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Studies of the Chemiluminescence of Several Xanthene Dyes. I. Kinetic Studies of Uranine and Eosine Chemiluminescence

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A kinetic study of the chemiluminescence in alkaline solutions of several xanthene dyes with hydrogen peroxide was carried out in the hope of gaining some insight into the mechanism of the luminescent reaction. When the concentrations of sodium hydroxide and hydrogen peroxide were kept constant, the decay curve of the luminescence was represented by $I_t = A\exp(-at)$, where I_t is the light intensity at time t ; a is equal to the rate constant of the decomposition of the dye (first order with respect to the dye concentration) and A is a parameter proportional to the initial dye concentration. The lifetime of the luminescent reaction was prolonged by the addition of such organic solvents as alcohol and dioxane; the a value decreased, while the A value remained unchanged. Both the a value and the A value increased with the elevation of the reaction temperature.

In 1924, Keaney[1] observed a glow when ozonized oxygen was passed through a solution of aesculin in water, methyl alcohol, ethyl alcohol or propyl alcohol. A short time later, Biswas and Dhar[2] investigated a similar glow, using fifty different fluorescent and non-fluorescent dyes; they concluded that the luminescent intensity was roughly proportional to the fluorescence efficiency of the dyes. Moreover, they found that the same glow was obtained when the dyes were oxidized by hydrogen peroxide, using ferrous sulfate as a catalyst. Strehlar and Shoup[3] have discovered the chemiluminescence of riboflavin and showed that the spectral energy distribution of the luminescence is very similar to that of the fluorescence emission of riboflavin.

Thus, it is very probable that the light emitted in the chemiluminescence of a fluorescent dye is the fluorescence emission of the dye, although little is known as to how the dye can be excited to emit fluorescence in the chemiluminescent reaction. It was with this in mind that the present authors[4] began to study the chemiluminescence of several xanthene dyes.

It has been established that, when the dyes are oxidized by hydrogen peroxide, hydroxyl ion, iodide ion, hypochlorite ion or lead acetate acts as a catalyst for the light production, as does ferrous sulfate. Moreover, when a hydrogen peroxide solution is added to alkaline solutions of the dyes, the emission is very much enhanced and is quite reproducible.

For the present paper, kinetic studies of uranine and eosine chemiluminescence were carried out in order to establish the mechanism for light production.

Experimental

The Preparation of the Luminescent System.—Two milliliters of a sodium hydroxide solution was added to 6 ml. of a uranine or eosine solution; the mixed solution was then kept at a definite temperature. After 1 ml. of a hydrogen peroxide solution has been added to the mixed solution, a portion of the reacting solution (4—5 ml.) was transferred quickly into a cell (2.8 cm. high, 2.0 cm. wide and 1.0 cm. thick); then the cell was placed in a thermostat, with water circulating at a constant temperature.

Uranine and eosine were recrystallized from a 60—70% ethanol solution. One gram of each dye was dissolved into 100 ml. of water or a water-organic solvent mixture in order to prepare the original solution; when necessary, this was further diluted to 1/2, 1/4, 1/8, and so on. The sodium hydroxide, the hydrogen peroxide solution (30 %) and organic solvents of an analytical grade were used without further purification.

Measurements of the Light Intensity versus the Time.—The light-measuring apparatus is shown diagramatically in Fig. 1. It was composed of a small thermostat and a photomultiplier-tube box, both of which were covered with a large dark box. The reaction cell in the thermostat was clamped on so as to face a glass window, through which the emitted light was

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[1] M. Kearney, *Phil. Mag.*, **47**, 648 (1924).

[2] N. N. Biswas and N. R. Dhar, *Z. anorg. u. allgem. Chem.*, **173**, 125 (1928); **186**, 154 (1930); **144**, 400 (1931).

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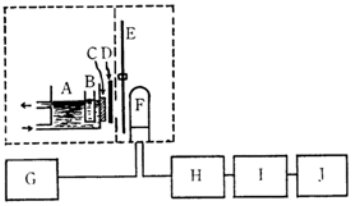

Fig. Diagram of measuring system.

A, Small thermostat. B, Reaction cell. C, Glass window. D, Shutter. E, Sector. F, Photomultiplier. G, High-voltage supply. H, Cathode follower. I, Linear amplifier. J, Self-recording micro-ammeter.

detected with a photomutiplier-tube (Matzuda MS9SY). The tube was coupled with a linear amplifier, and the output current was lead to a self-recording micro-ammeter, so that the curve of the light intensity versus the time could be recorded automatically.

Measurements of the Decomposition Rate of the Dyes.—The dyes were decomposed during the luminescent reaction; no emission was observed when the dye was not decomposed. In order to determine the decomposition rate of the dye, 1 ml. of the reacting solution was withdrawn at a definete interval in order to mix it with 250 ml. of water (the reaction was stopped upon dilution); the concentration of unoxidized dye was determined spectro-photometrically with a Shimadzu QR-50-type electro-spectrophotometer, at the wavelength of 492 mμ for uranine and of 517 mμ for eosine.

Experimental Results

In general, the curve of the emission intensity versus the time (the *I-t* curve) rises to reach a maximum at the initial stage and then decays slowly. The *I-t* curve was reproducible except in the initial stage. (The initial curve was not quite reproducible because hydrogen peroxide decomposed after a short induction period.)

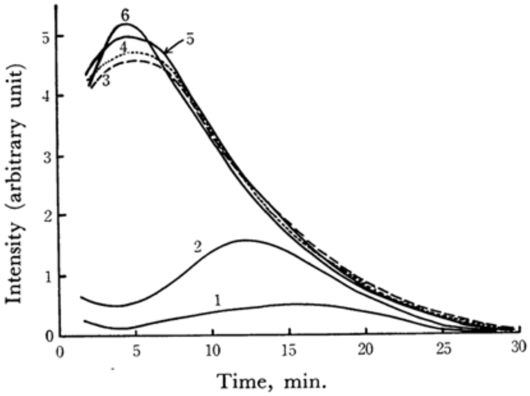

Fig. 2. Effect of hydrogen peroxide concentration on *I-t* curve for uranine chemiluminescence. (Original solution, 2.5 N NaHOaq., 30°C)
(1) 5%, (2) 10%, (3) 15%, (4) 20%, (5) 25%, (6) 30%

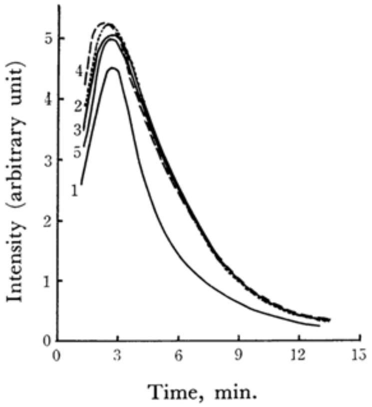

Fig. 3. Effect of hydrogen peroxide concentration on *I-t* curve for eosine chemiluminescence. (Original solution, 0.5 N NaOHaq., 41°C)
(1) 10%, (2) 15%, (3) 20%, (4) 25%, (5) 30%

The Effect of the Hydrogen Peroxide Concentration on the *I-t* Curve—The results are shown in Figs. 2 and 3. It is there demonstrated that the decay curve is little affected by the change in the concentration of the added hydrogen peroxide solution when its concentration is comparatively high (20—30%). When the concentration is low, however, the *I-t* curve becomes lower and is greatly affected by a change in the concentration of the added hydrogen peroxide solution. The optimum concentration of the added hydrogen peroxide solution for the kinetic studies is found to be 20—30%.

The Effect of the Sodium Hyroxide Concentration on the *I-t* Curve.—The correlation between the *I-t* curve and the added sodium

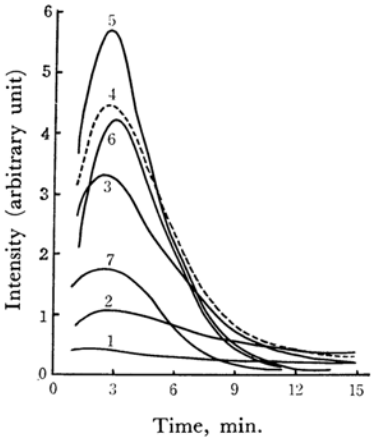

Fig. 4. Effect of sodium hydroxide concentration on *I-t* curve for uranine chemiluminescence. (1/4 conc. to original solution, 15% H_2O_2aq., 45°C)
(1) 0.5 N, (2) 1.0 N, (3) 1.5 N, (4) 2.0 N, (5) 2.5 N, (6) 3.0 N, (7) 4.0 N

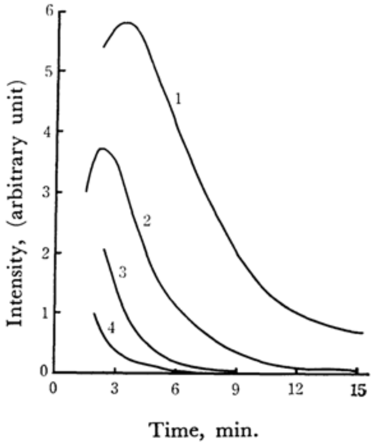

Fig. 5. Effect of sodium hydroxide concentration on *I-t* curve for eosine chemiluminescence.
(1/2 conc. to original solution, 30% H_2O_2aq., 41°C)
(1) 0.5 N, (2) 1.0 N, (3) 2.0 N, (4) 3.0 N

hydroxide concentration is not so simple, because the aspects of the *I-t* curve differ according to the concentration of hydrogen peroxide as well as according to the dye concentration. The effect is probably due to the fact that the alkali concentration controls both the rate of the decomposition of hydrogen peroxide and the luminescent reactivity of the dye. Typical examples are shown in Fig. 4 for uranine and in Fig. 5 for eosine. These figures show that the optimum concentration of the added sodium hydroxide solution should be 2—3 N for uranine and 0.5 N for eosine.

The Effect of the Dye Concentration on the *I-t* Curve.—*I-t* curves measured at 16°C in uranine chemiluminescent systems of different

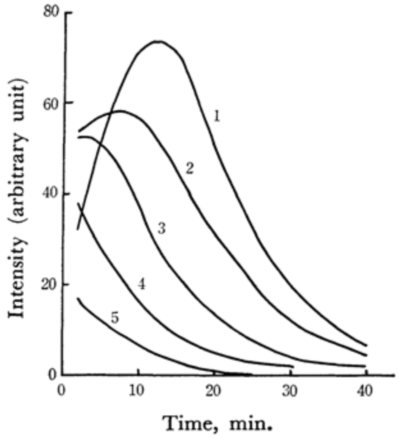

Fig. 6. Effect of uranine concentration on *I-t* curve for uranine chemiluminescence.
(3.0 N NaOHaq., 20% H_2O_2aq., 16°C)
(1) Original solution, (2) 1/2 conc. to original solution, (3) 1/4 conc. to original solution, (4) 1/8 conc. to original solution, (5) 1/16 conc. to original solution.

TABLE I. THE EFFECT OF VARYING URANINE CONCENTRATION ON THE INTENSITY-TIME RELATION
(3 N NaOHaq., 20% H_2O_2aq., 16°C)

Dye concn.	Original soln.		1/2 concn. to original soln.		1/4 concn. to original soln.		1/8 concn. to original soln.		1/16 concn. to original soln.	
	Eq. 1a		Eq. 1b		Eq. 1c		Eq. 1d		Eq. 1e	
Time min.	Calcd.	Obs.	Calcd.	Obs.	Calcd.	Obs.	Calcd.	Obs.	Calcd.	Obs.
2	32.3	32.0	42.0	53.0	51.6	54.0	39.3	35.5	17.6	16.8
4	42.7	44.0	49.4	57.0	50.3	51.0	32.3	30.0	15.2	14.5
6	53.6	54.5	54.9	59.0	46.1	47.0	26.3	25.0	11.3	10.5
8	65.9	64.2	58.7	60.0	41.5	41.5	21.5	20.0	9.1	8.5
10	75.0	71.0	57.5	57.5	34.7	35.0	17.7	16.5	7.3	7.0
12	77.2	74.2	52.5	52.5	29.2	29.5	14.4	13.5	6.5	5.5
14	76.1	71.0	48.6	48.0	24.7	24.0	11.8	10.5	4.8	4.5
16	67.9	65.2	43.8	43.0	20.2	19.5	9.7	9.0	3.7	3.0
18	59.5	59.5	37.9	37.0	16.5	16.0	7.0	7.0	2.5	2.2
20	50.5	52.0	32.0	32.0	13.5	13.5	6.5	6.0	2.2	2.0
22	43.3	43.3	28.0	28.0	11.5	11.5	5.3	5.0	0.9	1.5
24	36.3	36.5	23.0	23.5	8.2	6.9	4.4	4.5	0.7	0.5
26	29.6	33.2	18.0	18.5	6.1	6.0	3.9	3.5	—	—
28	24.3	30.0	16.0	15.0	5.1	5.0	2.9	2.8	—	—
30	20.0	24.0	13.4	12.5	4.1	4.0	2.4	2.4	—	—
32	16.3	16.8	11.2	11.5	3.3	3.0	—	—	—	—
34	13.4	13.5	9.4	9.8	2.7	2.5	—	—	—	—
36	10.9	10.5	7.6	7.5	—	—	—	—	—	—
38	8.9	8.5	6.4	6.5	—	—	—	—	—	—
40	7.3	8.0	5.4	4.5	—	—	—	—	—	—

dye concentrations are shown in Fig. 6, where the concentrations of the added sodium hydroxide and hydrogen peroxide solutions are 3.0 N and 20% respectively (optimum concentration). From numerical analysis, every curve can be represented by the following general equation (1):

$$I_t=A\exp(-at)/\{1+B\exp(-bt)\} \qquad (1)$$

where I_t is the emission intensity at time t; a is the decay costant, and A, B and b are parameters which differ according to the experimental conditions. The curves shown in Fig. 6 can be represented by the following equation:

Uranine concn. (ratio to the original soln.)	Equation
1	$I_t=400\exp(-0.10t)/\{1+16\exp(-0.28t)\}$
1/2	$I_t=200\exp(-0.09t)/\{1+5\exp(-0.25t)\}$
1/4	$I_t=100\exp(-0.10t)/\{1+\exp(-0.28t)\}$
1/8	$I_t=48\exp(-0.10t)$
1/16	$I_t=22\exp(-0.10t)$

Table I shows both the calculated values and the observed values. The calculated values are in fairly good agreement with the observed values except for a slight deviation at the initial stage. From the results, the following important conclusions are obtained:

i) Since the second term of the denominator in Eq. 1, $B\exp(-bt)$, can be disregarded except in the initial stage, the decay part of the *I-t* curve is represented by:

$$I_t=A\exp(-at) \qquad (2)$$

ii) The decay constant, a, is not affected by the dye concentration.

iii) The value of A is proportional to the initial dye concentration. This fact indicates that the luminescent decay is of the first order with respect to the dye concentration.

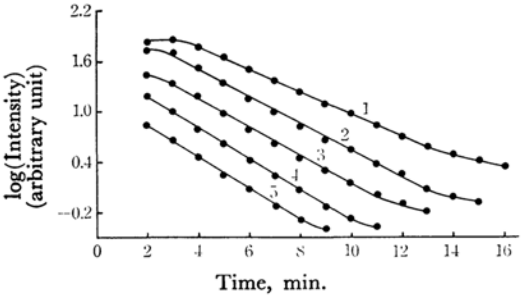

Fig. 7. Effect of eosine concentration on log *I-t* curve for eosine chemiluminescence.
(0.5 N NaOHaq., 30% H_2O_2aq., 41°C)
(1) Original solution, (2) 1/2 conc. to original solution, (3) 1/4 conc. to original solution, (4) 1/8 conc. to original solution, (5) 1/16 conc. to original solution

The intensities of emission measured at 41°C in eosine chemiluminescent systems of different dye concentrations are shown in Fig. 7, where the logarithm of I_t is plotted against the time. These plots are almost on straight lines except in the initial and final stages. Therefore, the decay curves may also be represented as follows:

Eosine concn. (ratio to the original soln.)	Equation
1	$I_t=180\exp(-0.28t)$
1/2	$I_t=150\exp(-0.41t)$
1/4	$I_t=78\exp(-0.41t)$
1/8	$I_t=39\exp(-0.41t)$
1/16	$I_t=20\exp(-0.41t)$

It is shown that, when the concentration of eosine is comparatively high, the values of A and a are smaller than the expected values. Moreover, the curves deviate from Eq. 2 at the final stages in both uranine and eosine chemiluminescence.

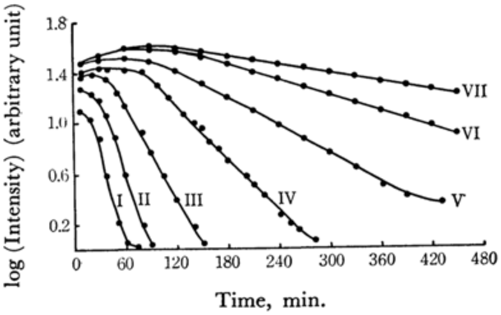

Fig. 8. Effect of methanol concentration on log *I-t* curve for uranine chemiluminescence.
(Original solution, 2.5 N NaOHaq., 20% H_2O_2aq., 20°C)
I 0%, II 15%, III 25%, IV 35%, V 45%, VI 55%, VII 65%

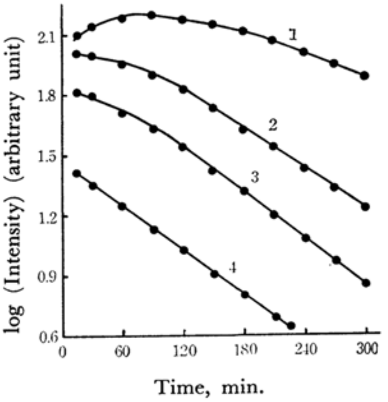

Fig. 9. Effect of uranine concentration on log *I-t* curve in 65% methanol system at 26°C.
(1) Original solution, (2) 1/2 conc. to original solution, (3) 1/4 conc. to original solution, (4) 1/8 conc. to original solution

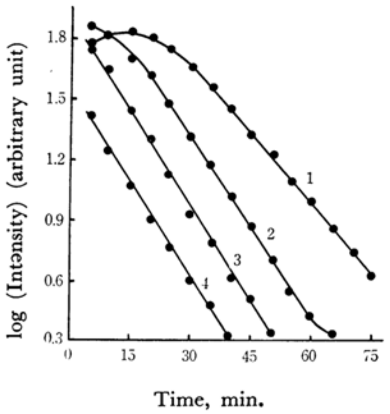

Fig. 10. Effect of uranine concentration on log I-t curve in 25% methanol system at 28°C. (1) Original solution, (2) 1/2 conc. to original solution, (3) 1/4 conc. to original solution, (4) 1/8 conc. to original solution

This deviation will be discussed in a succeeding paper.

The Effect of an Added Organic Solvent on the *I-t* Curve.—In general, the emission intensity was enhanced when such organic solvents as alcohol, dioxane and glycerol were added to the system. The I-t curves obtained from uranine chemiluminescence in water-methanol systems of different methanol concentrations at 20°C, with the uranine concentration kept constant, are shown in Fig. 8. The curves obtained from the systems with different uranine concentrations and with a constant methanol concentration are shown in Figs. 9 and 10. These curves were also represented as:

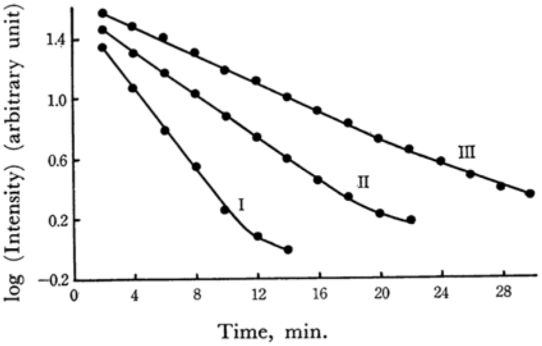

Fig. 11a. Effect of methanol concentration on log I-t curve for eosine chemiluminescence.
(1/8 conc. to original solution, 0.5 N NaOHaq., 30% H_2O_2aq., 41°C)
I 10%, II 30%, III 50%

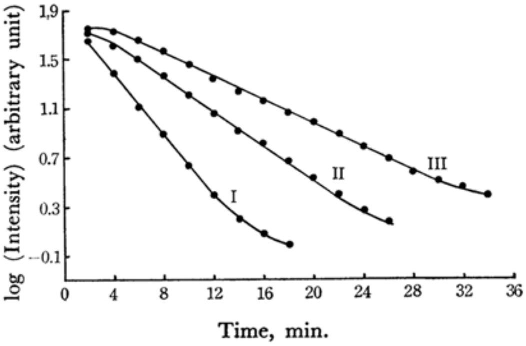

Fig. 11b. Effect of methanol concentration on log I-t curve for eosine chemiluminescence.
(1/4 conc. to original solution, 0.5 N NaOHaq., 30% H_2O_2aq., 41°C)
I 10%, II 30%, III 50%

TABLE II. EFFECT OF THE CONCENTRATION OF ADDED ORGANIC SOLVENT ON THE VALUES OF A AND a IN URANINE AND EOSINE CHEMILUMINESCENCE

Solvent	%	Dye	Concn.	NaOHaq., N	Temp., °C	A	a
Ethanol	20	Uranine	1/2	2.5	20	105	0.030
Ethanol	40	Uranine	1/2	2.5	20	105	0.015
Ethanol	60	Uranine	1/2	2.5	20	91	0.005
Isopropanol	20	Uranine	1/2	2.5	20	102	0.027
Isopropanol	20	Eosine	1/2	0.5	30	127	0.071
Isopropanol	40	Uranine	1/2	2.5	20	98	0.008
Isopropanol	40	Eosine	1/2	0.5	30	126	0.022
Isopropanol	60	Eosine	1/2	0.5	30	126	0.009
n-Propanol	20	Uranine	1/2	2.5	20	99	0.019
n-Propanol	40	Uranine	1/2	2.5	20	99	0.006
n-Propanol	60	Uranine	1/2	2.5	20	80	0.004
Dioxane	20	Uranine	1/2	2.5	20	109	0.030
Dioxane	20	Eosine	1/2	0.5	30	120	0.098
Dioxane	40	Uranine	1/2	2.5	20	104	0.016
Dioxane	40	Eosine	1/2	0.5	30	118	0.050
Dioxane	60	Eosine	1/2	0.5	30	107	0.021
Glycerol	20	Uranine	1/2	2.5	20	104	0.031
Glycerol	40	Uranine	1/2	2.5	20	105	0.021

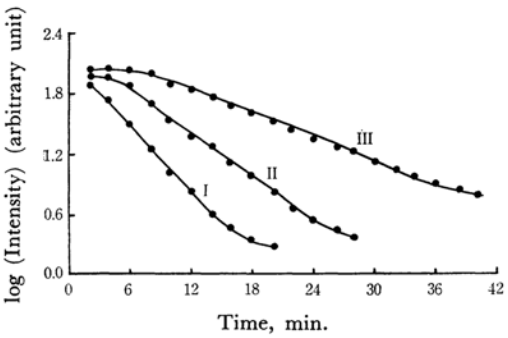

Fig. 11c. Effect of methanol concentration on log *I*-*t* curve for eosine chemiluminescence.
(1/2 conc. to original solution, 0.5 N NaOHaq., 30% H_2O_2aq., 41°C)
I 10%, II 30%, III 50%

Curves in Fig. 8 (original uranine soln.)

Methanol concn. (% in volume)	Equation
0	$I_t=100\exp(-0.078t)/\{1+7.4\exp(-0.11t)\}$
15	$I_t=100\exp(-0.051t)/\{1+6.0\exp(-0.10t)\}$
25	$I_t=100\exp(-0.030t)/\{1+3.9\exp(-0.067t)\}$
35	$I_t=100\exp(-0.016t)/\{1+3.2\exp(-0.050t)\}$
45	$I_t=91\exp(-0.0091t)/\{1+2.6\exp(-0.029t)\}$
55	$I_t=73\exp(-0.0047t)/\{1+2.0\exp(-0.024t)\}$
65	$I_t=68\exp(-0.0043t)/\{1+1.7\exp(-0.022t)\}$

Curves in Fig. 9 (65% methanol soln.)

Dye concn. (ratio to the original soln.)	Equation
1	$I_t=290\exp(-0.0045t)/\{1+1.4\exp(-0.019t)\}$
1/2	$I_t=210\exp(-0.0085t)/\{1+1.1\exp(-0.016t)\}$
1/4	$I_t=94\exp(-0.0085t)/\{1+0.35\exp(-0.021t)\}$
1/8	$I_t=30\exp(-0.0085t)$

Curves in Fig. 10 (25% methanol soln.)

Dye concn. (ratio to the original soln.)	Equation
1	$I_t=347\exp(-0.067t)/\{1+4.9\exp(-0.10t)\}$
1/2	$I_t=172\exp(-0.067t)/\{1+\ \exp(-0.10t)\}$
1/4	$I_t=84\exp(-0.074t)$
1/8	$I_t=37\exp(-0.074t)$

TABLE III. EFFECT OF TEMPERATURE ON THE VALUES OF *A* AND *a* UNDER SEVERAL EXPERIMENTAL CONDITIONS

1. Uranine chemiluminescence in aqueous solution
(1/4 concn. to original solution, 20% H_2O_2aq.)

NaOHaq., N	Temp., °C	*A*	*a*
3.0	21	155	0.13
3.0	31	800	0.35
3.0	41	3100	0.47
3.0	50	7700	1.02
3.0	59	17400	1.63
2.0	21	105	0.072
2.0	31	580	0.20
2.0	41	2640	0.40
2.0	50	6660	0.70
2.0	59	14000	1.05

2. Eosine chemiluminescence in aqueous solution
(1/4 concn. to original solution, 0.5 N NaOHaq,, 30% H_2O_2aq.)

Temp., °C	*A*	*a*
21	11	0.11
32	35	0.27
41	80	0.41
51	180	0.77
59	390	1.38

3. Uranine chemiluminescence in water-methanol system (25%).
(Original solution, 2.5 N NaOHaq., 20% H_2O_2aq.)

Temp., °C	*A*	*a*
22	147	0.031
30	412	0.060
39	1540	0.10
51	4980	0.28
57	13800	0.47

4. Eosine chemiluminescence in water-isopropanol system (40%)
(1/2 concn. to original solution, 0.5 N NaOHaq., 30% H_2O_2aq.)

Temp., °C	*A*	*a*
23	83.5	0.017
30	126	0.022
37	221	0.038
45	394	0.063
52	1338	0.14

I-*t* curves obtained from eosine chemiluminescence in water-methanol systems of different eosine and methanol concentrations at 41°C are shown in Fig. 11 (a—c); the corresponding equations are shown below.

Dye concn. (ratio to the original soln.)	Methanol concn. (% in volume)	Equation
1/2	10	$I_t=171\exp(-0.30t)$
1/2	30	$I_t=171\exp(-0.16t)$
1/2	50	$I_t=170\exp(-0.08t)$
1/4	10	$I_t=78\exp(-0.30t)$

1/4	30	$I_t = 72\exp(-0.14t)$
1/4	50	$I_t = 78\exp(-0.08t)$
1/8	10	$I_t = 40\exp(-0.31t)$
1/8	30	$I_t = 38\exp(-0.15t)$
1/8	50	$I_t = 38\exp(-0.08t)$

From these results, it can be seen that the decay constant, a, which is independent of the dye concentration, decreases with an increase in the concentration of added methanol, while A, which is proportional to the initial dye concentration, is little affected by the addition of methanol. It may, therefore, be concluded that the stimulation of luminescence by the addition of methanol is due to the prolongation of the luminescent lifetime. In other water - organic solvent systems, the same correlation is obtained between the values of A and a and the concentration of the added organic solvent. These results are shown in Table II.

The Effect of the Temperature on the *I-t* Curve.—*I-t* curves were also investigated at several different temperatures. Every curve can be represented by Eq. 2. The values of A and a obtained either in an aqueous system or in a water - organic solvent system are listed in Table III. It may be seen that in all of the systems both A and a increase with the increase in the temperature.

The Decomposition Rate of Dye in the Luminescent Reaction.—Typical decomposition curves obtained from uranine and eosine chemiluminescence are shown in Figs. 12 snd 13 respectively, where the logarithm of the dye concentration (in arbitrary units) is poltted against the reaction time. An almost straight line is obtained in each

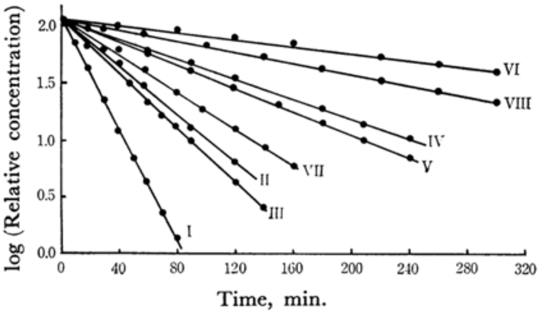

Fig. 12. Rate of uranine decomposition in water-organic solvent systems.
(Original solution, 2.5 N NaOHaq., 20% H_2O_2-aq., 20°C)

I 20% Methanol	V 20% *n*-Propanol
II 40% Methanol	VI 40% *n*-Propanol
III 20% Ethanol	VII 20% Isopropanol
IV 40% Ethanol	VIII 40% Isopropanol

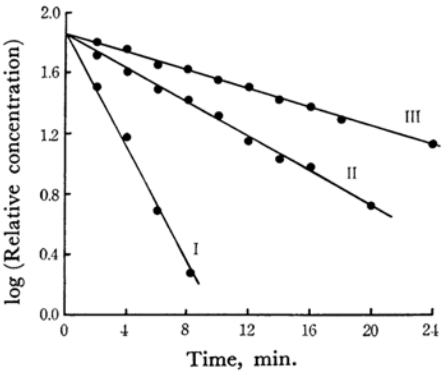

Fig. 13. Rate of eosine decomposition in water-methanol systems.
(1/8 conc. to original solution, 0.5 N NaOHaq., 30% H_2O_2aq., 41°C)
I 10%, II 30%, III 50%

TABLE IV. RATE CONSTANTS OF URANINE DECOMPOSITION AND OF EOSINE DECOMPOSITION UNDER SEVERAL EXPERIMENTAL CONDITIONS

Solvent	%	Dye	Conc.*	NaOHaq. N	H_2O_2aq. %	Temp. °C	Rate const.	a
Water	—	Uranine	1	3	20	16	0.07	0.10
Water	—	Uranine	1/2	3	20	16	0.07	0.09
Water	—	Uranine	1/4	3	20	16	0.07	0.10
Water	—	Uranine	1/8	3	20	16	0.08	0.10
Water	—	Uranine	1/16	3	20	16	0.08	0.10
Methanol	20	Uranine	1	2.5	20	20	0.05	0.050
Methanol	40	Uranine	1	2.5	20	20	0.02	0.025
Ethanol	20	Uranine	1	2.5	20	20	0.03	0.030
Ethanol	40	Uranine	1	2.5	20	20	0.01	0.015
n-Propanol	20	Uranine	1	2.5	20	20	0.01	0.019
n-Propanol	40	Uranine	1	2.5	20	20	0.004	0.006
Isopropanol	20	Uranine	1	2.5	20	20	0.02	0.027
Isopropanol	40	Uranine	1	2.5	20	20	0.005	0.008
Methanol	10	Eosine	1/8	0.5	30	41	0.4	0.31
Methanol	30	Eosine	1/8	0.5	30	41	0.2	0.15
Methanol	50	Eosine	1/8	0.5	30	41	0.08	0.08

* Ratio to the original solution.

case. These results indicate that the decomposition is first order with respect to the dye concentration. The decomposition rate constants under several different expermental conditions are tabulated in Table IV. The values of a (the decay constant) obtained under the same experimental conditions are also given in the last column. It is interesting to note that the decomposition rate constant is almost equal to the corresponding a value in every system.

Studies of several different aspects of chemiluminescence will be presented in a succeeding paper; the combined results, including those of the present experiment, will be discussed thereafter.