pure ether and 1:1 for the pure alcohol, thus can be explained.

The comparison of the present data with the average values reported by Gutowsky is generally good. The deviations that exist are probably due largely to medium effects since Gutowsky examined mainly pure organic liquids while our data are—for two of the compounds—for infinitely dilute solutions in the "spherical" solvent[8] CCl_4.

Acknowledgments.—It is a pleasure to thank Professor J. S. Waugh and Mr. Richard Fessenden for their coöperation and advice. This work was supported in part by the U. S. Atomic Energy Commission.

CAMBRIDGE 39, MASS.

[8] A. A. Bothner-By and R. E. Glick, THIS JOURNAL, **78**, 1071 (1956).

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENTS OF SYRACUSE UNIVERSITY AND BROOKHAVEN NATIONAL LABORATORY]

The Absorption Spectra and Decay Kinetics of the Metastable States of Chlorophyll A and B[1]

BY HENRY LINSCHITZ[2] AND KYOSTI SARKANEN

RECEIVED APRIL 7, 1958

Using flash-illumination apparatus with improved time-resolution, the absorption spectra and decay kinetics of the metastable states of chlorophyll A and B have been studied in pyridine and benzene solution at 25°. The spectra are peculiarly diffuse and a measurable absorption extends beyond 7500 Å. The decay kinetics obey the following rate law: $-dC^*/dt = k_1C^* + k_2(C^*)^2 + k_3C^*C_g$ (C^* = concentration of the metastable, C_g = ground state). Values of the k's are presented and the results are discussed. The fast k_2 process does not appear to be due simply to effects of paramagnetic molecules on the triplet–singlet conversion.

Introduction

The formation of a long-lived excited state of chlorophyll and its function as the energy-storing and catalytic agent in photosensitization have been postulated for some time, on the basis of much indirect evidence. This has included data on the kinetics of sensitization, fluorescence quenching and steady-state bleaching.[3,4] Recently, direct demonstrations of this metastable state (probably the triplet) have been given by flash-excitation techniques, and preliminary estimates of its absorption spectrum and lifetime have been obtained.[5–10] It is desirable to extend the spectral data, both in wave length coverage and precision, particularly in view of recent ideas on the mechanism of energy accumulation and transfer in photosynthesis.[11,12] Moreover, the study of the kinetics of conversion of the triplet to the ground state bears directly on the general and important problem of the nature of radiationless transitions in complex molecules.

Existing kinetic data on the deactivation reactions of the metastable state are very rough. This uncertainty is due mainly to the relatively long duration of the exciting flashes which have been used so far, comparable in fluid solvents to the lifetime of the metastable state itself. The overlapping of excitation and deactivation not only greatly complicates the kinetic analysis, but with oscillographic recording of absorption changes, necessitates large scattered-light corrections over most of the decay curve, with consequent further loss of accuracy.

In this paper, we present new data on the metastable states of chlorophyll A and B, taken with apparatus providing an exciting flash of much shorter duration than those previously used. Absorption spectra have been measured over the range 3500–7500 Å. The kinetic data establish that only one metastable species of chlorophyll is present in pyridine or benzene solution, and permit the evaluation or estimation of three rate constants involved in the transition to the ground state. The improved time resolution also makes feasible the direct study of bimolecular reactions between the metastable state and added quenchers.

Experimental

1. **Apparatus.**[13]—Changes in absorption of a steady light beam, passed through the sample, were measured by a photocell and oscilloscope as function of time after excitation, the flash being triggered at a suitable interval after starting the sweep. The apparatus is shown diagrammatically in Fig. 1.

Mullard "Arditron" lamps, Type LSD-2, were used as the flash source.[14] These are short, wide tubes, with a straight 40 mm. spark gap, filled to 1 atm. with argon containing a small addition of hydrogen and, when fresh, have a breakdown potential near 11,000 volts. This permits the use of high-voltage low-capacity storage condensers to minimize flash duration and obviates the need for auxiliary spark gaps. Triggering of these high-pressure lamps is facilitated by using copper electrodes, activated by impregnation with

---

[1] This work was assisted by a grant from the U. S. Atomic Energy Commission to Syracuse University (Contract No. AT-(30-1)-820), and was carried out in part while H. L. was on sabbatical leave at Brookhaven National Laboratory.

[2] Department of Chemistry, Brandeis University, Waltham, Mass.

[3] E. Rabinowitch, "Photosynthesis," Interscience Publishers, Inc., New York, N. Y., 1956, Vol. III, Chap. 35, and earlier volumes.

[4] J. Franck, *Ann. Rev. Plant Physiol.*, **2**, 53 (1951).

[5] R. Livingston and V. A. Ryan, THIS JOURNAL, **75**, 2176 (1953).

[6] R. Livingston, G. Porter and M. Windsor, *Nature*, **173**, 485 (1954).

[7] E. W. Abrahamson and H. Linschitz, *J. Chem. Phys.*, **23**, 2198 (1955).

[8] R. Livingston, THIS JOURNAL, **77**, 2179 (1955).

[9] H. Linschitz and E. W. Abrahamson, "Research in Photosynthesis," Interscience Publishers, Inc., New York, 1957, p. 31.

[10] R. Livingston, ref. 9, p. 3.

[11] J. Franck, ref. 9, p. 142.

[12] F. L. Allen and J. Franck, *Arch. Biochem. and Biophys.*, **58**, 124 (1955).

[13] This apparatus was developed and built at the Brookhaven National Laboratory, in collaboration with Mr. Lloyd Nevala and Mr. Fred Merritt, of the Instrumentation Section. It is a pleasure to acknowledge their indispensable assistance, as well as advice from Mr. W. Higinbotham.

[14] J. W. Mitchell, *Trans. Illum. Eng. Soc.* (*London*), **14**, 91 (1949).

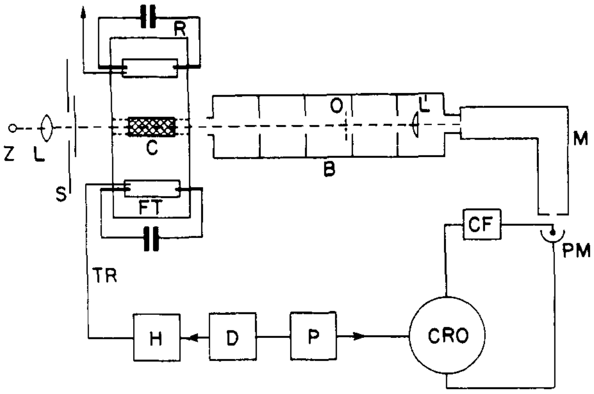

Fig. 1.—Diagram of flash-illumination apparatus: Z, d.c. zirconium arc; L, collimating lens; S, shutter; C, cell containing test solution; FT, flash tube; R, elliptical reflector; B, baffle box; O, mounting for filter or calibrated screen; L', condensing lens; M, monochromator; PM, photomultiplier tube; CF, cathode follower; CRO, oscillograph; P, timing-pulse generator; D, delay; H, high-voltage firing-pulse generator; Tr, trigger lead.

potassium. In our apparatus, the storage capacity (per flash) was further decreased by distributing the necessary energy among four Arditrons, which were mounted, respectively, at the outer foci of a four-leaved elliptical reflector. The cylindrical absorption cell, containing the solution to be irradiated, was supported at the common central focus of the four-leaved ellipse, with its axis parallel to those of the surrounding flash-tubes. Each lamp was powered by its own low-inductance, 0.5 μf. condenser, isolated from the others by resistors, and operated at 9,000–10,000 volts from a common high-voltage supply. The reflector unit was fixed in a rigid metal frame between the storage capacitors, which were lamp terminals with short strap leads. The lamps were mounted in the reflector on bases which were adjustable for optimum focus.

A powerful firing pulse was found necessary to ensure simultaneous flashing of all four lamps. This was generated by discharging a 0.01 μf. capacitor, charged to 9000 v., through a hydrogen thyratron and the primary of a special pulse transformer. The hydrogen thyratron was itself fired by an initiating trigger pulse from the timing circuit. The resulting output firing pulse (greater than 30 kilovolts, with rise time less than 0.1 μsec.) was then distributed to each of the four lamp trigger electrodes through 100 $\mu\mu$f. high-voltage condensers. At 9000 volts or higher, using fresh lamps, firing of all four lamps occurred with a time spread less than 0.2 μsec. The output trigger circuitry was mounted in the frame directly above the reflector assembly, while the timing pulses were provided by a Grass "Stimulator" (Model S4B).

The measuring light source was a 100-watt zirconium arc (Sylvania, Type T-100), operated from a d.c. generator. Its light was collimated by a short-focus achromatic lens and passed through a shutter, the absorption cell, through a cylindrical lens and into a Bausch and Lomb 250 mm. grating monochromator. To increase the ratio of collimated measuring light to scattered light from the flash, the distance between the absorption cell and monochromator condensing lens was made large (70 cm.) and a series of baffles were placed in this interval. Calibrated screens and filters could also be inserted in this portion of the optical path. Light from the monochromator exit slit was measured by an end-window photomultiplier tube (RCA, Type 6217), whose output was d.c. coupled through a cathode-follower to a wide-band oscilloscope (Tektronix, Type 531). With the cathode follower, photo-cell load resistors up to 10 Kohms could be used without serious distortion of the flash profile (2 μsec. decay time). In the kinetic measurements, involving much longer decay times, load resistors of 25 or 50 Kohms were used. The photomultiplier power supply was designed to render negligible any shifts in dynode bias during the measurement.

Absorption cells were made of Pyrex tubing, 50 mm. optical length and 13 mm. i.d. A narrow side-arm, placed at one end of the cell, led to an ampoule in which the degassed dye solution was prepared and sealed off from the vacuum line. For work at high concentrations, smaller cells were used.

2. **Profile of Exciting Flash.**—At 10,000 volts and 0.5 μf., with low-inductance circuitry, the Arditron lamp provides a flash of about 2 μsec. duration, measured to the half-decay point, in the blue region of the spectrum (Fig. 2A). Somewhat longer durations are obtained at longer wave lengths (2B). The profile also shows lesser peaks, corresponding to the oscillatory discharge. For comparison, we note that in their original work on this subject,[5] Livingston and Ryan used 200 μsec. flashes (measured to half-decay point) while Porter and Windsor's equipment for studying triplet states of aromatic hydrocarbons[15] gave 35 μsec. flashes at discharge energies comparable to those used here. However, for *precise* kinetic work, the minimum resolution time is *much* longer than any of these figures indicate, since rare-gas discharge lamps all show a very long tail, particularly at longer wave lengths (Fig. 2C). While the use of dissociable gases in the filling renders this tail relatively weak, the absolute intensity is still such that appreciable light is emitted at times as long as 20-fold half-decay time, and the integrated light output for the long tail may be a considerable fraction of that in the main peak.

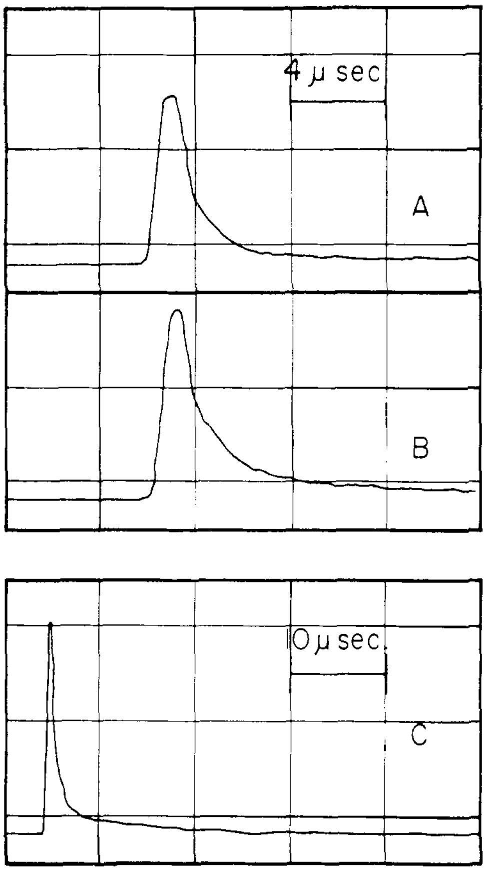

Fig. 2.—Light-flash profiles at various wave lengths: A and B, 4μsec. per grid; C, 10μsec. per grid; A, 400 mμ; B, 650 mμ; C, 650 mμ.

3. **Procedure.**—The measuring procedure was generally similar to that described previously.[7,9] Sweeps (and flashes) were photographed with the shutter in the measuring beam first open, then closed. Since scattered or fluorescent light from the flash is the same for the two sweeps, the "closed shutter" trace, including the flash profile, represents the base line (zero transmission) for the measurement, and changes in the vertical separation of the two sweeps measure changes in transmission. For times longer than about 20 microseconds after flash initiation, no scattered light cor-

---

(15) G. Porter and M. Windsor, *Faraday Soc. Disc.*, **17**, 178 (1954).

rection was necessary at all, except in the very far red, and the base-line could be taken to be entirely level.

Calibration of the oscilloscope deflection in terms of per cent. transmission was initially done by recording blank (no flash) sweeps, in which a solvent-filled cell and a calibrated neutral filter were substituted for the test solution. Since closely spaced neutral filters may be used for calibration, this procedure allows small changes in transmission to be spread over the full oscillograph scale, by suitable adjustment of slit width and gain and permits measurement at high optical densities. Transmissions obtained in this way generally agreed well with those given by conventional spectrophotometers. However, small differences in placement of absorption cells, window quality, etc., led occasionally to considerable errors, due to the long optical path between absorption cell and detector. Further small errors were caused by variation in intensity of the measuring source and in sweep position during the time required to change cells and filters. In establishing the spectra of the metastable state, more consistent results were obtained when the oscilloscope scale readings on a single sweep, immediately before and after the flash, were used to determine only the relative changes in transmission. Let R_i and R_t be the oscillograph deflections measuring transmitted light intensity before the flash and at time t after the flash, respectively. Thus, the change in absorbance is $\Delta D = \log (R_i/R_t)$. Adding this ΔD to the original absorbance of the sample, as measured independently (Cary, Model 11) then gave the absorbance of the excited solution at time t after the flash. The scale reading corresponding to initial excitation (representing maximum conversion) was found by a short extrapolation of the deflected trace, back to the instant of triggering the flash. To facilitate this, measurements intended to establish the metastable spectra were made with relatively fast sweeps, to flatten the decay curve. This technique permits good precision in measuring ΔD's, up to about 0.8.

The spectra of the excited solutions immediately after the flash were corrected to 100% conversion by assuming that the new absorption had no peak in the region of the original red band. A straight line was drawn between experimental points near or at the isosbestic points lying on either side of the original peak and the residual red absorption attributed to unchanged chlorophyll. This fractional conversion was then used to correct the observed spectrum. To establish the metastable spectrum in the blue and green regions, dilute chlorophyll solutions ($\sim 10^{-6}$ M) were used, for which the initial conversion was over 90%. The correction was thus small, and the calculation converged immediately. The spectra in the red were obtained at somewhat higher concentrations. Within the precision of these measurements, the metastable spectrum obeys Beer's law.

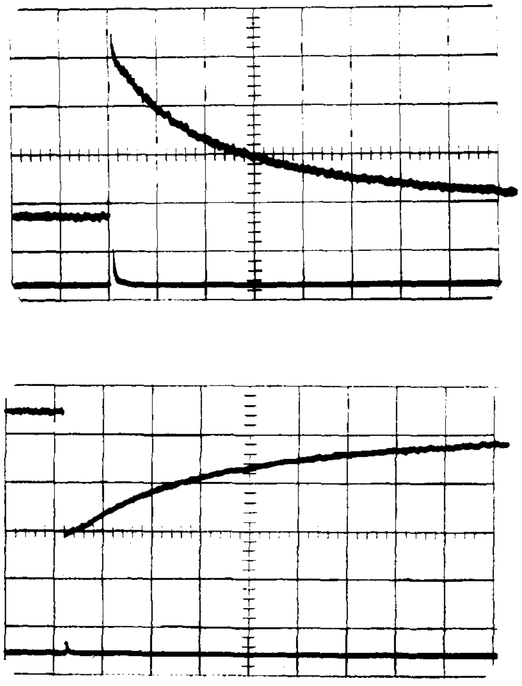

Fig. 3.—Typical oscillograms: total sweep time, 10^{-3} sec.; slit width, 2 mμ. Upper picture, Shot No. 26-11, chlorophyll A in pyridine; 1.73×10^{-6} M; wave length, 442 mμ. Lower picture, Shot No. 35-3, chlorophyll B in pyridine; 3.25×10^{-6} M; wave length, 513 mμ. In each picture, upper trace is with shutter open, lower trace with shutter closed (base-line). Sharp break marks instant of occurrences of flash.

Sample concentrations and gain were adjusted in the final runs on the basis of preliminary measurements to give maximum on-scale deflections at each wave length and at reasonable monochromator slit widths. Linearity of the photometric system and time base was checked frequently, using calibrated screens and a precision time-mark generator.

For measurement, enlarged images of the oscillograms were projected onto graph paper and traced. A smooth curve, averaging out noise, then was drawn through each sweep profile, and deflections determined from this.

Solution temperatures were maintained at 25 ± 1°, by equilibrating the absorption cell in a thermostat immediately before each shot. Heating effects due to the flash were slight.

To give a general idea of apparatus performance, typical oscillograms are shown in Fig. 3. At 480 mμ, for example, full-scale deflections could be obtained covering the transmission range 0-5%, with a monochromator slit width of 40 Å. and a signal-to-noise ratio of about 20. At higher transmissions, more favorable conditions were of course possible. For the points at longest wave length (7500 Å.) slit widths of 100 Å. were required.

In degassed pyridine solution, the photo-bleaching reaction was found to be highly reversible, less than 5% decomposition (judged from absorption spectrum) resulting after 100 flashes. In degassed benzene, the bleaching reaction is somewhat less reversible.

4. **Materials.**—Thiophene-free benzene (Mallinckrodt) was shaken with H_2SO_4, water, dried with Na_2SO_4 and distilled from CaH_2. General Chemical reagent-grade pyridine was allowed to stand over BaO and distilled from fresh BaO. These solvents were degassed on the vacuum line by cycles of freezing, pumping and thawing, and then several trap-to-trap distillations over CaH_2 and BaO. Chlorophyll A and B were prepared from fresh spinach by the method of Jacobs, *et al.*,[16] and stock solution in purified toluene stored at −20°. Toluene was used rather than ether to minimize peroxide contamination. To prepare a test sample, a suitable volume of stock solution was placed in the ampoule carrying the absorption cell, the stock solvent taken off on the vacuum line, freshly degassed benzene or pyridine distilled in, and the preparation sealed off. The lifetimes (k_1 below) were found to be highly sensitive to the quality of the vacuum (oxygen quenching) and only solutions sealed off under "sticking-vacuum" were used. Final concentrations were measured spectrophotometrically, using molar extinction co-efficients determined in separate experiments.

Results and Discussion

1. **Spectra of Metastable Forms.**—The absorption spectra of the metastable states of chlorophyll A and B are shown in Figs. 4 and 5, and extinction coefficients are given in Table I. Based on the reproducibility of our results, the extinction coefficients probably are accurate to 5% or better in the green region of the spectrum. Under the Soret band and in the orange and red, accuracy is somewhat less. The spectra of A and B are closely similar, the main peaks (at 462 and 485 ± 5 mμ, respectively) lying just at the long-wave side of the corresponding Soret bands. In the green, both spectra show broad plateaus, tailing off into gradually weakening absorption which extends into the

---

[16] E. E. Jacobs, A. E. Vatter and A. S. Holt, *Arch. Biochem. and Biophys.*, **53**, 228 (1954).

red at least as far as these measurements can follow. The most remarkable feature of these spectra is their extremely diffuse character. In view of the sharpness of the original bands, this can hardly be due to vibrational structure, and presumably several overlapping electronic bands are involved.

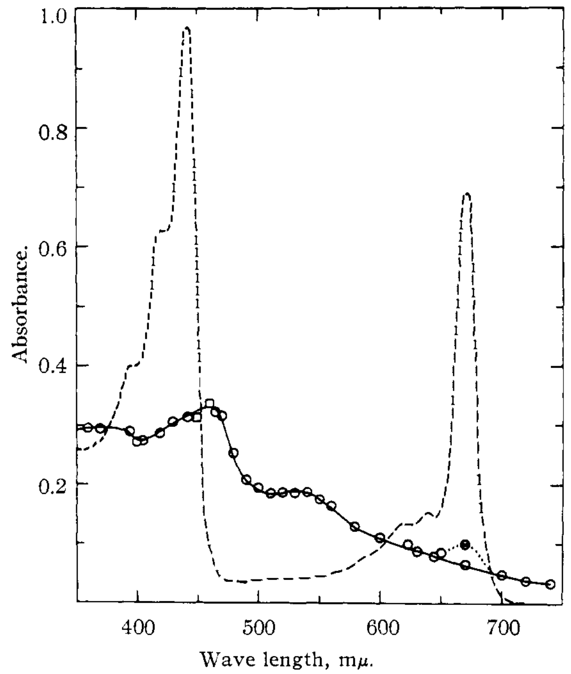

Fig. 4.—Absorption spectra of chlorophyll A and its metastable state, in pyridine; 2.1 × 10^{-6} *M;* cell length, 5 cm.: O, corrected spectrum, using relative calibration of oscillograph deflection; □, corrected spectrum, using absolute (neutral screen) calibration; ⊗, original uncorrected absorbance, immediately after flash.

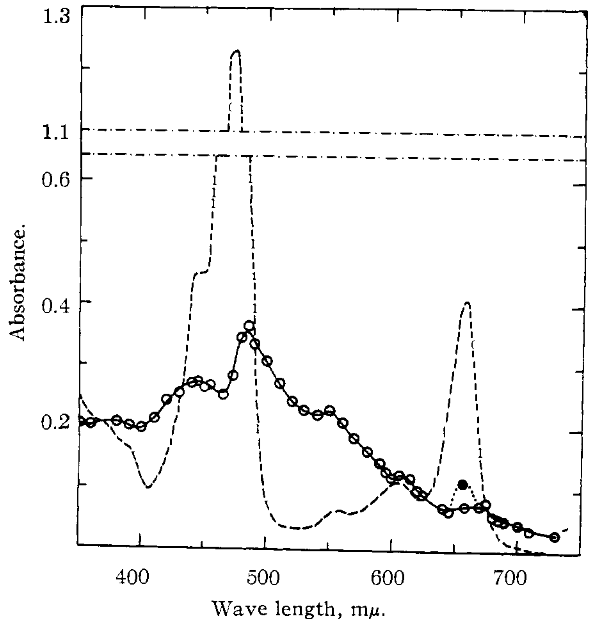

Fig. 5.—Absorption spectra of chlorophyll B and its metastable state, in pyridine; 2.1 × 10^{-6} *M;* cell length, 5 cm.: O, spectrum corrected to 100% conversion; ⊗, original uncorrected absorbance, immediately after flash.

TABLE I

SPECTRA OF METASTABLE CHLOROPHYLL A AND B, IN PYRIDINE

Molar extinction coefficients: $\alpha = (1/cl)\ \log_{10}\ (I_0/I)$

Chlorophyll A			Chlorophyll B		
λ, mμ		$\alpha \times 10^{-4}$	λ, mμ		$\alpha \times 10^{-4}$
400	min.	2.57	380	max.	1.97
462	max.	3.20	400	min.	1.89
505	min.	1.74	445	max.	2.60
530	max.	1.78	465	min.	2.41
			485	max.	3.47
			550	max.	2.15
			605	max.	1.21

In the "B" spectrum, the indications of a small peak at 605 mμ are outside our experimental error. However, the suggested peak near 675 mμ is somewhat more suspect, since the shape of the spectrum in this region is sensitive to the assumptions made in correcting to 100% conversion. There is no question whatever of the enhanced absorption in the far red.

These new data permit a review of earlier suggestions[17,12] that certain general relationships exist between the spectra of the metastable forms and the phase-test intermediates ("enolate ions") of chlorophyll. The phase test products[17] show diffuse absorption in the same regions found here for the metastable molecules, and it is striking that the enolate ion of chlorophyll-B has a very broad and weak absorption extending from the main peak in the green to about 7800 Å.[18] At low temperature, this sharpens somewhat to a broad flat band at 7400 Å.[18] While these similarities are marked, differences also appear. The far red absorption band of the chlorophyll-A phase-test product is much sharper than the long, flat tail of the corresponding metastable form, and the relative intensities of the blue and green peaks of the two intermediate forms do not agree.

2. Decay Kinetics; Evaluation of Rate Constants.—Typical examples of the extensive kinetic data taken in this work are given in Table II. Trace deflections above the base line, as measured on the projected oscillograms, are listed for various concentrations, wave lengths and times t, after an arbitrary zero time t_0. This was taken at 50–150-μ sec. after the flash to avoid any possible tailing effects and scattered light corrections. For use in calculation, Table II also gives values of a_λ, defined below. The data are conveniently handled in terms of a plot of log $\Delta D_0/\Delta D$ *vs.* t, in which ΔD_0 and ΔD are the changes in absorbance at times t_0 and t, respectively, relative to the absorbance of the solution before flashing. Figure 6 shows such typical curves for chlorophyll B in benzene.

Since ΔD is proportional to the concentration of metastable molecules, it is evident from Fig. 6 that the kinetics of the recovery reaction deviate markedly from first order. Experiments in which flash intensity and total dye concentration were varied indicated that higher-order terms involving the *excited* state were mainly responsible for this behavior. The data therefore were fitted to a rate expression as

---

(17) A. Weller, THIS JOURNAL, **76**, 5819 (1954).

(18) H. Linschitz, in preparation.

TABLE II

FLASH BLEACHING OF CHLOROPHYLL A AND B. TYPICAL EXPERIMENTAL DATA

	Chlorophyll A in pyridine				Chlorophyll B in benzene			
Run no.	16-13	26-11	26-8	31-17	30-17	31-3	30-16	29-11
λ (mμ)	475	442	671	480	458	647	500	500
Concn., $M \times 10^6$	3.00	1.73	1.73	110	2.16	2.16	2.16	11
t_0, μsec.	100	100	100	100	150	100	100	100
Time, μsec.	Deflection from zero transmission line, R[a]							
Before flash	9.01	2.83	3.82	9.77	2.82	5.74	9.63	10.26
0	5.19	7.27	9.26	6.69	11.08	10.82	6.44	3.19
25	..	6.86	8.75	7.33	9.33	10.30	6.65	..
50	5.69	6.50	8.36	7.79	8.12	9.86	6.84	4.82
75	..	6.22	..	8.11	7.23	9.49	7.01	..
100	6.07	5.98	7.70	8.33	6.60	9.16	7.17	5.93
150	6.39	5.58	7.20	8.67	6.08	8.65	7.43	6.67
200	6.64	5.24	6.79	8.91	5.69	8.27	7.64	7.23
250	..	4.96	6.46	9.07	5.08	7.97	7.81	7.67
300	7.05	4.73	6.20	9.19	4.68	7.72	7.96	8.00
400	7.36	4.39	5.75		4.40	7.36	8.20	8.48
500	7.60	4.13	5.40		4.14	7.08	8.39	8.82
600	7.80	3.93	5.11			6.87	8.52	9.07
700	7.96		4.92			6.70	8.64	9.26
800	..	..	..	..		6.59	..	..
$a_\lambda \times 10^6$[b]	8.51	3.21	3.35	9.72	1.92	5.28	7.78	7.78

[a] All measurements in 5 cm. cells, except 31-17 (1.5 cm.). [b] For 5 cm. cell.

Let C_0 = total chlorophyll concn.
C^* = concn. of metastable chlorophyll
C_g = concn. of unexcited chlorophyll
α^*, α_g = molar extinction coefficients = $\frac{1}{Cl}\log_{10} I_0/I$
l = length of absorption cell

We assume

$$-\frac{dC^*}{dt} = k_1C^* + k_2(C^*)^2 + k_3C^*C_g \qquad (1)$$

But

$$C_g = C_0 - C^*$$

and

$$C^* = a_\lambda \Delta D$$

where

$$a_\lambda = \frac{1}{(\alpha^* - \alpha_g)l}$$

The rate expression then is

$$-\frac{d(\Delta D)}{dt} = (k_1 + k_3C_0)\Delta D + a_\lambda(k_2 - k_3)(\Delta D)^2 \qquad (2)$$

Letting

$$A = k_1 + k_3C_0$$

and

$$B_\lambda = a_\lambda(k_2 - k_3)$$

the integrated rate law is then

$$\ln\frac{\Delta D_0(A + B_\lambda\Delta D)}{\Delta D(A + B_\lambda\Delta D_0)} = At \qquad (3)$$

A and *B* may be separated by first evaluating *A* from the asymptotic first-order slope, as ΔD goes to zero. However, the second-order term is relatively large, and very long cells must be used to obtain sufficiently accurate data. For uniform illumination under these conditions lamp size must be increased, with accompanying increase in flash duration and loss of time resolution. Accordingly we write equation 2 as

$$\frac{d}{dt}\ln(\Delta D_0/\Delta D) = A + B_\lambda\,\Delta D \qquad (4)$$

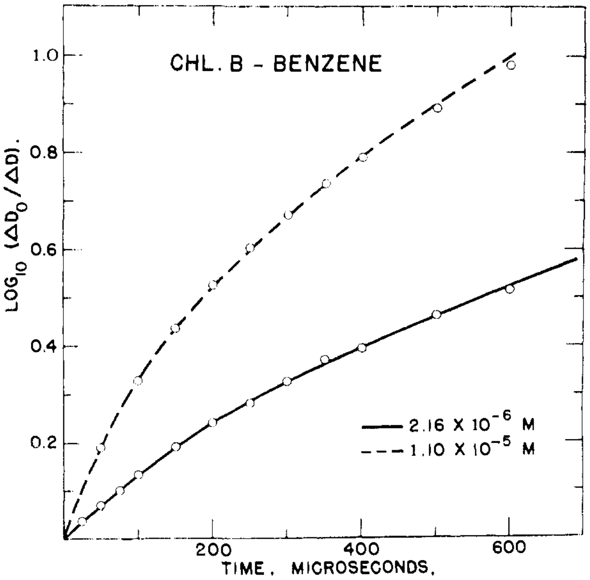

Fig. 6.—Plot of log $(\Delta D_0/\Delta D)$ *vs.* time for typical decay: chlorophyll B in benzene: Shots No. 30-16 and 29-11; λ 500 mμ.

The time derivative is obtained directly by drawing tangents to the plot of log $(\Delta D_0/\Delta D)$ *vs.* *t* (Fig. 6). These rates are then plotted against the corresponding values of ΔD to obtain A and B_λ. For any given sample, measurements at various wave lengths should be represented by a family of straight lines having the same intercept *A*, at $\Delta D = 0$, but with different slopes corresponding to the wave length dependence of a_λ. Conversely, measurements at a single wave length but with increasing C_0 should yield a family of lines of constant slope but increasing intercept. The variation of *A* with C_0 thus gives k_1 and k_3. Then, since the spectral data (corrected to 100% conversion) give a_λ at the wave length of the measurement, and B_λ at the same wave length is known, k_2 can be calculated.

Representative results of such treatment are shown in Figs. 7 and 8, in which eq. 4 is applied to data on chlorophyll A and B in pyridine and benzene respectively. Among others, runs given in Table II and Fig. 6 are included in Figs. 7 and 8. In agreement with the proposed kinetic scheme, satisfactorily converging straight lines are obtained for measurements at various wave lengths and constant concentrations. This has been verified throughout the entire visible spectrum and constitutes very strong evidence that, at least in pyridine and benzene, only a single long-lived intermediate is formed by light excitation.

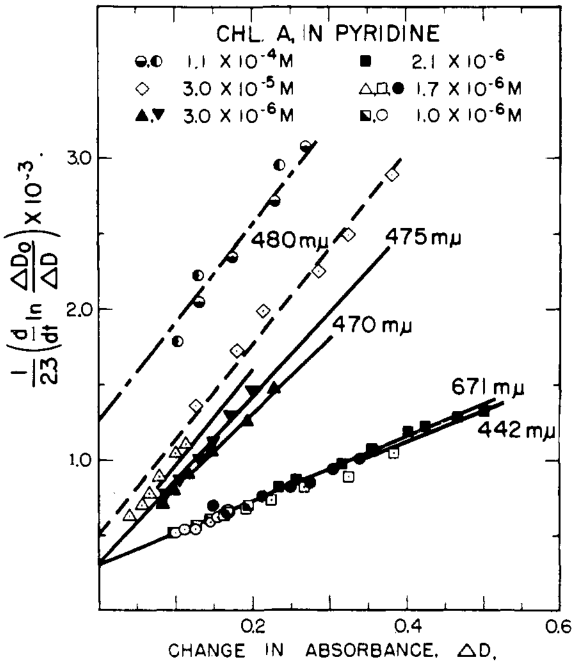

Fig. 7.—Decay kinetics of chlorophyll A in pyridine; application of eq. 4.

Runs at constant wave length give generally parallel lines, displaced to larger intercepts with increasing concentration (Figs. 7, 8) again in agreement with eq. 4. The consistency of the data with the kinetic scheme can be checked further by comparing k_2's obtained from measurements at different wave lengths. Satisfactory agreement is found. The rate constants are summarized in Table III.

TABLE III

SUMMARY OF RATE CONSTANTS

	Chlorophyll A				Chlorophyll B			
	Pyridine		Benzene		Pyridine		Benzene	
k_3, l. mole^{-1} sec.$^{-1}$	2×10^7		5×10^7		2×10^7		9×10^7	
k_1, sec.$^{-1}$	670		440		310		330	
	$k_2 \times 10^{-9}$, l. mole^{-1} sec.$^{-1}$							
	λ	k_2	λ	k_2	λ	k_2	λ	k_2
	442	1.51	433	1.98	473	1.61	458	2.23
	671	1.48	665	1.95	657	1.54	647	2.09
	470	1.54	470	2.33	480	1.53	500	2.26
					510	1.64		
Av. k_2, l. mole^{-1} sec.$^{-1}$	1.51		2.07		1.58		2.19	

To obtain appreciable shifts in intercept requires such "high" concentrations that, with minimal flash energies, one is soon limited by incomplete conversion and resulting concentration gradients. Since only small changes in A with C_0 can be observed, and k_2 is much larger than k_3, the error in k_3 is large, the indicated values being little better than order-of-magnitude estimates. However, we believe that the increase in k_3 in going from pyridine to benzene is real. In obtaining k_1, most reliance for the extrapolation was placed on the line of smallest slope (Figs. 7, 8). The convergence of the other lines is good, however, and we estimate that the error in the k_1's is probably not worse than ± 20%. In the case of chlorophyll B in benzene, a rather large correction for the k_3C_0 term is involved, and the error in k_1 may be somewhat greater. The effects of strongly quenching impurities, most seriously oxygen, will be to increase the observed k_1, so that the values given may, if anything, be too high. The various values of k_2 check well for each solution, inasmuch as they include also errors in measurement of a_λ for the different wave lengths. The relative values of the k_2's are probably reliable to ±10%, and the absolute error should not be much larger.

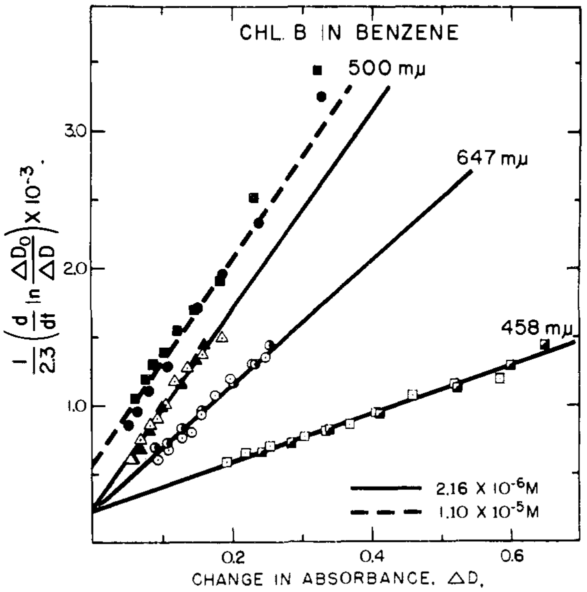

Fig. 8.—Decay kinetics of chlorophyll B in benzene; application of eq. 4.

Previous measurements on triplet-state decay in fluid solvents have been of too low precision to establish clearly the higher-order terms in the rate law.[5-10,15] These terms are not unique to chlorophyll.[19] Similar kinetics, in particular similar values for k_2, are found in the decay of triplet states of anthracene and porphyrins.[20]

The rate constant k_1 of course measures, in principle, the sum of radiative and radiationless transition rates. However, even in rigid media at low temperature, the phosphorescence of chlorophyll B is weak and that of chlorophyll A is doubtful.[21] It is thus most likely that in fluid pyridine

---

(19) Private communications from Profs. G. Porter and R. Livingston indicate that recent independent measurements in their respective laboratories give results in general agreement with those presented here.

(20) H. Linschitz and L. Pekkarinen, in preparation.

(21) R. S. Becker and M. Kasha, THIS JOURNAL, **77**, 3669 (1955).

or benzene, the triplet–singlet conversion is essentially radiationless, and we assume that k_1 refers to this latter process.

Making rough estimates of the collision frequency in solution, k_2 corresponds to a quenching efficiency per collision of about 0.1, an excited molecule being much more effective than an unexcited one in inducing transitions to the ground state. The slight increase in k_2 in going from pyridine to benzene can be most simply explained by the slightly lower viscosity of benzene. Kinetically, one cannot distinguish between mutual quenching of both metastable molecules in collision or the quenching of only one. If the excited states are triplets, mutual quenching would appear reasonable on the basis of the possibility of over-all spin-conservation in the triplet–singlet reaction. Alternatively, the high quenching efficiency of a collision with a triplet compared to that with a singlet might be attributed to the paramagnetism of the quencher. To check this point, lifetime measurements have been made on metastable chlorophyll and anthracene in solutions containing paramagnetic metal ions.[22] While strong quenching is found for some ions (Ni^{++}, Co^{++}, Cu^{++}), others show very much weaker interactions (Mn^{++}, Nd^{+++}). In addition, measurements on quenching rate constants by Fujimori and Livingston[23] show that various non-paramagnetic substances interact with the triplet as strongly as does oxygen. It is evident that other factors than paramagnetism must be invoked to explain these results, and it is likely that different mechanisms will apply to different specific situations. In the particular case of quenching by excited states, one may suggest, for example, in addition to the mechanisms already cited, a reversible dismutation reaction leading back to the ground state, or a collision of the second kind, in which one excited molecule passes to the ground state while the other is raised to a still higher excited state.

Under certain conditions, the large value of k_2 may have important consequences, apart from its theoretical interest. For example, in radiation chemistry, high yields of triplet states are expected, due either to direct excitation or to recombination of ions and electrons.[24] At high excitation densities, along the track of a particle, mutual quenching by excited states may be expected, and consequent decrease in chemical yield. Similar effects may also apply to the interaction of two excited singlets. However, in ordinary photochemical situations, under steady illumination in solutions containing reasonably reactive substrates, the k_2 process will play no serious role.

With regard to k_3, our data do not permit any thorough comparison of the quenching reactions between ground state molecules and excited triplets or singlets, respectively. Thus, while the kinetics of fluorescence self-quenching have been studied with good precision,[25] we cannot, in the case of k_3, adequately distinguish between bimolecular collision processes and more complex interactions involving possible long-range energy exchange.[25,26] However, the order of magnitude of k_3 raises certain problems in connection with the function of chlorophyll in photosynthesis. Even a relatively small k_3, combined with the extremely high local chlorophyll concentrations *in vivo*, will mean rapid quenching. This of course has long been appreciated for excited singlets, but the problem now appears for triplets as well. The explanation of fluorescence self-quenching as an enhancement of singlet–triplet conversion offers no solution since the resulting triplet will itself be quenched. If one cares to extrapolate from these dilute solution measurements to highly condensed chlorophyll, a lifetime of about 10^{-7} sec. may be estimated for the triplet. These measurements thus further emphasize the long-standing problem of how self-quenching is avoided in the chloroplast. Other mechanisms for efficient energy-transfer presumably must be involved in the pigment "crystal."

Acknowledgment.—It is a pleasure to thank our colleague, Dr. E. W. Abrahamson, for his assistance in the initial stages of this research.

SYRACUSE, N. Y.

---

(22) A more detailed report on this work will be published shortly.

(23) E. Fujimori and R. Livingston, *Nature*, **180**, 1036 (1957).

(24) H. Linschitz, M. G. Berry and D. Schweitzer, THIS JOURNAL, **76**, 5833 (1954).

(25) W. F. Watson and R. Livingston, *J. Chem. Phys.*, **18**, 802 (1950).

(26) T. Förster, "Fluoreszenz Organisches Verbindungen," Chap. XI, Vandenhoeck and Ruprecht, Göttingen, 1951.