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# Intermediates in the Photoreduction of Eosine as Revealed by a Flash-Photolysis Study

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Besides the triplet state, semi-reduced eosine (Rd) and semi-oxidized eosine (Ox) were observed as transient intermediates in an aqueous solution; the former has a peak at 408 m$\mu$, and the latter, one at 456 m$\mu$. These two species are produced by the reaction $D^T+D \rightarrow Rd+Ox$ or $D^T+D^T \rightarrow Rd+Ox$. The sum of the rate constants for the reactions $D^T+D \rightarrow Ox+Rd$ and $D^T+D \rightarrow 2D$ is about $8.0\times10^8$ M$^{-1}$ sec$^{-1}$, approximately equal to the sum of the rate constants for $D^T+D^T \rightarrow 2D$ and $D^T+D^T \rightarrow Ox+Rd$. Triplet eosine, by reacting with ATU or EDTA, produces two kinds of half-reduced eosine, Rd' and Rd'', which have absorption spectra similar to those of Rd but with different life-times. These are perhaps loose compounds of half-reduced dye and a half-oxidized reductant. The rate constants for $D^T+ATU \rightarrow Rd'$ and $D^T+EDTA \rightarrow Rd''$ are $3.4\times10^5$ M$^{-1}$ sec$^{-1}$ and $5\times10^5$ M$^{-1}$ sec$^{-1}$ respectively. Rd disappears bimolecularly by means of the $Rd+Ox \rightarrow 2D$ reaction with the rate constant of $8.8\times10^7$ M$^{-1}$ sec$^{-1}$. Ox, by reacting with EDTA or ATU, regenerates the original dye with the rate constants of $1\times10^6$ and $8\times10^5$ M$^{-1}$ sec$^{-1}$ respectively. The results enable us to conclude that the D-D mechanism occurs under suitable conditions.

It has been proposed by two of the present authors⁽¹⁾ (Koizumi and Ohno) that, in the continuous-light photoreduction of eosine by allylthiourea (ATU), the reaction proceeds by one of two alternative paths, depending on the experimental conditions. Thus, in the case of higher ATU concentrations, the primary step is the reaction between the triplet dye ($D^T$) and a reducing agent (the D-R mechanism), while at lower concentrations of ATU the primary step is the electron transfer between $D^T$ and D (the D-D mechanism). The rate constants of some elementary processes have also been evaluated.

The first evidence for the electron transfer between two $D^T$ molecules or between $D^T$ and D was presented by Lindqvist in his extensive study of the transient intermediates of fluorescein by means of a flash technique⁽²⁾; further evidence was presented by Kato et al. in the case of methylene blue.⁽³⁾

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1) T. Ohno, Y. Usui and M. Koizumi, This Bulletin, **38**, *1022 (1965)*.

2) L. Lindqvist, *Arkiv för Kemi*, **16**, 79 (1960).

3) S. Kato, M. Morita and M. Koizumi, This Bulletin, **37**, *117 (1964)*.

Meanwhile, Grossweiner and Zwicker reported,4) in their paper dealing with a flash-photolytic investigation of the eosine-phenol system, that there is no evidence for the production of semi-reduced and semi-oxidized eosine in an evacuated aqueous eosine solution. These quite different aspects of the behavior of fluorescein and eosine seemed to us rather odd, although Grossweiner himself attributed them to their different electronic configurations.

In view of our findings in steady-illumination experiments and the above disagreement between the two groups of investigators, we made flash-photolytic studies of aqueous solutions of eosine in connection with our steady-light studies.

The purpose of the present paper is to report that half-reduced and half-oxidized eosine does come into existence in the flash-illuminated aqueous eosine solution.

In addition, we have investigated the behavior of these intermediates as well as that of the triplet dye.

## Experimental

**Apparatus.**—The flash apparatus reported on in a previous paper3) was used to record the transient spectra.

Another apparatus, with a shorter duration of the photolysis flash, was used to trace the change in the transient absorption. It was assembled from a grating monochrometer (Narumi RM-23), a synchroscope (Iwasaki SS-5151) and a 150W. xenon short arc lamp (Ushio UXL-150). Two quartz flash lamps filled with xenon were set parallel and were connected, in series, with an air gap for triggering and a carbon rod (15 cm. long and 4.5 mm. in diameter) for electric damping. The lamps were powered by a 1 μF condenser and operated at 10 kV. The half-height duration of the flash was 4.5 μsec.

The relative light doses of flashes with and without the carbon rod were compared by the use of a ferrioxalate actinometer; it was found that the rod reduced the light dose to 55 per cent of the value without it. Thus, the flash energies recorded in this paper are 55 per cent of the input electrical energies.

The green plastic plates, always used as filters were transparent from 420 to 600 mμ and had a maximum transmittance of 60 per cent at 520 mμ. To change the light intensity, darkened photographic plates with a known transmittance were employed as neutral filters, instead of changing the electrical conditions. Accordingly, the profile of the incident flash light was always of the same shape.

**Materials.**—Eosine (Merck) was recrystallized three times from an ethanol solution. ATU, β-sulfonylanthraquinone, disodium hydrogen phosphate, potassium dihydrogen phosphate and $NaClO_3$, all of a G. R. grade, were recrystallized three times from aqueous solutions. Benzoquinone was recrystallized two times from ethanol and then washed with water. The other substances, all of a G. R. grade, were used without further purification.

All the sample solutions were prepared in a 0.1 M phosphate buffer solution, pH=7.2. The samples were degassed by cycles of freezing, pumping and thawing.

## Results

### General Features of the Reaction and the Assignment of the Transient Absorption Spectra.

—It was established that the permanent bleaching of dye by a single flash (19J) is less than 1%. The transient spectra at 65, 360 and 1410 μ sec. after the excitation flash are shown in Fig. 1. Four maxima are apparent, at 580, 456, 408 and 325 mμ, of which the first one and the last one

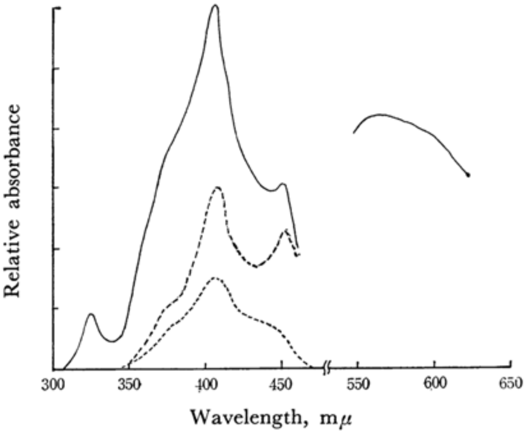

Fig. 1. Absorption spectra of transient species in the phosphate buffer solution (pH 7.2). Eosine concentration is 13 μM.
—— 65 μ sec. after flashing (input-energy 19J)
---- 360 μ sec. (upper curve) and 1410 μ sec. (lower curve) after flashing (input-energy 19J)

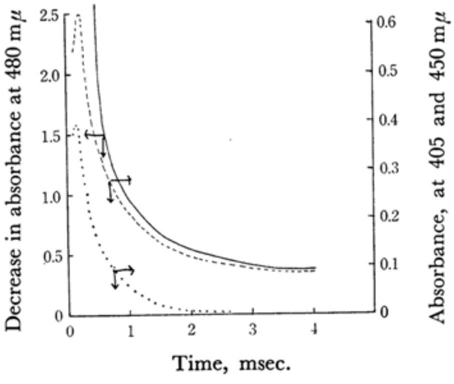

Fig. 2. Change in the absorbance of transient species and eosine. Eosine concentration is 10 μM.
—— 480 mμ (eosine) ---- 405 mμ
...... 450 mμ

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4) E. F. Zwicker and L. I. Grossweiner, *J. Phys. Chem.*, **67**, 549 (1963).

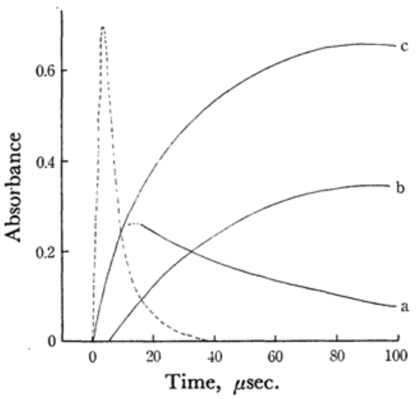

Fig. 3. Rise and fall of transient species during and after flash irradiation. Eosine concentration is 10 μM.
Dotted line; flash (55J), a, 610 mμ; b, 450 mμ; c, 405 mμ

completely disappear at 360 μsec., of the two remaining peaks, 408 mμ has the longer life. This is clear from Fig. 2, in which the decay at 405 and at 450 mμ in a later stage is shown. The recovery of the main absorption band of the original dye (480 mμ) is also included in this figure.

The rise and fall of each absorption maximum in an early stage are shown in Fig. 3. There is no doubt from these three figures that 325 mμ and 508 mμ, which appear to be produced only during illumination and which decay at the same rate, belong to a primary intermediate. These two bands may most plausibly be assigned to the triplet state of the dye. The peaks at 405 and 450 mμ, which grow with the decline of 325 and 580 mμ, can be attributed to the second-step intermediates.

When ATU, EDTA, $NaClO_3$, $NaHAsO_4$ or oxygen was added, a more or less permanent bleaching of the dye resulted from a single flash. The extent of bleaching is particularly large in the cases of EDTA and ATU.

In the cases of β-sulfonylanthraquinone and *p*-benzoquinone, there is scarcely any permanent bleaching observed. The triplet absorption spectra were not observable when an ample quantity of any of the above-mentioned substances, except $NaClO_3$ and $NaHAsO_4$, were added.

The transient spectra from the system containing ATU or EDTA have only a 408 mμ peak, as is shown in Fig. 4, whereas the only observable peak in the case of the system to which β-sulfonylanthraquinone or oxygen has been added is the one at 456 mμ. $NaClO_3$ and $NaHAsO_4$ scarcely affect the transient spectra at all.

The spectra for the system containing *p*-benzoquinone are complicated, as can be seen in Fig. 5. Besides the 450—460 mμ band, four peaks are apparent, at 430, 410, 380 and 355 mμ; these may most plausibly be attributed to the half-reduced forms of benzoquinone, in ionic and neutral forms.

To sum up the effect of the added substances, there is scarcely any doubt that the peaks at 408 and 456 mμ can be assigned to semiquinone (Rd) and half-oxidized eosine (Ox) respectively, because the addition of oxidants removes the 405 mμ peak, while reductants eliminate the 456 mμ absorption. This conclusion will be further substantiated below.

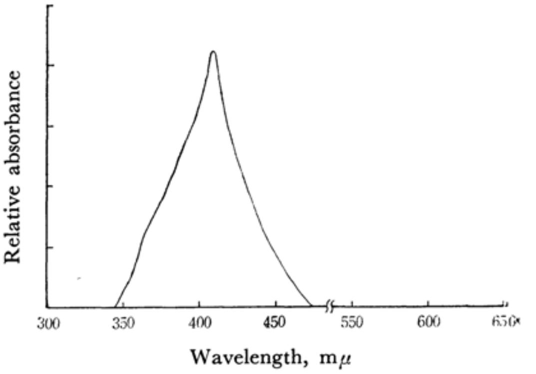

Fig. 4. Absorption spectra of transient species in the presence of $3\times10^{-2}$ M allylthiourea at 65 μsec. after flashing. Eosine: 13 μM

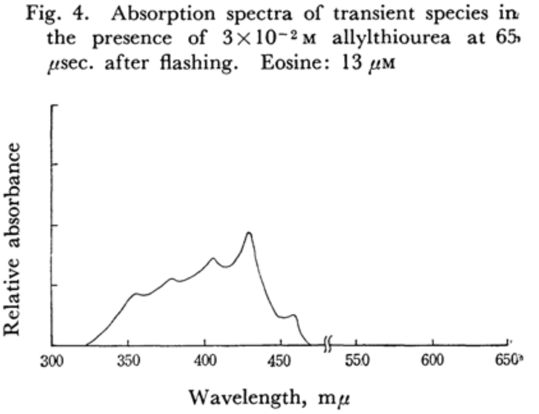

Fig. 5. Absorption spectra of transcient species in the presence of $10^{-3}$ M *p*-benzoquinone at 360 μ sec. after flashing. Eosine: 13 μM

**Some Observations Concerning the Behavior of Transient Intermediates.**—The relation between the absorbances at 450 and 408 mμ, due, respectively, to the existence of Ox and Rd at 80 μsec. after flashing, is shown in Fig. 6; the concentrations of these intermediates were varied by changing the intensity of the light flash. It is certain from this figure that the two intermediates are always produced in the same ratio, irrespective of the experimental conditions. In order to determine this ratio, one must know the molar extinction coefficients of Rd and Ox. These were derived in the following way.

As is evident from Fig. 2, only Rd survives at a later stage. If one assumes that both Rd and Ox return completely to the ground-state dye under any circumstances, the molar extinction coefficient

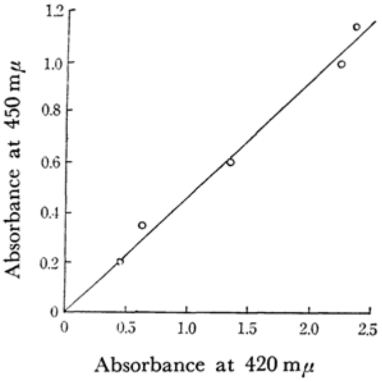

Fig. 6. Relation between the formation of Ox and Rd at 80 μsec. after flashing. Eosine concentration is 13 μM.

of Rd can easily be calculated, since its concentration in the later stage can be equated to that of the ground-state dye recovered after the corresponding time point. This, although not conclusive, appears to be justified if one recalls that the fluorescein semiquinone produced from the interaction of leuco- and oxidized-forms returns completely to the ground-state dye, probably by losing molecular hydrogen.[5] The molar extinction coefficient of Rd at 408 mμ thus obtained is $2.5\times10^4$. The molar extinction coefficient of Ox can, then, be obtained from the absorbance at 458 mμ in the stage where there is no triplet absorption remaining, by using the concentration data of the ground-state eosine and of Rd, both of whose coefficients are known. The coefficient of Ox thus obtained at 458 mμ is $1.2\times10^4$.

By the use of the above $\varepsilon$-values, the ratio of Rd to Ox was found to be about unity. This implies that Rd and Ox are produced by the reaction:

$$D^T + D \rightarrow Rd + Ox \qquad (1)$$

or

$$D^T + D^T \rightarrow Rd + Ox \qquad (2)$$

Although the procedure used above is somewhat ambiguous, the coefficients so obtained are used tentatively in the present paper. Grossweiner's value[4] for $\varepsilon_{408}$ is $3.8\times10^4\pm0.4$, which is not very different from ours.

The relation between the yield of Rd and the dye concentration was examined using a flash light of such an intensity that most of the dye was converted to the triplet state, while the production of the triplet state was considered to be roughly proportional to the dye concentration.

In Fig. 7 the absorbances at 408 mμ measured 80 μ sec. after flashing are plotted against the dye concentration. The shape of the curve favors the view that the predominant reaction is $D^T+D^T\rightarrow Rd+Ox$, but it does not obviate the possible participation of the process $D^T+D\rightarrow Rd+Ox$.

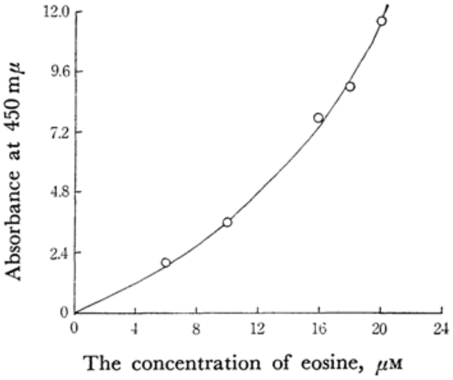

Fig. 7. Formation of Ox at various eosine concentrations at 80 μsec. after flashing. (55J, 77.5J)

To investigate further which reaction, 1 or 2, is the more efficient, the dependence of the yield of Rd on the triplet dye concentration was examined. Since the decay of the triplet state is exponential with the rate constant, independent of the light intensity (see below), the quantities of the triplet state produced by single flashes of various intensities are proportional to the absorbance density at 610 mμ at a fixed time point after flashing. Figure 8 gives the plot of the absorbance at 610 mμ, $A_{610}$, against the relative light intensity.

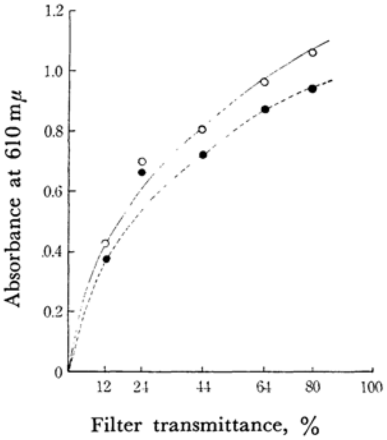

Fig. 8. Formation of triplet state by various light energies observed at various time interval.
—○— 30 μsec. after flashing
—●— 40 μsec. after flashing

Then, at various light intensities, the maximum values of $A_{408}$ after flashing were compared with the values of $A_{610}$. As is shown in Fig. 9, $A_{408}$ is approximately proportional to $A^2_{610}$. Although exact analysis is difficult, the results strongly suggest

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[5] Y. Momose, K. Uchida and M. Koizumi, This Bulletin, 38, 1601 (1965).

the predominance of the reaction 2 under the present experimental conditions.

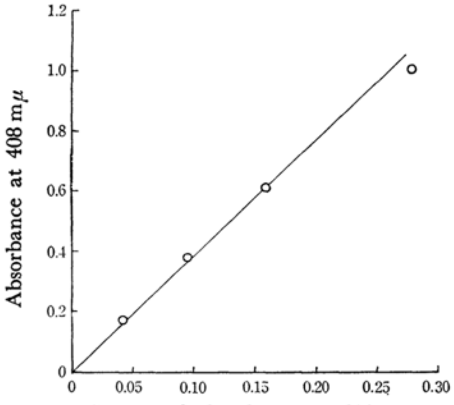

Fig. 9. Relation between the formation of Rd and triplet state.

When ATU or EDTA is added, the yield of Ox decreases with the increase in the reductant concentration. The yield of Rd, on the other hand, is not affected to any large extent. However, the decay of Rd, which will be treated quantitatively later, shows a sharp breaking point, after which the rate becomes very slow; yet the shape of the absorption spectrum remains the same. The phenomenon is similar in both reductants. It is reasonable to explain this break by assuming two somewhat different species, species which have the same absorption spectra but which differ in life time. The one with the shorter life time and that with a longer life time will conventionally be denoted as Rd and Rd′ respectively in the case of ATU, and as Rd and Rd′′ respectively in the case of EDTA. Both Rd′ and Rd′′ increase with the concentration of reductants. Tables I and II give some of the results; one example is shown in Fig. 10.

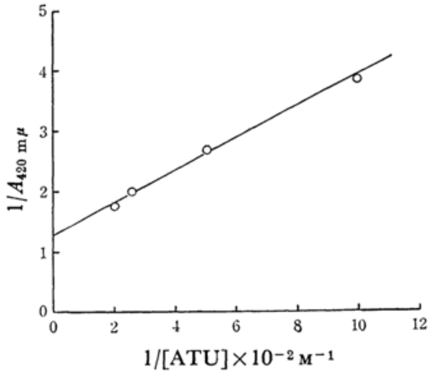

Fig. 10. Formation of Rd′ for various allylthiourea concentration.

TABLE I

| [ATU] ×10² | Rd+Rd′ (Op. dens.) | Rd (Op. dens.) | Rd′ (Op. dens.) | Ox |
|---|---|---|---|---|
| 0 | 0.670 | 0.670 | 0 | 0.360 |
| 2 | 0.609 | 0.385 | 0.244 | 0.12 |
| 4 | 0.560 | 0.243 | 0.316 | 0 |
| 5 | 0.652 | 0.286 | 0.366 | 0 |

TABLE II

| [EDTA] ×10² | Rd+Rd′′ | Rd | Rd′′ | Ox |
|---|---|---|---|---|
| 0 | 0.670 | 0.67 | 0 | 0.360 |
| 1 | 0.49 | 0.26 | 0.24 | 0.24 |
| 2 | 0.95 | 0.56 | 0.39 | 0 |
| 3 | 0.97 | | | 0 |
| 5 | 1.14 | 0.76 | 0.38 | 0 |

At first sight there appear to be two ways in which the nature of Rd′ and Rd′′ can be explained. One is to consider, as has been mentioned above, that they are slightly different species from Rd and are produced by a direct reaction between $D^T$ and the reductants; the other interpretation is to consider that they are the same species as Rd but that, since the reductants eliminate Ox, the remaining Rd, with no Ox to react, behaves in quite a different way. The data in the above table show that quite a large quantity of Rd still exists after the Ox has been exhausted; hence, the first interpretation is more plausible. The problem will be discussed later, after the studies of the decay of the transient intermediates have been described.

The quantity of Rd′ is proportional to the quantity of the permanent bleaching of the dye; if one assumes that the extinction coefficients of Rd′ and Rd′′ are same as that of Rd, then the quantity of Rd′ is exactly the same as the permanent bleaching. Thus Rd′ is completely converted to leuco eosine. Rd′′ is also proportional to the quantity of permanent bleaching, but the ratio of the former to the latter is 1 : 2.3 in this case. (Fig. 11)

When $10^{-3}$ M of $p$-benzoquinone is added, the absorption spectra of the triplet dye do not appear

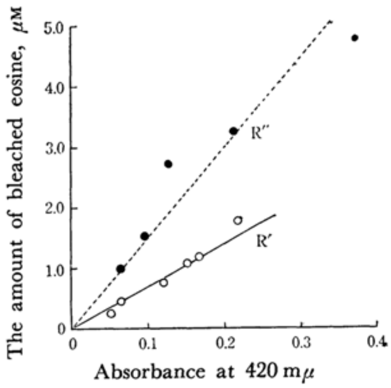

Fig. 11. Relation between the formation of Rd′ or Rd′′ and permanent bleaching of eosine.

at all, and the quantity of Ox reaches its maximum value immediately after the flashing. The quantity of Ox is exactly proportional to the absorbance at 430 m$\mu$ which is due to benzosemiquinone. This is shown in Fig. 12. Hence, one may say that the reaction between benzoquinone and $D^T$ yields Ox and the half-reduced form of $p$-benzoquinone very efficiently.

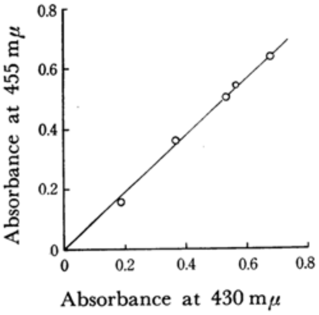

Fig. 12. Relation between the formation of Ox and that of benzosemiquinone ion.

**The Decay of the Intermediates.**—The decay of the triplet state was measured at 580 m$\mu$.

As Fig. 13 shows, the decay rate is scarcely influenced at all by a variation in the intensity of the exciting light. This suggests that the decay is not affected by the concentration of $D^T$. The decay is well expressed as a first-order process, seemingly suggesting the absence of any bimolecular processes. However, this is not the case, because the first order decay constant increases with the concentration of dye, as is shown in Fig. 14. Hence, the rate formula for the decay should be written as follows:

$$\frac{-\mathrm{d}[D^T]}{\mathrm{d}t}=\{k_{sp}+k[D]_0\}[D^T] \tag{3}$$

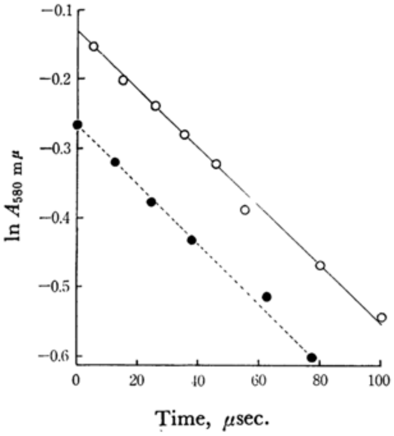

Fig. 13. Decay of triplet state for various light energies. Eosine concentration is 25 $\mu$M.
—○— Input-energy 55J
—●— Input-energy 2.5J

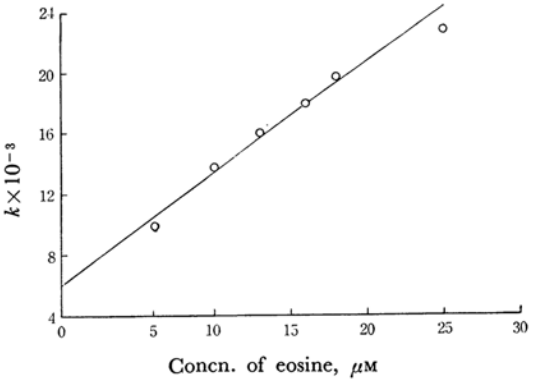

Fig. 14. Decay constant of triplet state for various eosine concentrations.

where $k_{sp}$ is a unimolecular rate constant and $[D]_0$ is the concentration of the dye. From the intercept, $k_{sp}\approx6\times10^3\ \mathrm{sec}^{-1}$, and from the slope, $k=8.0\times10^8\ \mathrm{M}^{-1}\ \mathrm{sec}^{-1}$.

The above results imply that, along with the reaction between two triplet molecules, deactivation by the ground-state dye takes place at about the same efficiency, for at an initial stage, where no appreciable quantities of Rd and Ox exist, the sum of $[D^T]$ and $[D]$ is equal to $[D]_0$.

In a later stage of the decay curves in Fig. 14, quite a large quantity of Rd and Ox may be expected to exist, so there is a great possibility that these species are equally efficient quenchers of $D^T$. From the above results, one may approximately put $k_{T-T}=k_{T-S}=8.0\times10^8\ \mathrm{M}^{-1}\ \mathrm{sec}^{-1}$, where $k_{T-T}$ and $k_{T-S}$ are the second-order rate constants for, respectively reaction $D^T+D^T\rightarrow$ and the $D^T+D$ $\rightarrow$reaction.

The decay of Rd was measured at 405 m$\mu$. As Fig. 15 shows, the decay is a second-order process with regard to Rd, but deviation becomes apparent at a later stage. A second-order rate constant is 8.8—$10^7\ \mathrm{M}^{-1}\ \mathrm{sec}^{-1}$.

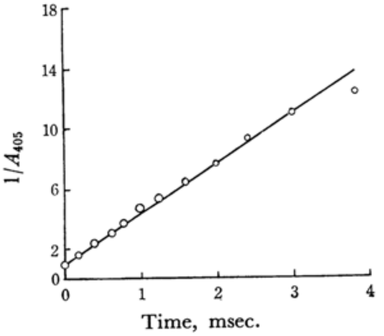

Fig. 15. Decay of Rd at 20 $\mu$M eosine.

The decay of Ox was measured at 450 m$\mu$. The decay is complicated and cannot be expressed by a simple rate formula. It seems that first-order and second-order processes are superimposed. At higher concentrations of Ox, the participation of the second-order process seems to increase.

If one puts

$$-\frac{\mathrm{d}C}{\mathrm{d}t}=k_1C+k_2C^2$$

then one can estimate the values of $k_1$ and $k_2$ by analysing the experimental data. They are $k_2=8.7\times10^8\ \mathrm{M}^{-1}\,\mathrm{sec}^{-1}$ and $k_1=9\times10^2\ \mathrm{sec}^{-1}$. The value of $k_2$, although not so reliable, agrees with the value of the decay constant for Rd, supporting the occurrence of the process Rx+Od→2D.

The nature of the superimposing first-order process is unknown.

As for the decay of the triplet state of the dye, it is accelerated more or less by the addition of oxygen, β-sulfonylanthraquinone, *p*-benzoquinone, ATU and EDTA. The air-saturated solution ($10^{-4}$ M) and the solution containing $10^{-3}$ M *p*-benzoquinone do not give any appreciable triplet absorption spectra.

Figure 16 shows the relation between the first-order decay constant and the concentrations of EDTA and ATU.

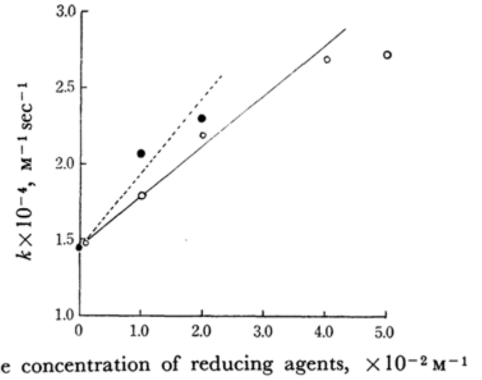

Fig. 16. Decay constant of triplet state for various reductant concentrations. Eosine: 10 μM

—○— Allylthiourea
--●-- EDTA

From the plots, one obtains, as the second-order rate constant for $D^T$+ATU→ and $D^T$+EDTA→ respectively, $3.4\times10^5\ \mathrm{M}^{-1}\,\mathrm{sec}^{-1}$ and $5\times10^5\ \mathrm{M}^{-1}\,\mathrm{sec}^{-1}$.

The addition of ATU or EDTA promotes the decay of Ox. The decay curve is expressed by a simple first-order rate formula, as is shown in Fig. 17. From the plots, the rate constants for Ox+EDTA→D+EDTA$^+$ and Ox+ATU→D+ATU$^+$ are found to be respectively, $8\times10^5\ \mathrm{M}^{-1}\cdot\mathrm{sec}^{-1}$ and $1.0\times10^6\ \mathrm{M}^{-1}\,\mathrm{sec}^{-1}$.

In the air-saturated solution, the absorption spectra near 450 mμ appear slightly only with quite a large input of energy. The addition of

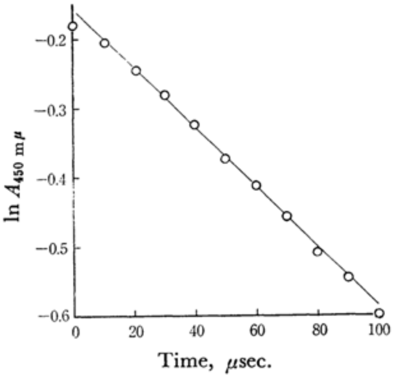

Fig. 17

$\mathrm{NaClO_3}$ or $\mathrm{NaHAsO_4}$ slightly lengthens the life of Ox. In the system containing *p*-benzoquinone, the life of Ox is only about 20 μsec., and the decay cannot be analyzed.

In the system containing $\mathrm{NaClO_3}$ or $\mathrm{NaHAsO_4}$, the decay of Rd at an earlier stage is a second-order process, while in a later stage it is a first-order process. The latter process may be attributed to the reaction between Rd and oxidants. Its rate constant is below $10^4\ \mathrm{M}^{-1}\,\mathrm{sec}^{-1}$.

In the case of ATU and EDTA, the decay consists of two parts, as has already been described. The decay for Rd'' (in the case of EDTA) is a first-order process, as Fig. 18 shows.

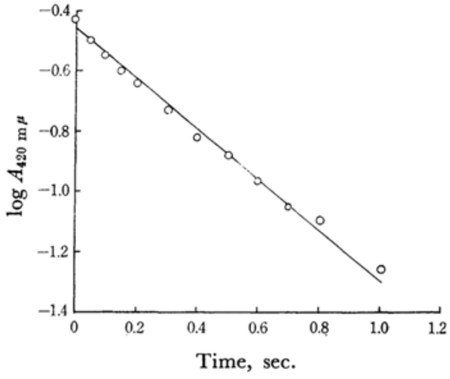

Fig. 18

The first-order rate constants is $1.0\ \mathrm{sec}^{-1}$, independent of the concentration of EDTA. For ATU we did not succeed in getting a reliable value because of the bad reproducibility of the experiment.

## Summary and Discussion

The elementary reactions established in the present paper may be summarized as follows:

$D^T$

$$\begin{cases} D^T \rightarrow D & k \approx 6\times 10^3\ \mathrm{sec}^{-1} \\ \left.\begin{array}{ll} D^T+D\rightarrow Ox+Rd & (1a) \\ D^T+D\rightarrow 2D & (1b) \end{array}\right\} & \begin{array}{l} \text{For the sum of the two} \\ k=8.0\times 10^8\ \mathrm{l.mol^{-1}\,sec^{-1}} \end{array} \\ \left.\begin{array}{ll} D^T+D^T\rightarrow Ox+Rd & (2a) \\ D^T+D^T\rightarrow 2D & (2b) \end{array}\right\} & \begin{array}{l} \text{For the sum of the two} \\ k=8.0\times 10^8\ \mathrm{l.mol^{-1}\,sec^{-1}} \end{array} \\ D^T+ATU\rightarrow Rd' \quad (3) & k=3.4\times 10^5\ \mathrm{l.mol^{-1}\,sec^{-1}} \\ D^T+EDTA\rightarrow Rd'' \quad (3') & k=5\times 10^5\ \mathrm{l.mol^{-1}\,sec^{-1}} \end{cases}$$

The rate constants for 3 and 3′, which had been obtained from the steady-light experiments,1) are, respectively, $k=3.1\times 10^5$ and $4.1\times 10^5$; these values are in excellent agreement with the above values.

The rate of reaction 2a is probably larger than that of 2b, but no comparison can be made between 1a and 1b.

*Rd.* Rd produced by 1a or 2a disappears by a second-order process, at least in the initial stage;

$$k=8.8\times 10^7\ \mathrm{l.mol^{-1}\,sec^{-1}} \tag{4}$$

$$\left.\begin{array}{l} Rd' \rightarrow \text{leuco eosine} \\ Rd'' \rightarrow \text{leuco eosine} \end{array}\right\} \text{(not certain)}$$

*Ox* The reaction of Ox is rather complicated; it seems to be a superimposition of a first-order and a second-order reaction. The rate constants are, respectively:

$$k_5=9\times 10^{-2}\ \mathrm{sec}^{-1} \tag{5}$$

$$k_6=8.7\times 10^8\ \mathrm{M^{-1}\,sec^{-1}} \tag{6}$$

These data are not very reliable.

$$Ox + EDTA \rightarrow D + (EDTA)^+$$
$$k=1\times 10^6\ \mathrm{M^{-1}\,sec^{-1}} \tag{7}$$

$$Ox + ATU \rightarrow D + (ATU)^+$$
$$k=8\times 10^5\ \mathrm{M^{-1}\,sec^{-1}} \tag{7}$$

From the above rate constants, one can say definitely that the D–D mechanism (in the present case, the reaction between two $D^T$ molecules) occurs under certain experimental conditions. Thus, from the rate constants for reactions 1, 2 and 3, the rate for the reaction between two dye molecules (the D–D-mechanism) is of the same order as that for the reaction between eosine and a reductant (ATU or EDTA) when the concentration of [D] is $\sim 10^{-5}$ M and that of the reductant is $\sim 10^{-2}$ M. If the reductant concentration is lower, the reaction due to the D-D mechanism will predominate.

The Ox thus produced will then react with the reductant or disappear according to 5 or 6. From the rate constant for 6, $8\times 10^5\ \mathrm{M^{-1}\,sec^{-1}}$, and for 7, $1\times 10^6\ \mathrm{M^{-1}\,sec^{-1}}$, the reaction 7 will predominate if $[Ox]=[Rd]\leqq 10^{-8}$ M and if EDTA $\approx 10^{-2}$ M. Hence, in such situations Rd will survive and will turn into leuco dye. This is just what may be expected via a D–D mechanism. Thus, the discussion so far, we believe, thoroughly confirms a D–D mechanism.

Most of the other results obtained in the present paper can be understood in terms of this scheme. Thus, the finding that $[Rd]\propto[D^T]^2$ can be interpreted on the assumption that the rate of the reaction is proportional to $D^T\times D^T$. No comment is needed for the fact that the yield ratio of Rd and Ox is 1 : 1.

However, the extent to which the back reaction, $Rd+Ox\rightarrow 2D$, occurs, is not yet clear enough, since some of Rd and some of Ox react independently of each other. The answers to such questions must await further study.

As to the nature of Rd′ and Rd″, they are probably loose compounds of a half-reduced dye and a half-oxidized reductant. This conclusion depends on the following reasons: Firstly, from the rate constant of 1a on the one hand and that of 3 and 3′ on the other, one can say that D–D and D–R mechanisms occur as competing processes under the experimental conditions in which Rd′ and Rd″ are produced (see Tables I and II). Secondly, the fact that Rd″ obeys the first-order decay with the same rate constant, irrespective of the concentration of EDTA, can be interpeted by the above model, but perhaps not so well by other models.

It is very interesting that such a compound has a life as long as 1 sec., and that its absorption spectra are quite similar to those of free semiquinone. There is no assurance, however, that its molar absorbance is the same as that of the latter. Hence, the statement given regarding the ratio of Rd′ or Rd″ to leuco dye is not decisive.