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Physical Aspects of the Electron Transport and Photophosphorylation in Green Plants

Von

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The fractionation of the two subsequent reviews on the primary processes of photosynthesis into a physical and a biochemical subdivision is intended to characterize two technically different approaches to the subject. However, it will become apparent that the application of different methods led to almost identical concepts.

On Physical Methods in Photosynthesis Research

A variety of physical methods were applied e. g. X-ray small angle scattering (KREUTZ 1970), dielectric dispersion (GORDON 1973), electrostatic induction (FOWLER and KOK 1974b), polarography (JOLIOT 1966, VATER et al. 1968) and a couple of spectroscopic methods (ESR [BEINERT and KOK 1964], IR [KATZ and NORRIS 1973], CD [PHILLIPSON et al. 1972], light scattering [DEAMER and PACKER 1967]) among which the spectroscopy in the visible and the UV spectral region was dominating. It relies on the influence of chemical reactions and changes in the microenvironment of a pigment molecule on its absorption or emission behaviour. Thus changes in absorption or emission of pigments intrinsically incorporated in or artificially added to photosynthetic membranes were used as indicators for molecular events ranging from the primary photochemistry over the electron transport and the concomitant ion transport phenomena to photophosphorylation.

The kinetic resolution of these events was favoured by the fact that the primary processes of photosynthesis are to be stimulated by flash light which today allows for a time resolution in the range of picoseconds (NETZEL et al. 1973). The principle of a flash spectrophotometer is illustrated in fig. 1. The sample, a suspension of algae or of isolated chloroplasts is contained in an absorption cell mounted in a photometer. The intensity of the interrogating light is kept low to avoid excitation of the sample. The primary processes of photosynthesis are stimulated by high energy flashes. Changes in transmission (or emission) are recorded and kinetically analysed. Compared to the first set-ups (WITT 1955) flash photometry was refined, especially by the introduction

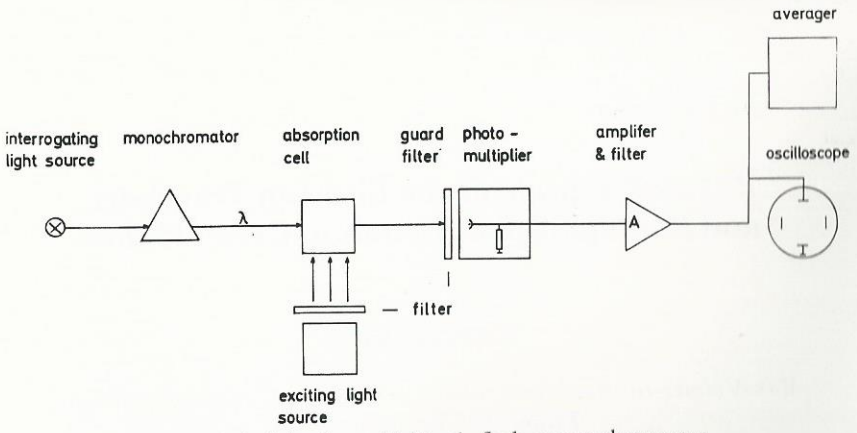

Fig. 1. Principle of a rapid kinetic flash spectrophotometer.

of repetitive excitation and averaging (WITT 1967a, RÜPPEL and WITT 1969), to overcome the high noise level which is caused by the necessity to keep the intensity of the interrogating light low (for recent reviews on instrumentation see KE 1972, JUNGE 1975).

The major advantage of flash spectrophotometry is its extremely high time resolution at a reasonably high sensitivity (for quantitative figures, see RÜPPEL and WITT 1969, JUNGE 1975). The crux of the method lies in the attribution of certain absorption changes to one and only one molecular event. Flash excitation of chloroplasts initiates a complex pattern of events. Since several of these may influence the spectroscopic properties of pigments somehow, control experiments have to be designed to make the attributions unequivocal. Usually these require physically or chemically modified chloroplasts. Since these modifications are more readily imposed on chloroplasts of the broken type (outer envellope broken) the majority of flash spectrophotometric results pertains to broken chloroplasts. It is probable, although not proven, that the principle mechanisms of the primary processes of photosynthesis which work in broken chloroplasts are operative under physiological conditions, too.

The Linear Electron Transport Chain

The concept of two photochemical reactions cooperating in series to drive electrons from water to NADP is part of textbooks. The lift in the free energy of an electron during the photochemical and subsequent dark steps is illustrated in fig. 1 of the subsequent review by Dr. HAUSKA. Flash photometric studies contributed to the characterization of the sequence of electron carriers. But more specific was the kinetic evaluation of the electron transport chain. Nine components, including the reaction center chlorophylls, were spectroscopically and kinetically resolved: chlorophyll-a_I or P 700 (KOK 1957, KOK 1961, RUMBERG and WITT 1964), chlorophyll-a_{II} or P 680 (DÖRING et al. 1967, 1969, GLÄSER et al. 1974, VAN GORKUM et al. 1974, cytochrome-b_{559} (KNAFF and ARNON 1969a, MATHIS et al. 1974), cytochrome-f (WITT et al. 1961a, HILDRETH et al. 1966, MARSHO and KOK 1970), C 550 (KNAFF and ARNON 1969b, VAN GORKUM et al. 1974), plastocyanin (KATOH and TAKAMIYA 1963, HAEHNEL 1975), plastoquinone (BISHOP 1959, RUMBERG et al. 1963, AMESZ 1964, SCHMIDT-

MENDE and WITT 1968), P 430 (HIYAMA and KE 1971, KE 1973) and X 320 (STIEHL and WITT 1968).

The dynamic interaction of some of these components is illustrated in fig. 2. Let us follow the sequence of events from absorption of light quanta to the production of the final reducing equivalents.

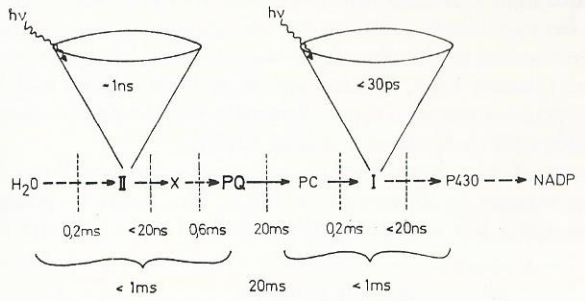

Fig. 2. Some kinetic constants of the resonant energy transfer within the antennae and the linear transport chain.

Both photochemically active reaction centers (denoted I and II in fig. 2) are surrounded by an antennae system of in the order of 300 chlorophyll molecules. It is still open whether or not these antennae units are all interconnected with each other (for a review, see BORISOV and GODIK 1973). And it is still open under which conditions the spill-over of photic energy from antennae belonging to reaction center I to those belonging to II and vice versa is efficient or not (for references, see SUN and SAUER 1972).

A quantum of light absorbed by one of the antennae systems is transferred into the respective photochemical reaction center within extremely short times. The total transfer time is less than 30 psec for photosystem I and less than 1 nsec for photosystem II (BORISOV and ILINA 1973). Excitation of the reaction centers rapidly initiates the primary charge separation. The halftime of the photooxidation of chlorophyll-a_I was determined via its absorption changes at 700 nm to be less than 20 nsec (K. WITT and WOLFF 1970). The same rapidity was concluded for the photooxidation of chlorophyll-a_{II} from an indirect argument based on the velocity of the primary charge separation (WITT 1971 based on WOLFF et al. 1969).

That the negative directed absorption changes at 700 nm indicate an univalent photooxidation of a chlorophyll-a is firmly established both by redox titration (KOK 1961) and ESR (BEINERT and KOK 1964, BEARDEN and MALKIN 1972, NORRIS et al. 1971). That they indicate a primary photochemical step was suggested by their appearance even at —150 °C (WITT et al. 1961b). Doubt as to the attribution of the absorbancy changes of chlorophyll-a_{II} at 682 nm (DÖRING et al. 1969) is almost eliminated in favour of an analogous interpretation by recent experiments (GLÄSER et al. 1974, VAN GORKUM et al. 1974).

The reducing and the oxidizing equivalents created by both reaction centers are transferred to neighbouring carriers within less than 1 msec, as illustrated in fig. 2. Both reaction centers are thus ready for another shot within less than 1 msec. The rate limiting step for the overall process, the reoxidation of plasto-

hydroquinone by plastocyanin is more than one order of magnitude slower (for a review of kinetic data, see WITT 1967b). From these kinetic data the following *dynamical behaviour* becomes evident:

Under excitation with flashlight of sufficiently short duration (halftime much less than 200 μsec) each reaction center transfers at most one electron to its primary acceptor. While under excitation with a step of light each reaction center performs several turnovers until it exhausted its donor or its acceptor pool. Then both light reactions turn over with a slower rate determined by the limiting step between them. Limiting for the number of rapid turnovers in the initial phase is the size of the plastoquinone pool for light reaction II (capacity 6—8 electrons [JOLIOT 1965, MALKIN and KOK 1966, STIEHL and WITT 1969]) and the donor pool for reaction center I, respectively (capacity about 3 electrons [STIEHL and WITT 1969, MARSHO and KOK 1970]).

An example of how these dynamic properties can be read out from records of absorption changes is illustrated in fig. 3. The negative going absorption changes in the upper left and in the lower half of fig. 3 indicate the reduction

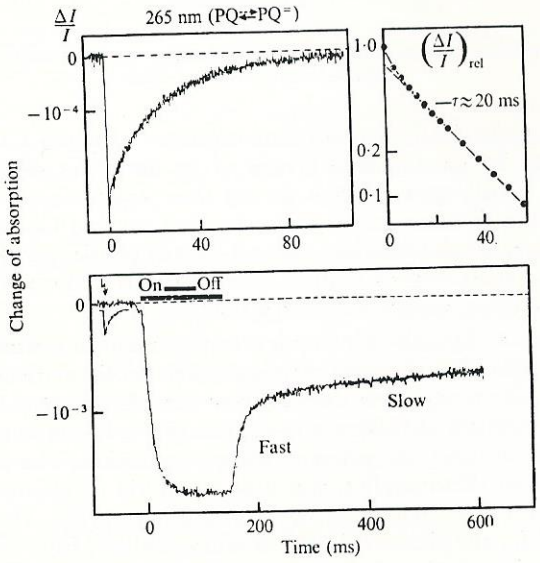

Fig. 3. Time course of the absorption changes of plastoquinone at 265 nm after excitation with a short flash of light (above) and under excitation with a light gate (below) after STIEHL and WITT (1969) (with permission from WITT, 1971).

of plastoquinone interrogated at a wavelength of 265 nm. The upper trace reflects the rapid reduction and the slower reoxidation on excitation of chloroplasts with a short flash of light. In the lower half of the figure (at a compressed ordinate scaling) the flash induced absorption change is compared with the one induced by excitation with a light gate. The duration of the gate is indicated by a bar. The extent of the flash induced change corresponds to the transfer of one electron. It is obvious that more electrons are accepted by plastoquinone on excitation with the light gate. The pool size is about 6 e⁻. From the relaxation which is biphasic it can be concluded that the pool of oxidizing equivalents created by excitation of reaction center I is of a smaller sice (about 3 e⁻), so

that it cannot accept all the electrons from the fully reduced plastoquinone-pool.

Flash spectrophotometric studies on chloroplasts in the presence of the poison DCMU, which is specific for photosystem II, revealed that the linear electron transport chains are not independent of each other. Instead, at least 10 of them are interconnected between the two reaction centers, possibly via a common pool of plastoquinone (SIGGEL et al. 1972). This certainly increases the reliability of the system in comparison to an isolated-chain-operation. Under conditions where the light intensity is not limiting the collaps of one reaction center II can be compensated by several turnovers of a neighbouring one during the relaxation time of the rate limiting step.

The sequence and the kinetics of the linear electron transport chain are largely known. However, some important *white spots* still deserve further studies. The major ones are as follows:

The water splitting enzyme system which accumulates four oxidizing equivalents before reacting with two molecules of water to yield one molecule of oxygen and four protons. Although kinetically well characterized (JOLIOT et al. 1969, KOK et al. 1970) its mechanism is still open (for a hypothetical model, see RENGER 1970).

The function of some *components between the light reactions*, namely C 550 (e. g. VAN GORKUM et al. 1974), cytochrome-b 559 (e. g. MATHIS et al. 1974) and cytochrome-f (HAEHNEL 1973) is not fully understood, yet.

The acceptor system of reaction center I is not fully resolved, although a few of its components are chemically and spectroscopically characterized (for a recent review, see KE 1973).

The cyclic electron transport around reaction center I (e. g. BÖHME and CRAMER 1972) especially its sequence and its relative efficiency in intact chloroplasts has not yet been studied with flash spectrophotometric methods.

Finally, *the mechanism* of the various electron transfer reactions deserves further investigation. It is open, whether the electron transfer proceeds by diffusion and collision between mobile components or by tunnelling between locked reaction partners.

The Electrochemical Generator

So far, the discussion of the electron transport did not require to mention of the membrane which carries the redox components. If one did not know better, one might argue that the electron transport from water to NADP could occur at a solubilized multi-enzyme-complex. That it does not is related to the splitted conservation of photic energy. While one part is used for the production of reduced NADP another one is used to generate an electrochemical potential of the proton across the thylakoid membrane. The generation of a "protonmotive force" in photosynthesis and respiration was first hypothesized by MITCHELL (1961, 1966) and only later experimentally confirmed by various experimental techniques. In the following I will first review the current concept of the electrochemical potential generation and thereafter some of the experimental evidence for it.

Physical and biochemical studies led to the conclusion that the electron transport chain crosses the thylakoid membrane twice. As illustrated in the upper part of fig. 4 both reaction centers translocate an electron from the inner to

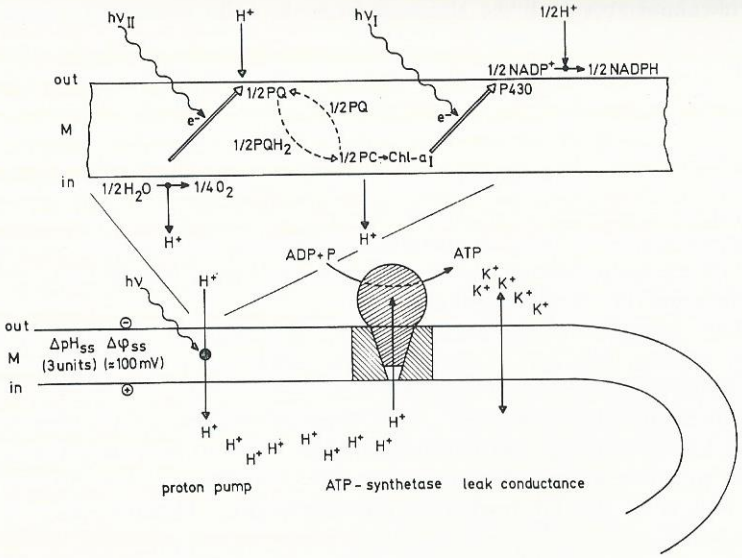

Fig. 4. Scheme of the electrochemical generator (above) and for the use of the electrochemical free energy for photophosphorylation (for details, see text).

the outer side of the thylakoid membrane. This electrically charges the membrane's electric capacitance. Both light reactions are linked by an electrically neutral step, the transfer of hydrogen via plastoquinone from the reducing site of light reaction II to the oxidizing one of light reaction I in the opposite direction across the membrane. Due to the fact that the oxidation of water and the oxidation of plastohydroquinone are causing the release of one proton per electron at physiological pH-values, two protons are released into the inner phase per electron transferred through the chain. On the other hand, the reduction of plastoquinone by light reaction II and the reduction of the terminal acceptor (NADP together with PGA, in the secondary reactions) are accompanied by the uptake of two protons per electron from the outer aqueous phase. Thus the initial charge separation due to a translocation of electrons is finally transformed into the equivalent of two protons translocated from outside to inside across the membrane per electron transferred from water to NADP. In consequence there are *two coupling sites* where free energy of redox couples is partly channelled into the formation of an electrochemical potential of the proton, each associated with one of the light reactions.

The above vectorial electron transport scheme is based on three independent lines of evidence; a first one proving the existence of a light induced electrochemical potential across the membrane, a second one correlating the redox reactions and the protolytic reactions and a third one identifying the location of electron carriers on either side of the membrane. While the third line, the topological one will be followed in the subsequent review by Dr. HAUSKA, I will focus on the evidence for the electrochemical potential and on the kinetic aspects of its generation.

First evidence for the generation of an electric potential across the thylakoid came from flash spectrophotometry (JUNGE and WITT 1968), however, it is now confirmed by other independent techniques as electrostatic induction (FOWLER and KOK 1974b, WITT and ZICKLER 1973), delayed light emission (BARBER and KRAAN 1970, FLEISCHMANN 1971, WRAIGHT and CROFTS 1971) and even by an indirect microelectrode technique (VREDENBERG 1974). Since the flash spectrophotometric method has proven most appropriate for kinetic studies it will be reviewed in some detail.

It was postulated that certain absorption changes observed on flash excitation of chloroplasts are a response of chloroplast pigments to a light induced electric field across the thylakoid membrane. This was corroborated by studies on the difference spectrum (EMRICH et al. 1969) which was analogous to the electrochromic response of chloroplast bulk pigments in vitro (SCHMIDT et al. 1971). Moreover, similar absorption changes were observed if chromatophores of bacteria were subjected to an artificially induced diffusion potential (JACKSON and CROFTS 1969). Similar experiments with chloroplasts (STRICHARTZ and CHANCE 1972) probably yielded artifactous results because of large changes in light scattering which might have mimicked absorption changes.

The physical basis of electrochromic changes is well understood (LABHARD 1961, LIPTAY 1969). Electrochromic effects of chloroplast pigments were studied in vitro by several groups (MALLEY et al. 1968, KLEUSER and BÜCHER 1969, SCHMIDT et al. 1971, SCHMIDT and REICH 1972). Quantitative studies on these effects in chloroplasts revealed that the observed absorption changes are a linear indicator of the voltage across the membrane (see WITT and ZICKLER 1974).

An *example* for the time course of the electrochromic absorption changes on excitation of chloroplasts with flash light is presented in fig. 5. The rise of absorption after firing of the flash indicates the build-up of the electric potential, the subsequent relaxation its decay via the electric conductivity of the thylakoid membrane. The three traces in fig. 5 were obtained under different conditions. While the upper one resulted in the absence, the lower two were

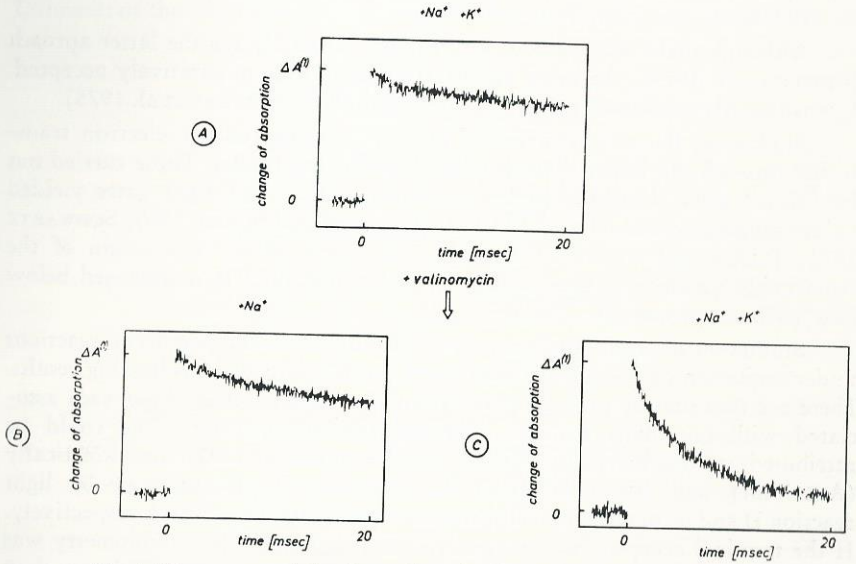

Fig. 5. Time course of the electrochromic absorption changes at 524 nm in the absence (above) and in the presence of the potassium carrier valinomycin.

observed in the presence of the potassium specific carrier valinomycin. This antibiotic increases the permeability of bimolecular liped membranes for potassium but not for sodium. It is obvious that this specificity is observed too for the thylakoid membrane. The decay of the electrochromic absorption change is accellerated by valinomycin only, if potassium is present.

Studies on the properties of the electrochromic absorption changes around 520 nm revealed:

1. Both reaction centers contribute about equally to the electric potential on excitation with a short flash of light (SCHLIEPHAKE et al. 1968).
2. The onset of the electric potential occurs within less than 20 nsec after flash excitation (WOLFF et al. 1969).
3. In the time domain of milliseconds the electric field is a collective property of a unit containing at least 10^5 chlorophyll molecules (JUNGE and WITT 1968), which is the minimum size of one thylakoid. Although, at a subnanosecond time scale, the electric field is certainly generated as a local dipole field in the membrane, the above result demonstrates its delocalization. An upper limit for the time required for delocalization has been estimated to be 100 nsec (JUNGE 1974).

First evidence for the generation of a pH-difference across the thylakoid membrane came from JAGENDORF and HIND's (1963) report of a proton uptake from the suspending medium by illuminated chloroplasts (NEUMANN and JAGENDORF 1964). An acidification of the inner phase of thylakoids by between 2 and 3 pH-units was demonstrated by three indirect techniques:

a) by the influence of the pH on the rate of the electron transport (RUMBERG and SIGGEL 1969),
b) by the influence of illumination on the activation of a chloroplast enzyme with a sharp pH-maximum more acidic than the pH of the usual suspending media for chloroplasts (HAGER 1969),
c) by the uptake of fluorescent probes into the inner space (SHULDINER et al. 1972a).

Although there was methodical criticism, especially for the latter aproach (FIOLET et al. 1974), the inner acidification is at least qualitatively accepted. It was recently confirmed even for intact chloroplasts (WERDAN et al. 1975).

Studies on the stoichiometry of protons translocated per electron transferred through the linear chain produced conflicting results. Those carried out by "slow" glass electrodes under excitation with broad light gates yielded values ranging between 1 and 2 H^+/e^- (RUMBERG and SIGGEL 1969, SCHWARTZ 1971, TELFER and EVANS 1972, HOPE and SHOW 1974). The origin of the remarkable variance in these results is well understood. It is discussed below (see "dynamic properties ...").

Studies on the stoichiometry and of the kinetics of the protolytic reactions under excitation of chloroplasts with short flashes yielded the following results. There are two sites of proton uptake from the outer aqueous phase, each associated with one light reaction (SCHLIEPHAKE et al. 1968). The could be attributed stoichiometrically (JUNGE and AUSLÄNDER 1973) and kinetically (AUSLÄNDER and JUNGE 1974) to the reduction of plastoquinone by light reaction II and of the terminal electron acceptor by light reaction I, respectively. If the terminal acceptor was oxygen via benzylviologen the stoichiometry was 1 H^+/e^- for each site. The velocity of proton binding usually is slower than the velocity of the corresponding redox reaction even when monitored with

rapidly responding pH-indicating dyes. The apparent delay, however, could be attributed to the existence of a diffusion barrier for protons shielding the redox reaction sites at the outer side of the membrane against the outer aqueous phase. This barrier was removable by appropriate treatment of chloroplasts (AUSLÄNDER and JUNGE 1974).

In addition to the above two sites of proton binding from outside two sites of proton release into the inner phase were identified (JUNGE and AUSLÄNDER 1973). One of these was unequivocally attributed to the oxidation of water (FOWLER and KOK 1974a), while the other one was attributed to the oxidation of plastohydroquinone (JUNGE and AUSLÄNDER 1973, AUSLÄNDER et al. 1974).

The dynamic properties of the electrochemical potential under excitation with a light gate are illustrated qualitatively in fig. 6. The duration of the exciting light is indicated on top of the figure. Below, the time course of the velocity of the electron transport is sketched. On switching on the square light pulse the electron transport first raises up to the "uncoupled" value to decline to the much lower "coupled" rate. The rate of the electron transport is tuned mainly by the internal pH, it slows down with increasing acidification. This was demonstrated by RUMBERG and SIGGEL (1969) and later confirmed with some corrections by ROTTENBERG et al. (1972).

The electric potential is shown in the third row of fig. 6. It rapidly rises in freshly isolated chloroplasts (full line), but then declines due to the synergistic effect of a decrease in the rate of the electron transport (and thus of the rate of the electrogenic pump, too) and of the increasing acidification of the inner phase which causes an increase of the proton leakage across the membrane (BOECK and WITT 1971). The magnitude of the electric potential on excitation of chloroplasts with a short flash of ligh was estimated to about 50 mVolt (SCHLIEPHAKE et al. 1968), the maximum observed under excitation with a step of light is about four times that high (SCHLIEPHAKE et al. 1968, BARBER 1972). Estimates of the electric potential under continuous illumination are at variance.

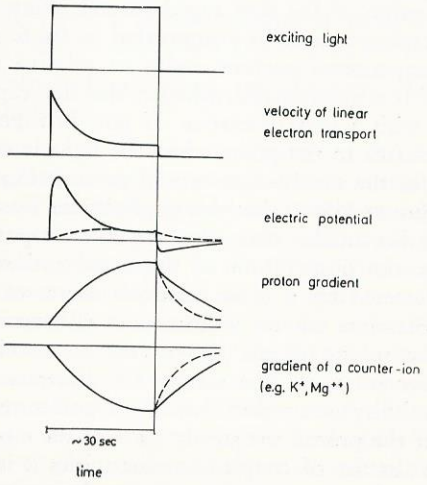

Fig. 6. Qualitative scheme for the dynamic behaviour of the electron transport, the electric potential, the proton gradient and the gradient of a counter ion.

A voltage in the order of 100 mVolts was reported for *Chlorella* (GRÄBER and WITT 1974). However, for isolated spinach chloroplasts, studies on the redistribution of ions after illumination (DILLEY and VERNON 1965, MUHLE 1973) led to the conclusion that the steady voltage is in the order of 10 mVolts, only (MUHLE 1973). Spectrophotometric determination of the steady state voltage is difficult because of large changes in light scattering superimposed to the electrochromic absorption changes under these conditions.

The time course of the proton gradient and of the gradient of a counter ion are depicted in the lower two traces in fig. 6. There is evidence that the dominating counter ion is Mg^{++} in intact chloroplasts (KRAUSE 1974, BARBER et al. 1974). In the steady state the number of protons accumulated and of course buffered in the inner phase is in the order of 100 per electron transport chain (NEUMANN and JAGENDORF 1964). The majority of these protons is electrically compensated by outwardly translocated counterions, since the electric potential after flash excitation is equivalent to two protons transported inwardly only (SCHLIEPHAKE et al. 1968) and it does not rise above this value in the steady state.

What happens if the light is switched off? Just before, the electric potential is positive inside and probably small, say 10 mVolt. After switching off the light the driving force of the electron transport and thus of the proton pump fades away within a few milliseconds. The magnitude of the electric potential is then solely dependent on the diffusion rates of the permeant ions. Two cases have to be distinguished:

1. the proton permeability is larger than the permeability of any other ion,
2. the permeability of one other ion exceeds the permeability for protons.

Let us consider case no. 1 first. It is illustrated in fig. 6 by full lines. If the light is switched off, the electrogenic pump is stopped and a diffusion potential builds up which is dominated by the rapid outwardly directed diffusion of protons. Thus the electric potential is inverted in polarity. The rapid initial inversion is followed by a slower coupled exchange diffusion of protons and counter ions, which is rate limited by the lower permeability of the latter. The relative proportion of the first rapid proton efflux which causes the inversion of the electric potential is exaggerated in fig. 6. Since it involves the electrically non-compensated protons, only, its relative magnitude should be in the order of 1 %. It is questionable, whether this first rapid blip is detected by pH-measurements with a glass electrode. If not, extrapolation of the rate of proton efflux thereafter to the point when the light is switched off will lead to underestimates for the steady-state-rate of proton efflux. It has been argued by RUMBERG and SIGGEL (1969) that this might be the reason why some authors reported H^+/e^--ratios smaller than 2, while others reported 2. The argument is strengthened by the observation of the same authors that the apparent H^+/e^--ratio as detected by a glass electrode increases from 1 to 2 in the presence of the potassium carrier valinomycin (RUMBERG and SIGGEL 1969). Let us consider the second of the above two cases, which can be installed by valinomycin, for instance. The results are illustrated by broken lines in fig. 6. If the permeability of another ion, say of potassium, is made to override the permeability of the proton the steady state of the electric potential will be unaffected. In the absence of coupled conductivities it is solely dependent on the dynamic balance between the rates of the electrogenic proton pump and the proton leaks in the membrane. Then any other permeant ion should adjust its concentration gradient into equilibrium with the membrane potential. In the presence of valinomycin potassium will accumulate outside. If the light is switched off, the dominating inwardly directed diffusion of potassium backs the light induced membrane potential which thus persists as a diffusion potential in the dark. Thus there will be no biphasicity of the proton efflux, and the rate detected by a glass electrode can be extrapolated to the point where the light is switched off. Since the hindrance on the rate of proton efflux by the low permeability of the counter ion is removed, the rate will be higher as in the first case. In consequence higher H^+/e^--ratios result which according to RUMBERG and SIGGEL (1969) approached the value 2 in agreement with the stoichiometries obtained under flash excitation (SCHLIEPHAKE et al. 1968, JUNGE and AUSLÄNDER 1973).

The above results in the absence of valinomycin suggest that the proton permeability exceeds the permeability of any other ion *in the steady state* (light). In contrast to this, in the dark or under flash excitation the electric potential relaxes within about 100 msec while the pH-difference only in the range of 10 sec (e. g. JUNGE and AUSLÄNDER 1973). Therefore, one has to conclude that the permeability of some other ion exceeds the one of the proton, under these conditions.

The topology of the electrochemical generator is discussed in the subsequent review by Dr. HAUSKA. Its characteristics are the location of the donors of both light reactions at the inner side and the location of the respective acceptors at the outer side of the thylakoid membrane (see fig. 4, above). There are two important questions as to the finer structure of the electrochemical generator:

1. How does hydrogen cross the thylakoid membrane? Is it by diffusion of plastoquinone across the membrane or by hopping within a plastoquinone lattice which extends over the membrane?
2. How does the extremely rapid electron transfer occur between the reaction center chlorophylls and their primary acceptors at the other side of the membrane? In chloroplasts this transfer occurs within less than 20 nsec (WOLFF et al. 1969) and in chromatophores of bacteria supposedly even in less than 10 psec (NETZEL et al. 1973, LEIGH et al. 1974).

These questions cannot be answered, yet. However, there is some marginal information on both of them.

1. The first question has to do with the fluidity of the thylakoid membrane. Electron micrographic studies revealed that surface proteins as the coupling factor of photophosphorylation aggregate if the membrane is treated with bifunctional agents, e. g. glutaraldehyde (WANG and PACKER 1973) or antibodies (BERZBORN et al. 1974). This suggests that at least some parts of the membrane may be in a fluid state. The exciton interaction of the antennae chlorophylls, on the other hand, requires tight packing if not quasicristalline arrays. Thus it seems reasonable to visualize the thylakoid membrane as a mosaic of fluid and quasicristalline domains, as first proposed by KREUTZ (for a review, see KREUTZ 1970) based on his X-ray small angle work. This concept is backed by independent kinetic evidence. Studies on the conduction behaviour of the thylakoid membrane in the presence of ionophores revealed that both valinomycin and nonactin act as mobile carriers, however, only a very small fraction of the mem-

brane bound molecules is active in ion transport, while a majority is inactivated, probably by immobilization (SCHMID and JUNGE 1975).

2. The second question has to do with the location and mutual orientation of the reaction center chlorophylls and their primary acceptors. ESR-studies revealed that the photochemically active species of the reaction centers is a dimer of two chlorophyll-a molecules which rapidly exchange the unpaired electron after photooxidation (NORRIS et al. 1971). Very probably, in addition to charge transfer, the dimer is under exciton interaction, as suggested by CD-studies (PHILLIPSON et al. 1972). Kinetic considerations led to the conclusion that the porphyrin rings of the dimer of reaction center I are located close to the inner surface of the thylakoid membrane (JUNGE 1974). According to linear dichroism studies by photoselection the porphyrin rings of the dimer are oriented rather in parallel than perpendicular to the membrane (JUNGE and ECKHOF 1973, 1974). Possible internal configurations of the dimer were studied in model experiments with chlorophylls in vitro (for a review, see KATZ and NORRIS 1973).

Photophosphorylation

The mechanism of photophosphorylation was subject of controversy in recent years. The major efforts were directed to the pathway of the energy flow from photochemically indiced redox energy into the free energy of the ATP/ADP-couple. The discussion was governed by three hypotheses on the nature of the obligatory intermediate between the electron transport and ATP;

1. a chemical intermediate (SLATER 1958),
2. an electrochemical potential difference of the proton (MITCHELL 1961, 1966),
3. and a high energy conformational state of an enzyme or of the membrane (GREEN et al. 1968) were postulated.

The discussion started at a time when none of these hypothetical intermediates was identified experimentally. Only later evidence was provided for the existence of a pH-difference across the functional membrane of photosynthesis in green plants (JAGENDORF and HIND 1963) and in bacteria (JACKSON et al. 1968) as well as in mitochondria (MITCHELL and MOYLE 1965). Then evidence for the existence of an electrical potential across the same membranes was published (JUNGE and WITT 1968, JACKSON and CROFTS 1969, BAKEEVA et al. 1970). Finally it was shown that there are conditions where energy stored in the form of an electrochemical potential difference across the functional membrane of photosynthesis can be used for phosphorylation while its dissipation competes with the synthesis of ATP (e. g. JAGENDORF and URIBE 1966, CROFTS 1967, JUNGE et al. 1970, BOECK and WITT 1971, SHULDINER et al. 1972). The former evidence for the existence and the possible use of electrochemical energy reduced the above discussion to its salient point, whether or not the electrochemical potential is "the obligatory intermediate" between redox reactions and ATP or whether it is located of a side path, only.

Although most of the mechanistic questions of photophosphorylation are still open, the present evidence is sufficient to favour a chemiosmotic mechanism as postulated by MITCHELL (1961, 1966). I will illustrate the argument in a somewhat formal way (see figs. 7 and 8).

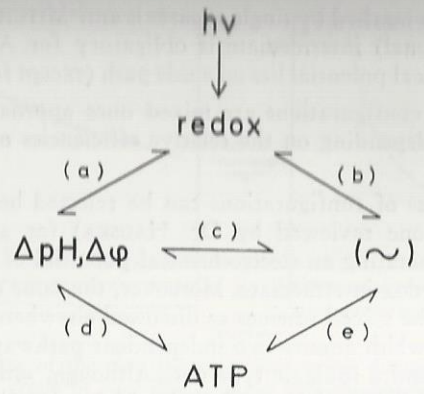

Fig. 7. The elements under discussion for the energetic question of photophosphorylation and the pathway of energy flow.

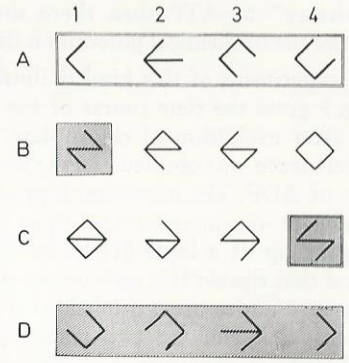

Fig. 8. Possible configurations for photophosphorylation. The topology underlying to the graphs is the same as in figure 7. For details, please see text (JUNGE 1972).

For the unbiased spectator at least four types of elements are necessary to characterize the energy flow in photophosphorylation; redox-couples, chemical or conformational intermediates (~), an electrochemical potential of the proton ($\Delta\psi$, ΔpH) and the ATP/ADP-couple. The possible pathways of energy flow between these elements are illustrated in fig. 7. The scheme is not intended to match the full complexity of events, e. g. there may be more than one chemical intermediate involved. It just gives the minimum number of elements required. All interconnections except for a direct link between redox reactions and the formation of the phosphoester bond were discussed in the literature. Being unbiased one ends with 16 possible reaction schemes as illustrated in fig. 8. They fall into three classes:

1. One marked by double squares which is essentially on line with MITCHELL's hypothesis in that the electrochemical potential difference is a direct consequence of the electron transport and that there is no other way leading from redox free energy to ATP except the one via the electrochemical potential of the proton.

2. The second class marked by single squares is anti-MITCHELL since a chemical (or conformational) intermediate is obligatory for ATP-synthesis while the electrochemical potential lies on a side path (except for B1).
3. The remaining configurations are mixed ones approaching either of the former classes depending on the relative efficiencies of the various pathways.

The second class of configurations can be rejected because of the above evidence (plus the one reviewed by Dr. HAUSKA) for a vectorial electron transport scheme generating an electrochemical potential of the proton without involving any nonredox intermediate. Moreover, the same evidence practically eliminates most of the mixed schemes as discussed elsewhere (JUNGE 1972), except for scheme B4 which admits two independent pathways for phosphorylation, a Mitchellian and a squiggle type one. Although, such a twofold way to ATP is rather improbable, the question should be experimentally answered, whether or not there is any "squiggle" phosphorylation *in addition* to the chemiosmotic one.

A qualitative answer can be based on kinetic experiments which compare the dissipation of electrochemical energy with the ATP-production. If there were any "squiggle pathway" to ATP then there should be some residual phosphorylation even if the electrochemical potential is dissipated.

An example for an experiment of this kind is illustrated in figs. 9 and 10 (JUNGE et al. 1970). Fig. 9 gives the time course of the electrochromic absorption change at 524 nm after excitation of chloroplasts with a group of four short flashes. While the left trace was obtained in the absence the right one was measured in the presence of ADP. The repetition frequency of the flash groups (0.1 cps) and the intensity of the interrogating light (45 erg/cm^2×sec) were kept low to avoid dumping up of a large pH-difference. Recent experiments on the same line indicated that the electric part of the electrochemical potential dominated the pH-difference under these conditions (GRÄBER and WITT, personal communication). Inspection of the two traces in fig. 9 shows that the decay of the electric potential is accellerated if ATP is synthesized. This fits into the expectation for the action of an ATP-synthetase which translocates protons downhill their electrochemical gradient, in order to gain the free energy necessary for the synthesis. However, the accelleration might represent a side effect, only. Thus it has to be questioned, whether ATP-synthesis is inhibited if the electrochemical potential is at least partly dissipated via some leak conductivity of the thylakoid membrane. Fig. 10 shows the time course of the electrochromic absorption change at 524 nm in the presence of ADP without (slow decay) and with (accellerated decay) the potassium carrier valinomycin. The dissipative conductivity induced by valinomycin is about as effective as the other conductivity induced by the activity of the ATP-synthetase, as evident from the about doubled decay rate of the electric field. The ATP-yield per flash group, which was determined for the same samples, was reduced to about one half on addition of valinomycin at this concentration (JUNGE et al. 1970). Addition of valinomycin at higher concentrations abolished the ATP-yield. That this inhibition was due to the dissipative action of valinomycin but not to an eventual side effect on the enzyme was clearly demonstrated in similar experiments with another conductivity increasing agent, gramicidin, which halfed the ATP-yield already when presented at a relative concentration of 1 molecule on more than 10^5 molecules of chlorophyll (BOECK and WITT 1971).

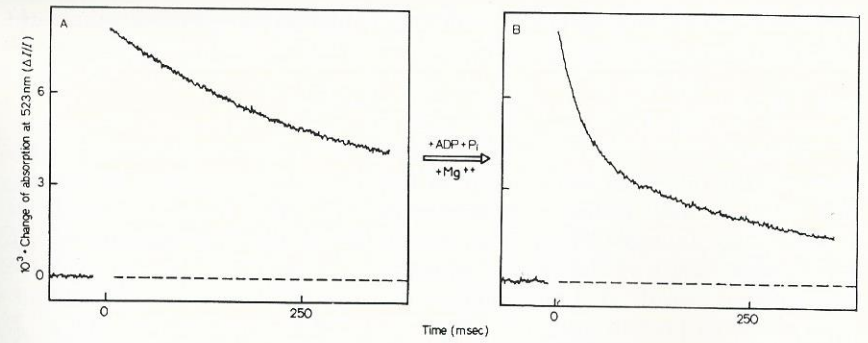

Fig. 9. The time course of the electrochromic absorption change at 523 nm under excitation with a short group of four flashes under non-phosphorylating (left) and under phosphorylating conditions (right) (JUNGE et al., 1970).

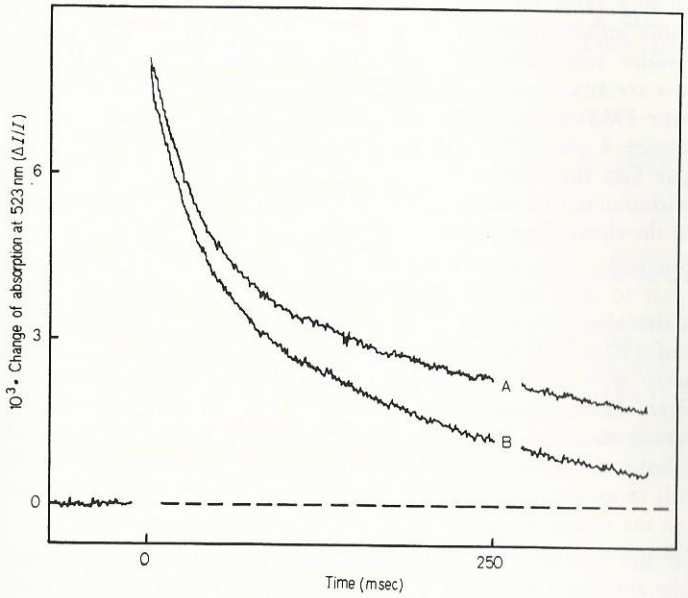

Fig. 10. The time course of the electrochromic absorption change at 523 nm under excitation with a group of four short flashes in the presence of ADP, P and Mg^{++}. Trace A: In the absence of valinomycin. Trace B: In the presence of valinomycin.

This clearly has demonstrated that there is no efficient non-chemiosmotic pathway in phosphorylation under these conditions.

Despite a lot of qualitative evidence for the competition between dissipative channels and ATP-synthesis for the electrochemical potential across the thylakoid membrane (JAGENDORF and URIBE 1966, CROFTS 1967, JUNGE et al. 1970, BOECK and WITT 1971, SHULDINER et al. 1972b) a quantitative correlation of sufficient precision is still missing. Such a correlation involves three elements; the ATP/ADP-ratio sustained by chloroplasts under continous illumination, the stoichiometry of protons translocated per ATP formed and the steady state level of the electrochemical potential difference. According to

MITCHELL (1966) these elements should be in equilibrium with each other provided that there are no consumers of ATP.

$$\Delta G^{01} + RT \ln \frac{[ATP]}{[P_i]\,[ADP]} = \nu \left(RT \ln \frac{[H^+_i]}{[H^+_0]} + F\Delta\psi \right)$$

Determination of the free energy of the ATP/ADP-couple in the steady state yielded values between 14 and 16 kcal/mole for chloroplasts (KRAAYENHOF 1969) as well as for mitochondria (SLATER 1969) and chromatophores (CROFTS and JACKSON 1970). The ambiguities in the determination of the steady state values of the electric potential and the pH-difference were discussed above, already. The most controversial element, however, is the stoichiometry between protons and ATP. MITCHELL (1966) postulated a value of 2. The values determined experimentally by correlation of proton fluxes with ATP-synthesis range from 2 (SCHWARTZ 1968) via about 3 (URIBE and JAGENDORF 1968, IZAWA 1970, JUNGE et al. 1970, SCHRÖDER et al. 1972) to 4 (SCHRÖDER 1974) and even 5 (GALMICHE et al. 1967). The difficulties in determining the proton flux rates were discussed above, already. One might hope to get more reliable values from the ATP/2e-ratios, which are more readily to determine, under consideration of the H^+/e^--stoichiometry 2. Unfortunately these values are at variance, too. Ratios between 1 (SCHRÖDER et al. 1974) over 1.3 (WINGET 1965) and 1.7 (REEVES and HALL 1973) equivalent to H^+/ATP-ratios between 4 and 2.35, respectively were reported in the literature. One might argue that the smaller values for the H^+/ATP-stoichiometry reflect the "true" stoichiometry of enzyme, while the higher values contain partial dissipation of the electrochemical energy via leaky channels.

In conclusion, the quantitative evaluation of the above equation is still missing. Just to give some figures for the influence of the H^+/ATP-stoichiometry on the above equation — a pH-difference of 3 units plus an electric potential of 150 mVolt were required to balance the ATP/ADP-couple if the stoichiometry were 2 — if it were 4 just a pH-difference of 2.75 units would be sufficient. In chromatophores, where the electrochemical potential in the steady state is easier to determine for several reasons the above equation was found to hold (CASADIO et al. 1974) although there are some aspects which are difficult to understand, yet (e. g. the influence of valinomycin on the electric potential in the steady state).

Under the impression that the energetic question of photophosphorylation soon will be answered definitely in favour of a, possibly modified, chemiosmotic mechanism several groups concentrate on mechanistic questions as to the enzyme which translocates protons in order to form phosphoester bonds. The major efforts are up to the biochemists, who partly resolved the enzyme and characterized some of its subunits (see the review by Dr. HAUSKA). One structural aspect was tackled by physical methods. A chemiosmotically operating ATP-synthetase should have access to both aqueous phases separated by the thylakoid membrane. In fact, evidence was provided (MCCARTY and RACKER 1967, GIRAULT et al. 1974, SCHMID and JUNGE 1974) that extraction of the coupling factor (CF1) for photophosphorylation opens a proton conducting channel in the membrane, which after recondensation of the coupling factor can be stopped up again. The channel opened up has been tentatively identified with the binding protein of the coupling factor (SCHMID and JUNGE 1974). Thus the location of the coupling factor may be visualized as illustrated in the lower part of fig. 4; its head protruding into the outer phase (for EM-evidence, see

HOWELL and MOUDRIANAKIS 1967) but its leg in contact with the pH of the internal phase via a proton well provided by its binding protein.

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