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Failure of Energy Transfer between Identical Aromatic Molecules on Excitation at the Long Wave Edge of the Absorption Spectrum

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Abstract. Electronic energy transfer among identical molecules has been followed by the depolarization of the fluorescence in concentrated solutions as well as in dimers, polymers, and micelle systems. In the many aromatic fluorophores examined, unlike a few nonaromatic ones, transfer is much decreased or altogether undetectable on excitation at the red edge of the absorption spectrum. The phenomenon is not due to the transfer taking place during a small fraction of the total fluorescence lifetime, nor is it explainable by a decrease in overlap of absorption and emission upon edge excitation.

Electronic energy transfer among identical aromatic molecules—called here homotransfer—has received considerable attention since the first observations of Gaviola and Pringsheim[1] in 1924. It is most easily revealed, as it was in the initial observations, by the depolarization of the fluorescence observed in concentrated solutions as compared with dilute solutions of the same molecule. The dependence of degree of polarization upon concentration has been used to estimate the intermolecular distances involved in the energy transfer process.[2–4]

(1) **Fluorescence Depolarization Anomalies in Concentrated Solutions.** In 1960, Weber[5] reported that 0.2 *M* indole solutions in propylene glycol at −70°C did not show depolarization when excited at wavelengths longer than 300 nm, although the depolarization was clearly evident and approximately constant in the range of excitation wavelengths of 240–300 nm. Following a similar observation on 1-anilino-8-naphthalenesulfonate in the same solvent or when adsorbed to bovine albumin in aqueous solution,[6] we measured and compared the polarization spectra of a series of aromatic compounds in dilute and concentrated solutions. The failure of depolarization upon excitation at the long wave edge of the absorption spectrum was found to be a completely general phenomenon without a single exception among the aromatics investigated (Table 1). Figures 1 and 2 show, as examples, the polarization spectra of acid fluorescein and 1-naphthylamine. The concentrated solutions (0.01–0.5 *M*) were examined in propylene glycol at −50 to −60°C in layers of 30 to 50 μ thickness. The absorbance of the layers in the region of fluorescence emission was sufficiently low so that radiative transfer of the fluorescence, known to result in increased lifetime and depolarization[7–10] was absent. This point was checked by measurements of fluorescence lifetime[11] in cases in which the reabsorption at the emitted wave-

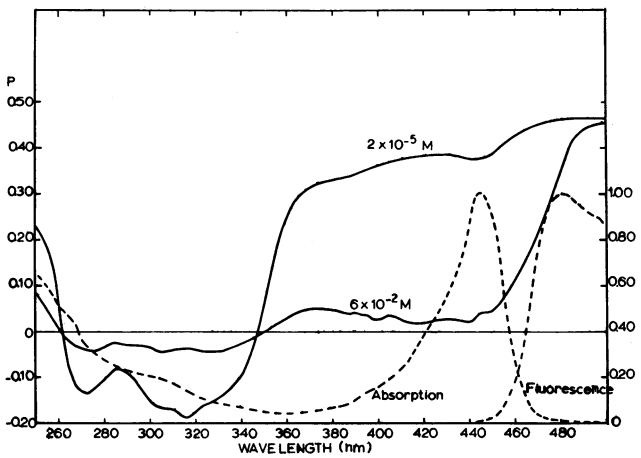

FIG. 1.—Fluorescence polarization spectra of dilute and concentrated solutions of acid fluorescein in propylene glycol at −50°C. The broken lines show the normalized absorption and emission spectra. The latter was found to be virtually independent of excitation wavelength (see Table 3). P = degree of polarization of the fluorescence emitted at wavelengths longer than 500 nm.

lengths was greatest. The optical quality of the layers was excellent and the polarization of appropriately dilute solutions were identical with those observed in 2-mm square-section cuvettes. The fluorescence spectra of the layers of concentrated solution were determined upon excitation with monochromatic light (0.5–2.5 nm bandwidth) in the range of wavelengths over which the variation in depolarization was observed. In many cases, a small displacement (1–2 nm) of the blue edge of the emission toward longer wavelengths was seen and in a few compounds a displacement of even four times this magnitude was recorded. These experiments were sufficient to exclude trivial reabsorption, optical artifacts, fluorescent contaminants, and evident aggregation as a possible cause of the phenomenon, but could not rule out the presence in the concentrated solutions of molecular pairs or other ground state aggregates of a type not hitherto characterized and responsible in principle for the observed effects.

(2) **Observations in Fluorescent Dimers and Polymers.** For the purpose of ruling out the latter possibility, we made observations upon synthetic dimers, pseudo dimers, and polymers. The dimers were of the type R—$(CH_2)_n$—R where R is an aromatic fluorophore and n a number of methylene links which ranged in the different cases from 0 to 6. The list of dimers and pseudo dimers is

TABLE 1. *Aromatic fluorophores which showed failure of depolarization upon edge excitation. Concentration solvent and temperature are given in parentheses in each case. Solvents: PG = propylene glycol, G1 = glycerol, TA = triacetin.*

(1) Naphthalene (0.156 *M*, PG, −50°C); (2) Anthracene (0.05 *M*, TA, −50°C); (3) Aniline (0.5 *M*, PG, −50°C); (4) *p*-Toluidine (0.5 *M*, PG, −50°C); (5) *N,N*-Dimethylaniline (0.5 *M*, PG, −50°C); (6) *N,N*-Dimethyl-*p*-toluidine (0.5 *M*, PG, −50°C); (7) 1-Naphthylamine (0.3 *M*, PG, −50°C); (8) 2-Naphthylamine (0.3 *M*, PG, −50°C); (9) 1-Aminoanthracene (0.1 *M*, PG, −50°C); (10) 9-Aminoanthracene (0.1 *M*, PG, −50°C); (11) 2-Aminonaphthalene-5-sulfonate (0.21 *M*, PG, −50°C); (12) 2-Aminonaphthalene 6-sulfonate (0.21 *M*, PG, −50°C); (13) 1-Dimethylaminonaphthalene-7-sulfonate (0.21 *M*, PG, −50°C); (14) 1-Anilinonaphthalene-8-sulfonate (0.125 *M*, PG, −50°C; 0.06 *M*, G1, 0°C); (15) Phenol (0.5 *M*, PG, −50°C); (16) Indole (0.2 *M*, PG, −50°C); (17) 3-Methyl indole (0.2 *M*, PG, −50°C); (18) *N*-Ethylcarbazole (0.2 *M*, TA, −50°C); (19) 9-Aminoacridine base (0.1 *M*, PG, −50°C); (20) 9-Aminoacridine cation (0.1 *M*, PG, −50°C); (21) *N*-Methylacridinium chloride (0.088 *M*, PG, −50°C); (22) Fluorescein acid (0.012 *M*, 0.06 *M*, PG, −50°C).

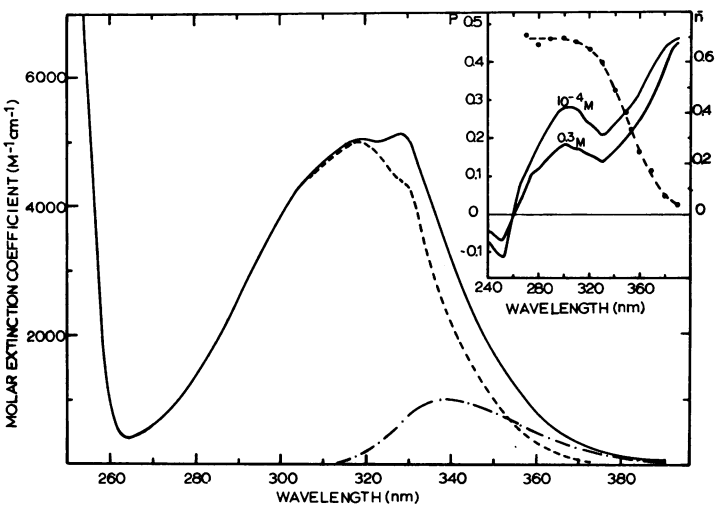

FIG. 2.—Inset: Fluorescence polarization spectra of dilute and concentrated solutions of 1-naphthylamine in propylene glycol −50°C. The average number of "forward" transfers $\bar{n}$ (·----·) was calculated from the polarization curves. The main figure shows the absorption spectra of 1-naphthylamine in propylene glycol −50°C (——) divided into an "inactive-transfer" component (-·-·) and an "active-transfer" component (- - -) according to the change of $\bar{n}$ with excitation wavelength. The maxima of the two components are some 1300 cm^{-1} apart. The fluorescence spectrum was virtually independent of excitation wavelength (Table 3).

given in Table 2. Observations were carried out in solutions of 10^{-4} to 10^{-6} M concentration. Possible ground state interactions are therefore limited to the two members of the pair, and in cases when n was 0, 1, and 2, these can in no way involve the close approach and mutual orientation required to modify conspicuously the electronic properties. Only small differences were seen in the absorption or emission spectrum of the dimers as compared with that of the monomers. Homotransfer as revealed by depolarization was always present on excitation at wavelengths other than the edge of the spectrum. In many of these cases multiple transfer within the pair could be assumed as the depolarization reached the value that could be computed if one half of the emission came from a molecule that preserved the excitation orientation and half from a molecule having an almost random orientation. When excited at the edge of the absorption, almost all depolarization disappeared just like in the concentrated

TABLE 2. *Dimers, pseudo-dimers, and polymers of aromatic fluorophores in which failure of depolarization on the edge excitation was observed. MR = mole residues, DMF = dimethyl formamide. (Other conventions as in Table 1.)*

(1) Methylene-di-*p*-aniline (10^{-4} MR, PG, −50°C); (2) α,α′-di-*p*-toluidine (10^{-4} MR, PG, −50°C); (3), (4) *N,N′*-(9-acridyl)-hexamethylene-diamine, protonated and free-base forms (2×10^{-5} MR, PG, −50°C); (5) 1,4-di-*N*-quinolinium butane (1.7×10^{-4} MR, PG, −50°C); (6) Di-dansyl-L-lysine (5×10^{-5} MR, PG, −50°C); (7) 1,1′-bi-2-naphthol (10^{-4} MR, PG, −50°C); (8) L-tryptophyl-L-tryptophan (5×10^{-5} MR, PG, −50°C); (9) 1,6-di-(9-carboxamido-anthracene)-hexane (10^{-5} MR, PG, −50°C); (10), (11) Poly ε-(9-acridyl)-L-lysine, protonated and free-base forms (2×10^{-5} MR, PG, −50°C); (12) Poly DL-tryptophan (5.10^{-5} MR, DMF-PG 1:9, −50°C); (13) Poly *N*-vinyl-carbazole (2.10^{-5} MR, CH_2Cl_2-PG 1:9).

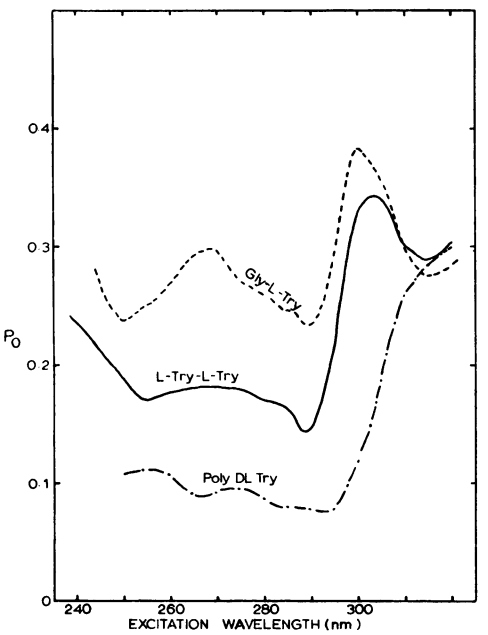

FIG. 3.—Fluorescence polarization spectra of glycyl L-tryptophan and L-tryptophyl-L-tryptophan in propylene glycol at −50°C, and of poly DL-tryptophan in dimethylformamide-propylene glycol 1:9 at −50°C, each at a concentration of 2×10^{-5} MR.

solutions. A series of polymers carrying aromatic fluorescent residues were also investigated (Table 2). Here again the disappearance of depolarization by transfer on edge excitation was uniformly found. Figure 3 shows the effects in tryptophyl-L-tryptophan and in poly DL-tryptophan.

(3) **Observations in Micelles.** Fluorescent micelles may be obtained in aqueous solutions of charged fluorophores carrying a long aliphatic chain. Figure 4 shows the behavior of micelles of 2-hexadecyl-amino-6-naphthalenesulfonate. As a result of brownian rotation, the molecularly dispersed material in alcohol has low polarization at room temperature, and a characteristic high polarization spectrum in propylene glycol at −50°C. The micelles, formed at concentrations of 5×10^{-5} *M* in water, exhibit low polarization at all wavelengths of excitation except the long wave edge of the absorption spectrum, where the polarization is comparable to the low-temperature propylene glycol solution. The micellar system permits one to perform an experiment in which the failure of homotransfer upon edge excitation may be inferred from a property other than polarization: The fluorescence of amino-naphthalenesulfonates was found to be quenched by pyridinium compounds. Mixed micelles were prepared containing 1–7 per cent hexadecyl pyridinium residues amid a large number of fluorophores (2-hexadecyl-amino-naphthalenesulfonate). Hexadecyl pyridinium absorbs at much shorter wavelengths than amino-naphthalenesulfonate and therefore its quenching action is a short range one limited to the few fluorophore molecules in its immediate vicinity. Homotransfer among the naphthylaminesulfonate residues will extend the range of action of the quencher through the migration of the excitation. The quenching of the fluorescent micelles by hexadecyl pyridinium followed a "Stern-Volmer" dependence upon the average number of quenchers per micelle and as shown in Figure 4, edge excitation resulted in a

decrease of the quenching constant by nearly an order of magnitude, as expected if failure of homotransfer followed edge excitation.

(4) Questions as to the Possible Origin of the Phenomenon. (*a*) Does homotransfer take place exclusively during the vibrational decay that precedes the attainment of the fluorescent state? Although theoretically unexpected,[12] such an ad hoc hypothesis is not disproven by any observation known to us. As it seemed to provide a possible explanation for the failure of energy transfer upon edge excitation, we devised experiments to test it and made the following observations: (*i*) Shortening of the lifetime of the excited state by quenching of the fluorescence of alkaline or acid fluorescein by *KI* leads to a parallel decrease in energy transfer. (*ii*) The lifetime of the polarized components of the fluorescence in concentrated solutions of acid fluorescein and of 1-anilino naphthalene-8-sulfonate at −58°C in propylene glycol show the differences that would be expected if the rates of transfer and emission were comparable.[13] Furthermore, on excitation at the edge of the absorption spectrum, the lifetime of the polarized components were identical. If energy transfer took place during the first few per cent of the total lifetime it could not give rise to differences in the decay time of the polarized components. From these two kinds of experiments, it appears that the possibility of the transfer being active only at the very beginning of the emission may be safely dismissed.

(*b*) Is the failure of energy transfer a general property independent of the molecular structure? All the compounds investigated were aromatics, and it seemed that one could not claim the observed property to be general without examining some nonaromatics. From this less common class of compounds, we examined the antibiotic filipin,[14] biacetyl, 4-hydro-*N*-methyl-nicotinamide[15] and *N*-butyltriazoline dione.[16] Surprisingly, only a very small increase in the polarization of the fluorescence of the solutions was seen on edge excitation and

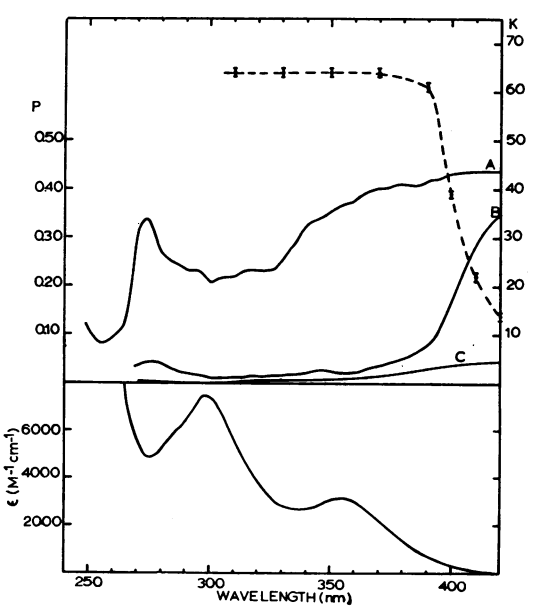

FIG. 4.—Fluorescence polarization spectra of 5×10^{-5} *M* 2,*N*-hexadecylamino-naphthalene-6-sulfonate. (*A*) in propylene glycol at −50°C, (*B*) in water at 23°C (micelles), (*C*) in ethanol at 23°C. The broken line shows the change in apparent Stern-Volmer constant *K* with excitation wavelength in micelles containing 1–7% hexadecylpyridinium as quencher. The *K* values were derived graphically from the equation $F_0/F = 1 + K([Q]/[A])$ where F_0 and F are the fluorescence intensities in the absence and presence of quencher, and $[Q]/[A]$ is the molar ratio of quencher to fluorescent residues in the system. The lower part of the figure shows the absorption spectrum of the micellar system.

this was small enough to be explainable by a trace of parasitic scattered light. A dimer molecule: bis-4-hydro-*N*-ethylene-nicotinamide in dilute solution behaved in a fashion similar to the concentrated solutions of the monomer, that is displayed no appreciable failure of energy transfer upon edge excitation. We thus concluded that in the "linear" conjugated systems examined, the decrease in energy transfer on edge excitation was—if at all present—of an altogether smaller order than that observed in aromatic fluorophores.

(*c*) Is the failure of energy transfer traceable to a decrease in overlap between the excitation and emission spectra? Since a small but nonetheless significant shift in the emission spectrum toward longer wavelengths was observed in many cases we examined this point in detail. The average number of effective or "forward" transfers $\bar{n}$ from directly excited molecules to molecules not directly excited is given by[2, 4] $\bar{n} = (1/P - 1/P_\infty)/(1/P_\infty - 1/3)$ where P is the observed polarization and P_∞ is the polarization of the individual monomer in a very dilute solution. The change in transfer with wavelength at fixed concentration and temperature can be characterized by $\bar{n}(\lambda)/\bar{n}(\lambda_0)$, where λ_0 is the absorption maximum. Similarly, if $J(\lambda)$[12] is the overlap integral of the absorption and emission spectra, $J(\lambda)/J(\lambda_0)$ gives the excitation wavelength dependence of the relative overlap. For the calculation of $J(\lambda)$, fluorescence and absorption were measured under conditions of concentration and temperature identical to those of the polarization measurements. In most of the cases studied, a wavelength of excitation λ_ϵ could be reached at which $\bar{n}(\lambda_\epsilon)/\bar{n}(\lambda_0) \ll 1$ with very small or even negligible decrease in $J(\lambda_\epsilon)/J(\lambda_0)$ (Table 3). These observations seem

TABLE 3. *Correlation of relative transfer* $(\bar{n}(\lambda_\epsilon)/\bar{n}(\lambda_0))$ *and relative overlap* $(J(\lambda_\epsilon)/J(\lambda_0))$ *of absorption and emission spectra.* λ_ϵ = *longest wavelength of excitation at which the fluorescence spectrum was measured,* λ_0 = *the absorption maximum.*

Fluorophore	Solvent, temperature	Concentration (mole residue/liter)	λ_ϵ (nm)	$\bar{n}(\lambda_\epsilon)/\bar{n}(\lambda_0)$	$J(\lambda_\epsilon)/J(\lambda_0)$
1-Naphthylamine	PG −50°C	0.3	380	0.05	1.04
1-Anilinonaphthalene 8-sulfonate	PG −50°C	0.125	420	0.20	0.65
Acid fluorescein	PG −50°C	0.012	460	0.20	1.05
Indole	PG −50°C	0.2	305	<0.02	0.96
Poly DL-tryptophane	DMF-PG (1:9) −50°C	5×10^{-5}	310	<0.02	0.92
Poly DL-tryptophane	DMF-PG (1:9) 23°C	5×10^{-5}	310	<0.02	0.97
N-ethylcarbazole	Triacetin −50°C	0.2	370	<0.02	~1.0
Poly *N*-vinyl-carbazole	Methylene chloride-PG (1:9) −50°C	2×10^{-5}	370	<0.02	~1.0
Poly *N*-vinyl-carbazole	Methylene chloride-PG (1:9) 23°C	2×10^{-5}	370	<0.02	~1.0
2-Hexadecylamino-naphthalene-6-sulfonate	Water 23°C	5×10^{-5} 5×10^{-4}	410	0.05	0.92

particularly important because they show that the failure on edge excitation cannot be reduced to the trivial case of perferential excitation of a molecular species with a fluorescence spectrum having negligible overlap with the absorption spectrum of the bulk of the fluorescent molecules.

Conclusions. All the observations described* led us to conclude that the excited state E^*, generated by excitation at the edge of the absorption band differs from the excited state B^* generated on excitation over the bulk of absorption. B^* and E^* may represent two distinct excited electronic states, which is not easy to justify on the basis of present day theory. It seems unlikely that E^* and B^* differ only in their vibrational energy, since the experiments described in 4(*a*) require that the difference in vibrational energy persist during a time of the order of the fluorescence lifetime in disagreement with current notions that vibrational equilibration in liquids is completed in times of the order of 10^{-11} sec. We are inclined to believe that E^* and B^* represent two distinct electronic states since preliminary observations indicate that, besides the variation in the emission spectrum already mentioned, other excited state characteristics such as quantum yield, lifetime, and sensitivity to collisional quenchers are also affected when excited at the edge of the absorption band. The evident lack of correlation between the spectral overlap and transfer (Table 3) when excitation is at the edge of the absorption proves that the phenonemon cannot be explained by just postulating that a different species, be it molecular aggregate, excited state tautomer, or ground state tautomer, is preferentially or uniquely excited at the edge of the absorption spectrum. That would merely shift the problem from failure of homotransfer to failure of heterotransfer with the added difficulty that the cases of the latter so far studied do not seem to show any excitation wavelength limitation. It seems, therefore, that a selection rule exclusive of, or at least very strongly effective in, aromatics must be responsible for the failure of homotransfer on edge excitation.

Failure of Energy Transfer and the Emerson Effect in Photosynthesis. The reported observations appear to offer an attractive and natural explanation of the Emerson effect in photosynthesis. As noted above, heterotransfer, that is, transfer of electronic energy to a different molecule, does not show the edge-excitation failure characteristic of homotransfer. Therefore, the transfer of electronic energy from excited chlorophyll molecules to the trap of system I, the long wave-absorbing pigment P700, will be largely independent of the excitation wavelength. On the other hand, if the trap for system II is a "colorless" energy sink, the situation for this will be entirely similar to that occurring in our mixed micelles containing a few per cent of pyridinium quencher molecules: edge excitation will result in failure of the homotransfer which is required for the transport of the excitation to a nearest neighbor to the quencher. The two systems, I and II, will thus compete on comparable terms for the excitation energy at all absorbing wavelengths except those at the edge of the absorption where activation of system II will be lacking with little or no loss in activation of system I.

* Observations on the polarization of the fluorescence made by others in L-tryptophyl-L-tryptophan,[17] poly tyrosine,[18] DNA-acridine orange complexes,[19] and phycocyanins[20] also indicate the failure of energy transfer on edge excitation.

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