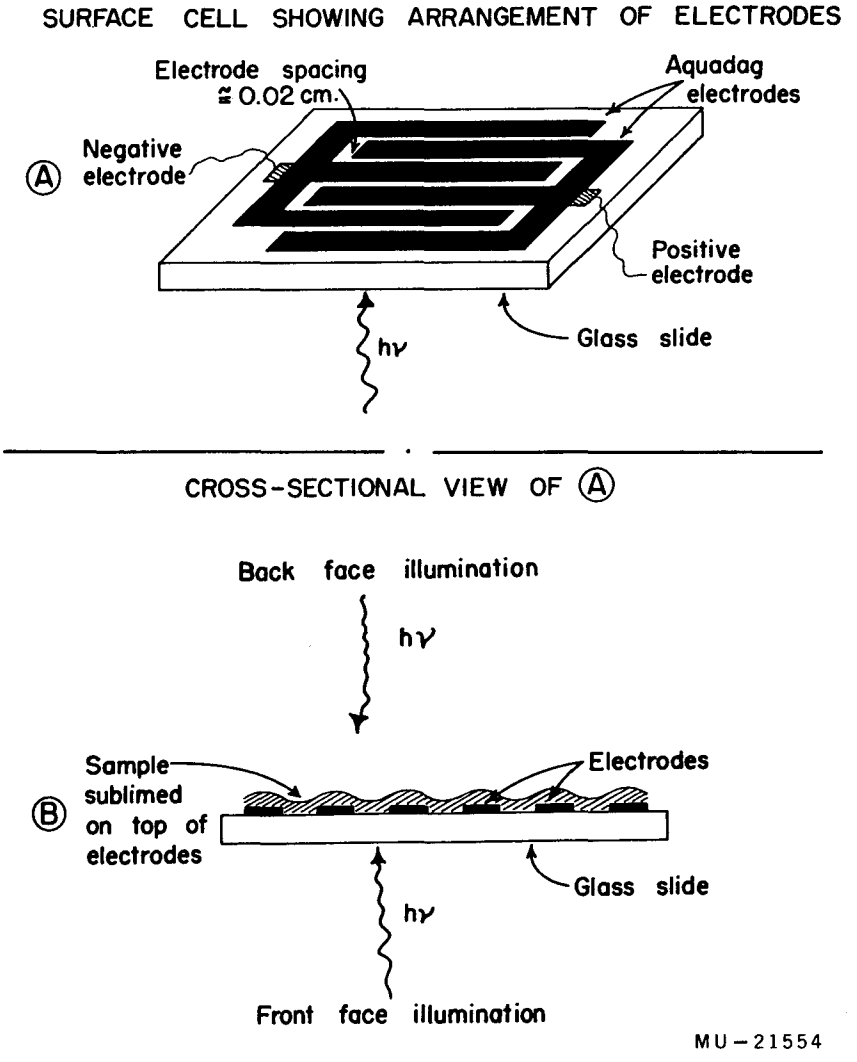

Fig. 7. Diagram of sample conductivity cells.

the work on the phthalocyanine and on the other aromatic systems (violanthrene, perylene, etc.) was done with electron acceptors as the top layer. (Kearns and Calvin, 1961, in press).

The results of such an experi ment are shown in Figure 8 in which we plot the log of the current flowing between the two electrodes (maintained at a 50 to 90 volt differential) as a function of the amount of electron acceptor which was put on top of the phthalocyanine layer. This, then, is the current flowing between the electrodes, i.e., through the phthalocyanine, as it is affected by the electron acceptor which is placed on top. The conductivity of this system rises very steeply as very small amounts of electron acceptor (o-chloranil) are added to the surface layer. This is true of the dark current and also of the photocurrent, which is the difference between the light current and the dark current. We are measuring the current that flows between the electrodes in the phthalocyanine layer. The o-chloranil (o-tetrachloroquinone) is a very good electron acceptor. As a small amount of the electron acceptor is placed above the phthalocyanine layer, the conductivity goes up by several powers of ten.

Apparently the acceptor pulls electrons out of the donor, putting electrons into orbitals of the o-chloranil and leaving behind electronic vacancies in the phthalocyanine molecules. By putting a potential between the two electrodes, it becomes possible to move charge much more readily between them because there are now low lying, unoccupied orbitals between which the electrons from the full orbitals can move. The electronic state in the organic solid after any particular move is the same as it was before, save for the passage of electrons from one electrode to the other. Without these vacancies for hole motion in the donor layer (electron motion in the acceptor layer), the conductivity would be very low. (Keppler, Biersted and Merrifield, 1960). A diagram representing this situation is shown in Figure 9. (Kearns and Calvin, 1961 in press).

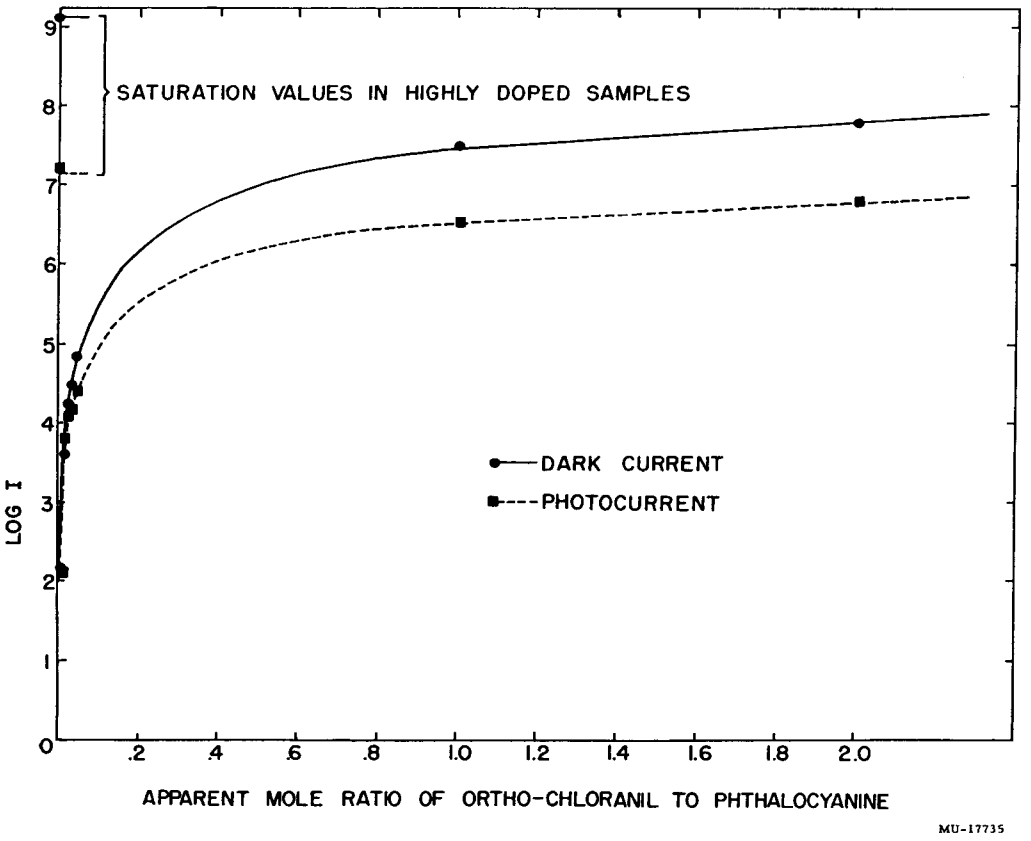

Fig. 8 Variation of dark conductivity and photoconductivity of phthalocyanine with amount of c-chloranil added.

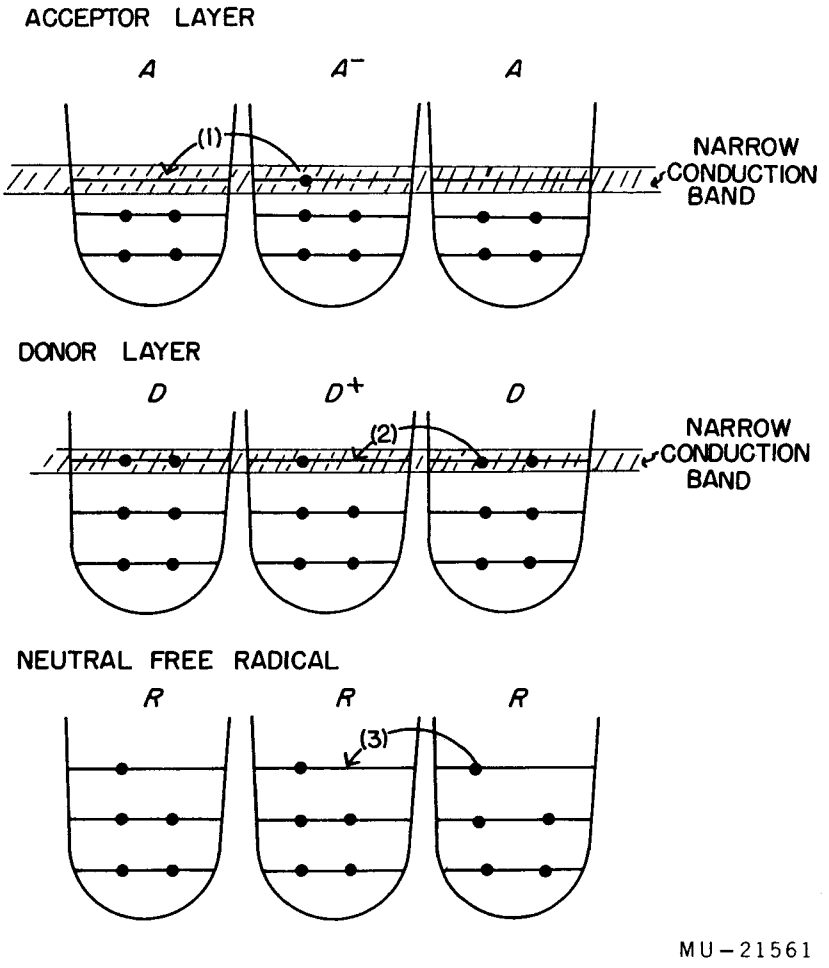

Fig. 9. Charge migration in a molecular lattice.

(See next page for descriptive caption.)

Figure 9

Schematic representation of donor and acceptor molecules and ions imbedded in a donor layer or an acceptor layer, respectively. From this diagram it is clear that process (1), the transfer of an electron from an acceptor negative ion to a neutral neighbor, produces a state of the system which is energetically identical with the initial state. Similarly, there is no net change in energy as a result of process (2) which rearranges charge in the donor layer. In the case of a neutral free radical, however, the electron transfer process (3) does not result in a state energetically equivalent to the initial state. Since processes (1) and (2) simply change the location of negative and positive charges respectively, with no net change in energy, we can consider the orbitals involved in the electronic rearrangements as forming conduction bands. If, however, the lattice were made up of A^- radical ions (no A's) irrespective of the cations, or entirely of D^+ radical ions (no D's) irrespective of the anions, there would be no identical vacant orbitals into which the charge carriers could move and hence no conduction bands (however narrow). This last situation would correspond to the completely filled free radical system as in process (3) above.

The light effect involved in the excitation of phthalocyanine to an excited state leads to a higher population of electrons in the acceptor molecules, making a higher population of electronic vacancies in the donor matrix so that the conductivity increases over that in the dark.

This is essentially the basic notion which we believe describes the model system as we now have it. We have used a wide variety of donor systems and a considerable variety of acceptor systems, and the behavior has fulfilled all of the expectations of such a description. (Kearns, Tollin and Calvin, 1960; Kearns and Calvin, 1961 in press).

There are various other properties of such a system which should follow, and we have measured them. For example, we have measured the kinetics of the photoconductivity -- how it grows and decays -- at various temperatures. One observation is particularly interesting, and it has to do with the fact that in a system of this kind, the electrons in the acceptor layer are, in effect, unpaired electrons. They may be considered as in very narrow conduction bands, or, if you like to think of them as a chemist would, they are in singly occupied orbitals in the molecules. The same things may be said of the unpaired electron which remains behind. One should see those unpaired electrons by virtue of their magnetic spin resonance and indeed we have seen them in that way. Figure 10 shows the electron spin resonance spectrum of o-chloranil 'doped' phthalocyanine; the g value is very close to that of a free electron. Figure 11 shows the change of that signal following illumination and darkening. When the light is turned on, the spin signal is decreased and when the light is turned off, the spin signal comes back. The reason for that in this particular situation is that almost all of the o-chloranil molecules adjacent to the phthalocyanine are already mono-negative ions in the dark, and when the light is turned on, a second

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