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Studies of the Chemiluminescence of Several Xanthene Dyes. V. Blue Emission from an Excited State of a Reaction Product

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We have found that the chemiluminescence emission of several xanthene dyes with hydrogen peroxide is comprised of two emission components; one is a greenish-yellow emission whose spectral distribution is similar to that of the fluorescence of the dye, and the other is a blue emission similar to the fluorescence band which appears during the course of the reaction. Summarizing the results, we can conclude that the chemical formation of an excited species from the oxidation of a dye, and an energy-transfer process from the species to an unoxidized dye, are likely to be the mechanism for the luminescent reaction. Certain dyes, uranin, eosin Y, eosin R, erythrosin B, 2′,7′-dichlorofluorescein, mercurochrome, rhodamine B, rhodamine 6G, and rhodamine S, showed similar characteristic chemiluminescence emissions, whereas phloxine, rose bengale, 4-nitrofluorescein, 4,5,6,7-tetrachlorofluorescein, sulfonfluorescein, and pyronine G did not chemiluminesce under comparable conditions.

In the present study, we have found that the chemiluminescence emission from the reaction of several xanthene dyes with hydrogen peroxide in an alkaline solution is comprised of two emission components; one is a greenish-yellow emission whose spectral distribution is similar to that of the fluorescence of the dye, and the other is a blue emission similar to the fluorescence band which appears during the course of the reaction.

In our previous papers, though only the greenish-yellow emission component could be detected in a concentrated uranin or eosin Y system where appreciable self-absorption occurred,[1] the possibility of such a component as the blue emission from an excited product was considered[2] because the behavior of the emission (the kinetic data[3] and the enhancement effect with a foreign dye[4]) gave support to a reaction scheme; an excited species is produced by the reaction of a dye with a radical produced by the decomposition of hydrogen peroxide, and energy is transferred from the species to an unoxidized dye, thereby exciting the dye to emit fluorescence.

Eosin R, erythrosin B, 2′,7′-dichlorofluorescein, mercurochrome, rhodamine B, rhodamine 6G, and rhodamine S showed a similar characteristic chemiluminescence, whereas such dyes as phloxine, rose bengale, 4-nitrofluorescein, 4,5,6,7-tetrachlorofluorescein, sulfonfluorescein, and pyronine G did not chemiluminesce under comparable conditions.

This paper will describe our experimental results in detail and will present a discussion based on these results.

Experimental

The luminescent systems were prepared by a method essentially identical with that described previously.[3] The reaction was performed by adding 0.5 m*l* of a 20% aqueous solution of hydrogen peroxide to a mixture of 3 m*l* of an aqueous solution or a 50% dimethylsulfoxide solution of a dye (about 1.3×10^{-3} mol/*l*) and 1 m*l* of a 2N or 1/2N aqueous sodium hydroxide in a quartz cell ($10\times10\times45$ mm). The chemiluminescence spectra were measured with a Hitachi MPF-2A type fluorescence spectrophotometer (using a R-104 photomutiplier tube) equipped with a thermostatic cell assembly with water circulating at a constant temperature (40°C). The fluorescence spectra of the reaction systems were also measured by lowering the voltage of the photomultiplier, with the same apparatus with the exciting done at 380 mμ. The slit width for the chemiluminescence measurements was about 40 mμ, whereas that for the fluorescence measurements was about 10 mμ. The scan speed was 5 sec per 10 mμ, the recorded spectral curves were corrected if necessary by means of decay curves measured under the same experimental conditions.

Results

Figure 1 shows the chemiluminescence spectrum in an eosin Y system measured at several different reaction times. The results indicate that the spectrum varies during the course of the reaction. At

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3) I. Kamiya and R. Iwaki, *ibid.*, **39**, 257 (1966).
4) I. Kamiya and R. Iwaki, *ibid.*, **39**, 269 (1966).

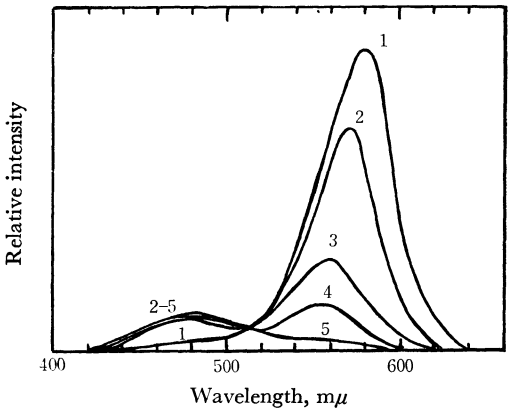

Fig. 1. Variation of the chemiluminescence emission spectrum in eosin Y system (aqueous solution) with reaction time.
1: after 2 min, 2: 4 min, 3: 6 min, 4: 9 min, 5: 15 min

the initial stage, the apparent emission is composed of one intense peak at about 580 mμ, as has been reported previously.[1] During the course of the reaction, however, the intensity of the peak gradually decreases with the blue shift, and simultaneously a new peak appears at about 480 mμ until only the blue emission is observed at the final stage.

The change in the fluoresence spectrum of the chemiluminescent eosin Y system after oxidation was measured*2 with exciting at 380 mμ. The results are illustrated in Fig. 2. It is demonstrated that the fluorescence of the system shows the same spectral change with the reaction time as does the chemiluminescence emission. When we compare

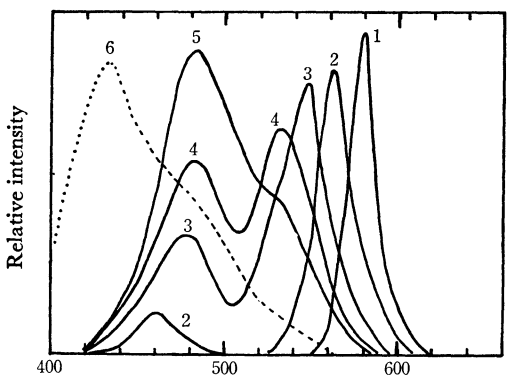

Fig. 2. Variation of the fluoresence emission spectrum in eosin Y system (aqueous solution).
1: before oxidation, 2: after 2 min, 3: 6 min, 4: 12 min, 5: 20 min, 6: 5 hr

*2 In these measurements, no photocurrent caused by feeble chemiluminescence was detected at the photomutiplier-sensitivity at which the fluorescence was detected.

the spectral change in the chemiluminescence with that of the fluorescence, it is apparent that the greenish-yellow emission component is to be attributed to the fluorescence emission from an excited dye, and the blue-emission component to the emission from an excited species produced in the reaction.

The peak at 480 mμ of the blue-fluorescence emission was further blue-shifted to 430 mμ by successive oxidation, as is shown by the dashed line in Fig. 2, but no chemiluminescence emission appeared at this oxidation stage. Hence, it can be said that the primary excited species is an intermediate product.

The changes in both the chemiluminescence and the fluorescence spectra with the reaction time in a uranin system are shown in Figs. 3 and 4 respectively. In the system, the blue emission com-

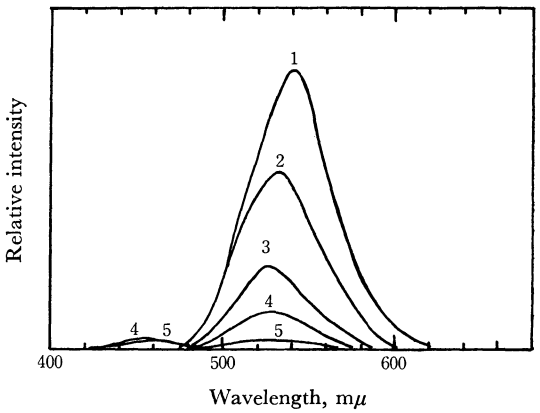

Fig. 3. Variation of the chemiluminescence emission spectrum in uranin system (aqueous solution) with reaction time.
1: after 2 min, 2: 4 min, 3: after 6 min, 4: 9 min, 5: 15 min

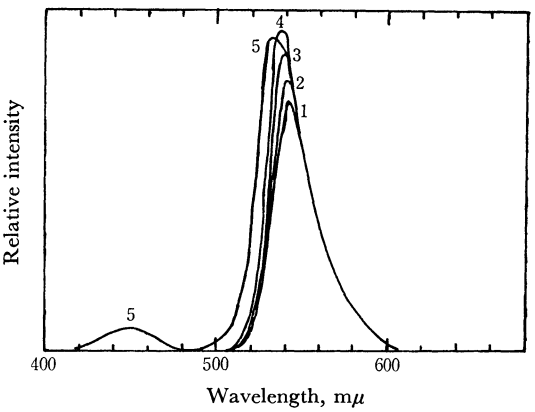

Fig. 4. Variation of the fluorescence emission spectrum in uranin system (aqueous solution) with reaction time.
1: after 2 min, 2: 4 min, 4: 6 min, 4: 12 min, 5: 20 min

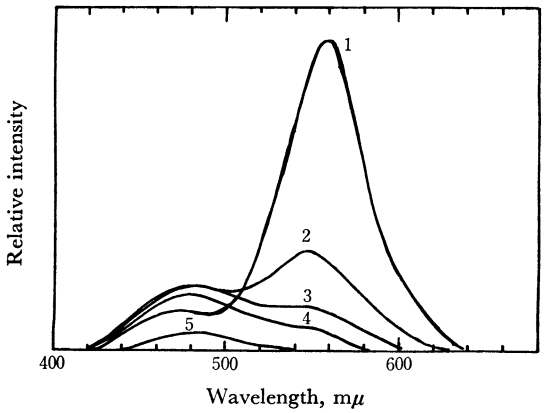

Fig. 5-a-1. Variation of the chmiluminescence emission spectra in the systems of several xanthene dyes with reaction time.
Eosin R system (aqueous solution)
1: after 2 min, 2: 4 min, 3: 6 min, 4: 9 min, 5: 15 min

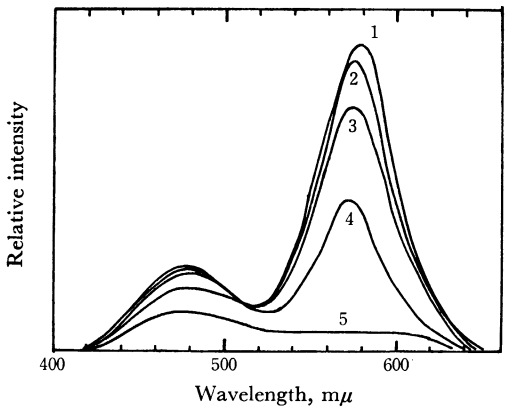

Fig. 5-a-2. Erythrosin B system (50% DMSO solution).
1: after 2 min, 2: 4 min, 3: 6 min, 4: 9 min, 5: 15 min

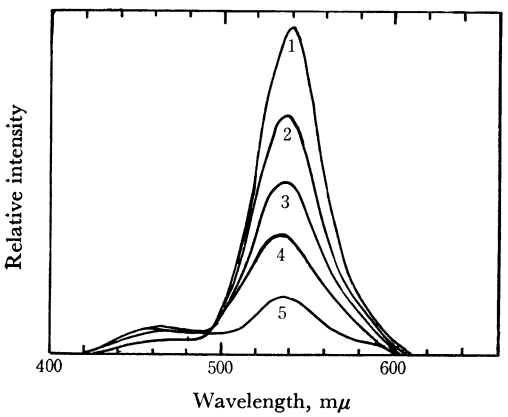

Fig. 5-a-3. 2′,7′-Dichlorofluorescein system (aqueous solution).
1: after 2 min, 2: 4 min, 3: 6 min, 4: 9 min, 5: 15 min

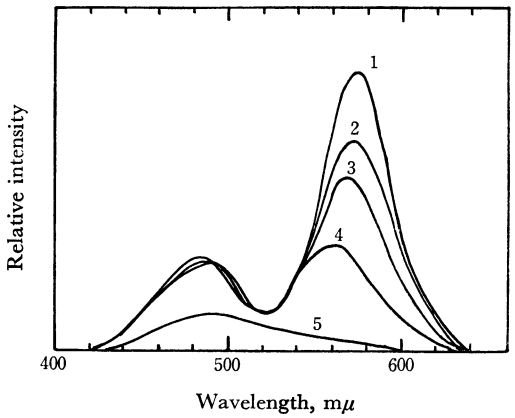

Fig. 5-a-4. Rhodamine B system (50% DMSO solution).
1: after 2 min, 2: 5 min, 3: 8 min, 4: 11 min, 5: 15 min

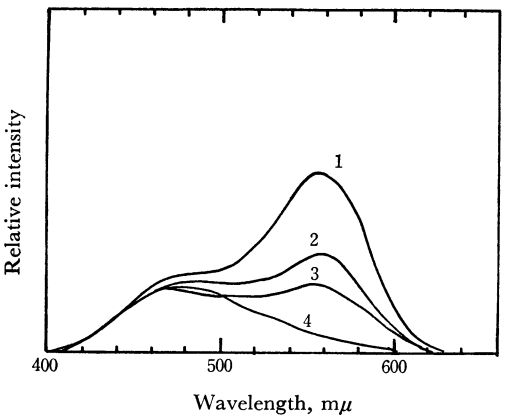

Fig. 5-a-5. Rhodamine S system (50% DMSO solution).
1: after 2 min, 2: 4 min, 3: 6 min, 4: 15 min

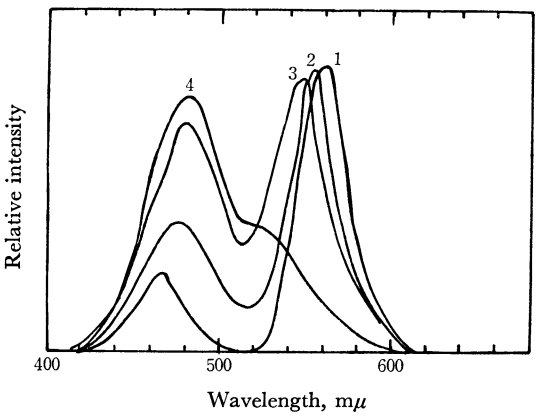

Fig. 5-b-1. Variation of the fluorescence emission spectra in the chemiluminescent systems of several xanthene dyes with reaction time.
Eosin R system (aqueous solution)
1: after 2 min, 2: 4 min, 3: 6 min, 4: 12 min, 5: 20 min

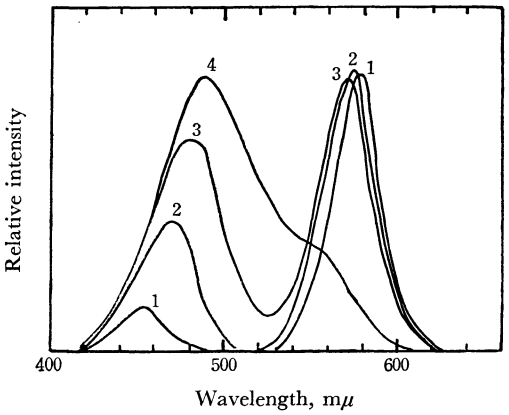

Fig. 5-b-2. Erythrosin B system (50% DMSO solution).
1: after 2 min, 2: 4 min, 3: 8 min, 4: 20 min

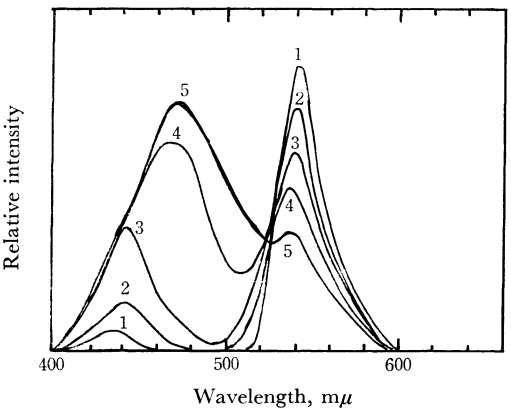

Fig. 5-b-3. 2′,7′-Dichlorofluorescein system (aqueous solution).
1: after 2 min, 2: 4 min, 3: 6 min, 4: 12 min, 5: 20 min

ponent was not very appreciable. However, with regard to the intense fluorescence of unoxidized uranin at the final stage, we may say that the self-absorption of the dye prevents any blue emission from the excited product.

Eosin R, erythrosin B, 2′,7′-dichlorofluorescein, mercurochrome, rhodamine B, rhodamine 6G, and rhodamine S chemiluminesced with the same two emission peaks as were observed in the eosin Y system. Typical examples of the results are illustrated in Fig. 5-a, where they are compared with the changes in the fluorescence spectra with the reaction time (Fig. 5-b). In each case, we can clearly find a spectral correspondence between the chemiluminescence and the fluorescence emissions.

On the other hand, such fluorescent dyes as phloxine, rose bengale, 4-nitrofluorescein, 4,5,6,7-tetrachlorofluorescein, sulfonfluorescein, and pyronine G did not actually chemiluminesce under comparable conditions. However, when one of

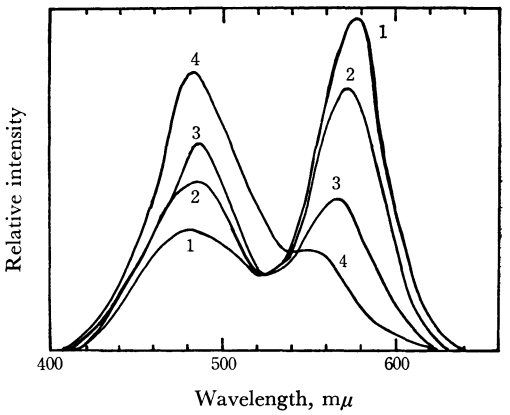

Fig. 5-b-4. Rhodamine B system (50% DMSO solution).
1: after 2 min, 2: 5 min, 3: 8 min, 4: 15 min

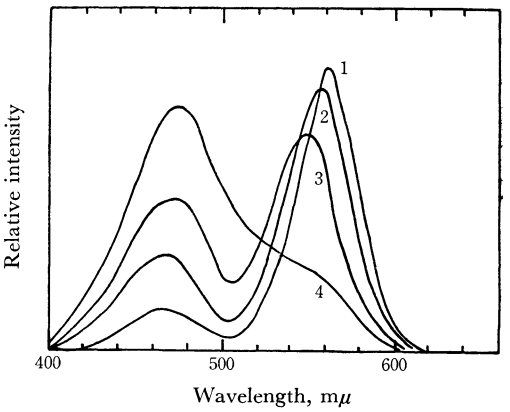

Fig. 5-b-5. Rhodamine S system (50% DMSO solution).
1: after 2 min, 2: 4 min, 3: 8 min, 4: 20 min

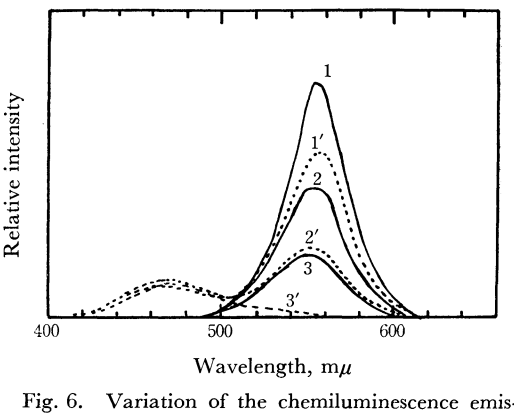

Fig. 6. Variation of the chemiluminescence emission spectra in eosin Y-sulfonfluorescein system (———) and eosin Y system (.....) with reaction time. eosin Y (4×10^{-4} mol/l), sulfonfluorescein (5×10^{-4} mol/l).
1, 1′: after 2 min, 2, 2′: 5 min, 3, 3′: 12 min

these dyes, such as sulfonfluorescein, was added to eosin Y, the emission corresponding to the fluorescence of the added dye appeared in place of the blue emission at the final stage, as is shown in Fig. 6. This experimental evidence can best be explained in terms of an energy-transfer process from an excited product to the added dye.

Discussion

All the findings presented above give support to the following reaction scheme, suggested in the previous paper; an excited species is produced from the reaction of the dye with a radical which is itself produced by the decomposition of hydrogen peroxide, and the energy is transferred from the species to the unoxidized dye, thereby exciting the dye to emit fluorescence.

An analogous mechanism has been proposed for the chemiluminescence of lucigenin.[5] The *N*-methylacridon produced is primary the emitting species because a blue emission corresponding to the fluorescence of *N*-methylacridon was observed in a hot dilute system, whereas a greenish-yellow emission whose major component was similar to that of the lucigenin fluorescence was observed in a cold, concentrated system.[6]

On the other hand, two different types of the chemiluminescence of several dyes have been reported. The first type is the induced-chemiluminescence of anthracene, acridine, eosin Y, fluorescein, quinoline sulfate, and aesculin, which is observed when these dyes are present in the reaction of alkaline hydrogen peroxide with chlorine gas or the hypochlorite ion.[7] The suggested mechanism involves the formation of excited (singlet) molecular oxygen, which then forms an excited dimer through collision, and an energy-transfer from the dimer to these added fluorescers.[8] The second type is the visible light emission, which builds up and decays over a period of microseconds, this emission is induced when deaerated solutions of certain dyes, rhodamine B, acriflavin, fluorescein, and eosin are electron-pulse radiolysed.[9] The emission corresponds to the fluorescence of each dye. The proposed mechanism involves the processes by which the OH-adduct, DOH, reacts with the hydrated electron, e^-, to give the excited dye, D*:[10]

$$DOH + e^- \longrightarrow D^* + OH^-$$

Neither of these two mechanism is likely, for if the excited dimer of oxygen and the blue-fluorescent product were involved, as a donor and an acceptor respectively, in an energy-transfer process, the intensity of the peak at 480 mμ would increase with the increase in the concentration of the fluorescent product.[11] (We can see from Figs. 1 and 2 that the intensity of the peak of the blue component of the chemiluminescence is kept almost constant, whereas that of the fluorescence increases with the increase in the concentration of the reaction product). Moreover, a significant emission would also be observed in the systems of such fluorescent dyes as 4-nitrofluorescein, 4,5,6,7-tetrachlorofluorescein, and sulfonfluorescein. Furthermore, if the dye were primarily excited, we would not observe a blue-emission component which changes to greenish-yellow emission when sulfonfluorescein or 4-nitrofluorescein is added.

In summarizing the above results, we can conclude that the chemical formation of an excited species from the oxidation of dye, and an energy-transfer process from the species to an unoxidized dye, are most likely to compose the mechanism for the chemiluminescent reaction. Reconsidering the present experimental findings, we can develop the previously-proposed reaction scheme:[2]

Radical (R·) formation:

$$H_2O_2 \longrightarrow R^\cdot (O_2H^\cdot \text{ or } OH^\cdot)$$

The production of an excited species (M_E^*) by the reaction of R· with a dye, for example, eosin Y (E):

$$E + R^\cdot \longrightarrow M_E^* \quad \text{(reaction rate, } k_1\text{)}$$

An energy-transfer process:

$$M_E^* + E \longrightarrow M_E + E^* \qquad (k_2)$$

Blue fluorescence emission from M_E^*:

$$M_E^* \longrightarrow M_E + h\nu_1 \qquad (k_3)$$

Greenish-yellow fluorescence emission from E*:

$$E^* \longrightarrow E + h\nu_2 \qquad (k_4)$$

Self-quenching:

$$E^* + E \longrightarrow E + E \qquad (k_5)$$

According to the above scheme, the intensity of the chemiluminescence, I_E, is given by:

$$I_E = k_3[M_E^*] + k_1[E^*] \qquad (1)$$

Using the steady-state approximation, $d[M_E^*]/dt = 0$ and $d[E^*]/dt = 0$, I_E can be written as:

$$I_E = k_1k_3[E][R^\cdot]/(k_2[E]+k_3) + k_1k_2k_4[E]^2[R^\cdot]/(k_2[E]+k_3)(k_4+k_5[E]) \qquad (2)$$

The experimental results indicate that the intensity of blue emission is almost constant. This

5) H. Kautsky and K. H. Kaiser, *Z. Naturforsch.*, **56**, 353 (1950); A. Spruit van der Burg, *Rec. Trav. Chim. Pays. Bas*, **69**, 1526 (1950).

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9) W. Prutz, K. Sommermeyer and E. J. Land, *Nature*, **212**, 1045 (1966).

10) L. I. Grossweiner and A. F. Rodde, Jr., *J. Phys. Chem.*, **72**, 756 (1968).

11) E. A. Ogryzlo and A. E. Pearson, *ibid.*, **72**, 2913 (1968).

means that the first term of Eq. (2) can be reduced to (k_1k_3/k_2) [R·] by assuming that $k_2[E]>k_3$, since [E] decreases with the reaction time. On such an assumption, I_E is finally given by:

$$I_E = (k_1k_3/k_2)[R^\cdot] + k_1[E][R^\cdot]\phi_E \quad (3)$$

where ϕ_E is the fluorescence efficiency of E and is given by:

$$\phi_E = k_4/(k_4+k_5[E])$$

As has been shown, the greenish-yellow emission is so intense, compared to the blue emission, except for the final stage, that the I_E in the decay stage can be given as approximately:

$$I_E = k_1[E][R^\cdot]\phi_E \quad (4)$$

When a foreign dye such as sulfonfluorescein (S) is added to the eosin Y system, the following steps are also present: An energy-transfer process from M_E^* to S:

$$M_E^* + S \longrightarrow M_E + S^* \quad (k_6)$$

Fluorescence emission from S*:

$$S^* \longrightarrow S + h\nu_3 \quad (k_7)$$

Quenchings:

$$S^* + S \longrightarrow S + S \quad (k_8)$$

$$E^* + S \longrightarrow E + S \quad (k_9)$$

$$S^* + E \longrightarrow S + E \quad (k_{10})$$

Taking all of the steps into account, we can write the intensity of the emission in the eosin Y-sulfonfluorescein system as follows, since no blue emission appears:

$$I_{ES} = k_4[E]^* + k_7[S^*]$$

Under stationary conditions, we can write:

$$[E^*] = k_2[M_E^*][E]/(k_4+k_5[E]+k_9[S])$$

$$[S^*] = k_6[M_E^*][S]/(k_7+k_8[S]+k_{10}[S])$$

$$[M_E^*] = k_1[E][R^\cdot]/(k_2[E+k_6[S])$$

Assuming that the quenching with a foreign dye is not appreciable compared to the self-quenching, we can write that:

$$\phi_E = k_4/(k_4+k_5[E]) \fallingdotseq k_4/(k_4+k_5[E]+k_6[S])$$

Hence,

$$I_{ES} = k_1[E][R^\cdot](k_2[E]\phi_E+k_6[S]\phi_S)/(k_2[E]+k_6[S]) \quad (5)$$

where

$$\phi_S = k_7/(k_7+k_8[S]+k_{10}[E]).$$

From Eqs. (4) and (5), we obtain:

$$\Delta = \int (I_{ES}-I_E)dt$$

$$= k_1 \int \{[R^\cdot](k_2[E]^2\phi_E-k_6[E][S])\phi_S/$$

$$(k_2[E]+k_6[S])-[E]\phi_S\}dt \quad (6)$$

Since an intense fluorescence of the dye is observed in the reaction system, we prefer the approximations that $k_4 \gg k_5[E]+k_9[S]$ and that $k_7 \gg k_8[S]+k_{10}[E]$ to the approximation used previously. Using these approximations, and recalling the results that [R·] is independent of the reaction time and that $[E]=[E]_0 \exp(-K_E t)$, we can write:

$$I_E = k_1[R^\cdot][E]_0 \exp(-K_E t) \quad (7)$$

$$\Delta = (k_1k_6[R^\cdot](\phi_S-\phi_E)[S]/k_2K_E)$$

$$\times \ln(1+k_6[S]/k_2[E]_0) \quad (8)$$

We have already shown in the previous paper that the experimental results of I_E and Δ can be reasonably explained by Eqs. (7) and (8) respectively.

However, neither the exact nature of the emitting product nor the reason why some dyes produce chemiluminescence emission, whereas others do not, is at present known. Much further work, including the chemical identification of the blue-fluorescent product, is required.