An Attempt to Determine Fluorescein's S*–T Transition Probability and a Preliminary Investigation of the Behavior of Its Semiquinone in an Alkaline Solution

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Having previously reported on the determination of φ_{S-T}* for eosine,[1] we will report here on a similar attempt with fluorescein. Although a value of 2.01×10^{-2} for ψ_{S-T} at pH 7.0 has been reported by Oster et al.,[2] it seems desirable to repeat this experiment and to examine more critically the conditions necessary for this method to be applicable. For example, a more detailed investigation of the rate formula and of the steps in the reaction following the formation of semiquinone seems to be required. Moreover, the pH value at which their experiment was made may have been the cause of some ambiguity, because in this pH region divalent and monovalent anions exist simultaneously.[3,4] We have, therefore, chosen a pH >10.9 and used disodium ethylenediaminetetraacetate (EDTA) and allylthiourea (ATU) as the reducing agents. In addition, it must be recalled that leuco-fluorescein can be oxidized photochemically by using fluorescein as a sensitizer in the evacuated state.[5,6] If the product of the photoreduction with EDTA or ATU is really a leuco compound, a similar phenomenon may be expected; therefore, this fact must be taken into consideration in order to obtain an exact kinetic treatment. Hence, the behavior of the reaction product was investigated; the effect of the addition of the reducing agents on the sensitized oxidation of leucofluorescein was also examined to some extent.

It was established that semiquinone is formed as a rather stable intermediate product and that the behavior of the final product differs from that of the product obtained by the photoreduction in an alcoholic solution. It was further found that most of the semiquinone is gradually converted to the oxidized form in the dark and, quite astonishingly, in the ratio of 1 : 1.

A simple rate formula could not always be given, and even a rather tentative value of φ_{S-T} was determined only under special conditions.

Experiment

Materials.—*Allylthiourea.*—Tokyo Kasei G. R. 1-allyl-2-thiourea was recrystallized three times from the aqueous solution.

Disodium Ethylenediaminetetraacetate, Dihydrate (DOTITE 2 Na).—The special grade reagent of the Dojindo Pharm. Ltd., was used.

Fluorescein.—Commercial material was repeatedly recrystallized from ethanol.

Leuco-fluorescein.—Fluorescein was reduced with sodium amalgam and, after repeated recrystallizations from ethanol solution, desiccated in a high vacuum.[5]

Sodium Hydroxide.—A saturated solution was prepared from the G.R. reagent of the Wako Junyaku Co. The supernatant liquid was diluted to the desired concentration.

Disodium Hydrogen Phosphate.—A G. R. reagent of Wako Junyaku Co. was recrystallized three times from the aqueous solution.

Apparatus.—The apparatus is shown in Fig. 1.

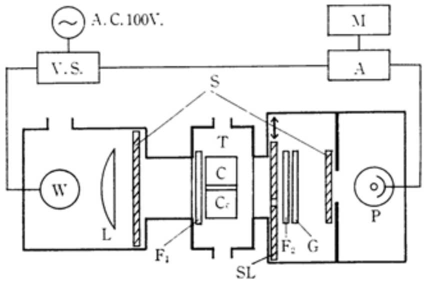

Fig. 1.

V. S.	Voltage stabilizer		
W	Tungsten lamp	L	Collimating lens
S	Shutter	T	Thermostat
C	Sample cell	C_0	Reference cell
F_1	Cut-off filter ($>$430 mμ)		
F_2	Interference filter (peak 490 mμ)		
SL	Slit for changing light path		
G	Frosted glass	P	Photoelectric tube
A	Amplifier	M	Microammeter

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* φ_{S-T} is the S*→T transition probablity. As to the general purpose for which the present investigations have been made, seen Ref. 1.

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

[2] A. H. Adelman and G. Oster, *J. Am. Chem. Soc.*, **78**, 3977 (1956).

[3] V. Zanker and W. Peter, *Chem. Ber.*, **91**, 572 (1958).

[4] L. Lindqvist, *Arkiv Kemi*, **16**, 79 (1960).

[5] K. Uchida, S. Kato and M. Koizumi, This Bulletin, **35**, 16 (1962).

[6] K. Uchida and M. Koizumi, ibid., **35**, 1871 (1962).

Procedure.—The light source, L, was a tungsten lamp (Kondo Projection Lamp 100 V.-500 W.); this was always operated at 50.0 V. All the experiments were carried out at 25°C in a thermostat. The fluorescein solution was ordinarily made up in a 1×10^{-5} M aqueous solution. The concentration of reducing agents (ATU or EDTA) was 3×10^{-2}—3×10^{-3} M. The sample solutions were made up in a sodium hydroxide - disodium hydrogen phosphate buffer at pH 10.95 or in a 1.0×10^{-1} N, 5.0×10^{-2} N, or 1.0×10^{-3} N sodium hydroxide solution. The solution was usually degassed five times by using a mercury diffusion pump, melting and freezing being repeated each time. After degassing, the solution was poured into a reaction cell 1 cm. thick. The fading and coloring of the solution was followed with a photoelectric tube (Toshiba 7211). The absorption spectra of the solutions were measured with a Hitachi EPU-2A spectrophotometer or a Hitachi EPS-2 recording spectrophotometer. The absolute intensity of the light falling on the reaction cell was determined with a calibrated thermopile (Kipp & Zonen, type E 5) with a sensitivity of 1.02×10^{-5} V./W. of radiant energy.

Results and Discussion

Preliminary Observation.—A check was made to ensure that the addition of EDTA or ATU did not essentially affect the absorption spectra of the dye, and that no dark reaction occurred. When the sample was illuminated with visible light, the absorption spectra in the visible region always declined monotonously, without any even slight shift. When EDTA was used as a reducing agent, the absorption due to semiquinone was readily apparent near 394 mμ, and at the same time, the decrease in the absorbance near 300 mμ was somewhat less. The last phenomenon[7] is considered to be due to the formation of leuco-uranine. Figure 2 shows a typical example. In the case of ATU, the absorption spectrum of the product could not be obtained in detail, because ATU absorbs up to $\lambda=310$ mμ. The increase in the absorbance near 394 mμ, however, could be clearly detected, although the intensity of the absorbance near 394 mμ was much less than in the case of EDTA.

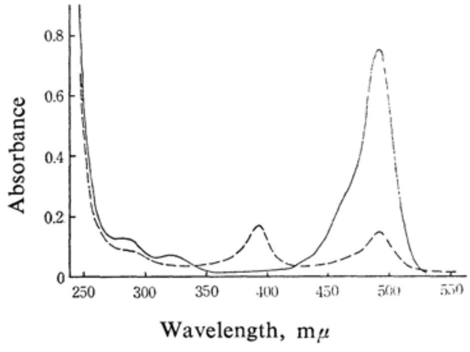

Fig. 2. Change of the absorption spectra of fluorescein by irradiation.
$[\mathrm{U}]_0=1\times10^{-5}$ M, $[\mathrm{EDTA}]_0=4.0\times10^{-3}$ M, $[\mathrm{NaOH}]=0.1$ N
—— before irradiation
---- immediately after 28.3 min. irradiation

Studies of the Reaction Products.—*The Formation of Semiquinone and Its Kinetic Behavior.*—Since semiquinone was found to be formed as a rather stable product both when EDTA and when ATU were used, its behavior was investigated in some detail. Figure 3 shows a typical example of the change in the absorption spectra when the irradiated solution involving EDTA was kept in the dark. It shows that the gradual dercrease in the height of the peak at 394 mμ is accompanied by an increase in the main absorption of the dye. An isosbestic point is seen at 430 mμ. The behavior agrees quite well with that, in ethanol-water or in isopropanol-water, observed by Imamura.[7] The molar absorbance of semiquinone at $\lambda=394$ mμ was determined in the following way.

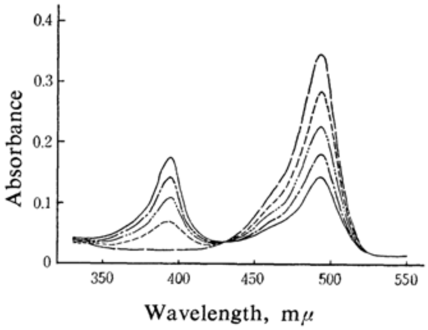

Fig. 3. Change of the absorption spectra when the solution having the spectra --- in Fig. 2, is kept in the dark.
—— after 2 min. 35 sec.
—-- after 20 min. 25 sec.
---- after 45 min. 15 sec.
---- after 129 min.
— — after 1000 min.

After the absorbances at 394 and 490 mμ of the partially-bleached solution had been measured, air was introduced into the solution and changes in the absorbances were examined immediately. Since it was confirmed that only semiquinone is converted to the original dye by this procedure, ε_{394} can easily be obtained by using the ε_{490} value of uranine (7.80×10^4 at pH 9). Table I gives some of the results.

The value, 5.0×10^4, obtained from the mixture of uranine and leuco-uranine is a little

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7) M. Imamura, This Bulletin, 31, 962 (1958).

TABLE I. THE DETERMINATION OF ε_{394} OF SEMIQUINONE

A)

$[L]_0$* M	$[U]_0$* M	[NaOH] N	Change in absorbance at 490 mμ	Change in absorbance at 394 mμ	ε_{394}
30.3×10^{-6}	7.7×10^{-6}	1.0	1.003	0.650	5.05×10^{4}
31.5×10^{-6}	6.5×10^{-6}	5.0×10^{-2}	0.702	0.438	4.88×10^{4}
					Av. 5.0×10^{4}

B) $[U]_0=1\times10^{-5}$ M, [NaOH]=0.1 N

Concn. of EDTA M	Change in absorbance at 490 mμ	Change in absorbance at 394 mμ	ε_{394}
3.0×10^{-2}	0.238	0.147	4.82×10^{4}
1.0×10^{-2}	0.247	0.142	4.98×10^{4}
1.0×10^{-2}	0.261	0.148	4.42×10^{4}
6.0×10^{-3}	0.256	0.146	4.45×10^{4}
6.0×10^{-3}	0.217	0.128	4.60×10^{4}
6.0×10^{-3}	0.242	0.150	4.84×10^{4}
4.0×10^{-3}	0.261	0.180	5.38×10^{4}
4.0×10^{-3}	0.256	0.144	4.38×10^{4}
3.0×10^{-3}	0.265	0.151	4.44×10^{4}
3.0×10^{-3}	0.254	0.145	4.45×10^{4}
6.0×10^{-3}**	0.272	0.154	4.42×10^{4}
			Av. $4.6_5\times10^{4}$

* $[L]_0$ and $[U]_0$ are the initial concentrations of leuco-uranine and uranine.
** $[NaOH]=5.0\times10^{-2}$ N.

larger than that (4.7×10^{4}) found for the solution containing EDTA. Since, however, the former agrees quite well with the published date ($\varepsilon_{394}=5\times10^{4}$ (Lindqvist[4]; 0.01 N NaOH), $\varepsilon_{395}=4.9\times10^{4}$* (Imamura; water-ethanol or water-isopropanol alkaline solution)), it may be accepted as reliable.

Using $\varepsilon_{394}=5.0\times10^{4}$, the quantities of semiquinone produced under various conditions were evaluated. It was found that when the alkaline solution, 0.1 N sodium hydroxide, containing 1×10^{-5} M fluorescein and various amounts of EDTA was illuminated for twenty minutes, about one-half of the bleached dye was present as a semiquinone. Some of the results are shown in Table II.

TABLE II. THE CHANGE IN THE ABSORBANCE AT 490 mμ ($-D_{ox}$) AND AT 394 mμ ($+D_{sem}$) DURING THE PHOTOREDUCTION BY EDTA

Concn. of EDTA, M	$-D_{ox}$	$+D_{sem}$	$R(-\frac{D_{sem}}{D_{ox}})$	f_{sem}
1×10^{-2}	0.506	0.142	0.28	
1×10^{-2}	0.462	0.148	0.32	
6×10^{-3}	0.562	0.146	0.26	
6×10^{-3}	0.563	0.128	(0.22)	
4×10^{-3}	0.544	0.180	0.33	
4×10^{-3}	0.546	0.144	0.32	
3×10^{-3}	0.544	0.151	0.27	
3×10^{-3}	0.433	0.145	0.33	
			Av. 0.29±0.03	0.45±0.05

The ratio of the quantity of the produced semiquinone to that of the consumed dye, f_{sem}, is 0.45±0.05.

In the case of ATU, the quantity of produced semiquinone is much smaller and appears to change according to the experimental conditions. Table III gives the values of f_{sem} obtained, when 0.1 N aqueous sodium hydroxide solutions of fluorescein, 1×10^{-5} M, containing various amounts of ATU were irradiated for twenty minutes.

TABLE III. f_{sem} FOR ATU

Concn. of ATU, M	$-D_{ox}$	f_{sem}
3.0×10^{-2}	0.534	0.04
1.6×10^{-2}	0.532	0.03
1.0×10^{-2}	0.532	0.07
6.0×10^{-3}	0.530	0.06
(4.0×10^{-3}	0.631	0.02)

Thus, f_{sem} value for ATU is only few hundredths for EDTA. Furthermore, in the mixed solvent of ethanol and water containing 3×10^{-2}—4×10^{-3} M ATU and 5×10^{-2} N sodium hydroxide, f_{sem} reaches 0.34 (the average of four determinations). From these results, one may characterize the reaction as follows. The reducing agent, RH_2, attacks the triplet dye, D^t, and forms a transient intermediate consisting of semiquinone and a half-oxidized reducing agent. If the intermediate simply dissociates into its two components, then the semiquinone would remain as a stable product,

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* Imamura's value was recalculated since the method of evaluation was found to involve some misunderstanding.

while if the cleavage involves a hydrogen transfer, then leuco-uranine or a leuco-type compound would be produced. Schematically, this may be written as follows:

$$D^{t}+RH_2 \rightarrow DH\cdots RH$$

$$DH\cdots RH \rightarrow DH+RH \qquad (a)$$

$$\hookrightarrow DH_2+R \qquad (b)$$

Thus, the behavior of the D-R redox-intermediate is quite different in EDTA and ATU; the reaction in the former case proceeds along both paths, a and b, with equal probabilities, while in the latter case the principal path is b.

When a solution which had been illuminated and which contained some semiquinone was kept in the dark, semiquinone gradually returned to the oxidized form. For example, when the solution containing about 3×10^{-6} M of semiquinone, obtained by illuminating the 0.1 N sodium hydroxide solution containing 1×10^{-5} M uranine and 3.0×10^{-2} M EDTA, was kept in the dark, 2.58×10^{-6} M of semiquinone disappeared and 3.07×10^{-6}M of dye was recovered in about 220 min. The ratio is close to one, indicating that 1 M semiquinone is converted into the 1 M oxidized form. This is quite unexpected, because the disproportionation of the semiquinone is generally considered to be the normal process.

Analogous results were obtained for the irradiated solution containing 5.0×10^{-2} N sodium hydroxide, 9.0×10^{-6} M leuco-uranine and a small quantity of uranine. The illumination of this solution for about 20 min. produced 5.04×10^{-6} M of semiquinone. When the solution thus obtained was kept in the dark for five hours, the decrease in semiquinone and the increase in the oxidized form were, respectively, 3.46×10^{-6}M and 3.15×10^{-6}M; after an additional twelve hours, the two quantities increased, respectively, to 5.04×10^{-6} M and 4.87×10^{-6}M. The ratios of the two quanties in the two cases are, respectively, 1.10 and 1.03, again indicating that the main reaction is $2DH \rightarrow 2D+H_2$. Even in the presence of ATU or EDTA, illumination converts leuco-uranine into semiquinone (see below) and the ratio of the decrease in semiquinone to the increase in uranine is also about one. All these results suggest that semiquinone, once produced, is not affected very much by EDTA, ATU, leuco or oxidized dye.

This view was further supported by the fact that semiquinone in all cases disappears according to the second order rate formula, although the rate constant itself is affected moderately by the presence of the reducing agent. Some of the rate constants in various conditions are given in Table IV. Figure 4 gives some examples of the second order plots.

Although the data are still preliminary, it can be said that the rate constant is very sensitive to the pH value. Further, it appears that the addition of EDTA or ATU increases the rate costant to some extent.

The Behavior of the Leuco Dye.—As has already been reported (in 1962),⁵⁾ leuco-uranine is oxidized to uranine by a photosensitized reaction using uranine as a photosensitizer.

TABLE IV. SECOND ORDER RATE CONSTANTS FOR THE DISAPPEARANCE OF SEMIQUINONE

$[U]_0$ M	[NaOH] N	$[L]_0$ M	[EDTA] or [ATU], M	Wavelength used for analysis, mμ	k, sec^{-1} M^{-1}
1.0×10^{-6}	1.0	37.0×10^{-6}	0	394	8.1
6.4×10^{-6}	1.0	31.6×10^{-6}	0	394	1.1
1.0×10^{-6}	5.0×10^{-2}	9.0×10^{-6}	0	394	19.9
7.1×10^{-6}	5.0×10^{-2}	1.9×10^{-6}	0	394	24.6
1.0×10^{-6}	5.0×10^{-2}	37.0×10^{-6}	0	394	9.73
3.8×10^{-6}	5.0×10^{-2}	34.2×10^{-6}	0	394	6.22
3.5×10^{-6}	1.0×10^{-3}	34.5×10^{-6}	0	394	4280
4.1×10^{-6}	1.0×10^{-3}	33.9×10^{-6}	0	394	4000
4.2×10^{-6}	1.0×10^{-3}	33.8×10^{-6}	0	394	4760
4.3×10^{-6}	1.0×10^{-3}	33.7×10^{-6}	0	394	4270
1.0×10^{-5}	1.0×10^{-1}	0	E 3.0×10^{-2}	394	181
1.0×10^{-5}	5.0×10^{-2}	0	E 1.0×10^{-2}	490	49.5
1.0×10^{-5}	5.0×10^{-2}	0	E 6.0×10^{-3}	490	90.1
1.0×10^{-5}	1.0×10^{-1}	0	E 4.0×10^{-3}	394	83.4
1.0×10^{-6}	5.0×10^{-2}	9.0×10^{-6}	A 4.0×10^{-3}	394	86.1
1.0×10^{-6}	5.0×10^{-2}	9.0×10^{-6}	A 4.0×10^{-3}	394	296
1.0×10^{-6}	5.0×10^{-2}	9.0×10^{-6}	E 4.0×10^{-3}	394	911
4.5×10^{-6}	5.0×10^{-2}	5.5×10^{-6}	E $4\ \times10^{-3}$	394	866

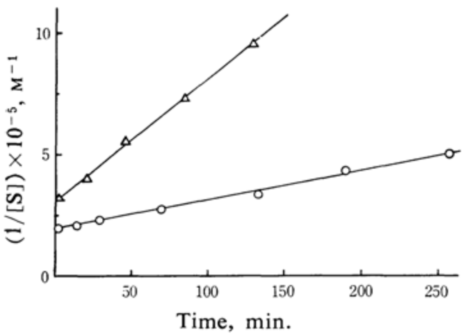

Fig. 4. Decay of the semiquinone as the second order process.

○ for the solution $[L]_0=9.0\times10^{-6}$ M, $[U]_0=1.0\times10^{-6}$ M, [NaOH]=0.05 N, irradiated for 19.5 min. $k=19.9$ M^{-1} sec^{-1}

△ for the solution $[U]_0=1\times10^{-5}$ M $[EDTA]_0=4.0\times10^{-3}$ M, [NaOH]=0.1 N, irradiated for 28.3 min. $k=83.4$ M^{-1} sec^{-1}

In order to compare the behavior of the photoproduct (other than semiquinone) obtained in the present experiments with that of leuco dye, the above photooxidation of leuco-uranine was reinvestigated. The results obtained in the present investigation were somewhat different from those that had been obtained before.

For instance, in the previous experiments, leuco-uranine was photooxidized to the dye, with the peak at 490 mμ rising monotonously, whereas in the present experiments the absorbance at 490 mμ showed a distinct maximum in the course of irradiation. For example, when a 5.0×10^{-2} N aqueous sodium hydroxide solution of 9.0×10^{-6} M leuco-uranine containing 1.0×10^{-6} M uranine was irradiated, the peak at 490 mμ rose at first, but soon, within a few minutes, it reached a maximum, equivalent to 3.38×10^{-6} M, and then fell to a constant value. After about twenty minutes' illumination, the concentrations of the oxidized dye and of the semiquinone reached, respectively, 2.11×10^{-6} M and 5.04×10^{-6} M. In another example using a similar solution, illumination caused the peak at 490 mμ to increase until, after 1.75 min., it reached a maximum value, equivalent to 2.64×10^{-6} M; after 50 minutes' illumination, the concentration of the oxidized form decreased to 0.90×10^{-6} M, while the concentration of semiquinone rose to 5.82×10^{-6} M.

These results imply that the oxidized form is produced in the initial stage, perhaps by the reaction c, $D^t+DH_2\rightarrow2D+H_2$, that at the same time, semiquinone is also produced, perhaps according to the reaction d, $D^t+DH_2\rightarrow2DH$, and, furthermore, that the latter reaction gradually supersedes the former, causing a decrease in the oxidized form. It is evident from the above results that the entire quantity of D produced by the reaction $D^t+DH_2\rightarrow2D+H_2$ is actually much larger than the increment of D at the maximum point, as long as one can consider that the formation of semiquinone is due to the reaction $D^t+DH_2\rightarrow2DH$. When the solution was kept in the dark, semiquinone was gradually converted to the oxidized form, as has been mentioned already. When this solution was illuminated again, a steady decrease in the peak at 490 mμ was observed. A typical series of experiments is shown in Fig. 5.

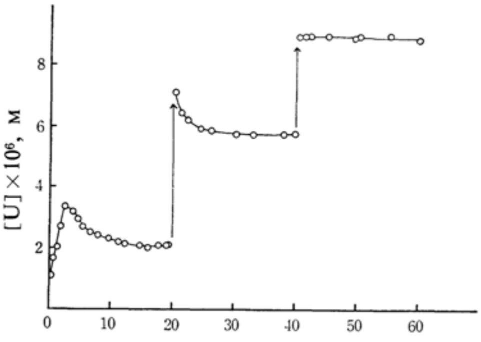

Fig. 5. Change of the dye concentration when the solution, $[L]_0=9.0\times10^{-6}$ M, $[U]_0=1.0\times10^{-6}$ M, [NaOH]=0.05 N, is irradiated.

↑ Change when the solution is kept in the dark for several days.

It appears that when the concentration of the oxidized dye exceeds a certain value, the peak at 490 mμ falls from the starting point instead of undergoing an initial increase; apparently a large excess of the oxidized form favors the reaction d. Although the present results are very curious and apparently differ from the previous results, the overall reaction is the same in both cases, i. e., $D^t+DH_2\rightarrow2D+H_2$.

The oxidation of leuco-uranine is observed even in the presence of a moderate quantity of ATU or EDTA. The quantum yields for the production of the oxidized form at the initial stage are 3.3×10^{-2}, 1.6×10^{-2}, and 8.4×10^{-3} when the concentrations of added ATU are zero, 4.0×10^{-3} and 1.0×10^{-2} M respectively. In the case of 4.0×10^{-3} M EDTA, the quantum yield is 2.0×10^{-2} under otherwise similar conditions. The behavior during the course of illumination is at least qualitatively similar to that in the absence of any reducing agent. Thus the quantity of the oxidized form usually reaches a maximum after a few minutes and then decreases. The quantity of semiquinone produced during irradiation varies, however, according to the experimental conditions. When the solution is kept in the dark for a long time, about the same quantity of the oxidized form as that of semiquinone is produced.

The cause of the discrepancy between these results and those obtained previously is at present unknown.

The Final Products when the Reducing Agent is EDTA.—It was established that when EDTA is used as a reducing agent in an alkaline solution, the reaction product consists mainly of semiquinone and leuco-uranine. The evidence will be given below.

Firstly, the absorbances for uranine, leuco-uranine and semiquinone were determined at two wavelengths in order to make the analysis spectroscopically. The extinction coefficients given in Table V were obtained by the following procedures.

TABLE V. THE EXTINCTION COEFFICIENTS OF URANINE, LEUCO-URANINE AND SEMIQUINONE AT 285 AND 300 mμ

	285 mμ	300 mμ
Uranine : ε_U	1.3×10^4	7.2×10^3
Leuco-uranine : ε_L	6.1×10^3	6.1×10^3
Semiquinone : ε_S	5.8×10^3	4.9×10^3

ε_U : from the alkaline solution of uranine at a known concentration.
ε_L : from the solution of leuco-uranine at a known concentration.
ε_S : from the irradiated solution of (leuco+uranine) at a known concentration $([L]+[U])_0$.

The concentration of semiquinone produced by the reaction $D^t + DH_2 \rightarrow 2DH$ was evaluated from ε_S at 394 mμ, where ε_U and ε_L are practically nil. The concentration of uranine was obtained from the absorbance at 490 mμ. The concentration of leuco-uranine was calculated from $[L]+[S]+[U]=([L]+[U])_0$. Two examples of analysis by the use of the above data are given in Table VI.

Thus, the sum of [L] and [S] agrees with the total concentration of bleached dye within the range of experimental error. The above data confirm the afore-mentioned result that semiquinone forms about one-half of the total dye that has disappeared.

A remarkable fact that was found is that the leuco dye produced in this case goes back to the oxidized form, although very slowly. When the solution is kept in the dark, the reaction proceeds as a second order reaction in regard to [L]. This may be due to the reaction between leuco dye and the oxidized product of EDTA. A few examples of the second order plot are given in Fig. 6.

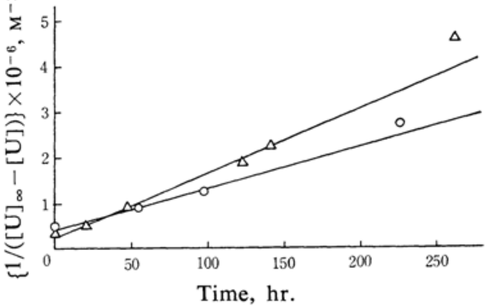

Fig. 6. Second order plot for the recovery of dye from the final product obtained by illuminating the solution containing uranine and EDTA.
○ for the solution, $[U]_0=1\times10^{-5}$ M, $[EDTA]_0=3.0\times10^{-2}$ M, $[NaOH]=0.1$ N, $k=2.7$ sec^{-1} M^{-1}
△ for the solution, $[U]_0=1\times10^{-5}$ M, $[EDTA]_0=1.0\times10^{-2}$ M, $[NaOH]=0.05$ N, $k=4.0$ sec^{-1} M^{-1}

Some of the rate constants obtained are:

i) $[NaOH]=1.0\times10^{-1}$ N; $[U]_0=1\times10^{-5}$ M; $[EDTA]_0=3.0\times10^{-2}$ M;
$k=2.7$ sec^{-1} M^{-1} (181 sec^{-1} M^{-1})

ii) $[NaOH]=5.0\times10^{-2}$ N; $[U]_0=1\times10^{-5}$ M; $[EDTA]_0=1.0\times10^{-2}$ M;
$k=4.0$ sec^{-1} M^{-1} (49.5 sec^{-1} M^{-1})

The values in parentheses refer to the second order rate constant for the decay of semiquinone. The order of magnitude for the two rate constants being different, one can treat the two processes separately. The total dye recovered from the solution usually reached about 90% of the original quantity.

The Product when ATU was Used. — The absorption spectra of the reaction product could not be investigated, because ATU absorbs

TABLE VI. ANALYSIS OF THE PRODUCTS

Irradiated solution			[L], M	[S], M	Total of the bleached dye, M
1.0×10^{-1} N [NaOH],	1×10^{-5} M $[U]_0$,	4.0×10^{-3} M $[EDTA]_0$,	6.1×10^{-6}* 6.0×10^{-6}**	3.5×10^{-6}	7.8×10^{-6}
1.0×10^{-1} N [NaOH],	1×10^{-5} M $[U]_0$,	6.0×10^{-3} M $[EDTA]_0$,	5.4×10^{-6}* 4.9×10^{-6}**	3.4×10^{-6}	8.7×10^{-6}

* From ε_{285} ** From ε_{300}

up to 310 mμ. Hence, the reaction product, other than semiquinone, was examined by means of a paper chromatograph. As a developer, a mixture of water (75 vol.%), ethanol (15 vol.%) and a 28% ammonia solution (10 vol.%) was used. The spots after development were detected by the emitted fluorescence.[5] Table VII gives the results obtained in one experiment.

TABLE VII. THE RESULTS FOR A PAPER CHROMATOGRAPH

Sample	R_f	
	Uranine	Leuco
1) Aq. soln. of uranine	0.79 (yellow)	
2) Aq. soln. of (ATU+ Leuco)	0.79 (faint yellow)	0.85 (dark yellow)
	0.78 (faint yellow)	0.85 (dark yellow)
3) Leuco -uranine	0,78 (faint yellow)	0.84 (dark yellow)
4) The irradiation product of uranine (buffer soln.)	0.77 (weak)	0.95 (?)

The product does not seem to be leuco-uranine. ATU and leuco dye might form some kind of intermolecular complex. The solution, after being kept in the dark for a day, showed no change in the absorption spectra.

In connection with the nature of the photoproduct in the case of ATU, it is important to note that if the main product were leuco-uranine (the quantity of semiquinone forms only a small percentage, as has already been stated), the photoreduction should cease at a certain point, since the rate of the reverse reaction is quite large. However, this is not the case, as is evident from Fig. 7. This is additional evidence that the photoproduct is not leuco-uranine in the case of ATU.

Concluding Remarks.—When EDTA is used as a reducing agent, the photoproduct consists of semiquinone and leuco dye in the ratio of about 1. Semiquinone is rather indifferent to EDTA and very slowly returns to the oxidized form, mainly by the $2DH \rightarrow 2D + H_2$ reaction but this does not affect the rate of the photoreduction under ordinary conditions. Leuco-uranine, on the other hand, reacts according to $D^t + DH_2 \rightarrow 2D + H_2$ or $D^t + DH_2 \rightarrow 2DH$; either process would affect the overall reduction rate in the opposite direction. It may, therefore, be inferred that the effect of the product on the rate would not be very large.

When ATU is used as a reducing agent, the quantity of semiquinone forms only a small percentage of the total product and the main product is some substance other than leuco-uranine. The effect of this substance on the rate is quite unknown at present.

Kinetic Studies and the Determination of φ_{S-T}.*—Most of the experiments concerning the effect of various factors upon the rate were made using ATU as a reducing agent in a sodium hydroxide - disodium hydrogen phosphate buffer solution (pH=10.94). However, the simple rate formula did not generally hold for this solution, while it did hold when a strong alkaline solution of sodium hydroxide was used. Hence, the later experiments for determining φ_{S-T} were performed in such a solution.

Rate Formula.—The following two rate formulas were tested:

a) First order rate formula with respect to the quantity of absorbed light:

$$-\frac{dc}{dt} = \Phi \frac{I_0}{d}(1-e^{-\alpha c d}) \times 1000$$

By integration:

$$\ln(e^{\alpha c d}-1) = \ln(e^{\alpha c_0 d}-1) - 1000\Phi \alpha I_0 t$$

where Φ, α, d, c and I_0 are, respectively, the quantum yield, the mean absorption coeffecient, the length of the light path, the concentration of dye, and the number of input photons (in mole) per cm^2. The above relation corresponds to the situation where bleaching is simply proportional to the absorption of light.

b) Second order rate formula;

$$\frac{1}{C} - \frac{1}{C_0} = \mathrm{kt}$$

Although neither a) nor b) can reproduce the general bleaching curve when ATU is present in the buffer solution, it seems that when the concentration of ATU is large enough, the relation a holds approximately; it seems further that the relation holds better at lower dye concentrations. On the other hand, at lower concentrations of ATU and higher concentrations of dye the relation b seems to hold better. Some examples are shown in Fig. 7.

These results suggest that the D-R mechanism[1] predominates in the higher concentration region of ATU, whereas the D-D mechanism[1] begins to play a part in its lower concentration region. This suggestion is consistent with

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* Details of the method were described in a previous paper. See Ref. 1.

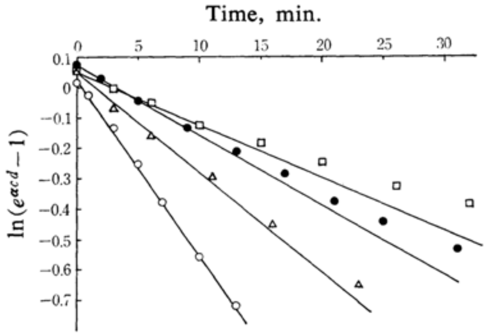

Fig. 7. Plot of $\ln(e^{\alpha cd}-1)$ vs. time for the buffer solution (pH 10.94), $[U]_0=1\times10^{-5}$ M. The concentration of ATU is;
○ 3.0×10^{-2} M; △ 1.5×10^{-2} M;
● 7.0×10^{-3} M; □ 3.5×10^{-3} M

the following rate constants reported by Lindqvist[4]:

$$D^t+D \rightarrow 2D \qquad 5\times10^7\ \mathrm{M^{-1}\,sec^{-1}}$$

$$D^t+D \rightarrow D^-+D^+ \qquad 5\times10^7\ \mathrm{M^{-1}\,sec^{-1}}$$

$$D^t+\mathrm{ATU} \begin{cases} \nearrow D^-+(\mathrm{ATU})^+ \\ \searrow D+\mathrm{ATU} \quad 2\times10^5\ \mathrm{M^{-1}\,sec^{-1}} \end{cases}$$

(all in the alkaline region)

Intensity Effect.—Using a buffer* solution of 1×10^{-5} M uranine containing 6.0×10^{-3} M ATU, the effect of the intensity of the exciting light was examined. When the metallic net with a transmission coefficient of 39.3% was inserted into the light path, the rate decreased to 36% of the original value. The rate is thus proportional to the intensity of light.

The Effect of the Dye Concentration on the Rate.—Using a solution containing 4.0×10^{-3} M ATU, and 5.0×10^{-2} N sodium hydroxide the concentration of the dye was varied from 4.6 to 23×10^{-6} M. The $1/\Phi$** vs. $[U]_0$ plot given in Fig. 8 clearly demonstrates that, in contrast to the case of eosine, the concentration of fluorescein has little effect on the quantum yield.

This different behavior may be accounted for by the value of the rate constant for:

$$D^t+D \begin{cases} \nearrow 2D \\ \searrow D^-+D^+ \end{cases}$$

and for:

$$D^t+\mathrm{ATU} \begin{cases} \nearrow D^-+(\mathrm{ATU})^+ \\ \searrow D+\mathrm{ATU} \end{cases}$$

(The values for fluorescein are given above; the values for eosine are, respectively, $8.8\times10^8\ \mathrm{M^{-1}\,sec^{-1}}$ and $3.4\times10^5\ \mathrm{M^{-1}\,sec^{-1}}$)

The Effect of the Reaction Product.—Using a buffer solution containing 5.0×10^{-3} M ATU and 1×10^{-5} M uranine, the effect of the reaction product on the rate was examined. The solution was irradiated and the initial rate was measured. After about 70% of dye had been bleached, the concentration of uranine was again increased to 1.2×10^{-5} M and the experiment was repeated. The rate in the second run was found to be about one-half of that in the first run.

The Determination of φ_{S-T} under Various Conditions.—The rate formula a holds practically in 5.0×10^{-2} N and 1.0×10^{-1} N sodium hydroxide alkaline solution when the concentration of ATU is in the 4×10^{-3}—3×10^{-2} M range. The plot of $1/\Phi$ against $1/[\mathrm{ATU}]_0$ as shown in Fig. 9, gives a satisfactorily straight line.

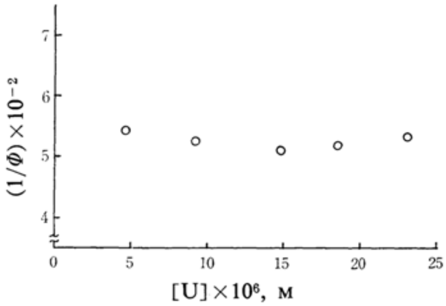

Fig. 8. $1/\Phi$ vs. $[U]_0$ plot for the solution containing $[\mathrm{ATU}]_0=4.0\times10^{-3}$ M, $[\mathrm{NaOH}]=0.05$ N.

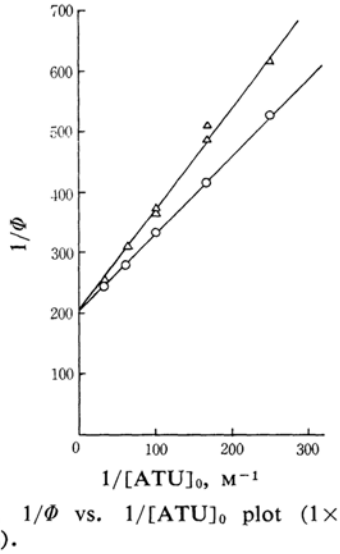

Fig. 9. $1/\Phi$ vs. $1/[\mathrm{ATU}]_0$ plot (1×10^{-6} M $[U]_0$).
△ 0.1 N [NaOH]; ○ 0.05 N [NaOH]

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* A sample solution with a pH value of 10.9 was usually prepared from a 1/5 M Na_2HPO_4 - 1/10 N NaOH solution.

** is evaluated from the initial slope of the $\ln(e^{\alpha cd}-1)$ vs. t plot.

TABLE VIII. QUANTUM YIELDS UNDER VARIOUS CONDITIONS

a) ATU

$[ATU]_0$ M	1.0×10^{-3} N [NaOH]	5.0×10^{-2} N [NaOH]	1.0×10^{-1} N [NaOH]
3.0×10^{-2}	2.8×10^{-3}	4.1×10^{-3}	3.9×10^{-3}
1.6×10^{-2}		3.6×10^{-3}	3.2×10^{-3}
1.0×10^{-2}	1.5×10^{-3}	3.0×10^{-3}	2.7×10^{-3}
6.0×10^{-3}	0.99×10^{-3}	2.4×10^{-3}	2.1×10^{-3}
4.0×10^{-3}		1.9×10^{-3}	1.6×10^{-3}

b) EDTA

$[EDTA]_0$ M	5.0×10^{-2} N [NaOH]	1.0×10^{-1} N [NaOH]
3.0×10^{-2}	7.2×10^{-3}	11×10^{-3}
1.0×10^{-2}	4.6×10^{-3}	7.2×10^{-3}
6.0×10^{-2}	3.8×10^{-3}	5.7×10^{-3}
4.0×10^{-3}	3.4×10^{-3}	4.2×10^{-3}
3.0×10^{-3}	2.5×10^{-3}	3.2×10^{-3}

TABLE IX. A SUMMARY OF THE φ_{S-T} VALUES

Reducing agent	$[U]_0$, M	[NaOH], N	φ_{S-T}	k_7/k_6
ATU	1.0×10^{-5}	5.0×10^{-2}	4.9×10^{-3}	6.2×10^{-3}
ATU	1.0×10^{-5}	1.0×10^{-1}	4.9×10^{-3}	8.2×10^{-3}
EDTA	1.0×10^{-5}	5.0×10^{-2}	8.7×10^{-3}	7.2×10^{-3}
EDTA	1.0×10^{-5}	1.0×10^{-1}	1.5×10^{-2}	10.8×10^{-3}

As for EDTA, the rate formula a also holds for 5.0×10^{-2} N and 1.0×10^{-1} N sodium hydroxide solutions. However, we could not get a satisfactory plot of $1/\Phi$—$1/[EDTA]_0$, as Fig. 10 shows. The φ_{S-T} value obtained from these plots is, therefore, not very accurate.

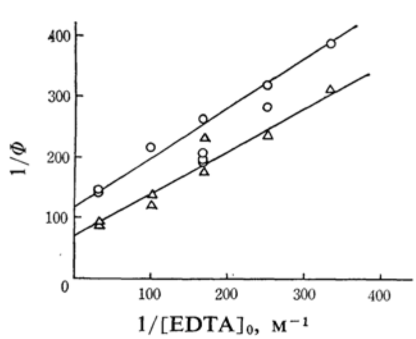

Fig. 10. $1/\Phi$ v. s. $1/[EDTA]_0$ plot (1×10^{-5} M $[U]_0$).
△ 0.1 N [NaOH]; ○ 0.05 N [NaOH]

Table VIII gives a summary of the quantum yields under various conditions.

Table IX gives the value of φ_{S-T} obtained in this paper. The value of k_7/k_6 was evaluated from the inclination and the intercept.

Since the validity of the present method for obtaining φ_{S-T} values has by no means been established beyond doubt, only a brief additional comment will be made on them. As is evident from the present studies, the main reaction product in the case of ATU is believed to be some leuco-like compound which is produced directly by the interaction of D^t and ATU and which is believed to have little effect on the reduction rate at the initial stage. In view of the fact that the rate is simply proportional to the absorption of light until about 50% of dye is bleached, the value of φ_{S-T} for ATU is considered to be more reliable.

On the other hand, the product in the case of EDTA consists mainly of semiquinone and leuco-dye. The former will not affect the reduction rate very much, but the latter will do so to some extent, probably by increasing it. In any case, these facts must be taken into consideration if we try to obtain a reliable φ_{S-T} value. Moreover, it is not unlikely that the two reducing agents cause simple deactivation of D^t. The value of φ_{S-T} obtained for ATU is, therefore, still tentative.

Summary

An attempt to obtain a reliable value of φ_{S-T} for fluorescein in an alkaline solution has been unsuccessful since the values for EDTA and ATU were quite different. It has been found that a large quantity of semiquinone is produced when EDTA is used as a reducing agent. In the case of ATU, the quantity of semiquinone produced is very small. Semiquinone in an alkaline solution reverts to the oxidized dye by the second-order thermal reaction and, quite astonishingly, mostly by the reaction $2DH \rightarrow 2D+H_2$. The photochemical behavior of leuco-fluorescein has been invesigated to some extent in the presence of and in the absence of a reducing agent.

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