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PRIMARY QUANTUM CONVERSION IN PHOTOSYNTHESIS

Berkeley, California

DISCLAIMER

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PRIMARY QUANTUM CONVERSION IN PHOTOSYNTHESIS

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ABSTRACT

The primary quantum conversion act in photosynthesis is defined as that event during which electronic excitation is converted into chemical species which are separable and which may be isolable. As yet no unambiguous assignment for such an event has been made.

There appear to be two major alternative approaches. The first is that a molecular event occurs leading directly to a chemical species from the excited molecule. While a great deal of work has been done over the years toward this end, no assignment of this sort has yet been achieved and work from this point of view continues. The second approach, involving the transfer of an electron from one molecule to another as the primary event, gives rise to a one-electron oxidant and a one-electron reductant in two different sites physically separated from each other as the primary chemical storage products. It is this latter point of view which is now being most actively sustained and it is the evidence for this viewpoint which is examined in the present paper. While a good many different kinds of evidence have been brought to bear, we will discuss only two, namely, that derived from the optical changes induced in the photosynthetic apparatus by actinic light and the magnetic changes induced by the actinic light.

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* National Science Foundation Predoctoral Fellow, 1961-64.

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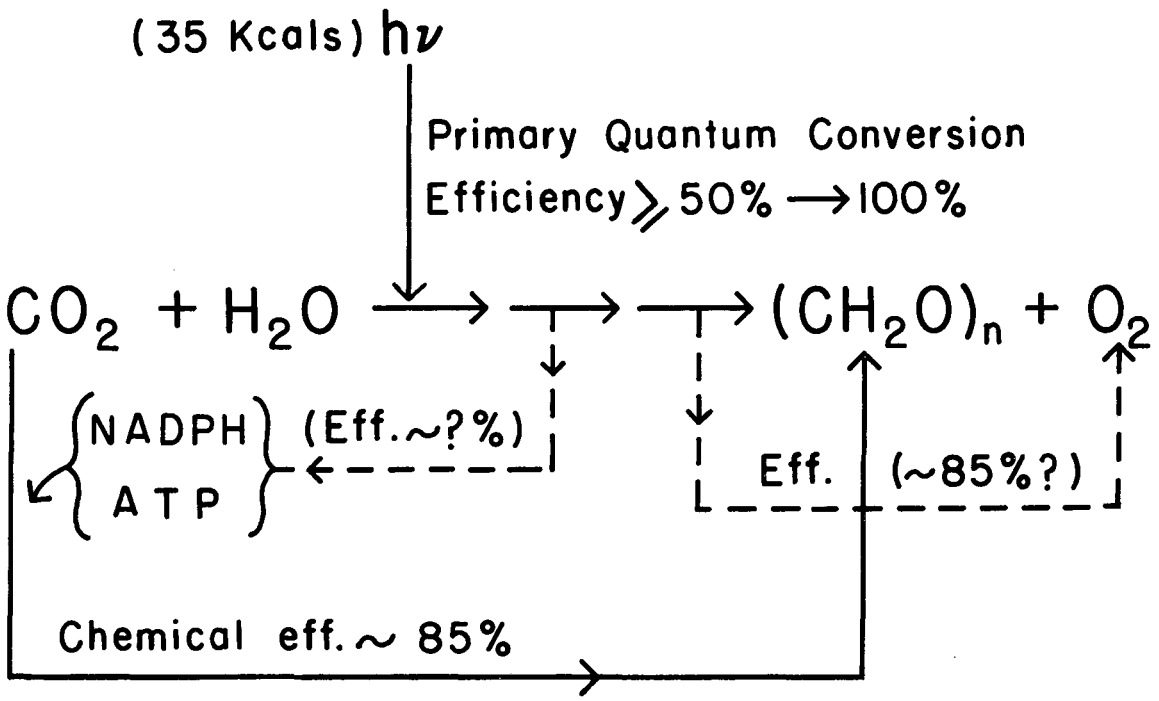
An examination of the variety of these changes and the kinetics of their formation and disappearance, as well as the dependence of these kinetics on physical and chemical environmental variables, leads to the supposition that the sequence of events is: absorption of light; migration of the exciton to the point of electron transfer; electron transfer producing a trapped electron and mobile hole, or, conversely, a trapped hole and mobile electron; followed by migration of the mobile particle to another point in the photosynthetic apparatus to a corresponding trap of opposite type. The reactions succeeding these events, such as the formation of adenosine triphosphate and the reduction of pyridine nucleotide, are dark enzymatic chemical reactions, some of which have been separately achieved.

INTRODUCTION

The overall process of photosynthesis in green plants is simply represented by Chart I. The energy stored in this process is of the order of 110 kcal per mole of oxygen liberated. The question before us is how the energy of the quantum, which is of the order of 7000 Å (35 kcal per quantum) is stored in chemical form. The maximum efficiency with which this reaction can be carried out is quite high. The measurements vary from lower than 30% to as high as 80%.

We know a good deal about the chemical reactions involved in going from carbon dioxide to carbohydrates. Our knowledge of the path of carbon (4) and the efficiency of the various steps involved in passing from the carbon dioxide to the carbohydrates, indicates that the efficiency of this chemical conversion is not more than 85%, beginning with the agents which are required to carry this out, i.e., carbon dioxide, reduced pyridine nucleotide as well as some selected number of collaborative energy sources. Thus, starting with CO_2 and these energy-rich molecules, the efficiency is of ther order of 85%. The efficiency of the chemical reactions from the primary photoproducts to the energy-rich molecules used in this reaction (NADPH and ATP) is not known, but is presumed to be of the same order of magnitude. A corresponding uncertainty exists about the nature and efficiency of the chemical reactions leading from the primary photoproducts to molecular oxygen. If we take this also to be 85%, the chemical efficiency following primary quantum conversion would amount to 60%.



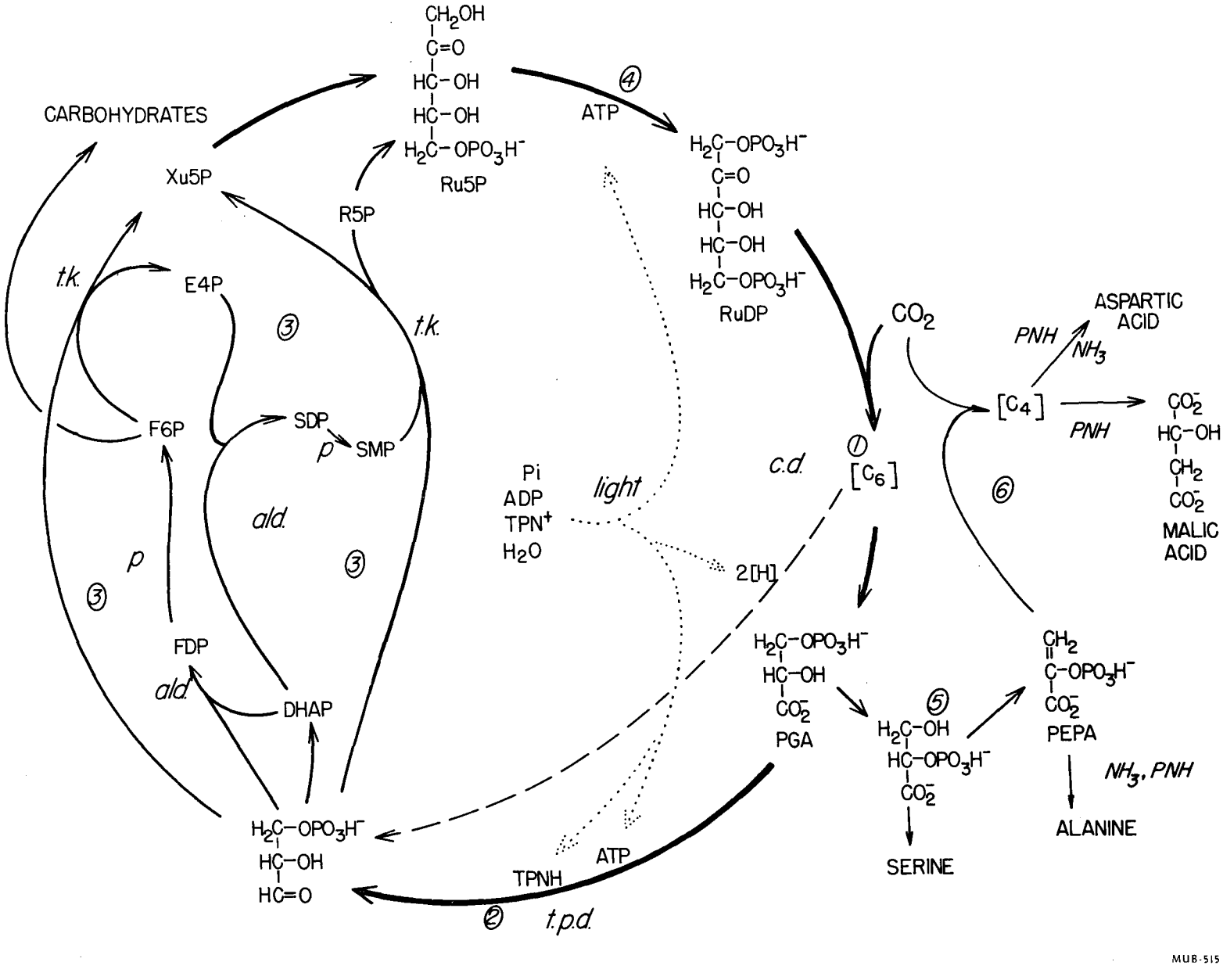
$\Delta F = +110$ Kcals/mole O_2

Overall efficiency measured between 30% and 80%

MUB-2966

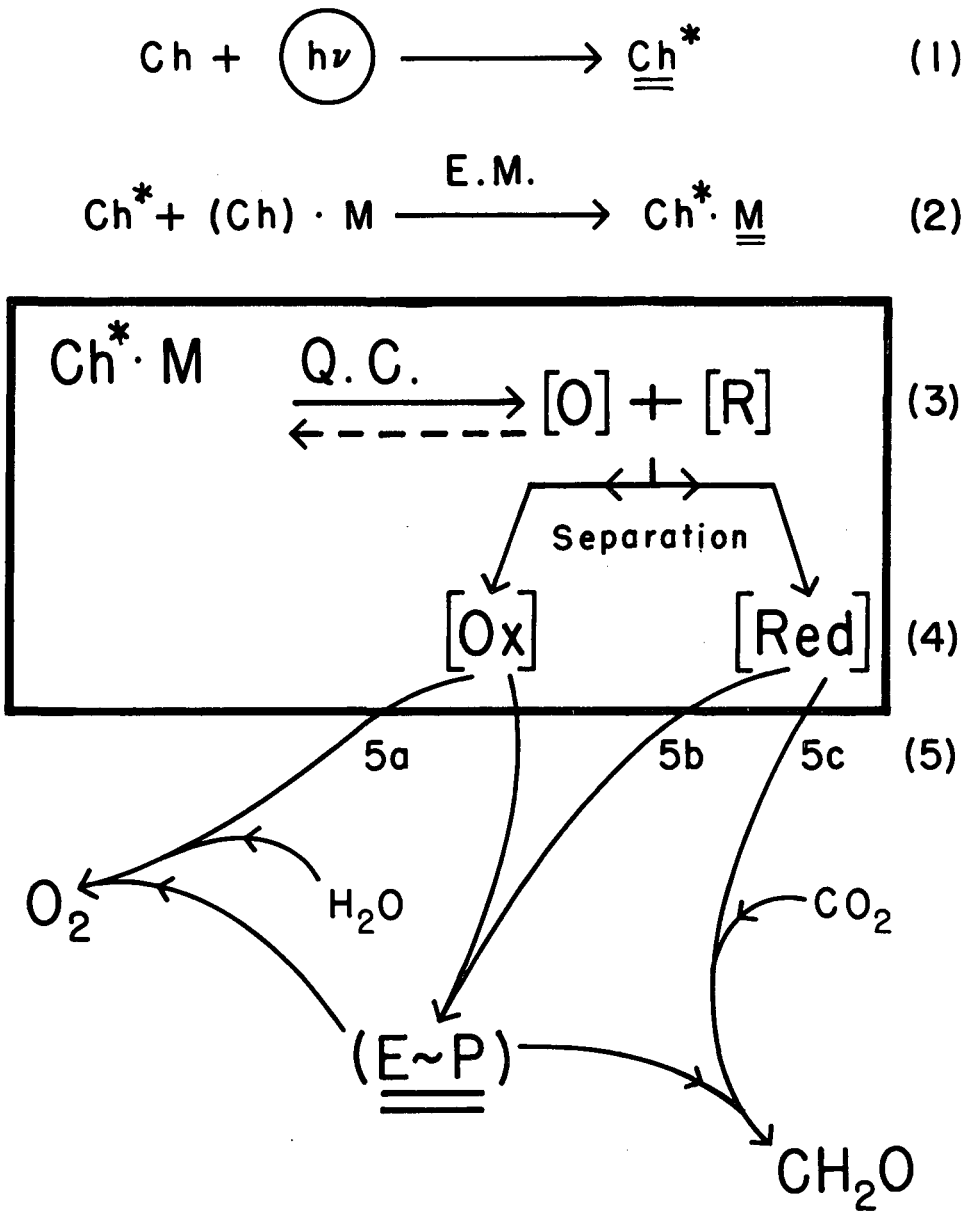
Thus if the overall efficiency of the raction may be as low as 30%, the efficiency of the primary quantum conversion cannot be less than 50%. If we allow the maximum overall efficiency to be any higher, then the quantum conversion act will have to be correspondingly greater, reaching more than 90% efficiency, particularly if we allow anything much less than 100% efficiency for the chemical path from the primary photoproducts to molecular oxygen.

Before defining what we mean by the quantum conversion act, let us look at the series of reactions from CO_2 to carbohydrate which we know something about and point out where that quantum conversion act plays its role. FIGURE 1 shows this in a schematic fashion. The carbon dioxide enters, reacting with a sugar molecule, giving an intermediate. The light produces the reducing power and other high energy molecules which are required to run this carbon cycle. The 85% efficiency figure which I gave you was the efficiency figure for actually running the carbon cycle, using the reduced pyridine nucleotide and ATP to keep it going. (There is a postscript to this which I would like to mention: We now know a little more about the nature of this carboxydismutase reaction (5) and it seems to be a carboxylation of thiolenediol formed from a sulfhydryl group in the carboxydismutase enzyme and ribulose diphosphate.)


CARBOHYDRATES
Xu5P
Ru5P
R5P
E4P
F6P
SDP
SMP
FDP
DHAP
t.k.
t.k.
ald.
ald.
p
p
③
③
③
④
ATP
RuDP
CO2
①
[C6]
c.d.
light
Pi
ADP
TPN+
H2O
2[H]
PGA
⑤
SERINE
PEPA
NH3, PNH
ALANINE
PNH
NH3
ASPARTIC ACID
[C4]
PNH
⑥
MALIC ACID
TPNH
ATP
②
t.p.d.
MUB-515

PHOTOCHEMISTRY OF CHLOROPHYLL

Let us return now to the question of the nature of the light reaction insofar as we can break it down. The first thing that happens is that the chlorophyll, on which the photosynthesis depends, first absorbs a quantum to form an excited chlorophyll molecule (Reaction (1), FIGURE 2). It appears that all of the chlorophyll molecules in the plant are not actually sites at which the quantum conversion occurs, but the excitation of one chlorophyll molecule allows the migration of that exciton among other chlorophyll molecules to a particular chlorophyll molecule, or particular pigment, located somewhere in the photosynthetic apparatus and possibly associated with some other unknown molecule (M) (Reaction (2), FIGURE 2). This is a migration of exciton energy still in the form of electromagnetic energy -- electronic excitation only -- from one molecule into a pattern of molecules to some particular site. Then comes the point, having reached this particular species associated with a particular type of pigment, at which the reaction occurs which leads to a product which must eventually produce an oxidized and a reduced form (Reaction (3), FIGURE 2). The overall reaction with which we are dealing is an oxidation-reduction reaction -- the CO_2 is reduced, the water is oxidized, so we know that at some point in the energy conversion scheme there must be an intermediate oxidant and reductant produced, which will ultimately lead chemically to these two kinds of final products.



MUB-2967

Fig. 2

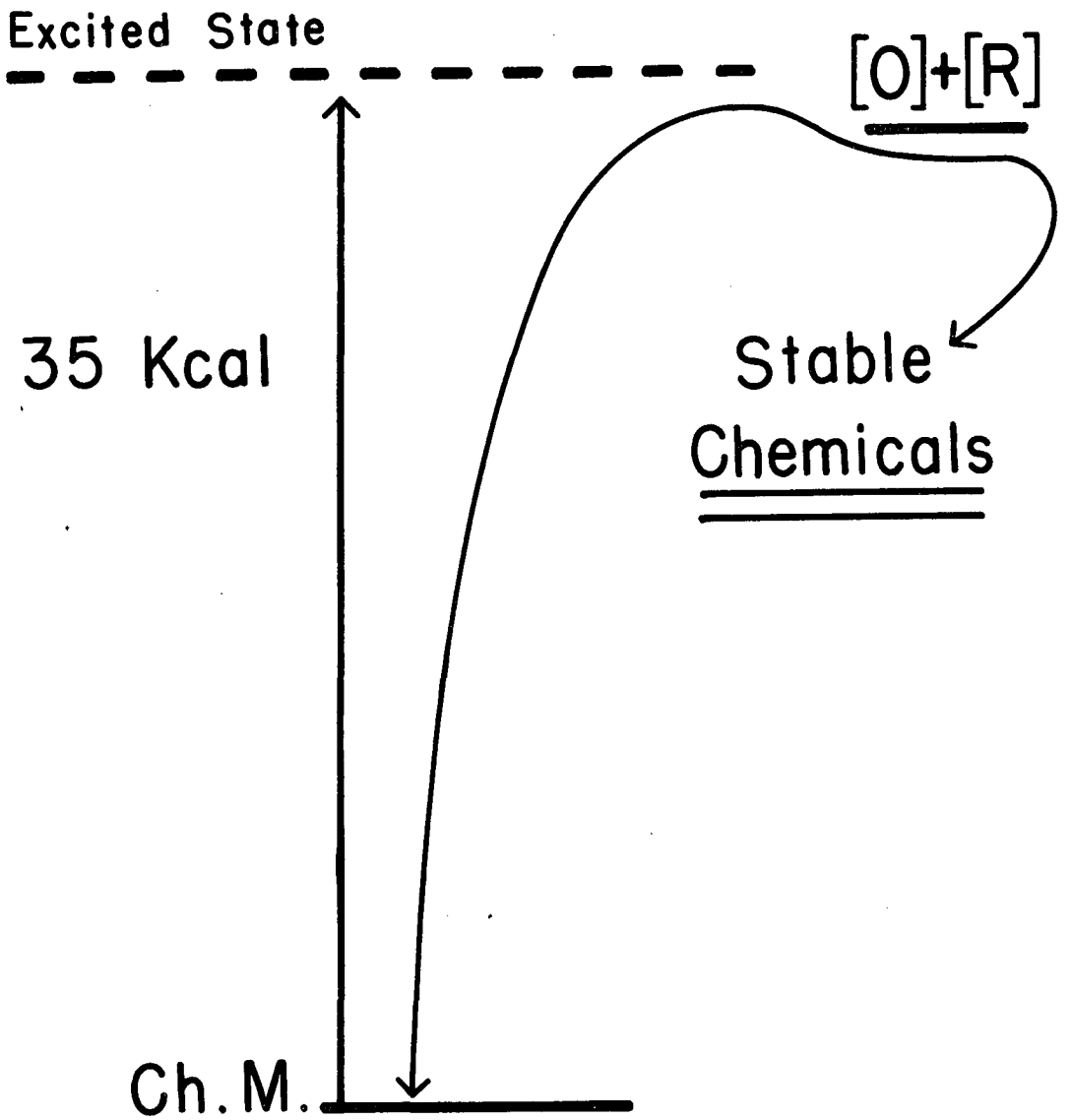
This is the beginning of the quantum conversion act, but it is not yet the whole act, because whatever these two or more species are, [O] and [R], they must be formed contiguous to each other, since they are formed from a single quantum. The next step in order to prevent the back reaction is the separation of these two, physically from each other (Reaction (4), FIGURE 2). This separation act is part of the primary quantum conversion act. From here on, the intermediate oxidant [O_x] undergoes a whole series of chemical transformations, leading ultimately to molecular oxygen (Reaction (5a), FIGURE 2). Water, of course, will be involved in this sequence at some point, and perhaps some additional energy may be introduced (possibly in the form of high energy phosphate). Similarly, the reduced species [Red] will go on and reduce carbon dioxide, and here, also, energy in the form of high energy phosphate will participate, giving carbohydrate (Reaction (5c), FIGURE 2). The high energy phosphate which we need for these reactions is acquired by a combination of the separated oxidant and reductant, giving energy-containing compounds, some in the form of ATP (Reaction 5b), FIGURE 2). The high energy containing compounds may play a role at various sites in the quantum conversion process. ATP may form during the passage of oxidant down in potential toward molecular oxygen. However, these steps remain to be determined. The only one we know anything about is the recombination reaction between the chemically separated intermediate oxidant and intermediate reductant.

The overall systems of Reactions (3) and (4) (as enclosed in FIGURE 2) is what we are now defining as the primary quantum conversion

act. You will note that what happens before that time is a physical transformation and transfer of energy, and what happens afterward is ordinary chemistry-biochemistry-enzymatic chemistry. It is in this region that we make the break between physical excitation and chemical species.

The high efficiency requirements for the primary quantum conversion act are for the following reasons: If we must convert the quantum with an efficiency even as high as 50%,(and maybe higher than 90%), it is clear that the initial products must have very nearly the same potential energy as the absorbed quantum -- it can only be less by the amount that the efficiency is less than 100%. If the energy content of the two products,(O + R, FIGURE 3) is very nearly the same as the energy of the excited state from which they are formed, there cannot be a potential energy barrier for their back reaction of any appreciable magnitude (FIGURE 3). This is really what the problem is. A very large bundle of energy -- 30 to 35 kcal -- is to be transformed into chemical species which we know to have a chemical potential to liberate very nearly thatsame amount of energy. Therefore, there cannot be a very high potential barrier for back reaction. If Reaction (3) FIGURE 2 is 90% efficient, then the barrier for back reaction cannot be more than 3 kcal (one-tenth of the total quantum). The alternative would be to allow a 15 kcal barrier to the back reaction, thus limiting the efficiency of the primary quantum conversion to 50% and the overall efficiency to less than 20%.

While many workers are willing to accept the requirement of at least 8 quanta per mole of oxygen produced, we ourselves (6) feel that we have demonstrated a long term requirement of less than 7, and perhaps as low



MUB-2968

Fig. 3

as 6, as have a number of others (7,8). There is one group which claims a long term requirement of 4 or less (9). It is our intention, therefore, to proceed here on the basis of a 90% efficiency requirement for the primary quantum conversion. Therefore the energy level of the [O] + [R] must not be more than 10% below the energy of the entire quantum (FIGURE 3). The quantum takes us up to a level, marked E.S. FIGURE 3, which is only 10% above the value of the potential energy of the products, if we are going to store 90% of the quantum energy. Therefore, the barrier over which the chemicals have to pass on the way back, down to the starting point, can be only of very small size.

There is no model in solution photochemistry that approaches this kind of an energy storage. There are many photochemical reactions known, but, in general, the energy of the products of the photochemical reactions is very low in relation to the quantum used to produce them. The quantum has 5-10 times the amount of energy that one can store in stable products, normally in ordinary solution photochemistry such as an isomerization reaction, tautomerization, dimerization, dissociation, etc. In ordinary photochemical reactions the products which can be separated, and the energy thus stored, is generally very small. In the quantum conversion act we must store 80-90% of the energy in "stable" chemical products.

We have not given up looking for chemical changes in the chlorophyll which might possibly be related to these stable chemical intermediates, but the tendency has been to seek ways and means in which this separation could occur other than in terms of solution photochemistry. The

principal types of raactions for which there are models for such a separation are electron transfer and electron migration reactions which can indeed achieve this kind of an energy storage. It appears that the evidence which is now accumulating is moving pretty definitely in this direction, although I must say it is not unequivocal.

For the present, we are going to discuss the evidence that an electron transfer reaction followed by electron-hole migration is the principal way in which the primary quantum conversion is achieved. The electron-hole migration separates the two primary products of the reaction, the formation of which is only part of the quantum conversion problem. We must not only get the intermediates, but they have to be separated so they don't back react in a random, nonproductive fashion; they should have the possibility of reacting under biochemically controlled conditions, so that useful products may result.

THE BIOLOGICAL PHOTOSYNTHETIC APPARATUS

What is the apparatus which performs the quantum conversion? Chlorophyll (FIGURE 4) is the principal energy-capturing agent, and I have already indicated to you that there is a good deal of chemistry being done on chlorophyll with the idea that it may be undergoing some kind of observable chemical change in which nuclei are moved. As yet no unequivocal evidence that such has indeed occurred in biological systems is at hand. The actual biological apparatus which performs the quantum conversion is in the chloroplasts, which contain in them a high degree of structure. FIGURE 5 shows the structure of one lamellar piece of a chloroplast,

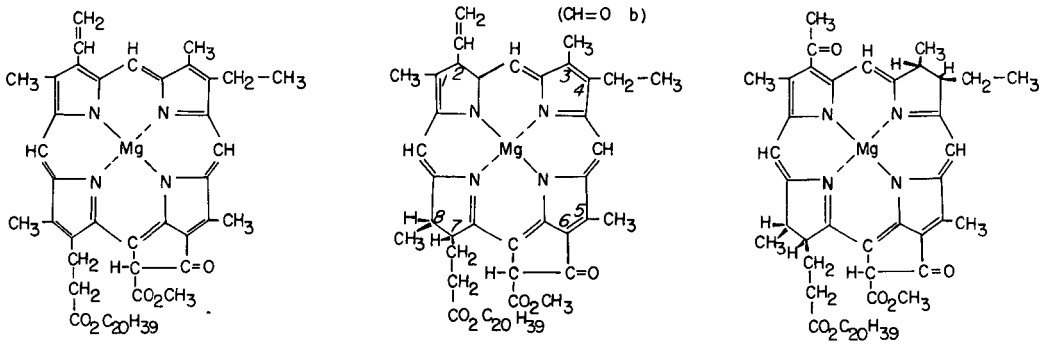
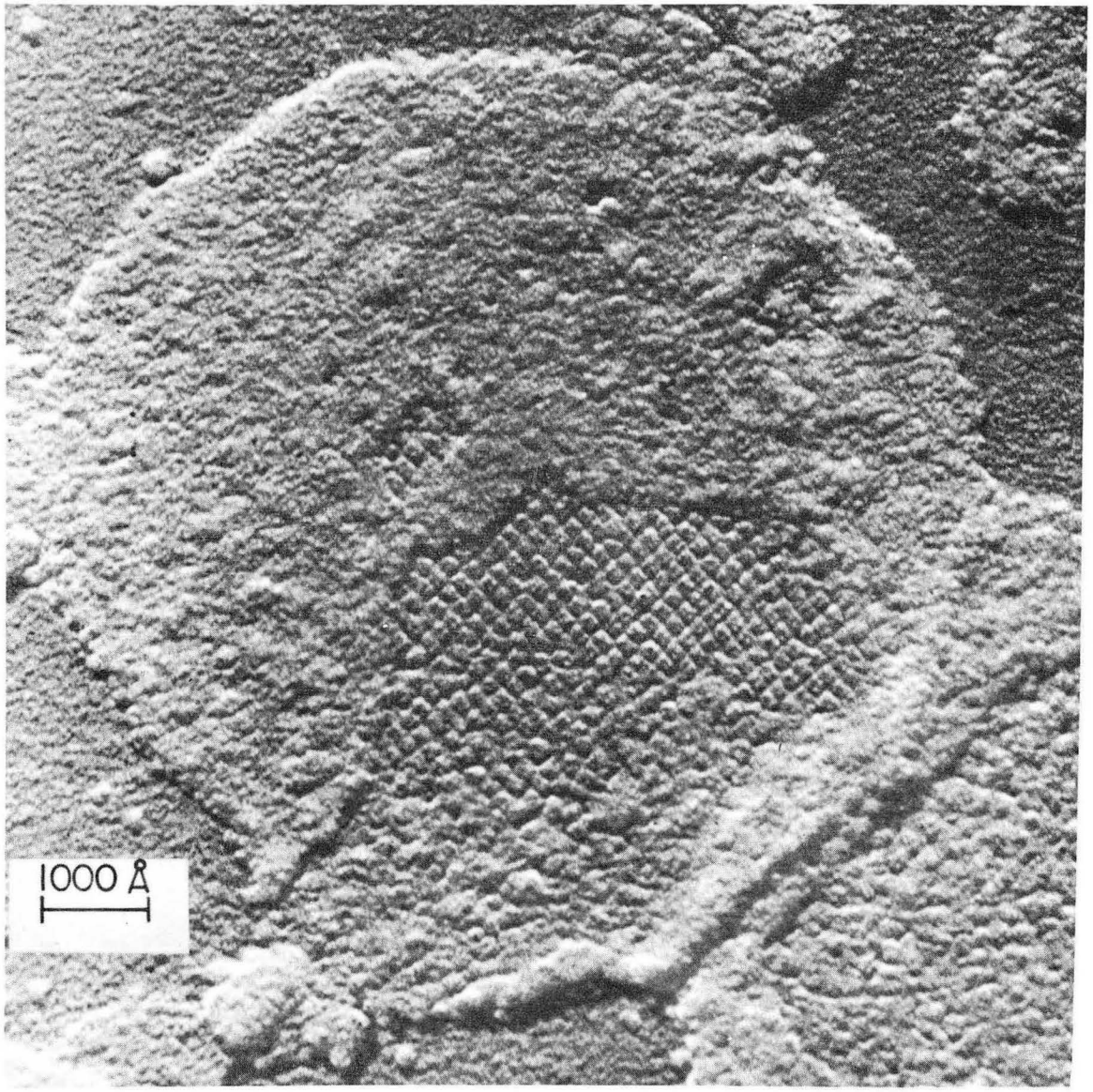

(CH=O b)
PROTOCHLOROPHYLL
CHLOROPHYLL a (b)
BACTERIOCHLOROPHYLL
MU-22388

Fig. 4



ZN-4304

Fig. 5

Aspidistra chloroplast lamina
Weier, 1962.

ZN-4500

Fig. 6

QUANTASOME COMPOSITION (10c)

Lipid (composition in molecules per quantasome)		Molecular Weight
230 chlorophylls		206,400
160 chlorophyll a	143,000	
70 chlorophyll b	68,400	
48 carotenoids		27,400
14 β-carotene	7,600	
22 lutein	13,600	
6 violaxanthin	3,600	
6 neoxanthin	3,600	
46 quinone compounds		31,800
16 plastoquinone A	12,000	
8 plastoquinone B	9,000	
4 plastoquinone C	3,000	
8-10 α-tocopherol	3,800	
4 α-tocopherylquinone	2,000	
4 vitamin K_1	2,000	
116 phospholipids (phosphatidylglycerol)		90,800
144 digalactosyldiglyceride		134,000
346 monogalactosyldiglyceride		268,000
48 sulfolipid		41,000
? sterols		15,000
unidentified lipids		175,600
	Total	990,000
Protein		
9,380 nitrogen atoms as protein		928,000
2 manganese		110
12 iron including two cytochrome		672
6 copper		218
	Total	930,000
Total lipid + protein		1,920,000

FIGURE 7

important role in the various electron transport reactions which are the chemical reactions following the quantum conversion act.

Is it possible to say anything about the molecular arrangement of these components in the quantasomes? There is a bit of evidence to the effect that a small fraction of the chlorophyll is oriented -- it is not randomly arranged in sdution. (11) FIGURE 8 shows the electric dichroism of the quantasome fragments. The dotted line shows the absorption spectrum and the solid line is the dichroic ratio. At about 7000 Å there is a relatively large electric dichroism which corresponds to a small fraction (5-15%) of the total chlorophyll with its absorption at this point. A similar conclusion was arrived at by Butler (12) from an examination of the fluorescence of chlorophyll in intact chloroplasts. He found that there was a fluorescence which was polarized and which seemed to be due to this particular kind of chlorophyll, the fluorescence seeming to be of somewhat longer wavelength than the fluorescence of the bulk of the chlorophyll.

Thus there seem to be two di-ferent kinds of evidence that in the green material there is at least one component of the chlorophyll which is highly ordered. The molecules are ordered enough so that they can be aligned in an electric field as well as a mechanical field (flow dichroism (11a)), and, furthermore, these ordered molecules seem to be the ones toward which all of the absorbed quanta in that particular pigment system migrate by exciton migration, and from which the fluorescence comes. The fluorescence is a competitive (wasteful) reaction to the quantum conversion act, which is also presumed to take place in these very special chlorophyll molecules.

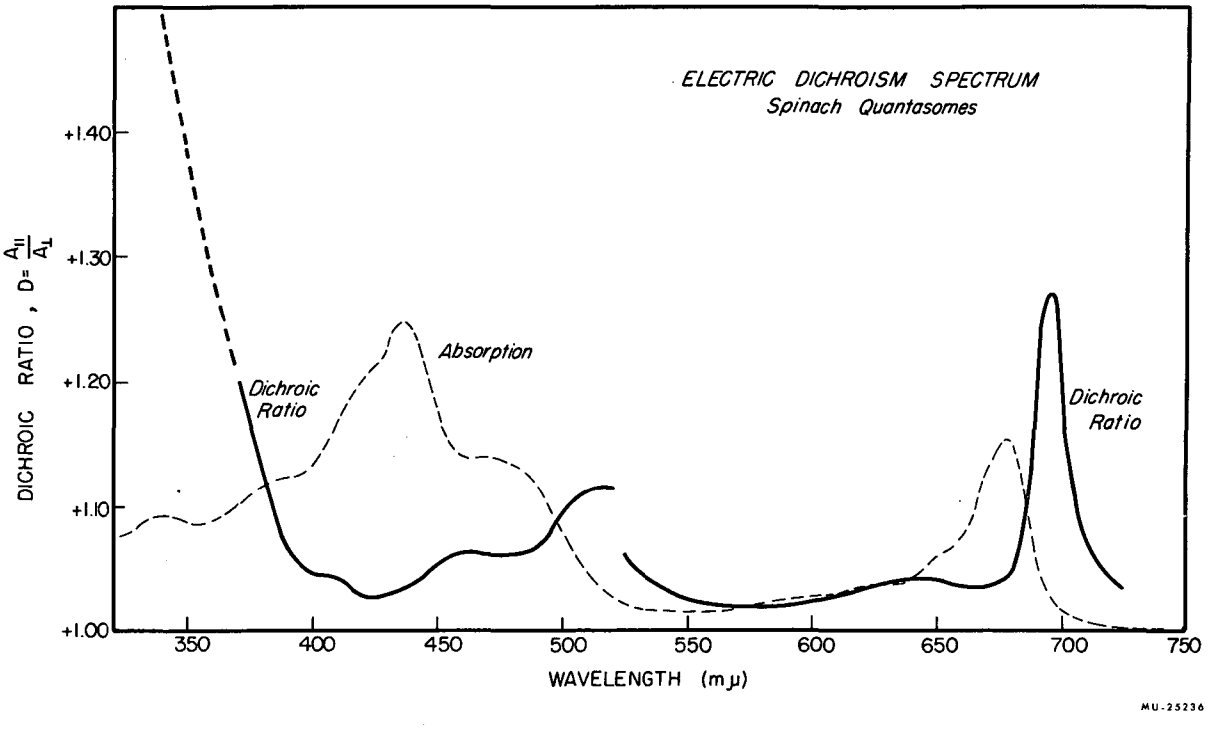

ELECTRIC DICHROISM SPECTRUM
Spinach Quantasomes
DICHROIC RATIO , D= A∥/A⊥
+1.40
+1.30
+1.20
+1.10
+1.00
Absorption
Dichroic Ratio
Dichroic Ratio
350
400
450
500
550
600
650
700
750
WAVELENGTH (mμ)
MU-25236

Fig. 8

The fluorescence evidence, together with the structural evidence, is part of the basis for our notion that following the primary absorption of the quantum anywhere in the bulk chlorophyll it (the quantum) migrates as the excited state to specific sites in the photosynthetic apparatus, and it is at these sites that the quantum conversion takes place.

THE SPECTROSCOPIC METHOD IN BIOLOGICAL MATERIALS

In order to find out what is happening at that site, which is defined as the site of (chlorophyll) pigment 705 (approximately the wavelength of the maximum dichroism), we must try to take the fragment apart still further, to see if we can find materials that have such an absorption and examine their individual photo- and biochemistry. It turns out that whenever this is attempted by going down to structural levels below that of the quantasome, the reactions which we recognize as either whole or partial reactions of photosynthesis fail. It is for this reason that we are confined, so far at least, to studying these primary quantum conversion acts in fairly complex chemical and relatively highly ordered physical systems. Those of you who have been accustomed to the successful approach which biochemistry has taken in the last 20-30 years, i.e., dismantling the biological apparatus to the individual molecules of which it is constructed and examining each of the reactions which the individual molecules can carry out and then reconstructing the system from that fragmentary study, will be somewhat skeptical or perhaps disappointed. So

far we have not succeeded in carrying the fragmentation of the photosynthetic apparatus down below this quantasome level and still retain the properties which we recognize as components of the photosynthetic process. Therefore we have to find other ways and means of examining them in this complex biological system.

There is really only one way in which such a complex system can be examined, so to speak (and I mean literally look at it) to find out anything about what is going on without destroying it. This is by a spectroscopic method of some sort. One has to examine the system with electromagnetic radiation of a suitable wavelength. One can use anything from radiofrequencies down to X-rays, but this is really the only way that we can examine directly what is going on inside this complex structure and analytically separate the various processes, one from another. We can do this by examining what happens inside the quantasome particles, using the principle of the double beam spectrophotometer (FIGURE 9). With a device which uses radiation (either radio, visible, infrared, microwave, etc.) as an analytical tool, we can look at the material in the two vessels and compare the emerging beam.

If we shine actinic light on one and have the other vessel in darkness, and compare the two emergent analytical beams by various devices, we are having a look at what the actinic light does to the one by comparing the change in the illuminated sample with the material in the dark. There are many variations of this type of experiment, but such a comparison is essentially what the spectroscopic examination is. The rest of the paper will deal with this subject, using two kinds of

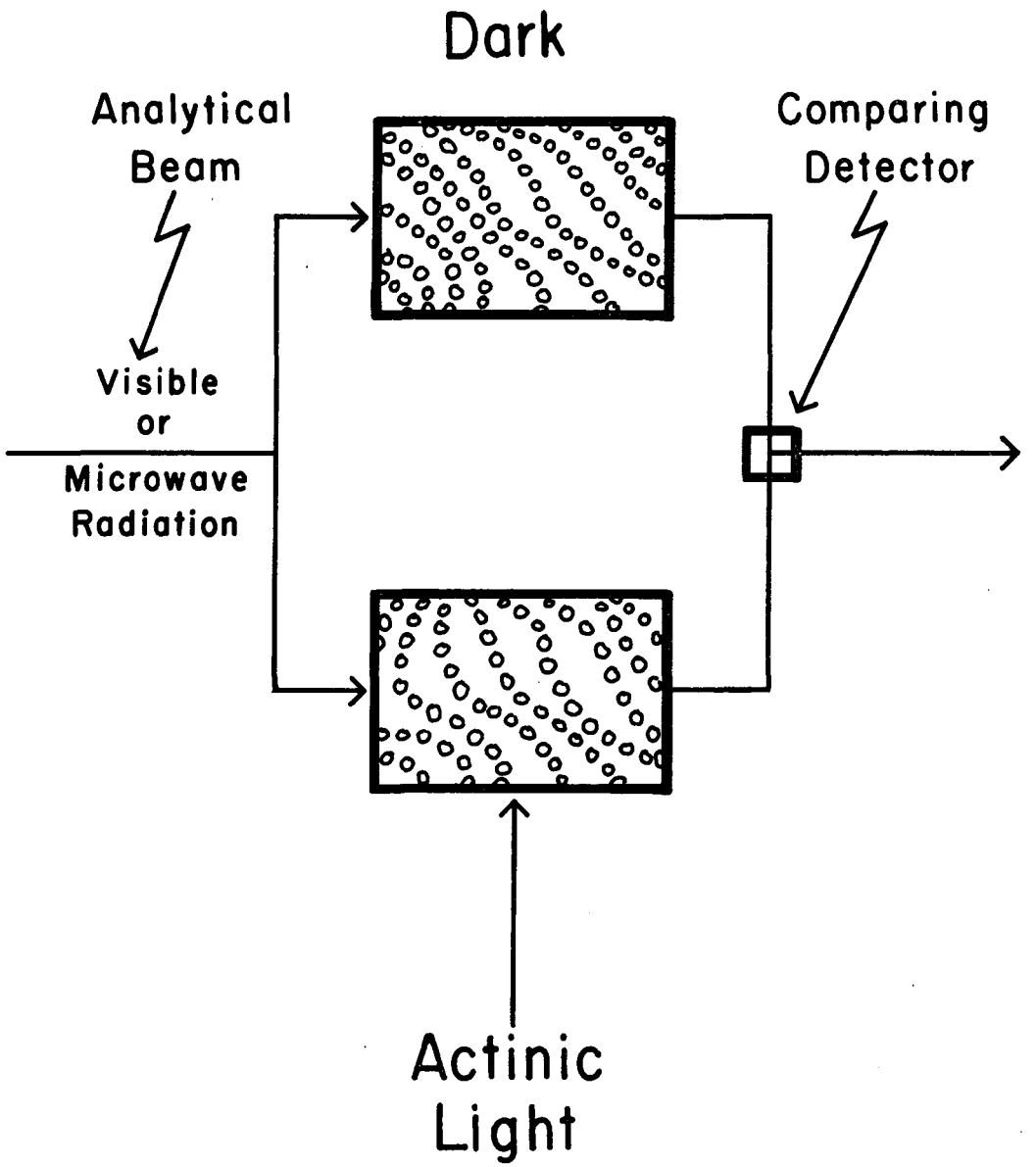

Dark
Analytical Beam
Comparing Detector
Visible or Microwave Radiation
Actinic Light
MUB-2969

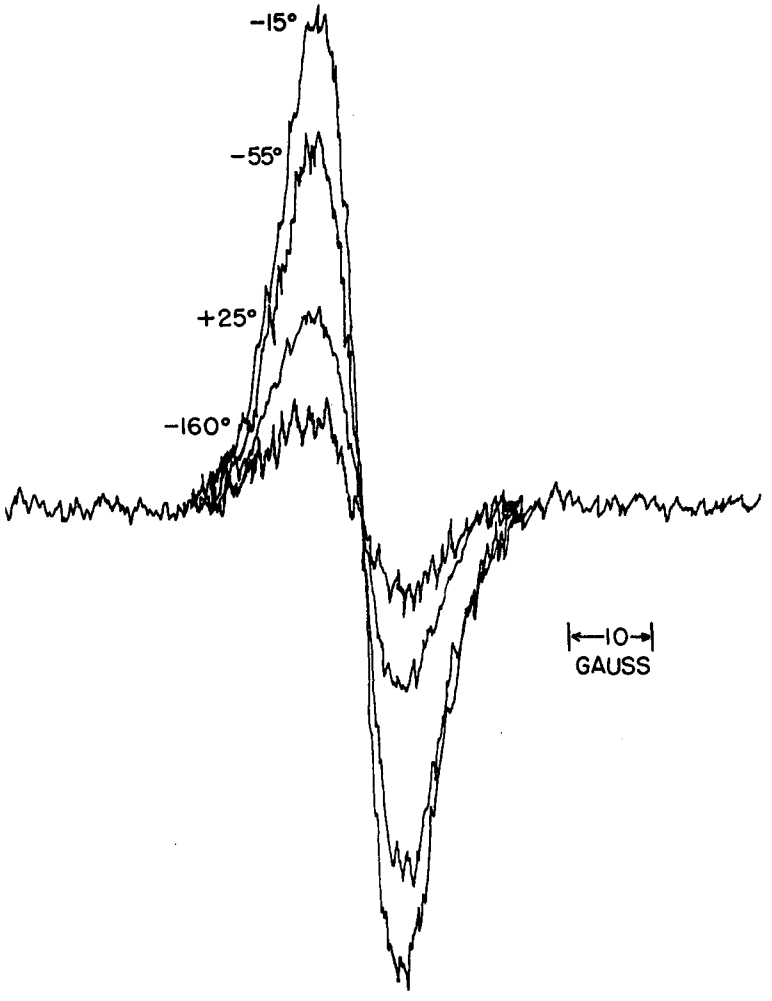
Fig. 9

electromagnetic energy as analytical tools, one of them visible light and the other one microwave frequencies. With these two methods, we can examine two different properties of the particulate material of the quantum conversion apparatus. A third type of examination would be to look at the fluorescence, but we are not going to discuss this in detail here. I have already brought out one of the basic pieces of information resulting from such a study, namely, that exciton migration occurs and that the quantum conversion act probably takes place in one, or possibly two, different kinds of traps for the quanta.

Let us now discuss the results obtained by the two methods: (1) change in the visible absorption spectrum induced by the actinic light and (2) changes in the microwave absorption spectrum, and compare the two.

EPR IN CHROMATOPHORES

FIGURES 10 and 11 show the result of an examination of bacterial material for photoinduced unpaired electrons. The photosynthetic bacteria seem to be, at least in one respect, somewhat simpler in their photochemical quantum conversion than the green plants. The bacteria do not have one of the very important photosynthetic functions, namely, the ability to make molecular oxygen. So, in a sense, they have less of a job to do than the green material of the plants. The next figures will show spectra of the chromatophores, the chlorophyll-containing fragments removed from the photosynthetic bacteria of various kinds. FIGURE 10 shows the production of unpaired electrons in the whole bacteria as a function of temperature. Here is what appears to be a single absorption band, and you can see that the reaction proceeds even at liquid nitrogen temperatures, just as it does at room temperature. The important fact is that we can't do very much ordinary biochemistry at -160°, so we can be confident that this reaction is something very close to the primary quantum conversion act. The apparent increase and subsequent decrease in the steady-state number of unpaired spins with falling temperature undoubtedly represents a freezing out of successive radical re-

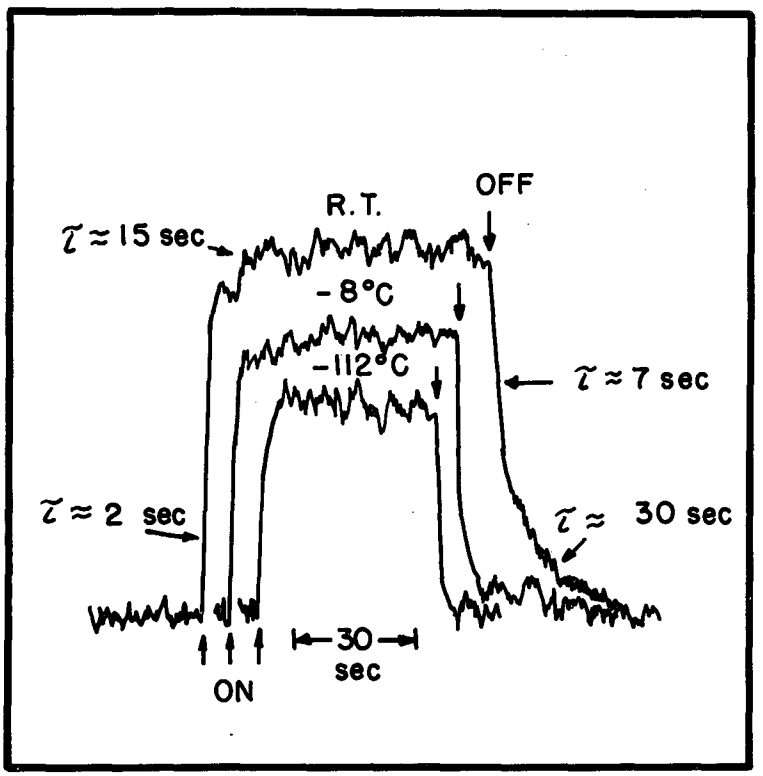


ESR SIGNALS FROM *RHODOSPIRILLUM RUBRUM*
5 MINUTES CONTINUOUS ILLUMINATION

MU-15137

Fig. 10

RISE AND DECAY OF THE EPR IN CHROMATOPHORES OF R. rubrum



MU-26351

Fig. 11

actions from the biochemical system. Most of the unpaired spins at 25° are surely secondary things, formed in a series of radical reactions after the primary quantum conversion act. When they freeze out, only the physical quantum conversion act itself is left behind.

If that is the case, you might expect that the growth and decay of the room temprature radicals would be slow, whereas the growth and decay of the radicals made at liquid nitrogen temperature would have a less slow component -- everything would be fast. This is shown in FIGURE 11 where you can see in the rates at room temperature both a rapid and slow rising component, as well as rapid and slow falling components. When the material is cooled, all the slow components freeze out and only the rapid components are left. Since this photograph was made, we have gone to faster times, using a different kind of equipment (flash photolysis) and have obtained a more detailed kinetic picture of the growth and decay curves. (15) The unpaired spins are formed even at liquid nitrogen temperatures and thus must be very close to the primary quantum conversion act itself.

OPTICAL CHANGES IN CHROMATOPHORES

There is another spectral region which we can examine, namely, the visible, in exactly the same way to see what changes in the visible absorption are introduced when we shine light on one-half of the experimental system. FIGURE 12 shows the visible absorption spectrum of particular particles from a bacterium, chromatophores of R. rubrum. The two bands at 880 and 375 mu are the two principal bacteriochlorophyll bands; the cytochrome bands are hidden underneath, particularly in the

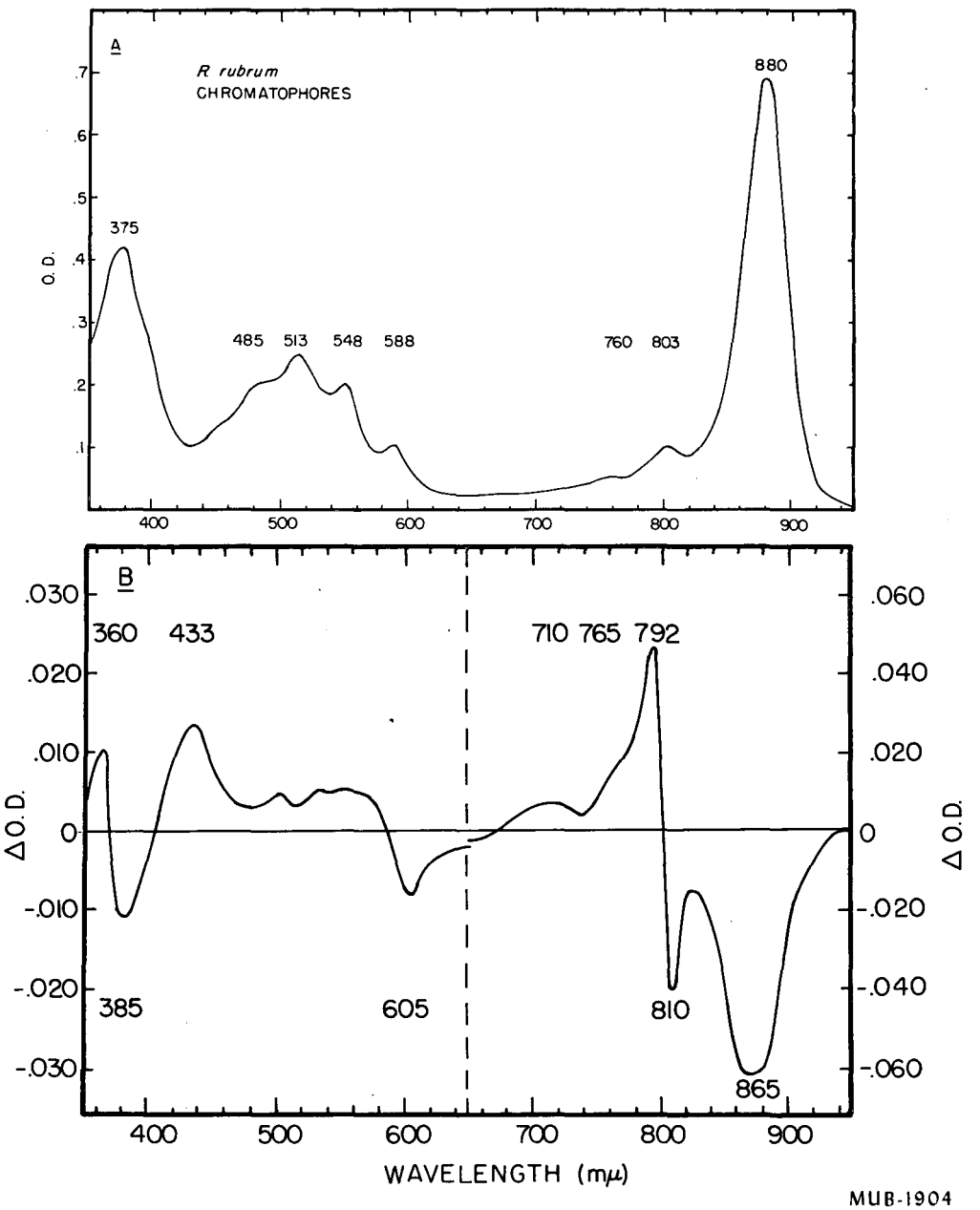

A
R rubrum
CHROMATOPHORES
O.D.
.7
.6
.5
.4
.3
.2
.1
375
485
513
548
588
760
803
880
400
500
600
700
800
900
B
.030
.020
.010
0
-.010
-.020
-.030
ΔO.D.
360
433
710
765
792
385
605
810
865
.060
.040
.020
0
-.020
-.040
-.060
ΔO.D.
400
500
600
700
800
900
WAVELENGTH (mμ)
MUB-1904

Fig. 12

carotenoid region. The lower spectrum shows the difference in optical density (change in absorption) between the illuminated set of chromatophores and the dark set of chromatophores. There are a large number of things happening: changes are occurring throughout the absorption spectrum of the R. rubrum. One of the big changes is a drop in the absorption of the chlorophyll at 865, and the -810 and the +790 peaks look like a shift of some sort at 803. Note that the scale on the right is larger than the one on the left by a factor of two. These many peaks and valleys represent optical changes taking place inside the R. rubrum chromatophores in different pigments. The question is: Are these changes in one or several systems, and how can this be recognized? How can we tell what these difference spectra represent, some of them being increases and some of them being decreases in absorption.

The identification of the changes that are occurring in this material is our principal objective. One can easily see changes in the 865 region, and these have been attributed to the chlorophyll itself. The -384 and the +360 change is probably due to a shift in the Soret band of the chlorophyll. There are other pigments also involved in this change as well.

There are at least two ways of identifying these various changes. One of them is, of course, to get the individual pigment out which is responsible for the changes in a form which corresponds to the form in which it exists in the chromatophores or quantasomes, and then to demonstrate the change in spectrum by some chemical method -- for example, introduce electrons or take electrons away. We have been able to do that with only one pigment, i.e., the cytochromes. The cytochrome can be

removed from the chromatophores or the chloroplasts and can be subjected to/or oxidation reduction by chemical methods. The spectral changes are observable and can be identified with a small part of the total difference spectrum, shown for Chromatium chromatophores in FIGURE 12b.

The rest of it, however, cannot be. The chlorophyll cannot be, because the moment the chlorophyll is removed from the plant as chlorophyll molecules in ordinary solution, its spectrum is completely different. Therefore, it is not possible to identify the chlorophyll by any other method. We can show that this is an oxidation of chlorophyll, or at least a destruction of it by oxidation, because we can produce the same spectral result by oxidation of the chromatophores by ferricyanide as is produced by light. So we say that the light is indeed oxidizing at least one small bit of the chlorophyll. (16) The light is also oxidizing the cytochromes, at least in the Chromatium chromatophores.

FIGURE 13 shows some of the low temperature evidence for the primary character of some of the light-induced spectral changes; this is some work of Arnold and Clayton (17) of several years ago showing that the absorption changes at 4200 Å for a purple bacterium go on at very low temperatures. The absorption at 4200 Å is increased when the light is turned on and decreased when it is turned off, and this takes place even at liquid helium temperatures. Therefore, one can be fairly sure that these changes correspond to no ordinary chemical reactions occurring at this temperature.

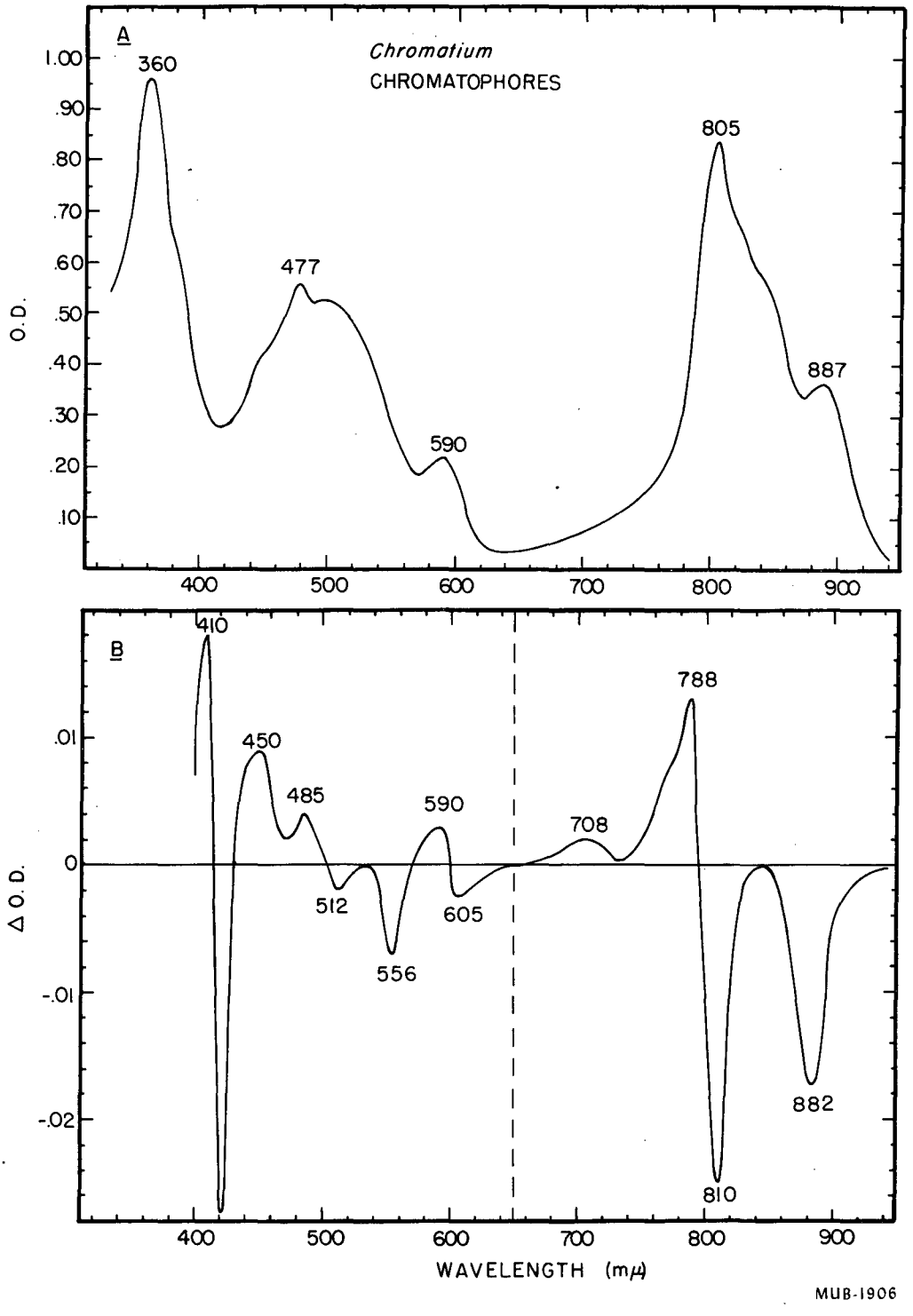
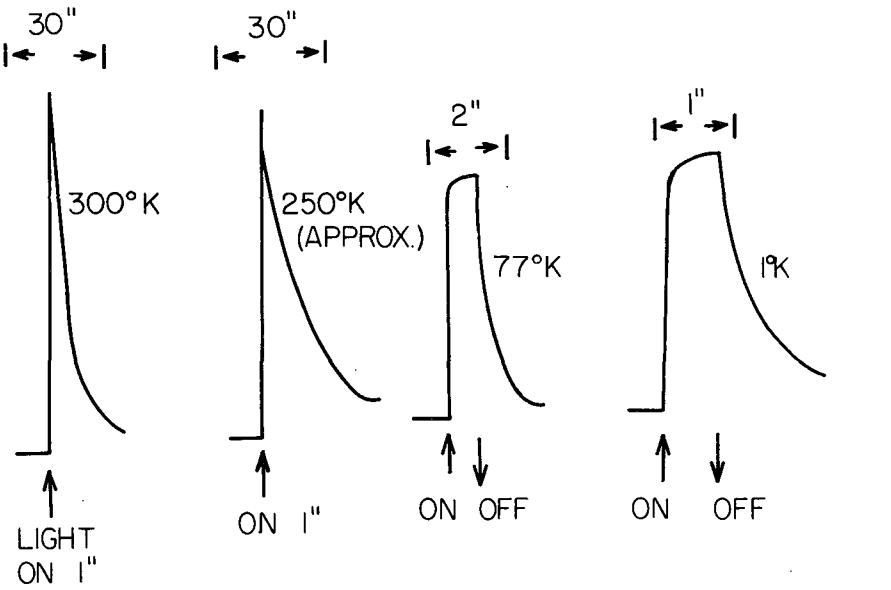

A
Chromatium
CHROMATOPHORES
360
477
590
805
887
O.D.
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
400
500
600
700
800
900
B
410
450
485
512
556
590
605
708
788
810
882
Δ O.D.
.01
0
-.01
-.02
WAVELENGTH (mμ)
MUB-1906

Fig. 12b



ABSORPTION CHANGES AT 4200Å INDUCED BY ILLUMINATION
($\lambda > 6400$ Å)

ARNOLD and CLAYTON

MU-22414

Fig. 13

KINETIC EXPERIMENTS ON CHROMATOPHORES

So far you have seen the total spectral changes when you shine light on one cell and keep the other one as a dark standard. We have tried to identify some of those changes with certain pigment changes, by isolating the pigments and performing some chemical analyses on them. However, the only one upon which this has been successfully accomplished is the cytochrome. The other changes have so far failed to yield to this method. Therefore, we have to use other methods for differentiating these changes; to determine whether these many changes are due to one and the same molecule or due to different molecular species.

One way of doing this would be to measure the rate at which the changes appear at the different points in the optical spectrum. If the rates are different, either for their appearance or disappearance, then quite obviously they are due to different molecule species. If the rates are the same, they may or may not be due to the same molecular species. We are now trying to distinguish between all the various changes which we see in the steady light by the rate at which they appear or disappear upon flash illumination. We are using the methods of repeated flash photolysis, shown here in FIGURE 14. The change in optical absorption produced by a single fast pulse of light is often so small that it is hidden in the noise. What we have done is put on a pulse of about one hundred milliseconds of light and recorded the growth and decay of the change, and we have done this repeatedly. We have had the apparatus coupled together in such a way that every time we go through

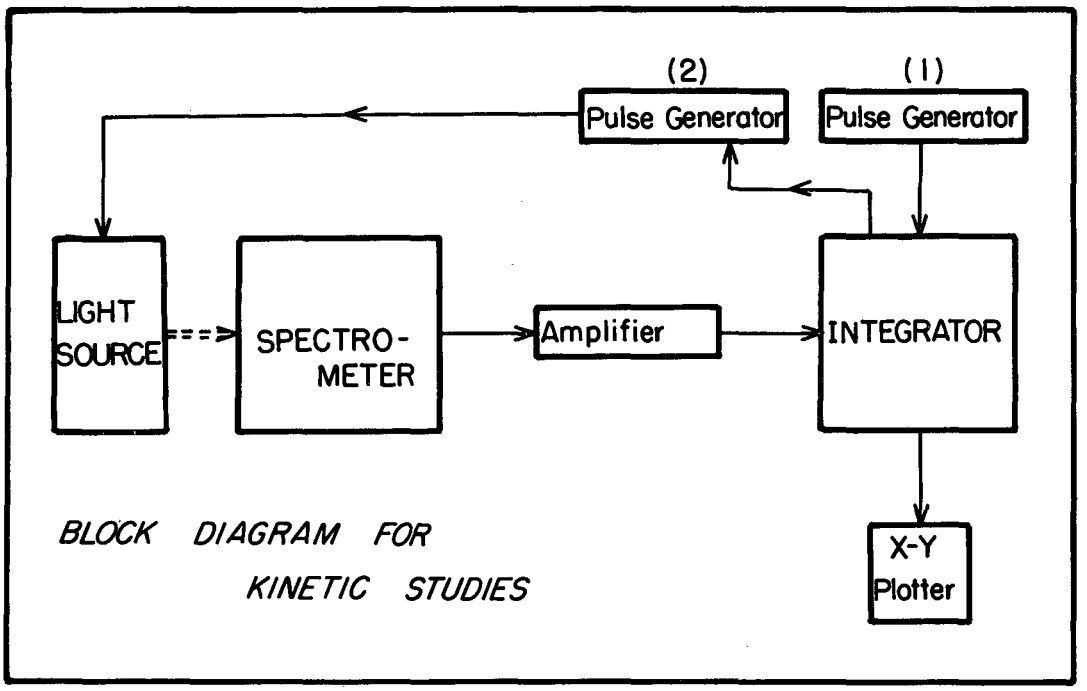

(2)
Pulse Generator
(1)
Pulse Generator
LIGHT SOURCE
SPECTRO-METER
Amplifier
INTEGRATOR
X-Y Plotter
BLOCK DIAGRAM FOR KINETIC STUDIES
MU-33079

Fig. 14

this scanning operation we start at the same point in time with respect to the light flash, and just kept adding up the figures. The noise is random and cancel itselffout.

FIGURE 15 shows some of the kinetic results of the flash photolysis. With R. rubrum chromatophores you can see that the change at 433 mu has quite a different decay rate than the change at 792 mu and 810 mu, which, in turn, is different from the decay rate at 865 mu. This already tells us that these three changes -- the 433, 792-810 and 865 -- are indeed due to three different species. They are disappearing at different rates and are therefore not due to the same species. This is an important piece of evidence because it looked at the start as though the 792-800 mu band was right on the side of the 865 mu band, and we thought they were vibrational components of the chlorophyll absorption band. It now appears that they are two different pigment changes -- they may both be chlorophyll, but, if so, they are two different chlorophyll molecules. The change at 433 mu is spectacularly different and quite obviously is not the Soret band associated with the 865 mu pigment. These are three different species, then.

With this kind of technique, in which we can measure accurately growth and decay rates of signals, we can now compare the growth and decay rates, say, in the R. rubrum chromatophores, of the oxidized chlorophyll, which is measured by the change at 865 mu, with the growth and decay rate of the unpaired spins in the same organism, which we can produce with the same light. We now have two different ways of looking at the material. We can observe the optical absorption at 865 mu, which seems almost certainly to be chlorophyll. We can examine it in the 3 cm microwave

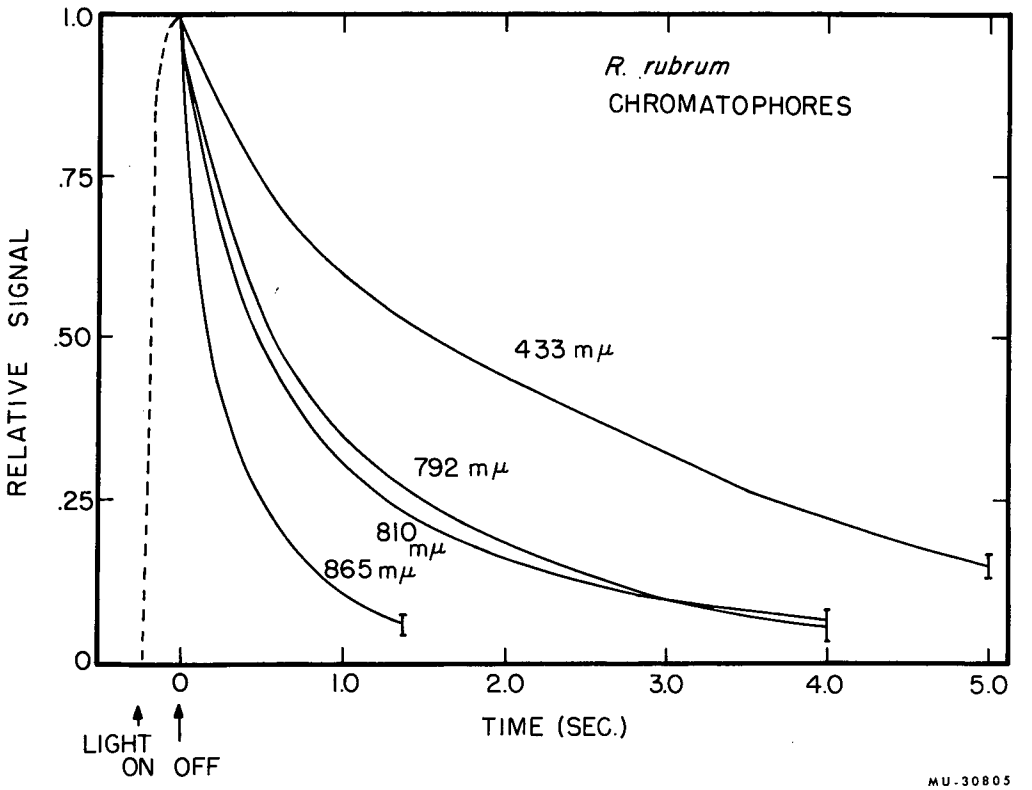

1.0
.75
.50
.25
0
RELATIVE SIGNAL
R. rubrum
CHROMATOPHORES
433 mμ
792 mμ
810 mμ
865 mμ
0
1.0
2.0
3.0
4.0
5.0
TIME (SEC.)
LIGHT ON
OFF
MU-30805

Fig. 15

region, which "sees" an unpaired electron, and we can determine whether the oxidized chlorophyll shows its unpaired electron, which it should show if a single electron transfer has been made. FIGURE 16 shows that comparison, and you can see the fine wavy line which is the growth and decay (performed by the flash photolysis method) of the paramagnetic resonance signal. It is quite evident that it does not correspond at all with the decay of the 865. The 865 change decays much more rapidly than does the unpaired electron. The unpaired electron, however, does decay at the same rate as the 433 change.

This information has one positive and one negative consequence. The positive fact is that it looks as though whatever is changing at 433 (i.e., whatever that change is due to) is very closely associated with, if not identical to, the species responsible for the unpaired electron. It also tells us, negatively, that the oxidation of chlorophyll by the removal of one electron, which we can demonstrate with ferricyanide corresponds to that 865 change, does not show an unpaired spin. This is an important negative conclusion, and is one of the few cases in which a negative observation may be important. The situation may be represented by the following change, showing the electron transfer <u>from</u> the excited chlorophyll molecule in its special location (FIGURE 17). We recognize the chlorophyll positive ion radical by its 865 spectrum, but no EPR can be identified with it yet. If this were an ordinary chlorophyll, either by itself in solution or attached to the protein molecule, and was indeed a single chlorophyll molecule not interacting with other chlorophyll molecules, we would and should see an unpaired electron on it as we do with other organic free radicals, but we don't. Thus, although the presence of the chlorophyll positive ion radical can be

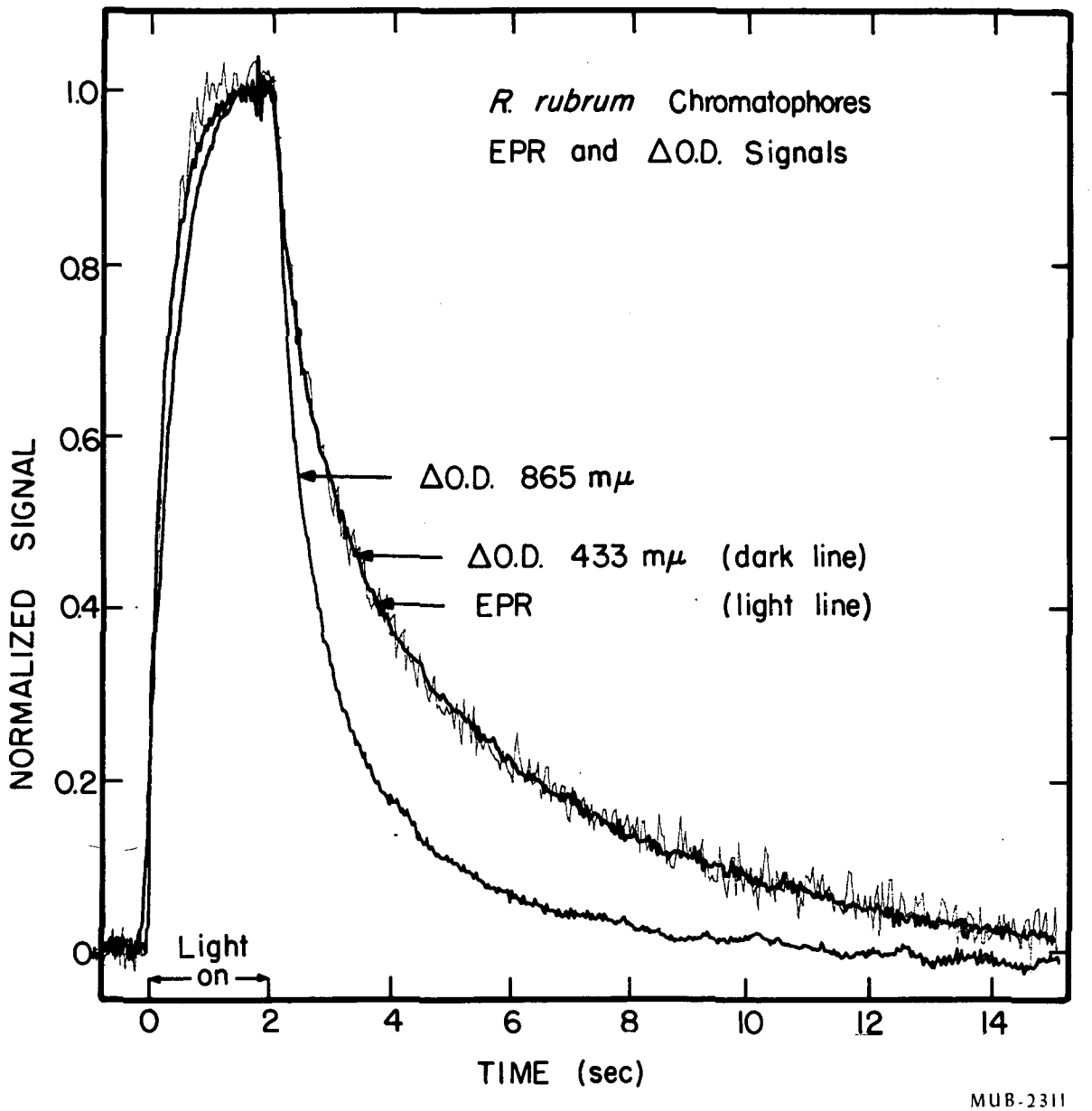
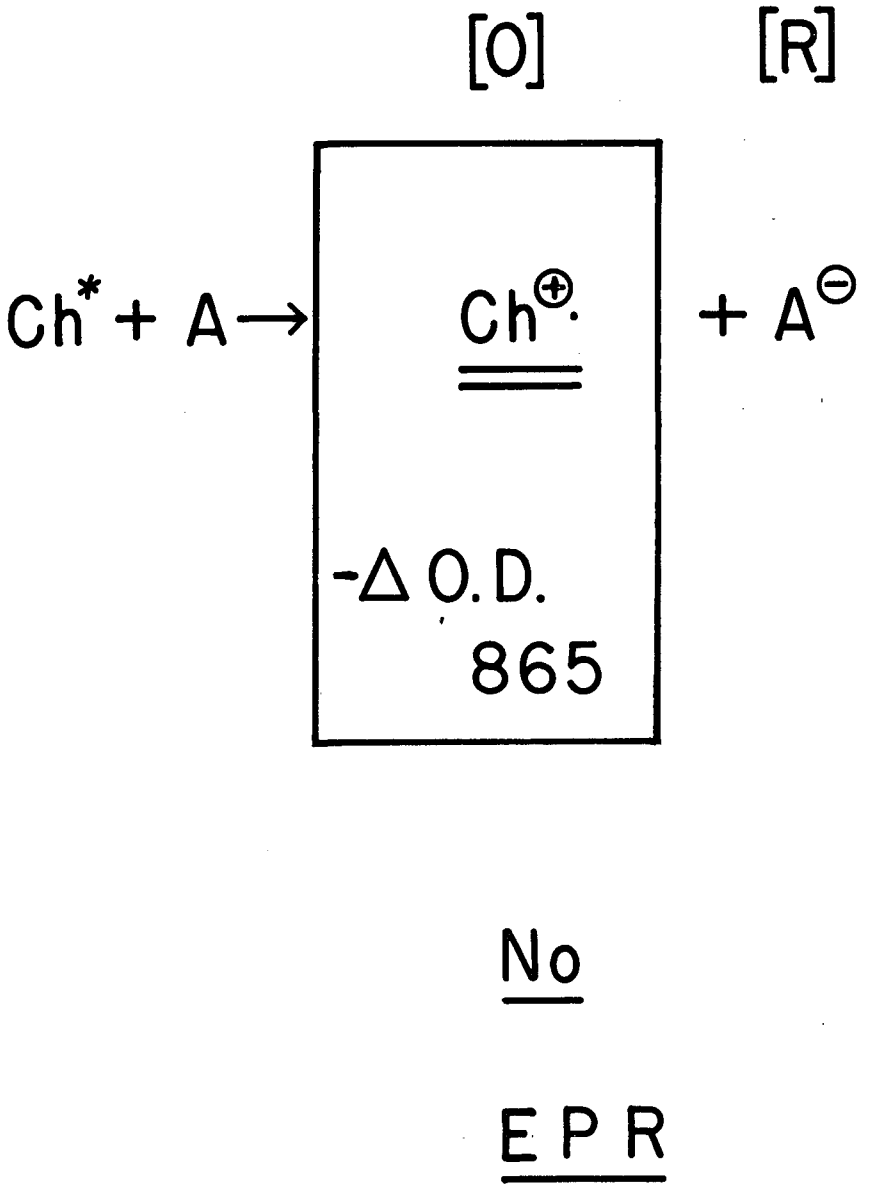

R. rubrum Chromatophores
EPR and ΔO.D. Signals
ΔO.D. 865 mμ
ΔO.D. 433 mμ (dark line)
EPR (light line)
Light on
NORMALIZED SIGNAL
TIME (sec)
1.0
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
MUB-2311

Fig. 16



MUB-2996

Fig. 17

demonstrated by the optical density change at 865 as an oxidized chlorophyll by a ferricyanide titration with one electron missing, it shows no unpaired spin. This may mean that the unpaired spin is not localized on that chlorophyll molecule.

The unpaired spin is presumably delocalized over the whole bed of chlorophyll molecules in one of the chromatophore pigment particles. This delocalization may very well be the essential process for the separation of the intermediate oxidant and the intermediate reductant. Since this is delocalized and can, in effect, move from one chlorophyll to another, it can be conceived as a charge migration in an array of chlorophyll molecules. This is what we suggest as the heart of the energy conversion process: that the separation occurs by virtue of electron delocalization in an array of chlorophyll molecules.

SPECTROSCOPY IN QUANTASOMES FROM GREEN PLANTS

In green plants we can also do microwave and optical studies. FIGURE 18 shows a result of the examination with microwave frequency. It is an absorption spectrum in the 9 kmc region which is induced in the chloroplasts by shining visible actinic light on them and shows the presence of unpaired electrons in the green materials. Two kinds of spectrum are clearly visible (FIGURE 18 (a)). The separation of the two different kinds of unpaired electrons can be achieved by separating the chloroplast into the nongreen component (c) and the green component (d). We have separated out most of the broad band, which is present in the intact chloroplast, and have left only a very sharp, narrow, very rapidly decaying signal which is characteristic of the green component

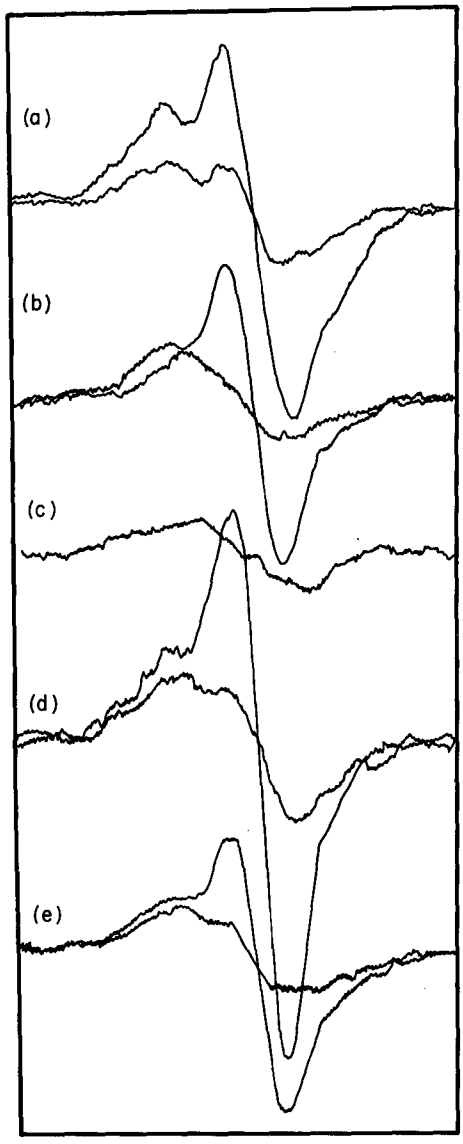


Fig. 18

of the chloroplast. We thus have evidence that light has introduced unpaired electrons into the green material of the plant. This is the first clue which we had that the light was actually unpairing electrons rather than producing an isomerization, tautomerization, etc. -- that it was actually separating paired electrons from each other. (13,14)

FIGURE 19 shows schematically, on the left where it is labeled "bacteria", a model of the process as we now hypothesize it: Light absorption by the chlorophyll molecule, exciton migration from one chlorophyll molecule to another in this array until the exciton finds its way to a specific localized pigment, at which place specific electron transfer occurs, followed by hole migration to another point at which the cytochrome is oxidized. This process -- absorption, exciton migration, electron transfer and hole migration -- can take place even at liquid nitrogen temperatures. From that point on, it is chemistry, in both plants and bacteria. The essential feature is exciton migration following absorption, electron transfer perhaps in a charge-transfer complex as the initiation of the quantum converting act, but the quantum conversion is not complete until the hole is separated from the electron. That kind of separation has to be achieved in some way, and we are proposing that it is achieved in this array by charge migration.

The difference spectrum (light-minus-dark) for some plants (Scenedesmus) is shown in FIGURE 20 and it exhibits the same kinds of effects that we saw with the bacteria, except it doesn't go out beyond 7000-8000 Å. The main difference lies at about 7000 Å. That change can be made the dominant one (at 705 mu), presumed to be at the ordered pigment which shows the electric dichroism. FIGURE 21 shows the absorp-

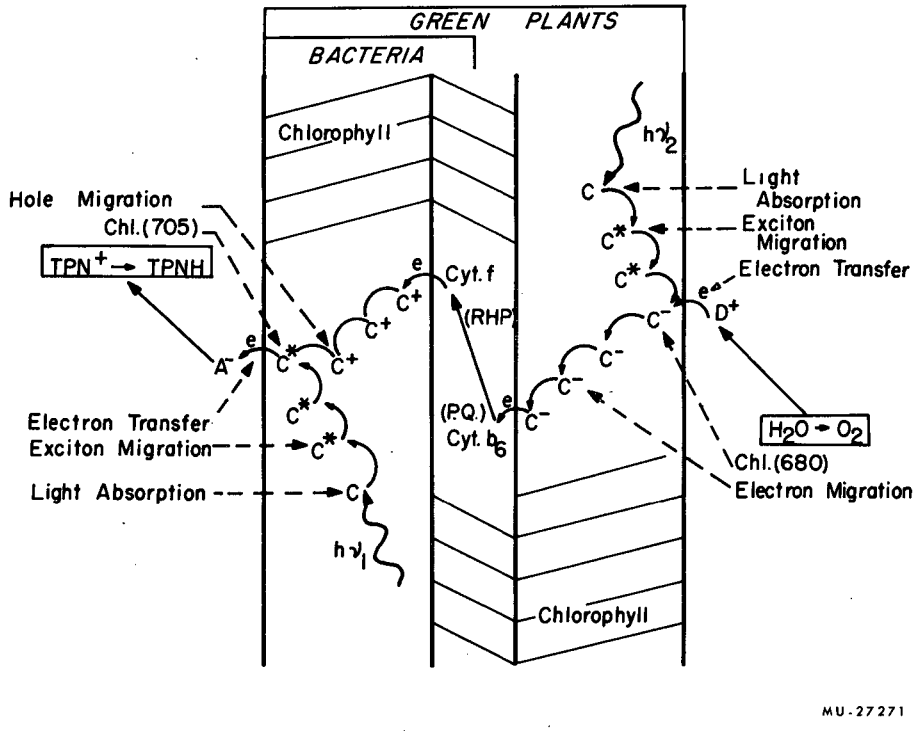
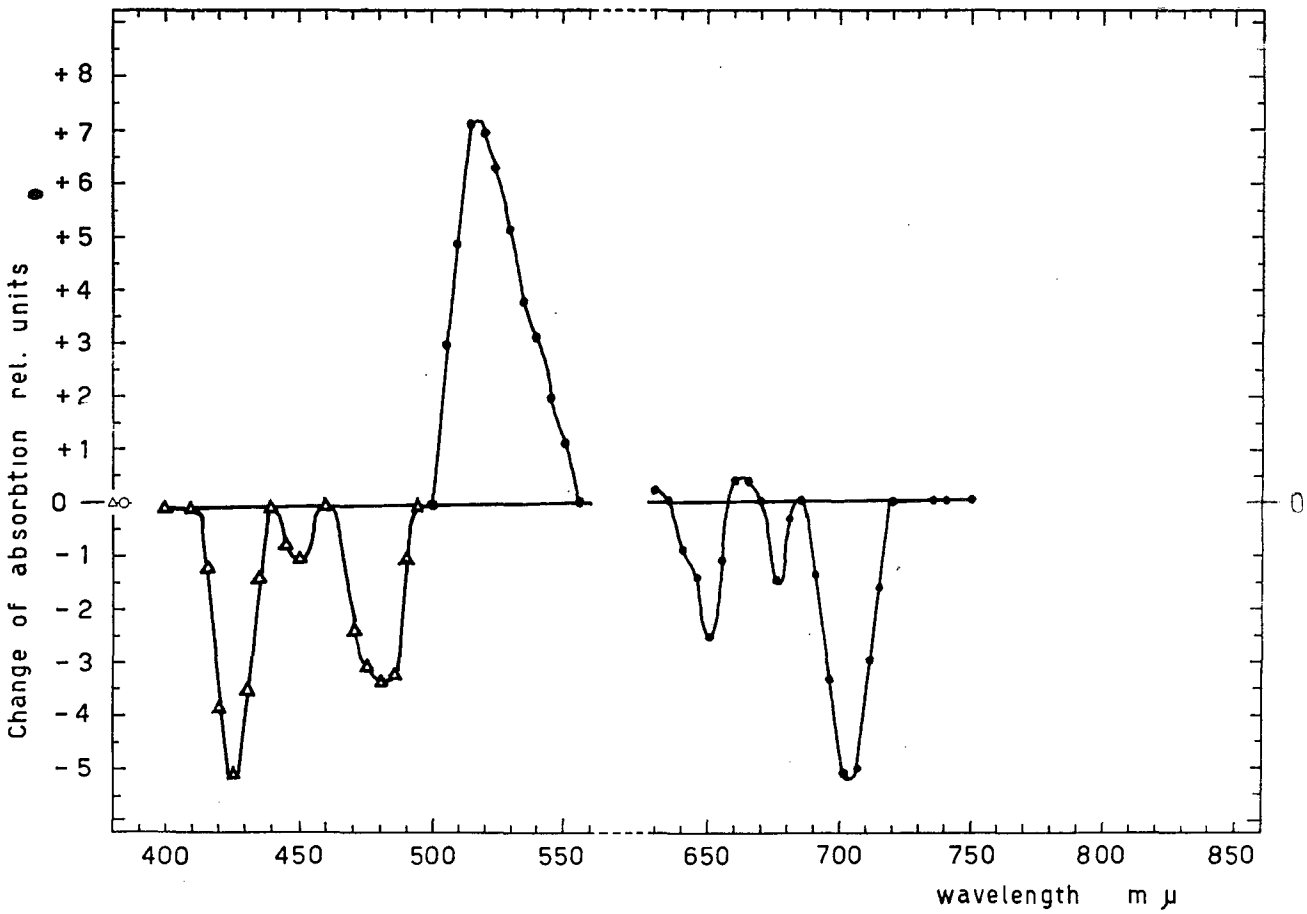

GREEN PLANTS
BACTERIA
Chlorophyll
Hole Migration
Chl.(705)
TPN+ → TPNH
Cyt. f
(RHP)
(P.Q.)
Cyt. b6
Electron Transfer
Exciton Migration
Light Absorption
Light Absorption
Exciton Migration
Electron Transfer
H2O → O2
Chl.(680)
Electron Migration
Chlorophyll
MU-27271

Fig. 19



Scenedesmus Difference Spectra (after Kok)

MUB-4239

Fig. 20

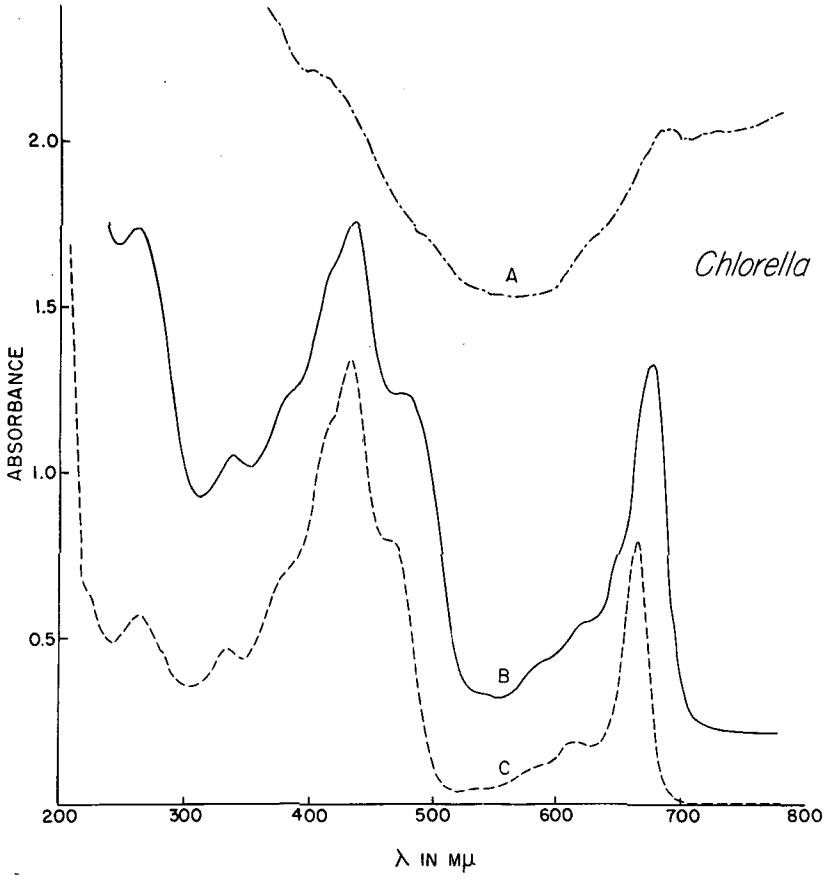

2.0
1.5
1.0
0.5
ABSORBANCE
A
Chlorella
B
C
200
300
400
500
600
700
800
λ IN Mμ
MU-7238

Fig. 21

tion of the Chlorella cells corrected for light scattering, and the spectrum is shifted from what it is in chlorophyll in solution. In FIGURE 22 is given a more highly resolved difference spectrum, taken on Chlorella, obtained with the flash photolysis, in which more significant detail can be discerned. It shows a very large positive change at 520 and a negative change at 480. This particular change is dominating in most green plants, while it is present only to a very small extent, if at all, in the bacteria or blue-green algae. The identity of that change is not yet clear, although it is believed to be due to the presence of the plastoquinone in the chloroplasts, and may be due to a charge-transfer complex between the quinone and the chlorophyll. However, this has not yet been established. It may also be a charge-transfer complex between the carotenoid and the chlorophyll.

The kinetics of the absorption changes in the green plants can also be determined, and we are just beginning to do that on the same scale that we did it with the bacteria. FIGURE 23 shows some of the kinetics with intact Scenedesmus cells. It shows the time course of the changes at 525 mu only. The time course of that change is dependent on the wavelength of the light used to induce the change, i.e., actinic light of different wavelengths produces a different time course of change at 525. The 525 obviously cannot be due to a single kind of change, a single kind of electron transfer reaction; it must have at least two, and perhaps more. It looks as though one of the component changes is brought about by the long wavelength at 720 mu, and both of them are brought about by the shorter wavelength at 650 mu. Thus, there would be two different electron transfer reactions showing an absorption change at 525 in the green plant, one of them brought about by 720 mu light and both of them by the 650.

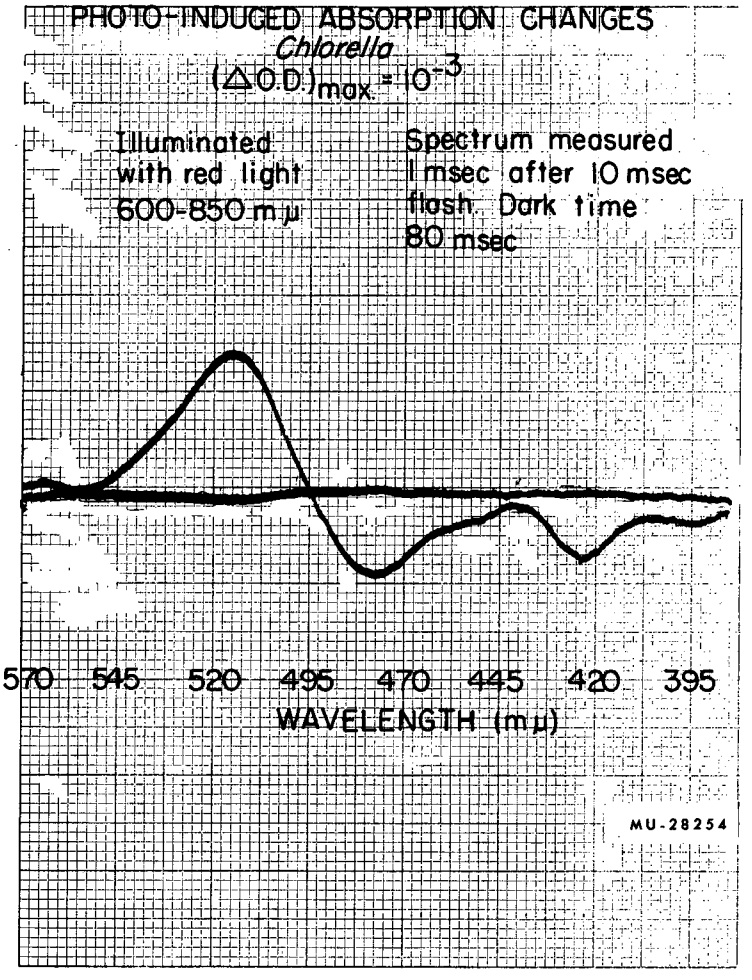


PHOTO-INDUCED ABSORPTION CHANGES
Chlorella
$(\Delta O.D.)_{max.} = 10^{-3}$
Illuminated with red light 600-850 mμ
Spectrum measured 1 msec after 10 msec flash. Dark time 80 msec
570 545 520 495 470 445 420 395
WAVELENGTH (mμ)
MU-28254

Fig. 22

RISE TIME DEPENDENCE ON EXCITATION WAVELENGTH

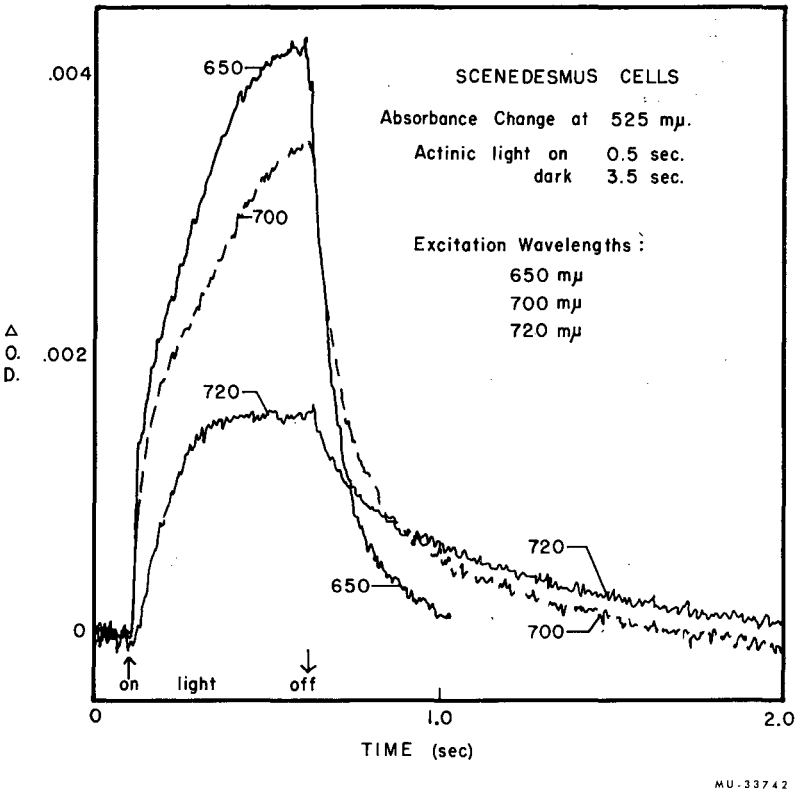


Fig. 23

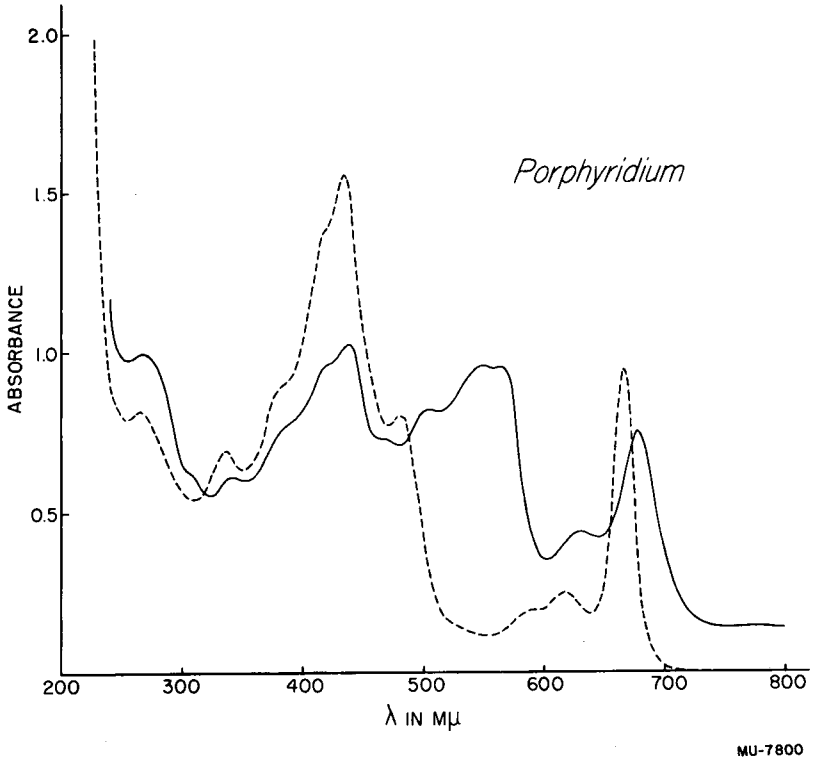
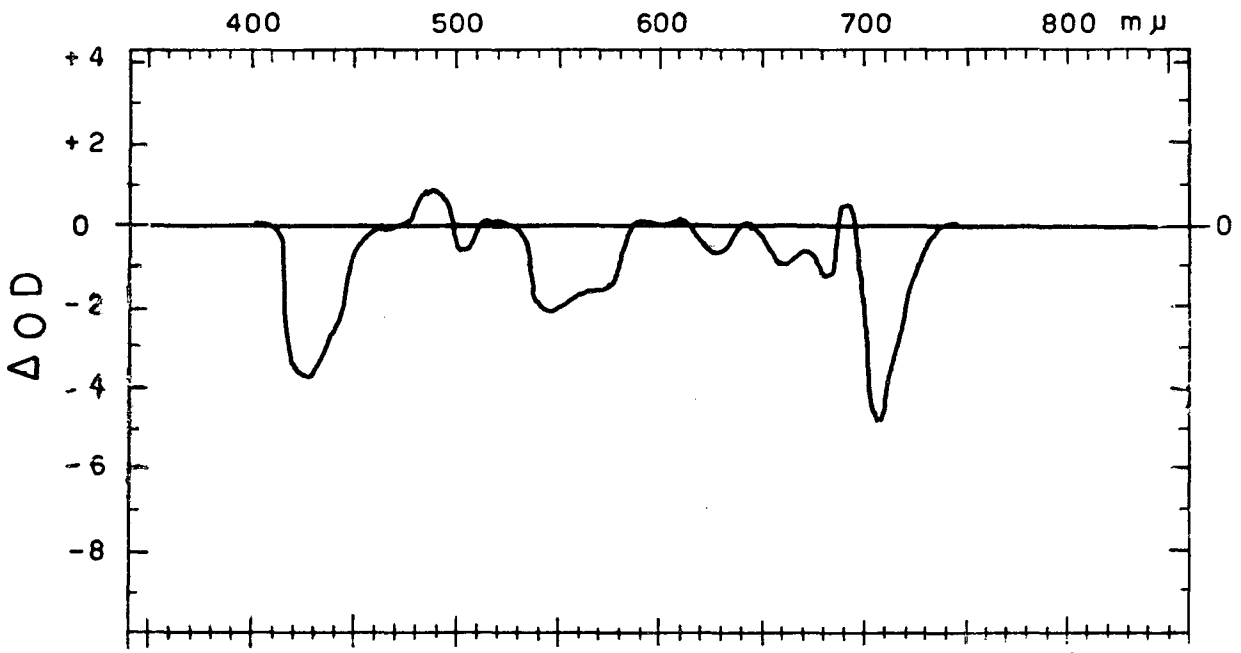

Porphyridium
ABSORBANCE
2.0
1.5
1.0
0.5
200
300
400
500
600
700
800
λ IN Mμ
MU-7800

Fig. 24 a



Porphyridium Difference Spectra (after Kok)

MUB-4240

Fig. 24 b

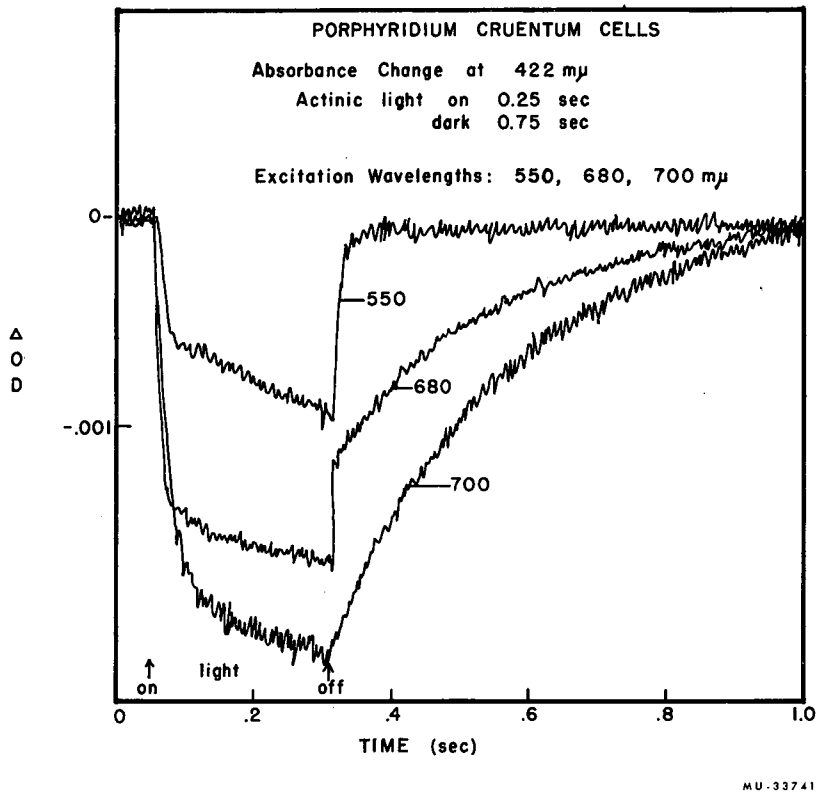

PORPHYRIDIUM CRUENTUM CELLS
Absorbance Change at 422 mμ
Actinic light on 0.25 sec
dark 0.75 sec
Excitation Wavelengths: 550, 680, 700 mμ
0
-.001
Δ O D
550
680
700
light
on
off
0
.2
.4
.6
.8
1.0
TIME (sec)
MU-33741

Fig. 25

optical evidence that the light is really transferring an electron from one molecule to another, but so far all we have seen with the light are the molecules from which the electrons are being removed, and we have not yet seen unequivocally anything of where the electrons are going.

QUANTUM CONVERSION IN PHOTOSYNTHESIS

The foregoing is a confirmation of the notion that there are two different kinds of quantum conversion acts in the green plants, which was first really hinted at by the work of Emerson about ten years ago. (22), when he found that he could increase the quantum yield of xoygen in a green plant by light of longer than 7000 Å if he had, in addition to that, light at about 6000 Å. In other words, the sum of the two quantum conversion acts, when two lights were shining on the plant together, was greater than when they were used on the plant separately. The products of these two different quantum converting acts could collaborate at producing higher efficiency than either one along could do. This is the extra complication in photosynthesis in green plants. This is now a well established notion -- that there are two different quantum converting acts in green plants, whereas it appears there is only one in bacteria. The nature of that collaboration has yet to be unequivocally established.

It is clear that information about the collaboration mechanism may be obtained from a detailed study of the kinetics of the production and decay of the optical density changes as they are effected by different wavelengths of actinic light applied in a variety of temporal and intensity relations to each other. Such work has already begun in various

laboratories, relating different optical density changes with different actinic wavelengths. (23,24) Now we are relating variable kinetics at one analyzing wavelength as a function of different actinic wavelengths, with two or more different molecular changes manifesting themselves at single wavelength.

The general tone of some current ideas is contained in FIGURE 26 which shows one way in which to set up the two different quantum converting acts (25). The first one, called here h_1 is suggested, in the case of the bacteria, as taking place with light in the 879-890 mu region, and in the green plant the changes are at 700 mu. This is presumed to be the first quantum converting act. A chlorophyll transfers an electron to some acceptor. The oxygen redox level is only at .8 volt, and the pyridine nucleotide level is only at -.4 volt, and in between them lies about 1.2 volts, which is just about what one quantum could do by itself. It appears that the products of the two quanta can cross-react. FIGURE 26 shows one current view of how they cross-react, with the plastoquinone pool in between, and most of the high energy phosphate is created in the flow of the electrons from one act into the product or vacancies created by a second act. It looks as though, at the moment, most of the schemes which you will find in the literature involve two acts of this kind, with plastoquinone, cytochrome f, cytochrome b_6 and plastocyanin, all in the intermediate region, coupling the two different quantum converting acts.

However, the nature of the two quantum converting acts would be the same in principle: exciton migration followed by charge migration in the final separation procedure. Both quantum converting acts as they now appear are shown in FIGURE 19: the absorption of light by

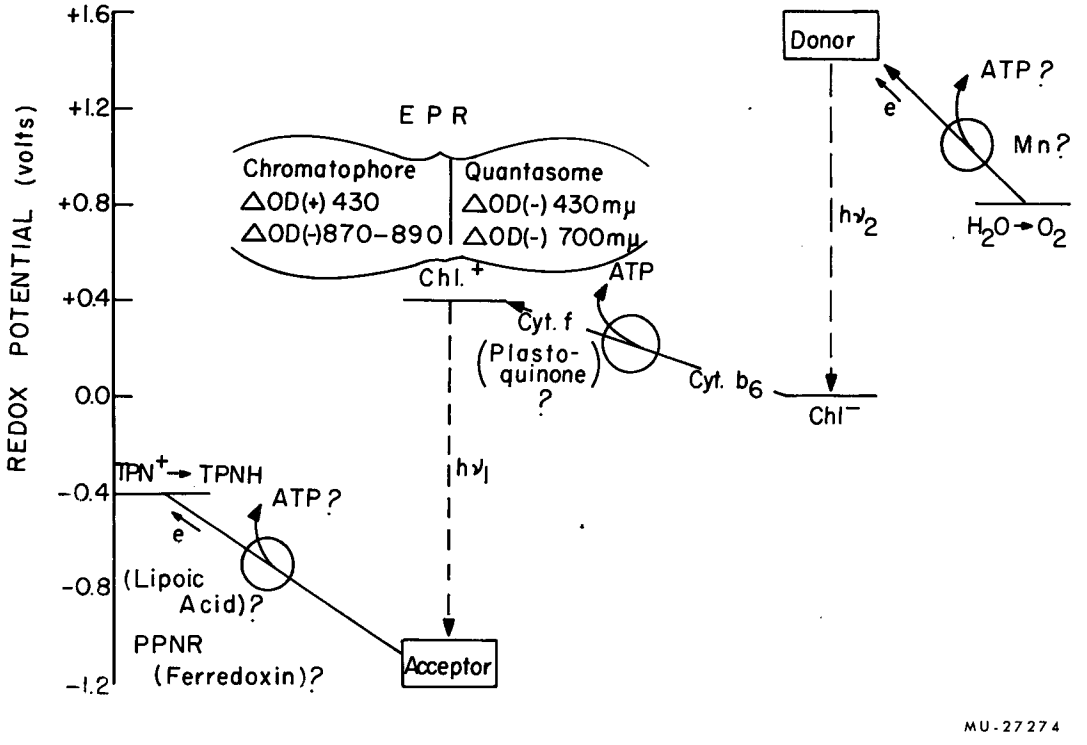

REDOX POTENTIAL (volts)
+1.6
+1.2
+0.8
+0.4
0.0
-0.4
-0.8
-1.2
EPR
Chromatophore
ΔOD(+) 430
ΔOD(-)870-890
Quantasome
ΔOD(-) 430mμ
ΔOD(-) 700mμ
Chl.+
ATP
Cyt. f
(Plasto-quinone ?)
Cyt. b6
Chl−
Donor
e
ATP ?
Mn?
H2O→O2
hν2
hν1
TPN+→TPNH
ATP ?
e
(Lipoic Acid)?
PPNR
(Ferredoxin)?
Acceptor
MU-27274

Fig. 26

chlorophyll to give an excited chlorophyll; the exciton migration from absorbed chlorophyll to particular sites in the quantasome or chromatophore; the charge-transfer operation at that particular site, giving a hole and an electron; and one or the other of these (perhaps both of them in the two separate pigment systems as indicated on the left and on the right of FIGURE 19, can move by delocalization amongst the chlorophyll array so that the oxidant and reductant are separated. In one pigment system the oxidant (hole) moves by delocalization, and in the other pigment system, in the green plant, the reductant (electron) moves by delocalization. This, then, is what we are now using as our current hypothesis for the primary quantum conversion act as earlier defined. From this point on the process is biochemistry, involving ordinary enzymological reactions of the standard type, and the recombination can take place in a manner very similar to that which is occurring in oxidative phosphorylation to give high energy phosphate.

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