MASTER

ENERGY RECEPTION AND TRANSFER IN PHOTOSYNTHESIS

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ABSTRACT

The basic information about the path of carbon in photosynthesis is reviewed, together with the methods that were used to discover it. This has led to the knowledge of what is required of the photochemical reaction in the form of chemical species. Attention is then directed to the structure of the photochemical apparatus itself insofar as it is viewable by electron microscopy, and some principles of ordered structure are devised for the types of molecules to be found in the chloroplasts. From the combination of these, a structure for the grana lamella is suggested and a mode of function proposed. Experimental test for this mode of function is underway; one method is to examine photoproduced unpaired electrons. This is discussed. (auth)

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You just heard a rather detailed and excellent description of how a cell can get energy by the combustion of carbohydrate, for example, and you also heard of the remaining (or at least outstanding) problems that remain in the mobilization problem. What I am about to describe to you is the reverse process (or something about the reverse process), namely, how the green cells of plants are able to transform electromagnetic energy into chemical energy, that is, by absorption of carbon dioxide and water, which are the end products of the animal cell, and by the absorption of light, how they produce the foodstuffs which are the beginning of the process which you just heard described. The first figure will illustrate diagrammatically what I would like to tell you about.

The starting points in this case (Figure 1) are carbon dioxide and water which contain the elements carbon, hydrogen and oxygen in their lowest energy forms with respect to biological processes. The chemical energy which is accumulated is here represented in the form of oxygen (molecular oxygen), on the one hand, and a carbohydrate, on the other. The process itself has been divided, both theoretically and physically, into two rather well separable stages. The first of these is the absorption of light by chlorophyll or some related pigments and the subsequent separation of water into a reducing agent, here represented by H, and some oxidizing fragment not specifically designated here but presumably one of the A, B, C series. The oxidizing agent, or the primary oxidant, ultimately becomes molecular oxygen. In the second stage, the reducing agent can be used (and is used) to reduce carbon dioxide to the level of carbohydrate and other plant materials.

Now in order to see how the energy of the light is actually accumulated in chemical form, it seemed to me wise to describe for you what we know about the right-hand side of that sequence -- the sequence from carbon dioxide to carbohydrate-- and to determine at what point in that sequence the energy ultimately derived from the light enters, and from that point on to recognize and define the problem of the primary quantum conversion into its first recognizable chemical form. Therefore, I am going to take a few minutes to describe to you in some detail what we know about the path of carbon so that we can define more precisely into what sort of energy the light must be converted in order to carry out that process.

With the ready availability of radiocarbon (carbon-14) from the nuclear reactors some 15 years ago, it became possible for us to trace this sequence in some detail. I am very briefly going to outline for you how we did it. The plant material used in most of the experiments was the unicellular green alga, Chlorella, and occasionally the alga, Scendesmus; we have also used higher plants as well as separated photosynthetic material. Figure 2 shows a photomicrograph of the algae cells we commonly use; these are the Chlorella cells and you can see the green stuff contained in a cup-shaped chloroplast. It is well illustrated by one of the cells in the upper right-hand corner.

Rather than describe for you step by step the operations that we performed, I have brought with me a film for your entertainment. This film constitutes a sequence of scenes illustrating all of the operations of the laboratory so as to give you some feel for the way in which most of our experiments, until recently, have been done. (It's a silent film and I will be the sound for it.) You will now see the sequence of events.

Narration of the Film:

The first thing we do is to show you where the work is done by means of our usual trademark which some of you will very quickly recognize (laughter). This first operation constitutes a selection of cultures, and the next stage will be the growth of those cultures in 200 cc flasks followed by the transfer of the 200 cc cultures into much larger continuous one-liter culture flasks. These we call shake flask cultures in which algae have been maintained for years at a time.

The most recent type of culture device that we use is this continuous tube culture in which the density of the cells is monitored by a photoelectric cell which controls the automatic feeding of the medium, so we always have the cells in a steady-state of growth available for our work. Being chemists, we like to have materials which would do the same thing on Tuesday as they did on Monday as this was what we were really used to doing. We couldn't go outside the laboratory and pick up leaves (which we did at first) because they didn't always behave the same way. As a matter of fact, occasionally these laboratory-grown algae don't behave, either.

Next we see the harvesting of an algal sample to be used now for the feeding of radiocarbon which is done in a special 'hot box.' There we place the cells in a little vessel (lollipop) between the lights and adapt them with the concentration of normal carbon dioxide at which we are going to work. The cells being adapted are now ready for the experiment.

Then we administer radioactive carbon dioxide to the cells for a suitable length of time in order to trace the paths that the carbon atoms take. The radioactive carbon is usually injected in the form of a solution of sodium bicarbonate. It is kept in contact with the cells for a period of time (in this case five seconds), after which the cells are killed by a variety of methods. (This has been subject to some discussion.) In this case, we drop the cells into methanol at room temperature. The cell extract is then analyzed for the radioactive compounds which it may contain by the method of paper chromatography. In order to achieve this analysis we must concentrate the extracts, and a vacuum evaporated is used in a routing fashion to reduce the volume from 200 cc, or a liter, down to a cubic centimeter or so.

From this concentrated extract we now take an aliquot and place it on the corner of a piece of filter paper for chromatographic separation. After the compounds have been placed on the corner of the filter paper, that filter paper is then hung in a box, in a trough, and a solvent is placed in it which will pass over the filter paper and spread the compounds down the side of the paper. (Before chromatography we count the origin. We usually do this in a quantitative way so that we know how much radioactivity is placed on the corner of the paper to start with and we can determine the material balance at the end of the experiment.) The filter paper goes into the trough, the trough is placed in a box and a solvent is placed in the trough. The solvent rises over the edge and flows down over the filter paper. This spreads the compounds according to their relative solubilities in the solvent, those that are the most soluble running the most rapidly. This procedure will result in a set of spots down the side of the filter paper, depending on the properties of the compound being analyzed. Some of these compounds will overlap each other in one solvent system. The paper is then removed from the box and dried. After drying overnight, we come back the next morning and pull out the paper to see if it's dry. The paper is then turned through 90° and placed in another trough, in another box, with another solvent. In a sense what we have now as our starting point is a whole series of spots along the top edge of the paper. Another solvent is put in the trough and it spreads those spots out again in another operation of similar type. After this operation is completed the paper is dried a second time. The next problem would be to find out where the radioactive materials are on the paper. They are, in fact, not colored and they are not easy to find except by virtue of their radioactivity.

Now in order for you to see how this separation has occurred, we have made a time-lapse film of a special kind of ink which will demonstrate for you how the compounds are separated in this procedure of paper chromatography. We place the special ink in the corner of the filter paper, the solvent comes down over and begins to separate the ink into its various components. This is the first dimension and you see how the original spot is now spread along one edge of the filter paper, giving a whole series of spots. Some of them are still overlapping spots. So when we turn the paper around we start all over again with a new set of spots, and the second solvent will pull some of those spots out which were not separated in the first dimension. You can see that big spot coming out, and the various others separating quite well.

The coordinates of the material with respect to the origin constitute a physical property which is useful in the identification of the compound being analyzed. Our problem is to locate the compound on the paper. The compounds are not colored, as I said, and the only property that we can use to find them is their radioactivity. We do this by placing the paper in contact with a sheet of photographic single coat blue sensitive X-ray film which is exposed by the radioactivity on the paper. Wherever there is a radioactive spot on the paper there will be an exposed area on the film after a suitable period of time. As you can see, there are a number of radioactive compounds on the paper defined by the black spots on the film. For quantitative work we place the film down on an X-ray viewer, the paper over it and explore the paper with a Geiger counter. This will show you that where the spots are black, you get the highest count; where the spots are weak, you get the lower count. This can be done in a quantitative way. You can tell not only what compound it is by its coordinates but also much there is by the amount of radioactivity found in the spot.

We can get a greater or lesser resolution depending upon the nature of the solvent systems we use and the time that is used for the chromatography. The next sequence will show two films of two bits of paper (two different films of the same extract; (one of them has been run for 48 hours and the other for only 12 hours). (The one on the left, 12 hours, the one on the right, 48 hours). One spot in the lower right-hand corner of the 12-hour film has now broken up into three spots by running the chromatogram a good deal longer (48 hours). (Here ends narration of film.)

Let us see what we can do with this kind of information. Figure 3 will show you a chromatograph picture of the extract of a 60-second illumination of Chlorella. (The film only gives us the black spots. The names we have to put on ourselves, and this identification takes anywhere from 15 minutes to 15 years. Some of the spots we don't have names on yet.') You can see that 60 seconds illumination is much too long to find the earliest compounds into which the carbon enters in the course of its conversion from carbon dioxide to carbohydrate.

Figure 4 shows a chromatogram of a shorter illumination (10 seconds). You see here one compound dominating the scene, that is, phosphoglyceric acid. I might say we have now been able to get the same type of sequence of events with isolated chloroplasts plus a number of co-factors. The phosphoglycerate appears no matter how the chloroplasts or the algae are killed: whether they are killed hot or cold, whether they are killed in alcohol, acetone, etc. We always get the same compound, phosphoglyceric acid.

This, then, gives us our initial clue that the first isolatable stable compound that we can get by these methods, or at least that we can identify by these methods, is phosphoglyceric acid. (I will write the formula for phosphoglyceric acid for those of you who aren't familiar with it. It's a three-

$$CH_2 - CHOH - CO_2H$$
$$|$$
$$OPO_3H$$
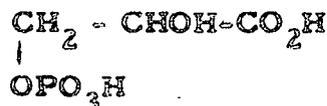
carbon compound containing a phosphate group, one of the low-energy phosphates that Lehninger spoke of.) The next problem is to determine which of these carbon atoms has the radioactivity in it, and this was done by chemical degradation methods. Taking the compound (phosphoglyceric acid) apart, one carbon atom at a time, we found that the carboxyl group became radioactive first and the other two later. From this, together with the degradation of sugar molecules that came out in the same experiment, we were able to determine how the sugar molecule was constructed.

Figure 5 will show what was supposed to have occurred. The phosphoglyceric acid is shown as PGA. This, by reduction, goes to triose phosphate. Isomerize some of this ketose phosphate, then combine them head-to-head to make a hexose diphosphate, putting the radioactive carbon atoms in the middle of the molecule. It gives us the information we need for putting the three carbons together into a six-carbon molecule, but it doesn't tell us where the three-carbon compound comes from. Simple arithmetic says that if you add one to something and get three, that something must be two. So we start looking for two-carbon containing molecules. After considerable time we found a lot of things but we didn't find two-carbon compounds. Figure 6 will show what we did find.

In addition to the PGA we found a five-carbon atom compound, a sugar, (ribulose diphosphate), a seven-carbon atom sugar (sedoheptulose diphosphate), and, of course, the six-carbon atom sugars. The stars on Fig. 6 give you some idea of the way in which the radioactivity was distributed in these various sugar compounds. Without going into the gory details of how we figured it out, we finally came up with the way in which these five- and seven-carbon sugars could be made from the triose. You have already seen how the hexose was made, and from that we can make the heptose and the pentose as shown in Figs. 7 and 8.

Figure 7 illustrates the method by which the heptose may be produced. You see from one molecule of hexose and one molecule of triose (taking off the top two carbon atoms of the hexose) we can make a pentose and tetrose; and the tetrose is labeled in the top two carbon atoms. The tetrose can then combine with a triose to make a heptose with the proper distribution of radioactive carbon which we are seeking.

Figure 8 shows the way in which the pentose is put together, by combination of a heptose and a triose, in the same kind of a reaction (the transketolase reaction) leading to two different pentoses which are in equilibrium with each other. And our analysis, of course, doesn't distinguish between the two pentoses; we got the proper labeling of pentoses this way. All of these rearrangements, as you can see, are done at the sugar level; triose, tetrose, pentose, hexose and heptose are all at the same redox level. They are all very nearly at the same energy level and there is thus practically no energy required for these rearrangements. However, no experiments of this type gave us the information which we sought, namely, the origin of the three-carbon piece in the first place. This awaited a quite different kind of an experiment, an experiment in which a steady-state was first established in the organism, after which some environmental variable was suddenly changed. We then examined the transients that resulted from changing some of these variables.

Figure 9 shows the results of such an experiment. We establish a steady state by feeding the radiocarbon long enough to the plant to saturate the phospho-glyceric acid and the other compound mentioned. Then suddenly we turn off the lights. When we do that, the transient ensues. The phosphoglyceric acid rises suddenly and the ribulose diphosphate falls precipitously. This complementary behavior gave us the clue we needed for the relationship between ribulose diphosphat and phosphoglyceric acid. It seemed as though the ribulose diphosphate was dis-appearing by combining with the carbon dioxide (that is, five carbons plus one, making a total of six carbon atoms) to produce two molecules of phosphoglyceric acid. If this is the case, then we can show diagrammatically the relationships of the various compounds, as in Fig. 10.

In Fig. 10 we have the ribulose diphosphate combining with carbon dioxide to form phosphoglyceric acid. The phosphoglyceric acid would be reduced by light to triose, triose then goes through this series of sugar rearrangements (shown in Figs. 6, 7, and 8) back again to the pentose. When we turn off the light we stop this reaction. When we stop the reduction reaction, phosphoglyceric acid builds up and the ribulose diphosphate disappears. Figure 10 simply expresses in a scheme what the transient experiment told us a moment ago.

But this scheme (Fig. 10) predicts another type of transient. If we keep the light on and stop the CO_2, we should get a different kind of transient, namely, the ribulose disphosphate should suddenly build up and the amount of the phosphoglyceric acid should fall. We have done this experiment (it's a much more difficult one to do) with the results shown in Fig. 11.

In Fig. 11 you see, at the left, the steady state for ribulose diphosphate and the steady state for phosphoglyceric acid with the CO_2 at a concentration of 1%. At the line, the CO_2 concentration is shifted by turning the stopcocks, from 1% to 0.003%. Under those circumstances, the predicted changes were observed, at least in the initial phase of the transient. The amount of phosphoglyceric acid fell and the amount of ribulose diphosphate rose. We got a number of kinetic oscillations here which are reminiscent of the kinds of oscillations one gets in circuitry, and indeed I think they are quite analogous. We made one or two mild attempts to reproduce these oscillations by putting first-order rate constants into the various reactions that are involved here and putting them through a digital computer. We can get this kind of oscillation but we have not yet pursued this work beyond the elementary stage of the first kind of transient. I believe this kind of study will lead to much more detailed knowledge of the mechanism of cellular response to changes in external or internal environments. It is a very simple system to use and one which is amenable to complete analysis, both experimentally in terms of the compounds involved and theoretically in terms of the simple kinetics involved. (Perhaps Alberty will say a little more about that later.)

Now, having done this, we can put together the completed system which is represented in Fig. 11 by only four terms (four compounds) although there are actually a good many more, and I believe Fig. 12 shows this.

Figure 12 shows the completed photosynthetic cycle in which we put together all the rearrangements of hexose and triose through heptose and pentose, back again to the ribulose diphosphate which then picks up carbon dioxide to make two molecules of phosphoglyceric acid.

Now in trying to visualize this particular step, I have set up a proposed mechanism for this reaction shown in Fig. 13. Here we have the ribulose diphosphate written as the ene-diol, combining with bicarbonate ion to form an intermediate, hypothetical up to this point, an alpha-hydroxy-beta-keto acid, which is then hydrolyzed to give two molecules of phosphoglyceric acid. This alpha-hydroxy-beta-keto acid would be, according to our chemical knowledge, very unstable either to decarboxylation,in which case it would lead back to ribulose diphosphate, or to hydrolysis, in which case it leads the other way. So for some years I didn't even institute a search for the unknown intermediate until fortunately I had a visitor in the laboratory who didn't know enough chemistry not to look for it. So he set out to look for it and found a close relative of it and probably some of the compound itself. He decided, on theoretical grounds, that one might expect a compound of this sort to appear down in the diphosphate area of the chromatogram. Figure 14 shows that chromatogram as you saw it in the film which ran solvent for 48 hours in both coordinates. What was originally a single spot, which is dominantly ribulose diphosphate, now breaks up into at least three spots. The principal spot is the ribulose diphosphate; another one is hexose diphosphate and heptose diphosphate; and the last spot turned out to be a keto acid diphosphate. I can't trouble you with all the evdience which leads to the identification

of a keto acid diphosphate. It wasn't the beta-keto acid, it was the gamma-keto acid diphosphate which apparently is an artifact of the method of killing, but it does come from the beta-keto acid diphosphate which is still a labeled compound and does show its presence in small amounts.

Figure 15 shows this. We see the diphosphate plus some dephosphorylated compounds, particularly the gamma-keto acid diphosphate. And here we see a trace of the beta-keto acid in its lactone form because it is stabilized as a lactone, enough to catch it on a chromatogram. Racker has carried out this whole sequence (the New York Central Railroad System which you saw in Fig. 12) by collecting all the enzymes that were indicated in that figure, and by putting in the suitable substrates, ribulose and carbon dioxide, he was able to pull out glucose phosphate from the CO_2. The immediate sources of energy are the two compounds adenosine triphosphate (ATP) and reduced pyridine nucleotide (TPNH). Figure 16 will show the relationships that we need.

Here is the photosynthetic carbon cycle in a simplified form. Carbon dioxide enters to make the beta-keto acid which then goes to the phosphoglyceric acid. Now the only points of entry of energy into this system the way it's written now, is where we need ATP and where we need reduced pyridine nucleotide. (These are the points of entry of energy into this wheel.) These points are the gears which drive the cycle in a forward direction. Clearly the ATP and the TPNH (energy sources for the photosynthetic cycle) must come ultimately from the light. It is of this, now, that I would like to speak in the remaining minutes. Of course, there are some problems left in the carbon cycle, but I don't think that we want to spend any more time with those today.

The big problem is: how does the light, which is absorbed by the chlorophyll, produce these two substances (ATP and TPNH) which we know are required for carbon reduction. Before going into the details of a possible answer, let's see some pictures of the apparatus which does it. Figure 17 shows a photomicrograph of liverwort tissue; you can see the cells, the cell walls and the chloroplasts in which the chlorophyll is very nicely distributed inside the cells. Figure 18 will show isolated chloroplasts from spinach (these are bigger chloroplasts and they have been isolated, I think, in sucrose solution). As I said earlier today, all of this carbon reduction-oxygen evolution-phosphate production can be done with these chloroplasts removed from their natural habitat inside a cell. However, in order for that to be possible at anything approaching the rates at which it occurs in the living cell, we have to add cofactors, some of which are heat stable, some of which are heat labile and some of which are unknown but which are obtained out of the sap of the cells. In any case, this whole process can be done outside the cell.

Figure 19 shows one further magnification of this type of thing with an electron microscope. On the left we have an electron microscope picture of a chloroplast and on the right it is shown at a still higher magnification. The outstanding features of the chloroplast structures are these lamellae, and I would now like to discuss the nature of these things.

Before I do that, let me say one other thing about the reactions which can be carried out by these separated cellular components. Most of you are familiar with the fact that for some twenty years now it has been possible to carry out the

photochemical evolution of oxygen by isolated chloroplasts using a suitable hydrogen acceptor such as ferrocyanide or quinone. This is called the Hill reaction. In the last five years, by preparing the chloroplasts in a manner (i.e., preparing the chloroplasts in salt or sugar solutions) which presumably does not destroy a chloroplast membrane or perhaps precipitates enzymes from the cytoplasm onto the chloroplast, we have been able to carry out two other reactions with the chloroplasts. These reactions are the reduction of CO_2 (which I mentioned earlier) as well as the evolution of oxygen, and, finally, the production of ATP by illumination of the chloroplasts. I would like to add one other bit of quantitative information: these three reactions, carbon dioxide reduction or, going one step further back, the production of reduced pyridine nucleotide rather than CO_2 reduction, ATP production and oxygen production are the three things that we can now do with the chloroplasts. You already can see that the reduction of CO_2 requires two of the items and the evolution of oxygen may or may not require ATP.

Now, how many of these things can be done simultaneously by the chloroplasts? In a recent conference (Brookhaven National Laboratory, June 1958) it became evident that all pair combinations of these processes could be demonstrated. But so far as I know, all three of them (i.e., of CO_2 reduction, ATP production, and oxygen production) at the same time had not yet been demonstrated; but you will see from what I say in a moment that this is likely to be so.

It has been demonstrated that one could make one mole of pyridine nucleotide for every atom of oxygen produced. Simultaneously one can demonstrate the production of one mole of ATP for every equivalent of reduced pyridine nucleotide produced. (One can demonstrate one mole of ATP created for every equivalent of oxygen produced, simultaneously now.) So this is something beyond the oxidative phosphorylation which Dr. Lehninger spoke about, that is, the oxidative phosphorylation would be the production of ATP by a recombination of TPNH and intermediate oxidant. But what I am saying now is that it appears that all three of these things can be produced equivalently at the same time.

The apparatus which does this in the plant (or which can do this) we saw in three magnifications -- the whole chloroplasts in the cells, the chloroplasts outside the cells and, finally, the lamellar structure of the chloroplasts as seen by electron microscopy. Out of this lamellar structure (out of the electron microscopy I might say) there has come, it seems to me, one particular conclusion which is sufficiently general to be stated; namely, that these chloroplast lamellae seem to be (no matter what plant cell is investigated) disc-like in character. They seem to be connected at the edges to form a hollow disc; this is the lamella. The lamellae are quite long; about 2000 Å in spinach chloroplasts. The lamellae do not appear in the chloroplast in the absence of chlorophyll or protochlorophyll. If one in some way prevents either the formation of protochlorophyll or of chlorophyll, one prevents the appearance of well developed lamellae. Protochlorophyll alone will induce in cells which are normally capable of making them, structures which look like these lamellae.

Now we shall discuss the possible function of this lamellar structure of the chloroplasts. The basic problem of photosynthesis is now reduced to the problem of converting a 35-40 Kcal quantum into some chemical potential. In order to do this, you presumably have to find a reaction, let us say, which will take up 35 Kcal at

one time. The products of this reaction must not back-react. Remember now, that it they do, you'll get back 35 Kcal so there's a great driving force for the back reaction. But there must be some mechanism provided in the apparatus to prevent this back reaction.

There are a number of other difficult requirements which must be fulfilled in this quantum conversion process with respect to the time constant that are involved. For example, following the absorption of the quantum, there must be a very efficient way in which that excited state of chlorophyll can be converted into long-lived chemical potential very quickly because of the efficiency of the overall process, regardless of whether you believe the maximum efficiency to be 30% or 60%. There must be some way in which this energy conversion can be accomplished.

There are a number of approaches to this problem which are based upon ordinary statistical solution photochemistry; I, myself, sometimes approached it in this way. I was looking for reactions of chlorophyll that were unique to chlorophyll that might conceivably be used to store this 35 Kcal of energy, such a reaction, let us say, as the reduction of chlorophyll to bacteriochlorophyll (that is, adding two more hydrogens to the chlorophyll molecule). Perhaps we can do it the other way around, by taking off two hydrogens from the chlorophyll molecule and making protochlorophyll and hanging the hydrogen atoms onto something else. These are possible reactions of chlorophyll.

As a matter of fact, our laboratory demonstrated both these reactions some years ago. More recently, and more elegantly, they have been demonstrated by Krasnovskii in systems that are more nearly related to those which one finds in the living organism.

As a result of a variety of requirements and as a result of the recognition of this highly organized apparatus in which chlorophyll occurs in the chloroplast, and as a result of the failure to solve the problem with solution photochemistry, I have turned (in our group at least) to the notions of cooperative phenomena of organized systems such as those which are represented by barrier layer cells in physics. I am trying to visualize how a lamellar structure such as this might conceivably be an unsymmetrical layer in which one could generate, by the absorption of light, an oxidant and a reductant, on opposite sides of the layer, so they couldn't back-react easily and could persist for a long period of time. These substances (reductant and oxidant) should live long enough, by chemical standards, to be efficiently taken up by electron acceptors, on the one hand, to go on to make the reduced pyridine nucleotide, and by electron donors, on the other, to make molecular oxygen. (I don't want to take any more time discussing the lamellar structure in chloroplasts because I think Dr. Hodge will give a more detailed description of that, and further discussion of the possible molecular structure of the lamella may be deferred until then. See also proceedings of the Brookhaven National Laboratory Symposium, June 1958.) I simply want to bring out here a proposal which fulfills all of the requirements of molecular interactions which we must have together with the need for conductivity (electrical conductivity) in certain parts of the lamellae and consequent separation of charges.

The basic notion that I want to introduce is really given in Fig. 20. I will then point out how it works on this lamellar array. Figure 20 suggests the basic notion of how these lamellae achieve this energy conversion. Chlorophyll in the ground state absorbs light which brings it to its lowest singlet excited state. This excited state can move around amongst the chlorophyll molecules by resonance transfer

(exciton migration) until a point is reached where ionization occurs. Then charge separation can occur. The exciton can be visualized as a charge-pair which cannot move separately -- a positive charge and a negative charge which must move together. (This would be another way of naming or describing an exciton.) When a suitable point in the chlorophyll lattice is reached, where the charges can be uncoupled so they can move separately, we have a conduction band. The electrons can move in one direction and the holes, or positive charges, can move in another. The electrons and holes move around until they find suitable places of lower potential energy into which they fall, and there they can sit for times long enough so that suitable chemicals can come up and take off electrons, on the one hand, and the positive holes, on the other, leading to chemical reactions which then produce stable chemicals such as pyridine nucleotide and perhaps hydrogen peroxide, or something else of that sort, ultimately going on to the final products.

With this concept, let's see how the structure of the lamella may be interpreted in terms of the molecular constitution. I am suggesting that this layer is made up of at least four components (see Fig. 21b). The protein enzymes, which are the ones involved in carbon dioxide reduction, are on the outside of the disc. The proteins on the inside of the disc would contain the enzymes involved in oxygen evolution. The separation of the two processes (carbon dioxide reduction and oxygen evolution) would be achieved by a layer of chlorophyll packed in the characteristic aromatic way. (This can take a lot of discussion all by itself. Perhaps we will have time to do it later.) This is a very characteristic pattern of pacing which was very briefly mentioned the other day. The aromatic rings do not pile flat-on themselves; they lie at an angle, approximately 45° to the stacking axis. This type of packing for chlorophyll is what I am suggesting here.

Figure 21a represents chlorophyll molecules tipped this way. Packed between them are carotenoids and the phospholipids. (I would like to add that chlorophyll is photoconductive, but that is beside the point.) The proposal is that after absorption, the exciton can migrate around amongst a few of these chlorophyll molecules to find a suitable point of ionization where the electrons may move in one direction and the positive holes in the other. Thus one side will lead to oxygen production and the other to the reduction of carbon.

What kind of experimental evidence can we think of which might detect this kind of a system? Well, we can't actually put electrodes on these lamellae; they are too small. But there is one part of this scheme which is susceptible to experimental observation, namely, the trapped holes and trapped electrons. These trapped electrons would be single, trapped electrons and would be detectably by paramagnetism. We have set out to search for photoinduced paramagnetism in the chloroplasts. Figure 22 will show the results of that search. This is an illustration of electron spin resonance signals for illuminated whole spinach chloroplasts at 25° and at -150°. (Similar signals, at least at room temperature, were reported from the St. Louis laboratory by Townsend.) The fact that we can get the signals at -150°, either in chloroplasts or in algae, indicates that their production is not an enzymatic process.

The next question that may be asked is how fast can the signals be produced at -150° compared to 25°? This is shown in Fig. 23. Here you see the situation at 25°, and when the lights are turned on this is as fast as the instrument will respond. The signals grow faster than we can follow them. At -150° the signals grow just as fast. The difference lies in the rate of decay of the signals. They have a complex decay -- partly rapid decay and partly slow decay. At room temperature, the decay is rather rapid. At -150° there may be a rapid decay, but most of it is slow. This

eliminates the possibility of the signals resulting from enzymatic formation, at least. The question remains could the signal result from a triplet state, that is, a paramagnetic excited 'chlorophyll', or could the signal be the result of a photo-dissociation of chlorophyll, or something very closely associated with chloro-phyll, to form chemical radicals, which process can take place at -150°? The answer to that will have to wait until the next lecture.

LEGENDS

Fig. 1. Elementary photosynthesis scheme.

Fig. 2. Photomicrograph of Chlorella cells.

Fig. 3. Chromatogram of extract from algae indicating uptake of radiocarbon during photosynthesis (60 seconds).

Fig. 4. Chromatogram of extract from algae indicating uptake of radiocarbon during photosynthesis (10 seconds).

Fig. 5. Path of carbon from CO_2 to hexose during photosynthesis.

Fig. 6. Distribution of radioactive carbon in certain sugars.

Fig. 7. Formation of a heptose from triose and hexose.

Fig. 8. Proposed scheme for labeling of pentose.

Fig. 9. Light-dark changes in concentrations of phosphoglyceric acid and ribulose diphosphate.

Fig. 10. Formation of phosphoglyceric acid from ribulose diphosphate.

Fig. 11. Transients in the regenerative cycle.

Fig. 12. The photosynthetic carbon cycle.

Fig. 13. Mechanism of carboxylation reaction.

Fig. 14. Chromatogram of extract of Chlorella after 3 minutes of photosynthesis in the presence of radiocarbon. The solvents were allowed to run for 48 hours in each dimension.

Fig. 15. Unknown sugar phosphate after treatment with acid phosphatase.

Fig. 16. Suggested cyclic scheme for relationships in photosynthesis.

Fig. 17. Photomicrograph of chloroplasts from liverwort.

Fig. 18. Photomicrograph of spinach chloroplasts.

Fig. 19. Ultrastructure of chloroplasts.

Fig. 20. Proposed scheme for various photochemical processes in photosynthesis.

Fig. 21. Schematic representation of possible molecular structure for a lamella.

Fig. 22. Light signals from whole spinach chloroplasts.

Fig. 23. Signal growth and decay time curves of whole spinach chloroplasts at $25°$ and $-150°$.

$$O_2 \qquad \{CH_2O\}$$

$$\uparrow$$

$$C \qquad \uparrow Z$$

$$\uparrow$$

$$B \qquad [H] \qquad Y \rightarrow H_2O$$

$$A \qquad \uparrow X$$

LIGHT ENERGY

$$2H_2O \qquad CO_2$$

MU-11418-A

Fig. 1

ZN-1750

Fig. 2.

ALANINE

PHOSPHOENOLPYRUVATE

PHOSPHOGLYCOLATE

PHOSPHOGLYCERATE

DIHYDROXYACETONE PHOSPHATE

RIBULOSE PHOSPHATE

RIBOSE PHOSPHATE

FRUCTOSE PHOSPHATE & MANNOSE PHOSPHATE

GLUCOSE PHOSPHATE & SEDOHEPTULOSE PHOSPHATE

NUCLEOTIDES

60 SEC. PS CHLORELLA

RIBULOSE DIPHOSPHATE & HEXOSE DIPHOSPHATE

ZN357

Fig. 3.

PHOSPHOENOLPYRUVATE

DIHYDROXYACETONE PHOSPHATE

PHOSPHOGLYCERATE

RIBULOSE PHOSPHATE

RIBOSE PHOSPHATE

FRUCTOSE PHOSPHATE & MANNOSE PHOSPHATE

GLUCOSE PHOSPHATE & SEDOHEPTULOSE PHOSPHATE

RIBULOSE DIPHOSPHATE & HEXOSE DIPHOSPHATE

IO SEC. PS SCENEDESMUS

ZN373

Fig. 4.

PGA

$CH_2O\,\textcircled{P}$
|
$CHOH$ $\xrightarrow{2\,[H]}$
|
$*COOH$

DHAP

$CH_2O\,\textcircled{P}$ $CH_2O\,\textcircled{P}$
| |
$CHOH$ $\rightleftharpoons$ $C=O$
| |
$*CHO$ $*CH_2OH$

$*CO_2$

C_2

?

$CH_2O\,\textcircled{P}$
|
$C=O$
|
$*CHOH$ FDP
|
$*CHOH$
|
$CHOH$
|
$CH_2O\,\textcircled{P}$

MU-15628

Fig. 5.

Fig. 6.

MU-6665-A

$$\begin{array}{c} CHO^* \\ | \\ HCOH \\ | \\ H_2CO\text{-}\textcircled{P} \end{array} \quad + \quad \begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ HOCH^* \\ | \\ HCOH^* \\ | \\ HCOH \\ | \\ H_2CO\text{-}\textcircled{P} \end{array} \quad \xrightarrow{\substack{trans-\\ketolase}} \quad \begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ HOCH^* \\ | \\ HCOH \\ | \\ H_2CO\text{-}\textcircled{P} \end{array} \quad + \quad \begin{array}{c} CHO^* \\ | \\ HCOH^* \\ | \\ HCOH \\ | \\ H_2CO\text{-}\textcircled{P} \end{array}$$

$$\begin{array}{c} H_2CO\text{-}\textcircled{P} \\ | \\ C=O \\ | \\ H_2COH^* \end{array} \quad + \quad \begin{array}{c} CHO^* \\ | \\ HCOH^* \\ | \\ HCOH \\ | \\ H_2CO\text{-}\textcircled{P} \end{array} \quad \xrightarrow{aldolase} \quad \begin{array}{c} H_2CO\text{-}\textcircled{P} \\ | \\ C=O \\ | \\ HOCH^* \\ | \\ HCOH^* \\ | \\ HCOH^* \\ | \\ HCOH \\ | \\ H_2CO\text{-}\textcircled{P} \end{array}$$

MU-7275

Fig. 7.

$$
\begin{array}{l}
\text{CH}_2\text{OH} \\
\text{C=O} \\
\text{HOCH}^* \\
\text{HCOH}^* \\
\text{HCOH}^* \\
\text{HCOH} \\
\text{H}_2\text{CO-}\text{\textcircled{P}}
\end{array}
+
\begin{array}{l}
\text{CHO}^{**} \\
\text{HCOH} \\
\text{H}_2\text{CO-}\text{\textcircled{P}}
\end{array}
\xrightarrow[\text{ketolase}]{\text{trans-}}
\begin{array}{l}
\text{CH}_2\text{OH} \\
\text{C=O} \\
\text{HOCH}^{**} \\
\text{HCOH} \\
\text{H}_2\text{CO-}\text{\textcircled{P}}
\end{array}
+
\begin{array}{l}
\text{CHO}^* \\
\text{HCOH}^* \\
\text{HCOH}^* \\
\text{HCOH} \\
\text{CH}_2\text{O-}\text{\textcircled{P}}
\end{array}\Big\}
\begin{array}{l}
\text{C}^* \\
\text{C}^* \\
\text{C}^{***} \\
\text{C} \\
\text{C}
\end{array}
$$

SMP   Phospho-
glyceraldehyde   Xylulose
Monophosphate   Ribose
Monophosphate

MU-6664

Fig. 8.

Fig. 9.

$$RuDP \xrightarrow{\quad CO_2 \quad} PGA \longrightarrow Triose\ phosphate$$

Light

RuMP ⟵ B ⟵ A ⟵

Sugar rearrangements

MU-6660-A

Fig. 10.

TRANSIENTS IN THE REGENERATIVE CYCLE

MU-7422

Fig. 11.

Fig. 12.

RuDP
(Ene-diol form)   Intermediate   3-PGA
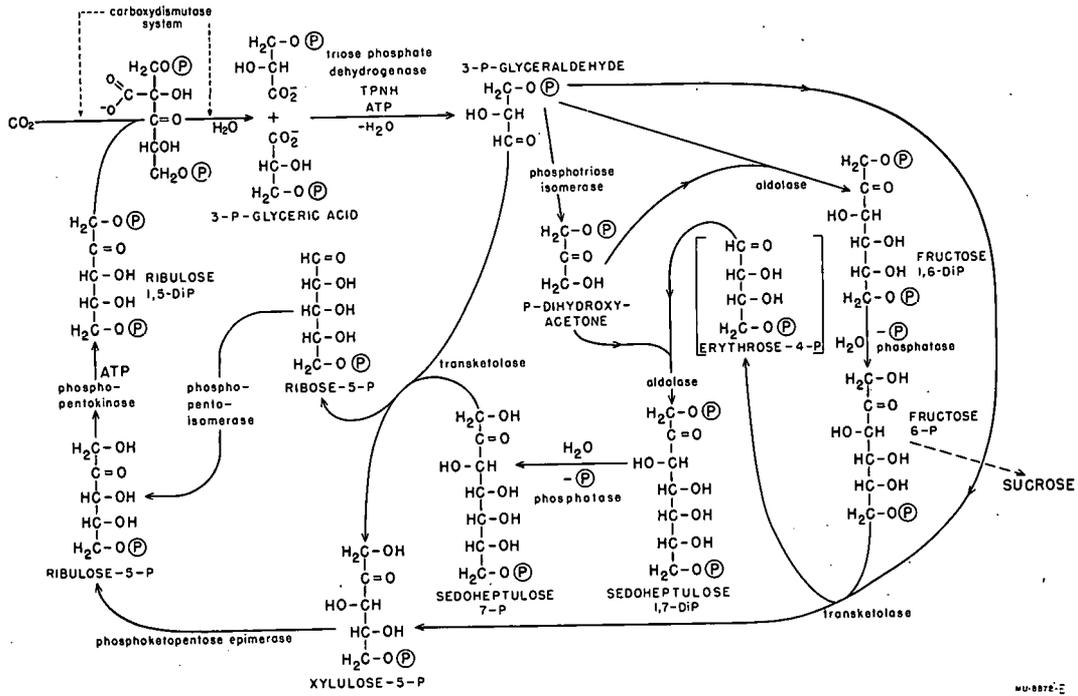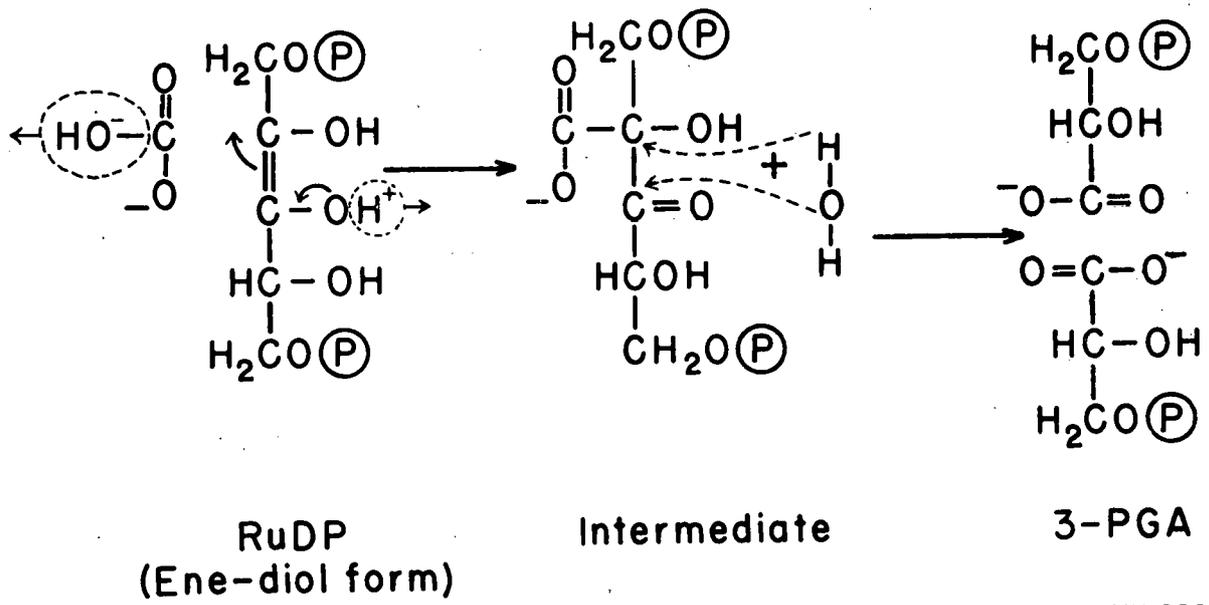
MU-9288

Fig. 13.

ZN-1865

Fig. 14.

SUSPECTED β-KETO ACID LACTONE

RIBULOSE

BUTANOL-
PROPIONIC ACID-
WATER
(8 HOURS)

II

SUSPECTED
γ-KETO ACID

UNKNOWN DIPHOSPHATE
AFTER TREATMENT WITH
ACID PHOSPHATASE

PHENOL-WATER (8 HOURS) ← I
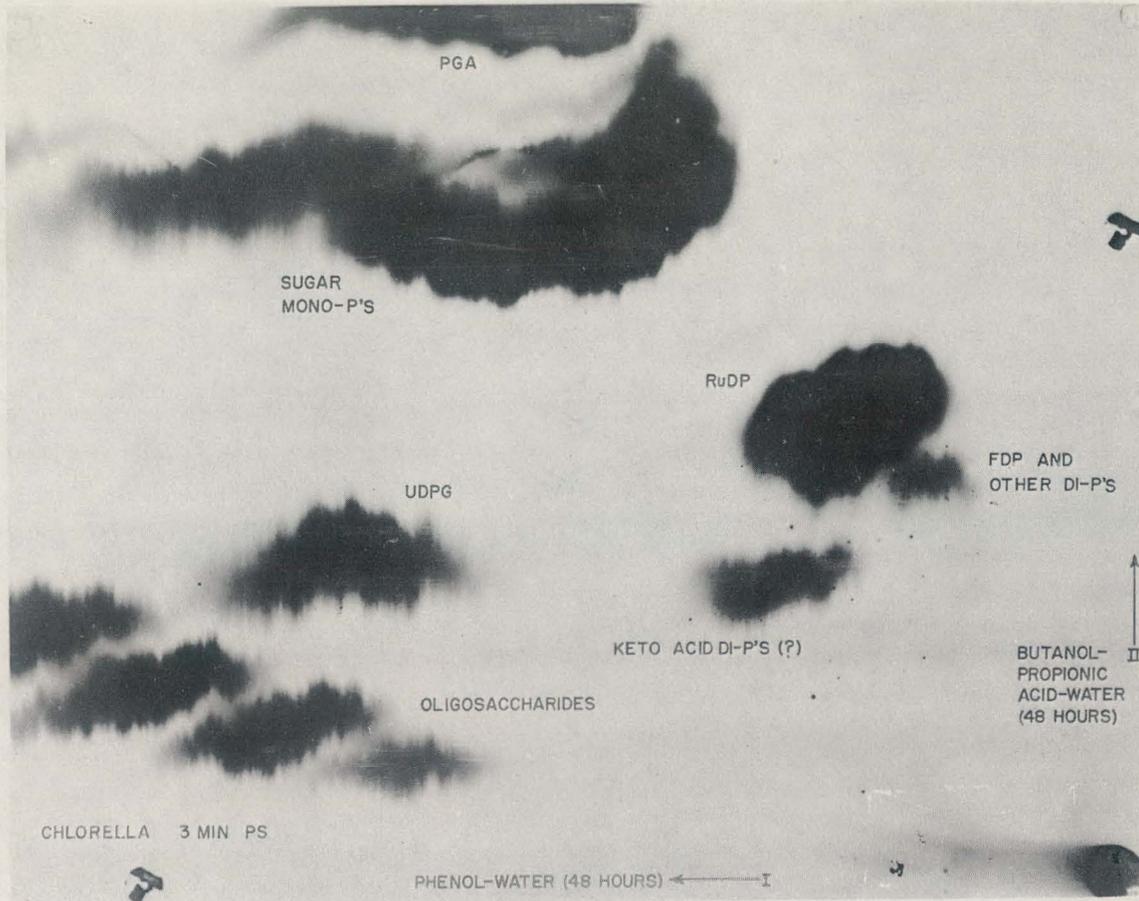
ZN-1866
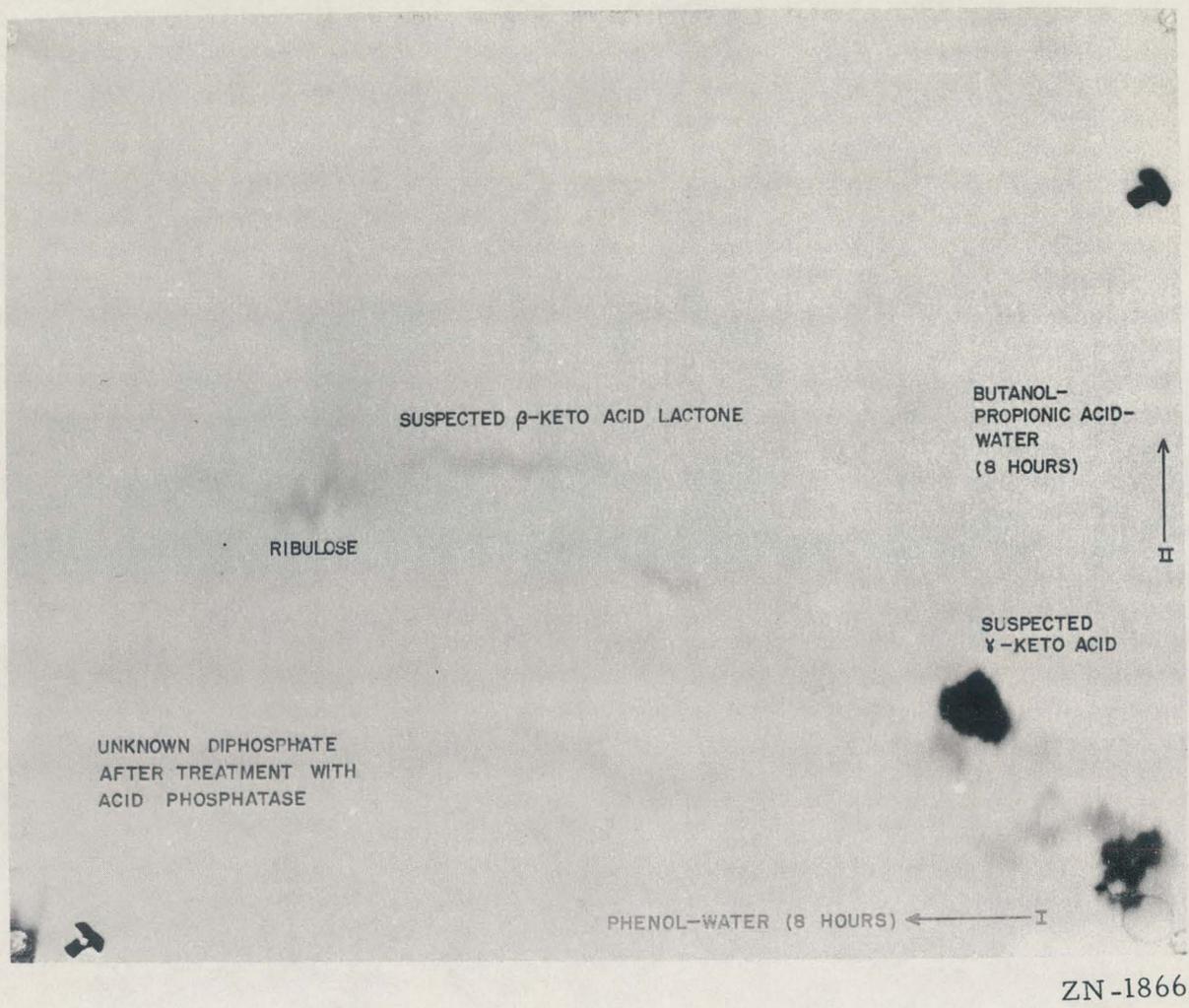
Fig. 15.
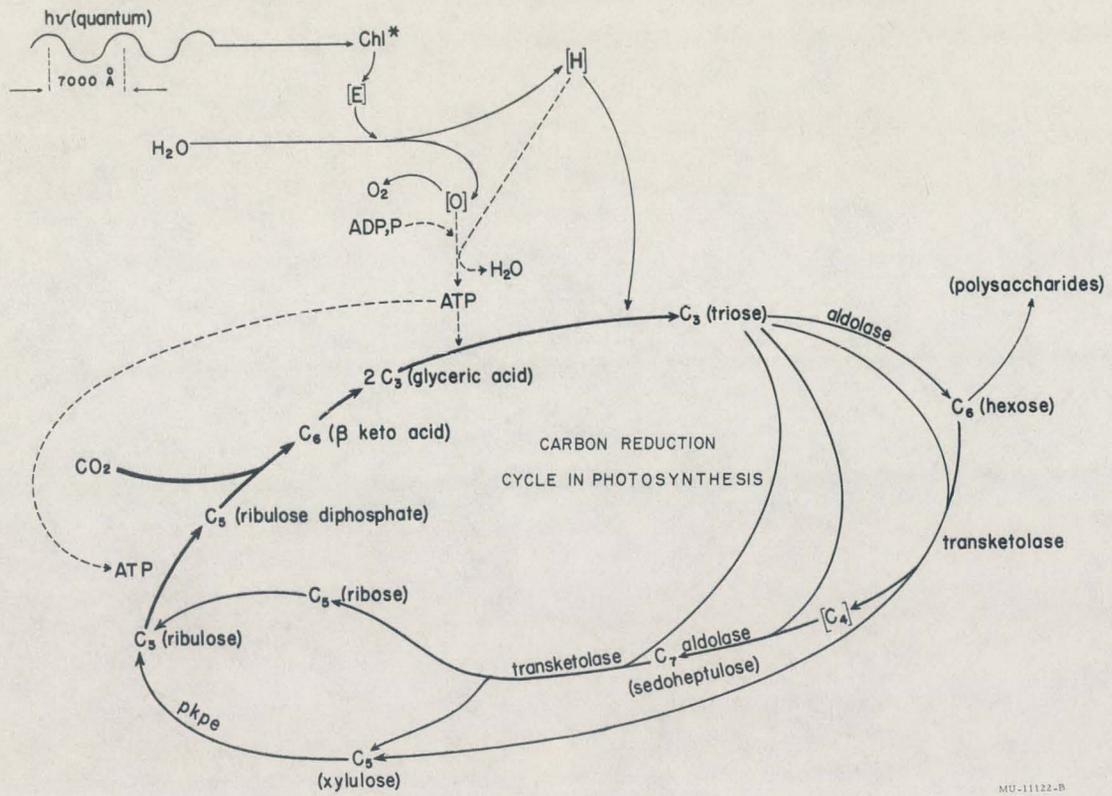
Fig. 16.

ZN-2071

Fig. 17.

40 μ
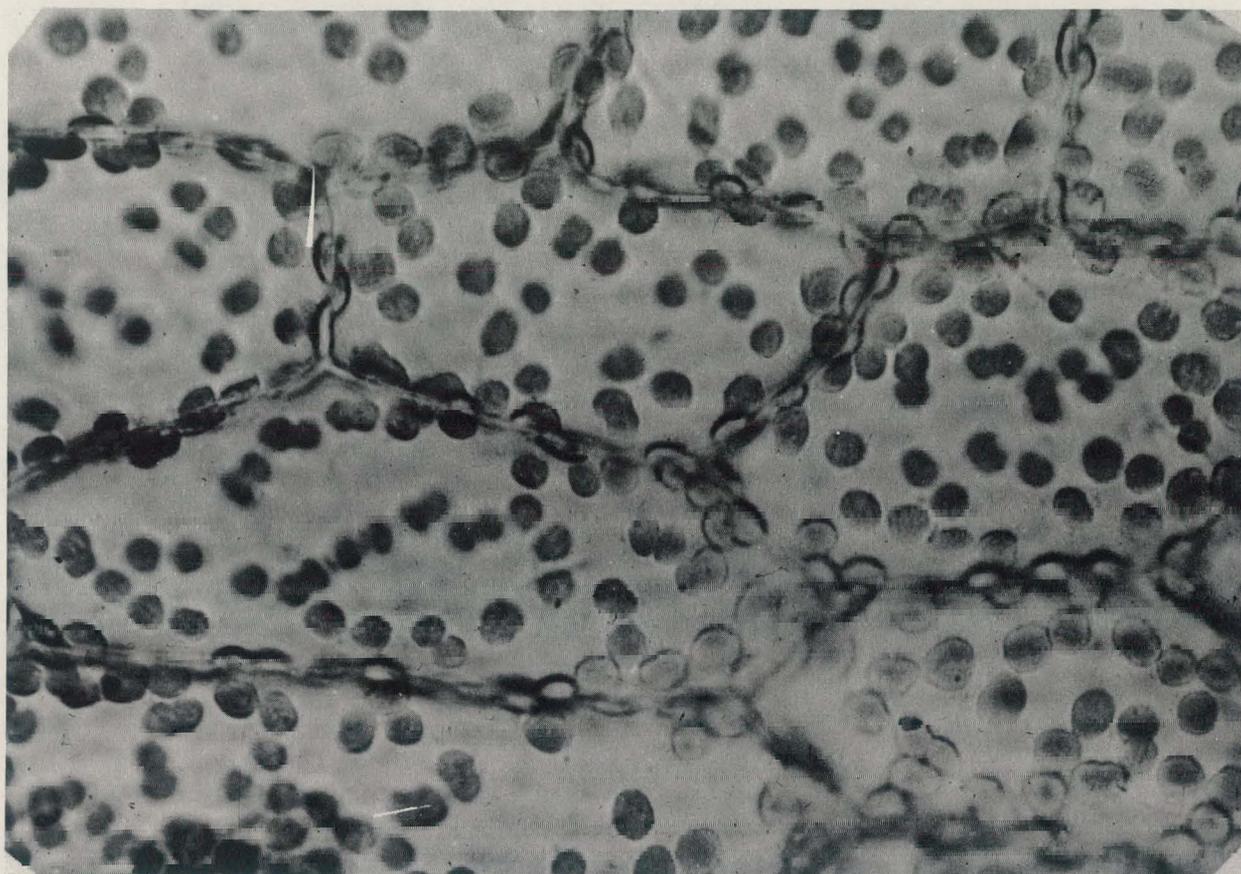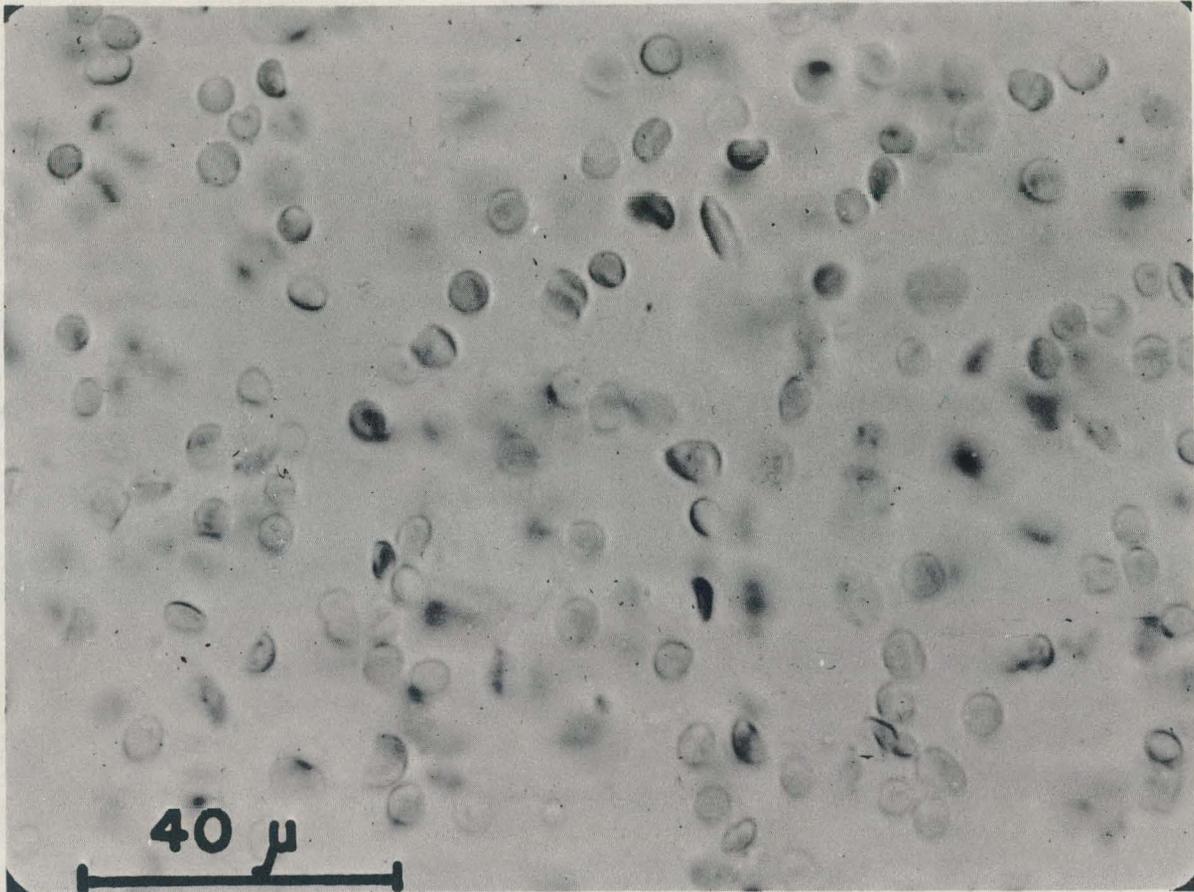
ZN-2072

Fig. 18
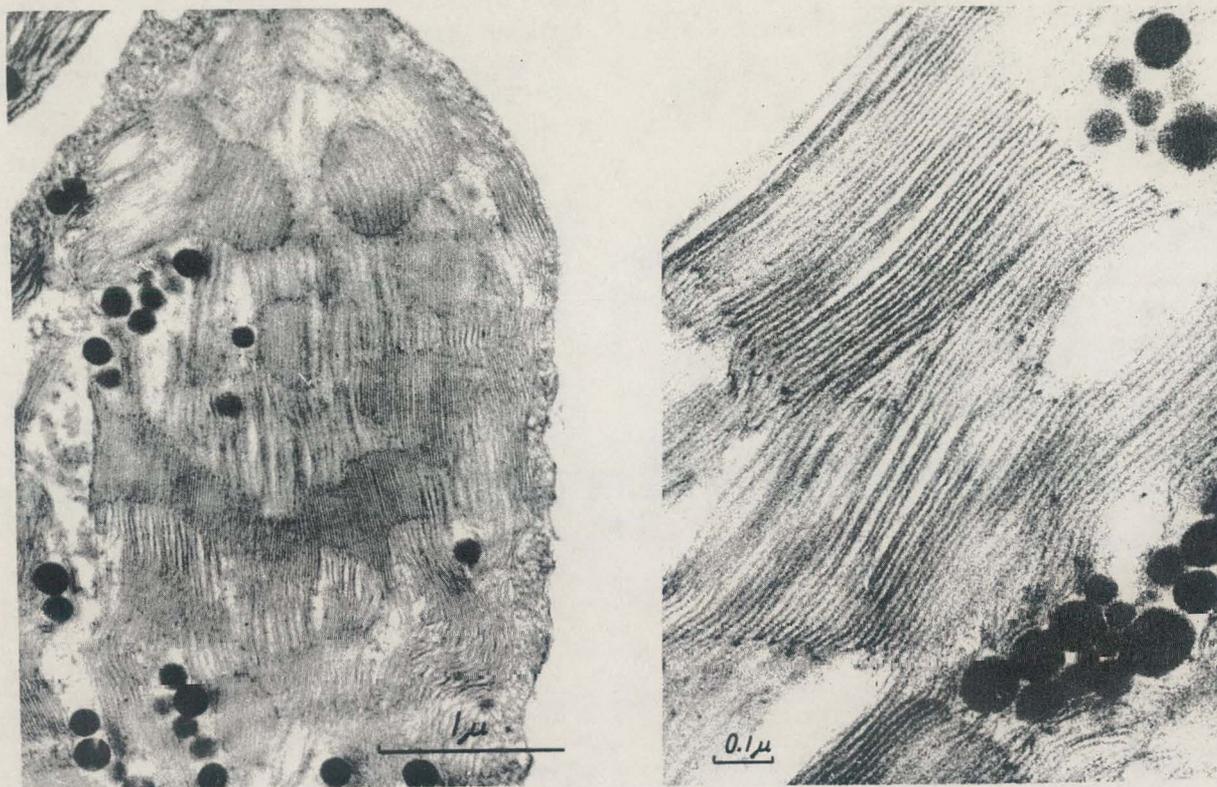
STEINMAN and SJÖSTRAND

ZN-1749

Fig. 19.
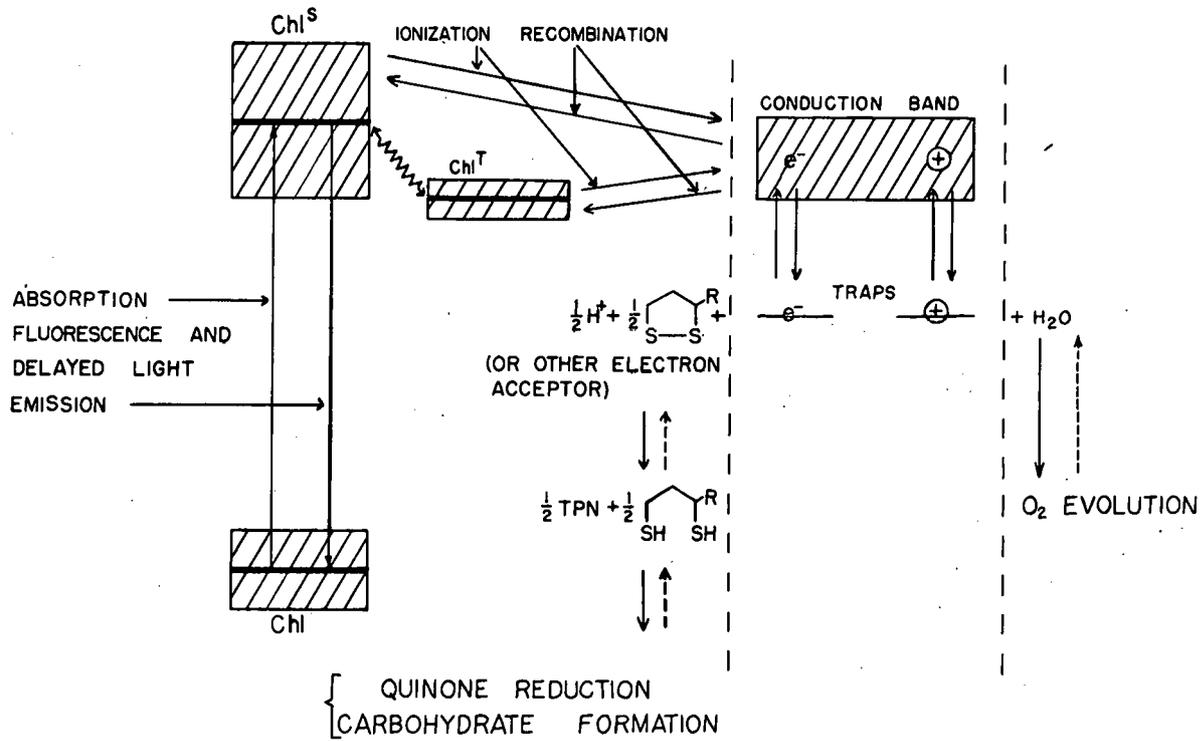
PROPOSED SCHEME FOR VARIOUS PHOTOCHEMICAL PROCESSES IN PHOTOSYNTHESIS

MU-14256-C

Fig. 20.
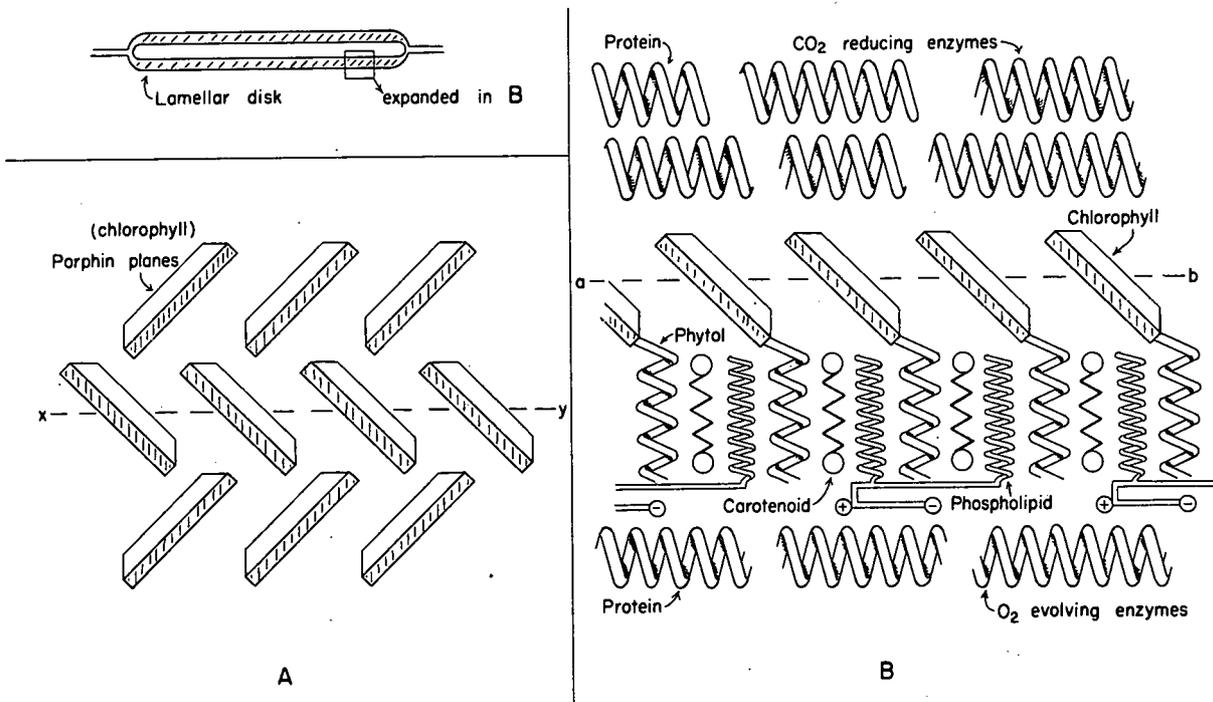
Fig. 21.
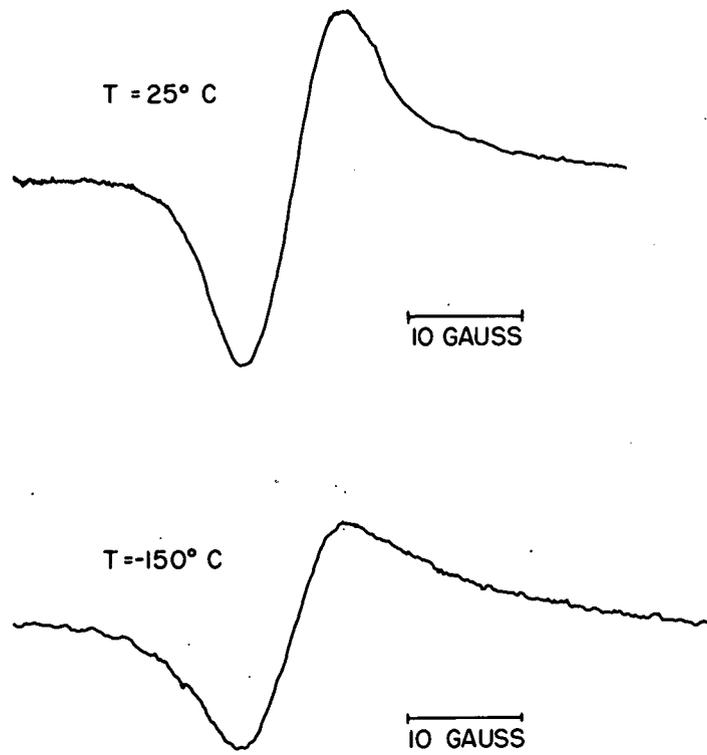
LIGHT SIGNALS FROM WHOLE SPINACH CHLOROPLASTS

MU-14534

Fig. 22.

T = 25°C

30 SEC

LIGHT ON   LIGHT OFF

T = −150°C

LIGHT ON   LIGHT OFF   30 SEC

WHOLE SPINACH CHLOROPLASTS

MU-14535

Fig. 23.