Aust. J. Plant Physiol., 1995, **22**, 131–60

Viewpoint

Sixty-three Years Since Kautsky: Chlorophyll *a* Fluorescence

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Abstract. In 1931, using their eyes as instruments, H. Kautsky and A. Hirsch related the time course of chlorophyll *a* fluorescence with photosynthesis in a less-than-one-page article in *Naturwissenschaften* (see Kautsky's photograph). Chlorophyll *a* fluorescence is now being used by hundreds of investigators as a probe for various aspects of photosynthesis—from excitation energy transfer in picosecond time scale to CO_2 fixation in minutes. It is not only a much used, but also a much abused, tool. It is used because of it being a non-invasive, rapid and a highly sensitive probe, and misused because it is sometimes not recognised that it is affected by various photosynthetic and other reactions. I submit that, like any other technique, if it is used with care and with due regard for its time dependence and competing parameters, it will remain as the one-most powerful tool for probing excitation energy transfer, primary photochemistry, electron flow on both the donor and the acceptor side of photosystem

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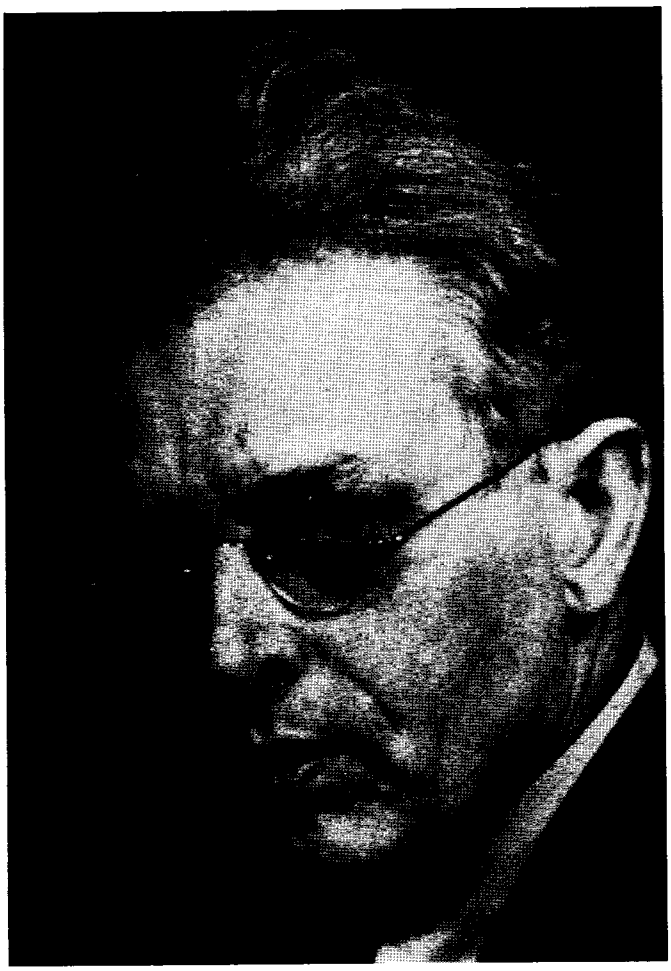

H. Kautsky (*ca* 60 years old) taken in Marburg, Germany,
(photo courtesy of H. K. Lichtenthaler, Karlsruhe).

II (PSII) of oxygenic PSII. Further, it is very useful in the quick assay of PSII mutations, and down-regulation and other adjustments to stress (excess light, heat, heavy metal, nutrients and certain herbicides). In this paper, I will present my viewpoint, not a review, on the conceptual and experimental developments in this field. Whenever appropriate, and without any shame and humility, I will include some of my involvement in the excitement surrounding this field. I hope that this paper will serve as a starting point for further discussion of not only the history, but the practical use of chlorophyll *a* fluorescence as an intrinsic probe of stresses to plants, as well as individual reactions of oxygenic photosynthesis, when combined with other parallel measurements.

Introduction

Chlorophyll (Chl)* *a* fluorescence is red and beautiful, and is no longer in the purview of specialists alone as it has become a routine probe for information, sometimes misinformation, on the various aspects of photosynthesis. If used properly, it provides information on the identity of the various pigments and pigment complexes, their organisation, excitation energy transfer among them, and on the various electron transfer reactions, specifically of photosystem II (PSII).

It must be stated at the very outset that the quantum yield of Chl *a* fluorescence (Φ_f) of a single species is related to the rate constants (k's) of various pathways of de-excitation (f for fluorescence, h for heat dissipation, t for excitation energy transfer, q for quenching by quenchers (e.g. carotenoids, O_2, triplets, etc.), and p for photochemistry) as follows:

$$\Phi_f = \frac{k_f}{k_f + k_h + k_t + k_q + k_p}. \qquad (1)$$

The absolute quantum yield of fluorescence (Φ_f) is obtained from the total number of photons emitted (F) divided by the total number of photons absorbed (I_a), or the lifetime of fluorescence (τ) divided by the theoretical intrinsic lifetime of fluorescence (τ_o) when the only pathway of de-excitation is fluorescence. Measurements of F/I_a will not agree with Φ_f from τ measurements if there is a change in absorption cross-section of the fluorescent pigment bed, such as caused by the formation of non-fluorescent complexes. Further, in view of the homogeneous emission of fluorescence in all directions only a portion of fluorescence is measured with constant intensity of incident light, and thus, only *relative* Φ_fs are reported.

In view of the fact that Chl *a* fluorescence is so rich in information (affected by several de-excitation pathways), it also becomes an ambiguous signal. It is for this very reason, it must be used with caution and in combination with other signals to fully tap its power. In this article, I plan to provide my viewpoint, not a review, on Chl *a* fluorescence and its use in photosynthesis, particularly PSII since about 90% of Chl *a* fluorescence at room temperature originates in that system. For reviews on Chl *a* fluorescence, readers may consult, among others, Rabinowitch (1951, 1956), Wassink (1951), Butler (1966), Govindjee *et al.* (1967), Govindjee and Papageorgiou (1971), Goedheer (1972), Papageorgiou (1975), Lavorel and Etienne (1977), Govindjee and Jursinic (1979), Govindjee *et al.* (1986) (see various chapters), Lichtenthaler (1988), Krause and Weis (1991), Karukstis (1991) and Holzwarth (1991).

In the Beginning

'It is a noble employment to rescue from oblivion those who deserve to be remembered'—Pliny the Younger, *Letters V.* Althanius Kircher (1646) was the first one to discuss, at length, the bichromatic appearance of an extract of 'lignum nephriticum', that had been recommended for curing kidney ailments. It was yellow in transmitted light and blue in reflected light; the blue light must have been its fluorescence. Sir David Brewster (1834), a Scottish preacher, while discussing his concept of the colour of natural bodies, remarked, almost in passing 'In making a strong beam of the sun's light pass through the green fluid, I was surprised to observe that its colour was a brilliant red, complementary to the green. By making the ray pass through greater thickness in succession, it became first orange and then...'. The green fluid in Brewster's experiment was an alcohol extract of Laurel leaves. It must have contained *chlorophyll*, the pigment of green leaves, as named by Pelletier and Caventou (1818). I consider it likely that this was not only the discovery of (chlorophyll) fluorescence in solution, but also of the reabsorption of this phenomenon in thick samples. However, the clearest discovery of the phenomenon of fluorescence has been that by Sir John Herschel (1845*a*, 1845*b*) in a clear solution of quinine sulfate; it was of 'celestial blue' colour, but was, unfortunately, called *epipolic dispersion*. Brewster (1846) designated it as *internal dispersion*. Professor G. G. Stokes (1852), Professor of Mathematics at Cambridge University,

*Abbreviations used: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; LHCI, light-harvesting complex I; LHCII, light-harvesting complex II; PQ, plastoquinone; PSI, photosystem I; PSII, photosystem II; NPQ, non-photochemical quenching.

who is known for his discovery that emission bands are shifted to wavelengths longer than the absorption bands (the so-called *Stokes' shift*), used the term *dispersive reflexion*, but quickly added a footnote: 'I confess I do not like this term. I am almost inclined to coin a word, and call the appearance *fluorescence*, from fluor-spar, as the analogous term opalescence is derived from the name of a mineral'. Stokes is the first one to recognise this phenomenon as light emission. Askenasy (1867) has credited Stokes also for the discovery of both phycobilin and Chl *a* fluorescence in fresh red algae. I recommend the book by Harvey (1957) for history of fluorescence prior to 1900.

It was Müller (1874), among others, who noticed that a green living leaf had a much weaker red Chl fluorescence than a dilute Chl solution. Although Müller had predicted an inverse relationship between Chl fluorescence and photosynthesis, his experiments were not done with proper controls. Since both duration of experiment and temperature changed during his measurements, I find it difficult to credit him with the discovery of Chl fluorescence transient (or induction). Further, his concepts on absorption bands cannot be accepted because he used acoustic analogy—vibrations of strings; he expected absorption at all the overtones. Transition dipoles are not strings.

Kautsky's 1931 Paper

'Everything reasonable has been thought of before. We have just to try to think it once anew'—Goethe. On 19 October 1931, Hans Kautsky and A. Hirsch at the Chemisches Institut der Universität in Heidelberg, Germany, submitted a less-than-one page 'Kurze Originalmitteilungen' titled (as translated in English) 'New experiments on carbon dioxide assimilation'. Following illumination of dark-adapted leaves, the time course of Chl fluorescence, observed with the authors' eyes, was correlated, although qualitatively, with the time course of CO_2 assimilation, published earlier by Otto Warburg. The main conclusions illustrated in Fig. 1 were that:

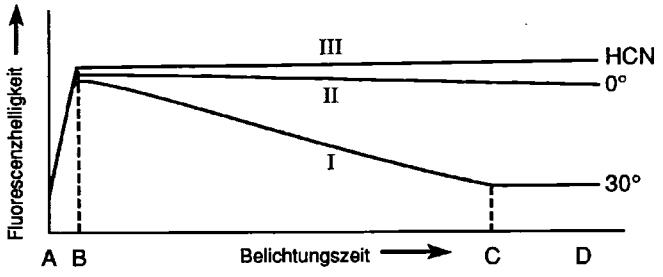

Fig. 1. Schematic representation of Chl *a* fluorescence intensity changes in leaves as observed after turning on the excitation light. I, at 30°C; II, at 0°C; III, poisoned with HCN. Data from Kautsky and Hirsch (1931).

(*a*) Chl fluorescence rises rapidly to a maximum, then declines and finally reaches a steady level, all within a matter of minutes.

(*b*) The rising portion of the curve was considered to reflect the primary photochemical reaction of photosynthesis, as it was unaffected by temperature (0 and 30°C) and by a poison (HCN). If the light was turned off at the maximum, the fluorescence transient recovered fast.

(*c*) The decline in the fluorescence curve was found to be inversely correlated with the increase in the rate of CO_2 assimilation; this suggested to the authors that more chemical energy is produced from photons when less Chl fluorescence is seen.

(*d*) The long lag in the carbon assimilation was considered rather strange—it seems that 'light-dependent' processes are required for the full development of the carbon assimilation process; also unexplained was the long time needed for the recovery of fluorescence transient if the light was turned off after the transient was completed.

I consider these observations to be a landmark in the history of photosynthesis that has led to such a 'mess' today that we have to gather 'Downunder' to sort the basics and interpretations of Chl fluorescence at this Robertson Symposium 63 years after the Kautsky and Hirsch paper. Lichtenthaler (1992) has already provided further details about Kautsky and his work on Chl fluorescence induction kinetics.

It is indeed remarkable to note that only one year after this paper, two classical papers by Robert Emerson and William Arnold (1932*a*, 1932*b*) appeared in which the concept of a 'photosynthetic unit' (2500 Chl molecules cooperating to do photosynthesis) was highlighted with highly sophisticated and elegant experiments (see Myers 1994). I shall discuss this concept later in the paper.

The Transient

A dark-adapted leaf or a suspension of higher plant, algal or cyanobacterial cells, shows characteristic changes in Chl *a* fluorescence intensity when illuminated with continuous light. These changes have been called fluorescence induction, fluorescence transient or simply the Kautsky effect. Basically, they can be classified as *fast* (up to 1 s) or *slow* (up to several minutes). These fluorescence transients have been the subject of a vast number of studies and continue to be used as probes for various qualitative effects on photosynthesis. The fast changes have been a bit easier to understand and interpret than the slower changes. However, caution must be exercised even in their interpretations. I remind the readers of a statement by Lavorel and Etienne (1977), 'Any given (fluorescence) response is initially directly related to the closest photochemical step, but subsequently becomes determined by a network of interactions of increasing complexity

ultimately involving the entirety of the apparatus. The analysis of the fluorescence phenomenon is thus made difficult not only because of the number of controlling factors but also because the mode of action of these factors may not simply be reduced to elementary concepts of quenching or non-radiative (heat) dissipation'. Although I fully appreciate this statement, I am not as pessimistic because of the tremendous progress currently being made in integrated approaches in physiology and medicine, in neural networks and in cognitive sciences. These have begun to be applied to Chl *a* fluorescence research.

Nomenclature

In the early literature, the Chl *a* fluorescence transients had been described by inflection points labelled as A, B, C, D, E, or D1, M1, D2, M2, etc. (see e.g. Rabinowitch 1951). However, the currently popular nomenclature of *OIDP* for the fast transient is based on those by Lavorel (1959, 1963; *O→P)*, and Munday and Govindjee (1969*a*,1969*b*; *O→I→D→P*) and of PSMT for the slow transient by Papageorgiou and Govindjee (1968*a*); a schematic diagram is shown in Fig. 2. The fluorescence increases from *O* (that stands for origin) to *I* (inflection or intermediary peak), decreases to *D* (dip) followed by an increase to *P* (peak) and then a decrease to *S* (quasi-steady state) (Table 1). This is also called the first wave. In the second wave, fluorescence rises from *S* to *M* (a maximum) and declines to *T* (terminal steady state). If there are several additional waves in between, they have been labelled as, e.g. S_1 M_1 S_2 M_2 *T* for the slow transient (Yamagishi *et al.* 1978, who partly based this nomenclature on an alternative terminology of Bannister and Rice 1968), or I_1 D_1 I_2 D_2 *P* for the fast transient (Neubauer and Schreiber 1987; Schreiber and Neubauer 1987). During weak illumination, the transient rises from *O* to '*Pl*', where *Pl* stands for plateau (Forbush and Kok 1968) and is considered equivalent to the *O* to *ID* phase. Since during these transient measurements, exciting intensity is kept constant, the fluorescence intensities at

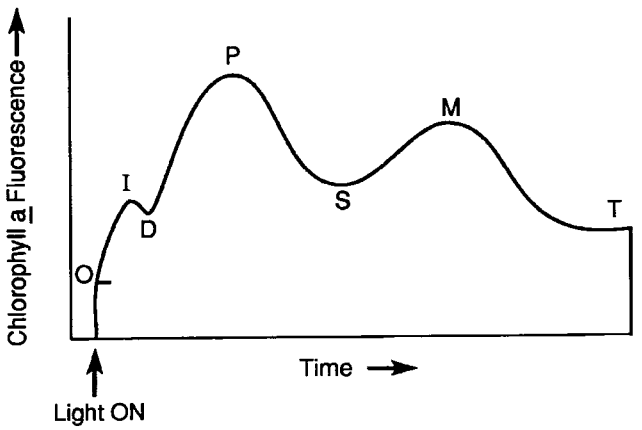

Fig. 2. Nomenclature of the Chl *a* fluorescence transient as used during 1960–1980. Data from Govindjee and Papageorgiou (1971).

these 'inflection' points, F_O, F_I, F_P, etc., may simply be labelled as ΦF_O, ΦF_I, ΦF_D, ΦF_P representing quantum yields of fluorescence. However, such nomenclature should be used with great caution especially during the slow transients (*PSMT*) since changes in absorption cross-section of fluorescent PSII pigments have been suggested to occur in this time scale.

Some Correlations

As a plant physiologist, I had learnt to accept, for pragmatic reasons, correlations that work under defined experimental conditions. Some examples follow.

Complementarity

Kautsky and Hirsch (1931) had already mentioned the antiparallel relationship between Chl *a* fluorescence and photosynthesis. This complementary relationship during the *DPS* transient was quantitatively established by MacAlister and Myers (1940) (Fig. 3). Delosme *et al.* (1959) showed

Table 1. Nomenclature of inflections and in fluorescence transients

	The Fast Transient: the first wave		
Origin	'*O*' (Lavorel)	≡	F_o
Inflection	'*J*' (Strasser	≡	*I* (Delosme) ≡ I_1 (Schreiber)
Intermediary hump	*I* (Munday)	≡	I_2 (Schreiber)
Plateau	*ID* (*D* for dip; Munday)	≡	*Pl* (Kok/Joliot)
Peak	*P* (Lavorel)	≡	F_m (if maximum)
	The Slow Transient: the second wave		
Quasi-steady state	*S* (Lavorel)	≡	S_1, S_2
A maximum	*M* (Papageorgiou)	≡	M_1, M_2
Terminal steady state	*T* (Papageorgiou)		

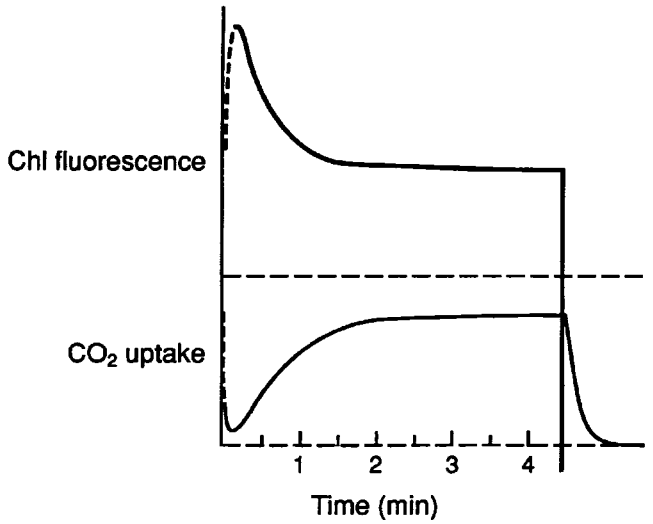

Fig. 3. Simultaneous recording of Chl *a* fluorescence intensity (top curve) and rate of photosynthesis (measured as CO_2 uptake, bottom curve) in a wheat leaf up to 4 min. Note the antiparallel relationship. Atmosphere: 0·03% CO_2 in N_2. Data from McAlister and Myers (1940).

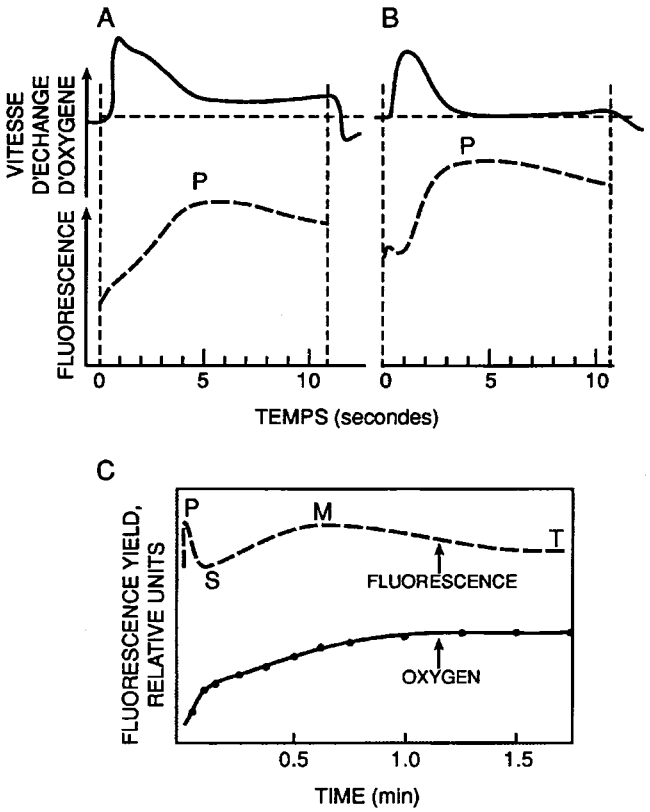

Fig. 4. Simultaneous recording of Chl *a* fluorescence intensity and rate of photosynthesis in algal cells dark-adapted for several minutes. (*A*) Fast transient, aerobic sample; (*B*) sample (*A*), after 3 min of anaerobiosis; (*C*) slow transient, aerobic sample. Note the initial parallel relationship between O_2 evolution and fluorescence, then antiparallel, followed by parallel (during *S* to *M* rise), and then constancy of O_2 evolution when fluorescence declines. (*A*) and (*B*) data from Delosme *et al.* (1959), the original O_2 curves were turned 180° in this figure; (*C*) data from Mohanty *et al.* (1971*a*); also see Papageorgiou and Govindjee (1968*a*)

that both O_2 evolution and Chl *a* fluorescence increased during the *OI* phase, and then they were antiparallel during the *DPS* phase; thus, *OID* was an 'activation' phase before O_2 was evolved. Papageorgiou and Govindjee (1968*a*, 1968*b*) and Mohanty *et al.* (1971*a*) showed the parallel increase in fluorescence during *SM* phase, and constancy of O_2 evolution during *MT* decline (Fig. 4). Thus, it is clear that the antiparallel relationship is observed *only* under certain experimental conditions. Looking back at Eqn (1), this happens when *only* two of the parameters change. When other rate constants also change, the antiparallel relationship breaks down. Kautsky and Hirsch (1931) have mentioned that it took a long dark time to restore the transient if the light was turned off at long time after illumination. Duysens and Sweers (1963) showed that the *OPS* transient was not restored if light was turned off at the '*S*' level and turned back on immediately. The hypothesis of *Q* (now called Q_A) was that Chl *a* fluorescence increased when Q_A was reduced and decreased when Q_A^- was oxidised. If this was the *only* factor controlling *OPS* transient, the transient should have been restored right away. Mohanty and Govindjee (1974) and Briantais *et al.* (1986) discuss the dual nature of this phase extensively: one related to Q_A, and the other to some 'high energy state'. Papageorgiou and Govindjee (1971) showed a relationship of the suspension pH, whereas Briantais *et al.* (1979) showed a relationship of proton gradient changes with the *P* to *S* decay. In terms of Eqn (1), this implies that another rate constant (perhaps, k_h), besides $k_p[Q_A]$, is affected by pH changes.

PQ pool size

The *OP* rise is mostly due to the net decrease in the concentration of the quencher, Q_A, and, thus, the net accumulation of Q_A^-. The area over the Chl *a* fluorescence rise curve of the fast transient (*OIDP*) has been used to measure the size of the electron acceptor pool of PSII, the plastoquinone (PQ) pool size, provided, e.g. the same area can be measured, under similar experimental conditions, when the PQ pool is 'isolated' from the PSII reaction centre by the addition of inhibitors, such as 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU). The earliest papers on the calculations of the acceptor side pool were by Malkin and Kok (1966) and Murata *et al.* (1966*a*). The area over the DCMU curve approximates one electron equivalent on Q_A^-, and the area without DCMU is the total number of electron equivalents on the electron acceptor pool. Such experiments have, in general, provided good estimates

(5–10 PQ molecules) of the PQ pool size. However, see Trissl *et al.* (1993) and Trissl and Lavergne (1995) for a discussion of potential problems.

Sites of inhibition/mutation

A simple-minded, but an effective, use of Chl *a* fluorescence transient has been to use it as a probe for finding out if an inhibitor or a mutation has caused a lesion on the electron donor or the electron acceptor side of PSII. For example, inhibitors or mutations that cause a block in the electron flow beyond PSII, e.g. after the electron acceptor Q_A (Duysens and Sweers 1963), cause a faster fluorescence rise ($O \rightarrow P$) and the fluorescence yield remains high. In course of such experiments, Vernotte *et al.* (1979) discovered that Chl fluorescence was often about 10–20% higher when the PQ pool was all reduced (saturating light, no DCMU). This suggests that oxidised PQ pool can also directly quench antenna Chl *a* fluorescence, an alternative mechanism that does not involve Q_A—a concept that often comes as a surprise to many students. A block in the electron flow on the donor side of PSII, e.g. between H_2O and P680 (the reaction centre Chl *a* of PSII), leads to a slower Chl *a* fluorescence rise and fluorescence remains low. This condition is, however, restored to normal if the cause of the block is removed (see e.g. Mohanty *et al.* 1971*b*; Critchley *et al.* 1982; Metz *et al.* 1989). Fig. 5 shows an example of inhibition on the donor side by chloride depletion.

For application of slow fluorescence changes to CO_2-fixation reactions, see e.g. Horton (1985) and Lichtenthaler (1988).

The OJIP Transient

A majority of fluorescence transient literature has used the term *OIDP* for the *fast* fluorescence transient, and it has been tacitly assumed that the *OI* phase, measured during transients by all investigators, is equivalent to the photochemical phase *OI*, recorded at high intensity excitation, and with fast measuring instruments (Morin 1964; Delosme 1967). I prefer to call this *I*, $I_{Delosme}$—the reason will become clear soon. The *I* in the Chl *a* fluorescence transient curve was the name given to the first inflection, observed on linear scale plots from measurements with camera shutter instruments (Munday and Govindjee 1969*a*, 1969*b*). However, using a LED Walz fluorometer, originally developed by Schreiber *et al.* (1986), and extremely high intensity excitation light, Neubauer and Schreiber (1987) and Schreiber and Neubauer (1987) discovered that the *OIDP* should be represented as OI_1 (D_1) I_2 (D_2) *P* transients since there were two, instead of one, inflection(s) between *O* and *P*. Using a commercial LED Hansatech instrument PEA (Plant Efficiency Analyser), Strasser and Govindjee (1991, 1992) recently observed two inflections between *O* and *P*, and labelled them as *J* and *I*,

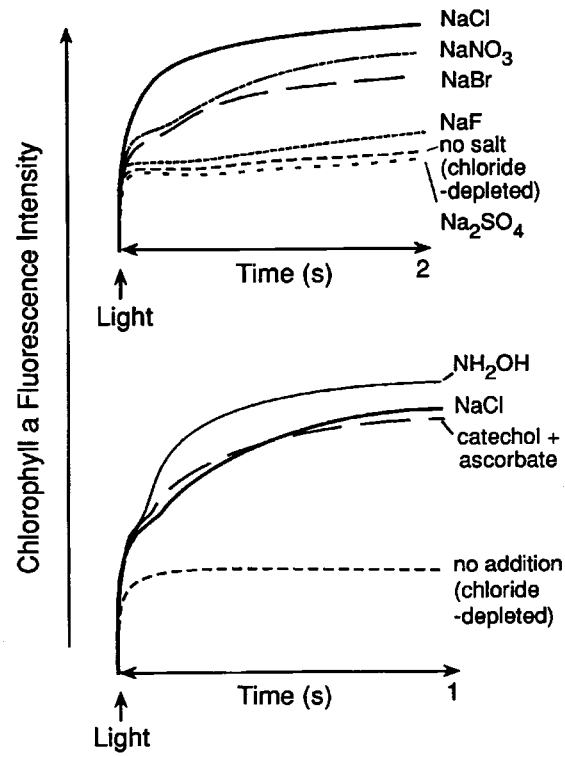

Fig. 5. Chl *a* fluorescence transients in halophyte (*Avicennia germinans*) thylakoids depleted of chloride, and then supplied with various salts or electron donors. Chloride depletion leads to loss of variable fluorescence which is recovered by electron donors (NH_2OH, catechol and ascorbate) or by NaCl, $NaNO_3$ and NaBr, but not by NaF or Na_2SO_4. Data from Critchley *et al.* (1982).

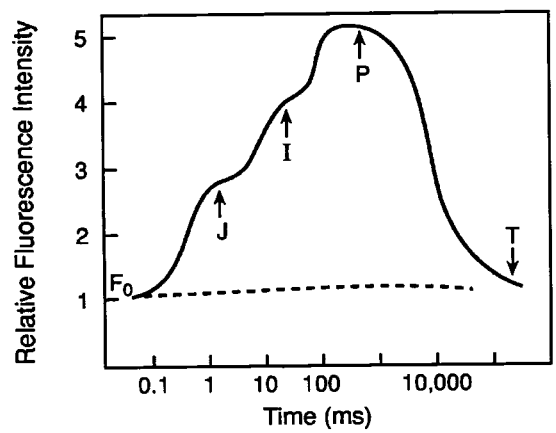

Fig. 6. Chl *a* fluorescence transient of an attached pea leaf, excited with 650 W m^{-2} 650 nm light, plotted on a logarithmic time scale. Two inflections, labelled as *J* and *I*, are observed between the levels *O* and *P*. Data from Strasser and Govindjee (1992).

not I and J, or I_1 and I_2. This, of course, may cause some confusion in the literature. However, the reason for our nomenclature was: (*a*) a comparison of the same fluorescence transient plotted on a linear and a logarithmic scale identified the I of the transient, and, the newer inflection was at a time shorter than that of this I; thus we gave it a new name—that of 'J'; and (*b*) since we had not proven the identity of these inflections with I_1 and I_2, we considered it safer to keep different names. The only reason, we think, why these inflections were revealed is very simple: with true 'O' level (F_o) obtained by careful use of the LED instrument, it was just the use of the logarithmic plot that allowed us to visualise clearly the two inflections on the same plot (see Fig. 6). However, our earlier work on Dl mutants of *Chlamydomonas reinhardtii* had neither revealed the two inflections nor the true 'O' level as the latter two were faster than the opening time of the camera shutter used (Govindjee *et al.* 1991). Measurements of Strasser *et al.* (1995) on the intensity dependence of the quantum yield of fluorescence at O, J, I and P reveal that J behaves like the I of Delosme, and is different from the I of usual fluorescence transients. If we had labelled the transient *OIJP*, it would have been consistent with Delosme (with J being the new inflection) but inconsistent with most of the transient literature. We, therefore, favour the use of *OJIP* with the understanding that J is equivalent of I_{Delosme}. However, $OI_1\ I_2\ P$ is another alternative, but is a bit cumbersome due to the use of the subscripts and the implication that I_1 and I_2 may have similar origin. Personally, I like the sound of '*OJIP*'.

Our current understanding of *OJIP* transient rise, that Kautsky and Hirsch could not have observed 60 years ago, is as follows: it reflects, in the first approximation, the successive but overlapping filling-up (i.e. reduction) of the electron acceptor pool of PSII. This pool includes not only the electron carriers in the main pathway (Q_A, Q_B, PQ, where Q_A is the one-electron acceptor-bound PQ, Q_B is the two-electron acceptor-bound PQ, and PQs are mobile PQ molecules), but also Q-400 (non-heme iron). The hypothesis of Duysens and Sweers (1963) that Q_A^- is the determining factor governing the increase in Chl *a* fluorescence is implicitly accepted here. The inflections represent the heterogeneity of the process. The *OJ* rise is the photochemical phase, the inflection J represents the momentary maximum of $[Q_A^-]$, perhaps, because of limitation in electron acceptance by Q_B, and I reflects the heterogeneity of the PQ pool, fast-reducing and slow-reducing PQ pools, an idea that is really not new (see Lavorel and Etienne 1977). Thus, the *OJIP* transient can be used as a quick monitor of the electron acceptor side reactions, the pool heterogeneity and pool sizes, and the effects of inhibitors and mutations on these processes, as well as on the donor side. Hsu (1993) has confirmed the earlier conclusion from the Joliot–Delosme laboratory that the fast fluorescence rise is influenced by the S-states. I do not recommend that you throw away your fluorometers (Holzwarth 1993). However, at the moment, we may not be able to easily obtain any quantitative information on the individual rate constants since the secondary reactions of both photosystem I (PSI) and PSII are slow compared with the single-turnover of the PSII reaction centre leading to the overlapping and complex effects (also see Trissl *et al.* 1993). Thus, we should wait for more sophisticated measurements of parallel transients of individual reactions and components, as well as for the evolution of more sophisticated deconvolution procedures. In the meantime, I recommend the use of kinetics of reactions following single-turnover flashes to monitor individual reactions of PSII.

Two-light-reaction and Two-pigment-system Concept

Kautsky Again

The concept of two light reactions through Chl *a* fluorescence studies was first considered by Kautsky and U. Franck (1943). They attributed the observed rise and fall of fluorescence to two light reactions succeeding one another almost immediately, one responsible for the rise and the other for the fall. Wassink (1951), however, pointed out that the quenching of fluorescence may have been caused by a side reaction. On the other hand, Rabinowitch (1945) discussed a two-light-reaction scheme (see scheme 7.V, on p. 162) to explain the requirement of eight quanta per O_2 (Fig. 7). And in 1956, Rabinowitch suggested (see p. 1862, par. 2) that one light reaction may transfer electrons from water to cytochrome, and another from cytochrome to a final acceptor. Kautsky *et al.* (1960), based on newer experiments on Chl *a* fluorescence *in vivo*, reiterated the suggestion that two consecutive light reactions worked in photosynthesis.

We shall now discuss the history of the evolution of the concept of the quencher, Q_A. Kautsky *et al.* (1960) discussed the concept that the oxidised state of a compound, A, a member of the electron transport chain, determined the quenching of fluorescence: when A was oxidised (A_O), Chl fluorescence was quenched, but when A was reduced (A_I), it was not. During Chl fluorescence transient, the rise was due to conversion of A_O to A_I, whereas the successive decline (presumably P to S?) was due to oxidation of A_I by the next member of the chain, B, in its oxidised state (B_O) —the latter was formed from B_I (reduced state) by another light reaction. The absence of fluorescence decline when the inhibitor phenylurethane was present was explained to be due to a block of reoxidation of A_I. In their model, A was closer to the O_2-evolving process, and B to the CO_2-fixation reactions. Although the above model is quite revealing, it lacked the impact because: (*a*) it ignored the existence of the

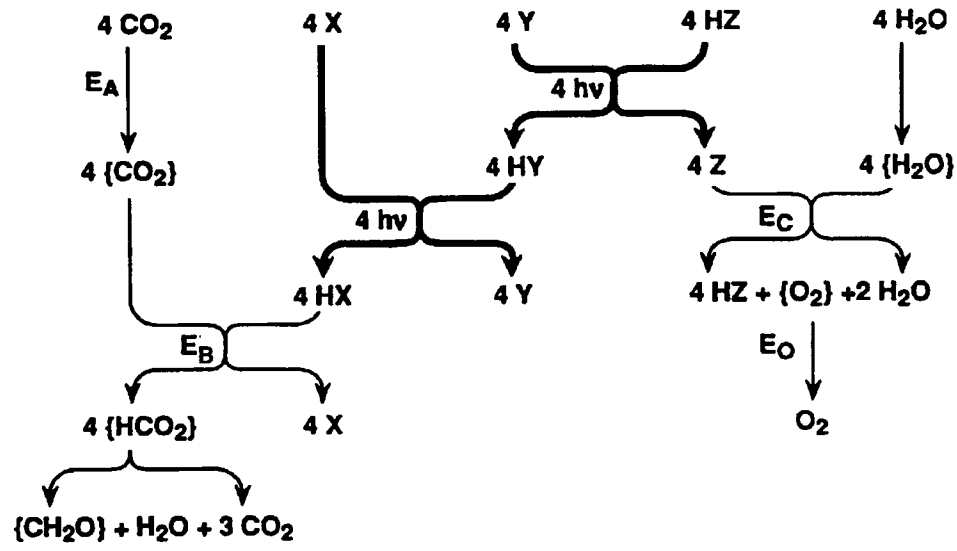

Fig. 7. Oxygenic photosynthesis with oxidation–reduction reactions between three intermediary catalysts (X, Y and Z) utilising two primary photochemical reactions, suggested as one of the several schemes to explain the eight quanta per O_2 measurements. One light reaction (bold lines) functions to transfer an electron (or hydrogen atom) from HZ to Y; the oxidised product Z reacts with H_2O to evolve O_2 (right side). Another light reaction (bold lines) functions to transfer an electron (or hydrogen atom) from HY to X; the reduced product HX reduces CO_2 to carbohydrate (CH_2O) (left side). The E's with subscript indicate that they are enzymatic reactions catalysed by different enzymes. In current language, electron transfer from HZ to Y involves PSII, and that from HY to X involves PSI. Now, Z could be equated with a specific tyrosine residue in PSII, Y with cytochrome *f* and X with $NADP^+$ or an equivalent intermediate. Original diagram from Rabinowitch (1945).

two-pigment-system concept already evolved by the work of Robert Emerson; (*b*) it was *not* the correct explanation of the fluorescence decline observed; and (*c*) it was published in a journal that many scientists did not read. On the other hand, Hill and Bendall (1960) proposed a scheme of two light reactions that had the additional possibility of providing energy for ATP synthesis during a downhill process between the two light reactions (see Duysens 1989, for the historical perspective of the discovery of the two-light-reaction scheme).

Govindjee *et al.* (1960) discovered that far-red light (absorbed in Emerson's longwave system, later known as system I, Duysens *et al.* 1961) quenched the high Chl *a* fluorescence (excited by blue or 670 nm light, Emerson's short-wave system, system II) in *Chlorella* cells. This antagonistic effect of light I and II on Chl *a* fluorescence yield was considered the fluorescence evidence for the two-light-reaction–two-pigment-system concept of photosynthesis. Butler (1962) demonstrated the same phenomenon in anaerobic leaf with red (650 nm, system II) and far-red (>720 nm, system I) beams. However, it was Duysens and Sweers (1963) who provided the current explanation: light II, absorbed in PSII, reduces a quencher of Chl *a* fluorescence, labelled as Q, and light I, absorbed in PSI, oxidises Q^- back to Q. The herbicide DCMU was shown to block the reoxidation of Q^-, but not the reduction

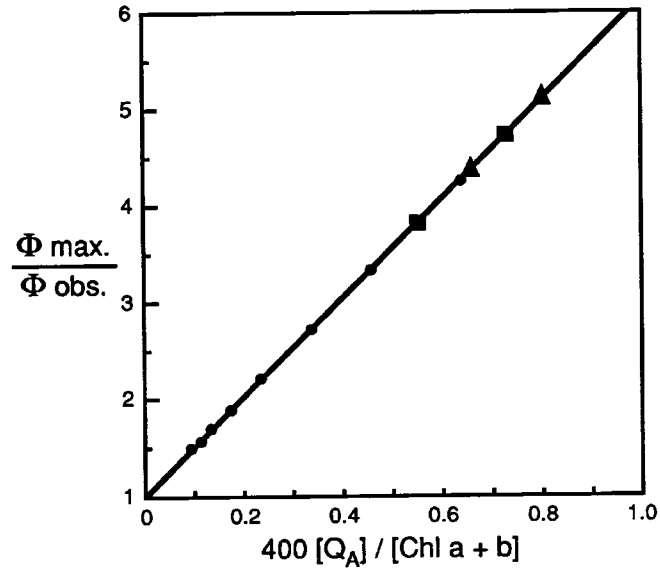

Fig. 8. Chl *a* fluorescence changes in Tris-washed thylakoids measured as a ratio of $F_{maximum}$ (labelled as Φ_{max}) to $F_{observed}$ (labelled as Φ_{obs}), plotted as a function of Q_A (400[Q_A]/[Chl *a* + Chl *b*]). [Q_A^-] was measured by absorbance changes at 320 nm due to the formation of plastosemiquinone. Changes were induced by a series of non-saturating flashes; different symbols refer to different experiments. These data suggest a linear relationship between Chl *a* fluorescence parameters and [Q_A]. Data from Van Gorkom *et al.* (1978).

of Q. Today, this Q is known as Q_A, and is shown to be a PQ molecule (see Van Gorkom *et al.* 1978) (Fig. 8). The antagonistic effect of light I and II on Chl *a* fluorescence yield is still a useful tool to investigate the site of an inhibitor between Q_A and P700, the reaction centre Chl *a* of PSI, as recently shown for bicarbonate-reversible formate inhibition (Govindjee *et al.* 1993*a*).

The Two-pigment Systems

Different spectral varieties of Chl *a* (see French 1971) are present in different pigment–protein complexes of both PSI and PSII. As earlier mentioned, most of the Chl *a* fluorescence (approx. 90%) at room temperature originates in PSII complexes, PSI complexes being weakly fluorescent. Further, it is only the PSII fluorescence that varies with changes in photochemistry, i.e. the variable Chl *a* fluorescence belongs strictly to PSII. Why is PSI weakly fluorescent, and why there is no variable fluorescence in it are important questions that have not been systematically dealt with yet. Among several others, the following speculations can be made:

(*a*) The reaction centre Chl *a* of PSI, the P700, is a deeper energy trap than the trap of PSII, the P680, and, thus, its photochemistry may not be 'trap-limited', i.e. energy transfer to it is more irreversible than to P680; the antenna fluorescence of PSI does not compete with PSI chemistry.

(*b*) The immediate electron acceptor or donor to P700 is not a chemical quencher of Chl *a* and, thus, variations in Chl *a* fluorescence do not occur.

(*c*) The physico-chemical nature of antenna Chl *a* of PSI, that absorb, on the average, longer wavelength of light than shorter wavelength, is such that k_h predominates over k_f. We know that the lifetime of PSI Chl *a* fluorescence is shorter than that of PSII Chl fluorescence, i.e. it traps energy faster than PSII (Holzwarth 1991). Of course, this means a lower quantum yield of fluorescence as $\phi_f = \tau/\tau_o$.

Room temperature

Although there is a heterogeneity in Chl *a* fluorescence at room temperature because of the existence of two photosystems (PSI and PSII), the major fluorescence band at 683–685 nm and its vibrational satellite at 720–735 nm originate mostly in the PSII antenna complexes (Table 2). I am unable to state as to exactly what proportion comes from which of the PSII complexes. I suspect that most of the variable Chl *a* fluorescence originates in CP43 and CP47 Chl *a* protein complexes, with CP47 being responsible for a small 693–695 nm emission when PSII reaction centres are *closed* either by strong light or by DCMU. The existence of a 693–695 nm emission at room temperature in different organisms was shown by Krey and Govindjee (1964, 1966), Papageorgiou and Govindjee (1967, 1968*a*, 1968*b*) and Govindjee and Briantais (1972). On the other hand, a PSI emission, that may be from an 'ordered' Chl *a*, is centered around 705–715 nm (Lavorel 1963; Wong and Govindjee 1979; Goedheer 1981). Fig. 9 summarises these concepts.

Low temperature

At 77K, Chl *a* *in vivo* produces, at least, four emission bands: F685, F695, F720 and F740 in addition to the long wavelength shoulders due to the various vibrational satellite bands. Brody (1958) discovered that cooling *Chlorella* cells to 77K leads to the formation of a new broad emission band at about 725 nm. It was shown by Govindjee and Yang (1966) and Cho and Govindjee (1970*a*) to be composed of, at least, two bands (Table 2). Mar *et al.* (1972) showed that

Table 2. Chlorophyll *a* fluorescence bands in oxygenic thylakoids

Emission range – peak or shoulder (nm)	Name	Possible origin of Chl *a*	Comments
		At room temperature	
683–687	F685	PSII core: CP43, CP47??	Major band
693–698	F695	PSII core: CP47?	Minor shoulder: with high $[Q_A^-]$ (1964)
705–712	F710	PSI core or antenna	Minor: F_o/F_m spectrum (1963)
720–760	F740	PSII + PSI	Broad vibrational satellite bands
		At low temperatures (4–120K)	
			Upon cooling
679–682	F680	LHCII (1979)	Minor: appears at 4K
683–687	F685	PSII core: CP43 (1984)	Major: increases below 20K (1966)
693–700	F695	PSII core: CP47 (1984)	Major: appears below 120K (1966); decreasese below 20K
705–715	F710	???	
715–730	F720	PSI reaction centre complex (1980)	Major: includes vibrational satellite bands
730–750	F740	LHCI (1980)	Major: includes vibrational sattelite bands

it could also be distinguished from F685 as it had a longer lifetime of fluorescence. Although Litvin and Krasnovsky (1958) had observed the existence of a band at 695 nm in etiolated leaf (originating in a chlorophyll precursor), it was not until 1963 that the existence of F695 was discovered and generally recognised to originate in PSII (Bergeron 1963; Brody and Brody 1963; Govindjee 1963; Kok 1963). Although it was recognised independently in three laboratories that F685 and F695 belong to PSII and F720 and F740 to PSI (Boardman *et al.* 1966; Cederstrand and Govindjee 1966; Govindjee and Yang 1966; Murata *et al.* 1966*b*), earlier assignments to particular protein complexes were in error. Contrary to earlier beliefs, F685 and F695 *do not* belong to light-harvesting complex II (LHCII) and reaction centre II, respectively. Although their complete assignment is still not fully established, most of F685 and F695 belong to Chl *a* in core PSII complexes (Gasanov *et al.* 1979; Rijgersberg *et al.* 1979), and F720 and F740 to PSI core (reaction centre I containing intrinsic antenna Chls) and light-harvesting complex I (LHCI), respectively (Mullet *et al.* 1980*a*, 1980*b*). Although Nakatani *et al.* (1984) turned out to be in error in assigning the function of reaction centre II to CP47, they are correct in assigning F685 to originate in CP43 Chl *a* and F695 to Chl *a* in CP47. PSI band F720 originates in a Chl *a* complex absorbing at 695 nm (Das and Govindjee 1967) and F740 in a Chl *a* complex absorbing at 705 nm (Butler 1961). On the other hand, a band at 680 nm (F680) appears at 4K only when LHCII is present (Rijgersberg *et al.* 1979). Thus, F680 belongs to Chl *a* from LHCII; it cannot be normally observed due to highly efficient transfer from it to other complexes (see Fig. 9). In addition, Shubin *et al.* (1991) have observed a new emission band in a cyanobacterium *Spirulina platensis* at 758 nm (F758), at 77K, which originates in a Chl complex with an absorption band at 735 nm (Chl^{758}_{735}). Interestingly, this complex transfers its excitation energy to the oxidised form of the reaction centre of PSI, $P700^+$, and thus, quenching F758 during photo-oxidation of P700.

Photosynthetic Unit and Excitation Energy Transfer

Photosynthetic Unit

Gaffron and Wohl (1936*a*, 1936*b*) interpreted the results of Emerson and Arnold (1932*a*, 1932*b*) stating that a collection of 2500 Chl molecules somehow cooperates to evolve, with high quantum efficiency, one molecule of O_2, as follows: light energy, absorbed anywhere in this *unit*, the *photosynthetic unit*, migrates by excitation energy transfer to the *photoenzyme* where several excitons cooperate to initiate photosynthesis. This is in contrast to diffusible chemicals being formed at each site, and then diffusing to the *photoenzyme*. This concept of a photosynthetic unit composed of many pigments serving a photoenzyme has been conceptually supported by the discovery of excitation energy transfer and of the reaction centre chlorophylls P700 (Kok 1956) and P680 (Döring *et al.* 1967) and the many pigment–protein complexes that contain only antenna or bulk pigments.

Robinson (1967) coined the terms *lake* versus *puddles* for the organisation of antenna and reaction centre chromophores. In the lake model, also called the statistical or the matrix model, the exciton may freely visit territories surrounding reaction centres. In contrast, in the isolated puddles, the separated units, or the restricted model, the exciton can visit only its own reaction centre. However, the situation may be 'in-between', i.e. there may be some probability of energy exchange between the different puddles. Looking at the existence of various pigment–protein complexes, it is quite likely that a 'pebble-mosaic' model (Sauer 1975) is the real picture. It remains a challenge to provide a complete mathematical and physical model for exciton migration in photosynthesis.

Whether there is a directed or a random exciton migration must depend on many factors including the relative energy levels of the donors and the acceptors. The directed model (the *funnel* model) seems to be appropriate for heterogeneous energy transfer in phycobilisomes, or even when one deals with transfer from shortwave to long

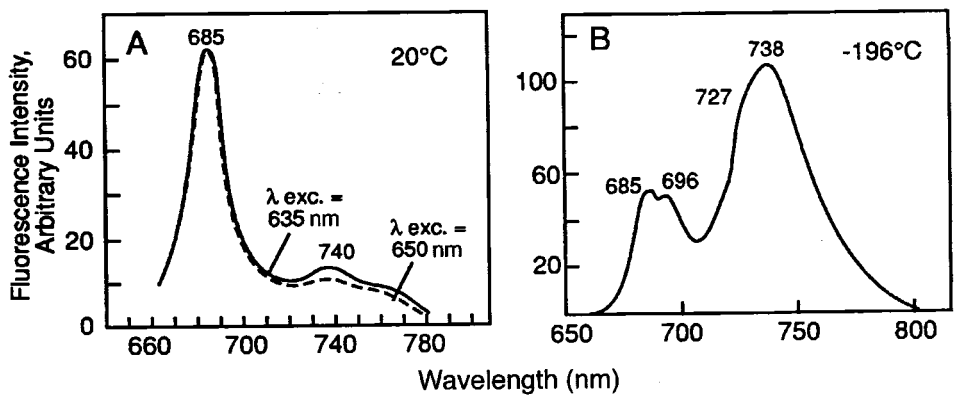

Fig. 9. Chl *a* fluorescence spectrum of spinach thylakoids at room temperature (*A*) and at 77K (*B*). Presented by Govindjee at the 10th International Botanical Congress, Edinburgh 1964. Data from Govindjee and Yang (1966).

wavelength forms of Chl *a* (Govindjee *et al.* 1967; Seely 1973). However, a random migration is more appropriate for homogeneous energy transfer among isoenergetic pigment molecules (see Discussion in Pearlstein 1982).

Butler and Strasser (1977), Strasser and Butler (1977), Butler (1978) and Strasser (1978) have discussed various *bipartite* or *tripartite* and *grouping* models of organisation of pigments. These concepts have been extensively used in the literature, but will not be discussed here.

Excitation Energy Transfer

A detailed and mechanistic picture of excitation energy (exciton) transfer is only possible when the detailed distances and orientations of the chromophores are known. A major breakthrough in this direction has been the recent electron diffraction picture of LHCII by Kuhlbrandt *et al.* (1994). It shows the detailed arrangement of individual Chl *a* and Chl *b* molecules, their orientation and distances (approx. 5Å edge-to-edge). From Förster's resonance theory (Förster 1948), one can calculate excitation energy transfer from one molecule to another—the rate of this transfer is dependent upon three crucial parameters:

(*a*) $1/R^6$, where R is the distance between the donor and the acceptor molecules;

(*b*) $(\kappa)^2$, where κ(orientation factor) = $\cos\alpha - 3\cos\beta_1 \cos\beta_2$, with α and β being angles between the acceptor and donor dipoles and between the vector that connects the dipoles and each dipole, respectively; and

(*c*) the overlap of energy levels, as calculated by the overlap integral between the fluorescence spectrum of the donor and the absorption spectrum of the acceptor molecule (see e.g. Van Grondelle and Amesz 1986).

In Förster's theory, excitation energy transfer from a donor to an acceptor occurs after there is a relaxation by internal conversion within the donor molecule. The overlap integral between donor and acceptor molecules is temperature dependent. Thus, temperature dependence of energy transfer had been predicted. Cho *et al.* (1966) and Cho and Govindjee (1970*a*) observed changes in emission spectra of *Chlorella* cells as they decreased temperatures down to 4K; similar changes were observed in cyanobacterium *Anacystis* (Cho and Govindjee 1970*b*). Although other interpretations are possible, these results were taken to support the Förster theory for energy transfer from phycobilins to Chl *a* and for transfer from Chl *a* fluorescing at 685 nm to that fluorescing at 695 nm.

Excitation energy migration (homogeneous energy transfer) studies between Chl *a* molecules were pioneered by Arnold and Meek (1956) through observation of depolarisation of Chl fluorescence. These investigations were later pursued in my laboratory by Ted Mar and Daniel Wong (see Mar and Govindjee 1972; Wong and Govindjee 1981) and Whitmarsh and Levine (1974). A decrease in the polarisation of Chl fluorescence by closure of PSII reaction centres was taken as evidence of increased energy migration. However, due to a lack of detailed knowledge of the orientation of dipoles, and due to a possible lack of coherence of excitons even after one or two transfers, conclusions from such studies have been rather limited, and extraction of quantitative information about energy migration rather difficult (see e.g. Knox 1975).

The existence of excitation energy transfer (heterogeneous energy transfer), however, has been convincingly shown by the technique of steady-state sensitised fluorescence, from fucoxanthol to Chl *a* (Dutton *et al.* 1943), from phycobilins to Chl *a* (Duysens 1952; French and Young 1952) and from Chl *b* to Chl *a* (Duysens 1952). Excitation in the donor molecule shows a quenching of the donor fluorescence and a stimulation or enhancement of the acceptor fluorescence.

Müller (1874) had already commented on the lower fluorescence intensity of leaves over that in solution, implying the use of the absorbed energy in a leaf for photosynthesis. This concept has been quantitatively emphasised when it was noted that the quantum yield of Chl *a* fluorescence *in vivo* is 0·03–0·06 in contrast to 0·25–0·30 *in vitro* (Latimer *et al.* 1956)—the majority of the absorbed energy *in vivo* must be used in photosynthesis. Since the quantum yield of fluorescence (Φ_f) is directly proportional to the lifetime of fluorescence (τ), and since the latter can also provide unique information on the primary photochemical events of photosynthesis, a major advancement was made when Brody and Rabinowitch (1957) and Dmetrievsky *et al.* (1957), independently, and by independent methods (direct flash and phase shift), measured the lifetime of Chl *a* fluorescence *in vivo*. Even in the very first paper, Brody and Rabinowitch (1957) showed that there was a delay in observing Chl *a* fluorescence when phycoerythrin was excited, showing that energy transfer takes a finite time when it moves from phycobilins to Chl *a*. Tomita and Rabinowitch (1962) calculated this time to be about 300 ps and the efficiency of energy transfer to be 80–90%. The time of energy transfer from Chl *b* to Chl *a* was too fast to be resolved, but the efficiency of transfer was confirmed to be 100%, as earlier found by Duysens (1952) in steady-state measurements.

Indeed, when ultrashort (femtoseconds to picoseconds) flashes of light are used to excite donor molecules, one can measure precise times for the movement of excitation energy from the donor to the acceptor molecule: as the donor fluorescence subsides, the acceptor fluorescence appears. A beautiful cascade has now been observed in the red algae where one can follow precisely the excitation energy transfer by this technique, from phycoerythrin to phycocyanin to allophycocyanin (see e.g. Yamazaki *et al.* 1984). These events occur in picosecond time scale (see Fig. 10).

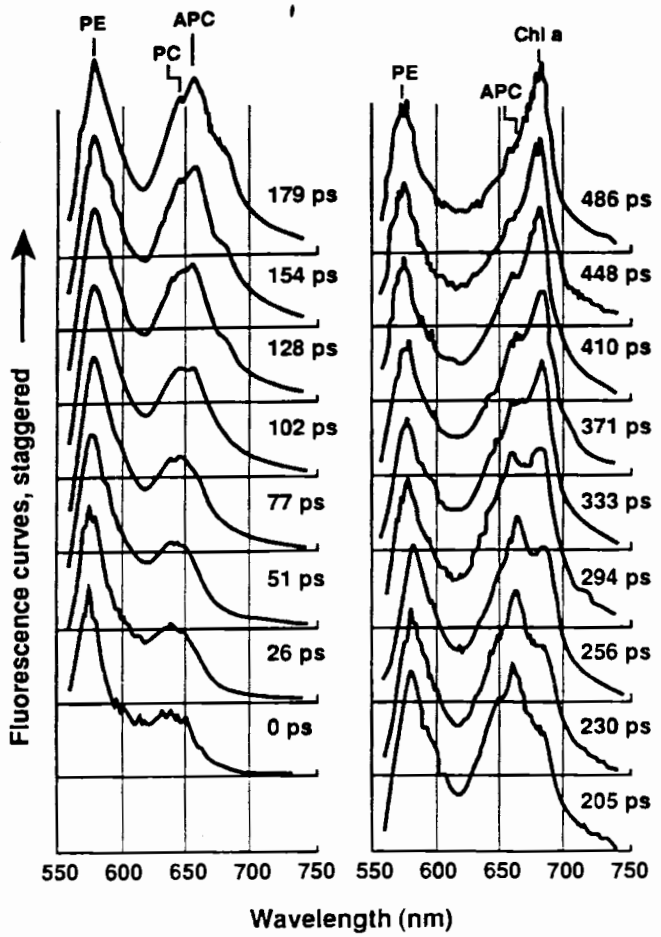

Fig. 10. Cascade of excitation energy transfer in picosecond time domain from PE (phycoerythrin) to PC (phycocyanin) to APC (allophycocyanin) to Chl *a* in the red alga *Porphyridium cruentum* after excitation with a 6 ps 540 nm flash. The vertical lines mark the maximum of fluorescence of various pigments. The time sequence starts at the bottom of the left, and, then again at the bottom of the right graph. Data from Yamazaki *et al.* (1984).

Probe of Photosystem II

Chlorophyll *a* fluorescence measurements have been most useful in probing PSII reactions since, as mentioned twice, most Chl *a* fluorescence at room temperature is from PSII.

Basic Relationship

Ignoring extraneous quenching processes, assuming that all energy transfer in PSII leads to photochemistry, and omitting terms for quenching by $P680^+$ (see later discussion) and those originating in *S*-state transitions, Eqn (1) can be simplified to:

$$\Phi_f = \frac{k_f}{k_f + k_h + k_i + k_q + k_p} . \qquad (2)$$

where it is assumed that we are only considering PSII, and $[Q_A]$ is the concentration of electron acceptor of PSII.

At low light intensities, when most $Q_A = Q_A$ (assume it is 1), i.e. its concentration is maximal, Chl *a* fluorescence yield is minimal:

$$\Phi_f^{min}(\text{at } F_o) = \frac{k_f}{k_f + k_h + k'_p} . \qquad (3)$$

On the other hand, at strong light intensities, when all $Q_A = Q_A^-$, $[Q_A] = 0$, Chl *a* fluorescence yield is ~~minimal~~ maximal:

$$\Phi_f^{max}(\text{at } F_{max}) = \frac{k_f}{k_f + k_h} . \qquad (4)$$

Dividing (3) by (4):

$$\frac{\Phi_f^{min}}{\Phi_f^{max}} = \frac{k_f}{k_f + k_h + k'_p} \times \frac{k_f + k_h}{k_f} = \frac{k_f + k_h}{k_f + k_h + k'_p} .$$

Adding and subtracting k'_p in the numerator:

$$= \frac{k_f + k_h + k'_p - k'_p}{k_f + k_h + k'_p} = 1 - \frac{k'_p}{k_f + k_h + k'_p} = 1 - \Phi_p^{max} .$$

Thus,

$$\Phi_p^{max} = 1 - \frac{\Phi_f^{min}}{\Phi_f^{max}} = \frac{\Phi_f^{max} - \Phi_f^{min}}{\Phi_f^{max}} = \frac{\Delta\Phi_f^{max}}{\Phi_f^{max}} , \qquad (5)$$

which is equivalent to $F^{max}_{variable} / F_{max}$. This is the origin of the well-known relationship between measured fluorescence parameters and the quantum yield of photochemistry of PSII. This relationship has the implicit assumption that not only are all the postulates given above true, but none of the rate constants k_f, k_h and k'_p change between F_o and F_{max}, i.e. the restrictions are indeed severe.

Shinkarev and Govindjee (1993) have re-emphasised Eqn (1) in which the rate constant kq cannot be ignored under many experimental conditions. It should, we believe, include the terms $kq[P680^+]$, and perhaps, $kq[Pheo^-]$ unless they are proven to be zero, and kp should include the concentration of the pair $P680Q_A$, not just Q_A, as was already done by Duysens (1979). Extending the concept to include a possible quenching by O_2 (see Discussion in Papageorgiou 1975), we may rewrite Eqn (1) as:

$$\Phi_f = \frac{k_f}{k_f + k_h + k_i + k_q[O_2] + kq[P680^+] + kq[Pheo^-] + k'_p[P680Q_A]} . \qquad (6)$$

If, however, we must deal with high intensity flashes, where the possibility of triplet formation exists, one has to include it as another quenching process (Sonneveld *et al.* 1979).

The concept of fluorescence yield as a monitor of only one process is obviously wrong and the early concepts of Kautsky and Hirsch (1931) and MacAlister and Myers (1940) about the inverse relationship between fluorescence and photosynthesis are specific but important cases when most of the other rate constants remain constant except for the major one that governs photosynthesis. Genty *et al.* (1989) have provided the most significant correlation between the measured quantum yield of photosynthesis and that calculated from fluorescence measurements (Fig. 11).

Connectivity Between Units

In the *lake* model, excitons migrate freely (random walk). If they encounter a closed reaction centre Chl *a*, they can just go to another centre (Knox 1975; Pearlstein 1982). Such a model predicts a linear relationship between lifetime of fluorescence, τ, and quantum yield of fluorescence, Φ_f, as the traps are closed, by varying the intensity of excitation or by addition of an inhibitor. Briantais *et al.* (1972) introduced a τ versus Φ_f diagram, and showed a proportionality between the two quantities throughout the entire range of measurements in *Chlorella* cells (Fig. 12). This result and the earlier results of Tumerman and Sorokin (1967) were taken to support the *lake* model. It did not support the strict 'isolated puddles' model, where exciton can visit *only* its own reaction centre, because fluorescence would have to be dealt with as a sum of fluorescence from open and closed units, leading to a significant nonlinearity in the τ–Φ_f curve.

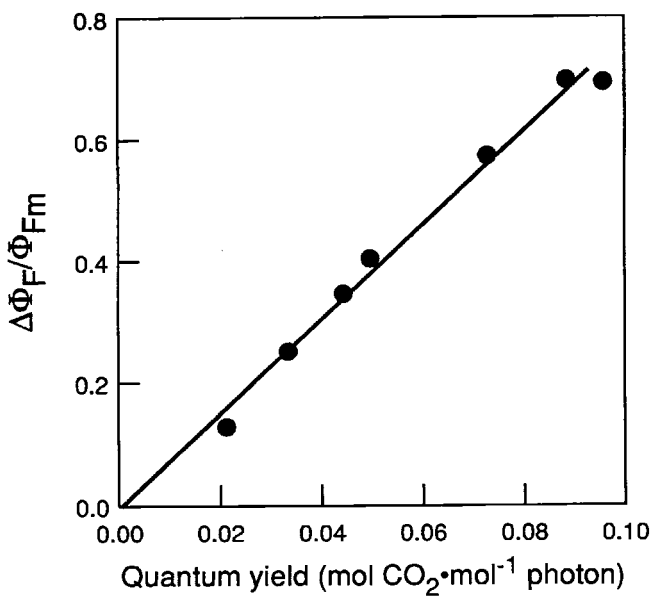

Fig. 11. Relationship between the photochemical yield of PSII, calculated from variable/maximum ($\Delta\phi_F/\phi_{Fm}$) Chl *a* fluorescence yield, and the quantum yield of CO_2 fixation in a barley leaf. Note the linear relationship. Data from Genty *et al.* (1989).

In reality, however, the picture may be 'in-between', i.e. there may be a certain probability of exciton migration from one unit to another, as if there were interconnected puddles or a pond. Joliot and Joliot (1964) had derived a relationship:

$$\frac{F_{(t)} - F_o}{F_{max} - F_o} = \frac{(1-p)q}{1-pq}, \qquad (7)$$

where, $F_{(t)}$ is the Chl *a* fluorescence yield at time *t*, F_o is the fluorescence yield when all Q_A is in the oxidised state, F_{max} is the maximum fluorescence yield when all Q_A is in the reduced state, p is a parameter related to the probability of intersystem energy transfer, and q is the fraction of closed reaction centres, counted in the coin of Q_A. Here $q = 1$, when Q_A^- is maximum. Joliots' calculated the parameter p, that depended solely on the variable Chl fluorescence. The calculated values of p have hovered around 0·5 in most cases.

Both Paillotin (1976, 1978) and Strasser (1978) have independently pointed out difficulties with this concept and have suggested modifications. As the centres close, the proportion of open centres decrease. Many scientists, including ourselves (Xu *et al.* 1989), have used Eqn (7) without questioning its validity. Paillotin (1976) suggested

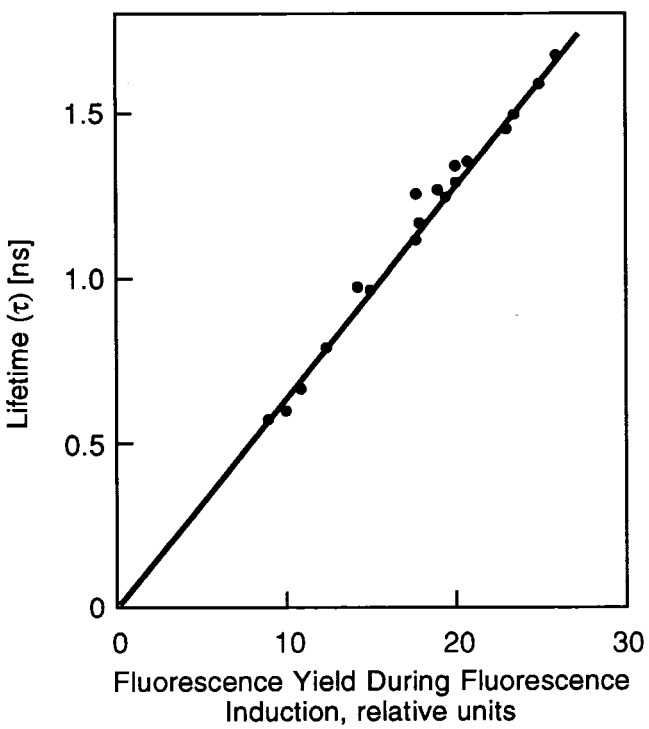

Fig. 12. τ (lifetime) versus Φ_f (yield) diagram for Chl *a* fluorescence of the green alga *Chlorella*. Φ_f was changed by varying the flow rate of algae suspension. The frequency of modulation for τ measurement was 102·207 MHZ. Wavelength of excitation was 632·8 nm. Note the linear relationship of τ with changes in Φ_f suggesting the 'lake' model for the organisation of pigments. Data from Briantais *et al.* (1972).

using a physical connection parameter P that depends only upon exciton migration from a closed to an open reaction centre; he relates it to Joliots' p as follows:

$$p = P(1-F_O/F_{max}) = P \times F_{variable}/F_{max}. \quad (8)$$

On the other hand, Strasser (1978) has proposed that the probability of exciton migration in Joliots' equation be corrected by the ratio of $F_{variable}/F_o$. For a relationship between the three equations, see Strasser *et al.* (1992).

In a rather effective manner, Trissl *et al.* (1993) have challenged many concepts and provided reasons for further caution in blindly using fluorescence induction measurements to make quantitative calculations. We need to look seriously at this paper and the follow-up paper (Trissl and Lavergne 1995) in which a parameter J, related to P, is explicitly given by a number of rate constants. I shall now discuss where Chl *a* fluorescence measurements have been highly useful in probing PSII reactions, but first I will present a detailed schematic of PSII reactions that is needed to appreciate the experiments.

The Photosystem II Reactions

Most of the PSII Chl *a* fluorescence in PSII preparations and in thylakoids that we measure is from antenna Chl *a* molecules, not reaction centre Chl *a* molecules. The variable Chl *a* fluorescence is created either from exciton equilibration between the antenna and the reaction centre Chl *a*, or from exciton/radical pair equilibration (see Renger 1992, for literature and discussion of PSII chemistry). It had been generally believed that all the PSII fluorescence was prompt fluorescence. Klimov *et al.* (1977) suggested that all of the variable Chl *a* fluorescence of PSII was recombinational luminescence from the back reaction of $P680^+$ with $Pheo^-$. Although there hasn't been a general acceptance of this concept (see Van Gorkom 1986), the exciton/radical pair equilibration recombination model (see Holzwarth 1991) seems capable of accommodating it. A good part of fluorescence from the *isolated* PSII reaction centre, however, originates in recombination of $P680^+$ with $Pheo^-$ (see e.g. Govindjee *et al.* 1990*a*).

Upon excitation of the reaction centre Chl *a* P680 of PSII, the following reactions occur (Eqn 9(1–13)).

Eqn (9):

$$\underset{(1)}{S_1Z\,P680^*\,Ph Q_A Q_B} \overset{3\text{ ps}}{\leftrightarrow} \underset{(2)}{S_1Z P680^+\,Ph^- Q_A Q_B} \overset{200\text{ ps}}{\leftrightarrow}$$

$$\underset{(3)}{S_1Z\,P680^+\,Ph Q_A^- Q_B} \overset{20\text{ ns}}{\leftrightarrow} \underset{(4)}{S_1Z^+\,P680\,Ph Q_A^- Q_B} \overset{200\ \mu s}{\leftrightarrow}$$

$$\underset{(5)}{S_1Z^+\,P680\,Ph Q_A Q_B^-} \overset{600\ \mu s}{\leftrightarrow} \underset{(6)}{S_2Z\,P680\,Ph Q_A Q_B^-} \overset{H^+}{\leftrightarrow}$$

Q_B^- has a long lifetime; its neighborhood gets protonated; after another excitation, the reaction continues:

$$\underset{(7)}{S_2Z\,P680^*\,Ph Q_A^- Q_B^-(H^+)} \overset{3\text{ ps}}{\leftrightarrow} \underset{(8)}{S_2Z\,P680^+\,Ph^- Q_A Q_B^-(H^+)} \overset{\sim 200\text{ ps}}{\leftrightarrow}$$

$$\underset{(9)}{S_2Z\,P680^+\,Ph Q_A^- Q_B^-(H^+)} \overset{50\text{ ns; }200\text{ ns}}{\leftrightarrow} \underset{(10)}{S_2Z^+\,P680\,Ph Q_A^- Q_B^-(H^+)} \overset{\sim 400\ \mu s}{\leftrightarrow}$$

$$\underset{(11)}{S_2Z^+\,P680\,Ph Q_A Q_B^{2-}(H^+)} \overset{800\ \mu s}{\leftrightarrow} \underset{(12)}{S_3Z\,P680\,Ph Q_A Q_B^{2-}(H^+)} \overset{H^+}{\leftrightarrow}$$

$$\underset{(13)}{S_3Z\,P680\,Ph Q_A Q_B H_2} \overset{PQ \rightarrow PQH_2}{\leftrightarrow} S_3Z\,P680\,Ph Q_A Q_B$$

Here, S_n represents the redox states of the O_2-evolving Mn complex, Ph is pheophytin, and other symbols have been defined earlier. Due to the imprecise knowledge of the exact times listed above, it is not clear which reactions control the relaxation of the *S*-states.

Analysis of primary photochemical reactions by lifetime of fluorescence measurements has led Van Mieghem *et al.* (1992) and Govindjee *et al.* (1993*b*) to conclude that PSII charge separation (Eqn 9(1)) is decreased if Q_A^- is present, but is increased if doubly reduced Q_A, Q_A^{2-}, is present. The prior redox state of the donor side may also have effects on the reactions presented above.

Z to P680$^+$ reaction

In the nanosecond to sub-microsecond time scale, Chl *a* fluorescence yield rise, after a brief ($\leqslant$ ns) actinic flash, measures the electron flow from Z to $P680^+$ (steps (3) and (9) in Eqn 9). This rise was discovered by Mauzerall (1972) and explained by Butler (1972) to be due to the removal of the quencher $P680^+$ (Fig. 13) Sonneveld *et al.* (1979) have elegantly measured this reaction, after correcting for quenching due to triplets, and showed that this reaction was faster (approx. 20 ns) during transition of S_0 and S_1, and slower and more complex during transitions of S_2 and S_3 (for a description of *S*-states, see Kok *et al.* 1970). This fluorescence rise can be observed even at longer times due to the equilibria reactions between $S_n \leftrightarrow Z \leftrightarrow P680$ (see e.g. Kramer *et al.* 1990).

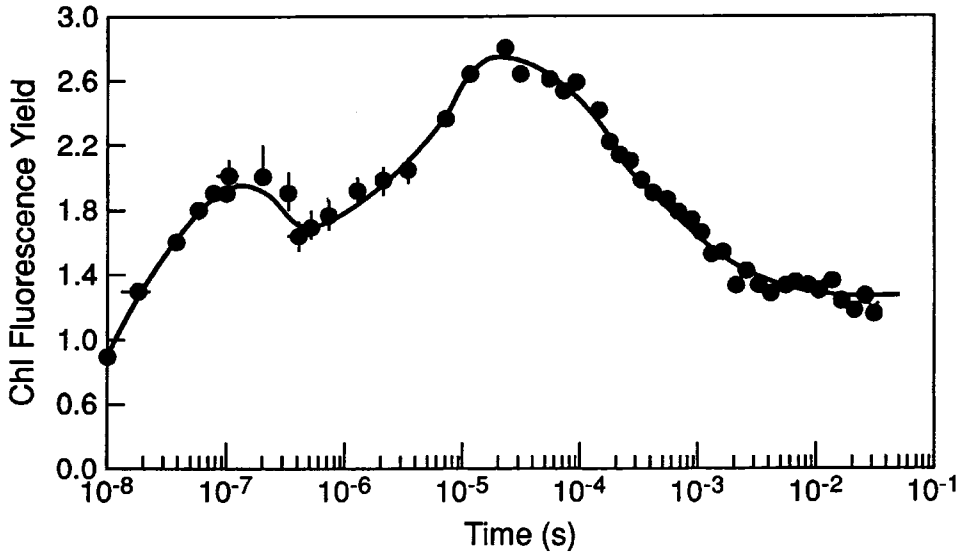

Fig. 13. Chl *a* fluorescence yield changes in the dark-adapted cells of the green alga *Chlorella* after a saturating nanosecond laser flash. The rise near 20 ns was ascribed by Butler (1972) to the reduction of the quencher $P680^+$ by *Z*; the rise in the microsecond range is due to the disappearance of the carotenoid triplets (see Sonneveld *et al.* 1979); the decrease in fluorescence yield in the 10 μs to millisecond range is due to the electron transfer from Q_A^- to Q_B (Forbush and Kok 1968). Data from Mauzerall (1972).

Q_A^- to PQ reactions

In the microsecond to millisecond time scale, Chl *a* fluorescence yield decay, after a brief flash, measures the electron transfer from Q_A^- to Q_B (steps (4) and (10) in Eqn 9). These measurements were presented, in a preliminary fashion, by Forbush and Kok (1968) who used a 1 ms (approx.) saturating flash to induce a single turnover of PSII reaction centre; they observed a fast decay phase of $t_{\frac{1}{2}} \sim 0{\cdot}6$ ms, which they correctly attributed to re-oxidation of Q_A^-; about 18 flashes were needed to fill completely the secondary acceptor PQ pool then called the *A* pool. They also remarked at the heterogeneity of this *A* pool. Although Mauzerall (1972) showed the microsecond to millisecond fluorescence decay (Fig. 13), the first detailed and reliable measurements on this decay was by Zankel (1973) who observed a phase of $t_{\frac{1}{2}} \sim 200$ μs and another of 1 ms, and related them to the equilibria between $Q^- \leftrightarrow A$ and $Q^- \leftrightarrow A \leftrightarrow A'$ (Q being Q_A, and A and A' being the two fractions of the *A* pool, the fast and the slow reducing pool).

Further, Zankel (1973) was the first one to observe that the Chl *a* fluorescence decay after first flash was faster than after the second flash (Fig. 14) as was rediscovered by Bowes and Crofts (1980). Instead of explaining this result on the basis of electron transfer on the acceptor side of PSII, Zankel related it incorrectly to the donor side since, I believe, he had just confirmed in the same paper the period 4 oscillation in the 35 μs fluorescence signal, originally discovered by Delosme (1971) and related to the *S*-states of the O_2-evolution steps.

The two-electron gate

The concept of the two-electron gate was elegantly demonstrated in an experiment, that I consider to be a major breakthrough, by Velthuys and Amesz (1974). In these experiments, the possible oscillations due to the donor side (the *S*-state cycling related to O_2-evolution steps) were eliminated by alkaline Tris-washing, and an external electron donor was added to run PSII. A series of preflashes were given and then the herbicide DCMU was injected and Chl *a* fluorescence yield monitored. There was an obvious binary oscillation in the Chl *a* fluorescence yield: high after the first and all uneven preflashes, and low after the second and all even preflashes (Fig. 15). This work provided, for the first time, information on how one electron acceptor, Q_A (then called Q), communicates with the two-electron-acceptor PQ molecule. The authors assumed that a carrier they had called *R* (now called Q_B) exchanges electrons one

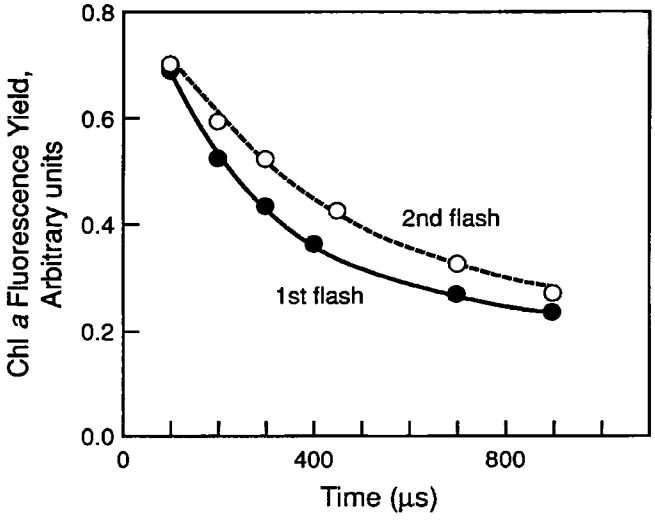

Fig. 14. Decay of variable part of Chl *a* fluorescence yield as a function of time after flash 1 and 2, spaced 2 s apart. Sample: dark-adapted spinach chloroplasts at 22°C. Note that the decay is faster after the first than after the second flash. Data from Zankel (1973).

by one with Q_A, but two by two with PQ. This is the meaning of, what we call today, the *two-electron gate.* Bowes and Crofts (1980) explained their results, in which Chl *a* fluorescence yield decays faster after the first than after the second flash, in terms of a slower electron flow from Q_A^- to Q_B^- (step (10)) than from Q_A^- to Q_B (step (4)) possibly because of electrostatic repulsion from Q_B^-.

The existence of a 'two-electron gate' somewhere between PSII and electron acceptance by methyl viologen from PSI was shown by Bouges-Bocquet (1973) in a paper that was submitted within a week or so of that by Velthuys and Amesz (1974). Bouges-Bocquet had called the carrier *B*, and shares the credit of independent discovery of the two-electron gate.

Kinetics of S-states

As mentioned earlier, electron transfer from Z to $P680^+$ can be measured through Chl *a* fluorescence rise in the nanosecond to sub-microsecond range after an actinic flash. However, this does not take into account the equilibria between the *S*-states and *Z*, and *Z* and P680. Ignoring the acceptor side, this can be written as:

$$S_n Z P680^+ \overset{K_{ZP}}{\leftrightarrow} S_n Z^+ P680 \overset{K_{SZ}}{\leftrightarrow} S_{n+1} Z P680 .$$

There are two possibilities of how *S*-states can control Chl *a* fluorescence yield: (*a*) a greater positive charge on the *S*-states can slow the positive charge transfer from *Z* to S_n and, thus, the latter can slow the positive charge transfer from $P680^+$ to *Z*, leading to a higher concentration of the natural quencher [$P680^+$]; and (*b*) a direct influence of *S*-states on the Chl *a* fluorescence yield. I have no idea as to how this will function. There also exists the possibility of O_2, *per se*, released during S_4 to S_0 transition to cause quenching of Chl *a* fluorescence (Shinkarev *et al.* 1994). Shinkarev *et al.* (1994) have measured the kinetics of the difference between the inverse of the fluorescence yield after the first flash (S_1 to S_2 transition, no O_2 evolution) and that after the third flash ($S_3 \rightarrow S_4 \rightarrow S_0$ transition, O_2 evolution; see Kok *et al.* 1970; Renger 1993). Analysis of this data shows that a quencher is produced with a lag of approximately 1 ms and a rise half-time of about 2 ms (Fig. 16). The amplitude of

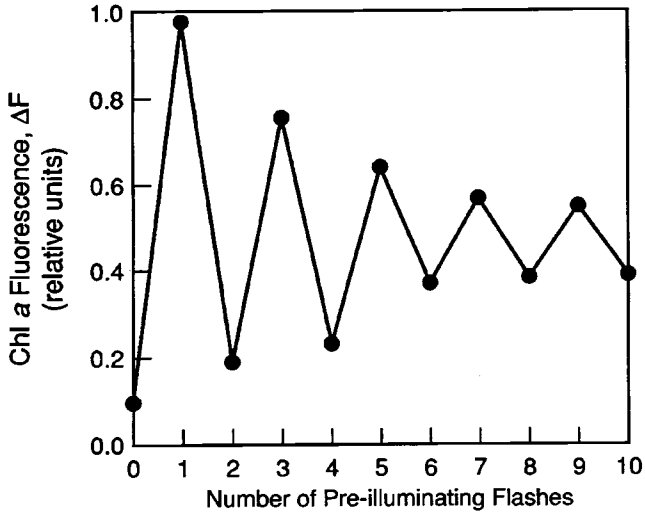

Fig. 15. Chl *a* fluorescence yield changes (ΔF) observed after a series of pre-illumination flashes, followed by DCMU addition. The units on the ordinate are equivalent to $F-F_o/F_o$ (or $(\phi_F-\phi_{F_o}/\phi_{F_o})$. The period 4 oscillation involved in O_2 evolution was abolished by alkaline Tris treatment, but PSII was operative due to the addition of an artificial electron donor. The binary oscillation, observed in this experiment, led to the concept of the *two-electron gate.* Data from Velthuys and Amesz (1974).

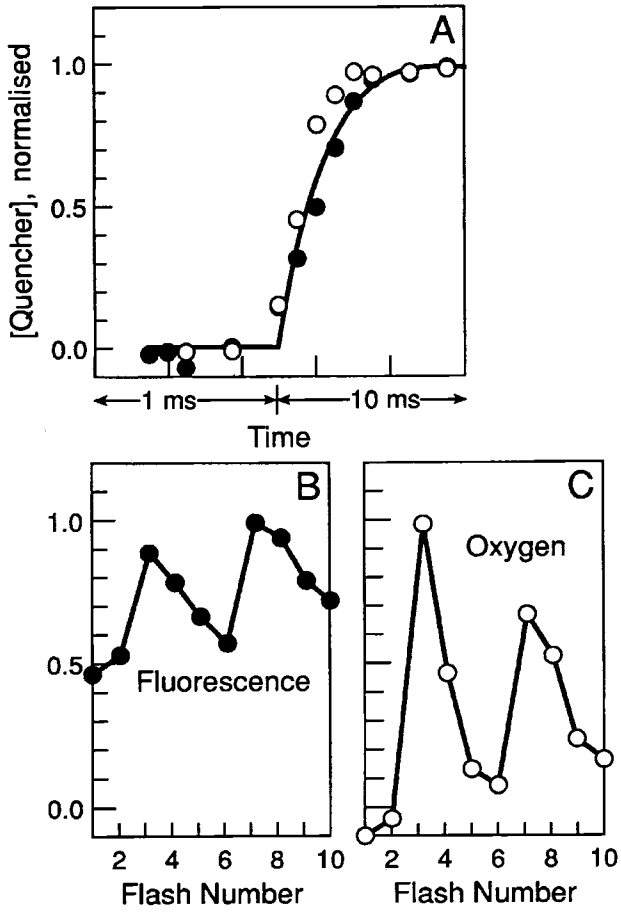

Fig. 16. (*A*) Kinetics of a quencher (or quenchers) of Chl *a* fluorescence evaluated from the difference of inverse of fluorescence yield after flash 3 from that after flash 1 as a function of time. Different symbols: different methods of calculation.
(*B*) Flash number dependence of quenching of Chl *a* fluorescence determined from the difference of reciprocal of fluorescence yield at 2 and 8 ms after flash.
(*C*) Flash number dependence of O_2 evolution, measured by the Joliot electrode. Sample: spinach thylakoids. Data from Shinkarev *et al.* (1994).

this quencher oscillates with a period of 4 in synchrony with O_2 evolution, but there are serious quantitative differences. In the same way, there are inconsistencies with the H^+ release patterns (Lavergne and Junge 1993). It is still tantalising to consider the possibility that this phase is a monitor of the kinetics of the $S_4 \rightarrow S_0$ O_2-evolving step. Whether it could be O_2 itself (for arguments regarding O_2 as a quencher of Chl fluorescence, see Papageorgiou 1975) is a valid question to ask. Since fluorescence can be measured in intact leaves, Chl *a* fluorescence kinetics could become an excellent probe for monitoring crucial steps of PSII *in situ*.

Understanding the Site of Bicarbonate in Photosystem II

Photosystem II, but not the purple or green bacterial or PSI, reaction centre shows a bicarbonate-reversible formate or NO inhibition of electron transfer from Q_A^- to the PQ pool (for reviews, see Govindjee and Van Rensen 1978, 1993; Blubaugh and Govindjee 1988; Diner *et al.* 1991; Govindjee 1991, 1993; Van Rensen 1992). Using Chl *a* fluorescence transient measurements, Wydrzynski and Govindjee (1975) were the first to demonstrate that a major *bicarbonate effect* was on the electron acceptor side of PSII: the effect of bicarbonate depletion was more like a block by DCMU than by a block on the donor side of PSII (Fig. 17).

Using Chl *a* fluorescence yield changes, after a series of repetitive flashes, Jursinic *et al.* (1976) concluded that electron flow out of Q_A^- to the PQ pool (steps (4), (10) and (13)) is reversibly affected by bicarbonate, but electron flow from *Z* to $P680^+$ (steps (3) and (9), etc.) is not (Fig. 18). Soon thereafter, Govindjee *et al.* (1976) showed that 160 ms after a series of individual 3 μs flashes, spaced 30 ms, the Chl *a* fluorescence intensity in bicarbonate-depleted thylakoids was high after flash 3 and beyond. Further, the binary oscillations, due to the existence of the two-electron gate, discovered by Velthuys and Amesz (1974), were abolished. These results, obtained with thylakoids thoroughly depleted of bicarbonate, suggested that the protonation and the exchange of Q_B^{2-} by the PQ pool (steps (12) and (13)) is drastically, but reversibly slowed down since addition of bicarbonate reversed all the effects (Fig. 19).

As noted above, *Z* to $P680^+$ reaction was shown by Jursinic *et al.* (1976) to be unaffected by bicarbonate-reversible formate. Govindjee *et al.* (1989) confirmed this result and showed that this result was independent of the *S*-states. However, this does not contradict (or disprove) the existence of a small, although statistically significant, effect on the donor side of PSII (Jursinic and Dennenberg 1990; Stemler and Jursinic 1993). In fact, under other experimental conditions (e.g. at low pH), a bicarbonate-reversible formate effect prior to Q_A has been observed also in our laboratory (El-Shintinawy and Govindjee 1989;

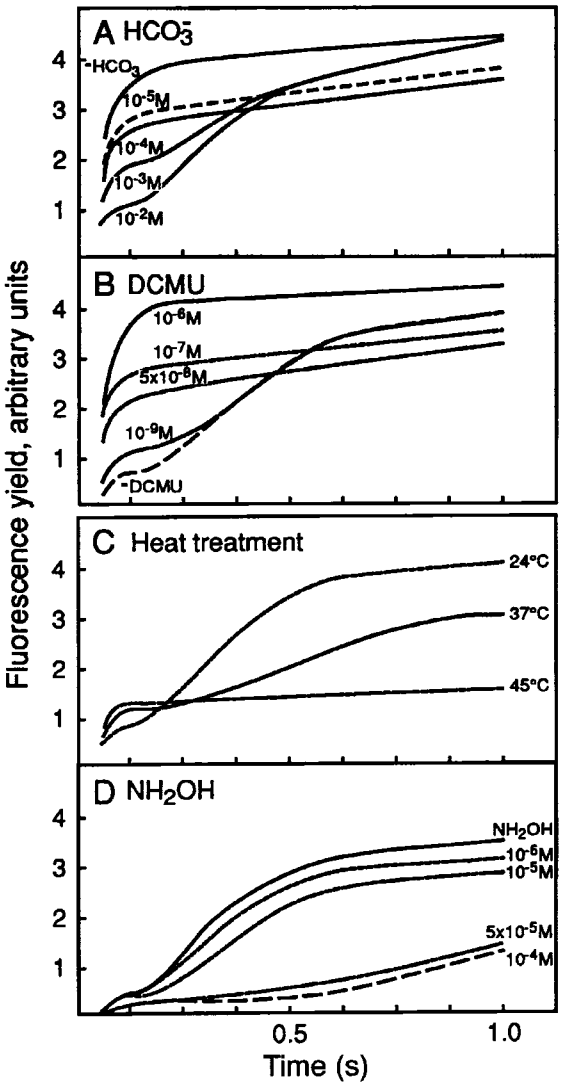

Fig. 17. Comparison of concentration dependence of variable Chl *a* fluorescence on bicarbonate with various System II inhibitory treatments. (*A*) HCO_3^--depleted thylakoid sample to which various $NaHCO_3$ concentrations were added. (*B*) Normal thylakoids at various DCMU concentrations. (*C*) Normal thylakoids heat-treated for 1 min at different temperatures. (*D*) Normal thylakoids at various NH_2OH concentrations. Preincubation time was 5 min in dark. In other experiments, it was shown that the bicarbonate effect persists in Tris-washed thylakoids with artificial H-donors (hydroquinone, $MnCl_2$, NH_2OH or diphenyl carbazide). These experiments established the role of bicarbonate on the acceptor side of PSII. Data from Wydrzynski and Govindjee (1975).

El-Shintinawy *et al.* 1990; Xu 1992, and citations therein). Further research is needed to investigate the significance of these observations.

On the other hand, there is indeed a major bicarbonate effect between Q_A and PQ, as discussed above. Eaton-Rye and Govindjee (1988*a*, 1988*b*) and Xu *et al.* (1991) showed a drastic bicarbonate-reversible slowing down, by formate, of electron transfer from Q_A^- to Q_B after the second and

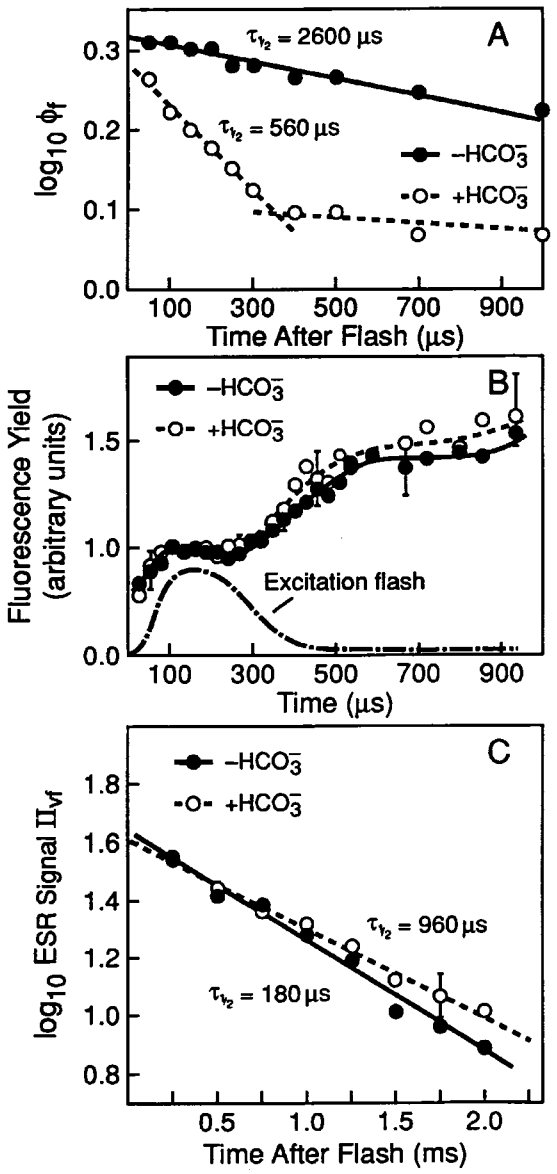

Fig. 18. (*A*) Semilog plot of the decay of Chl *a* fluorescence yield for Q_A^- to Q_B or Q_B^- reaction with and without 10 mM bicarbonate. (*B*) The rise in Chl *a* fluorescence yield (Z to P680$^+$ reaction) during and after an excitation flash with and without bicarbonate, normalised at 3 µs; a trace of the excitation flash is also shown. (*C*) Semilog plot of the ESR signal II very fast (vf) (S_n to Z^+ reaction) with and without bicarbonate. All experiments were made with spinach thylakoids. These experiments showed the effect of bicarbonate on Q^- reoxidation and the lack of effect between the *S*-states and P680. Data from Jursinic *et al.* (1976).

subsequent flashes, but not after the first flash (Fig. 20). This has been interpreted to suggest that it is not so much the electron flow (Eqn 9 (4)), but protonation of the site near Q_B^- (Eqn 9 (6)) that is inhibited by bicarbonate-reversible formate. A similar result was obtained by Diner and Petrouleas (1990) for the bicarbonate-reversible NO effect.

A role of HCO_3^- in protonation reactions has also been suggested from proton measurements by Van Rensen *et al.* (1988). Allakhverdiev *et al.* (1994) have suggested that such reactions may add an entropic factor to variable thermal emission, not detected by fluorescence.

The hypothesis of Blubaugh and Govindjee (1988) is that one of the functions of bicarbonate, suggested to be bound on (or H-bonded to) a particular arginine (D1-R269 and/or R257) and, perhaps, stabilised by other arginines, is to deliver a H^+ to a particular histidine to stabilise the negative charge on Q_B^- formed after the flash. In the absence of HCO_3^-, this is much slowed and, thus, electron transfer after the second and succeeding flashes is slowed (Xu *et al.* 1991). The importance of D2-R251 and D2-R233, but not D2-R139, for stabilisation of HCO_3^- was shown by Cao *et al.* (1991) (see Govindjee 1993) through the use of site-directed *Synechocystis* sp. PCC 6803 mutants D2-R251S, D2-R233Q and D2-R139H. However, we suspect that D1-R269 is one of the most crucial amino acids for the HCO_3^- effect (Fig. 21). Xiong *et al.* (1994) have constructed, in *Chlamydomonas reinhardtii*, a D1-R269G mutant. Interestingly, this mutant exhibits normal levels of D1 protein in dark-grown cells, does not show formate-induced Q_A^--Fe^{2+} EPR signal, has a very slow electron transfer rate beyond Q_A^-, and has a very low affinity for the herbicide terbutryn (Fig. 22). It remains to be seen if it has the non-heme iron or not, or if only the HCO_3^- binding site is lost. We consider it likely that D1-R269 may be one of the major binding sites of HCO_3^- in addition to the non-heme iron (Diner and Petrouleas 1990).

Chlorophyll *a* fluorescence measurements on several herbicide-resistant D1 mutants of the Q_B-binding niche (between helices IV and V) have revealed that different amino acids have different sensitivities to bicarbonate-reversible formate emphasising the role of a broad binding niche for bicarbonate ions (Govindjee *et al.* 1990*b*, 1991, 1992; Cao *et al.* 1992; Vernotte, Briantais and Govindjee, unpublished data). The (bi)carbonate binding niche in human lactoferrin (Anderson *et al.* 1989), the only other Fe-(bi)carbonate protein known to us, has served as a partial model for further investigations. Here (bi)carbonate is not only liganded to Fe, but is H-bonded to an arginine and several other amino acids.

Recently, Mäenpää *et al.* (1995) demonstrated that a mutant (CA1) of *Synechocystis* sp. PCC 6803 that lacks certain glutamic acids in the loop between helix IV and V of its D1 protein, shows a high resistance to bicarbonate-reversible formate treatment. Since the mutation is not in the Q_AFeQ_B niche, this result may suggest the importance of conformational changes. We are, obviously, far from an understanding of the bicarbonate binding and its function in PSII.

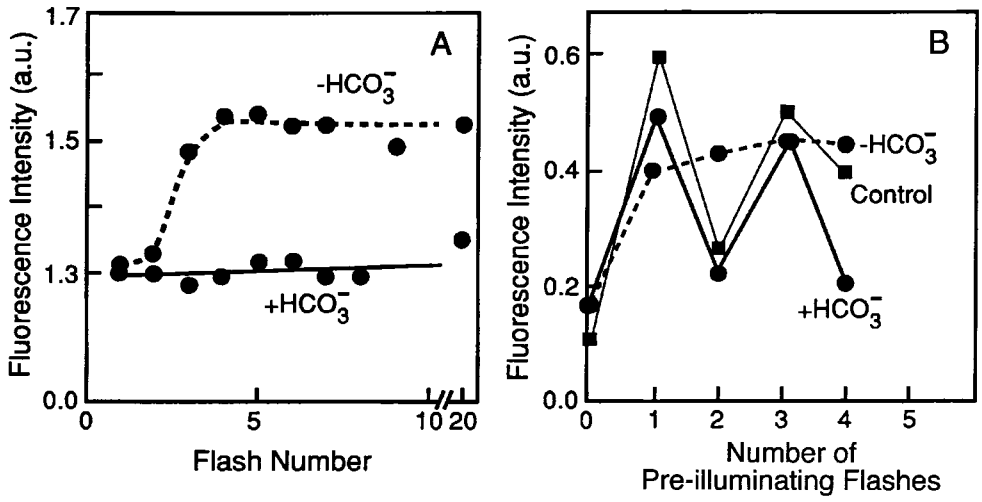

Fig. 19. (*A*) Chl *a* fluorescence intensity 160 ms after the last of a series of 3 μs saturating flashes, spaced at 30 ms, as a function of the number of flashes. Sample: spinach thylakoid suspension. (*B*) DCMU-induced fluorescence increase as a function of the number of pre-illuminating flashes (see Fig. 15). Sample: spinach thylakoid suspension. These experiments showed the role of bicarbonate at the *two-electron gate*. Data from Govindjee *et al.* (1976).

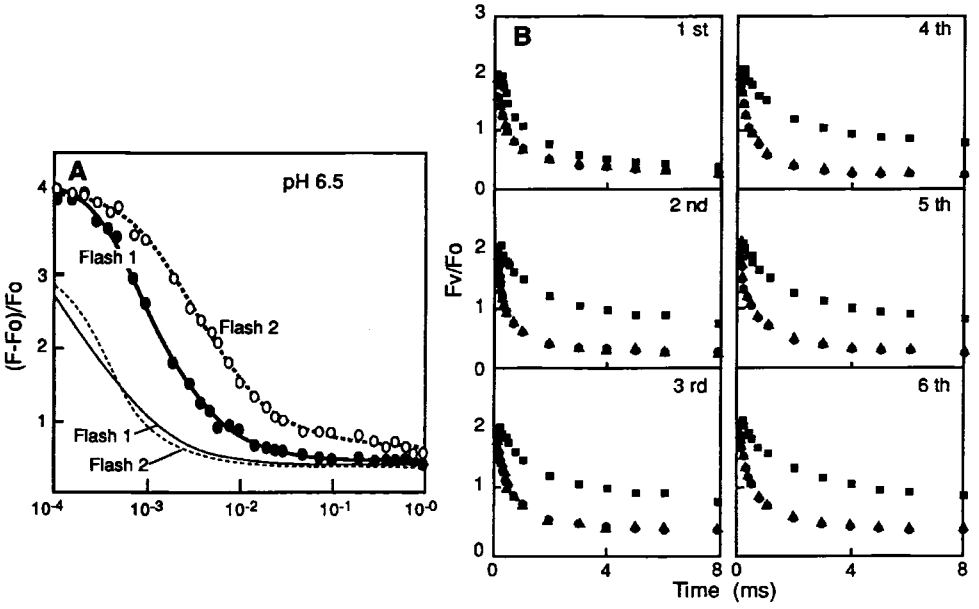

Fig. 20. (*A*) Decay of variable Chl *a* fluorescence yield with and without bicarbonate (formate-treated) after flash 1 and 2 up to 1 s. Slowing down by bicarbonate-depletion was much more after flash 2 than after flash 1. Data from Eaton-Rye and Govindjee (1988*b*).
(*B*) The same as above, after direct addition of formate, but plotted for flashes 1–6, and up to 8 ms only. Both these data suggested that bicarbonate may be involved in protonation of Q_B^-. Experiments by Xu and Govindjee, unpublished data; and data from Xu (1992).

Comments on Non-photochemical Quenching

Higher plants and algae can adapt to high light intensities by down-regulating PSII photochemistry (Demmig-Adams and Adams 1992). This down regulation is usually achieved by increasing the heat dissipation in the light-harvesting antenna complexes. The heat dissipation decreases the quantum yield of PSII photochemistry as well as Chl *a* fluorescence and, thus causes non-photochemical quenching (NPQ). Non-photochemical quenching of Chl *a* fluorescence simply implies that de-excitation pathways other than involved in photochemistry increase to quench Chl *a* fluorescence. The most obvious pathways are that by heat loss (k_h), as noted above, and/or by quenching (k_q) with physico-chemical quenchers (e.g. carotenoids, O_2, triplets, etc.). Thus, these include what we may call 'non-Q_A related' changes.

Louisa Yang and I (cited in Govindjee *et al.* 1967) observed a quenching of Chl *a* fluorescence by PMS (phenazine methosulfate) in DCMU-treated thylakoids. The explanation considered then was a change in k_t (rate constant of excitation energy transfer from strongly fluorescent PSII to weakly-fluorescent PSI). We never published this work as the explanation was not substantiated by low temperature fluorescence spectroscopy of the sample. Papageorgiou and Govindjee (1967, 1968*a*, 1968*b*) began looking at the effects of uncouplers of photophosphorylation, even in the presence of DCMU, on Chl *a* fluorescence of intact green and blue-green photosynthetic cells. We observed complex changes in both kinetics and emission spectra and it was evident that these changes are also 'non-Q_A-related' as was the experiment of L. Yang. We invoked cyclic changes around PSI as well as

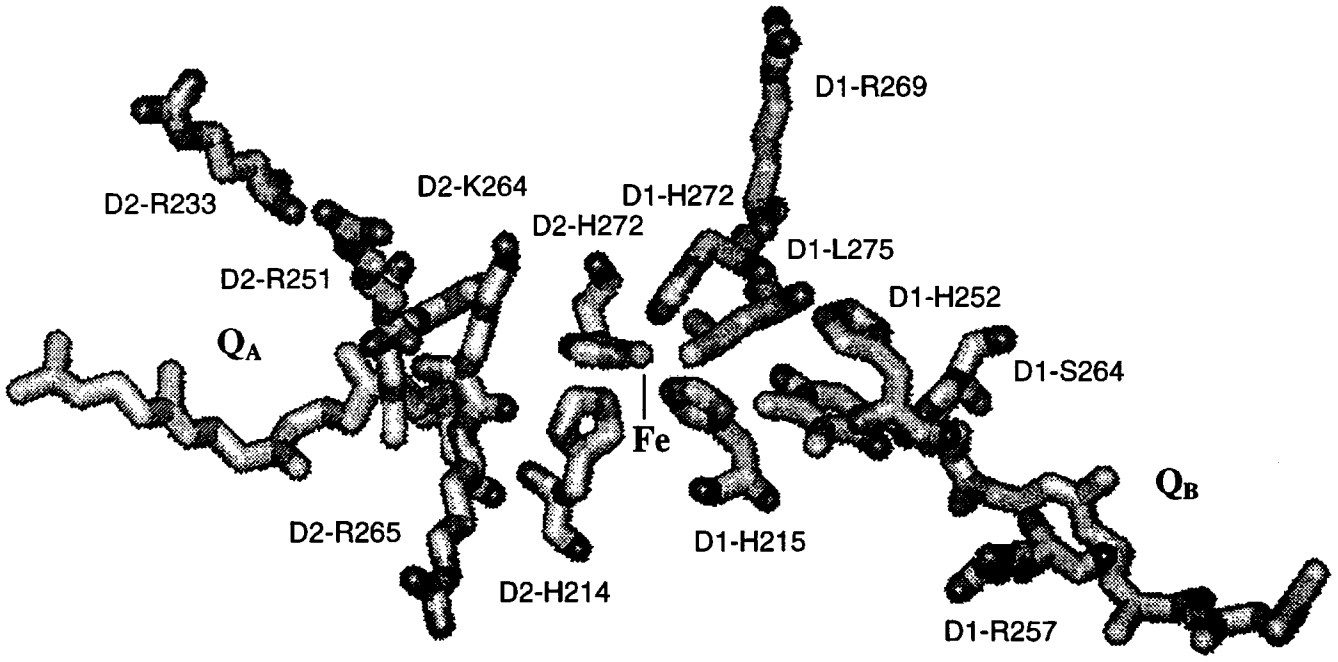

Fig. 21. A theoretical model of the acceptor side (Q_A-Fe-Q_B) of PSII reaction centre of *Synechocystis* sp. PCC 6803, constructed on QUANTA program (version 3·3). The model is based upon the sequence homology of PSII reaction centre proteins D1 and D2 with the bacterial reaction centre subunits L and M. Only the plastoquinones Q_A and Q_B, the non-heme iron and the important amino acid residues related to the bicarbonate effect are shown (unpublished model by Xiong, Subramaniam and Govindjee). Site-directed mutagenesis done by Cao *et al.* (1991) on D2-R233 and D2-R265, by Diner *et al.* (1991) on D2-K264 and D2-R265, and by Xiong *et al.* (1994) on D1-R269 have implicated the roles of these amino acid residues in stabilisation, binding, and functioning of bicarbonate in PSII.

structural changes to explain these results. The non-relationship of slow changes to photosynthesis was also obvious when we observed that the rate of O_2 evolution paralleled the SM fluorescence rise, and remained constant during the MT fluorescence decline (Papageorgiou and Govindjee 1968*a*, 1968*b*; Mohanty *et al.* 1971*a*).

Murata and Sugahara (1969) observed an uncoupler sensitive lowering of Chl *a* fluorescence yield when they added reduced PMS to DCMU-treated spinach chloroplasts. Wraight and Crofts (1970) showed a correlation between the protonation of the interior of the thylakoid, and the lowering of the Chl *a* fluorescence yield. When the quenching of fluorescence by Q_A was optimal at pH 6·5, the 'high energy state' (protonation) quenching was optimal at pH 8·5. Briantais *et al.* (1979, 1980) showed that the slow decline phase of Chl *a* fluorescence (the *SMT* phase) is correlated with the lumen [H^+] in isolated chloroplasts. This fluorescence lowering cannot be due to direct quenching by H^+'s as they cannot accept electronic excitation energy.

Papageorgiou (1975) has considered the possibility that some of the 'non-Q_A related' or 'high-energy-state, or X_E' quenching may occur through changes in structure that allow diffusion of quenchers (such as O_2) to the pigment site. Fixation of cells by glutaraldehyde does eliminate quenching of Chl *a* fluorescence by PMS (Mohanty *et al.* 1973). In view of the absence of PMS-induced effects on excitation energy transfer from PSII to PSI, and in view of the fact that fluorescence intensity changes paralleled lifetime of fluorescence changes, Mohanty *et al.* (1973) concluded that these changes were due to increases in rate constant of heat loss, k_h, not k_t, as originally thought. These were the beginnings of the observations on non-photochemical quenching of Chl *a* fluorescence of PSII.

Since the conclusions of Murata and Sugahara (1969), Wraight and Crofts (1970) and Mohanty *et al.* (1973) and of Briantais *et al.* (1979, 1980) on thylakoids and chloroplasts were more understandable than those we had earlier obtained on algal cells, we investigated the effects of salicylanalides, uncouplers of photophosphorylation, on DCMU-treated cyanobacterial cells (Mohanty and Govindjee 1973). We observed that these uncouplers abolished the time-dependent Chl *a* fluorescence increase, a sort of opposite effect to that observed with the PMS-system in thylakoids. Moreover, in both cases uncouplers of photophosphorylation caused drastic changes in 'non-Q_A-related' Chl *a* fluorescence changes. I hope that with the new theoretical and experimental framework available now, these early observations in intact cells can be reinvestigated and finally understood at a molecular level.

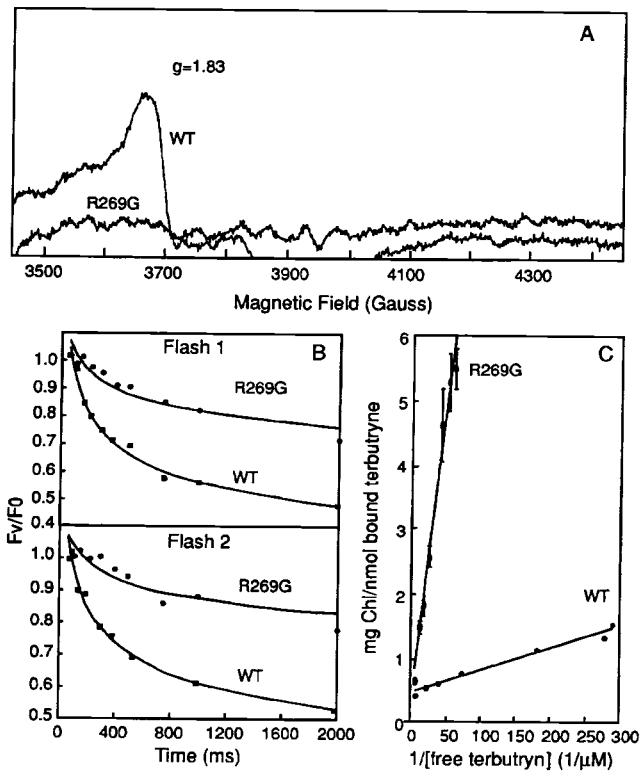

Fig. 22. Characterisation of the dark grown D1-R269G mutant of *Chlamydomonas reinhardtii*. (*A*) The EPR spectrum showing the absence of formate-induced $g = 1{\cdot}83$ signal. (*B*) The normalised fluorescence decay pattern showing the slowed electron transfer from Q_A^- to $Q_B(Q_B^-)$. (*C*) The herbicide binding assay showing an extremely low affinity of terbutryn binding. In addition, the mutant shows inability to evolve oxygen and low levels of D1 protein when grown in light. These data suggest the importance of the amino acid residue D1-R269 in several aspects of PSII including its role in the bicarbonate effect. Preliminary data of Xiong *et al.* (1994).

Seven years before the observations of Murata and Sugahara, Yamamoto *et al.* (1962) discovered what is called the *xanthophyll cycle*:

$$\text{violaxanthin} \overset{\frac{1}{2}O_2}{\leftrightarrow} \text{antheraxanthin} \overset{\frac{1}{2}O_2}{\leftrightarrow} \text{zeaxanthin}.$$

Harry Yamamoto, who has invested years of research characterising biochemistry of this cycle, concluded that it played an unknown but important regulatory role in photosynthesis (see Yamamoto 1979). It was only recently that Barbara Demmig-Adams and her coworkers suggested that the pigments of the Yamamoto cycle play a role in NPQ of Chl *a* fluorescence by increasing k_h (see e.g. Demmig-Adams *et al.* 1990). There is a general consensus among most of the researchers (for fear of missing any names, I do not list their names) that [H^+s] may not only activate the enzyme violaxanthin de-epoxidase to convert violaxanthin to antheraxanthin and zeaxanthin, but may also affect the conformational state of the antenna pigment protein complexes such that the quenching of Chl *a* fluorescence by zeaxanthin and antheraxanthin (Gilmore and Yamamoto 1993) is favoured. Quenching processes in fluorescence studies are best analysed by the well-known Stern–Volmer relationships (Stern and Volmer 1919; Papageorgiou 1975; Demmig-Adams *et al.* 1990):

$$\frac{F\,(\text{control})}{F\,(\text{with quencher})} - 1 = kp\tau\,[\text{Quencher}],$$

where F = fluorescence intensity, k = collision rate constant, p = probability of effective collisions and τ = lifetime of fluorescence in the absence of the quencher.

Using the Stern–Volmer relationship, Gilmore and Yamamoto (1993) have obtained a correlation between the Chl *a* fluorescence yield and the combined [H^+] and [zeaxanthin (Z) + antheraxanthin (A)]. Thus, the k_h, proposed earlier, may be equated most simply to k_q [H^+] [Z + A]. This does not *preclude* the existence of other quenching mechanisms. It becomes a matter of knowledge of which mechanism dominates when. However, the role of zeaxanthin in photoprotection *in vivo* has been emphasised by several, including Barry Osmond and coworkers (see Casper *et al.* 1993).

A decrease in fluorescence intensity, even when the number of absorbed quanta is kept constant, need not necessarily mean a decrease in quantum yield of fluorescence if the absorption cross-section of the fluorescent pigment bed decreases. Such a change would lead to what is called *static* quenching and would not reflect changes in rate constants of de-excitation pathways given in Eqn (1). However, if fluorescence intensity changes strictly parallel lifetime of fluorescence changes, we can be sure that these changes are in the quantum yield and, thus, in the rate constants of de-excitation. Gilmore *et al.* (1995) have observed an almost linear relationship (Fig. 23) between Chl *a* fluorescence intensity changes (measured by a Walz fluorometer) and the fraction of a short (approximately 0·4 ns) lifetime Lorentzian component of Chl *a* fluorescence (measured by a multifrequency phase fluorometer) during quenching of Chl *a* fluorescence that was dependent upon [H^+] and [zeaxanthin + antheraxanthin]. Gilmore *et al.* (1995), further, observed that the increase in the fraction of the short lifetime fluorescence component was accompanied by a decrease in the fraction of a 2 ns lifetime Lorentzian component. These data are interpreted in terms of the formation of a complex that has a low quantum yield of fluorescence and, thus, increased rate constant of heat loss within it. For a further discussion of the mechanism of non-photochemical quenching, and of how carotenoids may quench Chl *a* fluorescence, see Crofts and Yerkes (1994) and Frank *et al.* (1994), respectively.

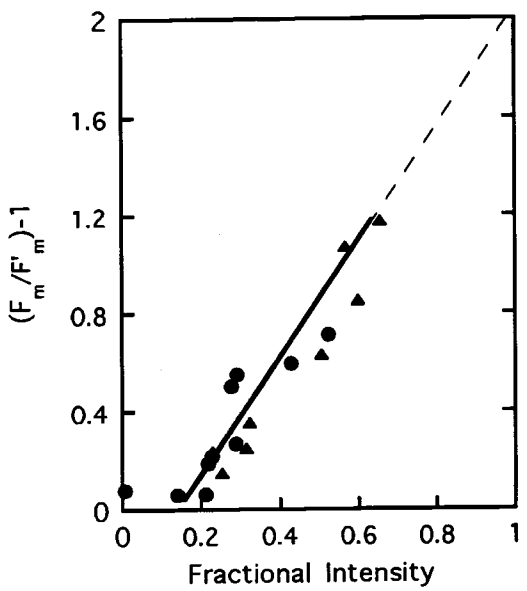

Fig. 23. The relationship between $(F_m/F'_m)-1$ and the fractional intensity of the shorter lifetime of Chl *a* fluorescence centre of a bimodal Lorentzian distribution in thylakoids. The fractional intensity (abscissa) is increased as the concentration of zeaxanthin + antheraxanthin was increased. The short lifetime centres for spinach (▲) and lettuce (●) thylakoids, respectively, were 0·42 ± 0·06 and 0·34 ± 0·10 ns. The fractional intensity of the longer lifetime centres that decreased in parallel (not shown) were 1·87 ± 0·08 and 2·14 ± 0·07 ns. F_m is the maximal fluorescence intensity and F'_m is the same in the presence of non-photochemical quenching. Measurements were made in the presence of 10 μM DCMU; fluorescence quenching was induced by ATP hydrolysis, as published by Gilmore and Yamamoto (1992). Data from Gilmore *et al.* (1995).

Concluding Remarks

I see no reason to throw away our fluorometers, but to further improve our fluorometers. We should, however, realise that it is essential to make parallel measurements on other parameters (absorption, heat changes, rates of electron transfer, etc.) before conclusions can be drawn from Chl *a* fluorescence measurements. Allakhverdiev *et al.* (1994) have provided parallel measurements on thermal and light emissions in PSII. To this, we should add measurements on lifetime of fluorescence and on absorption changes of key intermediates in order to obtain a thorough understanding of the phenomenon under investigation.

Two major areas that I have not covered in this viewpoint, both dear to me because of my personal involvement in them, are: use of Chl *a* fluorescence as a probe of dynamics of thylakoid membranes (see Barber 1982), and of dynamics of primary photochemical events of PSII (Dau 1994). I ask for forgiveness for not including these topics here.

An interesting future prospect is the possibility of using time-dependent and flash-number-dependent Chl *a* fluorescence yield changes to probe not only the acceptor side of PSII, as is being currently done in several laboratories, but also the donor side of PSII including the *S*-state transitions.

In spite of my own intellectual shortcomings and that of Chl *a* fluorescence, we have come a long way since Kautsky's paper 63 years ago. Chl *a* fluorescence has provided new and important information on the composition of the pigment systems, excitation energy transfer, physical changes in pigment–protein complexes, primary photochemistry, kinetics and rates of electron transfer reactions in PSII, the sites of various inhibitors, and activators, and of lesions in newly constructed mutants. The current use of Chl fluorescence as a probe of photosynthesis (Evans and Brown 1994) and stress (Lichtenthaler and Rinderle 1988) is evident from the listing of 599 papers in all of the *Current Contents* series during a period of 2·5 years (1992 to 23 April 1994). The *viewpoint* presented here is only a drop in the *lake* of Chl fluorescence research.

Acknowledgments

I thank Adam Gilmore, Vladimir Shinkarev and Jin Xiong for reading the text presented here. Financial support from the Campus Research Board (UIUC), the National Science Foundation (MCB 16838), University of Geneva (Switzerland), Wageningen Agriculture University (the Netherlands), and a DOE/NSF/USDA IPTR grant is gratefully acknowledged. My special thanks is reserved for Thomas J. Wydrzynski who gave me the opportunity to write this viewpoint.

References

Allakhverdiev, S. I., Klimov, V. V., and Carpentier, R. (1994). Variable thermal emission and chlorophyll fluorescence in photosystem II particles. *Proceedings of the National Academy of Sciences, USA* **91**, 281–285.

Anderson, B. F., Baker, H. M., Norris, G. E., Rice, D. W., and Baker, E. N. (1989). Structure of human lactoferrin: crystallographic structure analysis and refinement at 2·8 Å resolution. *Journal of Molecular Biology* **209**, 711–734.

Arnold, W., and Meek, E. S. (1956). The polarization of fluorescence and energy transfer in grana. *Archives of Biochemistry and Biophysics* **60**, 82–90.

Askenasy, E. (1867). Beiträge zur Kenntniss des Chlorophylls und einiger dasselbe begleitender Farbstoffe II. *Botanische Zeitung* **25**, 233–238.

Bannister, T., and Rice, G. (1968). Parallel time courses of oxygen evolution and chlorophyll fluorescence. *Biochimica et Biophysica Acta* **162**, 555–580.

Barber, J. (1982). Influence of surface charges on thylakoid structure and function. *Annual Review of Plant Physiology* **33**, 261–295.

Bergeron, J. A. (1963). Studies of the localization, physicochemical properties and action of phycocyanin in *Anacystis nidulans*. *National Research Council, USA, Publications* **1145**, 527–536.

Blubaugh, D., and Govindjee (1988). The molecular mechanism of the bicarbonate effect at the plastoquinone reductase site of photosynthesis. *Photosynthesis Research* **19**, 85–128.

Boardman, N. K., Thorne, S. W., and Anderson, J. M. (1966). Fluorescence properties of particles obtained by digitonin fragmentation of spinach chloroplasts. *Proceedings of the National Academy of Sciences, USA* **56**, 586–593.

Bouges-Bocquet, B. (1973). Electron transfer between the two photosystems in spinach chloroplasts. *Biochimica et Biophysica Acta* **314**, 250–256.

Bowes, J., and Crofts, A. R. (1980). Binary oscillations in the rate of reoxidation of the primary acceptor of photosystem II. *Biochimica et Biophysica Acta* **590**, 373–384.

Brewster, D. (1834). On the colours of natural bodies. *Transactions of the Royal Society of Edinburgh* **12**, 538–545.

Brewster, D. (1846). On the decomposition and dispersion of light within solid and fluid bodies. *Transactions of the Royal Society of Edinburgh* **16**, 111–123.

Briantais, J.-M., Merkelo, H., and Govindjee (1972). Lifetime of the excited state (τ) *in vivo*. III. Chlorophyll during fluorescence induction in *Chlorella pyrenoidosa*. *Photosynthetica* **6**, 133–141.

Briantais, J.-M., Vernotte, C., Krause, G. H., and Weis, E. (1986). Chlorophyll *a* fluorescence of higher plants: chloroplasts and leaves. In 'Light Emission by Plants and Bacteria'. (Eds Govindjee, J. Amesz and D. C. Fork.) pp. 539–583. (Academic Press: Orlando.)

Briantais, J.-M., Vernotte, C., Picaud, M., and Krause, G. H. (1979). A quantitative study of the slow decline of chlorophyll *a* fluorescence in isolated chloroplasts. *Biochimica et Biophysica Acta* **548**, 128–138

Briantais, J.-M., Vernotte, C., Picaud, M., and Krause, G. H. (1980). Chlorophyll fluorescence as a probe for the determination of the photo-induced proton gradient in isolated chloroplasts. *Biochimica et Biophysica Acta* **591**, 198–202.

Brody, S. S. (1958). New excited state of chlorophyll. *Science* **128**, 838–839.

Brody, S. S., and Brody, M. (1963). Aggregated chlorophyll *in vivo*. *National Academy of Science–National Research Council Publications* **1145**, 455–478.

Brody, S., and Rabinowitch, E. (1957). Excitation lifetime of photosynthetic pigments *in vitro* and *in vivo*. *Science* **125**, 555.

Butler, W. L. (1961). A far-red absorbing form of chlorophyll *in vivo*. *Archives of Biochemistry and Biophysics* **93**, 413–422.

Butler, W. L. (1962). Effects of red and far-red light on the fluorescence yield of chlorophyll *in vivo*. *Biochimica et Biophysica Acta* **64**, 309–317.

Butler, W. L. (1966). Fluorescence yield in photosynthetic systems and its relation to electron transport. *Current Topics in Bioenergetics* **1**, 49–73.

Butler, W. L. (1972). On the primary nature of fluorescence yield changes associated with photosynthesis. *Proceedings of the National Academy of Sciences, USA* **69**, 3420–3422.

Butler, W. L. (1978). Energy distribution in the photochemical apparatus of photosynthesis. *Annual Review of Plant Physiology* **29**, 345–378.

Butler, W. L., and Strasser, R. J. (1977). Tripartite model for the photochemical apparatus of green plant photosynthesis. *Proceedings of the National Academy of Science, USA* **74**, 3382–3385.

Cao, J., Ohad, N., Hirschberg, J., Xiong, J., and Govindjee (1992). Binding affinity of bicarbonate and formate in herbicide-resistance D1 mutants of *Synechococcus* sp. PCC 7942. *Photosynthesis Research* **34**, 397–408.

Cao, J., Vermaas, W. F. J., and Govindjee (1991). Arginine residues in the D2 polypeptide may stabilize bicarbonate binding in photosystem II of *Synechocystis* sp. PCC 6803. *Biochimica et Biophysica Acta* **1059**, 171–180.

Casper, C., Eickmeier, W. G., and Osmond, C. B. (1993). Changes of fluorescence and xanthophyll pigments during dehydration in the resurrection plant *Selaginella lepidophylla* in low and medium light intensities. *Oecologia* **94**, 528–533.

Cederstrand, C. N., and Govindjee (1966). Some properties of spinach chloroplast fractions obtained by digitonin. *Biochimica et Biophysica Acta* **120**, 177–180.

Cho, F., and Govindjee (1970*a*). Low temperature (4–77K) spectroscopy of *Chlorella*: temperature dependence of energy transfer efficiency. *Biochimica et Biophysica Acta* **216**, 139–150.

Cho, F., and Govindjee (1970*b*). Low temperature (4–77K) spectroscopy of *Anacystis*: temperature dependence of energy transfer efficiency. *Biochimica et Biophysica Acta* **216**, 151–161.

Cho, F., Spencer, J., and Govindjee (1966). Emission spectra of *Chlorella* at very low temperatures (–269° to –196°C). *Biochimica et Biophysica Acta* **126**, 174–176.

Critchley, C., Baianu, I. C., Govindjee, and Gutowsky, H. S. (1982). The role of chloride in O_2 evolution by thylakoids from salt-tolerant higher plants. *Biochimica et Biophysica Acta* **682**, 436–445.

Crofts, A. R., and Yerkes, C. T. (1994). A molecular mechanism for q_E-quenching. *FEBS Letters* **352**, 265–270.

Das, M., and Govindjee (1967). A long-wave absorbing form of chlorophyll *a* responsible for the 'red drop' in fluorescence at 298°K and the F723 band at 77°K. *Biochimica et Biophysica Acta* **143**, 570–576.

Dau, H. (1994). Molecular mechanisms and quantitative models of variable photosystem II fluorescence. *Photochemistry and Photobiology* **60**, 1–23.

Delosme, R. (1967). Etude de l' induction de fluorescence des algues vertes et des chloroplastes au debut d'une illumination intense. *Biochimica et Biophysica Acta* **143**, 108–128.

Delosme, R. (1971). Variations du rendement de fluorescence de la chlorophylle *in vivo* sous l'action d'eclairs de forte intensité. *Comptes Rendues des séances de l' Academie Sciences des Paris* **272**, 2828–2831.

Delosme, R., Joliot, P., and Lavorel, J. (1959). Sur la complémentarité de la fluorescence et de l'emission d'oxygène pendant la période d'induction de la photosynthèse. *Comptes Rendues des séances de l' Academie Sciences des Paris* **249**, 1409–1411.

Demmig-Adams, B., and Adams, W. W. III (1992). Photoprotection and other responses of plants to high light stress. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 599–626.

Demmig-Adams, B., Adams, W. W. III, Heber, U., Neimanis, S., Winter, K., Kruger, A., Czygan, F.-C., Bilger, W., and Björkman, O. (1990). Inhibition of zeaxanthin formation and of rapid changes in radiationless energy dissipation by dithiothreitol in spinach leaves and chloroplasts. *Plant Physiology* **92**, 293–301.

Diner, B., and Petrouleas, V. (1990). Formation by NO of nitrosyl adducts of redox components of the photosystem II reaction center. II. Evidence that HCO_3^-/CO_2 binds to the acceptor side non-heme iron. *Biochimica et Biophysica Acta* **1015**, 141–149.

Diner, B., Petrouleas, V., and Wendoloski, J. J. (1991). The iron quinone electron-acceptor complex of photosystem II. *Physiologia Plantarum* **81**, 423–436.

Dmetrievsky, O. D., Ermolaev, V. L., and Terenin, A. N. (1957). Direct measurement of the lifetimes of chlorophyll and analogous pigments in various media. *Doklady Akademii Nauk* **114**, 468–470.

Döring, G., Stiehl, H. H., and Witt, H. T. (1967). A second chlorophyll reaction in the electron transport chain of photosynthesis-registration by the repetitive excitation technique. *Zeitschrift für Naturforschung* **22b**, 639–644.

Dutton, H. J., Manning, W. M., and Duggar, B. B. (1943). Chlorophyll fluorescence and energy transfer in diatom *Nitzchia closterium*. *Journal of Physical Chemistry* **46**, 308–313.

Duysens, L. N. M. (1952). Transfer of excitation energy in photosynthesis. PhD Thesis, State University, Utrecht.

Duysens, L. N. M. (1979). Transfer and trapping of excitation energy in photosystem II. In 'Chlorophyll Organization Energy Transfer in Photosynthesis'. Ciba Foundation Symposium 61. pp. 323–340. (Excerpta Medica: Amsterdam.)

Duysens, L. N. M. (1989). The discovery of the two photosynthetic systems: a personal account. *Photosynthesis Research* **21**, 61–79.

Duysens, L. N. M., Amesz, J., and Kamp, B. M. (1961). Two photochemical systems in photosynthesis. *Nature* **190**, 510–511.

Duysens, L. N. M., and Sweers, H. E. (1963). Mechanism of two photochemical reactions in algae as studied by means of fluorescence. In 'Studies on Microalgae and Photosynthetic Bacteria'. (Eds Japanese Society of Plant Physiologists.) pp. 353–372. (University of Tokyo Press: Tokyo.)

Eaton-Rye, J. J., and Govindjee (1988*a*). Electron transfer through the quinone acceptor complex of photosystem II in bicarbonate-depleted spinach thylakoid membranes as a function of actinic flash number and frequency. *Biochimica et Biophysica Acta* **935**, 237–247.

Eaton-Rye, J. J., and Govindjee (1989*b*). Electron transfer through the quinone acceptor complex II after one or two actinic flashes in bicarbonate-depleted spinach thylakoid membranes. *Biochimica et Biophysica Acta* **935**, 248–257.

El-Shintinawy, F., and Govindjee (1989). Bicarbonate effects in leaf discs from spinach. *Photosynthesis Research* **24**, 189–200.

El-Shintinawy, F., Xu, C., and Govindjee (1990). A dual bicarbonate-reversible formate effect in *Chlamydomonas* cells. *Journal of Plant Physiology* **136**, 421–428.

Emerson, R., and Arnold, W. (1932*a*). A separation of the reactions in photosynthesis by means of intermittent light. *Journal of General Physiology* **15**, 391–420.

Emerson, R., and Arnold, W. (1932*b*). The photochemical reaction in photosynthesis. *Journal of General Physiology* **16**, 191–205.

Evans, E. H., and Brown, R. G. (1944). An appraisal of photosynthetic fluorescence kinetics as a probe of plant function. *Journal of Photochemistry and Photobiology B-Biology* **22**, 95–104.

Forbush, B., and Kok, B. (1968). Reaction between primary and secondary electron acceptors of photosystem II of photosynthesis. *Biochimica et Biophysica Acta* **162**, 243–253.

Förster, Th. (1948). Intermolecular energy transference and fluorescence. *Annalen Physik* **2**, 55–75 [in German].

Frank, H. A., Cua, A., Chynwat, V., Young, A., Gosztola, D., and Wasielewski, M. (1994). Photophysics of the carotenoids associated with the xanthophyll cycle in photosynthesis. *Photosynthesis Research* **41**, 389–395.

French, C. S. (1971). The distribution and action in photosynthesis of several forms of chlorophyll. *Proceedings of the National Academy of Sciences, USA* **68**, 2893–2897.

French, C. S., and Young, V. M. K. (1952). The fluorescence spectra of red algae and the transfer of energy from phycoerythrin to phycocyanin and chlorophyll. *Journal of General Physiology* **35**, 873–890.

Gaffron, H., and Wohl, K. (1936*a*). Zur Theorie der Assimilation. *Naturwissenschaften* **24**, 81–90.

Gaffron, H., and Wohl, K. (1936*b*). Zur Theorie der Assimilation. *Naturwissenschaften* **24**, 103–107.

Gasanov, R., Abilov, Z. K., Gazanchyan, R. M., Kurbonova, U. M., Khanna, R., and Govindjee (1979). Excitation energy transfer in photosystems I and II from grana and in photosystem I from stroma lamellae, and identification of emission bands with pigment–protein complexes. *Zeitschrift für Pflanzenphysiologie* **95**, 148–169.

Genty, B., Briantais, J.-M., and Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**, 87–92.

Gilmore, A., Hazlett, T. L., and Govindjee (1995). Xanthophyll cycle dependent quenching of photosystem II chlorophyll *a* fluorescence formation of a quenching complex with a short fluorescence lifetime. *Proceedings of the National Academy of Sciences, USA*, (in press).

Gilmore, A. M., and Yamamoto, H. Y. (1992). Dark induction of zeaxanthin-dependent non-photochemical fluorescence quenching mediated by ATP. *Proceedings of the National Academy of Science, USA* **89**, 1899–1903.

Gilmore, A., and Yamamoto, H. Y. (1993). Linear models relating xanthophylls and lumen acidity to non-photochemical fluorescence quenching. Evidence that antheraxanthin explains zeaxanthin-independent quenching. *Photosynthesis Research* **35**, 67–78.

Goedheer, J. H. C. (1972). Fluorescence in relation to photosynthesis. *Annual Review of Plant Physiology* **23**, 87–112.

Goedheer, J. H. C. (1981). Comparison of the long-wave

chlorophyll fluorescence in various green and blue-green algae and diatoms. *Photosynthesis Research* **2**, 49–60.

Govindjee (1963). Emerson enhancement effect and two light reactions in photosynthesis. *National Academy of Sciences–National Research Council Publications* **1145**, 318–334.

Govindjee (1991). A unique role of carbon dioxide in photosystem II. In 'Impact of Global Climatic Changes on Photosynthesis and Plant Productivity'. (Eds Y. Abrol, P. N. Wattal, A. Gnanam, Govindjee, D. R. Ort and A. H. Teramura.) pp. 349–369. (Oxford/IBH: New Delhi.)

Govindjee (1993). Bicarbonate-reversible inhibition of plastoquinone reductase in photosystem II. *Zeitschrift für Naturforschung* **48c**, 251–258.

Govindjee, Amesz, J., and Fork, D. C. (1986). 'Light Emission by Plants and Bacteria.' (Academic Press: Orlando.)

Govindjee, and Briantais, J.-M. (1972). Chlorophyll *b* fluorescence and an emission band at 700 nm at room temperature in green algae. *FEBS Letters* **19**, 278–280.

Govindjee, Eggenberg, P., Pfister, K., and Strasser, R. J. (1992). Chlorophyll *a* fluorescence decay in herbicide-resistant D1 mutants of *Chlamydomonas reinhardtii* and the formate effect. *Biochimica et Biophysica Acta* **1101**, 353–358.

Govindjee, Ichimura, S., Cederstrand, C., and Rabinowitch, E. (1960). Effect of combining far-red light with shorter wave light in the excitation of fluorescence in *Chlorella*. *Archives of Biochemistry and Biophysics* **89**, 322–323.

Govindjee, and Jursinic, P. (1979). Photosynthesis and fast changes in light emission by green plants. *Photochemical Photobiological Reviews* **4**, 125–205.

Govindjee, and Papageorgiou, G. (1971). Chlorophyll fluorescence and photosynthesis: fluorescence transients. *Photophysiology* **6**, 1–50.

Govindjee, Papageorgiou, G., and Rabinowitch, E. (1967). Chlorophyll fluorescence and photosynthesis. In 'Fluorescence, Theory, Instrumentation and Practice'. (Ed. G. G. Guilbault.) pp. 511–564. (Marcel Dekker: New York.)

Govindjee, Pulles, M. P. J., Govindjee, R., Van Gorkom, H. J., and Duysens, L. N. M. (1976). Inhibition of the reoxidation of the secondary acceptor of photosystem II by bicarbonate depletion. *Biochimica et Biophysica Acta* **449**, 602–605.

Govindjee, Robinson, H., Crofts, A. R., and Van Rensen, J. J. S. (1989). Bicarbonate does not influence electron transfer to the reaction center chlorophyll *a* of photosystem II: measurements by chlorophyll *a* fluorescence rise in microseconds. *Naturwissenschaften* **76**, 119–121.

Govindjee, Schwarz, B., Rochaix, J.-D., and Strasser, R. J. (1991). The herbicide-resistant D1 mutant L275F of *Chlamydomonas reinhardtii* fails to show the bicarbonate-reversible formate effect on chlorophyll *a* fluorescence transients. *Photosynthesis Research* **27**, 199–208.

Govindjee, Snel, J. F. H., deVos, O. J. and Van Rensen, J. J. S. (1993*a*). Antagonistic effects of light I and II on chlorophyll *a* fluorescence yield and P700 turnover as monitors of carbon dioxide depletion in intact algal and cyanobacterial cells. *Physiologia Plantarum* **89**, 143–148.

Govindjee, Van de Ven, M., Cao, J., Royer, C., and Gratton, E. (1993*b*). Multifrequency cross-correlation phase fluorometry of chlorophyll *a* fluorescence in thylakoid and PSII-enriched membranes. *Photochemistry and Photobiology* **58**, 438–445.

Govindjee, Van de Ven, M., Preston, C., Seibert, M., and Gratton, E. (1990*a*). Chlorophyll *a* fluorescence lifetime distributions in open and closed photosystem II reaction center preparations. *Biochimica et Biophysica Acta* **1015**, 173–179.

Govindjee, and Van Rensen, J. J. S. (1978). Bicarbonate effects on the electron flow in isolated broken chloroplasts. *Biochimica et Biophysica Acta* **505**, 183–213.

Govindjee, and Van Rensen, J. J. S. (1993). Photosystem II reaction center and bicarbonate. In 'The Photosynthetic Reaction Center'. Vol. 1. (Eds J. Deisenhofer and J. R. Norris.) pp. 357–389. (Academic Press: San Diego.)

Govindjee, Vernotte, C., Peteri, B., Astier, C. and Etienne, A.-L. (1990*b*). Differential sensitivity of bicarbonate-reversible formate effects on herbicide-resistant mutants of *Synechocystis* 6714. *FEBS Letters* **267**, 273–276.

Govindjee, and Yang, L. (1966). Structure of the red fluorescence band in chloroplasts. *Journal of General Physiology* **49**, 763–780.

Harvey, E. N. (1957). 'A History of Luminescence From the Earliest Times Until 1900.' (The American Philosophical Society: Philadelphia.)

Herschel, J. (1845*a*). On a case of superficial colour presented by a homogeneous liquid internally colourless. *Philosophical Transactions of the Royal Society of London* **135**, 143–145.

Hershel, J. (1845*b*). On the epipolic dispersion of light. *Philosophical Transactions of the Royal Society of London* **135**, 147–153.

Hill, R., and Bendall, F. (1960). Function of the two cytochrome components in chloroplasts: a working hypothesis. *Nature* **186**, 136–140.

Holzwarth, A. R. (1991). Excited state kinetics in chlorophyll systems and its relationship to the functional organization of the photosystems. In 'Chlorophylls'. (Ed. H. Scheer.) pp. 1125–1151. (CRC Press: Boca Raton.)

Holzwarth, A. R. (1993). Is it time to throw away your apparatus for chlorophyll fluorescence induction? *Biophysical Journal* **64**, 1280–1281.

Horton, P. (1985). Interactions between electron transfer and carbon assimilation. *Topics in Photosynthesis* **6**, 135–187.

Hsu, B. D. (1993). Evidence for the contribution of the *S*-state transitions of oxygen evolution to the initial phase of fluorescence induction. *Photosynthesis Research* **36**, 81–88.

Joliot, A., and Joliot, P. (1964). Etude cinétique de la réaction photochimique libérant l'oxygene au cours de la photosynthèse. *Comptes Rendus des sèances de l'Academie Sciences Paris* **258**, 4622–4625.

Jursinic, P., and Dennenberg, R. J. (1990). Oxygen release in leaf discs and thylakoids of peas and photosystem II membrane fragments of spinach. *Biochimica et Biophysica Acta* **1020**, 195–206.

Jursinic, P., Warden, J., and Govindjee (1976). A major site of bicarbonate effect in system II reaction: evidence from ESR signal II_{vf}, fast fluorescence yield changes and delayed light emission. *Biochimica et Biophysica Acta* **440**, 322–330.

Karukstis, K. K. (1991). Chlorophyll fluorescence as a physiological probe of the photosynthetic apparatus. In

'Chlorophylls'. (Ed. H. Scheer.) pp. 769–795. (CRC Press: Boca Raton.)

Kautsky, H., Appel, W., and Amann, H. (1960). Chlorophyllfluoreszenz und Kohlensäureassimilation. XIII. Die Fluoreszenzkurve und die Photochemie der Pflanze. *Biochemische Zeitschrift* **332**, 227–292.

Kautsky, H., and Franck, U. (1943). Chlorophyllfluoreszenz und Kohlensäureassimilation. XI. Die Chlorophyllfluoreszeng von *Ulva lactuca* und ihre Abhangigkeit von Narcotica, Sauerstoff und Kohlenoxyd. *Biochemisches Zeitschrift* **315**, 176–206.

Kautsky, H., and Hirsch, A. (1931). Neue Versuche zur Kohlensäureassimilation. *Naturwissenschaften* **19**, 964.

Kircher, A. (1646). Ars magna lucis et umbrae in X libras digesta, etc. Rome. 935 pp.

Klimov, V. V., Klevanik, A. A., Shuvalov, V. A., and Krasnovsky, A. A. (1977). Reduction of pheophytin in the primary light reaction of photosystem II. *FEBS Letters* **82**, 183–196.

Knox, R. (1975). Excitation energy transfer and migration: theoretical considerations. In 'Bioenergetics of Photosynthesis'. (Ed. Govindjee.) pp. 183–221. (Academic Press: New York.)

Kok, B. (1956). On the reversible absorption change at 705 nm in photosynthetic organisms. *Biochimica et Biophysica Acta* **22**, 394–401.

Kok, B. (1963). Fluorescence studies. *National Academy of Sciences—National Research Council Publications* **1145**, 45–55.

Kok, B., Forbush, B., and McGloin, M. (1970). Cooperation of charges in photosynthetic oxygen evolution. I. A linear four-step mechanism. *Photochemistry and Photobiology* **11**, 457–475.

Kramer, D., Robinson, H., and Crofts, A. R. (1990). A portable multi-flash kinetic fluorimeter for measurement of donor and acceptor reactions of photosystem 2 in leaves of intact plants under field conditions. *Photosynthesis Research* **26**, 181–193.

Krause, G. H., and Weis, E. (1991). Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 313–349.

Krey, A., and Govindjee (1964). Fluorescence changes in *Porphyridium* exposed to green light of different intensity: a new emission band at 693 nm and its significance to photosynthesis. *Proceedings of the National Academy of Sciences, USA* **52**, 1568–1572.

Krey, A., and Govindjee (1966). Fluorescence studies on a red alga, *Porphyridium cruentum*. *Biochimica et Biophysica Acta* **120**, 1–18.

Kuhlbrandt, W., Wang, D. N., and Fujiyoshi, Y. (1994). Atomic model of plant light-harvesting complex by electron crystallography. *Nature* **367**, 614–620.

Lavergne, J., and Junge, W. (1993). Proton release during the redox cycle of the water oxidase. *Photosynthesis Research* **38**, 279–296.

Latimer, P., Bannister, T. T., and Rabinowitch, E. (1956). Quantum yields of fluorescence of plant pigments. *Science* **124**, 585–586.

Lavorel, J. (1959). Induction of fluorescence in quinone-poisoned *Chlorella* cells. *Plant Physiology* **34**, 204–209.

Lavorel, J. (1963). Indications d'ordre spectroscopique sur l'heterogeneite de la chlorophylle *in vivo*. *Colloques Internationaux du Centre National de la Recherche Scientifique* **119**, 161–176.

Lavorel, J., and Etienne, A.-L. (1977). *In vivo* chlorophyll fluorescence. *Topics in Photosynthesis* **2**, 203–268.

Lichtenthaler, H. K. (1988). 'Applications of Chlorophyll Fluorescence in Photosynthesis Research, Stress Physiology, Hydrobiology and Remote Sensing.' (Kluwer Academic: Dordrecht.)

Lichtenthaler, H. K. (1992). The Kautsky effect: 60 years of chlorophyll fluorescence induction kinetics. *Photosynthetica* **27**, 45–55.

Lichtenthaler, H. K., and Rinderle, U. (1988). The role of chlorophyll fluorescence in the detection of stress conditions in plants. *Chemical Rubber Company Critical Reviews of Analytical Chemistry* **19**, 529–585.

Litvin, F. F., and Krasnovsky, A. A. (1958). Investigation of absorption spectra and of the fluorescence of plant leaves in the region 400–850 nm (English translation). *Doklady Akademii Nauk* **120**, 307–310.

MacAlister, E. D., and Myers, J. (1940). The time course of photosynthesis and fluorescence observed simultaneously. *Smithsonian Institution Publications Miscellaneous Collections* **99**, 1–37.

Maenpää, P., Miranda, T., Tyystjärvi, E., Tyystjärvi, T., Govindjee, Ducruet, J.-M., Etienne, A.-L., and Kirilovsky, D. (1995). A mutation in the D-de loop of D_1 modifies the stability of the $S_2Q_A^-$ and $S_2Q_B^-$ states in photosystem II. *Plant Physiology* **107**, 187–197.

Malkin, S., and Kok, B. (1966). Fluorescence induction studies in isolated chloroplasts. I. Number of components involved in the reaction and quantum yields. *Biochimica et Biophysica Acta* **126**, 413–432.

Mar, T., and Govindjee (1972). Decrease in the degree of polarization of chlorophyll fluorescence upon the addition of DCMU to algae. In 'Photosynthesis, Two Centuries After its Discovery by Joseph Priestley'. Proceedings of the 2nd International Congress on Photosynthesis Research, Stresa, 24–29 June 1971. Vol. I. (Eds G. Forti, M. Avron and A. Melandri.) pp. 273–281. (Dr W. Junk N. V. Publishers: The Hague.)

Mar, T., Govindjee, Singhal, G. S., and Merkelo, H. (1972). Lifetime of the excited state *in vivo*. I. Chlorophyll *a* in algae, at room and at liquid nitrogen temperatures, rate constants of radiationless deactivation and trapping. *Biophysical Journal* **12**, 797–808.

Mauzerall, D. (1972). Light-induced fluorescence changes in *Chlorella*, and the primary photoreactions for the production of O_2. *Proceedings of the National Academy of Sciences, USA* **69**, 1358–1362.

Metz, J. G., Nixon, P. J., Rogner, M., Brudvig, G. W., and Diner, B. A. (1989). Directed alteration of the D1 polypeptide of photosystem II; evidence that tyrosine is the redox component *Z*, connecting the oxygen-evolving complex to the primary electron donor, P680. *Biochemistry* **28**, 6960–6969.

Mohanty, P., Braun, B. Zilinskas, and Govindjee (1973). Light-induced slow changes in chlorophyll *a* fluorescence in isolated

chloroplasts: effects of magnesium and phenazine methosulfate. *Biochimica et Biophysica Acta* **292**, 459–476.

Mohanty, P., and Govindjee (1973). Light-induced slow changes in the fluorescence yield of chlorophyll *a* in *Anacystis nidulans*. I. Relationships of slow fluorescence changes with structural changes. *Biochimica et Biophysica Acta* **305**, 95–104.

Mohanty, P., and Govindjee (1974). The slow decline and the subsequent rise of chlorophyll fluorescence transients in intact algal cells. *The Plant Biochemical Journal* **1**, 78–106.

Mohanty, P., Mar, T., and Govindjee (1971*b*). Action of hydroxylamine in the red alga *Porphyridium cruentum*. *Biochimica et Biophysica Acta* **253**, 213–221.

Mohanty, P., Papageorgiou, G., and Govindjee (1971*a*). Fluorescence induction in the red alga *Porphyridium cruentum*. *Photochemistry and Photobiology* **14**, 667–682.

Morin, P. (1964). Études des cinétiques de fluorescence de la chlorophylle *in vivo* dans les premiers instants qui suivent le début de illumination. *Journal de Chimique Physique* **61**, 674–680.

Müller, N. J. C. (1874). Beziehungen zwischen Assimilation, Absorption und Fluoreszenz im Chlorophyll des lebenden Blattes. *Jahrbucher Wissenchaftliche Botanik* **9**, 42–49.

Mullet, J. E., Burke, J. J., and Arntzen, C. J. (1980*a*). Chlorophyll proteins of photosystem I. *Plant Physiology* **65**, 814–822.

Mullet, J. E., Burke, J. J., and Arntzen, C. J. (1980*b*). A developmental study of photosystem I peripheral chlorophyll proteins. *Plant Physiology* **65**, 823–827.

Munday, J. C. M. Jr, and Govindjee (1969*a*). Light-induced changes in the fluorescence yield of chlorophyll *a in vivo*. III. The dip and the peak in the fluorescence transient of *Chlorella pyrenoidosa*. *Biophysical Journal* **9**, 1–21.

Munday, J. C. M. Jr, and Govindjee (1969*b*). Light-induced changes in the fluorescence yield of chlorophylla *in vivo*. IV. The effect of pre-illumination on the fluorescence transient of *Chlorella pyrenoidosa*. *Biophysical Journal* **9**, 22–35.

Murata, N., Nishimura, M., and Takamiya, A. (1966*a*). Fluorescence of chlorophyll in photosynthetic systems. II. Induction of fluorescence in isolated spinach chloroplasts. *Biochimica et Biophysica Acta* **120**, 23–33.

Murata, N., Nishimura, M., and Tamiya, A. (1966*b*). Fluorescence of chlorophyll in photosynthetic systems. III. Emission and action spectra of fluorescence—three emission bands of chlorophyll *a* and energy transfer between two pigment systems. *Biochimica et Biophysica Acta* **126**, 234–243.

Murata, N., and Sugahara, K. (1969). Control of excitation transfer in photosynthesis. III. Light-induced decrease of chlorophyll fluorescence related to photophosphorylation system in spinach chloroplasts. *Biochimica et Biophysica Acta* **189**, 182–192.

Myers, J. (1994). The 1932 experiments. *Photosynthesis Research* **40**, 303–310.

Nakatani, H. S., Ke, B., Dolan, E., and Arntzen, C. J. (1984). Identity of the photosystem II reaction center polypeptide. *Biochimica et Biophysica Acta* **765**, 347–352.

Neubauer, C., and Schreiber, U. (1987). The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination. I. Saturation characteristics and partial control by the photosystem II acceptor side. *Zeitschrift für Naturforschung* **42c**, 1246–1254.

Paillotin, G. (1976). Movement of excitations in the photosynthetic domains of photosystem II. *Journal of Theoretical Biology* **58**, 237–252.

Paillotin, G. (1978). Organization of the photosynthetic pigments and transfer of excitation energy. In 'Photosynthesis 77'. (Eds D. O. Hall, J. Coombs and T. W. Goodwin.) pp. 33–44. (The Biochemical Society: London.)

Papageorgiou, G. (1975). Chlorophyll fluorescence: an intrinsic probe of photosynthesis. In 'Bioenergetics of Photosynthesis'. (Ed. Govindjee.) pp. 319–371. (Academic Press: New York.)

Papageorgiou, G., and Govindjee (1967). Changes in intensity and spectral distribution of fluorescence. Effect of light pretreatment on normal and DCMU-poisoned *Anacystis nidulans*. *Biophysical Journal* **7**, 375–390.

Papageorgiou, G., and Govindjee (1968*a*). Light-induced changes in the fluorescence yield of chlorophyll *a in vivo*. II. *Chorella pyrenoidosa*. *Biophysical Journal* **8**, 1316–1328.

Papageorgiou, G., and Govindjee (1968*b*). Light-induced changes in the fluorescence yield of chlorophyll *a in vivo*. I. *Anacystis nidulans*. *Biophysical Journal* **8**, 1299–1315.

Papageorgiou, G., and Govindjee (1971). pH control of the chlorophyll *a* fluorescence in algae. *Biochimica et Biophysica Acta* **234**, 428–432.

Pearlstein, R. M. (1982). Chlorophyll singlet excitons. In 'Photosynthesis'. Vol. 1. Energy conversion by plants and bacteria. (Ed. Govindjee.) pp. 293–330. (Academic Press: New York.)

Pelletier, J., and Caventou, J. B. (1818). Notice sur la matière verte des feuilles [chlorophylle]. *Annal de Chimique Physique* **IX**, 194–196.

Rabinowitch, E. (1945). 'Photosynthesis and Related Processes.' Vol. I. Chemistry of photosynthesis, chemosynthesis and related processes *in vitro* and *in vivo*. (Interscience Publishers Inc. (John Wiley): New York.)

Rabinowitch, E. (1951). 'Photosynthesis and Related Processes.' Vol. II. Part 1. Spectroscopy and fluorescence of photosynthetic pigments; kinetics of photosynthesis. pp. 740–820. (Interscience Publishers Inc. (John Wiley): New York.)

Rabinowitch, E. (1956). 'Photosynthesis and Related Processes.' Vol. II. Part 2. Kinetics of photosynthesis (continued); addenda to Vol. I and Vol. II, Part I. pp. 1375–1406. (Interscience Publishers Inc. (John Wiley): New York.)

Renger, G. (1992). Energy transfer and trapping in photosystem II. *Topics in Photosynthesis* **11**, 45–99.

Renger, G. (1993). Water cleavage by solar radiation—an inspiring challenge of photosynthesis research. *Photosynthesis Research* **38**, 229–247.

Rijgersberg, C. P., Amesz, J., Thielen, A. P. G. M., and Swager, J. A. (1979). Fluorescence emission spectra of chloroplasts and subchloroplast preparations at low temperature. *Biochimica et Biophysica Acta* **545**, 473–482.

Robinson, G. W. (1967). Excitation transfer and trapping in photosynthesis. In 'Energy Conversion by the Photosynthetic Apparatus'. Brookhaven Symposia in Biology. Vol. 19. (Ed. J. M. Olson.) pp. 16–48. (Brookhaven National Laboratory: Upton.)

Sauer, K. (1975). Primary events and the trapping of energy. In 'Bioenergetics of Photosynthesis'. (Ed. Govindjee.) pp. 115–181. (Academic Press: New York.)

Schreiber, U., and Neubauer, C. (1987). The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination. II. Partial control by the photosystem II donor side and possible ways of interpretation. *Zeitschrift für Naturforschung* **42c**, 1255–1264.

Schreiber, U., Schliwa, U., and Bilger, W. (1986). Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis Research* **10**, 51–62.

Seely, G. (1973). Energy transfer in a model of the photosynthetic unit of green plants. *Journal of Theoretical Biology* **40**, 189–199.

Shinkarev, V. P., and Govindjee (1993). Insight into the relationship of chlorophyll *a* fluorescence yield to the concentration of its natural quenchers in oxygenic photosynthesis. *Proceedings of the National Academy of Sciences, USA* **90**, 7466–7469.

Shinkarev, V. P., Xu, C., Govindjee, and Wraight, C. A. (1994). Kinetics of the oxygen evolution step *in situ* estimated from its quenching effect on flash-induced chlorophyll *a* fluorescence. *Biophysical Journal* **66**, A114.

Shubin, V. V., Murthy, S. D. S., Karapetyan, N. V., and Mohanty, P. (1991). Origin of the 77K variable fluorescence at 758 nm in the cyanobacterium *Spirulina platensis*. *Biochimica et Biophysica Acta* **1060**, 28–36.

Sonneveld, A., Rademaker, H., and Duysens, L. N. M. (1979). Chlorophyll fluorescence as a monitor of nanosecond reduction of the photo-oxidized $P680^+$ of photosystem II. *Biochimica et Biophysica Acta* **548**, 536–551.

Stemler, A., and Jursinic, P. (1993). Oxidation-reduction potential dependence of formate binding to photosystem II in maize thylakoids. *Biochimica et Biophysica Acta* **1183**, 269–280.

Stern, V. O., and Volmer, M. (1919). Über die Ablingungszeit der fluoreszeng. *Physik Zeitschrift* **20**, 183–188.

Stokes, G. G. (1852). On the change of refrangibility of light. *Philosophical Transactions of the Royal Society of London* **142**, 463–562.

Strasser, R. J. (1978). The grouping model of plant photosynthesis. In 'Chloroplast Development'. (Ed. G. Akoyunoglou.) pp. 513–542. (Elsevier/North Holland: Amsterdam.)

Strasser, R. J., and Butler, W. L. (1977). Energy transfer and the distribution of excitation energy in the photosynthetic apparatus of spinach chloroplasts. *Biochimica et Biophysica Acta* **460**, 230–238.

Strasser, R. J., Eggenberg, P., Pfister, K., and Govindjee (1992). An equilibrium model for electron transfer in photosystem II acceptor complex: an application to *Chlamydomonas reinhardtii* cells of D1 mutants and those treated with formate. *Archives de Science Genève* **45**, 207–224.

Strasser, R. J., and Govindjee (1991). The F_o and the *O-J-I-P* fluorescence rise in higher plants and algae. In 'Regulation of Chloroplast Biogenesis'. (Ed. J. H. Argyroudi-Akoyunoglou.) pp. 423–426. (Plenum Press: New York.)

Strasser, R. J., and Govindjee (1992). On the *O-J-I-P* fluorescence transients in leaves and D1 mutants of *Chlamydomonas reinhardtii*. In 'Research in Photosynthesis'. Vol. II. (Ed. N. Murata.) pp. 39–32. (Kluwer Academic: Dordrecht.)

Strasser, R. J., Srivastava, A., and Govindjee (1995). Polyphasic chlorophyll *a* fluorescent transient in plants and cyanobacteria. *Photochemistry and Photobiology* **61**, 32–42.

Tomita, G., and Rabinowitch, E. (1962). Excitation energy transfer between pigments in photosynthetic cells. *Biophysical Journal* **2**, 483–499.

Trissl, H.-W., Gao, Y., and Wulf, K. (1993). Theoretical fluorescence induction curves derived from coupled differential equations describing the primary photochemistry of photosystem II by an exciton–radical pair equilibrium. *Biophysical Journal* **64**, 974–988.

Trissl, H.-W., and Lavergne, J. (1995). Fluorescence induction from photosystem II: analytical equations for the yields of photochemistry and fluorescence derived from analysis of a model including exciton–radical pair equilibrium and restricted energy transfer between photosynthetic units. *Australian Journal of Plant Physiology* **22**, 183–193.

Tumerman, L. A., and Sorokin, E. M. (1967). Fotosinteticheskaya edinitsa: 'fizicheskaya' ili 'staticheskaya' model'? [The photosynthetic unit: a 'physical' or 'statistical' model?] *Molecular Biology, USSR* **1**, 628–638.

Van Gorkom, H. J. (1986). Fluorescence measurements in the study of photosytem II electron transport. In 'Light Emission by Plants and Bacteria'. (Eds Govindjee, J. Amesz and D. C. Fork.) pp. 267–289. (Academic Press: Orlando.)

Van Gorkom, H. J., Pulles, M. P. J., and Etienne, A.-L. (1978). Fluorescence and absorbance changes in Tris-washed chloroplasts. In 'Photosynthetic Oxygen Evolution'. (Ed. H. Metzner.) pp. 135–145. (Academic Press: London.)

Van Grondelle, R., and Amesz, J. (1986). Excitation energy transfer in photosynthetic systems. In 'Light Emission by Plants and Algae'. (Eds Govindjee, J. Amesz and D. C. Fork.) pp. 191–223. (Academic Press: Orlando.)

Van Mieghem, F. J. E., Searle, G. F. W., Rutherford, A. W., and Schaafsma, T. J. (1992). The influence of the double reduction of Q_A on the fluorescence decay kinetics of photosystem II. *Biochimica et Biophysica Acta* **1100**, 198–206.

Van Rensen, J. J. S. (1992). Regulation of electron transport at the acceptor side of photosytem II by herbicides, bicarbonate and formate. In 'Photosynthesis: Photoreactions to Plant Productivity'. (Eds Y. P. Abrol, P. Mohanty and Govindjee.) pp. 157–180. (Oxford/IBH: New Delhi; Kluwer Academic: Dordrecht.)

Van Rensen, J. J. S., Tonk, W. J. M., and de Bruijn, S. M. (1988). Involvement of bicarbonate in the protonation of the secondary quinone electron acceptor of photosystem II via the non-heme iron of the quinone-iron acceptor complex. *FEBS Letters* **226**, 347–351.

Velthuys, B. R., and Amesz, J. (1974). Charge accumulation at the reducing side of system 2 of photosynthesis. *Biochimica et Biophysica Acta* **325**, 138–148.

Vernotte, C., Etienne, A.-L., and Briantais, J.-M. (1979). Quenching of the system II fluorescence by the plastoquinone pool. *Biochimica et Biophysica Acta* **545**, 519–527.

Wassink, E. C. (1951). Chlorophyll fluorescence and photosynthesis. *Advances in Enzymology* **11**, 91–199.

Whitmarsh, J., and Levine, P. (1974). Excitation energy transfer and chlorophyll orientation in the green alga *Chlamydomonas reinhardtii*. *Biochimica et Biophysica Acta* **368**. 199–213.

Wong, D., and Govindjee (1979). Antagonistic effects of mono- and divalent cations on polarization of chlorophyll fluorescence in thylakoids and changes in excitation energy transfer. *FEBS Letters* **97**, 373–377.

Wong, D., and Govindjee (1981). Action spectra of cation effects on the fluorescence polarization and intensity in thylakoids at room temperature. *Photochemistry and Photobiology* **33**, 103–108.

Wraight, C., and Crofts, A. R. (1970). Energy-dependent quenching of chlorophyll *a* fluorescence in isolated chloroplasts. *European Journal of Biochemistry*. **17**, 319–327.

Wydrzynski, T., and Govindjee (1975). A new site of bicarbonate effect in photosystem II of photosynthesis: evidence from chlorophyll fluorescence transients in spinach chloroplasts. *Biochimica et Biophysica Acta* **387**, 403–408.

Xiong, J., Hutchison, R. S., Sayre, R., and Govindjee (1994). Construction and characteristics of a putative bicarbonate site mutant in the photosystem II D1 protein of *Chlamydomonas reinhardtii*. Abstracts of the 4th International Congress of Plant Molecular Biology, Amsterdam, 20–24 June, 1994.

Xu, C. (1992). Differential inhibition of the plastoquinone reductase activity by weak organic acids and its relationship to the bicarbonate effect in spinach thylakoids. PhD Thesis, University of Illinois.

Xu, C., Rogers, S. M. D., Goldstein, C., Widholm, J. M., and Govindjee (1989). Fluorescence characteristics of photoautotrophic soybean cells. *Photosynthesis Research* **21**, 93–106.

Xu, C., Taoka, S., Crofts, A. R., and Govindjee (1991). Kinetic characteristics of formate/formic acid binding at the plastoquinone reductase site in spinach thylakoids. *Biochimica et Biophysica Acta* **1098**, 32–40.

Yamagishi, A., Satoh, K., and Katoh, S. (1978). Fluorescence induction in chloroplasts isolated from the green alga *Bryopsis maxima*. III. A fluorescence transient indicating proton gradient across the thylakoid membrane. *Plant and Cell Physiology* **19**, 17–25.

Yamamoto, H. Y. (1979). Biochemistry of the violaxanthin cycle in higher plants. *Pure and Applied Chemistry* **51**, 639–648.

Yamamoto, H. Y., Nakayama, J. O. M., and Chichester, C. O. (1962). Studies on the light and dark interconversions of leaf xanthophylls. *Archives of Biochemistry and Biophysics* **97**, 168–173.

Yamazaki, I., Mimuro, M., Murao, T., Yamazaki, I., Yoshihara, K., and Fujita, Y. (1984). Excitation energy transfer in the light harvesting antenna of the red alga *Porphyridium cruentum* and the blue-green alga *Anacystis nidulans*. Analysis of the time-resolved fluorescence spectra. *Photochemistry and Photobiology* **39**, 233–240.

Zankel, K. (1973). Rapid fluorescence changes observed in chloroplasts: their relationship to the O_2 evolving system. *Biochimica et Biophysica Acta* **325**, 138–148.

Manuscript received 5 July 1994, accepted 18 November 1994

Sixty-three years since Kautsky: chlorophyll *a* fluorescence.
Govindjee
Volume 22, Number 2 (1995), 131–160

Page 140, right column, line 23. 'Fig. 9 summarises' should read 'Table 2 summarises'.

Page 143, equation 2. The correct equation is as follows:

$$\Phi_f = \frac{k_f}{k_f + k_h + k'_p[Q_A]},$$

Page 143, right column, line 7. 'Chl *a* fluorescence yield is minimal' should read 'Chl *a* fluorescence yield is maximal'.

Page 151, Fig. 21 caption, line 5. 'and D2-R265, by Diner *et al.*' should read 'and D2-R251, by Diner *et al.*'