Switch-over of the Mechanism of the Primary Processes in the Photo-oxidation of Xanthene Dyes as Revealed by the Oxygen Consumption Experiments

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As early as 1933 Blum and Spealman[1] studied the photo-oxidative bleaching of xanthene dyes by oxygen. Some of their findings on aerated 2.8×10^{-4} M aqueous solutions of eosine were, 1) production of hydrogen peroxide, 2) existence of an induction period in the bleaching course of the dye, 3) decrease of the rate at high concentrations of oxygen. They inferred from the findings 1 and 2, that the reaction may proceed according to the following scheme, $D^*+O_2 \rightarrow D+O_2^*$, $O_2^*+H_2O \rightarrow OH+HO_2$ followed by the decomposition of dye by OH and HO_2. Since then no kinetic studies had been made on similar systems until. Imamura and one of the present authors (M. K.) investigated the aerated 1×10^{-5} M solution of dye.[2] They found that all the above phenomena were absent at this concentration, the rate of reaction being simply proportional to the absorbed light and increasing monotonously to a certain constant value as the concentration of oxygen increased. They concluded therefore, that the primary process of the photo-oxidation at this concentration of dye is attack of the oxygen on the triplet dye, resulting in destruction of the dye structure. They did not, however, attempt to interpret the differing results of Blum et al., only attributing them to the experimental conditions, particularly to the different concentration of dye.

Now the flash photolytic investigations made by Lindqvist[3] and Grossweiner et al.[4], the former on fluorescein and the latter on eosine,

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[1] H. F. Blum and C. R. Spealman, *J. Phys. Chem.*, **37**, 1123 (1933).

[2] M. Imamura and M. Koizumi, This Bulletin, **28**, 117 (1955); M. Imamura, *J. Inst. Polytech. Osaka City Univ.*, **5**, 85 (1956).

[3] L. Lindqvist, *Arkiv Kemi*, **16**, 79 (1960); V. Kaoshe and L. Lindqvist, *J. Phys. Chem.*, **68**, 817 (1964).

[4] E. F. Zwicker and L. I. Grossweiner, ibid., **67**, 549 (1963).

have afforded kinetic data for the various elementary reactions and have revealed much of the details of the photochemical behaviors of these dyes. In particular, Lindqvist proposed an epoch-making view that electron transfer can take place between the triplet dye and the ground state dye of fluorescein. Following this work, Koizumi et al. presented strong evidence for the occurence of such a process in the actual photochemical reduction of thiazine dyes.[5,6] Further, Ohno and Koizumi[7] found that in the photoreduction of eosine by allyl thiourea, a switch-over of mechanism does take place; the attack of reducing agent on triplet dye (D-R mechanism) predominates at lower concentrations of dye, whereas electron transfer between the triplet and the ground-state dyes (D-D mechanism) becomes the main process at higher concentrations.

The purpose of the present investigation is to provide additional evidence of a switch-over from D-O* to D-D mechanism by studying the photo-oxidative behavior of rather concentrated aqueous solutions of xanthene dye, and further, to give a reasonable correlation between Blum's and Imamura's results.

For this purpose the oxygen up-take of the irradiated aqueous solutions has been investigated by means of Warburg's apparatus and the results are compared with those obtained previously. The results, which are somewhat similar to those of Blum et al., can now be interpreted very satisfactorily and the differences in the results of the two groups of investigators are well interrelated with each other on the basis of the switch-over of the mechanism from a D-O mechanism at low concentrations of dye to a D-D mechanism at higher concentrations. A reinterpretation of Imamura's data is also given.

Experimental

Sample. — *Eosine.* — Merck's G. R. sample was recrystallized twice from ethanol. The maximum molar extinction, ε_{max} for an aqueous solution is 9.6×10^4.

Erythrosine.— Merck's G. R. reagent was used without further purification. ε_{max} was 6.7×10^4.

Fluorescein.—The Sodium salt of Tokyo Kasei was recrystallized three to four times from ethanol containing a small quantity of sodium hydroxide. ε_{max} in the phosphate buffer solution was 6.8×10^4 (pH 7.65).

Apparatus and Procedure.—Warburg's apparatus was the one supplied by Ōtake Co. The light source consisted of three 30W. daylight fluorescent lamps 60 cm. in length placed below the reaction cells with a suitable filter in between. The filters were 30×100 cm. plastic plates; the one for eosine and erythrosine cuts off below 480 mμ, and the one for fluorescein cuts off below 340 mμ. Reaction cells, each containing 20.0 ml. of the dye solution 5.0×10^{-5}—8.0×10^{-3} M in concentration, were immersed in a Warburg thermostat at 30.0±0.02°C and after 20—25 min. the illumination was started. The depth of the solutions being about 2 cm., the absorption of light is practically complete in the wavelength region of the visible absorption spectra. The cell constants of all cells are about 2. The quantity of absorbed gas was measured by a push-up method. Correction of atmospheric pressure and temperature was made by means of a thermobarometer. This is necessary, because of the long duration of irradiation and the rather small quantity of absorbed gas. The quantity of bleached dye was measured spectroscopically, after diluting to about 10^{-5} M, on a Hitachi EPU-2A spectrophotometer or EPS-3 autorecording spectrophotometer.

The saturated solution of oxygen under 1 atm. was prepared as follows. The sample solution was flushed for fifteen minutes with 1 atm. of oxygen and 20.0 ml. of it was poured into the reaction cell, the operation being done in the current of oxygen.

Results

Effect of the Dye Concentration on the Rate of Oxygen Consumption. — Using the plain aqueous solutions of eosine and erythrosine and the phosphate buffer solutions of fluorescein (pH 7.65), all at various dye concentration, two series of experiments were performed for each dye, one series with the air-saturated solutions ($[O_2]\approx2.5\times10^{-4}$ M) and the other with the solutions saturated with 1 atm. of oxygen ($[O_2]\approx1.2\times10^{-3}$ M).

Eosine.—As seen from Fig. 1, oxygen consumption by the air-saturated solution takes place linearly with time. The slope which gives the rate, increases with the dye concentration up until about $\sim5\times10^{-4}$ M is reached but becomes practically constant beyond 10^{-3} M of dye.

The results are similar for the oxygen-saturated solutions except for the fact that the limiting rate is attained at a somewhat higher concentration of dye. Figure 2 gives the plots of the rate against the dye concentration. It is worth while to note that the limiting value is practically the same for the air-saturated and the oxygen-saturated solutions.

Erythrosine.—Figure 3 shows the results for erythrosine. In this case the limiting value

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[5] S. Kato, M. Morita and M. Koizumi, This Bulletin, 37, 117 (1964).

[6] M. Koizumi, H. Obata and S. Hayashi, ibid., 37, 108 (1964).

[7] T. Ohno, Y. Usui and M. Koizumi, ibid., 38, 1022 (1965).

[*] O denotes oxidant (oxygen). In this case oxygen attacks the triplet dye instead of th reducing agent.

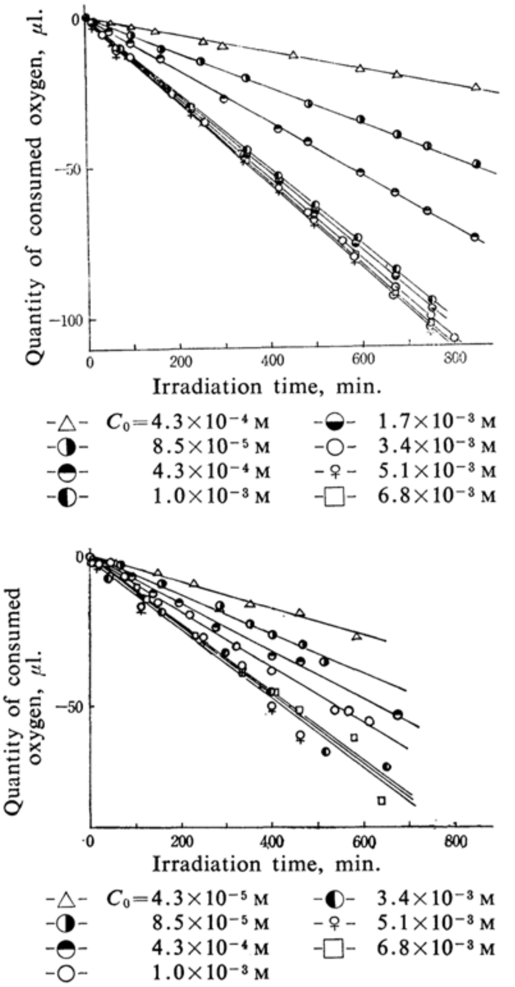
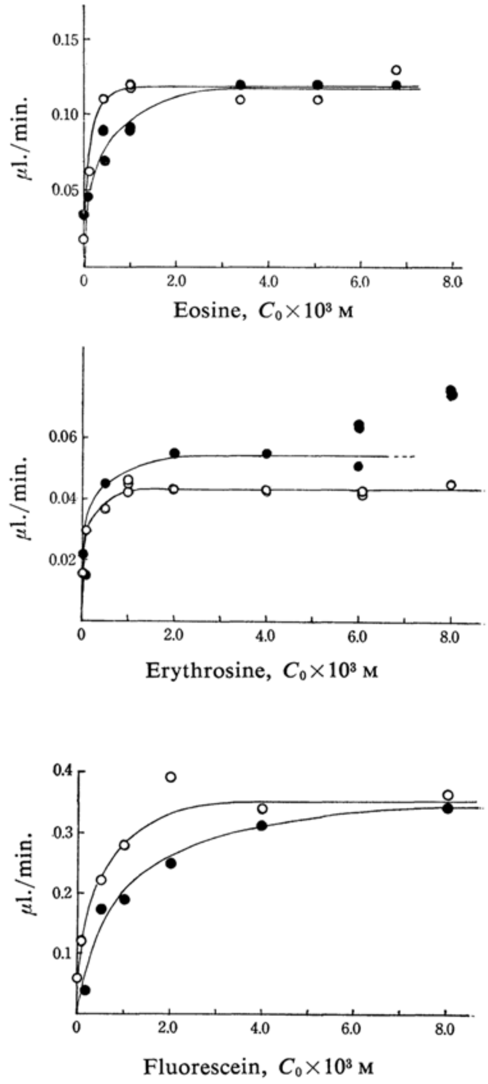


Fig. 1. Examples of oxygen consumption in the air saturated (upper), and oxygen saturated (under) solution of eosine.



Figs. 2, 3 and 4. Dependence of the rate of oxygen consumption on the dye concentration. -●- oxygen saturation, -○- air saturation

for the oxygen-saturated solution seems to be a little higher than that for the air-saturated solution, although its magnitude lies close to the experimental error.

Fluorescein.—Figure 4 gives the results for fluorescein at pH 7.65. The main features of the curve are essentially the same as those in the case of eosine except that the attainment of the critical value seems to be realized at a somewhat higher concentration of dye.

Summing up the above results, one can say that all the xanthene dyes behave essentially the same in the neutral region and that the rate of oxygen consumption is constant when the dye concentration is much larger than that of oxygen but begins to fall rather sharply in the region $[D]\approx[O_2]$.

The Bleaching Rate of the Dye.—Measurements were made choosing dye concentrations in the region 10^{-5} to 10^{-4} M, since accurate experiments with more concentrated solutions, which undoubtedly are very desirable, require quite a new technique and, besides, Imamura has pointed out[2)] that a simple rate formula ceases to hold near 10^{-4} M of dye. A 75V.-500W. tungsten projection lamp was used as a light source and the experiments were made at 30.0°C. A spacer 9.0 mm. in width was used for measuring the optical density of the 10^{-4} M solutions. The results are given in Fig. 5 and in Table I. In Fig. 5, the linearity of the curves implies that the rate is simply proportional to the absorption of light. As this figure shows, the linear relation holds as long as $r=[D_0]/[O_2]$ is less than ~0.2. The results at lower *r*-values are in excellent

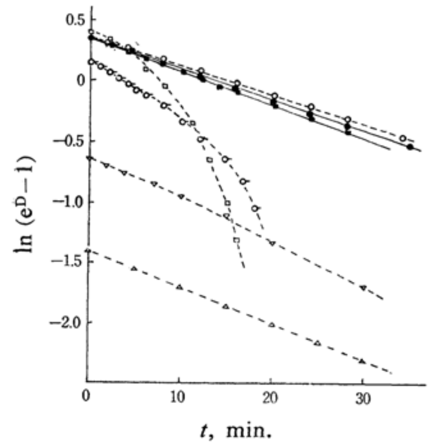


Fig. 5. Photobleaching of eosine at various concentration. (C_0)

	Sign	C_0 (M)	r
Air saturation (2.5×10^{-4} M)	□	1.0×10^{-4}	0.40
	○-	8.0×10^{-5}	0.32
	▽	4.3×10^{-5}	0.17
	△	2.3×10^{-5}	0.09
Oxygen saturation (1.2×10^{-3} M)	●	1.0×10^{-4}	0.08
	■	1.0×10^{-5}	0.008

Table I

Oxygen saturated	D_0 (M)	r	Quantum yield
1	1.0×10^{-4}	0.08	3.2×10^{-4}
2	1.0×10^{-5}	0.01	3.5×10^{-4}
Air-saturated			
1	1.0×10^{-5}	0.04	3.0×10^{-4}*
2	2.3×10^{-5}	0.09	3.0×10^{-4}
3	4.3×10^{-5}	0.17	3.0×10^{-4}
4	8.0×10^{-5}	0.32	—
5	1.0×10^{-4}	0.40	—

* Koizumi and Imamura[2)]

agreement with those of Imamura. In two experiments on the air-saturated solutions for 8.0×10^{-5} and 1.0×10^{-4} M of dye, for which r is respectively 0.32 and 0.40, the initial rate is somewhat larger and, besides, the simple rate formula does not hold, the rate increasing as the reaction progresses. It is estimated that the final rate is several times larger than that at the initial stage. It is remarkable that an oxygen-saturated solution of the same concentration of dye still obeys the simple rate formula with the same slope as the others. There is no doubt, therefore, that the above anomalous behavior is due to the higher value of r.

Figure 6 gives the relation between the initial rate and the concentration of oxygen when the dye concentration is fixed at 10^{-4} M. It is seen from the figure that the rate increases with the oxygen concentration, reaching a maximum at about 1.5×10^{-4} M of oxygen and then falls to a constant value beyond $\sim2.5\times10^{-4}$ M of oxygen.

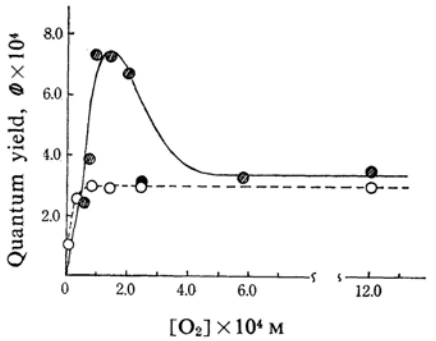


Fig. 6. Oxygen concentration effect on the quantum yield of bleaching.
Eosine concn.:
-●- 1.0×10^{-4} M, -○- 1.0×10^{-5} M

From the above results one can say that at high concentrations of dye, the rate of the oxydative bleaching of dye decreases when the oxygen concentration exceeds a certain value which is close to the dye concentration.

Such a phenomenon had not been observed in Imamura's experiment on 1×10^{-5} M of dye. This was reconfirmed by the present authors, as shown by a dotted curve in Fig. 6.

Quantum Yield of the Oxygen Up-take and the Ratio of Oxygen Consumption and the Dye Bleaching.—The quantity of light absorption in the oxygen consumption experiment was determined by measuring the oxidative photobleaching of dye in the Warburg apparatus under conditions similar to those used in Imamura's experiment and by using the value for the quantum yield reported by Imamura[2)] and confirmed by Grossweiner.[4)] Then the quantum yields for oxygen consumption are easily calculated from the results given in Figs. 2, 3, 4 and 5. The ratio of the consumed oxygen to the bleached dye was determined by measuring the dye concentration after the oxygen up-take experiment. Table II gives the results.

Although the data for $\Delta[O_2]/\Delta[D]$ scatter to a large extent, it seems that they are from 1 to 2 for eosine and fluorescein, while for erythrosine they are about 1.

There is no doubt from this table, that the value for the photobleaching at $10^{-4}-10^{-3}$ M of dye, as judged from the quantum yield for oxygen consumption and $\Delta[O_2]/\Delta[D]$, is in most cases a few times larger than that at 10^{-5} M.

Production of Hydrogen Peroxide and Its Effect on the Rate.—The quantity of hydrogen peroxide produced was analyzed by the ordinary iodometry. Some of the results are given in Table III.

TABLE II. QUANTUM YIELDS OF OXYGEN-UP-TAKE AND THE VALUES OF $\Delta[O_2]/\Delta[D]$

$C_0\times10^3$ M	Air-saturation $\gamma_{O_2}\times10^2$	Air-saturation $\Delta[O_2]/\Delta[D]$	O_2-saturation $\gamma_{O_2}\times10^2$	O_2-saturation $\Delta[O_2]/\Delta[D]$
Eosine				
6.8	1.24	—	1.14	—
5.1	1.05	—	1.14	—
3.4	—	—	1.14	—
1.0	1.14	1.4	0.85	1.9
0.43	1.05	1.8	0.85	2.9
0.085	0.59	2.4	0.44	(4.6)
0.043	0.17	1.9	0.32	—
Erythrosine				
8.0	0.43	—	0.72	—
6.0	0.41	—	0.5	—
4.0	0.41	—	0.50	—
2.0	0.41	—	0.50	—
1.0	0.41	1.0	0.41	1.3
0.50	0.36	0.7	0.43	1
0.10	0.28	0.6	0.20	0.7
0.050	0.14	0.4	0.18	—
Fluorescein				
8.0	1.06	—	1.01	—
4.0	1.01	—	0.92	—
2.0	1.15	2.35	0.71	1.72
1.0	0.82	2.05	0.57	1.34
0.50	0.65	1.88	0.50	1.49
0.10	0.35	2.70	0.09	—
0.050	0.19	(3.4)	—	—

TABLE III. PRODUCTION OF HYDROGEN PEROXIDE

Dye	Dye-concentration M	Quantity of bleached dye µM/300 min.	Quantity of produced H_2O_2 µM/300 min.
Fluorescein (phosphate-buffer)	2.0×10^{-3}		2.4
	1.0×10^{-3}	2.5	2.2, 3.0
	1.0×10^{-4}	(4.4)	0
Eosine	1.0×10^{-3}	1.4	2.6
	1.0×10^{-4}	0.5	1.4
Erythrosine	1.0×10^{-3}	0.7	0
	1.0×10^{-4}	0.4	0

It can be said that hydrogen peroxide is really produced when the dye concentration is high, and its quantity is of about the same order as that of the bleached dye. To examine the effect of hydrogen peroxide on the rate, hydrogen peroxide in concentrations as high as 1×10^{-3} M was added to the 1×10^{-3} M solution of eosine. The result was that the rate was the same within the experimental error.

Discussion

Reinterpretation of Imamura's Results.— According to Lindqvist,[3)] the triplet state (D^T) of fluorescein dianion is deactivated by oxygen or the ground state dye, as follows.

$$(1)\quad D^T+O_2\rightarrow D+O_2 \qquad k_1=1.7\times10^9\ \mathrm{M^{-1}\,sec^{-1}}$$

$$(2)\quad D^T+D\rightarrow D+D \qquad k_2=1\times10^8\ \mathrm{M^{-1}\,sec^{-1}}$$

Ohno and Koizumi estimated the rate constant of the process 2 for eosine dianion to be $5.5\times10^8\ \mathrm{M^{-1}\,sec^{-1}}$. These results imply that the lifetimes of the triplet state of these dianions are greatly reduced at 10^{-5} M of dye by the bimolecular deactivation process.

In 1955, when nothing was known about the rate constants of various elementary reactions, Imamura and Koizumi supposed that the genuine triplet lifetime is so short that the process 2 need not be taken into account. Hence their data must now be reinterpreted in such a way that, instead of self-deactivation, competitive deactivation is taking place with oxygen and the ground state dye. By slightly modifying the method given by Imamura, one can easily evaluate the ratio k_1/k_2 from the previous data for the oxygen concentration effect on the oxidative photobleaching of dye. The results are given in Table IV. These data refer to the solutions with pH near 7. From the above data one can say that the two deactivation processes have in general the same order of magnitude and hence it is very plausible that they are approximately diffusion controlled. A value of k_1/k_2 larger than 1 also supports this view.

TABLE IV

Dye	Temp, °C	k_1/k_2
Fluorescein	30	1.8
Eosine	30	1.4*
	40	5.3
Erythrosine	30	3.4
(Methylene blue)**	30	1.3

* Evaluated from the new data.

** The result for methylene blue is added for comparison.

Lindqvist's result for fluorescein at pH= 0—5.5, $k_1=1.2\times10^9\ \mathrm{M^{-1}\,sec^{-1}}$ and $k_2=2.5\times10^9\ \mathrm{M^{-1}\,sec^{-1}}$, as well as the above data for the alkaline region are not inconsistent with the above view. Grossweiner's value for k_1 of eosine is rather small, being $2.3\times10^7\ \mathrm{M^{-1}\,sec^{-1}}$. This was evaluated by analyzing the data on the competitive reaction of D^T with oxygen and phenol and it is suspected that this small value of k_1 has resulted from neglections to consider the simple deactivation of D^T by phenol.

Interpretation of the Oxygen Consuming Reaction. — Assuming the triplet dye to be simultaneously deactivated by oxygen and the ground state dye, and assuming the occurrence of a genuine reaction between D^T and D such that $D^T+D\rightarrow R+X$ where R and X are the half-reduced and half-oxidized dyes, respectively, then, during oxidative photobleaching, the D-O mechanism would occur when $[D] \ll [O_2]$ and the D-D mechanism would prevail in the opposite situation.

Now the present experimental conditions are such that the above switch-over of the mechanism should naturally occur. Hence the data will be treated along this line. The reaction scheme for lower concentrations of dye is

$$\text{A}\left\{\begin{array}{llr} D \rightarrow D^* & & \text{(i)} \\ D^* \rightarrow D^T & \varphi_{sT} I_{ab} & \text{(ii)} \\ D^T \rightarrow D & k_d & \text{(iii)} \\ D^T + O_2 \rightarrow D^T \cdots O_2 & \left.\begin{array}{l} k_{O_2} \\ k_{O_2}{}^{(d)} \end{array}\right\} k^s{}_{O_2} & \text{(iv)} \\ D^T + O_2 \rightarrow D + O_2 & & \text{(v)} \end{array}\right.$$

while with higher concentrations of dye, one plausible scheme after the step ii may be written as follows.

$$\text{B}\left\{\begin{array}{llr} D^T + D \rightarrow D^+ \cdots D^- & \left.\begin{array}{l} k_D \\ k_D{}^{(d)} \end{array}\right\} k^s{}_D & \text{(vi)} \\ D^T + D \rightarrow D + D & & \text{(vii)} \\ D^+ \cdots D^- \rightarrow D + D & k'_d & \text{(viii)} \\ D^+ \cdots D^- + O_2 \rightarrow \text{Prod.} & \left.\begin{array}{l} k'_{O_2} \\ k'_{O_2}{}^{(d)} \end{array}\right\} k'^s{}_{O_2} & \text{(ix)} \\ D^+ \cdots D^- + O_2 \rightarrow 2D + O_2 & & \text{(x)} \end{array}\right.$$

In the general case where both A and B are taking place simultaneously, the rate of oxygen consumption (expressed by the concentration dissolved oxygen), R_{O_2}, is easily derived as follows,

$$R_{O_2} = \{k_{O_2}[D^T][O_2] + k'_{O_2}[D^+ \cdots D^-][O_2]\}$$
$$= \frac{\varphi_{sT} I_{ab} k_{O_2}[O_2]}{k_d + k^s{}_{O_2}[O_2] + k^s{}_D[D]} \times \left\{1 + \frac{k_D k'_{O_2}[D]}{k_{O_2}(k'_d + k'^s{}_{O_2}[O_2])}\right\} \qquad (1)$$

The scheme B is still provisional but it is thought that the resultant expression will not be essentially affected by the details of the scheme. Quantum yield $\gamma_{O_2} = R_{O_2}/I_{ab}$ is given as follows after the neglection of k_d which is very small compared with the competitive terms.

$$\gamma_{O_2} = \frac{\varphi_{sT} k_{O_2}[O_2]}{k^s{}_{O_2}[O_2] + k^s{}_D[D]} \left\{1 + \frac{k_D k'_{O_2}[D]}{k_{O_2}(k'_d + k'^s{}_{O_2}[O_2])}\right\} \qquad (2)$$

In an extreme case where $[D] \gg [O_2]$

$$\gamma_{O_2}(\text{D-O}) = \frac{\varphi_{sT} k_{O_2}}{k^s{}_{O_2}} \qquad (2a)$$

and in the other extreme case where $[D] \gg [O_2]$

$$\gamma_{O_2}(\text{D-D}) = \varphi_{sT} \frac{k_D}{k^s{}_D} \frac{k'_{O_2}[O_2]}{k'_d + k^s{}_{O_2}[O_2]} \qquad (2b)$$

The condition for 2b to hold is fully satisfied when the concentration of dye is large enough, and quite reliable values for γ_{O_2}(D-D) can be evaluated directly from Figs. 2, 3 and 4. But this is not so with γ_{O_2}(D-O). To estimate this value the following procedure is conventionally adopted. If one puts $\alpha = k^s{}_{O_2}/k^s{}_D$, Eq. 2 can rewritten as follows,

$$\{\alpha[O_2] + [D]\} = \frac{\varphi_{sT} k_{O_2}[O_2]}{k^s{}_D} \times \left\{1 + \frac{k_{O_2}[D] k'_{O_2}}{k_{O_2}(k'_d + k^s{}_{O_2}[O_2])}\right\} \qquad (3)$$

By using the α-values given in Table V a plot of the left-hand side of Eq. 3 against [D] can be drawn and this should give a straight line. One can evaluate γ_{O_2}(D-O) and γ_{O_2}(D-D) from the slope and intercept respectively, although the former is not so reliable as the latter. As Fig. 7 shows, this method is fairly satisfactory and the results are given in Table V. It was checked that moderate change of α does not affect the result so much (cf*). For comparison, Imamura's data on the quantum yield of photobleaching at $[D] = 10^{-5}$ M are added in the last column. It is interesting that γ_{O_2}(D-D) for eosine and fluorescein is the same for the air-saturated and oxygen saturated solutions, while this is not so for erythrosine. This may be due to the difference in stability of the D-D redox intermediate.

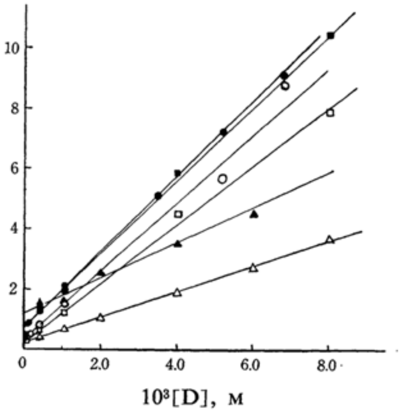


Fig. 7. Estimation of γ_{O_2}(D-O) and γ_{O_2}(D-D). Dark point is to the case of oxygen saturation and blank point to air saturation.
□■ uranine, ○● eosine, △▲ erythrosine

Interpretation of the Bleaching Curve.—As Fig. 5 shows, the rate of bleaching begins to increase when $\gamma = [D_0]/[O_2]$ exceeds about 0.2, and in such cases, the rate increases rapidly with the progress of the reaction. On the basis of the present view that a switch-over from the D-O to the D-D mechanism takes place, the above phenomena are just what is expected, because the D-D mechanism is considered to begin to contribute to the whole

TABLE V. QUANTUM YIELDS OF OXYGEN CONSUMPTION FOR D-O AND D-D MECHANISM

	α	Air-saturation		Oxygen saturation		$\gamma_{\text{dye-bleach}}$
		γ_{O_2}(D-O)	γ_{O_2}(D-D)	γ_{O_2}(D-O)	γ_{O_2}(D-D)	
Eosine	1.4	4×10^{-3}	1.2×10^{-2}	6×10^{-3}	1.3×10^{-2}	2.7×10^{-4}
Erythrosine	1.5	2.8×10^{-3}	4.3×10^{-3}	2.2×10^{-3}	6×10^{-3}	5.0×10^{-4}
	2.4*	2.5×10^{-3}	4.5×10^{-3}	3.4×10^{-3}	7×10^{-3}	—
Fluorescein (pH 7.65)	1.8	4.4×10^{-4}	1.1×10^{-2}	3.7×10^{-4}	1.2×10^{-2}	1.3×10^{-4}

bleaching reaction when $[D_0]$ approaches $[O_2]$, and further, because the oxygen concentration decreases as the reaction proceeds (the experimental condition is static in the bleaching experiment), thus increasing the contribution of the D-D process. Broadly speaking, the bleaching reaction is thought to proceed according to a scheme similar to that of the oxygen consumption reaction, hence it is very plausible that the bleaching rate via the D-D mechanism is several times larger than that via the D-O mechanism, and this is also just what is observed in Fig. 5.

The above view is further supported by the experiments in which the rate of bleaching is measured at a constant dye concentration of 10^{-4} M, and at various oxygen concentrations. As Fig. 6 shows, the rate increases with $[O_2]$ in the low $[O_2]$ region, reaches a maximum and finally falls to a constant value which of course corresponds to γ_{O_2}(D-O). The different behavior at 1×10^{-5} M of dye will be considered later.

Interpretation of Blum's Results and General Discussions.—Our results reconfirm Blum's observations mentioned at the beginning of this paper and make them more quantitative. Blum in addition, suggested that the formation of hydrogen peroxide is not directly connected with the decomposition of dye. This is clearly understood on the basis of a D-D mechanism. Thus at higher concentrations of dye, the reaction proceeds as follows:

$$D^T+D \rightarrow D^+\cdots D^-$$

$$D^+\cdots D^-+O_2+H^+ \rightarrow HO_2\cdot+D^++D$$

Alternatively,

$$D^T+D \rightarrow D^++D^-$$

$$D^-+H^++O_2 \rightarrow HO_2\cdot+D$$

and hydrogen peroxide is produced from $HO_2\cdot$, while most of D^+ is converted into the colorless form. Lindqvist proposed the view that dimerization of D^+ occurs. D^+ may, however, alternatively lose its dye structure by reacting with oxygen or it may revert to the original dye upon the attack of $HO_2\cdot$ as suggested by Lindqvist. Thus the formation of hydrogen peroxide and the destructive bleaching of dye proceed independently of each other in the later stage. The magnitude of oxidative bleaching and accordingly $\Delta[O_2]/\Delta[D]$ are thought to depend upon the fate of the half-oxidized dye. On the other hand, at low dye concentrations, oxygen directly attacks the triplet state leading to the decomposition of dye without the production of hydrogen peroxide. In this case the kinetic features of the bleaching are very simple.

Lastly, some speculative discussions will be added on the lifetime of the redox intermediate and on the general features of the photooxidative bleaching of the dye. In Imamura's experiments at $[D]=10^{-5}$ M, and as reconfirmed by the present investigation, the rate of the oxidative photobleaching decreases monotonously with the decrease of oxygen concentration below $[O_2]=10^{-5}$ M. But under such circumstances, the D-D mechanism should become appreciable, and if oxygen effectively attacks the D-D redox intermediate, a larger rate than that for the D-O mechanism should be expected. The experimental results thus require the D-D redox intermediate to have such a short lifetime that it has no chance to react with oxygen. Putting $[O_2] \sim 10^{-6}$ M and assuming that the reaction between oxygen and the intermediate is diffusion controlled, the decay constant of the D-D intermediate should be larger than $10^9\times10^{-6}=10^3\,\text{sec}^{-1}$. When $[O_2]$ is 10^{-4} M, the D-D intermediate of eosine should have enough chance to be attacked by oxygen, because γ_{O_2}(D-D) is independent of the oxygen concentration. Thus the decay constant of the D-D intermediate must be far less than $10^5\,\text{sec}^{-1}$. From the above argument, the decay constant of the D-D intermediate for eosine should be of the order of $10^4\,\text{sec}^{-1}$. In case of erythrosine this is a little larger since the dependence on $[O_2]$ is really observed. If one assumes that instead of the formation of $D^+\cdots D^-$, the reaction $D^T+D \rightarrow D^++D^-$ occurs very effectively, yielding free semiquinone and semioxidized dye, then the steady concentration of these species in the present experimental condition is estimated to be of the order of 10^{-9} M assuming that the reaction between these species is diffusion-controlled. Hence the lifetime of such species should be of the order of 1 sec. The present results completely reject this possibility and one can safely conclude that most of the redox intermediate $D^+\cdots D^-$ reverts to the original dye via the reverse reaction without forming separate radical ions.

The lifetime of $D^+\cdots D^-$ and its tendency to form free radical ions will depend sensitively on the experimental conditions as well as on the nature of the dye, and this is perhaps one of the reasons why dyes of the same group show sometimes very different behavior and why the general nature of the reaction is often difficult to understand.

Summary

The oxygen up-take of irradiated aqueous solutions of xanthene dyes has been investigated by means of Warburg's apparatus, and evidence for a switch-over from a D-O to a D-D mechanism is obtained from the variation of dye-concentration. This view is supported by the photobleaching rate and the oxygen concentration effect at high eosine concentrations ($\sim 10^{-4}$ M).

The quautum yields (γ_{O_2}) of the consumption are estimated and it is found that γ_{O_2} (D-D) is larger than γ_{O_2}(D-O).

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