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Studies of the Chemiluminescence of Several Xanthene Dyes. III. The Effect of Added Foreign Dyes on Light Production

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The intensity of uranine chemiluminescence decreased when trypaflavine was added to the system. The enfeebling action seemed to be due to external quenching. On the contrary, the intensity of eosine chemiluminescence was enhanced by the presense of uranine. The values of Δ and σ are defined by the following equations:

$$\Delta=\int_0^T (I_{UE}-I_U-I_E)dt \qquad \sigma=\Delta\Big/\int_0^T I_E dt$$

where I_{UE}, I_U and I_E are the intensities measured in uranine-eosine, uranine and eosine systems respectively, and where T is the duration of eosine chemiluminescence. Δ increased with the increase in either the concentration of uranine or that of eosine, while σ increased only when the ratio of the uranine concentration to the eosine concentration increased. Δ increased with the increase in the temperature, while σ was little affected by the temperature. Δ increased upon the addition of organic solvents, while σ was little affected by the addition of the organic solvents. The enhancing action is due neither to the production of a stable compound which emits strongly nor to the increase in the rate of the decomposition of the dye. One possible mechanism of the enhancing action is that energy is transferred from an energy-rich intermediate produced by the reaction to an unoxidized uranine or eosine.

The intensity of chemiluminescence is affected more or less by the addition of foreign substances. Biswas and Dhar[1] have studied the subject extensively with a number of foreign substances and have found that all of them were inhibitors of the luminescent reaction. Bersis,[2] however, has succeeded in showing that the intensity of the chemiluminescence of polyphenol is remarkably enhanced by the presence of rhodamine B. In the present paper, the chemiluminescence of uranine and eosine will be investigated in the presence o foreign dyes, in the hope that a full examination of the effect of foreign substances will give some insight into the mechanism of the luminescent reaction. The results may be summarized as follows:

1) The intensity of uranine chemiluminescence decreases in the presence of trypaflavine (an enfeebling action). This has also been found to be true in uranine-erythrosine and in eosine-erythrosine systems.

2) The intensity of eosine chemiluminescence

1) N. N. Biswas and N. R. Dhar, *Z. anorg. u. allgem. Chem.*, **173**, 125 (1928).

2) D. S. Bersis, *Z. phys. Chem.* (*Neue Folge*), **26**, 359 (1960).

increases in the presence of uranine, and (an enhancing action).

Experimental

The luminescent systems were prepared, and both the luminescence intensities and the rates of the decomposition of the dyes were measured in the ways described in a preceding paper.[3]

Results and Discussion

I) Enfeebling Action.

Figure 1 shows the effect of added trypaflavine on the intensity of uranine chemiluminescence; curves I, II, III and IV represent, respectively, the results obtained from the uranine-trypaflavine sample solutions of I, II, III and IV listed in Table I. Each luminescent reaction was started by the addition of 1 ml. of a 20% hydrogen peroxide solution to a mixture of 7 ml. of the sample solution and 2 ml. of a 2.5 N sodium hydroxide solution at 40°C. We can see an "enfeebling action" of the added trypaflavine. The higher the concentration of added trypaflavine, the stronger becomes the enfeebling action. By numerical analysis, the curves can be represented as:

$$I_t = A\exp(-at)/\{B + Q\exp(-qt) + C\exp(-ct)\} \quad (1)$$

where I_t is the intensity of the emission at time t and A, a, B, Q, q, C and c are all parameters which differ according to the experimental conditions. Curves I, II, III and IV of Fig. 1 can be represented as the following equations:

Curve

$$\text{I:}\quad I_t = 500\exp(-0.46t)/\{1 + 6.0\exp(-0.80t)\} \quad (1a)$$

$$\text{II:}\quad I_t = 460\exp(-0.46t)/\{1.14 + 5.5\exp(-0.80t) + 6.3\exp(-0.53t)\} \quad (1b)$$

$$\text{III:}\quad I_t = 430\exp(-0.46t)/\{1.29 + 5.2\exp(-0.80t) + 16.2\exp(-0.51t)\} \quad (1c)$$

$$\text{IV:}\quad I_t = 357\exp(-0.46t)/\{1.53 + 4.5\exp(-0.80t) + 26.4\exp(-0.47t)\} \quad (1d)$$

Both the calculated values and the observed values are tabulated in Table II. The calculated values are in fairly good agreement with the observed values except for a slight deviation at the initial stage.

In the reaction system of uranine-trypaflavine, no emission was observed as resulting from the decomposition of trypaflavine. It is difficult to determine the decomposition rate of trypaflavine in the uranine-trypaflavine system by measuring the absorbancy of the dye at 452 mμ, because uranine absorbed light appreciably at that wavelength. However, as the rate of the decomposition of trypaflavine was found to be almost independent of the amount of uranine, its rate in the system could be estimated from a separate experiment in an isolated trypaflavine system. The rate of the decomposition of trypaflavine was measured in a system composed of 7 ml. of a trypaflavine solution, 2 ml. of a 2.5 N sodium hydroxide solution, and 1 ml. of a 20% hydrogen peroxide solution. The results are shown in Fig. 2, where the concentrations of trypaflavine in II', III' and IV' are equal to those in II, III and IV of Fig. 1 respectively. These decomposition curves can be represented as:

$$[D]_t = \alpha + \beta(-k_t t) \quad (2)$$

where $[D]_t$ is the concentration of trypaflavine at time t, both α and β are parameters which differ according to the experimental conditions, and k_t is the rate constant. The curves of Fig. 2 can be expressed as:

$$\text{Curve II'}\quad [D]_t = 0.036 + 1.74\exp(-0.53t) \quad (2a)$$

$$\text{Curve III'}\quad [D]_t = 0.076 + 4.27\exp(-0.53t) \quad (2b)$$

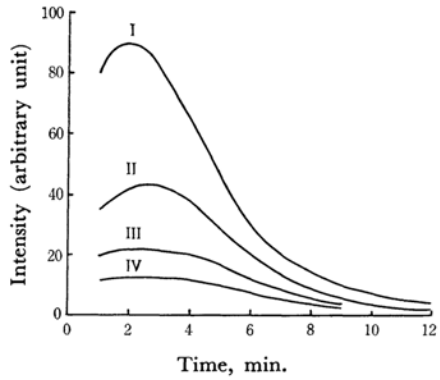

Fig. 1. *I-t* curves in uranine and uranine-trypaflavine systems.
(2.5 N NaOHaq., 20% H_2O_2 aq., 40°C)

TABLE I. THE RATIOS OF URANINE, TRYPAFLAVINE AND WATER IN VOLUME

Sample No.	Uranine solution (10 g./l.)	Trypaflavine solution (10 g./l.)	Water
I	3.5	0.0	3.5
II	3.5	0.5	3.0
III	3.5	1.0	2.5
IV	3.5	1.5	2.0

3) I. Kamiya and R. Iwaki, This Bulletin, **39**, 257 (1966).

TABLE II. QUENCHING REACTION BY TRYPAFLAVINE ON THE CHEMILUMINESCENCE OF URANINE AT 40°C

Time min.	Sample No. I Eq. 1a Calcd.	I Eq. 1a Obs.	II Eq. 1b Calcd.	II Eq. 1b Obs.	III Eq. 1c Calcd.	III Eq. 1c Obs.	IV Eq. 1d Calcd.	IV Eq. 1d Obs.
1.0	85.4	79.2	39.0	36.0	20.3	19.5	11.8	12.9
1.5	89.9	86.8	40.9	39.0	20.8	21.2	11.9	12.7
2.0	90.0	90.0	41.5	41.6	22.2	21.8	11.9	12.6
2.5	87.6	88.0	41.1	42.8	20.9	20.9	11.8	11.9
3.0	81.7	81.7	39.7	42.2	20.6	20.4	11.3	11.5
3.5	73.0	73.0	37.6	40.0	19.9	19.6	10.9	11.2
4.0	63.7	63.0	34.5	36.8	18.9	18.2	10.3	10.8
4.5	54.2	53.2	31.0	32.3	17.8	17.0	9.8	10.0
5.0	45.0	45.0	27.0	28.0	16.2	16.0	9.1	9.2
5.5	37.4	36.0	23.8	24.0	14.7	13.2	8.4	8.4
6.0	29.9	29.0	20.0	19.0	12.6	11.3	7.5	7.3
7.0	19.6	19.0	14.0	12.6	9.8	8.0	5.9	5.2
8.0	12.8	12.2	9.7	8.4	7.2	5.2	4.3	3.6
9.0	8.0	8.0	6.2	6.4	4.7	4.3	3.1	2.6
10.0	5.1	5.0	3.8	4.0	—	—	—	—
11.0	3.2	3.0	2.5	2.4	—	—	—	—
12.0	2.0	2.0	1.6	1.6	—	—	—	—

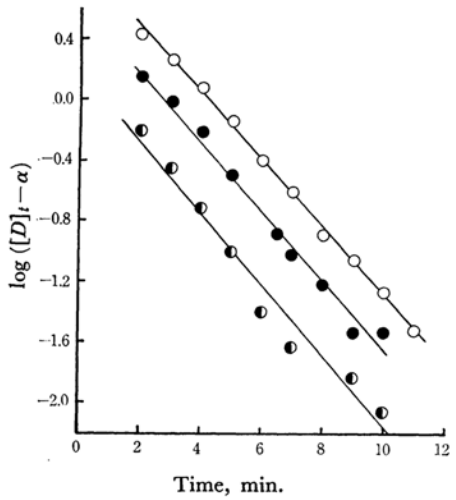

Fig. 2. Decomposition of trypaflavine at 40°C.
◐ II′ ● III′ ○ IV′

Curve IV′ $[D]_t = 0.141 + 6.92\exp(-0.53t)$ (2c)

The results indicate that the decomposition of trypaflavine is first order with respect to its concentration.

We shall first discuss the physical meaning of the parameters, *a*, *A*, etc.

Parameter a.—It was shown in a previous paper[3] that all the curves of the intensity versus the time measured in uranine chemiluminescence could be represented by a single equation in the form of Eq. 1a, in which *a* was found to correspond to the rate of the decomposition of uranine. Perhaps this may also be the case with Eqs. 1b—1d. We may also assume that parameter *a* is the rate of the decomposition of uranine, which is not affected by the addition of trypaflavine.

Parameter A.—From the same argument, *A* should be a value proportional to the initial concentration of uranine. Since an equal amount of uranine was added to each solution, the values *A* should be equal to one another. However, the values of *A* in the equations of 1b—1d are not constant. This deviation can be accounted for by the following consideration.

When a large amount of trypaflavine was added to a uranine solution, a small amount of a white precipitate was produced. Assuming that the amount of the precipitate is proportional to the product of the initial concentrations of uranine $[U]_0$ and trypaflavine $[T]_0$, the final concentration of uranine is given as $[U]=[U]_0(1-p[T]_0)$, where p is a proportionality constant. In the present case, [U] is given by the following equations, since the ratio of $[T]_0$ is 0 : 1 : 2 : 3.

Sample	Equation
I	$[U]=[U]_0$
II	$[U]=[U]_0(1-p')$
III	$[U]=[U]_0(1-2p')$
IV	$[U]=[U]_0(1-3p')$

where p' is a new constant. If $[U]_0$ and p' are put equal to 500 and 0.08 respectively in arbitrary units, the [U] values in the four specimens are

calculated to be 500, 460, 420 and 380 respectively. These values agree with the corresponding A value in Eqs. 1b—1d. Therefore, it may be concluded that A is a value proportional to the initial concentration of uranine.

Parameters c and C.—The third term of the denominator in Eq. 1, $C\exp(-ct)$, is found only in Eqs. 1b, 1c and 1d, not in Eq. 1a. This would indicate that the term corresponds to the effect of the addition of trypaflavine. If we assume the values of c in Eqs. 1b, 1c and 1d (0.53, 0.51 and 0.47 min^{-1}) to be approximately equal to one another, c is very likely to be the rate constant of the decomposition of trypaflavine (0.53 min^{-1} under the same experimental conditions). Moreover, the values of C in Eqs. 1b, 1c and 1d are almost proportional to the values of β in Eqs. 2a, 2b and 2c. Hence, it may be concluded that C is a value proportional to the initial concentration of trypaflavine. The actual concentration of trypaflavine may decrease upon the addition of uranine, but it is still proportional to the initial concentration (Table I).

Parameter B.—The mutual ratio of (B-1) values (0.14 : 0.29 : 0.53) is approximately equal to the ratio of α values in Eqs. 2a, 2b and 2c. The value of (B-1), therefore, is proportional to the initial concentration of trypaflavine.

From the same argument, parameters q and Q seem to correspond, respectively, to the decomposition rate constant and the initial concentration of a substance inhibiting light production.

On the basis of this discussion, Eq. 1 can be rewritten as:

$$I_t = A'[\mathrm{U}]_0 \exp(-k_\mathrm{U}t)/[1 + Q\exp(-qt) + [\mathrm{T}]_0 \times \{1 + B'\exp(-k_\mathrm{T}t)\}] \quad (3)$$

where A' and B' new constants, $[\mathrm{U}]_0$ and $[\mathrm{T}]_0$ are the initial concentrations of uranine and of trypaflavine respectively, and K_U and K_T are the rate constants of the decomposition of uranine and trypaflavine respectively.

At the initial stage of the reaction, Eq. 3 becomes:

$$I_t = A'[\mathrm{U}]_0/\{1 + Q + (1 + B)[\mathrm{T}]_0\} \quad (4)$$

This equation is similar to the Stern-Volmer equation for the quenching of fluorescence, if Q and $[\mathrm{T}]_0$ are the concentrations of the quenching substances. According to Wilhelmann, Lumry and Eyring,[4] the fraction of excited molecules which emit light in the chemiluminescence is given by:

$$\phi = k_1/(k_1 + k_2 + \sum_i k_i q_i)$$

where k_1, k_2 and k_i are the rates of the emission process, the internal quenching and the external quenching respectively, and q_i is the concentration of the ith quencher. The equation is very similar to Eq. 4.

From the above considerations, it may be concluded that the enfeebling action of trypaflavine is probably the external quenching.

II) Enhancing Action.

Figure 3 shows the curves of the intensity versus the time measured in a reaction system composed of 1 ml. of a 20% hydrogen peroxide solution, 2 ml. of a 3 N sodium hydroxide solution and 6 ml. of a uranine, eosine or uranine-eosine mixed solution, where the notation I_{UE} (3-1-2), for example, refers to a system composed of 3 ml. of a uranine

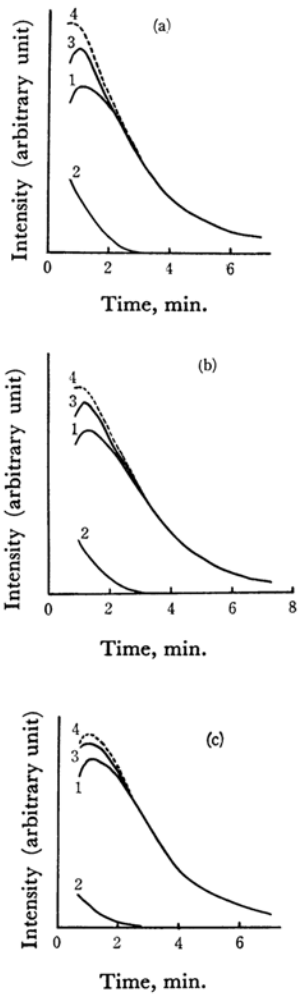

Fig. 3. I-t curves in uranine-eosine systems. (3 N NaOHaq., 20% H_2O_2aq., 45°C)

(a) 1: I_U (3-0-3); 2: I_E (0-3-3); 3: I_UE (3-3-0); 4: $I_\mathrm{U}+I_\mathrm{E}$

(b) 1: I_U (3-0-3); 2: I_E (0-2-4); 3: I_UE (3-1-2); 4: $I_\mathrm{U}+I_\mathrm{E}$

(c) 1: I_U (3-0-3); 2: I_E (0-1-5); 3: I_UE (3-1-2); 4: $I_\mathrm{U}+I_\mathrm{E}$

4) P. C. Wilhelmann, R. Lumry and H. Eyring, "The Luminescence of Biological Systems," John Wiley & Sons, New York, N. Y. (1955), p. 75.

solution (0.5 g./100 ml.), 1 ml. of an eosine solution (0.5 g./100 ml.) and 2 ml. of water. Similarly, I_U (3-0-3) and I_E(0-3-3), for example, refer respectively to the systems of (3 ml. of a uranine solution)+(3 ml. of water) and (3 ml. of an eosine solution)+(3 ml. of water). In every case, the I_{UE}-curve is lower than the corresponding (I_U+I_E) curve (shown by a dotted line); that is, an enfeebling action was found under these experimental conditions. Figure 4 involves similar intensity-time curves, which have been measured with the systems except that the 3 N sodium hydroxide solution is replaced by a 1.5 N solution. In this case, the I_{UE}-curve is slightly higher than the (I_U+I_E)-curve; that is, the enhancing action is exhibited under the conditions employed. This enhancing

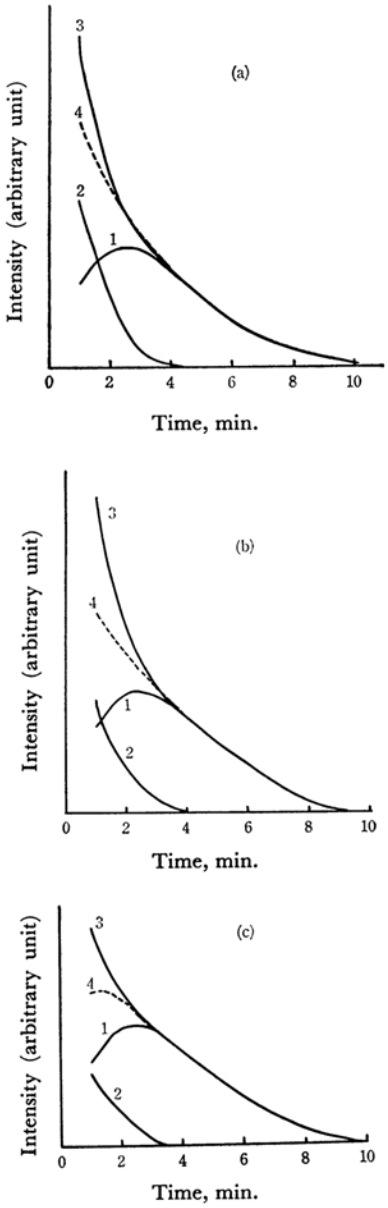

Fig. 4. *I-t* curves in uranine-eosine systems. (1.5 N NaOHaq., 20% H_2O_2aq., 45°C)

(a) { 1: I_U (3-0-3); 2: I_E (0-3-3); 3: I_{UE} (3-3-0); 4: I_U+I_E }
(b) { 1: I_U (3-0-3); 2: I_E (0-2-4); 3: I_{UE} (3-2-1); 4: I_U+I_E }
(c) { 1: I_U (3-0-3); 2: I_E (0-1-5); 3: I_{UE} (3-1-2); 4: I_U+I_E }

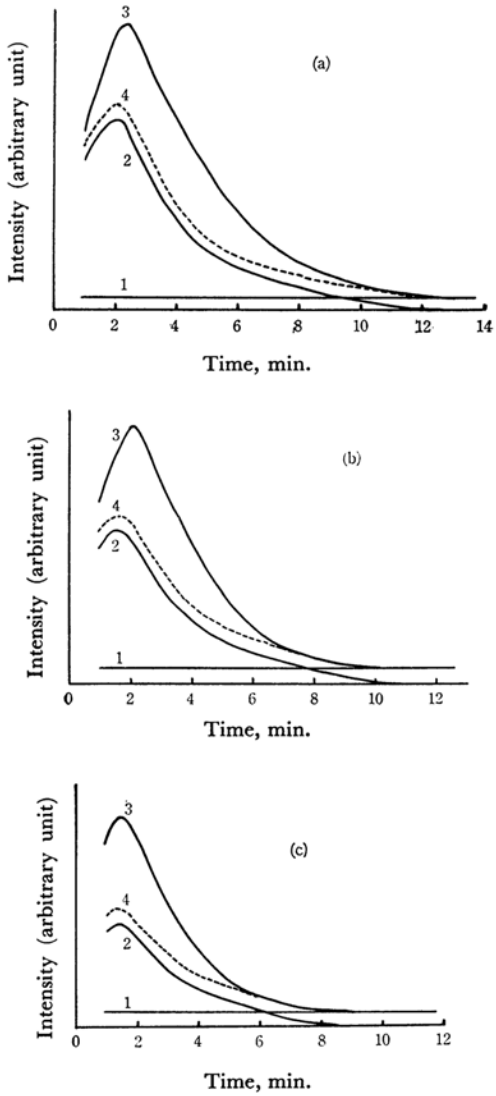

Fig. 5. *I-t* curves in uranine-eosine systems. (0.5 N NaOHaq., 20% H_2O_2aq., 45°C)

(a) { 1: I_U (3-0-3); 2: I_E (0-3-3); 3: I_{UE} (3-3-0); 4: I_U+I_E }
(b) { 1: I_U (3-0-3); 2: I_E (0-2-4); 3: I_{UE} (3-2-1); 4: I_U+I_E }
(c) { 1: I_U (3-0-3); 2: I_E (0-1-5); 3: I_{UE} (3-1-2); 4: I_U+I_E }

TABLE III. THE VALUES OF Δ AND σ AS A FUNCTION OF DYE CONCENTRATION AT 40°C
(0.3 N NaOHaq., 20% H_2O_2aq.)

Dye concentration (Ratio to original solution)						
Uranine	Eosine	$\int I_U dt$	$\int I_E dt$	$\int I_{UE} dt$	Δ	σ
1/2	1/2	1.5	35.5	80.0	43.0	1.3
1/2	1/4	1.5	17.5	50.0	31.0	1.8
1/2	1/8	1.5	9.1	33.0	22.4	2.5
1/2	1/16	1.5	3.5	16.3	11.3	3.2
1/2	1/32	1.5	1.5	9.8	6.8	4.5
1/2	1/64	1.5	0.6	6.0	3.9	6.5
1/2	1/2	1.5	35.5	80.0	43.0	1.3
1/4	1/2	0.8	35.5	66.0	29.7	0.83
1/8	1/2	0.4	35.5	52.0	16.1	0.44
1/16	1/2	0.2	35.5	46.0	10.3	0.30
1/32	1/2	0.0	35.5	41.8	6.3	0.17
1/64	1/2	0.0	35.5	39.0	3.5	0.078

TABLE IV. EFFECT OF TEMPERATURE ON THE VALUES OF Δ AND σ IN AQUEOUS SYSTEM WITH $[U]_0/[E]_0=1$
(0.5 N NaOHaq., 20% H_2O_2aq.)

Temp., °C	$\int I_U dt$	$\int I_E dt$	$\int I_{UE} dt$	Δ	σ
37	0.0	8.0	17.5	9.5	1.2
29	0.0	5.2	11.4	6.2	1.2
20	0.0	3.5	7.75	4.25	1.2
9	0.0	1.6	3.47	1.87	1.1

TABLE V. THE EFFECT OF THE CONCENTRATION OF ADDED ORGANIC SOLVENTS ON THE VALUES OF Δ AND σ
($[U]_0/[E]_0=5$, 0.5 N NaOHaq., 20% H_2O_2aq., 45°C)

Solvent	% (In volume)	$\int I_U dt$	$\int I_E dt$	$\int I_{UE} dt$	Δ	σ
Water	—	192.4	43.1	324.3	88.8	2.0
Methanol	20	220.8	80.3	461.2	160.1	2.0
	30	398.4	124.2	768.3	245.7	2.0
	40	405.8	153.8	839.8	280.2	1.8
	60	632.8	207.7	1244.3	403.8	1.9
Ethanol	20	408.0	135.1	812.5	269.4	2.0
	30	547.8	169.0	1059.2	342.4	2.0
	40	796.8	223.6	1488.0	467.6	2.1
	60	1223.2	259.6	1999.6	516.8	2.0
n-Propanol	20	474.5	122.4	839.7	242.8	2.0
	30	481.4	131.8	882.2	269.0	2.0
	60	921.4	198.1	1495.0	375.5	1.9
Isopropanol	20	559.3	128.6	953.0	265.1	2.1
	30	654.0	131.1	1066.7	281.6	2.1
	40	880.0	165.2	1404.0	358.8	2.1
Glycerol	20	174.0	73.0	409.4	162.4	2.2
	30	184.1	96.2	491.6	211.3	2.2
	40	193.9	122.2	571.8	255.7	2.1
	50	250.4	168.3	739.3	320.6	1.9
Dioxane	20	472.7	156.6	898.0	268.7	1.7
	30	723.2	217.0	1314.2	374.0	1.7
	40	910.8	245.0	1560.6	404.8	1.7
	50	1626.6	258.6	2294.0	408.8	1.6

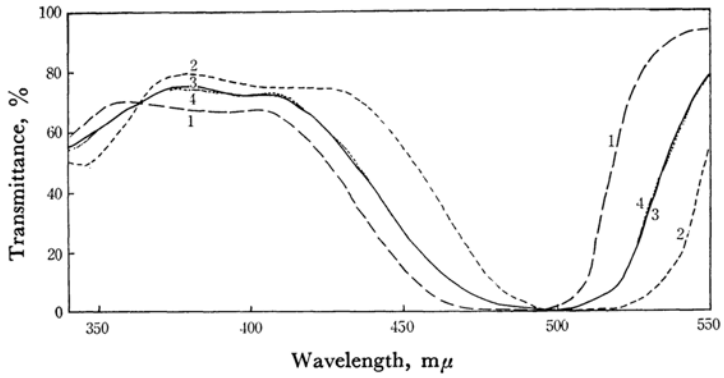

Fig. 6. Absorption spectra of uranine, eosine and uranine-eosine systems. (1/2 conc. to original solution, 0.5 N NaOHaq.)
1: T_U; 2: T_E; 3: T_{UE}; 4: $\sqrt{T_U \times T_E}$

action becomes stronger when 2 ml. of a 0.5 N sodium hydroxide solution is used instead. Hence, it may be concluded that the enhancing action arises only in a system of low alkali concentration.

It is worth noting that every I_{UE}-curve in Fig. 5 approaches the I_U-curve when the I_E-curve falls to zero. This allows the "increment of the total light emission" to be defined as:

$$\Delta = \int_0^T (I_{UE} - I_U - I_E)\mathrm{d}t \qquad (5)$$

where I_{UE}, I_U and I_E are the intensities of the emission measured in uranine-eosine, uranine and eosine systems respectively, and T is the duration of eosine chemiluminescence. Furthermore, the results indicate that the enhancing action can be seen only when eosine chemiluminescence takes place; in other words, the processes of eosine chemiluminescence seem to be necessary for the enhancing action. In a system of such a low alkali concentration, uranine decomposes so slowly that the intensity of its chemiluminescence is practically zero, so the ratio of Δ to the total light emission of eosine chemiluminescence can be taken as the value of the "relative increment," σ:

$$\sigma = \Delta \Big/ \int_0^T I_E \mathrm{d}t \qquad (6)$$

The values of Δ and σ were investigated under various experimental conditions. In order to obtain the pertinent values for I_{UE}, I_U and I_E, the I-t cruves were integrated graphically.

The Dependence of Δ and σ on the Concentration of the Dye.—The values of Δ and σ measured in systems with different uranine concentrations but with a constant eosine concentration are shown in Table III. The Δ and σ values in the systems with different eosine concentrations but with a constant uranine concentration are also shown in Table III. These results indicate that Δ increases with an increase in the initial concentration of either uranine ($[U]_0$) or eosine ($[E]_0$), while σ increases only when the ratio of $[U]_0/[E]_0$ increases. As will be discussed in a succeeding paper, Δ and σ are given by:

$$\Delta = M[U]_0 \log \{1 + N([E]_0/[U]_0)\}$$

$$\sigma = M'([U]_0/[E]_0) \log \{1 + N([E]_0/[U]_0)\}$$

where M, M' and N are the parameters.

The Temperature Dependence of Δ and σ.—Table IV illustrates the values of Δ and σ as measured in systems of (3-3-0), (3-0-3) and (0-3-3) at several different temperatures. It is there shown that Δ increases with an increase in the temperature, while σ is almost completely insensitive to the temperature.

The Dependence of Δ and σ on the Concentration of Added Organic Solvent.—It has been found that the total amount of emission is enhanced when organic solvents, such as alcohol, acetone, dioxane and glycerol, are added to the luminescent system. Therefore, Δ and σ have been determined in several water-organic solvent systems (Table V). We see that Δ increases as the concentration of the organic solvent increases, while σ remains constant. The results will be discussed in a succeeding paper.

The Absorption Spectrum of the Uranine-Eosine System.—The enhancing action arises from an interaction between uranine and eosine. To decide what kind of the interraction this is, the absorption spectrum of the uranine-eosine system was measured. The results are shown in Fig. 6, where T_U and T_E are the absorption curves of the solutions of uranine (0.5 g./100 ml.) and eosine (0.5 g./100 ml.), and T_{UE} is that of a uranine-eosine mixed solution (0.5 g. uranine and 0.5 g. eosine per 100 ml.). It can be seen that the I_{UE}-curve is very close to the $\sqrt{T_U \times T_E}$-curve (shown by a dotted line). This fact indicates that no stable substance is produced when the two dyes are mixed.

The Rates of the Decomposition of Uranine and Eosine in the Uranine-Eosine System.—

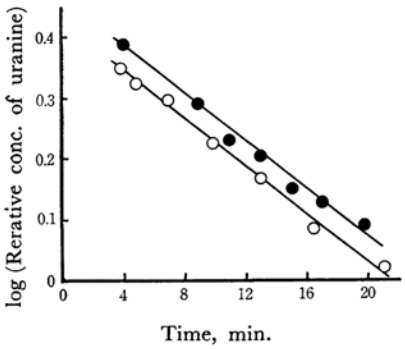

Fig. 7. Rates of uranine decomposition in uranine eosine system and in isolated uranine system.
(0.5 N NaOHaq., 20% H_2O_2aq., 41°C)
-●- Isolated uranine system
-○- Uranine-eosine system

The rate of the decomposition of uranine in the system of uranine-eosine can easily be determined by spectrophotometry at 490 mμ, bceause practically no eosine absorbed that light. The measurements were carried out in a system composed of 12 ml. of a (6-6-0) sample solution, 4 ml. of a 0.5 N sodium hydroxide solution and 2 ml. of a 20% hydrogen peroxide solution. The results are shown in Fig. 7. It shows that the decomposition of uranine in the uranine-eosine system is first order with respect to the uranine concentration. The rate constant is 0.048 min^{-1}, which is almost identical with the decay constant of uranine chemiluminescence in an isolated uranine system under the same experimental conditions. Hence, it may be concluded that the rate of uranine decomposition is not affected by the addition of eosine.

As has been shown before, the I_{UE}-curve approaches the I_U-curve when the I_E-curve falls to zero. This fact would mean that the duration of eosine chemiluminescence in mixed system is the same as that in an isolated system, because the enhancing action is found only when the chemiluminescent reaction of eosine takes place. Hence, it seems that the rate decomposition of eosine is little affected by the addition of uranine.

It follows from the above considerations that the enhancing action is due neither to the production of a stable substance which emits strongly nor to the increase in the rate of the decomposition of uranine or eosine.

One possible mechanism for the enhancing action would be that eosine is oxidized to an energy-rich intermediate at the first stage of the reaction; then the energy is transferred from the intermediate to an oxidized dye, thereby exciting both eosine and uranine to emit fluorescence. The same mechanism for the light production was proposed by Kautsky,[5] who studied the luminescent reaction of siloxen ($Si_6O_3H_6$) in the presence of rhodamine. He found that the spectral distribution of the luminescence was similar to that of the fluorescence of rhodamine, although the dye was not oxidized; he concluded that energy was transferred from siloxene to the dye by some sort of collision. Similar results have been reported by Schales,[6] Tamamushi[7] and Vassil'ev[8].

Spruit van der Burg[9] has reported that the emission of lucigenin chemiluminescence is blue in a hot, dilute solution and that it may arise from the energy-rich product, methyl acridone, while the emission is green in a cold, concentrated solution. This fact is suggestive of the same energy-transfer mechanism as that of the emission in the uranine-eosine system.

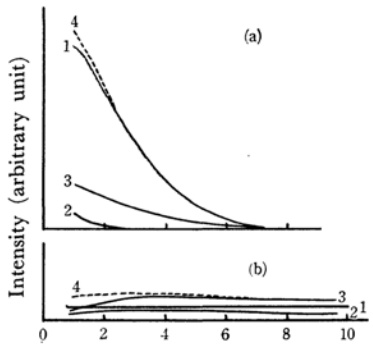

Fig. 8. *I-t* curves in uranine-erythrosine systems.
(a) 3 N NaOHaq., 20% H_2O_2aq., 45°C
(b) 0.5 N NaOHaq., 20% H_2O_2aq., 45°C
1: I_U (3-0-3) 2: I_{Er} (0-3-3)
3: I_{UEr} (3-3-0) 4: I_U+I_{Er}

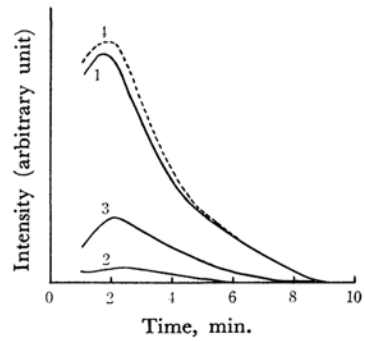

Fig. 9. *I-t* curves in eosine-erythrosine system.
(0.5 N NaOHaq., 20% H_2O_2aq., 45°C)
1: I_E (3-0-3) 2: I_{Er} (0-3-3)
3: I_{EEr} (3-3-0) 4: I_E+I_{Er}

Chemiluminescence in such other systems as uranine-erythrosine and eosine-erythrosine has also been investigated; the results are illustrated in Figs. 8 and 9. They exhibit no enhancing action.

5) H. Kautsky and H. Zocher, *Z. Phys.*, **9**, 267 (1922).
6) O. Schales, *Ber.*, **172**, 1155 (1939).
7) B. Tamamushi, *Naturwiss.*, **28**, 722 (1940).
8) R. F. Vassil'ev, *Nature*, **196**, 668 (1962).
9) A. Spruit van der Burg, *Rec. Trav. Chim.*, **69**, 1526 (1950).