Photodegradation of Chlorpromazine Hydrochloride

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The quantum efficiency of chlorpromazine hydrochloride (CLP HCl) at 253.5 mμ in aqueous media (pH 1.5) was 0.18 and 0.14 under normal and oxygen-free atmospheres, respectively. The quantum efficiency remained constant within the concentration range and periods of irradiation studied, but increased at pH values above 4.5. The photoreaction appears to follow zero-order kinetics. Reactivity toward acetic anhydride, partition studies, and infrared spectra of light degraded solutions of CLP HCl suggest ring hydroxylation occurs during irradiation. Spectral evidence indicated the formation of a semiquinone free radical intermediate on irradiation of aqueous solutions of CLP HCl at 253.5 mμ. Chlorpromazine-5-oxide hydrochloride (CLP-O HCl) forms by disproportionation of this free radical in aqueous media, both in the presence and absence of dissolved oxygen. The phenolic and other unidentified compounds formed on irradiation of CLP HCl apparently result from degradation of the photolabile CLP-O formed.

CHLORPROMAZINE, 2-chloro-10-(3-dimethyl-aminopropyl)-phenothiazine (I) represents a large group of antipsychotic agents which are photosensitive. However, photolytic studies reported on this compound have been limited. Nakagawa, *et al.* (1), irradiated CLP under both normal and nitrogen atmospheres. They reported that degradation occurred under both conditions with very little apparent difference in the resultant products. Raven, *et al.* (2), measured manometrically the oxygen uptake of an aqueous solution of CLP HCl subjected to ultraviolet radiation. Under these conditions, after a short induction period, the oxygen uptake was linear with time, but stopped as soon as the source of radiation was removed. Turse (3) subjected CLP HCl in acidic medium to ultraviolet radiation. He noted that very little degradation occurred when the wavelength of irradiation employed was above 365 mμ. He also noted that CLP and CLP-O degraded on exposure to ultraviolet radiation; on long exposure, both compounds appeared to degrade to the same product. Forrest, *et al.* (4), detected free radicals by ESR measurements on exposure of chlorpromazine to ultraviolet radiation. Under these conditions the ultraviolet spectrum of this radical showed no characteristic peaks. Fels and Kaufman (5) confirmed the presence of free radicals under similar conditions. Borg and Cotzias (6) on extended ultraviolet irradiation of a solution of CLP detected a weak ESR peak and concluded that while radicals were produced they were not the typical semiquinone free radical produced on chemical oxidation of CLP. It should be noted that except for the work reported in *References 1 and 3*, no attempt was made to restrict the wavelength

employed for irradiation. Ultraviolet lamps were employed without using filters or monochromators. None of this work served to establish a quantum efficiency or mechanism for the photodegradation.

The purpose of this work was to study photodecomposition of CLP HCl under carefully controlled conditions to evaluate the quantum efficiency at 253.5 mμ (the absorbance peak) and define the mechanism of this reaction better.

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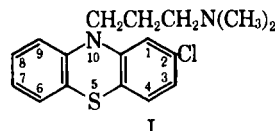
EXPERIMENTAL AND RESULTS

Instrumentation.—All solutions were analyzed using the Beckman model DU spectrophotometer. Ultraviolet absorption spectra were obtained using a Beckman ratio recording spectrophotometer model DK-2; infrared spectra were obtained using a Perkin-Elmer model 21 spectrophotometer. A Beckman model N pH meter was used for pH determinations.

The microirradiation studies, using concentrations in the range of 10^{-5} M, were conducted using the irradiation instrumentation described by Discher, *et al.* (7), except for a modification in the reaction box which permitted the use of standard size Beckman quartz cells. This consisted of a mercury arc light source operated from a voltage regulator. This light was passed through a Bausch & Lomb grating monochromator into the reaction box. Within the reaction box was a water coil connected to an externally placed constant temperature water bath, a thermopile, and a cell holder which accommodated three standard 1-cm. Beckman quartz cells. The cell holder was set so that two of the cells could be moved individually into the light beam. This permitted irradiation of the solvent and sample while a reference solution was maintained in the reaction box without being subjected to irradiation. The thermopile, which was positioned so that the light passing through the cell being irradiated would strike it, was attached through a direct current
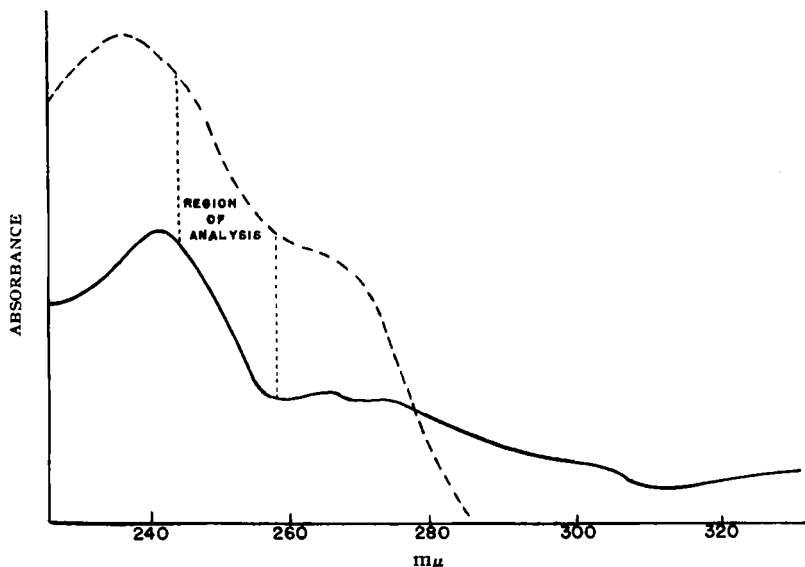
Fig. 1.—The ultraviolet spectra of compounds formed on irradiation of CLP HCl. Key: ———, spectrum obtained by a differential spectrophotometric technique; – – – –, spectrum obtained by an extraction procedure.

breaker amplifier to a direct current recording milliammeter. Thus, the incident radiant energy and that absorbed by the sample could be measured.

The macroirradiation studies, using concentrations in the range of 10^{-2} to 10^{-3} M, were conducted using a Hanovia ultraviolet lamp equipped with a heat filter and removable glass filter transmitting from 360 to 370 mμ. A 30-ml. capacity quartz cell was used in conjunction with this lamp. This portion of the work was qualitative in nature, and no attempt was made to determine the quantum efficiency or extent of degradation under these conditions.

Material.—The CLP HCl and CLP-O HCl were generously supplied by Smith Kline & French Laboratories. These compounds were used without further purification. All other chemicals were of reagent grade. The distilled water was ordinary distilled water, redistilled from permanganate and phosphoric acid using all glass equipment.

Preparation of Sample.—Solutions were prepared on the day they were to be irradiated. Hydrochloric acid, 0.05 N, was used as the solvent unless otherwise noted. When oxygen-free atmospheres were employed, the solutions were prepared using recently distilled nitrogen-deaerated water. The nitrogen

used for the deaeration was first passed through 0.05 N hydrochloric acid to prevent loss of HCl from the solvent. In the microirradiation studies these deaerated samples were measured and transferred to Beckman 1-cm. ground glass-stoppered quartz cells under a nitrogen atmosphere. A positive nitrogen pressure was maintained in the irradiation reaction box and the cell chamber of the Beckman DU. In both oxygen-free and normal atmosphere runs, the irradiation sample and a reference sample were analyzed immediately before and after irradiation.

All glassware was rinsed three times with distilled water prior to use to minimize reaction with trace metal ions.

Assay and Proof of Specificity.—To permit the analysis of CLP HCl in the presence of the interfering compounds formed during the irradiation, a three point spectrophotometric assay similar to that used by Flanagan, *et al.* (8), and Smith Kline & French Laboratories (3) was employed. An additional modification involving the selection of wavelengths was made in this study, since preliminary work indicated this change would increase the accuracy of the assay in the presence of light degraded materials.

TABLE I.—ANALYSIS OF CLP HCl IN THE PRESENCE OF INTERFERING COMPOUNDS

10^5 × CLP HCl Concn., mg./ml.	10^5 × CLP-O HCl Concn., mg./ml.	10^5 × Calcd. CLP HCl Concn., mg./ml., by Eq. 1	% Error	% of Total Compn. Present as CLP-O HCl[a]
800	13	795	0.40	1.6
800	25	801	0.13	3.1
400	13	403	0.75	3.2
800	50	799	0.13	5.8
800	50	795	0.40	5.8
800	100	789	1.40	11.0
800	100	808	1.00	11.0
800	100	796	0.50	11.0
800	100	782	2.30	11.0
800	100	782	2.30	11.0
400	50	400	0.00	11.0
400	100	394	1.50	20.0
533	371	528	1.00	41.0
267	371	257	4.00	51.0
400	742	386	3.50	65.0
0	400	0	0.00	100.0

[a] Represents either pure or light degraded chlorpromazine-5-oxide hydrochloride.

This assay is specific for CLP HCl as long as interfering compounds (light degraded products) present absorb linearly within the wavelengths selected for the analysis (9). The linearity of the absorbance of degraded products was demonstrated by the following procedures: (*a*) a differential spectrophotometric technique whereby the nondegraded CLP HCl present in an irradiated solution was compensated for by including an indentical amount in the reference cell. The resultant spectrum represented only the degraded compounds (Fig. 1); (*b*) by an extraction procedure whereby the nondegraded CLP HCl was extracted with chloroform from an irradiated solution, pH of 5 (Fig. 1).

The assay was further tested by analyzing solutions of known concentrations of CLP HCl to which had been added known amounts of CLP-O HCl or light degraded CLP-O HCl (Table I).

Assay Procedure.—The absorbance of a solution to be analyzed was determined at 246.5, 254.5, and 259.5 mµ. The absorbances at 246.5 and 259.5 mµ were plotted *versus* wavelength and the two points connected with a straight line. The absorbance at 254.5 mµ was then read off this line (A_{base}). The difference between the observed absorbance at 254.5 mµ ($A_{max.}$) and (A_{base}), that is ($A_{max.} - A_{base}$), was proportional to the concentration of nondegraded CLP HCl within the range studied. Thus, the concentration, c, of CLP HCl may be calculated using

$$c = K(A_{max.} - A_{base}) \qquad \text{(Eq. 1)}$$

TABLE II.—EVALUATION OF THE PROPORTIONALITY CONSTANT K^a

$10^5 \times$ CLP HCl Concn., mg./ml.	$A_{max.} - A_{base}$, Absorbance Units	$10^4 \times K$ Calcd. from Eq. 1
200	0.046	435
400	0.090	444
400	0.092	435
400	0.090	444
600	0.134	448
800	0.183	437
800	0.181	442
800	0.182	439
800	0.180	444
800	0.182	439
800	0.180	444
800	0.182	439
800	0.183	437
800	0.180	444
800	0.181	442
800	0.180	444
800	0.180	444
1000	0.225	444

^{a} Average $K = 0.0442$ S.D. ± 0.0005.

TABLE III.—QUANTUM EFFICIENCY AT 253.5 mµ UNDER NORMAL ATMOSPHERE AND pH 1.5

$10^5 \times$ Initial Concn. of CLP HCl, moles/L.	Time of Irradiation, Min.	Quantum Efficiency^a
1.13	20	0.17
1.13	15	0.18
1.13	10	0.19
2.25	15	0.19
2.25	13	0.18
2.25	10	0.17
	Av.	0.18 ± 0.02

^{a} The values in this column represent an average of at least three determinations.

TABLE IV.—QUANTUM EFFICIENCY AT 253.5 mµ UNDER OXYGEN-FREE ATMOSPHERE AND pH 1.5

$10^5 \times$ Initial Concn. of CLP HCl, moles/L.	Time of Irradiation, Min.	Quantum Efficiency^a
1.13	20	0.15
1.13	15	0.14
2.25	30	0.16
2.25	20	0.13
2.25	10	0.14
	Av.	0.14 ± 0.02

^{a} The values in this column represent an average of at least three determinations.

where K is a proportionality constant having the experimentally determined value of 4.42×10^{-2} $\pm 0.05 \times 10^{-2}$ mg./ml. (Table II).

Calculation of Quantum Efficiency.—The quantum efficiency, ϕ, may be defined

$$\phi = \frac{\text{No. of molecules decomposed}}{\text{No. of photons absorbed}} \qquad \text{(Eq. 2)}$$

The evaluation of this equation is described in detail by Discher, *et al.* (7). However, a brief discussion of the procedure will be covered here.

The numerator can be shown to be equal to

$$(M)(V)(N)(r) \qquad \text{(Eq. 3)}$$

where M is the molarity of the irradiated solution with respect to CLP HCl, V is the volume of the irradiated solution in liters, N is Avogadro's number (6.025×10^{23}), and r is the fraction of CLP HCl degraded during the irradiation.

To evaluate the denominator, the total radiant energy absorbed by the sample during the period of irradiation must first be determined.

The voltage output of the thermopile is proportional to the amount of radiation striking it per unit time. The recorder response is proportional to the voltage output of the thermopile. Thus, the radiant energy absorbed by the sample is proportional to the difference between the recorder response when a cell filled with solvent, I_o, is placed between the thermopile and the light source, and when a cell filled with a solution of the sample, I, is placed in this position, *i.e.*, $I_o - I$.

To calibrate the recorder response to irradiation in units of voltage, the recorder response, I_s, to a test signal of known voltage, s, is determined. Thus, the value of $I_o - I$ can be calculated in terms of volts.

The use of the thermopile constant, k, determined to be 0.0444 microvolts/microwatt/cm.^{2} (7) and the area of irradiation, A, determined to be 1.561 cm.^{2} (10) permit the calculation, in microwatts, of the radiant energy absorbed per second. The time of irradiation, t, in seconds permits calculation of the microwatts-seconds absorbed during the period of irradiation. Since 1 microwatt-second equals 10 ergs, the factor 10 is used to convert the energy to units of ergs. Thus, the total energy absorbed in ergs can be

$$\frac{10(I_o - I)(A)(t)(s)}{(I_s)(k)} \qquad \text{(Eq. 4)}$$

The energy, E, of a photon in ergs can be

$$E = hc/\tau \qquad \text{(Eq. 5)}$$

where h is Planck's constant (6.62×10^{-27} erg-sec.),

TABLE V.—SUMMARY OF EFFECT OF CHANGE IN pH ON QUANTUM EFFICIENCY AT 253.5 mμ UNDER NORMAL AND OXYGEN-FREE ATMOSPHERE

pH	Av. Quantum Efficiency Under Normal Atmosphere[a]	Av. Quantum Efficiency Under Oxygen-Free Atmosphere[a]
1.5	0.18	0.14
3.2	...	0.13
4.5	0.19	...
5.0	0.23	0.21
5.8	0.24	0.21
6.3	0.26	...
7.2	...	0.22

[a] The values in these columns represent an average of at least three determinations.

c is the velocity of light (3.00 × 10^{10} cm./sec.), and r is the wavelength in cm. The energy of the wavelength used for the determination of the quantum efficiency, 253.5 mμ, was calculated to be 7.82 × 10^{-12} ergs/photon using Eq. 5.

The number of photons absorbed can be calculated by dividing the total number of ergs absorbed (Eq. 4) by the energy per photon, 7.82 × 10^{-12} ergs/photon.

The quantum efficiency can now be

$$\phi = \frac{(M)(V)(N)(r)(I_s)(k)\ 7.82 \times 10^{-12}}{10\ (I_o - I)(A)(t)(s)} \quad \text{(Eq. 6)}$$

A typical calculation is $M = 1.13 \times 10^{-5}$ moles/L., $V = 3.00 \times 10^{-3}$ L., $r = 0.234$, $I_s = 35.4$ chart units, $I_o - I = 18.0$ chart units, $t = 1200$ seconds, and $s = 0.1$ microvolts. The other symbols represent constants with previously assigned values.

$$\phi = \frac{(1.13 \times 10^{-5})(3.00 \times 10^{-3})(6.03 \times 10^{23})}{(0.234)(35.4)(0.0444)(7.82 \times 10^{-12})}}{10(18.0)(1.56)(1200)(0.1)}$$

$$\phi = 0.177 = 0.18$$

Microirradiation—Determination of Quantum Efficiency.—A series of CLP HCl solutions was irradiated at 253.5 mμ for 10 to 30 minutes at selected pH values within the range of 1.5 to 7.2, under normal and oxygen-free atomspheres. The concentration range selected allowed both irradiation and analysis to be conducted in the same cell, eliminating the chemical degradation possible during transfer or dilution techniques.

At a pH of 1.5, the average quantum efficiency was 0.18 ±0.02 under a normal atmosphere (Table III), while under an oxygen-free atmosphere it was 0.14 ±0.02 (Table IV). The effect of changing pH under both normal and oxygen-free atmospheres is summarized in Table V. Typical examples of the change in absorbance taking place during irradiation are shown in Fig. 2. The spectrum obtained on irradiation under oxygen-free atmosphere is similar to that obtained during the early phase of irradiation under a normal atmosphere. Thus, the difference in the spectra in Fig. 2 is indicative of the more rapid rate of degradation which occurs in the presence of dissolved oxygen. This is also demonstrated by the determined quantum efficiencies.

Microirradiation—Detection of CLP Semiquinone Free Radical.—Since previous work indicated the semiquinone free radical of CLP was stabilized in 9 N sulfuric acid (11), CLP HCl dissolved in this solvent was irradiated for 2 hours at 253.5 mμ under

a normal atmosphere in an attempt to stabilize any free radical produced as a result of irradiation. During the irradiation the solution turned pink, typical of the semiquinone free radical. The absorbance spectrum of the solution at the end of the irradiation differed markedly from that obtained on irradiation of CLP HCl in 0.05 N hydrochloric acid. Comparison with the spectrum of a 9 N sulfuric acid solution of a crystalline semiquinone free radical of CLP (compound R), prepared as described by Felmeister, Discher, and Merkle (11), indicated a semiquinone free radical of CLP was a product of the irradiation (Fig. 3).

Macroirradiation—Effect of Irradiation on pH.—A solution of CLP HCl ($\approx 3 \times 10^{-2}$ M) in distilled water was irradiated for 20 hours under a normal atmosphere. A significant reduction in pH was noted. The results are recorded in Table VI.

It appeared from the irradiations performed under an oxygen-free atmosphere that the water present as the solvent was involved in the formation of CLP-O. If this were the case, it appeared likely that two protons might be released for each molecule of CLP-O formed. This should manifest itself by a decrease in pH following irradiation.

Macroirradiation—Effect of Irradiation on Polarity and Reactivity.—The products formed on extensive irradiation of CLP HCl appeared to be more hydrophilic than CLP HCl itself. CLP-O HCl did not demonstrate this property, but the formation of phenolic compounds, which had been suggested as oxidation products of CLP (4), would be expected to increase the hydrophilic nature of the compound. Attempts to separate pure compounds from the
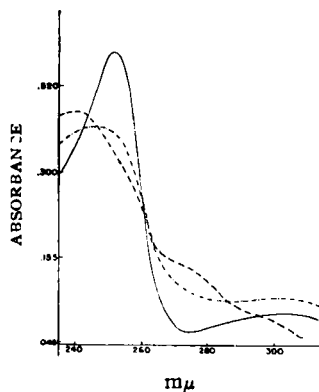

Fig. 2.—Ultraviolet absorbance spectrum of CLP HCl in 0.05 N HCl irradiated at 253.5 mμ for 4 hours. Key: ———, before irradiation;———, after irradiation under a normal atmosphere; —·—·—·—, after irradiation under an oxygen-free atmosphere.
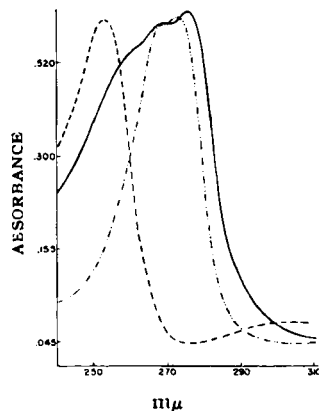

Fig. 3.—Ultraviolet absorbance spectra of a 9 N H$_2$SO$_4$ solution of CLP HCl before and after irradiation compared with a 9 N H$_2$SO$_4$ solution of compound R. Key: - - -, CLP HCl before irradiation; ——, CLP HCl after irradiation; —·—·—·—, compound R.

	pH of Soln. Before Irradiation	pH of Soln. After 20 Hr. Irradiation, 360 to 370 mμ
Run *1*	5.05	1.90
Run *2*	4.90	2.00

degraded mixtures were unsuccessful. The detection of phenols by use of Fe (III) was attempted, but interference from both the CLP-Fe (III) reaction and the color of the degraded solution made interpretation of the results doubtful.

Since the nitrogens in CLP are tertiary and would not react with acetic anhydride, it was felt that any reactivity toward this reagent should serve to indicate the presence of phenolic compounds. A solution of CLP HCl ($\approx 3 \times 10^{-2}$ *M*) was irradiated for 3 hours under a normal atmosphere. A typical dark brown developed during the course of the irradiation. The pH of the irradiated solution was adjusted to about 5 with 0.1 *N* sodium hydroxide solution and extracted three times with chloroform. The ultraviolet absorbance spectrum of the material extracted into the chloroform layer was typical of nondegraded CLP HCl. The dark brown degraded material, which remained in the aqueous layer, was treated with acetic anhydride. After 20 minutes it was noted the dark brown material readily partitioned into chloroform, and was extracted by two separate portions of chloroform. The chloroform was evaporated at room temperature and the residue dissolved in methanol. To remove excess acetic anhydride or acetic acid, the dark brown material was precipitated from the methanol by the addition of ether; this process was repeated three times. The material was then dried in a vacuum desiccator for 48 hours. The infrared spectrum of the residue was determined. A strong peak at 5.97 μ was noted which was absent in the spectrum of CLP HCl or its 5-oxide.

Macroirradiation—Effect of Dissolved Oxygen.— To note the effect of dissolved oxygen in the absence of water, solutions of CLP HCl in anhydrous methanol were treated as follows. One 20-ml. portion was placed in the 30-ml. quartz cell and flushed with nitrogen for 1 hour. The cell was sealed and irradiated for 2 hours. During this irradiation the solution turned a pale yellow. A second 20-ml. portion of the initial solution was placed in the quartz cell under normal atmosphere and irradiated for 2 hours. During this irradiation the solution developed a deep brown. The ultraviolet absorbance spectra of these solutions after irradiation indicated the 5-oxide does not form under anhydrous conditions in the absence of oxygen (Fig. 4).

The slight shift in the absorbance peak after irradiation in the absence of water and oxygen could not be explained on the basis of this work. However, a reaction at the alkyl chain or the ring at positions other than the sulfur could conceivably result in such a shift.

Macroirradiation—Irradiation of CLP-O HCl.— To demonstrate the photosensitivity of CLP-O HCl and to show that the photodegradation of CLP HCl probably results in the formation of CLP-O HCl, a solution of CLP-O HCl was irradiated for 2 hours.

The resultant spectrum (Fig. 5) strongly resembles that of photodegraded CLP HCl (Fig. 2).

DISCUSSION

Semiquinone free radicals which undergo disproportionation to yield their respective 5-oxides have been shown to be intermediates in the electrolytic oxidation of phenothiazine (12) and CLP HCl (13) and in the chemical oxidation of CLP HCl (6).
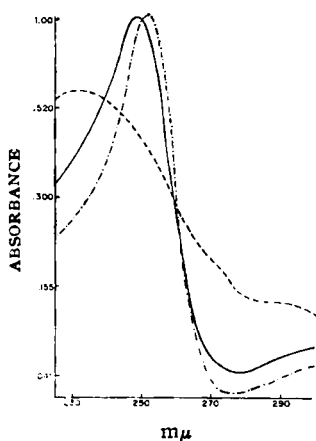
Fig. 4.—Ultraviolet absorbance spectra of anhydrous methanol solutions of CLP HCl irradiated for 2 hours under normal and oxygen-free atmospheres. Key: —·—·—, before irradiation; - - -, after irradiation under a normal atmosphere; ———, after irradiation under an oxygen-free atmosphere.

The formation of free radicals on photolysis of CLP has been demonstrated by electron spin resonance studies (4–6). However, identification of these radicals was not reported.

The ultraviolet absorbance spectrum which resulted on irradiation of CLP HCl in 9 *N* sulfuric acid at 253.5 mμ (Fig. 3) is quite similar to that of compound R, indicating that the intermediate formed on irradiation of CLP HCl is a semiquinone free radical. The observed difference in these spectra can be accounted for on the basis of the disproportionation of a small fraction of the photoproduced free radical, followed by photodegradation of the 5-oxide formed as a result of this disproportionation. The fact that the absorbance peak at 227 mμ, indicative of the semiquinone free radical, is not present in the spectrum of an irradiated solution of CLP HCl when 0.05 *N* hydrochloric acid is used as the solvent is not surprising (Fig. 2), since the semiquinone free radical of CLP HCl disproportionates rapidly at hydrogen ion concentrations below 4 *N* (11).

It appears from these data that ultraviolet irradiated CLP HCl degrades *via* a semiquinone free radical intermediate. Disproportionation of this free radical results in the formation of CLP-O HCl and apparently further changes result from degradation of this photolabile CLP-O (3) (Fig. 5) rather than from CLP itself.

The presence of phenolic compounds was demonstrated by Brown (14) in the reaction mixture of ultraviolet irradiated phenothiazine-5-oxide. The formation of similar compounds on extensive irradiation of CLP HCl, probably *via* the CLP-O HCl, are indicated in this work by (*a*) the increase in polar properties of CLP HCl after irradiation, demonstrated by the change in partitioning properties in a chloroform-acidic system, (*b*) the ability of the degraded compounds to undergo acetylation, (*c*) the

reduction of the polar properties as a result of this acetylation, (*d*) the appearance of an infrared absorbance peak at 5.97 μ indicative of a carbonyl group after acetylation.

It should be noted that the loss of an electron from a semiquinone free radical of phenothiazines, as results in disproportionation, yields first a phenazathionium ion (15), which on further reaction results in the 5-oxide. Work now in progress has shown that the semiquinone free radial of CLP disproportionates in aqueous media both in the absence and presence of dissolved oxygen, though the loss of free radical is slightly faster in the presence of oxygen. Thus, it would appear water and oxygen both may compete for the short lived CLP phenazathionium ion or the oxygen may react directly with the free radical, yielding the 5-oxide in both cases. Since the introduction of molecular oxygen into the system has only a minor effect on the rate of radical loss, it appears the water reaction is much more rapid than the oxygen reaction.

The significant increase in hydrogen ion concentration on irradiation of CLP HCl in water, shown in Table VI, can be explained on the basis of the reaction of the short lived phenazathionium ion with water to yield the 5-oxide, as shown below. It can be seen from this reaction mechanism that two hydrogen ions result for each molecule of 5-oxide formed. Thus, on the irradiation of a 10^{-2} M solution of CLP HCl (initial pH $\approx$ 5) a resultant pH of 2 is reasonable to expect.
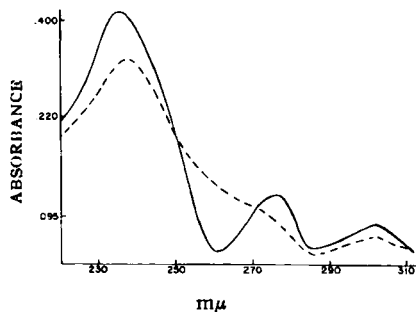

Fig. 5.—Ultraviolet absorbance spectrum of CLP-O HCl in 0.05 N HCl irradiated for 2 hours under a normal atmosphere. Key: ———, before irradiation; - - - - - -, after irradiation.

The fact that the irradiation of CLP HCl in the absence of water and dissolved oxygen did not yield the 5-oxide, but in the presence of either one of these species apparently did (Figs. 2 and 4), tends to support the postulation that water and/or oxygen are involved in the 5-oxide formation.

The increase in quantum efficiency with increase in pH as shown in Table V is not readily explained on the basis of this study, though it may be a measure of the greater instability of the free amine base (which increases in concentration with pH) compared to the amine salt.

If each photon absorbed resulted in the decomposition of a molecule of CLP HCl the quantum efficiency would have a value of 1. However, any process that tends to dissipate the energy of the absorbed photons before decomposition occurs will reduce the value of the quantum efficiency. The low quantum efficiency determined for this reaction no doubt is due to a number of such factors (*a*) fluo-

rescence, the emission by the excited molecule at a higher wavelength of part of the absorbed energy, (*b*) loss of absorbed energy through collisions with solvent molecules, and (*c*) the gaining of an electron by the photo produced radical, resulting in reformation of CLP. However, the proposed disproportionation reaction would also favor a low quantum efficiency, since this tends to reproduce the starting compound continually.
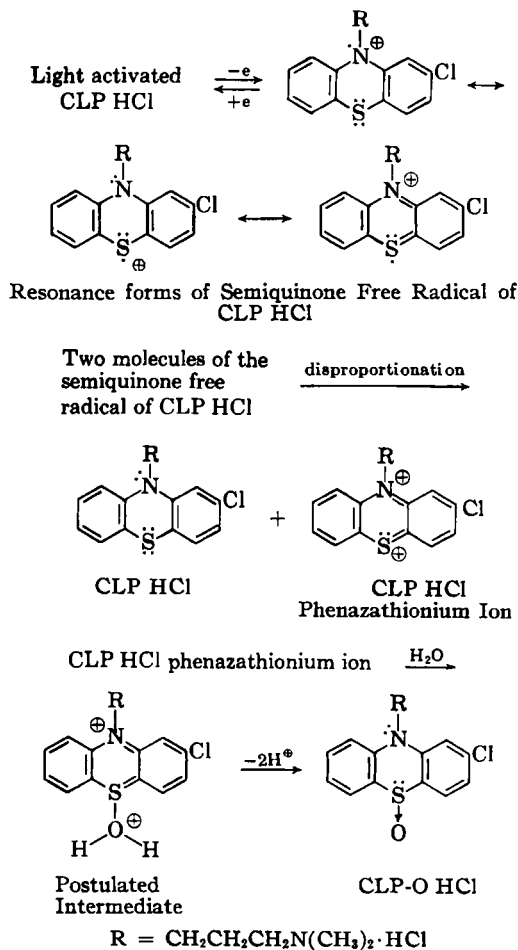
The small reduction in quantum efficiency on deaeration of the system demonstrated that the removal of oxygen is insufficient in itself to prevent the photodegradation of aqueous solutions of CLP HCl. This further supports the postulation that while apparently both water and/or oxygen take part in the reaction the water reaction proceeds at a more rapid rate.

From the quantum efficiency data it can be shown that the over-all photodegradation of aqueous solutions of CLP HCl follow zero-order kinetics as expressed by Eq. 7

$$A = A_o - \phi I_a t \qquad (\text{Eq. 7})$$

where A_o = the initial concentration of CLP HCl (molecules/ml.), A = the concentration of CLP at time t, t = the period of irradiation in seconds, I_a = the number of photons absorbed/ml. second, and ϕ = the quantum efficiency.

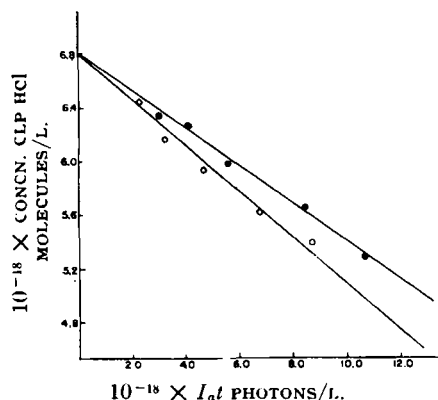A plot of concentration of CLP HCl, A, *versus*

Light activated
CLP HCl

Resonance forms of Semiquinone Free Radical of CLP HCl

Two molecules of the semiquinone free radical of CLP HCl

disproportionation →

CLP HCl   CLP HCl
Phenazathionium Ion

CLP HCl phenazathionium ion $\xrightarrow{\text{H}_2\text{O}}$

Postulated Intermediate   CLP-O HCl

$R = CH_2CH_2CH_2N(CH_3)_2 \cdot HCl$

Fig. 6.—A plot of concentration of CLP HCl *vs.* number of photons/liter absorbed showing the zero-order dependency of the photoreaction. Key: $\bigcirc$, under a normal atmosphere, slope = 0.18; $\bullet$, under an oxygen-free atmosphere, slope = 0.14.

total photons absorbed per milliliter, $I_a t$, is included (Fig. 6).

SUMMARY

The presence of a semiquinone free radical as an intermediate in the ultraviolet photolysis of CLP HCl was demonstrated. Disproportionation of this free radical intermediate yields the photolabile CLP-O HCl, which apparently is the precursor to phenolic and other unidentified degradation products formed on irradiation.

Photodegradation of CLP HCl seemed to be more dependent on the presence of water than dissolved oxygen, and it would not be expected that elimination of oxygen alone would have a marked effect on the degradation of ultraviolet irradiated solutions of CLP HCl at 253.5 mμ.

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Alterations in the Activity of Pentothal, Phenobarbital, Pentylenetetrazol, and Strychnine by Cholinesterase Inhibitors

By VERNON A. GREEN

Mice pretreated with anticholinesterases showed enhanced stimulation in the presence of pentylenetetrazol and strychnine, a decreased lag period with anesthetic doses of phenobarbital, and a prolongation of anesthesia with pentothal. An attempt was made to correlate these changes in response with the depression of acetylcholine hydrolyzing enzymes.

BECAUSE THE brain has higher concentrations of acetylcholinesterase than other areas of the body (1), attempts to potentiate drug action through cholinesterase inhibition should be accomplished more readily with agents which primarily affect the central nervous system.

The findings of potentiation of the rate of diffusion of acid fuchsin (2) in frog and dog (3), trypan red (4) in dog, and the potentiation of morphine (5) in cats, streptomycin on *Escherichia coli* (6) and rats (7), and barbital (8) in mice by neostigmine or physostigmine were indications that possibly the activity of other drugs could be potentiated.

In this study, physostigmine, neostigmine, and diisopropylfluorophosphate (DFP) were used as prior medication in mice in an attempt to potentiate the action of some central nervous system stimulants and central nervous system depressants. The basic criteria for the determination of potentiation were the ability of cholinesterase inhibitors to render a subconvulsive dose of a central nervous system stimulant convulsive (9)—