This research also points out the necessity for considering stability factors in equilibrium dialysis experiments of this type. Competitive binding or other effects imparted by the presence of degradation products can significantly alter experimental results. In addition, since results of dialysis experiments are calculated from concentration differences before and after equilibration, disappearance of solute due to unrecognized chemical instability could be mistaken for binding by the protein and introduce further error into the experiment.

REFERENCES

(1) Goldstein, A., *Pharmacol. Rev.*, **1**, 102(1949).
(2) Sandberg, A. A., Slaunwhite, W. R., and Antoniades, H. N., *Recent Progr. Hormone Res.*, **13**, 209(1957).
(3) Daughaday, W. H., *J. Clin. Invest.*, **35**, 1428(1956).
(4) Slaunwhite, W. R., Rosenthal, H., and Sandberg, A. A., *Arch. Biochem. Biophys.*, **100**, 486(1963).
(5) Brunkhorst, W. K., and Hess, E. L., *ibid.*, **111**, 54 (1965).
(6) Westphal, U., and Ashley, B. D., *J. Biol. Chem.*, **234**, 2847(1959).
(7) Daughaday, W. H., *Physiol. Rev.*, **39**, 885(1959).
(8) Daughaday, W. H., *J. Clin. Invest.*, **37**, 511(1958).
(9) Upton, C. V., and Bondy, P. K., *Arch. Biochem.*, **78**, 197(1958).
(10) Slaunwhite, W. R., and Sandberg, A. A., *J. Clin. Invest.*, **38**, 384(1959).
(11) Beisel, W. R., DiRaimondo, V. C., and Forsham, P. H., *Ann. Internal Med.*, **60**, 641(1964).
(12) Bush, I. E., *Pharmacol. Rev.*, **14**, 317(1962).
(13) Oesterling, T. O., and Guttman, D. E., *J. Pharm. Sci.*, **53**, 1189(1964).
(14) Guttman, D. E., and Meister, P. D., *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 773(1958).
(15) Mader, W. J., and Buck, R. R., *Anal. Chem.*, **24**, 666 (1952).
(16) Klotz, I. M., and Walker, F. M., *J. Am. Chem. Soc.*, **69**, 1609(1947).
(17) Westphal, U., Ashley, B. D., and Selden, G. L., *ibid.*, **80**, 5135(1958).
(18) Hughes, T. R., and Klotz, I. M., *Biochemical Methods*, **3**, 278(1956).
(19) Sorby, D. L., unpublished data.
(20) Velluz, L., Petit, A., and Berrett, R., *Bull. Soc. Chim. France*, **1947**, 123.
(21) Chulski, T., and Forist, A. A., *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 553(1958).
(22) Caspi, E., Schmid, W., and Wittstruck, T. A., *Tetrahedron*, **16**, 271(1961).
(23) Hamlin, W. E., Chulski, T., Johnson, R. H., and Wagner, J. G., *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 253(1960).
(24) Jensen, E. H., and Lamb, D. J., *J. Pharm. Sci.*, **53**, 402(1964).
(25) Klotz, I. M., in "The Proteins," vol. 1B, Academic Press Inc., New York, N. Y., 1953, chap. 8.
(26) Belman, S., *J. Am. Chem. Soc.*, **85**, 2154(1963).
(27) Karush, F., *ibid.*, **72**, 2705(1950).
(28) Kauzmann, W., *J. Cell Comp. Physiol., Suppl. 1*, **47**, 113(1956).
(29) Sogami, M., and Foster, J. F., *J. Biol. Chem.*, **238**, 2245(1963).
(30) Karush, F., *J. Phys. Chem.*, **56**, 70(1952).
(31) Westphal, U., *J. Am. Oil Chemists' Soc.*, **41**, 481 (1964).

Mechanistic Implication Between Quenching in Liquid Scintillation System and Photosensitivity with Respect to Energy Transfer

By C. T. PENG

A basic mechanism for energy transfer in photosensitive biological systems is postulated.

THE USE OF liquid scintillation system for radiation measurement has been widely accepted. Owing to its high counting efficiency, the internal sample method becomes the preferred mode of assaying radionuclides emitting β particles of low kinetic energy, particularly tritium, ^{14}C, ^{35}S, ^{45}Ca, *etc.* The introduction of a sample in the form of an organic compound into the liquid scintillation system frequently causes fluorescence quenching which manifests itself as a decrease in the counting efficiency of the system. The cause of quenching has been attributed to either a diminished light transmission owing to the color of the sample or a molecular interaction between the scintillator and the sample leading to thermal degradation of the excitation energy or a combination of both (1).

The liquid scintillation system is a sensitized fluorescent donor–acceptor system in which the solvent molecule, by virtue of its presence in an overwhelming number in the medium, absorbs the radiation energy and becomes excited; from the excited states, the solvent molecules may undergo de-excitation by fluorescence emission, nonradiative transition, or excitation transfer. In the presence of solute molecules which can accept the excitation energy from the donor solvent molecule, de-excitation can take place by emission of fluorescence of the solute. Because of the inherently greater fluorescence intensity, the spectrum of the solute characterizes that of the entire system. In the presence of a quencher, transfer of excitation energy is adversely affected. According to theory, energy transfer between unlike molecules is brought about by a resonance mechanism over long distances involving dipole–dipole interaction (2, 3) or dipole–quadrupole interaction (3) or by the exchange mechanism (3, 4) in which an overlap of the wave function of the molecules concerned is necessary.

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Similar mechanisms may also be evident in biological systems. Aside from photosynthetic processes, the presence of dyes and certain drugs in biological systems causes sensitization to light, presumably as a result of a more efficient localization and utilization of the radiant energy. This report emphasizes the general aspect of energy transfer process in quenching in liquid scintillation system and by analogy extends the principle to energy transfer in photosensitive biological systems with an evaluation of the possibility of the use of the former as a means to elucidate the mechanism involved in the latter.

EXPERIMENTAL

The composition of the liquid scintillator used and the procedure of obtaining the concentration quenching curves have been previously described (5, 6). The quenching constant, q, was obtained from the initial portion of the concentration quenching curve which showed less than 50% reduction of the initial count rate of the sample by quenching.

The excitation sources were toluene-^{14}C and toluene-^{3}H. The ultraviolet excitation was carried out with an Aminco-Bowmann spectrophotofluorometer. The activating wavelength and the fluorescent wavelength were adjusted for each sample to give the maximum meter reading.

RESULTS AND DISCUSSION

Energy Transfer in Quenching in Liquid Scintillation System—The relative abundance of solvent and solute molecules in a liquid scintillator is given in Table I. In such system, toluene is the solvent, 2,5-diphenyloxazole, the primary solute, and 1,4-bis-2(5-phenyloxazolyl)benzene, the secondary solute. The use of a secondary solute is to shift the fluorescence spectrum of the scintillator system to longer wavelength to match the spectral response of the multiplier phototube (7), and its role of energy transfer between solute and solute in the liquid scintillation system need not concern our discussion. Dissipation of radiation energy in the scintillator system under these conditions causes the formation of the excited solvent molecules from which the excitation energy is transferred to the scintillator or the solute molecules which decay by fluorescence emission. In the presence of a quencher, this energy transfer between the excited solvent and the scintillator molecules is interrupted.

Energy transfer by molecular encounter is not so important a process as that by inductive resonance in a liquid scintillation system. The latter mechanism predicts energy transfer over distances as large as 50 to 100 Å. According to Förster (2), resonance transfer requires an overlapping of the absorption spectrum of the acceptor and the fluorescence spectrum of the donor. Since polyatomic molecules have broad spectra in solution, it is likely that coupled transitions can occur between unlike species of organic molecules. This may afford an explanation to the observed fact that addition of an organic compound to a liquid scintillation system frequently causes quenching.

TABLE I—COMPOSITION OF A LIQUID SCINTILLATOR

Compd.	Concn., mole/L.	Abundance Ratio	Av. Radius of Separation Between Adjacent Molecules, Å.
Solvent: Toluene	10.9	345	
Primary solute:			
2,5-Diphenyloxazole	3.16×10^{-2}	1 232	23.2
Secondary solute:			
1,4-bis-2(5-Phenyloxazolyl)benzene	1.36×10^{-4}	1	142

Based on quantum mechanical considerations involving dipole–dipole interaction between molecules, Förster concluded that the rate constant of excitation transfer from donor to acceptor molecules at thermal equilibrium is inversely dependent to the sixth power of the intermolecular distance. By assuming a sharp critical transfer distance with instantaneous excitation transfer for shorter and no transfer for longer distance, a simple exponential formula can be used to approximate the donor fluorescence yield. Thus:

$$\frac{n}{n_0} = \text{exp. } (-\alpha c/c_0) \qquad \text{(Eq. 1)}$$

where n is the fluorescence yield at quencher concentration c; n_0, that in the absence of the quencher; α, a constant; and c_0, the quencher concentration corresponding to the critical transfer distance R_0. Their interrelationship is given by the following equation:

$$c_0 = \frac{3000}{4\pi N R_0^3} \qquad \text{(Eq. 2)}$$

where N is Avogadro's number.

Equation 1 resembles an empirical equation derived by Peng (5, 8) for quenching correction in liquid scintillation counting, based on an observation by Kerr *et al.* (9) that quenching varies exponentially with the concentration of the quencher. Thus:

$$S_a = S_0 \text{ exp. } (-qc) \qquad \text{(Eq. 3)}$$

$$= S_0 \text{ exp. } (-c/c_0) \qquad \text{(Eq. 3}a\text{)}$$

where S_a and S_0 are the apparent specific count rates in the presence and in the absence of the quencher, respectively; c is the quencher concentration, and q, the quenching constant which equals $1/c_0$ or $0.693/c_{1/2}$. c_0 and $c_{1/2}$ are the concentrations of the quencher that reduce the count rate to e^{-1} and $1/2$ of its initial value, respectively. The two equations differ by a constant exponent α which was found to have a value of 1.42 (2), although the theoretical value should be 1.000. It may also be pointed out that c_0, the "relative" critical transfer concentration, can also be obtained from statistical consideration of a random interaction between the excited molecules and the quencher molecules according to poisson distribution with an average of one interaction between them. Such a consideration is

TABLE II—CRITICAL TRANSFER CONCENTRATION (IN ARBITRARY UNITS) AND QUENCHING INDEX OF VARIOUS COMPOUNDS (^{14}C β-EXCITATION)

Compd.	Critical Transfer Concn., c_0, moles/L.	Critical Transfer Distance,[a] R_0, Å.	Quenching Index
CH_3OH	14.18	3.06	1
$HCON(CH_3)_2$	5.2	4.25	1.41
CH_3COOH	1.41	6.70	2.22
CH_3COCH_3	.852	7.75	2.58
CD_3COCD_3[b]	.845	7.78	2.59
$CHCl_3$	.41	9.90	3.29
$CDCl_3$[b]	.43	9.75	3.24
CCl_4	.061	18.65	6.20
C_6H_5NCS	.055	19.35	6.43
Hyamine hydroxide	.0294	23.80	7.90
Benzophenone	.026	24.80	8.23
Benzil	.0055	41.70	13.90

[a] Calculated by Eq. 2. [b] The perdeuterated acetone and chloroform were obtained from New England Nuclear Corp., Boston, Mass.

analogous to that used in deriving the e^{-1} surviving fraction by the target theory in radiobiology (10).

The use of Eq. 3 to obtain c_0 or $c_{1/2}$ in a liquid scintillation system gives only relative values. It has been pointed out that $c_{1/2}$ value depends on the pulse height and consequently varies with the instrument settings of the spectrometer used for the measurement (5). But at given instrument settings, the $c_{1/2}$ values are quite reproducible. It is therefore feasible to use either q or its related values as an index of the quenching property of the compound, provided that the measurement is carried out simultaneously against a selected standard. This index, in order to eliminate variations in instrument settings, should be expressed as a ratio of q or its related value of the compound to that of the standard. Such a ratio would allow a comparison of the quenching properties of various compounds. The absolute value can be calculated once the true critical transfer concentration of the standard is known. Table II shows the arbitrary values of critical transfer concentration c_0 of various compounds and the ratios of their critical transfer distance to that of methanol. It may be noted that perdeuterated acetone and chloroform show insignificant difference in quenching property from their protonated forms indicating an absence of intermolecular isotope effect. The relative magnitude of the quenching index of benzophenone and benzil as given is of interest because they are known to form a donor–acceptor pair (11).

Deviation from Eq. 3 is frequently observed and is attributed to the presence in solution of molecular aggregates of fluorescent molecules which exhibit different quenching properties (6). Figure 1 shows the quenching curves of 2,5-diphenyloxazole in two different concentrations in toluene and in n-hexane with ultraviolet excitation and with varying amounts of carbon tetrachloride as quencher. The composite nature of the quenching curve in toluene can be represented by a summation expression of Eq. 3a. Thus:

$$S = \Sigma S_{0i} \exp.(-c/c_{0i}) \qquad i = 1, 2, 3, \ldots .$$

Since toluene is a very efficient solvent for energy transfer owing to the availability of π electrons, its replacement with n-hexane under similar conditions gives more linear quenching curves. From the linearity of the quenching curve it may be interpreted that in inefficient energy transfer systems there is generally an absence of excimer formation (12) or photoassociation of solute molecules or formation of molecular aggregates of similar nature which frequently occurs in liquid scintillation systems of efficient excitation transfer. Formation of dimers or molecular aggregates in solution has been reported for naphthalene (13) and 2,5-diphenyloxazole (14).

The relationship between the extent of quenching and the concentration of the quencher is frequently governed by the Stern-Volmer equation (15). In liquid scintillation system, Stern-Volmer plots for a number of quenchers studied were found to exhibit a nonlinear relationship over wide concentration ranges. A notable exception is the straight line obtained for hyamine hydroxide as shown in Fig. 2.

The nonlinearity of the Stern-Volmer plot may be interpreted as a result of energy transfer between the triplet state of molecules. Such interpretation is not only in accord with the findings of Terenin and Ermolaev (16) but also with the theoretical evaluation by Inokuti and Hirayama (4) of energy transfer by the exchange mechanism in sensitized luminescence. They demonstrated the constancy of the value of α as 1.00 in Eq. 1 in the approximation of the donor luminescence yield. Approximation with a Stern-Volmer type equation, $n/n_0 = (1 + \beta c/c_0)^{-1}$, would be less meaningful under similar circumstances because the β value which by definition of the equation should remain constant was found to vary with changing values of c/c_0.

Recent studies by pulse radiolysis of various solutions of binary mixtures of anthracene, biacetyl, naphthalene, *cis*- and *trans*-stilbene, *etc.*, indicate that the order of intermolecular energy transfer among these compounds may be related to their triplet energy level (17). Because of the low energy

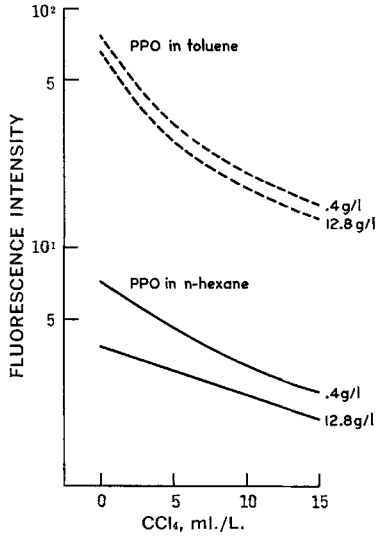

Fig. 1—Carbon tetrachloride quenching curves of 2,5-diphenyloxazole (PPO) solutions by U.V. excitation.

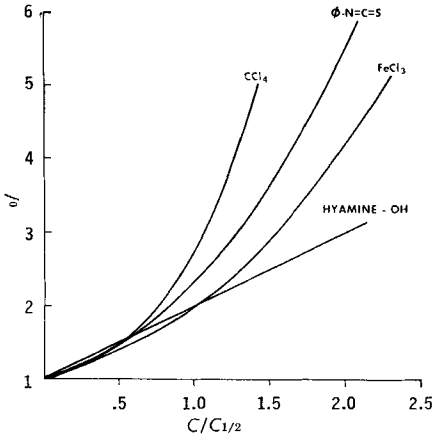

Fig. 2—Stern-Volmer plots for quenchers in liquid scintillation system. The quencher concentration on the abscissa is expressed in terms of $c_{1/2}$ for equivalent quenching effectiveness. The ordinate is the ratio of initial count rate to the quenched count rate.

level of the triplet state (E_T: 60.9 Kcal./mole) and an absence of the self-quenching effect, naphthalene has the property to suppress quenching in liquid scintillation systems (18) and to deactivate triplet benzophenone (E_T: 68.5 Kcal./mole) very efficiently (19). These observations constitute further evidence of support for our interpretation. The triplet–triplet energy transfer mechanism can also account for the observed change in the channels ratio which is a measure of the q values as the concentration of the quencher is increased, as shown in Fig. 2 of *Reference 20*. It is likely that at high concentrations, the molecules are in close proximity to each other, and consequently, energy transfer by the exchange mechanism becomes more probable.

Energy Transfer in Photosensitivity—Although the causation of hypersensitivity to light by the administration of drugs and dyes has been reported and exhaustively reviewed in the literature (21–24), the postulated mode of action has not been based on a consideration of energy transfer. As the conditions under which photosensitivity or photodynamic effect[1] occurs coincide in general with the conditions necessary for energy transfer, it is probable that a mechanism based on an intermolecular energy transfer of the radiant energy will be able to account inclusively for the observed facts such as the extremely dilute concentration of dyes that is sufficient to elicit a response, the diversity of the chemical structure of the active agents, the obligatory presence in the photosensitive biological system of compounds which can act as donors or sensitizers in energy transfer, the heavy atom effect, and the dependence of the end effect on light intensity.

A mechanism of energy transfer based on the consideration of similar processes in liquid scintillation system can be postulated to form a common basis for all observations on hypersensitivity to light; this consists of optical excitation of the sensitizer either directly or indirectly with intersystem crossing to the triplet state and formation of the triplet state of the acceptor from both the singlet and the triplet states of the sensitizer by energy transfer. The sequence of events may be represented by reactions *1*, *4*, *7*, *8*, and *10* in Scheme I. The intervening reactions represent a diversion of the excitation energy leading to a reduction of the quantum yield of the triplet acceptor. Once formed, the triplet acceptor will have a greater probability for participation in many biological reactions because of its long lifetime. In photodynamic action, the triplet acceptor can react with oxygen in its ground triplet state to yield the moleoxide according to reactions *12*, *13*, and *15*, similar to those postulated for photooxidation of anthracene (25) and other organic compounds (26).

Alternately, the peroxide can be formed by reaction with excited singlet oxygen molecule. The formation of singlet oxygen ($^1\Delta_g$) from the ground triplet state oxygen ($^3\Sigma_g^-$) is shown in Scheme II as involving the transfer of excitation energy either from singlet or from triplet, donor or acceptor molecules by the exchange mechanism or the triplet–triplet annihilation. In both instances, spin conservation is observed. The singlet oxygen will then react with the acceptor molecule or other oxidizable substances present in biological systems to form peroxides. This mode of reaction *via* singlet oxygen

1, $S + h\nu \rightarrow S^*$	Absorption of light
2, $S^* \rightarrow S + h\nu_f$	Emission of fluorescence
3, $S^* \rightarrow S$	Nonradiative transition
4, $S^* \rightarrow {}^3S$	Intersystem crossing to the triplet state
5, $^3S \rightarrow S + h\nu_p$	Emission of phosphorescence
6, $^3S \rightarrow S$	Nonradiative transition
7, $S^* + A \rightarrow A^* + S$	Energy transfer by resonance mechanism
8, $^3S + A \rightarrow {}^3A + S$	Energy transfer by the exchange mechanism
9, $A^* \rightarrow A$	Nonradiative transition
10, $A^* \rightarrow {}^3A$	Intersystem crossing to the triplet state
11, $^3A \rightarrow A$	Nonradiative transition
12, $A^* + O_2 \rightarrow {}^3AO_2$	Formation of the moleoxide
13, $^3A + O_2 \rightarrow {}^3AO_2$	Formation of the moleoxide
14, $^3AO_2 \rightarrow A + O_2$	Spontaneous decomposition of the moleoxide
15, $^3AO_2 + A \rightarrow AO_2 + A$	Formation of the stable peroxide

Scheme I

12a, A^* (or S^*) + $O_2(^3\Sigma_g^-) \rightarrow {}^3A$ (or ^3S) + $O_2(^1\Delta_g)$
Formation of singlet oxygen by the exchange mechanism

13a, ^3A (or ^3S) + $O_2(^3\Sigma_g^-) \rightarrow A$ (or S) + $O_2(^1\Delta_g)$
Formation of singlet oxygen by triplet–triplet annihilation

14a, $A + O_2(^1\Delta_g) \rightarrow AO_2$
Formation of the stable peroxide

Scheme II

[1] The term "photodynamic effect" is exclusively used for photosensitivity in the presence of oxygen because both oxygen and light are required for the hypersensitive response.

is in agreement with the evidence from recent photochemical studies (27, 28).

The importance of the triplet state in biological systems has been emphasized (29), especially for its long lifetime in which useful biological work can be done (30). The heavy atom effect which is uniquely linked to the triplet state has been observed in the killing of paramecia by fluorescein dyes as the efficacy decreases from erythrosin to eosin and to fluorescein (31). It is known that the presence of heavy atoms increases the rate of intersystem crossing leading to the population of the triplet state.

All sensitizers for photosensitive biological actions are fluorescent. The fluorescent state is an indication of the lifetime of the excited state of the molecule, as the fluorescence lifetime is usually approximately 10^{-8} sec. which is approximately 10^5 times longer than the lifetime for nonradiative transition. A long-lived state of the sensitizer is necessary to ensure a high probability of energy transfer. For effectiveness in excitation transfer, a maximum overlap of the absorption spectrum of the acceptor and the fluorescence spectrum of sensitizer is required. In riboflavin–cytochrome c system, the overlap integral for long distance resonance energy transfer has been evaluated (32).

The acceptor in photosensitive biological systems will be proteins, nucleic acids, and other normal tissue constituents. These are also fluorescent (33), and some even show phosphorescence. Protein phosphorescence was first observed in 1952 by Debye and Edwards (34). The triplet states of aromatic amino acids and proteins have also been observed and studied with electron spin resonance spectroscopy (35, 36). Together these observations lend credence to the formation of the triplet state by reactions *8* and *10*.

The postulated scheme for photosensitive action may also account for a lack of photosensitivity in many individuals by a quenching mechanism which diverts the excitation energy away from the target acceptor. Some of the strong quenchers such as carbon tetrachloride, pyridine, phenyl isothiocyanate, *etc.*, found in the liquid scintillation system are also toxicants (37). The exceptions are the carbonyl compounds. The presence of carbonyl groups, by their unique alignment in the condensed phase, gives the connective, skeletal, and keratinized tissues their luminescence (38). The strong interaction of the carbonyl group with the sensitizer would ensure a maximum utilization of the absorbed radiant energy. By the same principle, melanin or similar compounds may afford protection to photosensitive action by quenching since compounds with adjacent carbonyl groups such as benzil and biacetyl have been shown to be strong quenchers.

The parallelism in the basic mechanisms of energy transfer between a photosensitive biological system and a liquid scintillation system would allow the latter system to be used as a model for elucidation of the mechanism involved in the former, because the liquid scintillation system is more sensitive in detecting fluorescence quenching than the optical method. As already indicated, measurement in such a system gives only relative values. For absolute values, it needs to be standardized against reference compounds. To resolve color and chemical quenching in the system, a method by Ross (39) for color quenching may be used.

REFERENCES

(1) Peng, C. T., *Atomlight*, (No. 44) 1(1965).
(2) Förster, T., *Discussions Faraday Soc.*, **27**, 7(1959).
(3) Dexter, D. L., *J. Chem. Phys.*, **21**, 836(1953).
(4) Inokuti, M., and Hirayama, F., *ibid.*, **43**, 1978(1965).
(5) Peng, C. T., *Anal. Chem.*, **32**, 1292(1960).
(6) Peng, C. T., *Mol. Crystals*, to be published.
(7) Hayes, F. N., Ott, D. G., and Kerr, V. N., *Nucleonics*, **14** (No. 1), 42(1956).
(8) Peng, C. T., in "Liquid Scintillation Counting," Bell, C. G., Jr., and Hayes, F. N., eds., Pergamon Press, New York, N. Y., 1958, p. 198.
(9) Kerr, V. N., Hayes, F. N., and Ott, D. G., *Intern. J. Appl. Radiation Isotopes*, **1**, 284(1957).
(10) Fano, U., in "Radiation Biology," Hollaender, A., ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1954, vol. I, pp. 123–134.
(11) Bäckström, H. L. J., and Sandros, K., *Acta Chem. Scand.*, **14**, 48(1960).
(12) Stevens, B., *Nature*, **72**, 725(1961).
(13) Dammers-de Klerk, A., *Mol. Phys.*, **1**, 141(1958).
(14) Berlman, I. B., *J. Chem. Phys.*, **34**, 1083(1961).
(15) Stern, O., and Volmer, M., *Physik. Z.*, **20**, 183(1919).
(16) Terenin, A., and Ermolaev, V., *Trans. Faraday Soc.*, **52**, 1042(1956).
(17) Peng, C. T., and Dainton, F. S., unpublished data.
(18) Furst, M., and Kallmann, H., *Phys. Rev.*, **97**, 583 (1955).
(19) Porter, G., and Wilkinson, F., *Trans. Faraday Soc.*, **57**, 1686(1961).
(20) Peng, C. T., *Anal. Chem.*, **36**, 2456(1964).
(21) Blum, H. F., "Photodynamic Action and Diseases Caused by Light," Reinhold Publishing Co., New York, N. Y., 1941.
(22) Sollmann, T., "A Manual of Pharmacology," 8th ed., W. B. Saunders Co., Philadelphia, Pa., 1957, p. 201.
(23) Fitzpatrick, T. B., Pathak, M. A., Magnus, I. A., and Curwen, W. L., *Ann. Rev. Med.*, **14**, 195(1963).
(24) Spikes, J. D., and Glad, B. W., *Photochem. Photobiol.*, **3**, 471(1964).
(25) Livingston, R., in "Symposium on Photochemistry in the Liquid and Solid States," Daniels, F., ed., John Wiley & Sons, Inc., New York, N. Y., 1960, p. 76.
(26) Gollnick, K., and Schenck, G. O., *Pure Appl. Chem.*, **9**, 507(1964).
(27) Foote, C. S., and Wexler, S., *J. Am. Chem. Soc.*, **86**, 3880(1964).
(28) Wilson, T., *J. Am. Chem. Soc.*, **88**, 2898(1966).
(29) Reid, C., "Excited States in Chemistry and Biology," Butterworths, London, England, 1957, chap. 5, pp. 82–110.
(30) Becker, R. S., and Kasha, M., in "The Luminescence of Biological Systems," Johnson, F. H., ed., American Association for the Advancement of Science, Washington, D. C., 1955, p. 25.
(31) Giese, A. C., and Crossman, E. B., *J. Gen. Physiol.*, **29**, 193(1946).
(32) Karreman, G., and Steel, R. H., *Biochim. Biophys. Acta*, **25**, 280(1957).
(33) "Handbook of Chemistry and Physics," 41st ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1959–1960, p. 3002.
(34) Debye, P., and Edwards, J. O., *Science*, **116**, 143 (1952).
(35) Shiga, T., Mason, H. S., and Simo, C., *Biochemistry*, **5**, 1877(1966).
(36) Shiga, T., and Piette, L. H., *Photochem. Photobiol.*, **3**, 223(1964).
(37) Peng, C. T., unpublished data.
(38) Kallmann, H., Krasnansky, V. J., and Person, P., Abstracts. Second International Biophysics Congress, Abstr. No. 601, Vienna, Austria, 1966.
(39) Ross, H. H., *Anal. Chem.*, **37**, 621(1965).