EXCITATION ENERGY TRANSFER BETWEEN PIGMENTS IN PHOTOSYNTHETIC CELLS

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ABSTRACT The excitation lifetimes of photosynthetic pigments and the times needed for energy transfer between pigments in various algae, were determined *in vitro* and *in vivo*. For this purpose, the time curves of fluorescence rise and decay were measured by means of Brody's instrument (10), and compared with theoretical curves obtained by the method of "convolution of the first kind."[1]

I. INTRODUCTION

The primary process of photosynthesis is the absorption of light quanta by the various pigments. These pigments are present in the living cells in high concentration, in a condensed phase. The fluorescence bands of several pigments overlap strongly with the absorption bands of the same (or other) pigments, creating favorable conditions for resonance transfer of excitation energy between them, in accordance with Förster's theory (1).

Energy transfers of two types can occur: those between identical, and those between different pigment molecules ("homogeneous" and "heterogeneous" transfers, respectively). The latter give rise to sensitized fluorescence of the energy acceptor, and compete with the emission of fluorescence by the energy donor; because of this competition, the actual lifetime of the excited state (determined from the observation of fluorescence decay) can be used to calculate the efficiency of the excitation energy transfer.

Excitation energy migration between identical chlorophyll *a* molecules was first postulated by Gaffron and Wohl (2) to explain the results of experiments on photosynthesis in flashing light, carried out by Emerson and Arnold (3)—experiments which have lead to the concept of a "photosynthetic unit." The extent of this "homogeneous" transfer is difficult to estimate directly; but it was studied by various

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[1] See *e.g.*, Doetsch, G., Theorie und Anwendung der Laplace-Transformation, Berlin, Springer, 1937, 155; Handbuch der Laplace-Transformation, Basel, Birkhauser, 1950, 1, 121; Widder, D. V., The Laplace Transformation, Princeton University Press, 1941, 84.

indirect methods, including measurements of the self-quenching of fluorescence (*e.g.* by Watson and Livingston (4)), and of the concentration depolarization of fluorescence (*e.g.* by Arnold and Meek (5)). Theoretical calculations of the probability of this type of transfer were made by Förster (1). The phenomena have been discussed in some recent papers by one of the authors (6, 11).

The occurrence of "heterogeneous" energy transfer between unlike pigments *in vivo* was discovered by Dutton, Manning, and Duggar (7); the efficiency of this energy transfer was evaluated by Duysens (8), and by French and Young (9).

Brody and Rabinowitch (10, 11) undertook direct measurements of the lifetime of chlorophyll fluorescence *in vitro* and *in vivo*. They were able to obtain, on the oscilloscope, a direct display of the course of chlorophyll fluorescence excited by a very brief flash of hydrogen discharge lamp, and to calculate from this display the lifetime of chlorophyll in the excited singlet state. However, the time lag of the instrument was of the same order of magnitude (several millimicroseconds) as the decay constant of fluorescence. Therefore, the lifetimes could not be obtained, from the observed curves of fluorescence decay, without a substantial correction for this lag. To do so, as is known (12, the observed time curve, $F\ (t)$ can be assumed to be represented by the following expression ("superposition theorem"),

$$F(t) = \int_0^t f(\alpha)\ I\ (t - \alpha)\ d\alpha \qquad (1)$$

where $I(t)$ and $f\ (\alpha)$ are, respectively, the time course of the response of the system (lamp + detector), and the true time course of fluorescence; the product $f(\alpha)\ I\ (t - \alpha)$ is the contribution to the fluorescence, observed at time t, of fluorescence emitted at time α and only measured at time t because of the sluggishness of the instrument. Integrating over all values from $\alpha = 0$ to $\alpha = t$ gives the total fluorescence intensity observed at time t. To eliminate the influence of the instrumental response time from the observed decay curves, Brody used the so called "method of moments," assuming that the true fluorescence decay curves are exponential.

The time needed for energy transfer between unlike molecules (phycobilin and chlorophyll *a*) was determined by Brody from the horizontal distance between the rise curves of the directly excited and of the sensitized fluorescence of chlorophyll in red algae. However, these curves are not parallel, and the horizontal distance between them cannot provide a precise measure of the transfer time.

In order to examine more closely the above mentioned points, we undertook to evaluate numerically the curves given by equation (1), assuming plausible forms for the function $I(t)$. [Simple exponential decay in absence of energy transfer, *cf.* equation (5), and more complicated curves in the presence of energy transfer, *cf.* equation (6).] We found that the calculated function $F(t)$ can be made to represent satisfactorily the observed responses of the instrument. The lifetimes of fluorescence, and the times of the heterogeneous excitation energy transfer *in vivo* could thus be determined by comparing the observed response curves with the $F(t)$ curves calculated by using different time parameters; the transfer efficiencies could then be calculated from the lifetimes and the transfer times obtained.

II. EXPERIMENTAL PROCEDURE AND CALCULATION

A. *Experimental*

The response curves of fluorescence in various algae were recorded by means of the instrument used by Brody and Rabinowitch, with some improvements (for details of the instrument, see reference 10). In order to increase the precision of the instrument, the transit time spread of electrons in the photomultiplier was minimized by using only the center of the photosensitive surface of the photomultiplier (0.18 cm^2); to improve sensitivity, the voltages applied to the photomultiplier and to the hydrogen lamp were made as high as possible without producing excessive noise. The time courses of the lamp flash and of the fluorescence were observed on the "EG and G" type 2236 traveling wave oscilloscope. From the decay curve of the lamp flash, the time constant of the instrument as a whole (with a 50 ohm cable and no amplifier) was determined to be 2.3 mμsec. The hydrogen lamp, the fluorescent sample, and the photomultiplier used for measuring the fluorescence were placed in a straight line, so as to make the distance from the lamp to the detecting photomultiplier as short as possible. The thickness of the vessel containing the cell suspension was 1.5 mm. Complementary Corning glass filters or Farrand interference filters were used to prevent the flash light from reaching the fluorescence-measuring photomultiplier.

B. *Calculations*

(*a*) We assume that the system is composed of N different pigments, $I, II, \ldots\ldots\ldots,$ $J, \ldots\ldots, N$, in a transparent medium. The molecules of pigment J are denoted by $1_J, 2_J, \ldots\ldots\ldots, i_J, \ldots\ldots, l_J, \ldots\ldots\ldots$ Two types of energy transfers are possible — between identical, and between different molecules.

In the first case, transfer can be reversed, so that $F_{l_J, i_J} = F_{i_J, l_J}$ (where F_{l_J, i_J} is the number of energy transfers, $l_J \rightarrow i_J$ per unit time, and F_{i_J, l_J} is that of reverse transfers, $i_J \rightarrow l_J$).

In the second case, transfer is irreversible and excitation energy moves in one direction, from molecules with the higher to those with a lower excitation level.

We assume that pigment I has the highest excitation level and pigment N the lowest one, and that "heterogeneous" energy transfer can occur only between "adjacent" pigments in the series:

$$I \rightarrow II \rightarrow \cdots\cdots \rightarrow J \rightarrow \cdots\cdots\cdots \rightarrow N$$

(This assumption is plausible because the fluorescence band of a pigment usually overlaps strongly with the absorption band of only one other pigment.) When the system is excited with light absorbed only by pigment I, the time course of the probability, $\rho_{l_J}(t)$ of finding a molecule l_J of pigment J in the excited state can be obtained from the following equation:

$$\frac{d\rho_{l_J}(t)}{d(t)} = \sum_{i_J} F_{l_J,i_J}(\rho_{i_J}(t) - \rho_{l_J}(t))$$

$$+ \sum_{k_{J-I}} F_{k_{J-I},l_J}\rho_{k_{J-I}}(t) - \sum_{m_{J+I}} F_{l_J,m_{J+I}}\rho_{l_J}(t) - \frac{1}{\tau_J}\rho_{l_J}(t) \qquad (2)$$

where τ_J is the average excitation lifetime of pigment J and $F_{l_J,m_{J+I}}$, the number of energy transfers per unit time from molecule l_J of pigment J to molecule m_{J+I} of pigment $J + I$. The first sum in (2) refers to homogeneous energy transfers in pigment J, the second to heterogeneous transfers from pigment $J - I$ to pigment J, and the third to heterogeneous transfers from pigment J to pigment $J + I$; the last term represents the decay of the excited state of pigment J by mechanisms (fluorescence, internal conversion, etc.) not involving energy transfer.

For the totality of molecules of pigment J, we obtain, by summation, the following equation:

$$\frac{d\sum_{l_J}\rho_{l_J}(t)}{dt} = \sum_{l_J}\sum_{k_{J-I}} F_{k_{J-I},l_J}\rho_{k_{J-I}}(t)$$

$$- \sum_{l_J}\sum_{m_{J+I}} F_{l_J,m_{J+I}}\rho_{l_J}(t) - \frac{1}{\tau_J}\sum_{l_J}\rho_{l_J}(t) \qquad (3)$$

The terms referring to homogeneous transfer cancel each other out in the summation:

$$\sum_{l_J}\sum_{i_J} F_{l_J,i_J}(\rho_{i_J}(t) - \rho_{l_J}(t)) = 0. \qquad (3a)$$

Equation (3a) means that the decline of excitation in the total system is not affected by energy transfers between identical molecules.

The magnitude F_{k_{J-I},l_J} must decrease rapidly with increasing distance between the molecules k_{J-I} and l_J. If we postulate, as a first approximation, that the total probability of energy transfer from a molecule of pigment $J - I$ (or J) to any one of the molecules of pigment J (or $J + I$) is the same for each molecule of pigment $J - I$ (or J), equation (3) can be simplified:

$$\frac{d[\rho_J(t)]}{dt} = [F^{(J-I)}][\rho_{J-I}(t)] - [F^{(J)}](\rho_J(t)] - \frac{1}{\tau_J}[\rho_J(t)],$$

$$(J = I, II, III, \ldots\ldots, N), \qquad (4)$$

where

$$[\rho_{J-I}(t)] = \sum_{k_{J-I}}\rho_{k_{J-I}}(t), \qquad [\rho_J(t)] = \sum_{l_J}\rho_{l_J}(t),$$

$$[F^{(J-I)}] = \sum_{l_J} F_{k_{J-I},l_J}\ (= \sum_{l_J} F_{p_{J-I},l_J}),$$

$$[F^{(J)}] = \sum_{m_{J+I}} F_{l_J,m_{J+I}}\ (= \sum_{m_{J+I}} F_{q_J,m_J J_I}),$$

$$[F^{(0)}] = [F^{(N)}] = 0 \quad \text{and} \quad [\rho_0(t)] = 0.$$

If pigment I alone is excited initially, so that $[\rho_I(0)] = 1$, $[\rho_{II}(0)] = 0, \ldots\ldots\ldots,$ $[\rho_N(0)] = 0$, the general solutions of equation (4) are as follows:—
for $J = I$,

$$[\rho_I(t)] = \exp\,(-t/\tau_I^*), \tag{5}$$

for $J \neq I$,

$$\begin{aligned} [\rho_J(t)] = (-1)^J \exp\,(-t/\tau_J^*) \prod_{K=1}^{K=J-I} [F^{(K)}] \\ \times \sum_{K=I}^{K=J-I} \left[\{1 - \exp\,(-(1/\tau_K^* - 1/\tau_J^*)t)\} \Big/ \prod_{M=I,\, M\neq K}^{M=J} (1/\tau_K^* - 1/\tau_M^*) \right], \end{aligned} \tag{6}$$

where

$$1/\tau_J{}^* = [F^{(J)}] + 1/\tau_J.$$

The fluorescence yield, η_J is

$$\begin{aligned} \eta_J &= \frac{1}{\tau_J^0} \int_0^\infty [\rho_J(t)]\, dt \\ &= \frac{\tau_J^*}{\tau_J^0} \prod_{K=I}^{K=J-I} \tau_K^*[F^{(K)}], \qquad (J = I, II, \ldots\ldots\ldots, N) \end{aligned} \tag{7}$$

where $\tau_J{}^0$ is the natural lifetime of pigment J (*i.e.*, fluorescence lifetime in absence of all other energy dissipating processes).

When the system is composed of three different kinds of pigments (I, II, III), all of which can be excited by external radiation, the time courses ($[\rho_{III}{}^{(I)}(t)]$, $[\rho_{III}{}^{(II)}(t)]$ and $[\rho_{III}{}^{(III)}(t)]$) of direct and sensitized fluorescence of pigment III are obtained from the equations (5) and (6), respectively, as follows:—

$$\begin{aligned} [\rho_{III}^{(I)}(t)] = [(1/\tau_{t_I}\tau_{t_{II}})/(1/\tau_I^* - 1/\tau_{II}^*)] \\ \times [\{1/(1/\tau_{II}^* - 1/\tau_{III})\}\{1 - \exp\,(-(1/\tau_{II}^* - 1/\tau_{III}))t\} \\ + \{1/(1/\tau_{III} - 1/\tau_I^*)\}\{1 - \exp\,(-(1/\tau_I^* - 1/\tau_{III}))t]\, \exp\,(-t/\tau_{III}), \end{aligned} \tag{8}$$

$$\begin{aligned} [\rho_{III}^{(II)}(t)] = [(1/\tau_{t_{II}})/(1/\tau_{II}^* - 1/\tau_{III})] \\ \times [1 - \exp\,\{-(1/\tau_{II}^* - 1/\tau_{III})\}t]\, \exp\,(-t/\tau_{III}), \end{aligned} \tag{9}$$

$$[\rho_{III}^{(III)}(t)] = \exp\,(-t/\tau_{III}), \tag{10}$$

where τ_{t_I} and $\tau_{t_{II}}$ are the transfer times for the pigment pairs $I \rightarrow II$ and $II \rightarrow III$, respectively; while $\tau_{tI} = 1/[F^{(I)}]$ and $\tau_{tII} = 1/[F^{(II)}]$.

(*b*) As mentioned before, the time curves of the hydrogen lamp flash and of the fluorescence flash are distorted significantly by instrumental factors, making it impossible to determine the fluorescence decay constant and the transfer times directly from the observed fluorescence curves. However, by using the superposition theorem, the instrumental factors can be eliminated as follows:

We call the observed response of the instrument to the lamp flash $I(t)$ and the true time course of the flash, $l(t)$. We postulate that the response to the flash observed on the oscilloscope, $L(t)$ can be represented by the following equation:

$$L(t) = \int_0^t I(\alpha) l(t - \alpha)\, d\alpha \tag{11}$$

The true time course of fluorescence, $[\rho(t)]$, is modified by using the lamp flash with the time course $l(t)$, to $f(t)$, represented by the following expression:

$$f(t) = \int_0^t [\rho(\beta)] l(t - \beta)\, d\beta \tag{12}$$

The fluorescence curve, as observed on the oscilloscope, which we call $G(t)$, can be represented as follows:

$$G(t) = \int_0^t I(\gamma) f(t - \gamma)\, d\gamma \tag{13}$$

If t in equation (12) is replaced by $t - \gamma$, we obtain

$$f(t - \gamma) = \int_0^{t-\gamma} [\rho(\beta)] l(t - \gamma - \beta)\, d\beta \tag{14}$$

With the help of equations (13) and (14), $G(t)$ can be represented as follows:—

$$G(t) = \int_0^t L(t - \beta)[\rho(\beta)]\, d\beta \tag{15}$$

When any two functions in the above equation are known, the third one can be calculated.

If $G(t)$ is calculated numerically for different values of the parameters, using the observed curve $L(t)$ and the theoretical curve $[\rho(t)]$, the so called calculation of "convolution of the first kind" (see Note at the end of this paper), the lifetime of fluorescence and the transfer time of excitation energy between different pigments can be determined by comparing the observed $G(t)$ curves with the curves calculated for different values of the parameters.

III. RESULTS AND DISCUSSION

When, in a system containing three different pigments, one of them (I, II, or III) is excited, the fluorescence curve $G(t)$ of pigment III can be calculated from the observed time curve of the flash and equations (8), (9), (10), and (15). Some of these calculated curves are shown in Figs. 1–3. All the curves are normalized to a maximum height of unity.

First, the time constant of fluorescence of the pigment III, τ_{III}, can be determined by comparing the observed fluorescence—time curves for direct excitation with a set of calculated $G(t)$ curves, as exemplified in Fig. 1.

Next, the transfer time from pigment II to III, τ_{tII}, can be obtained from the

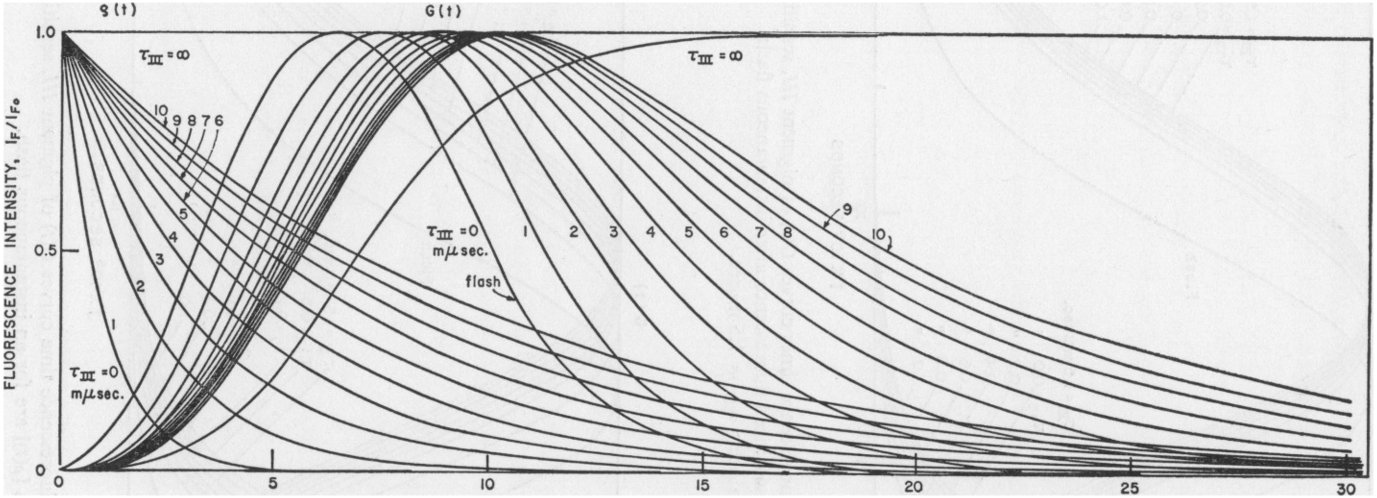

FIGURE 1 Fluorescence time curves $G(t)$ of pigment *III*, for direct excitation by the flash, calculated for different values of the decay constant τ_{III}. The $[\rho(t)]$ curves are for fluorescence decay after instantaneous excitation.

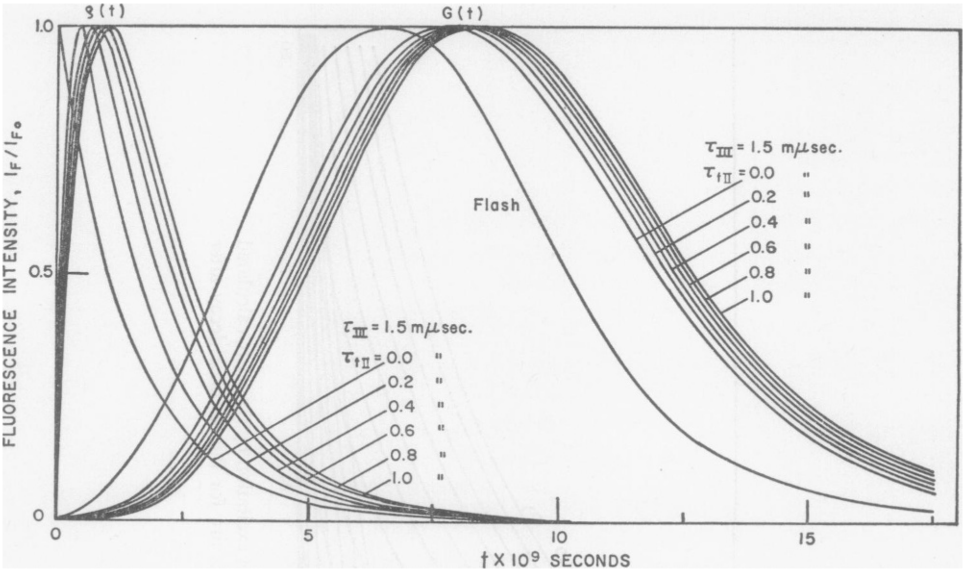

Figure 2 Calculated fluorescence times curves $G(t)$ of pigment *III*, sensitized by pigment *II*. Curves $[\rho(t)]$ are for sensitized fluorescence after instantaneous flash.
Parameters: $\tau_{II} = 1.8$ mμsec.; $\tau_{III} = 1.5$ mμsec.

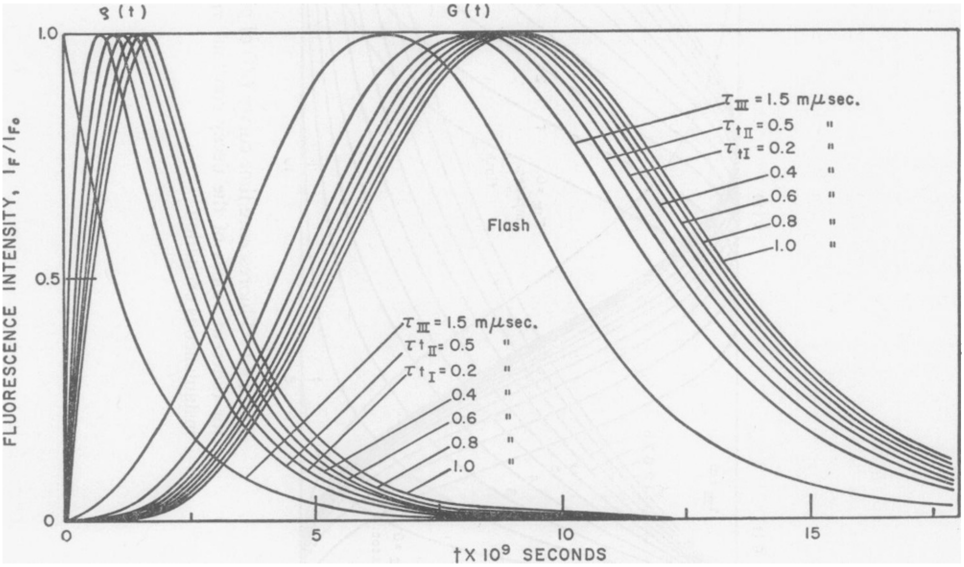

Figure 3 Calculated fluorescence time curves $G(t)$ of pigment *III*, sensitized by pigment *I via* pigment *II*; curves $[\rho(t)]$ are for an instantaneous flash.
Parameters: $\tau_I = 7.1$ mμsec.; $\tau_{II} = 1.8$ mμsec.; $\tau_{III} = 1.5$ mμsec.; $\tau_{t_{II}} = 0.5$ mμsec.

observed curves of pigment *II*-sensitized fluorescence of pigment *III*, and a set of curves calculated with the measured values of τ_{II} and τ_{III}, as shown in Fig. 2.

Last, the transfer time from pigment *I* to *II*, τ_{tI}, can be similarly determined from a set of curves calculated for the established values of τ_{I}, τ_{II}, τ_{III}, and τ_{tII}, as shown in Fig. 3.

The intervals of time between the peak of the lamp flash, and the peaks of direct and sensitized fluorescence, can be determined from the curves shown in Figs. 1–3. The variations of these intervals with the decay constant of pigment *III*, and the transfer times τ_{tI} and τ_{tII}, are shown in Fig. 4. Δt_m, $\Delta t_m'$, and $\Delta t_m''$ are, respectively, the intervals of time between the peak of the lamp flash and that of direct fluorescence; between that of direct and that of pigment *II*-sensitized fluorescence, and between that of pigment *II*-sensitized and that of pigment *I*-sensitized fluorescence. If these intervals were known, it would be possible to determine from them the average lifetimes and transfer times between pigments; but the peaks of flash and

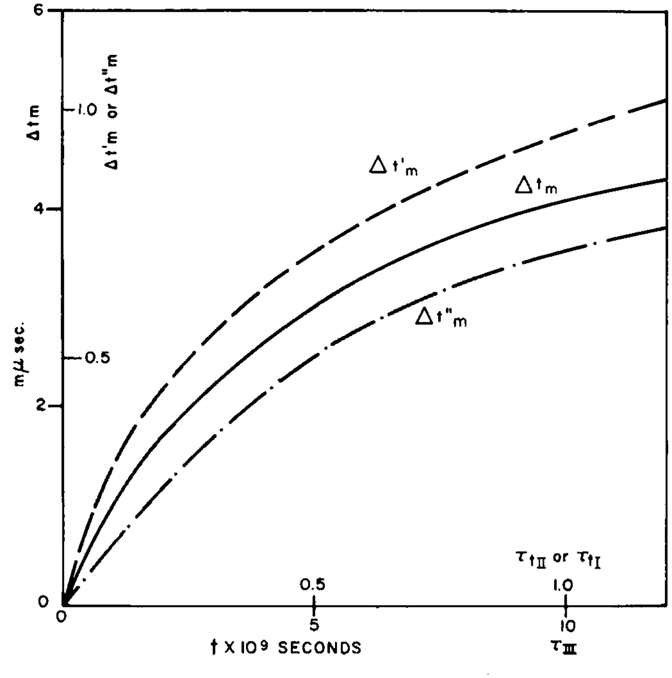

FIGURE 4 Variations of the shift of fluorescence peak as function of τ_{III} (the lifetime of the fluorescence of pigment *III*) and of the time of energy transfer from pigment *II* to *III* (or *I* to *II*), τ_{tII} (or τ_{tI}).

————, variation of the interval (Δt_m) between peaks of flash and fluorescence, for direct excitation.

– – – – – –, variation of the interval ($\Delta t_m'$) between peaks of directly excited fluorescence and pigment *II*-sensitized fluorescence.

–·–·–·–, variation of the interval ($\Delta t_m''$) between peaks of pigment *II*- and pigment *I*-sensitized fluorescence.

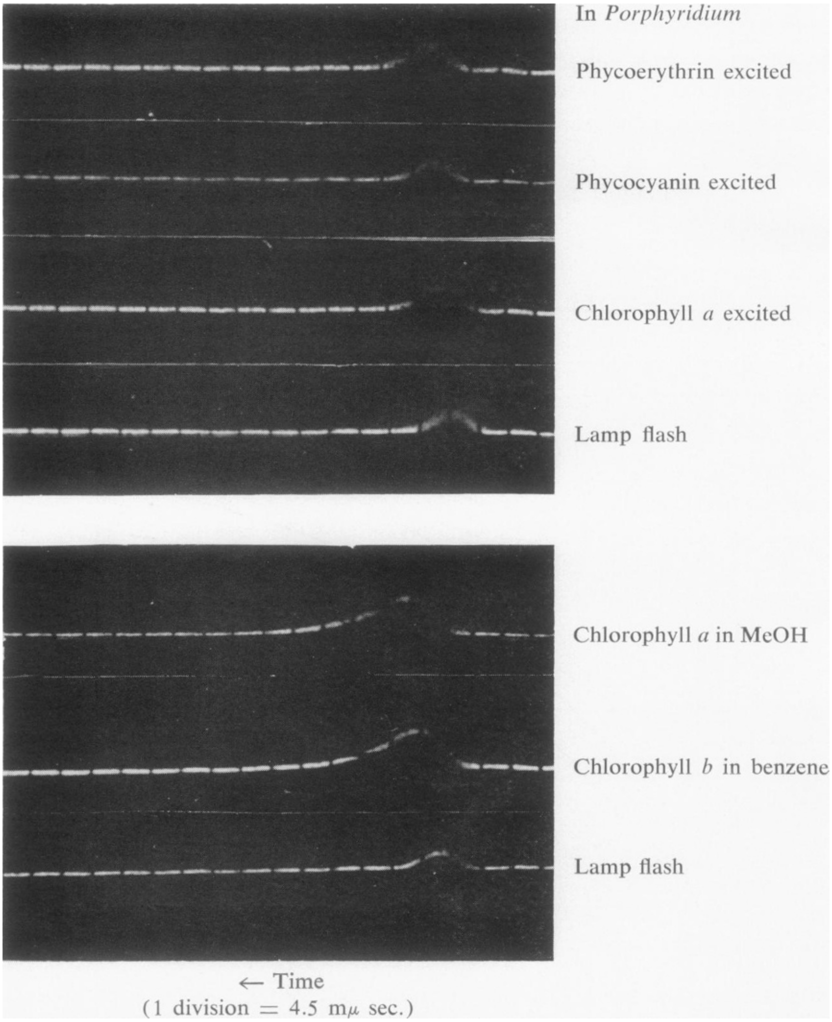

FIGURE 5 Records of lamp flash and chlorophyll fluorescence.

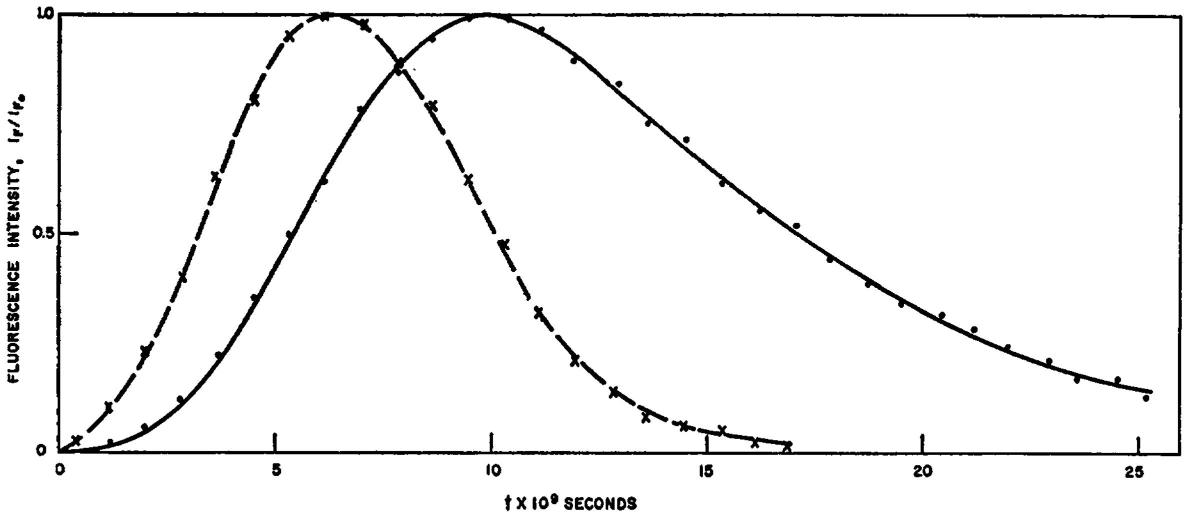

FIGURE 6 Time curves of lamp flash and fluorescence.

× lamp flash.

● fluorescence of chlorophyll *a* in MeOH (measured).

— the closest theoretical curve (calculated by assuming a decay constant τ_{III} of 6.8 mμsec.).

fluorescence are not sharp, and it is difficult to determine the positions of these peaks accurately. The curves shown in Fig. 4 were used therefore only to show the general trend of the change of the time intervals with the decay constant (or the transfer time). All curves tend to saturate with the increasing decay constant (or transfer time).

The average lifetimes of excitation of chlorophyll *a* and *b* were measured before by Brody (10) and by Terenin *et al.* (*cf.* reference 14). They were again measured by us in various solutions (ethyl ether, methanol, and benzene), as well as in several species of algae, by comparing the measured time curves of fluorescence with the calculated $G(t)$ curves in Fig. 1.

The chlorophylls used were prepared from fresh spinach by Zscheile and Comar's method (13). The species of algae used were *Porphyridium cruentum* (red alga), *Anacystis nidulans* (blue-green alga), and *Chlorella pyrenoidosa* (green alga), all in the dark-adapted state (the instantaneous intensity of the flash is very high, but its duration is so short that cells remain in the dark-adapted state).

The time curves of lamp flash and fluorescence were recorded on Kodak panatomic-X film, without changing the voltage on the photomultiplier tube. The lamp was flashed 60 times a second; the exposure time was 30 seconds. This means that 1800 sweeps were superimposed on the same film. Some of the recordings are shown in Fig. 5. To determine the decay constant, the time curves of the flash and of fluorescence were plotted together (all curves having been normalized so as to have the maximum height of unity). One of these plots is shown in Fig. 6. When Fig. 6 is superposed on Fig. 1, by making the flash curves coincide, the theoretical curve closest to the observed fluorescence curve can be easily picked out and the average lifetime τ_{III} determined. The closest theoretical curve is shown in Fig. 6. The results of these experiments are listed in Table I; they agree closely with those that Brody (10) obtained by the "method of moments."

The energy transfer times from phycoerythrin to phycocyanin, and from phycocyanin to chlorophyll *a* in *Porphyridium*, were determined as follows:—We measured the time courses of the hydrogen lamp flash (illumination filter: Corning glass filter 9782; observation filter: 2424), that of directly excited fluorescence of chlorophyll *a* (illumination filters: 5030 and 9782; observation filter: 2030), that of phycocyanin-sensitized fluorescence of chlorophyll *a* (illumination filters: Farrand interference filter 630 mμ and Corning 9788; observation filter: 2030); and that of phycoerythrin-sensitized fluorescence (illumination filters: 9782 and 3384; observation filter: 2030). Fig. 7 shows the transmission curves of the filters used, and the absorption and fluorescence spectra of chlorophyll *a*, phycocyanin, and phycoerythrin, as determined by French *et al.* (9, 15).

The results are plotted in Fig. 8, where all curves are normalized to unity in their peaks. The decay constant τ_{III}, was determined from the observed curve of directly excited fluorescence curve and the theoretical curves shown in Fig. 1, by the procedure described above. The curve of phycocyanin-sensitized fluorescence was compared with a set of theoretical curves, calculated by using the above determined τ_{III} value

TABLE I

FLUORESCENCE LIFETIMES OF PIGMENTS

Material	Average lifetime* $\tau \pm 0.2$	Fluorescence yield $\Phi = \tau/\tau^0$ (± 0.01)
	mμsec.	
Chlorophyll *a* (τ^0 = 15.2 mμsec.)‡		
in ether	5.2 (5.1)	0.34
in methanol	6.8 (6.9)	0.45
in benzene	7.8 (7.8)	0.51
in *Anacystis*	1.2 (1.2)	0.08
in *Porphyridium*	1.5 (1.5)	0.10
in *Chlorella*	1.7 (1.6)	0.11
Chlorophyll *b* (τ^0 = 23.0 mμsec.)‡		
in ether	4.0 (3.9)	0.17
in methanol	6.0 (5.9)	0.26
in benzene	6.4 (6.3)	0.28

* Figures in parenthesis are from Brody (10); Terenin *et al.* found τ = 5 mμsec. for chlorophyll *a* + *b* in ether; 0.6 mμsec. for chlorophyll in leaves of *Elodea*; and 1.0 mμsec. for chlorophyll in leaves of *Cyperus* (all in weak light).

‡ Natural lifetimes τ^0 determined by Brody (10) by integrating the absorption band of chlorophyll in ethyl ether.

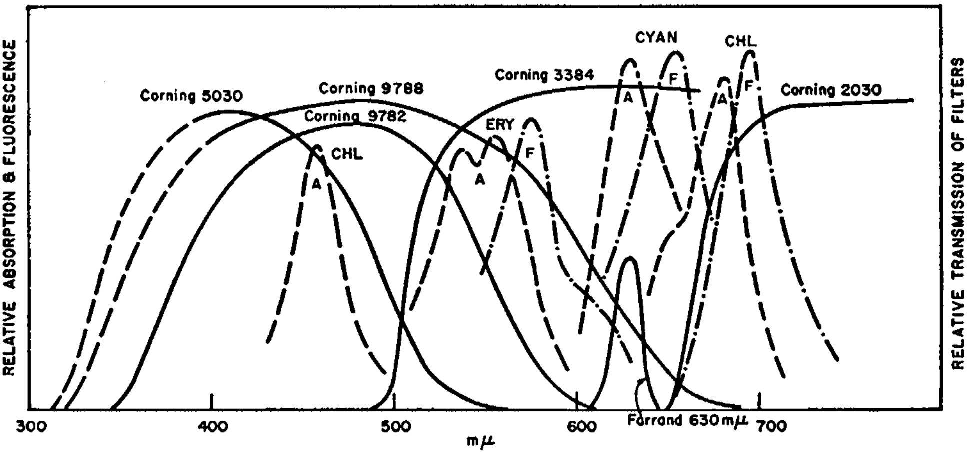

FIGURE 7 Absorption (A) and fluorescence (F) spectra of the pigments in *Porphyridium*, and transmission curves of optical filters.
Chl = Chlorophyll; Cyan = Phycocyanin; Ery = Phycoerythrin.
————————, filter transmission.
– – – – – –, absorption spectra.
–·–·–·–·–, fluorescence spectra.

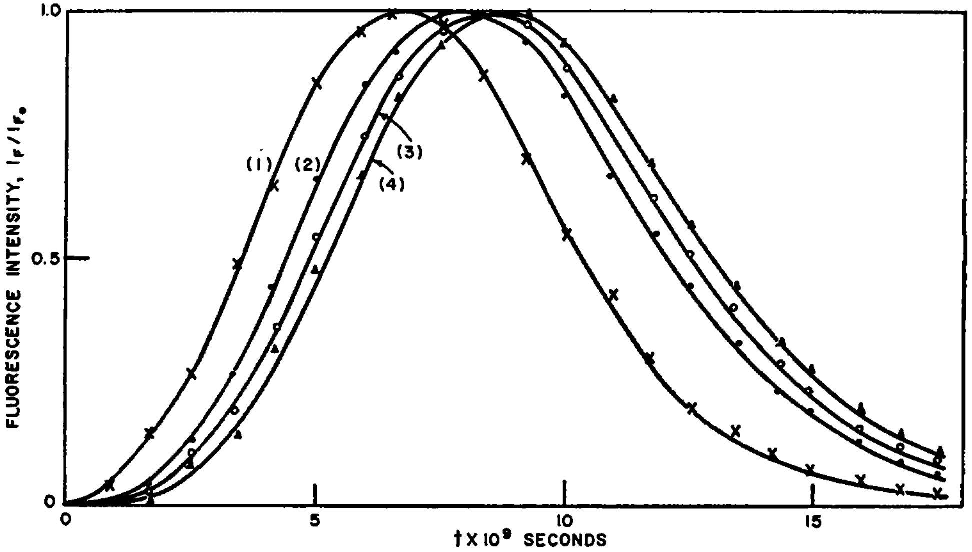

Figure 8 Time curves of direct and sensitized fluorescence of chlorophyll *a* in *Porphyridium*. (Experimental points and the closest theoretical curves.)
(1), Lamp flash; (2), direct fluorescence; (3), phycocyanin-sensitized fluorescence; (4), phycoerythrin-sensitized fluorescence.

and other known parameters, and the transfer time $\tau_{t_{II}}$ was determined. Fig. 2 shows a set of theoretical curves for $\tau_{III} = 1.5$ mμsec. The phycoerythrin-sensitized fluorescence was compared with a set of theoretical curves calculated by using the experimental values of τ_{III}, $\tau_{t_{II}}$, and other known parameters, and the transfer time τ_{t_I} was determined. Fig. 3 shows the theoretical curves calculated for $\tau_{III} = 1.5$ mμsec. and $\tau_{t_{II}} = 0.5$ mμsec. In the calculation of these theoretical curves, Brody's values (10) for the decay constants of phycocyanin (τ_{II}) and phycoerythrin (τ_I) were used. The phycocyanin-sensitized chlorophyll fluorescence in *Anacystis* was measured by the same experimental procedure as in the case of *Porphyridium*, and the transfer time from phycocyanin to chlorophyll *a* was determined. The transfer time from chlorophyll *b* to *a* in *Chlorella* also was estimated, from measurements of direct fluorescence (illumination filters: Farrand interference filter 410 mμ and Corning 9782; observation filter: 2030) and of chlorophyll *b*-sensitized fluorescence (illumination filters: Farrand interference filter 475 mμ and Corning 9782; observation filter: 2030).

The absorption bands of the several pigments present in plant cells overlap to some extent with each other, so that it is not possible to excite only one pigment, even by monochromatic illumination. In the case of *Chlorella* (16), the fractional absorptions of chlorophyll *a* and *b* at 475 mμ can be estimated (from the absorption spectra of extracted pigments and the absorption spectrum of a cell suspension) as "a few per cent" and "about 50 per cent," respectively; at 410 mμ they are "about 50 per cent" and "about 10 per cent." (The rest of the absorbed light goes to the carotenoids.) In *Porphyridium* and *Anacystis*, chlorophyll excitation through Corning filters 5030 and 9782 is accompanied with slight excitation of phycoerythrin, and phycocyanin excitation at 630 mμ is accompanied by some excitation of chlorophyll *a*. Moreover, the filter 2030, used in the measurement of chlorophyll fluorescence, transmits also a small fraction of the fluorescence of phycocyanin. For these reasons, the observed delay of the emission of sensitized fluorescence represents a weighted average of the delays caused by the several energy transfers involved. We did not attempt to take into account this complexity of the phenomenon; the corresponding corrections do not appear significant in consideration of the broad limits of error in our determination of the transfer times.

In equation (7), the term $\Pi_{K=I}^{K=J-I}\ \tau_K^*[F^{(K)}]$ represents the efficiency of energy transfer from pigment *I* to pigment *J*. Therefore, the transfer efficiency from phycoerythrin to phycocyanin (or from phycocyanin to chlorophyll *a*) is

$$\tau_I^*[F^{(I)}] = \frac{1}{\tau_{t_I}} \Big/ \left(\frac{1}{\tau_I} + \frac{1}{\tau_{t_I}}\right) \left(\text{or } \tau_{II}^*[F^{(II)}] = \frac{1}{\tau_{t_{II}}} \Big/ \left(\frac{1}{\tau_{II}} + \frac{1}{\tau_{t_{II}}}\right)\right).$$

According to Brody (10), the average lifetimes of excitation of phycoerythrin and phycocyanin in phosphate buffer are 7.1 mμsec. and 1.8 mμsec., respectively. Combining these data with the obtained transfer time, the efficiencies of energy transfer from phycoerythrin to phycocyanin and from phycocyanin to chlorophyll *a* in *Porphyridium* can be calculated from the above relations; the results are 96 per cent and 78 per cent, respectively. In the case of *Anacystis*, which contains more phycocyanin than *Porphyridium*, the transfer efficiency from phycocyanin to chlorophyll *a* was determined, in the same way, as 86 per cent. In *Chlorella*, the transfer efficiency from chlorophyll *b* to *a* is nearly 100 per cent. The results are in good agreement with Duysens' estimates (8). The observed transfer times and efficiencies are listed in Table II. In the case of *Porphyridium*, the transfer time from phycoerythrin to

TABLE II

TRANSFER TIMES AND EFFICIENCIES

	Transfer time $\tau_t \pm 0.2$	Transfer efficiency*
	mμsec.	*per cent*
in *Porphyridium:*		
Phycoerythrin → Phycocyanin	0.3	96 ± 3 (95)
Phycocyanin → Chlorophyll *a*	0.5	78 ± 8 (80)
in *Anacystis:*		
Phycocyanin → Chlorophyll *a*	0.3	86 ± 8
in *Chlorella:*		
Chlorophyll *b* → Chlorophyll *a*	~0.0	~100 (100)

* Figures in parentheses are from Duysens (8).

chlorophyll *a* was determined as about 0.5 mμsec. by Brody (10) from the horizontal distance between the rise curves of direct and sensitized fluorescence of chlorophyll *a*. However, as indicated by equations (8), (9), and (10), the horizontal distance between them does not correspond directly to the transfer time.

As suggested by M. Brody *et al.* (17), the transfer efficiency, even for a given type of pigment and a given species of cells, can vary with physiological conditions, such as relative and absolute concentration of the two pigments, pre-exposure of cells to light of various wavelengths and intensity, etc. The results given in Table II are therefore strictly applicable only to the particular cells used.

Reabsorption of primary fluorescence can contribute to sensitized fluorescence, especially when the intrinsic fluorescence yield of the sensitizing pigment is high. According to Brody and Rabinowitch (11), the estimated efficiency of energy transfer from phycoerythrin to phycocyanin by this mechanism *in vivo* is <40 per cent; for the transfer from phycocyanin to chlorophyll, it is <25 per cent. Since these efficiencies are much smaller than the actually observed ones which reach 80 to 90 per cent it appears that reabsorption of primary fluorescence is, at best, only a minor contributing mechanism of sensitization. Furthermore, as Brody and Rabinowitch (11) pointed out, the lifetime of excitation of phycoerythrin, phycocyanin, and chlorophyll *b in vitro* are 7.1, 1.8, and 4.0 mμsec., respectively; delays involved in the reabsorption of primary and emission of secondary fluorescence should therefore reach several millimicroseconds. This is much longer than the actually observed delays, which must therefore correspond to the times needed for resonance transfer of excitation energy.

NOTE

CALCULATION OF THE CONVOLUTION OF THE FIRST KIND

When functions $f_1(t)$ and $f_2(t)$ are as shown in Fig. 9a and the combined function $F(t)$ is given by the following equation,

$$F(t) = \int_0^t f_1(t)f_2(t - \xi)\, d\xi \left(= \int_0^t f_1(t - \xi)f_2(t)\, d\xi \right),$$

$F(t)$ can be calculated. The mirror image function, $f_1^*(t)$ of $f_1(t)$ is plotted in Fig. 9*a*. When the curve of $f_1^*(t)$ is moved by t to the right along the time axis, this curve is given as $f_1(t-\xi)$, as shown in Fig. 9*b*. Then, the curve, $f_1(t-\xi)f_2(t)$ can be obtained by calculating the products of $f_1(t-\xi)$ and $f_2(t)$ at the same ξ values. If this procedure is carried out at various values of t, a set of curves is obtained as shown in Fig. 9*c*. The areas between these curves and the time axis give the values of $F(t)$ at each t.

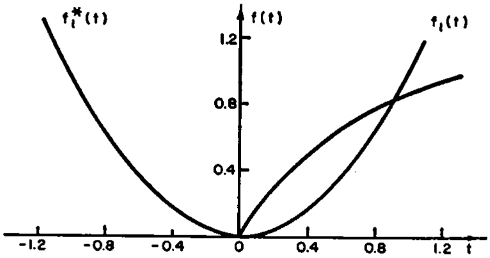

FIGURE 9a

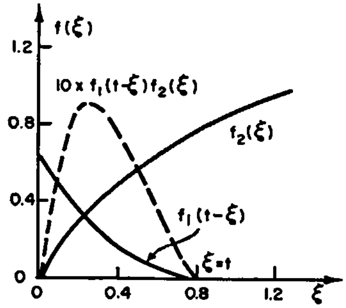

FIGURE 9b

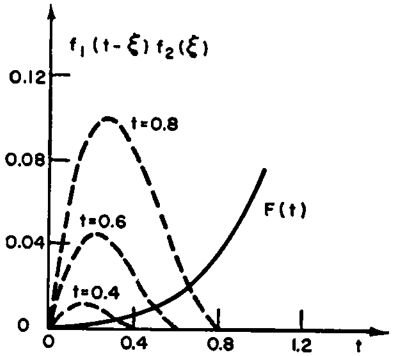

FIGURE 9c

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