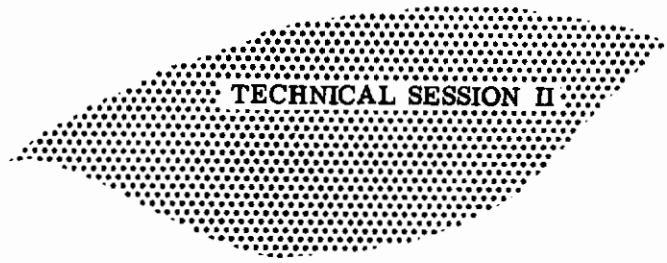

PHOTOSYNTHETIC MECHANISMS

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LIGHT CONVERSION IN PHOTOSYNTHESIS*†

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RESEARCH INSTITUTE FOR ADVANCED STUDY

INTRODUCTION

Notwithstanding recurrent claims to the contrary in the science news columns, the photosynthetic conversion of light into chemical energy is still largely a secret jealously guarded by the plant. Ingen Housz (1789), finding that light induced a leaf to produce oxygen, thought he found the solution. We think he only stated the problem. To the engineer who wants to design a gas exchanger and use photosynthesis as a tool, perhaps photosynthesis is solved - at least halfway. We have numbers, rates, products, organisms and there is great satisfaction in designing and building a tank which performs according to the slide rule. This session, however, is devoted to the basic phenomena behind this and we ask - how does the plant grasp and hold the tiny amount of energy contained in photons of some 680 mμ wavelength (1.7 E.volt). I will try to summarize present facts and speculations and for more details have to refer to, e.g., a recent review article by Dr. Hoch and myself (ref. 12).

METABOLIC ENERGY UNITS

Chlorophyll, as it occurs in solution, will feel the impact of a quantum (remains in the excited state) for about 10^{-8} seconds and then

*The following abbreviations are used: ADP, ATP (adenosine diphosphate, triphosphate; DCMU (3-[3,4-dichlorophenyl]-1, 1-dimethylurea); DCPIP (dichlorophenolindophenol); FMN (flavin mononucleotide); g (a constant times the ratio of microwave frequency over the magnetic field strength at which electron spin resonance signals are found); PMS (phenazine methosulfate); TPN, $TPNH_2$ (oxidized and reduced triphosphopyridine nucleotide).

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forgets the encounter, returning to its ground state. But in the plant, the quantum once caught is guided towards a suitable "sink", trapped and put to work. Electrons and protons are released, ultimately from water, passed on and locked tight in chemical bonds such as in $TPNH_2$ (reduced triphosphorpyridine nucleotide). $TPNH_2$ as most of you know is a common unit of reducing power in the cell. Its redox-potential* (-0.32 volt) is close to that of molecular hydrogen (-0.42 volt), but it is much more manageable in cellular metabolism. It is a relatively stable compound which can be stored in the freezer for months. This is a considerable gain in stability if we compare the 10^{-8} seconds' lifetime of the original excited chlorophyll molecule. To achieve this, of course, the plant pays a price: at most one-third of the absorbed energy ends up in the storage bin. It is probably undesirable and even dangerous to have a high concentration of the reactive $TPNH_2$ in the cell. Therefore excess of this material, produced in photosynthesis and not immediately used (for instance, to build cellular protein machinery), is reconverted to TPN and the reducing power is stored in the very stable bonds of carbohydrate or fat (with uptake of CO_2). These materials can be transported from cell to cell, stored during winter and remobilized at will to regenerate molecules of $TPNH_2$ out of TPN (with release of CO_2). The so called "carbon cycle", running up hill during photosynthesis and down hill during respiration, thus should be considered mainly as an energy storage device.

We now should mention another cell constituent which functions as an energy carrier: a labile pyrophosphate bond as occurring in adenosine triphosphate (ATP). A small amount of energy (10 kcal./mole) is released upon breakage of this bond (yielding ADP and inorganic phosphate). For instance, all our muscle contractions are energized by this type of metabolic fuel. Each large unit of reducing power $TPNH_2$ can be converted into three of these small energy "coins":

$$3PO_4^{\equiv} + 3\ ADP + TPNH_2 + 1/2\ O_2 \longrightarrow 3\ ATP + TPN + H_2O \qquad (1)$$

This process is called oxidative phosphorylation, it occurs in almost all aerobic organisms and is the main purpose of respiration. It is a rather efficient process, retaining in the ATP bonds formed some 30 kcal./mole of the 50 kcal./mole free energy of the reaction. Except in primitive organisms such as bacteria and blue-green algae, special subcellular particles called "mitochondria" are the site for this conversion. They must consist of an ordered array of enzymes with different redox potentials which constitute an oxidation-reduction "chain". Stepwise, electrons are passed singly or in pairs from $TPNH_2$ (-0.32 volt) to oxygen (+0.8 volt). Some of these steps are "coupled" to a phosphorylation: For instance, cytochrome b (a heme-protein compound with an oxidation-reduction potential of 0.0 volt) may transfer an electron to cytochrome c (a similar component with a potential of +0.25 volt) while at the same time an ADP molecule is converted to an ATP molecule and so retains part of the energy of the reaction.

It has been established that also in photosynthesis these high energy phosphate bonds are generated. Arnon and coworkers (ref. 1) showed that this happens directly and concomitant (coupled) to the reduction of TPN (or

*All oxidation reduction potentials quoted are E_o' values at pH 7.

other oxidants) and have determined a ratio of 1 ATP formed per one molecule of $TPNH_2$ reduced (i.e., per 1/2 O_2 evolved). On first sight the photophosphorylation of the chloroplast appears quite different from the oxidative phosphorylation occurring in the mitochondria; in the first case $TPNH_2$ is generated, and in the second case it is combusted. However, as we will discuss later, the possibility is not excluded that ATP formation in photosynthesis occurs in a reaction step very similar to one of the respiration steps coupled to phosphorylation.

CHLOROPLAST REACTIONS

Isolated chloroplasts, as was found by Hill in 1937, can still evolve oxygen and reduce an added substrate. Oxidants of widely varying redox potential and chemical nature can be reduced: ferricyanide, quinones, dyes, cytochromes. Only in recent years have optimal conditions been established for obtaining fast reduction of exogeneous TPN (which requires a special enzyme present in the chloroplast) and for photophosphorylation coupled with most (but not all) Hill reactions. "Coupling" between a reduction and phosphorylation is indicated if the addition of inorganic phosphate and ADP (i.e., the generation of ATP) increases the reduction rate. What must happen is that the intermediate step involved in both processes cannot operate optimally if deprived of its natural function. In the absence of ADP, etc., this "friction" can be relieved by so called uncoupling agents which destroy the ability of the involved reaction step to phosphorylate. Some oxidants - for instance, the dye 2-6 dichlorophenol indophenol (DCPIP) - are reduced with good rate without concurrent phosphorylation. Phenazin methosulfate (PMS) does not become reduced or yield evolution of oxygen but will still mediate photophosphorylation. Due to aging or specific poisons (for instance, DCMU), chloroplasts can lose their ability to evolve oxygen - while retaining their ability to produce reducing power in the light: As Dr. Vernon will discuss in more detail - the oxygen evolution step can be bypassed by the addition of a suitable reductant.

Finally we should mention that chloroplasts which are aged or treated in special fashion can photo-oxidize a number of reduced compounds such as dyes, cytochrome, and ferrocyanide. Oxygen is now taken up instead of released. Even more complex: one can select conditions such that the light conversion carried out depends upon the color of the light used. One wavelength may induce photo-oxidation, the other photoreduction. An example of this wavelength dependent behavior is shown in fig. 1; we will come back to this in greater detail later.

With such a multitude of conversions to be studied, chloroplasts appear the ideal material to learn more about the essential phenomena of photosynthesis.

ABSORPTION AND FLUORESCENCE

Basic laws of photochemistry are that light cannot act unless it is absorbed and that primarily a single photon interacts with a single pigment

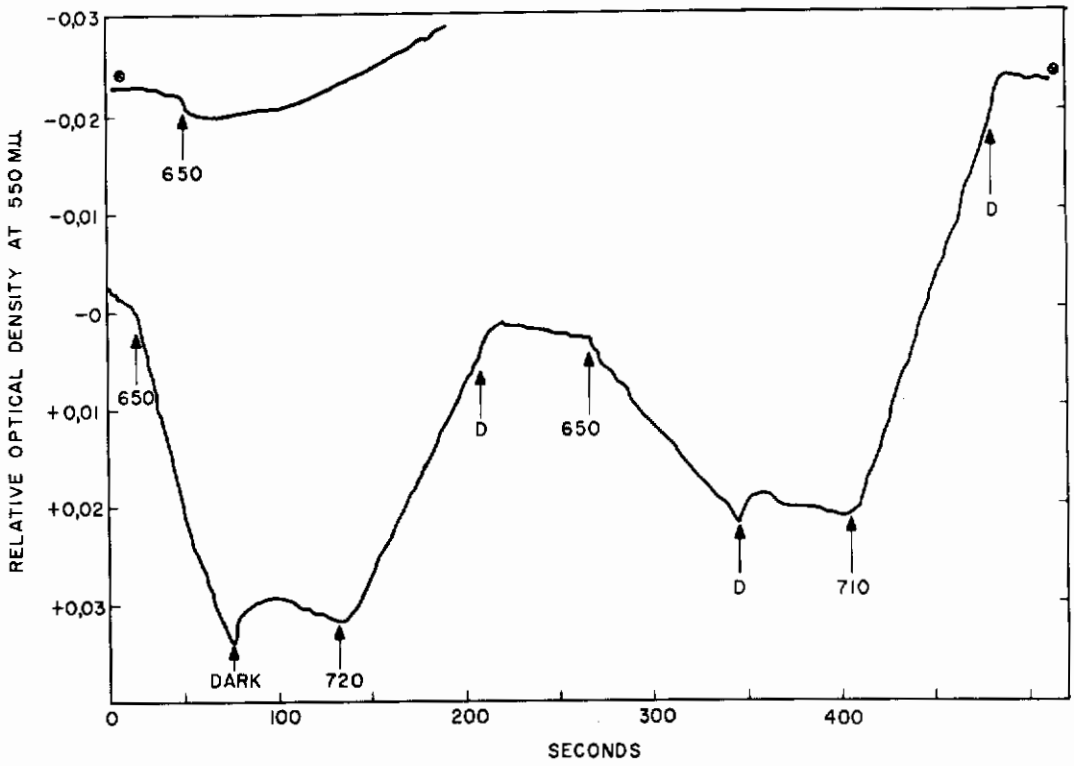

Figure 1. Reduction and oxidation of cytochrome c by spinach chloroplasts. Partially reduced cytochrome c is added to chloroplasts which were mildly treated with detergent. Light of 650 mμ wavelength (absorbed by chlorophyll b mainly) causes reduction of cytochrome c. Light of longer wavelengths (710-720 mμ, absorbed by chlorophyll a only) induces oxidation (decrease of optical density at 550 mμ).

molecule. Quite an array of pigments sensitizing photosynthesis occur in the plant kingdom. The most (and maybe only) essential ones are: bacteriochlorophyll found in photosynthetic bacteria - which do not evolve oxygen - and chlorophyll a, occurring in all plants capable of photosynthetic oxygen evolution. Carotenoid pigments are found abundantly in all these organisms, to a variable degree they act as sensitizers of photosynthesis, but their major function is as yet unclear.

Most aerobic organisms possess besides chlorophyll a one or more so called accessory pigments: green plants have chlorophyll b, red and blue algae several phycobilins. Especially the latter accessory pigments absorb most strongly in a wavelength area where chlorophyll a absorption is weak. It has often been proposed that this would help the plant to more fully utilize all wavelengths of the solar spectrum. Probably this speculation is still meritorius, since no evidence has yet come to light pointing to a specific photochemical role of accessory pigments: Fluorescence data have indicated that light absorbed by these pigments is efficiently transferred to chlorophyll a (cf. below). Several plant species are known which (besides carotenoid) do not have any other pigment than chlorophyll a and carry out photosynthesis just the same.

A chlorophyll molecule after absorbing a photon (of any wavelength) can dispose of it in several ways: (a) by passing it on to a neighboring chlorophyll molecule (resonance transfer); (b) by re-emitting a photon of red (680 mμ) light (fluorescence); (c) by returning to the ground state, wasting the excitation as vibrational energy; (d) by a return to the ground state coupled with a chemical conversion.

Fluorescence, although a loss process and in vivo occurring with relatively small yield (~3%) has been a useful tool to prove - among other things - the occurrence of the first mentioned event in photosynthesis: Light absorbed by phycocyanine or chlorophyll b can be re-emitted as fluorescence by chlorophyll a and depolarization studies have indicated that light absorbed by one chlorophyll a molecule can be re-emitted by another one after having passed through several.

A peculiar phenomenon especially noticeable in red and blue-green algae was observed by Duysens (ref. 5): Accessory pigment appears to be more efficient in exciting chlorophyll a fluorescence than chlorophyll a itself. The author had to conclude that there occurred two different groups of chlorophyll a: one associated with accessory pigment, free to fluoresce and active in photosynthesis (cf. later). The other - actually the largest - group was not connected with accessory pigment, non-fluorescent and presumably inactive in photosynthesis. As we will see later this hypothesis of two functionally different groups of chlorophyll a has proven to be quite useful.

THE PHOTOSYNTHETIC UNIT

In all measurements of substrate conversion such as of oxygen evolution, ferricyanide reduction or ATP formation, the information pertains only indirectly to the light reaction, being separated from it by many seconds and several reaction steps. To approach the photochemical events

one has to avoid interference of dark reactions and observe phenomena as close as possible to the pigment system in which it all starts. Although not the first, the most elucidating attempt to separate the "photo" part from the "synthesis" part was published in 1932 by Emerson and Arnold (ref. 7). For our present discussion the most important aspect of this work were experiments in which brief (10^{-5} sec.), bright flashes of light were alternated with dark times of sufficient length. Presumably so many photons were given in each flash that all available light traps were filled. Long enough dark periods were given to allow the various enzymes to perform their duty and present a fully "discharged" system to the next flash. It then was found that no more light could be caught in a single flash than one quantum per 200 to 300 chlorophyll molecules: (In a single flash at most one oxygen molecule was evolved per 2500 chlorophylls and 8-10 quanta are required per oxygen.) This entity of 300 chlorophyll molecules - often defined as the "photosynthetic unit" makes good sense from many aspects: It is a group - at least statistically - of pigment molecules arranged closely around a conversion center. A quantum falling in any one of these is transferred towards the center and caught. The center is the entrance to the enzyme chain, which - as shown by other experiments - is capable of performing a complete act of O_2 evolution and CO_2 reduction in about 10 milliseconds. An optimally designed system thus would require a quantum flux into the center of the same rate. But even in full sunlight a single chlorophyll molecule absorbs photons much less frequently than a hundred times per second: although one of the densest pigments known, its "cross section" is too small. Therefore the stacking of several hundred molecules - cooperating as if they were one - is a major feat of photosynthesis: with it the influx of light can be matched to the capability of the enzyme machinery. Actually one can observe that plants exposed to strong light, tend to make smaller pigment assemblies (to avoid overexposure) and that shadow plants have a large amount of pigment per unit. The unit thus functions as a funnel or a chemical lens, with variable aperture. The actual mode of operation of this collecting device has been disputed in recent years.

The first alternative in explaining energy migration towards a trapping center is resonance transfer, photons or rather excited states can wander as such from pigment to pigment molecule. The only rigid requirements for this mechanism are: that the molecules are close neighbors, and that their absorption and fluorescence bands overlap, i.e., this type of energy migration will take place from one absorption band to the other if the second band is located at a slightly longer wavelength (lower energy). The trap therefore should be a pigment and absorb at a longer wavelength than any other molecule of the assembly.

Transfer from one pigment molecule to another (for instance, from chlorophyll b or from carotene to chlorophyll a) has been observed in solution, which proves that in principle there is no need for a specific mutual orientation. In vivo, chlorophyll, concentrated in the lamellar structure of the grana, must occur in very high local concentration (> 0.1 molar). During the lifetime of the singlet excited state (10^{-8} sec.) there seems ample time for the photon to wander about and find the trap.

The fact, however, that such typical lamellar structures occur in all photosynthetic tissue has led to the speculation of an alternate mode of energy migration towards the trapping centers a concept, borrowed from

solid state physics: Excitation of any one pigment molecule yields a hole and an electron. Through what would be analogous to conduction bands these travel towards trapping centers (which now are not required to be pigments). In contrast to the first alternative - which as we shall see fits most present data - no convincing evidence has yet been brought forward to support the second one. Especially Arnold and coworkers have observed several phenomena reminiscent of "solid state" events. These, however, could as well pertain to reactions following energy transfer to the trapping centers - such as most likely is true for the unpaired electrons generated in the light (cf. next paragraph).

PHOTOCATALYST P700

Assuming energy migration via resonance transfer in the photosynthetic unit, the light trap - to be effective - should be a pigment and have an absorption band, slightly displaced towards longer wavelengths compared to the bulk of the chlorophyll (680 mμ). We have searched for this trap with rather specialized techniques: As in the Emerson-Arnold experiment photosynthetic material was exposed to recurrent brief and bright light flashes - the flash should "charge" the traps and the dark time allow the enzyme chain to discharge them. If excitation of the trapping center would change its absorption spectrum - this change should be restored during the dark. With sensitive spectrophotometry we have indeed found a flash induced and dark reversible bleaching: of an absorption band located at about 700 mμ. This band occurred in all oxygen evolving organisms tested. Fig. 2 shows a few of these so called light minus dark difference spectra, collected with chloroplasts. In this material the 700 mμ band undergoes a cyclic change of bleaching and restoration only in case a suitable substrate (such as TPN of FMN) is added. Since, we have studied this reversible photo-bleaching of "P700" in more detail: the quantum yield of the conversion was of the order of unity which proved its significance in the bulk of photosynthetic energy conversion. The conversion occurs as fast as we could measure on an oscilloscope, at room temperature as well as at -180°C, indicating the true photochemical nature of the bleaching. It appeared that the bleaching was sensitized by chlorophyll a (but poorly by "accessory pigment" - cf. later). We could purify the pigment system to some extent and remove more than 80% of the chlorophyll without impeding the reversible photobleaching. Such partly purified preparations facilitated a study of the oxidation reduction characteristics of the system P700 red (colored)/P700 ox (bleached): photobleaching could be substituted for by chemical oxidation. It appeared that a single electron transferring agent was involved with a redox potential of + 0.43 volt independent of pH (quite similar in fact to the system ferro/ferricyanide). Fig. 3 illustrates the identity of the difference spectra induced by either photo- or chemical bleaching and at the same time identifies P700 with a chlorophyll type pigment having its main absorption bands at 698 and 432 mμ. After losing a single electron, P700 remains as a free radical which appeared to yield a signal in the electron paramagnetic resonance apparatus (ref. 2). Table 1 shows that either photo- or chemical oxidation produced a number of unpaired electrons nearly equal to the number of P700 molecules in the sample. The signals were closely identical to those observed earlier by other workers upon illumination of photosynthetic material.

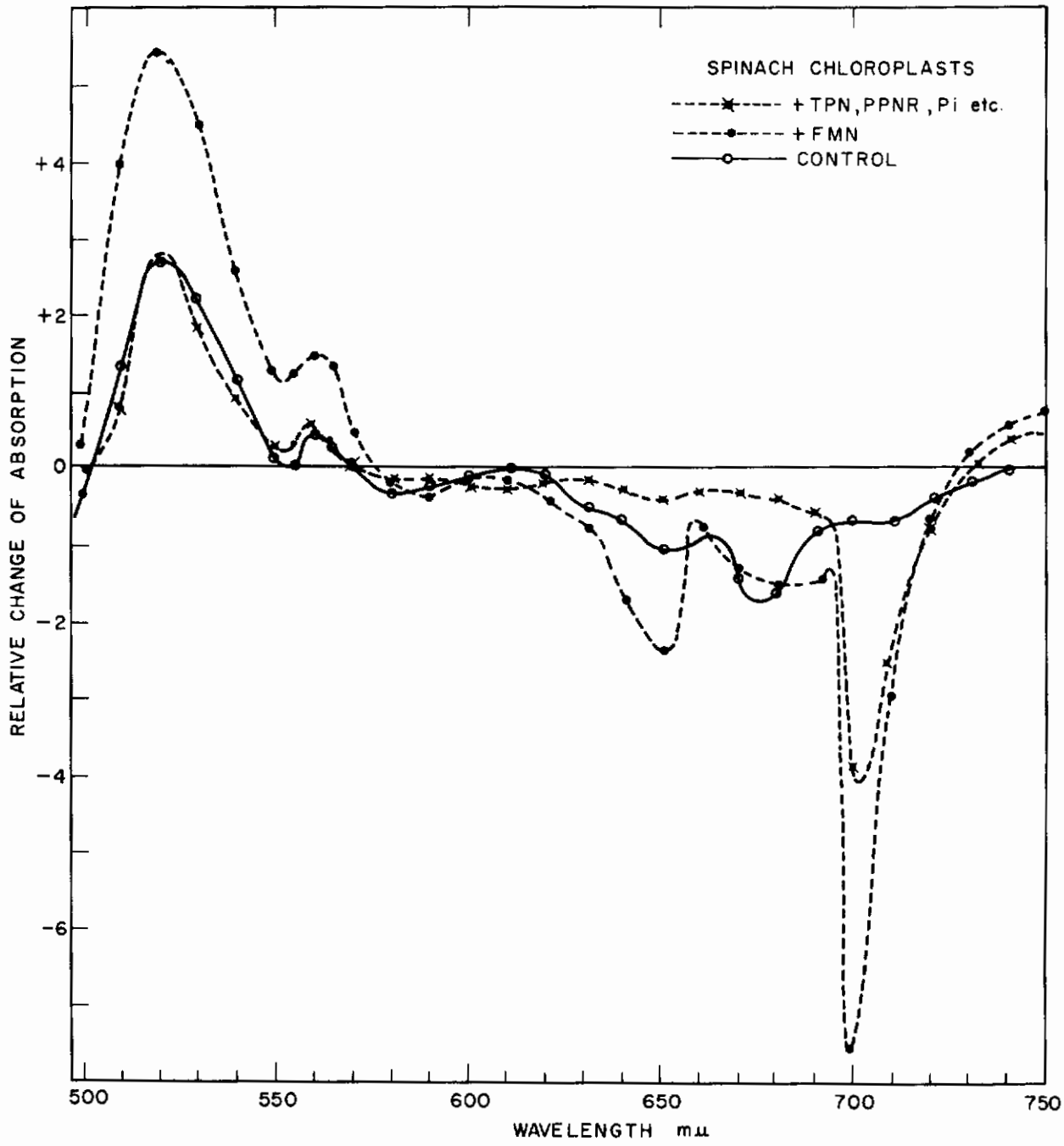

Figure 2. Cyclic absorption changes induced by light flashes in a chloroplast suspension. Flashes were given at a rate of 20 per second. The absorption was of the sample measured before and after each flash, the differences are plotted as a function of the monitoring wavelength. Note that the light minus dark difference bands - especially the one due to P700 - are affected in a typical fashion by added reagents. (Kok and Hoch to be published)

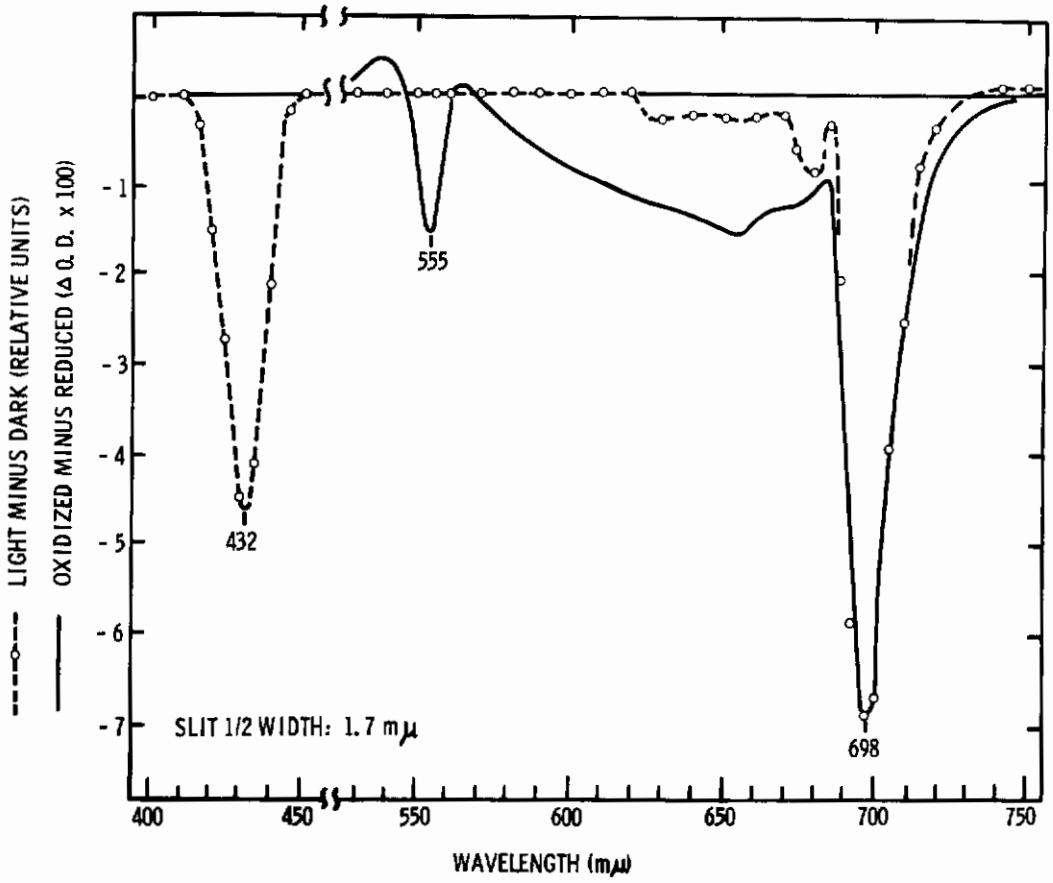

Figure 3. Two identical samples of chloroplasts - from which most of the chlorophyll but little P700 was removed - were placed in a double beam spectrophotometer. Illumination of one of the vessels induced the absorption difference drawn as a dashed line. Oxidation with ferricyanide induced the difference drawn as a full line. Note that in this material ferricyanide, but not light oxidized cytochrome f (as indicated by the band at 555 mμ). (From ref. 13)

Table 1

EPR Signal of Photocatalyst P700

Treatment	Concentration	
	Unpaired Electrons (from EPR)	Oxidized P700 (from absorption)
Oxidized minus reduced	$3.3(10)^{-6}$ M	$2.7(10)^{-6}$ M
Light minus dark	$3.3(10)^{-6}$ M	$2.7(10)^{-6}$ M
Extraction with 80% acetone	0	0

EPR signal:

center:	$g = 2.0025 \pm 0.0005$
half width:	$7.2 \pm .1$ g
fine structure:	none

THE FIRST PHOTOACT OF PHOTOSYNTHESIS

We have described how a quantum absorbed by a chlorophyll a molecule wanders toward P700, which upon excitation loses an electron. We have to assume that this electron is "caught" by a neighboring active group "X" on the enzyme surface, and thereby is reduced (cf. fig. 5):

$$X_{ox} + P700_{red} \xrightarrow{h\nu} P700_{ox} + X_{red} \qquad (2)$$

The light does work, by moving an electron against the chemical potential, that is, in the dark the reaction would run in opposite direction (in the absence of any activation barrier). The system X ox/X red therefore must have a potential lower than the system P ox/P red, the potential difference being a direct measure of the amount of chemical work done. So far we have been unable to identify X, but in an indirect way we have recently obtained information concerning its redox characteristics. It was already mentioned that "long wavelength chlorophyll a" (but not accessory pigment or the chlorophyll a associated with it) transfers its energy to P700: Activation spectra of the photobleaching of P700 showed a peculiar effectiveness of long wave red light in case chloroplasts were studied (cf. fig. 4 open circles). Using chloroplasts, exactly the same activation spectrum was found for the reduction of TPN in case the oxygen evolution step was circumvented by DCMU and ascorbate (cf. fig. 4 crosses) and also

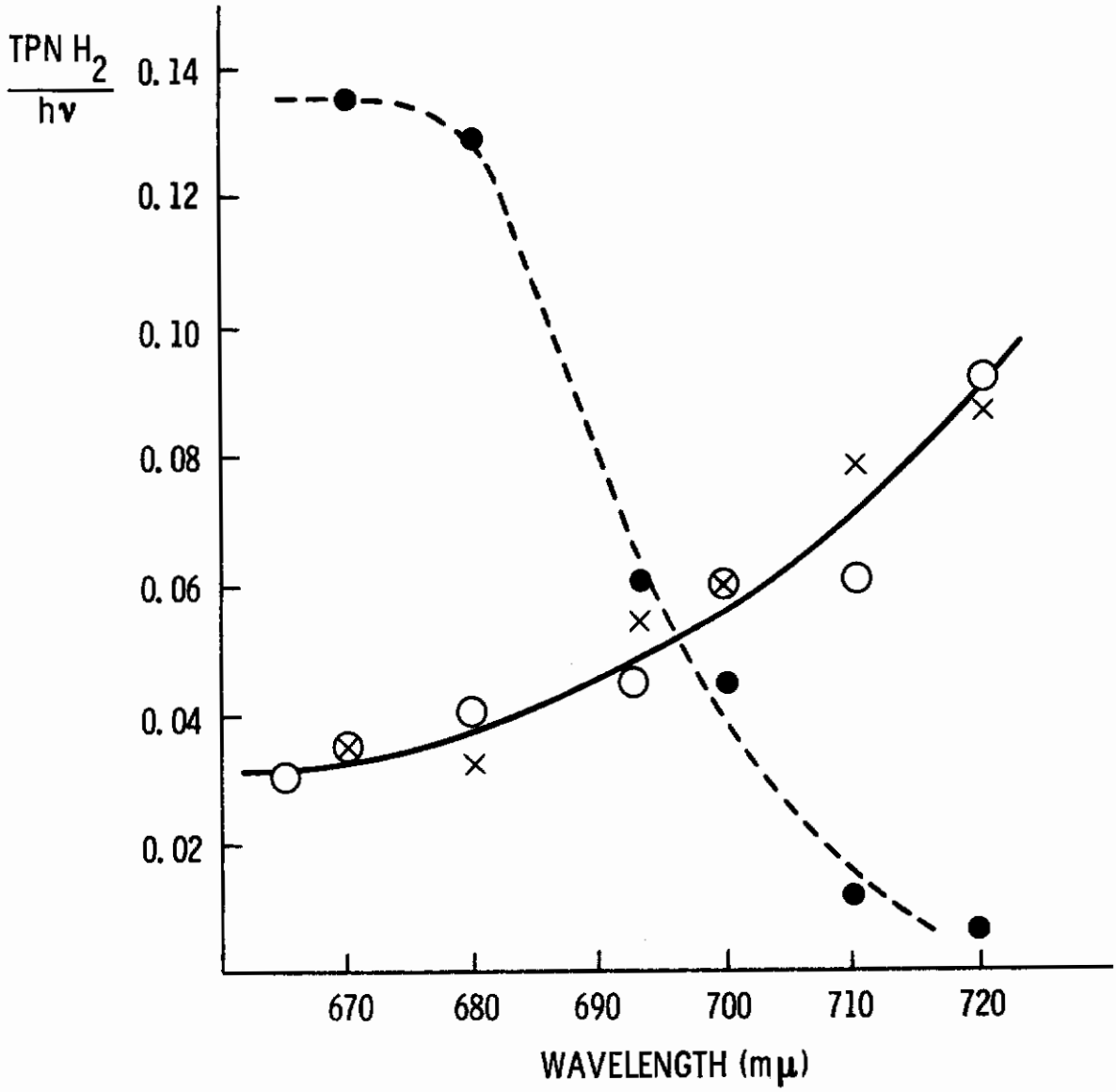

Figure 4. Activation spectra of chloroplast reactions. Dots: reduction of TPN with concomitant oxygen evolution. Crosses: reduction of TPN in the presence of DCMU ascorbate and dye - oxygen evolution step circumvented. Open circles: reversible photobleaching of P700, crossed circles: photo-oxidation of cytochrome c both measured with aged chloroplasts. Quantum yield plotted vs. wavelength, the first curve in absolute, the others in relative units. (Hoch and Kok unpublished)

for the limited reduction of a very low potential dye: benzyl viologen (-0.35 v.). We also observed that a high turnover rate of the P700 system accompanied these reductions.

Another instance of this long wavelength sensitization (crossed circles in fig. 4) proved the photo-oxidation of exogeneous cytochrome c or dye by chloroplast preparations. These latter reactions were stimulated by the addition of benzyl viologen, a very auto-oxidizable material.

By bringing the two components together we could show that oxidized P700 will oxidize reduced cytochrome c in the dark (according to eq. 3). This photo-oxidation process therefore might occur according to sequence (2-5):

$$X_{ox} + P700_{red} \xrightarrow{h\nu} X_{red} + P700_{ox} \qquad (2)$$

$$P700_{ox} + Cyt._{red} \longrightarrow P700_{red} + Cyt._{ox} \qquad (3)$$

$$X_{red} + Viol._{ox} \longrightarrow X_{ox} + Viol._{red} \qquad (4)$$

$$Viol._{red} + O_2 \longrightarrow Viol._{ox} + H_2O \qquad (5)$$

These data thus indicate that the primary electron acceptor of photoreaction 1 is a powerful reductant, the potential of the system X/XH being lower than -0.30 volt. Since we measured for the system $P700_{red}/P700_{ox}$ a potential of +0.43 volt, the work done by the light, or rather the energy retained in the products of photoact 1 can now be estimated. It amounts to about 20 kcal./mole - half of the energy which was originally available per Einstein of absorbed red light (40 kcal./mole). This primary conversion thus has an efficiency of about 50%.

EVIDENCE FOR TWO PHOTOREACTIONS IN PHOTOSYNTHESIS

Although the so-far-obtained elucidation of photoreaction 1 is quite a step forward - it does not describe photosynthesis. The reductant X_{red} is potent enough to do what we expect from it. But the oxidant $P700_{ox}$ is obviously too weak to oxidize water and yield oxygen. It is not far fetched to assume that a photoreaction similar to 1 is the sole process occurring in photosynthetic bacteria - which do not evolve oxygen. These organisms do make a potent reductant but require an external electron donor other than water.

Complete photosynthesis, thus needs another energy jump to bridge the potential gap between the P700 and the O_2/H_2O system (+0.8 volt) and most workers in the field think that this step is driven by a second photoreaction. The evidence for more than one photoreaction has accrued from three types of observations:

(1) Emerson and Lewis (ref. 8) determined the quantum yield of photosynthesis, as a function of wavelength, and found the efficiency dropped in an unexpected fashion at wavelengths beyond 680-690 mμ. This drop of quantum yield is illustrated in fig. 4 for the reduction of TPN by chloroplasts.

Far red light is absorbed but not converted. Later Emerson and coworkers observed that far red light could be used with better or even normal efficiency if an illumination of shorter wavelength light was given simultaneously. The rate of oxygen evolution produced by the two beams given simultaneously was greater than the sum of the rates produced by the individual beams and the effect since became known as the "enhancement" effect. Obviously photons absorbed by different pigments assist each other in the production of oxygen and the reduction of substrate.

(2) Blinks (ref. 3) found that if two light beams of different color - each of which yielded an equal steady state rate of oxygen evolution - were alternately thrown on a sample of algae, peculiar transient phenomena occurred ("chromatic transients"). For instance, when with a red alga 680 mμ light (absorbed by chlorophyll a) was replaced by 540 mμ light (absorbed by the accessory pigment phycoerythrin), the rate temporarily rose and then dropped again. The reverse phenomena occurred upon reswitching the beams. We presently think that changing the color of illumination, means a changed distribution of the photons over the two photoreactions which necessitates the cell to "change gears" and readjust the new rate.

(3) When in our own studies we tried to find out which colors were most effective in photo-oxidizing P700, it proved that, mainly light absorbed by chlorophyll a exerted the bleaching (cf. figs. 4 and 5). Peculiarly, light absorbed by accessory pigment (phycocyanin in experiment fig. 6) produced the opposite effect: it induced a formation of reduced pigment (a positive absorption change). A time delay between illumination and reduction indicated that this second, reductive, light effect was indirect and mediated by dark reactions (in contrast to the direct photobleaching of P700 sensitized by chlorophyll a).

All these phenomena could only be explained if more than one light reaction were involved sensitized by different pigments. The two steps exert a push and pull effect upon the intermediate P700 and collaborate in the evolution of oxygen. One of the most lucid demonstrations of the two photoreactions we have already presented in fig. 1.

THE SECOND PHOTOREACTION

The previous paragraph described ample evidence for the occurrence of (at least) two photoreactions. Little, if any, direct information concerning the second photoact is available. Before mentioning a few observations which have been claimed to pertain to it, we might first discuss: what requirements should this hypothetical step meet in order to complete the work done by the first photoreaction - which we presently think we know:

Firstly, it needs to yield oxygen, that is an oxidant with a potential higher than that of the system O_2/H_2O: +0.81 volt. Secondly, the first photoact leaves a weak oxidant ($P700_{ox}$), which should be re-reduced in order to allow it to again go through a cycle:

$$2H_2O + 4P700_{ox} \xrightarrow{\text{light}} 4P700_{red} + O_2 \quad \Delta F^\circ \approx 36 \text{ kcal.}$$

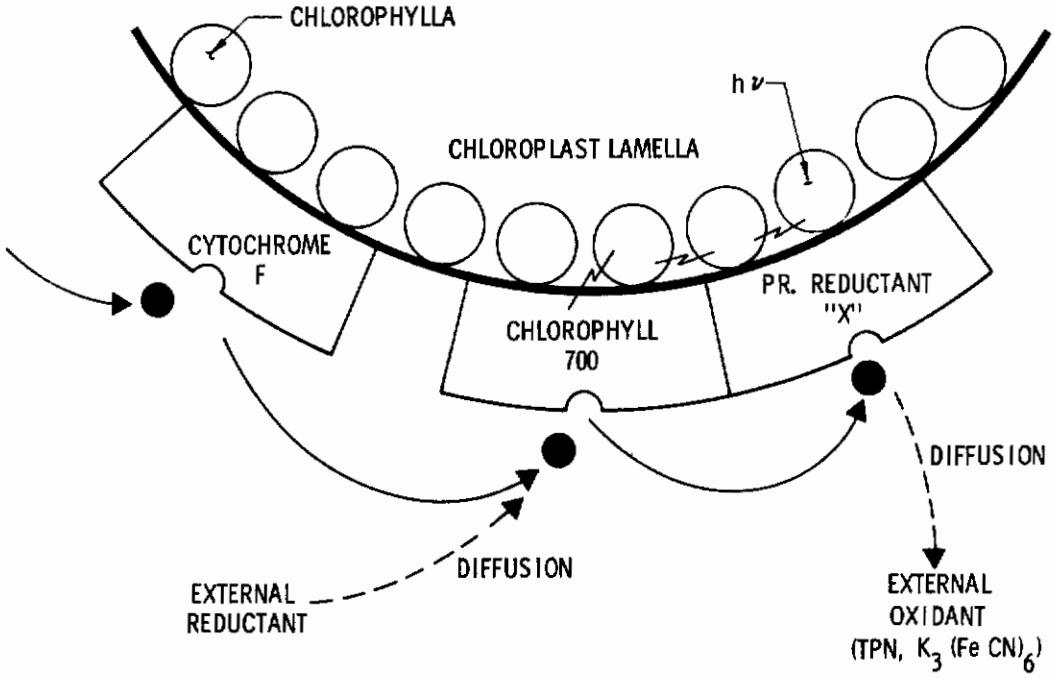

Figure 5. Illustration of photoreaction sensitized by long wavelength chlorophyll a. A photon guided toward the complex P700X induces the transfer of an electron. The remaining "hole" can be filled in the dark by reduced cytochrome f - a close neighbor on the particle - or by other reductants.

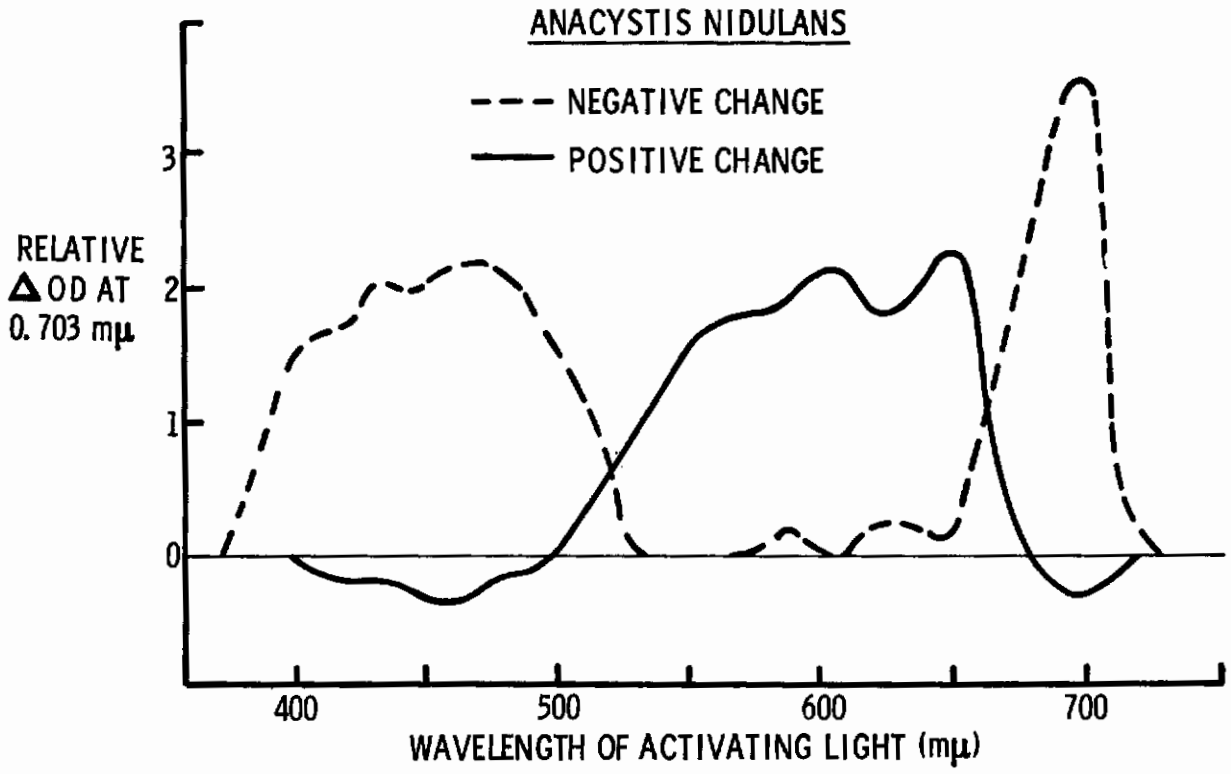

Figure 6. Activation spectra of the two light effects on P700. Dashed line: oxidation (negative absorption change), showing chlorophyll bands. Full line reduction (positive change), showing phycocyanin bands. Material: whole cells of the blue-green alga Anacystis.

The energy span between oxygen and P700 is rather small (~10 Kcal./eq.) and would only require one-fourth of the energy available per quantum if one electron were transferred per photon. As long as evidence to the contrary is lacking, one might as well assume that the second photoact has a similar efficiency as the first one - which as we recall of the 40 kcals./eq. available as light energy retained 20 kcals., or a span of 0.8 volt on the redox scale. This extra amount of energy would give some leeway, firstly, in assuming a potent oxidant and secondly, a stronger reductant (YH) than strictly necessary for the reduction of oxidized P700. If the system Y/YH had a normal potential of ~0.0 volts, there would be an extra amount of energy available corresponding to 0.4 volt in the reaction

$$Y_{red} + P700_{ox} \xrightarrow[ADP \longrightarrow ATP]{\text{cytochromes}} P700_{red} + Y_{ox} \qquad (6)$$

Hill and Bendall (ref. 10), who originally proposed this type of interaction between the two photoacts, assumed that reaction (6) was mediated by two electron transporting enzymes typical for the chloroplast cytochromes b and f (ref. 11). In analogy to what happens in respiration (cf. section 1), they proposed the oxidation of one cytochrome (b, 0.0 V) by the other (f, 0.36 V) was coupled to phosphorylation.

This scheme, which has become quite popular, is visualized in fig. 7. It shows the two photoacts as two batteries generating a redox voltage connected "in series" except for the stretch of "parallel" operation coupled to phosphorylation.

Recently Witt et al. (ref. 16), using a fast spectrophotometric method, could observe under certain conditions that P700, after being photo-oxidized, quickly (within a few milliseconds) recaptured an electron from cytochrome f - both at normal and at very low temperature (-150°C). In photosynthetic bacteria a similar temperature independent photooxidation of a cytochrome, sensitized by bacterio chlorophyll, had been observed earlier (ref. 4). These cases of temperature independent electron transport are a fascinating illustration of the peculiar arrangement of events in ordered living structures.

This interplay between P700 and cytochrome f (cf. also ref. 6) is an argument for the validity of scheme fig. 7, at least for the indicated site of photophosphorylation. However, it remains to be proven whether this is the exclusive or even the main path by which $P700_{ox}$ becomes reoxidized in photosynthesis. Also, no convincing evidence has come to light supporting the formation in the second photoact of a weak photoreductant (YH). Instead, our own data possibly indicate the formation of a strong one, comparable to XH made in the first photoact (Dr. G. Hoch pers. comm.). Therefore, to illustrate the cooperation between the two primary processes, the scheme of fig. 8 might be preferred. It tends to focus attention upon the difficulty which we feel underlies the necessity for two photoacts in photosynthesis: With 8 quanta available per molecule of oxygen produced, this is not a lack of energy. It is the kinetic problem in oxygen evolution of moving four electrons simultaneously against the thermochemical gradient. For this reason, in fig. 8 the energetic coupling between the two photoreactions is located in the oxidized moieties, rather than in the intermediate (single electron transfer) path of eq. (6). This is visualized by denoting $P700_{ox}$ or a dismutation product of it as a peroxide, functioning as the oxygen

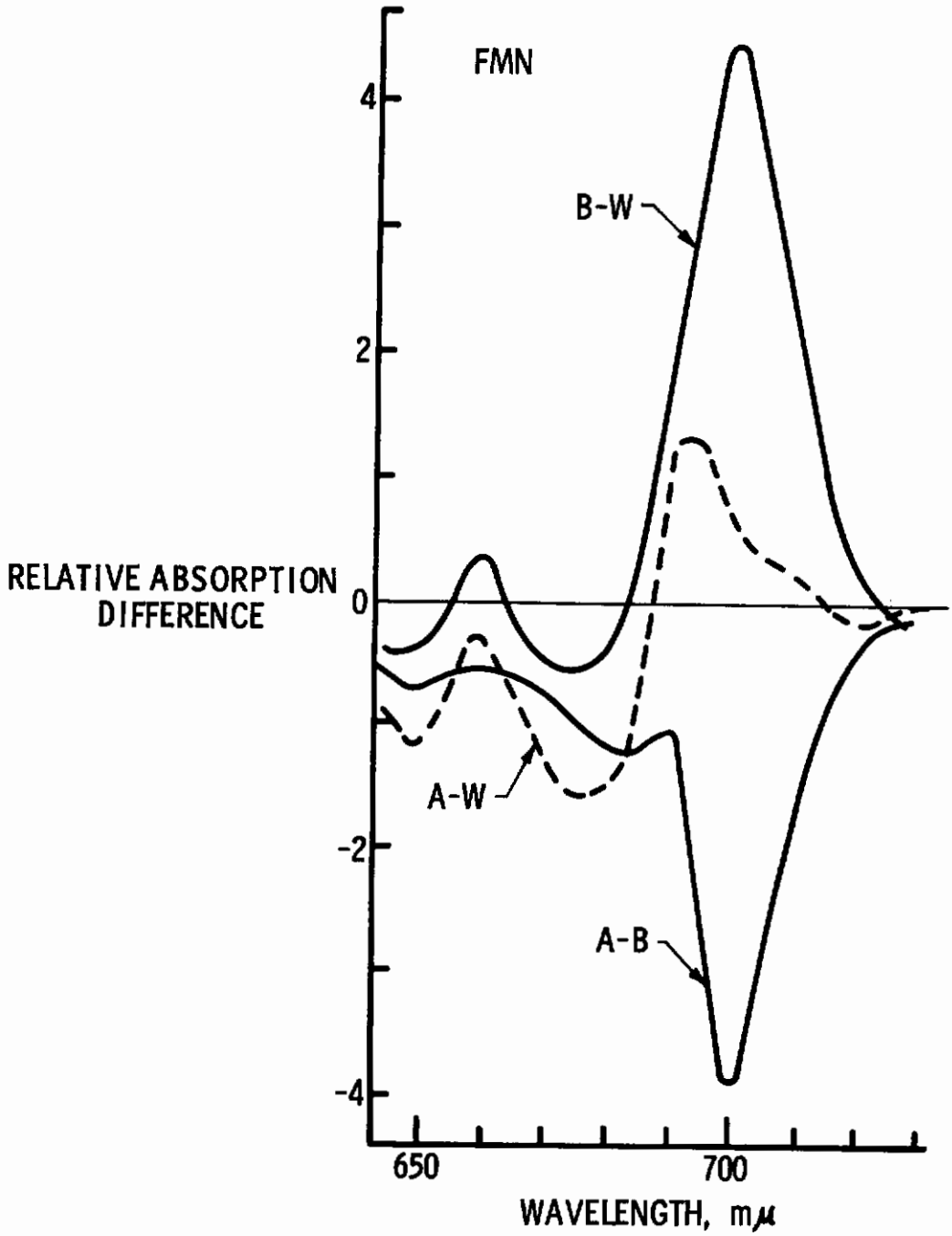

Figure 7. Hypothetical diagram of the two photoreactions as described in the text (ref. 10).

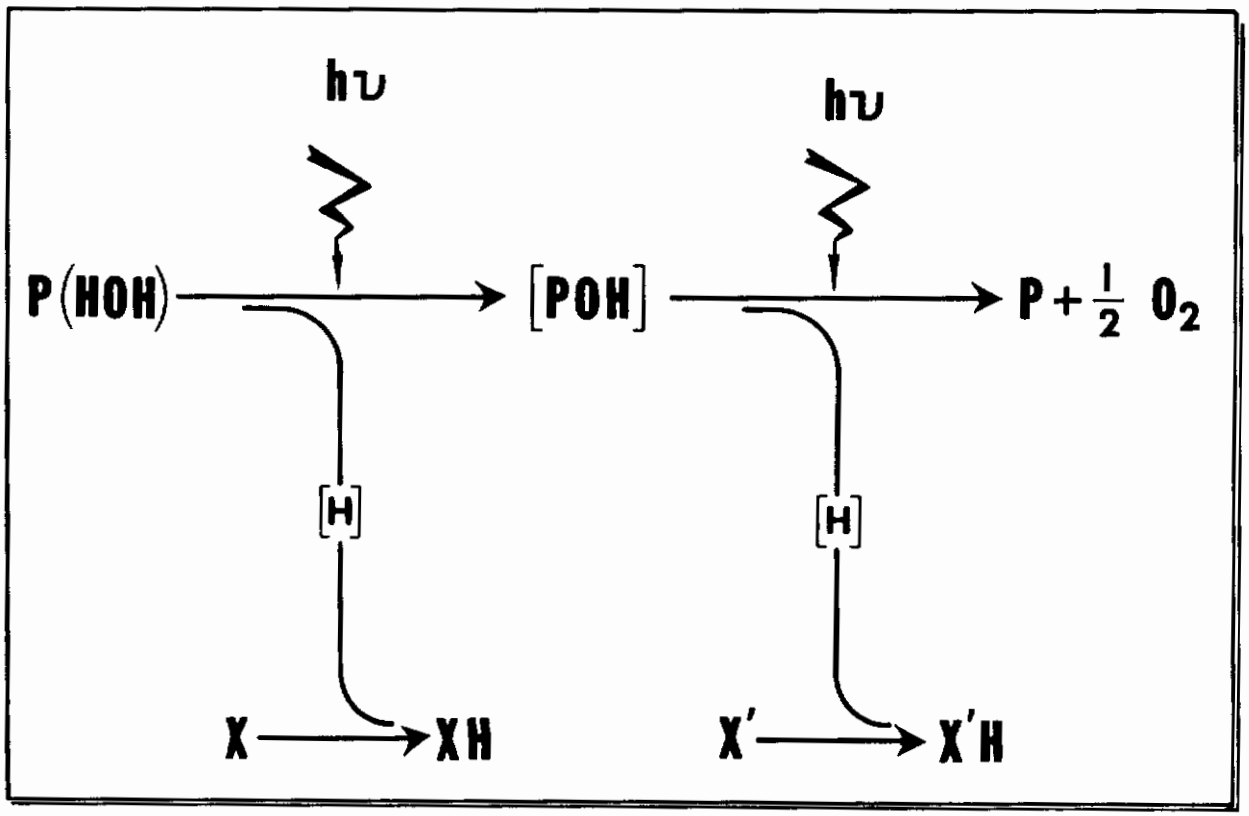

Figure 8. Hypothetical diagram of the two photoreactions visualizing "parallel" operation of the steps.

precursor for the second photoact.

The summary schemes such as the ones discussed are actually a reflection of our lack of knowledge especially about the mechanism of the oxygen evolution process. Not a single reaction step or intermediate of it has been characterized. This also leaves us largely ignorant about the interplay between the several steps: eight photochemical ones leading to one oxygen, two $TPNH_2$ and two ATP molecules in chloroplasts and to a whole cell in vivo.

Questions of great interest to this audience are, of course, where in this complex process is the rate limiting step and why have we as yet been unable to find an alga which can utilize strong light efficiently? Presently we have no satisfactory answer to these questions. The indications are that the rate determining reaction is located in the oxygen evolving system. But the conventional concepts of the rate limiting step as an enzymatic conversion, which cannot surpass a certain velocity, may be too simple. We should mention in this connection McLeod's (ref. 14) observation (with whole algae) that not only the quantum yield, but also the rate in strong light - the saturation rate - drops beyond 700 mμ. Off-hand, one would expect that the process will run with maximum velocity as long as all traps, "emptied" by the enzyme chain, are immediately re-excited - which should be the case in infinitely strong light (where even very weak absorption in one (or both) photoact would yield enough excitations). Therefore a color dependence of the light saturated rate indicates that two processes with different sensitization can counteract or at least be in the way of each other. This phenomenon must be closely connected to the problem discussed in the next paragraph.

THE DISTRIBUTION OF LIGHT BETWEEN THE TWO PHOTO STEPS

We finally should consider the question: how does the plant provide for an optimal distribution of absorbed photons between two light steps so that both will run synchronously, securing optimal overall efficiency. So far we have tacitly adhered to Duysens' original interpretation of his fluorescence data: that of two independent pigment groups both having chlorophyll a - one connected with accessory pigment, the other not connected and extending its absorption further in the red. Each group sensitizes a photoreaction carried out by its own trapping center and operates essentially independently of the other (cf. fig. 9). The only coupling then is between the final - relatively stable - reaction products.

Optimal performance of this model requires that the two photosystems receive equal quantum influx - i.e., the two sets of sensitizing pigment should absorb an equal portion of the light. Under natural conditions - in sunlight - it would be rather immaterial whether one pigment group absorbed only the green and the other only the red part of the spectrum or both groups had exactly the same absorption characteristics. In laboratory studies with monochromatic light, however, these two possibilities would yield entirely

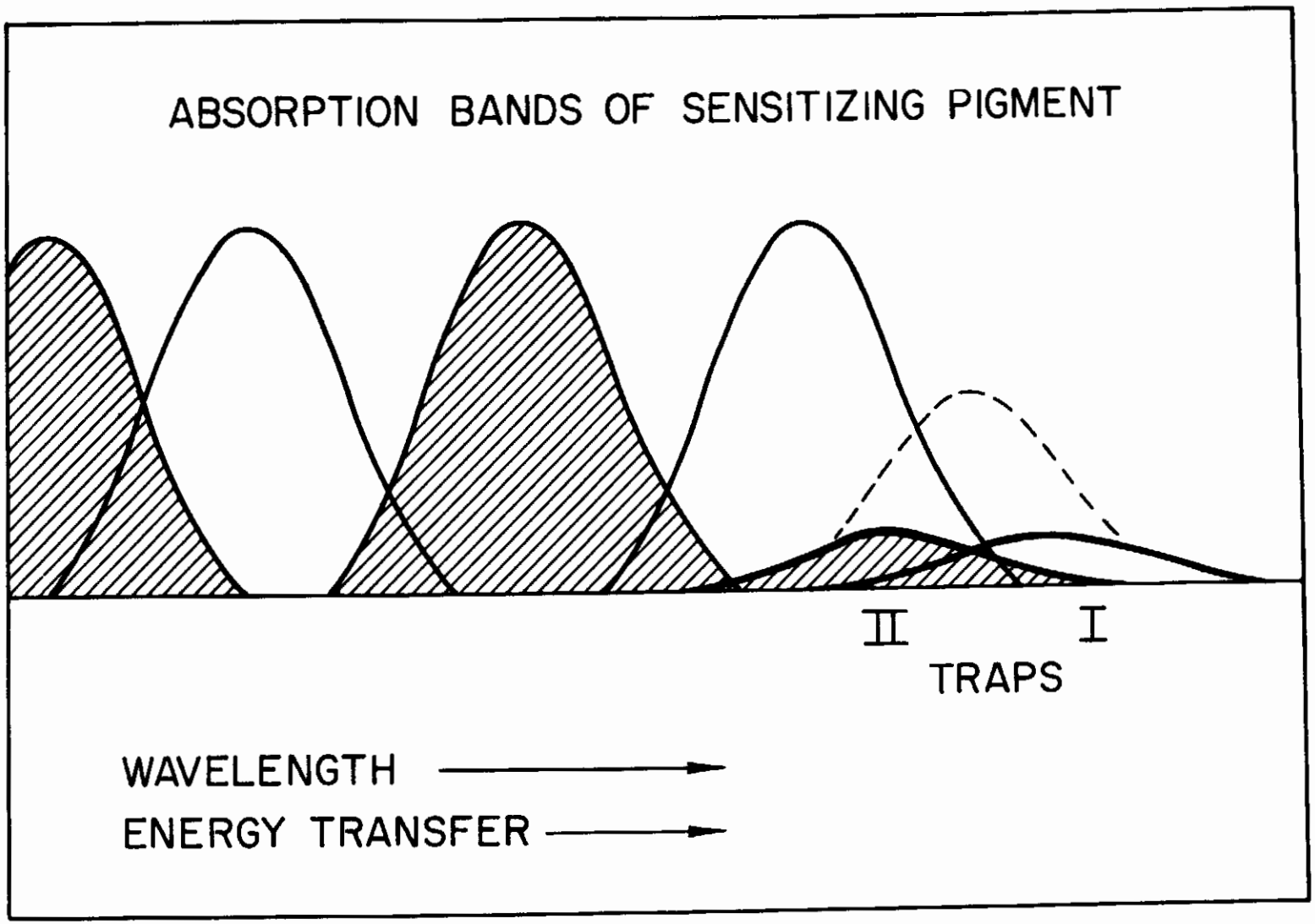

Figure 9. Schematic illustration of energy flow in photosynthetic pigment arrays. Two trapping pigments, present in small quantity are assumed. Either each trap has its own sensitizing pigment group (one shaded, the other not) or a more sophisticated mechanism exists to equally distribute the quantum flow. The number, location and height of the different chlorophyll bands are arbitrarily chosen. Dashed curve indicates a long wave pigment, connected to trap I.

different results: Only at wavelengths where both pigment groups absorb equally strong can a high yield be expected. Constancy of quantum yield over an extended spectral region then must indicate that the two pigment groups are not only equal in respect to amount but also in respect to absorption spectrum. Conversely, if the two systems contained pigments with different absorption spectra the quantum yield would fluctuate strongly with wavelength.

As an alternate hypothesis to explain a proper distribution of quantum flow, one could assume that most or all pigments are able to transfer their excitation towards the longest wavelength absorption band present. In this case essentially a single pigment group suffices to sensitize both photoreactions. This would require the same total amount of pigment as needed in the first discussed model, but there would be no concern about maintaining two units equal. The problem in this case is how to regulate the influx of quanta into the two steps. Franck (ref. 9) has long defended a two quanta hypothesis in which in essence a single chlorophyll molecule underwent two successive excitations. The first excitation brought the molecule into a metastable state, which presumably shifted its absorption band towards longer wavelengths and so made it an efficient trap for a second excitation.

An operationally similar mechanism can be based on the more likely assumption of two individual photochemical events of the type shown in eq. (2). Light will travel to the longest wavelength band available, initially this will be e.g. P700 (trap I in fig. 9). The first quantum received will bleach this band (initiating photoact 1) and now another sink (trap II in fig. 9) will be the longest wavelength band. This sink does not necessarily have to be bleached but it should yield the restoration of P700 so the third quantum will fall again in the proper "bucket". Such a servomechanism could insure a nearly complete independence of quantum yield upon wavelength.

Actually, studies of quantum yield as a function of wavelength, of enhancement effects (refs. 8A, 15) and of the complex absorption band of chlorophyll (ref. 3A) so far have not allowed a choice between the two discussed possibilities or pinpointed a still different mechanism. The data probably reveal traits of both. The constancy of the yield over a wide spectral range, covering several chlorophyll bands found in green plants, seems to comply with the second model. However, the decline of the yield at long wavelengths definitely indicates the existence of an independent long wave pigment sensitizing photosystem I exclusively.

You might realize that in this and the preceding paragraphs I have mainly attempted to confront you with present-day problems and thinking of people interested in the photoevents in photosynthesis. And if after reading this paper you feel more confused than before, you have come somewhat closer to being one of us.

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