Effect of Ultraviolet Irradiation on Chlorpromazine II

Anaerobic Condition

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In this report, ultraviolet light-catalyzed reaction of chlorpromazine under anaerobic condition is presented. It appears that chlorpromazine behaves differently to ultraviolet energy under aerobic and anaerobic conditions. Under the former condition oxidation products prevail, and the sulfoxide and *N*-oxide are formed; however, under the latter condition, the polymerization process predominates and the polymer was found to be the major product. The biological activity of this compound was tested on human volunteers; this study indicated that this compound caused a negligible skin irritation but produced a bluish-purple color resembling that of the skin discoloration observed in patients. Hydrogen chloride liberated in the initial phase of the photochemical reaction may be responsible for the skin rash reported in patients receiving daily maintenance dose of chlorpromazine. Formation of a polymer coupled with melanin may be the cause of the skin discoloration observed in patients under prolonged and high dose of chlorpromazine treatment.

SINCE THE introduction of chlorpromazine in the clinical treatment of psychosis, a number of cases of photosensitivity or photoallergy, namely, abnormal skin reactions (1–16) and ocular changes (8–19), associated with this drug have been reported. Concurrently, these investigators pointed out that skin discoloration occurred on the exposed portions of the body of patients receiving a long-term medication of large doses (300–500 mg. three times a day) of phenothiazines, especially chlorpromazine. The rate of occurrence of the skin discoloration is about 0.1–0.2% of the patients treated with chlorpromazine. The incidences of the ocular changes seem to precede the skin pigmentation in reference to the reports that many patients had ocular changes before skin pigmentation took place (20–22), and the majority of patients with skin changes also had ocular complications (23). It appears that these bizarre phenomena are directly associated with photo energy of short wavelength; however, the nature of these abnormal reactions is not well understood.

It is the purpose of this study to investigate the *in vitro* effect of ultraviolet energy on an aqueous solution of chlorpromazine which might furnish clues toward the nature of the photochemical reactions taking place in patients under chlorpromazine medication.

In a previous communication (24), the effect of ultraviolet (U.V.) irradiation (25–29) on an aqueous solution of chlorpromazine under aerobic conditions was presented; two oxidation products, chlorpromazine sulfoxide and chlorpromazine *N*-oxide, were identified, and the physical properties of 10 other compounds were described. Apparently, oxygen (30) present in the medium played an important role in the formation of oxidation products of chlorpromazine in this photochemical reaction. The site of attack by the oxygen was found to take place first on the S function at position 5 followed by the terminal N.

In this report, the photo-induced reaction products of chlorpromazine in an aqueous solution under anaerobic condition are presented. The chromatographic pattern of the products formed under the anaerobic condition was quite different from that of the aerobic condition. A polymerization reaction was found to be a major process taking place under these conditions, and a polymer and a dimer were isolated from the reaction mixture. Evidently, the cleavage of the chlorine function (31) at position 2 resulted in the formation of a free radical which prompted these reactions. In a preliminary test, an aqueous suspension of the polymer injected intracutaneously to two volunteers exhibited a discoloration resembling that of the photosensitivity observed in patients receiving chlorpromazine medication. The formation of a polymer in the skin might be a cause of photosensitivity reported in patients under a long-term medication of large doses (300–500 mg. three times a day) of chlorpromazine. Promazine and 2-hydroxypromazine were also isolated and identified. Negative tests with ninhydrin and nitroprusside reagents on the reaction products isolated in this experiment indicated that there is no demethylation occurring under these conditions (32).

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METHOD[1]

Chlorpromazine hydrochloride (5 Gm.) was dissolved in freshly boiled water (500 ml.) and placed in a glass ampul (10 ml.), which was previously filled with nitrogen gas. The ampul was replenished with nitrogen gas and sealed under nitrogen atmosphere. Fifty ampuls thus prepared were placed in a box with aluminum foil linings on inside walls and subjected to ultraviolet irradiation from a Spectroline B-100 lamp (120 w.) at a distance of 20 cm. At every 24-hr. interval, one ampul was removed from the box and the content examined for the extent of the photochemical effect. It was found that all the starting material was consumed completely within 5 days. The time sequential chromatographic patterns and the ultraviolet absorption spectra of the irradiated media are shown in Fig. 1.

At the end of 5 days, U.V. irradiation was terminated. The ampuls were removed from the box, and the contents emptied into a container filled with nitrogen. It was noted that the colorless solution at the beginning had changed to deep-brown at the termination of the irradiation, and the pH had shifted from 4.5 to 1.9 (33). The results of alkalimetry revealed that approximately 0.8 mole of hydrogen chloride per mole of starting material was liberated in the medium. The solution was adjusted to pH 7 with 10% NaOH solution and the yellowish green precipitate which appeared was collected by centrifugation. The precipitate was washed 3 times with water, and the washings were added to the supernatant liquid.

The supernatant liquid was tested for the presence of dimethylamine according to the procedure described by Yamamoto and Fujisawa (32). Although the product recovered from the reaction vessel (containing dichlorodiphenyldisulfimide reagent) acquired a light brown color at the end of the experiment, it gave the same melting point as that of the starting material, m.p. 211–213°. Therefore, it was concluded that no dimethylamine was present in the irradiated solution of chlorpromazine and no demethylation took place under these conditions.

Thick-Layer Chromatography.—The precipitate collected above was extracted with a large quantity of cold methanol. The methanol extract was condensed to a small volume and subjected to thick-layer chromatography. Approximately, 1.5 ml. of an aliquot specimen was placed linearly in a 0.5-cm. band about 4 cm. from the bottom of a 15 × 48-cm. plate (coated with a 0.5-mm. layer of Silica Gel G and activated at 100° for 1.5 hr.). The chromatogram was developed in a nitrogen atmosphere in a chamber containing a solvent system, ethyl acetate–ethanol–water (8:3:3), for 7 hr. to a height of approximately 31 cm. After the chromatogram was removed from the solvent system, it was dried, examined under a U.V. lamp, and covered with a sheet of plastic wrap (Saran wrap); this left about a 2-cm. strip on one side of the plate uncovered. The exposed strip was sprayed with 50% sulfuric acid to produce color. Twelve fractions with R_f 0–0.78 and the color varying from pink to purple

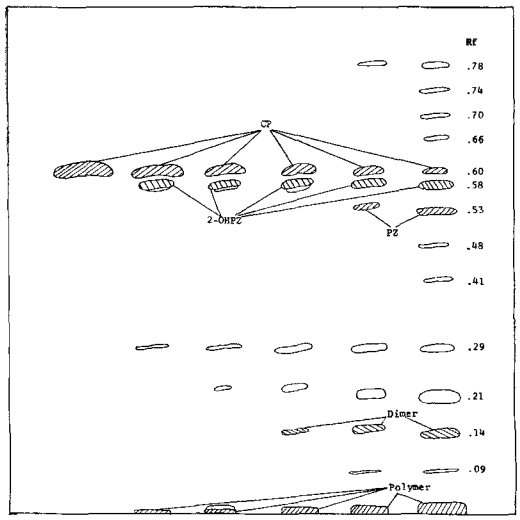


Fig. 1.—Sequential thin-layer chromatographic patterns of ultraviolet light-induced reaction products of chlorpromazine under anaerobic condition. Key: CP, chlorpromazine; 2PZ-OH, 2-hydroxypromazine; PZ, promazine.

were observed. Using this sprayed strip as a guide, the chromatogram was fractionated, and each fraction was removed from the plate and extracted with the above solvent system. The extract of each fraction was rechromatographed twice for further purification. Only a trace of the starting material was recovered from the medium after 5 days of irradiation.

Isolation of Promazine.—Fraction *6* was rechromatographed on a thick-layer plate several times for purification. After the final methanol eluate was evaporated to dryness, an oily residue was obtained. The physical properties of this compound—namely, R_f (0.46), color reaction with 50% sulfuric acid (orange), $\lambda_{max.}$ (208, 256, 300 mμ), and I.R. spectrum, were found to be identical with those of a reference promazine. The melting point of the picrate of this compound was found to be 143–144°, and the mixed melting point with an authentic promazine picrate did not show depression (142–144°).

Isolation of 2-Hydroxypromazine.—Fraction *4* was eluted with methanol from a thick-layer plate. The methanol extract was evaporated to dryness and the residue was rechromatographed three times for further purification. The purified fraction had an R_f value of 0.48 and a reddish-purple color reaction with 50% sulfuric acid. The melting point (132–133.5°), U.V. (212, 254 mμ), and I.R. spectra of this compound were found to be identical with those of a reference 2-hydroxypromazine. The color reaction of this compound compared with that of 2-hydroxypromazine (2-PZOH) is shown in Table I.

Isolation of the Dimer.—*Scheme I.*—Fraction *11* (R_f 0.09) was eluted with hot methanol. The methanol eluate was evaporated to dryness and the residue recrystallized from methyl ethyl ketone to a yellow

[1] Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Molecular weights were determined by using a Mechrolab vapor pressure osmometer. Ultraviolet absorption spectra were obtained in a Bausch & Lomb spectronic 505 and infrared absorption spectra were taken in a Perkin-Elmer 237 spectrophotometer.

TABLE I.—COLOR REACTION OF FRACTION 4 AND 2-PZOH

	Ehrlich's Reagent		$FeCl_3$		2,6-Dichloro-
	Preheating	Postheating	Reagent	$NaIO_4$	quinone Chlorimide
2-PZOH	Reddish-purple	Deep green	Brown	Green	Greenish-gray
Fraction 4	Pinkish-purple	Green	Brown	Green	Greenish-gray

powder. This material had a purple color reaction with 50% sulfuric acid. The melting point of this compound was above 300° (loses color reaction with 50% sulfuric acid at 250°). Ultraviolet absorption peaks were found at 262 and 313 mμ. A quaternary ammonium salt of this compound was prepared by reacting an acetone solution of this compound (1 mole) with methyliodide (1 mole) at room temperature. The mixture was shaken for a few minutes, and ether was added to precipitate the quaternary ammonium salt (m.p. > 300°). The molecular weight of this compound was found to be 816; this is equivalent to the molecular weight of the quaternary ammonium salt of a dimer (31).

Anal.—Calcd. for $(C_{18}H_{21}N_2SI)_2$: mol. wt., 848.7; C, 50.94; H, 4.99; N, 6.61; S, 7.55. Found: C, 50.44; H, 5.13; N, 6.71; S, 7.43; mol. wt., 816.

Isolation of the Polymer.—The greenish-yellow residue left after the methanol extraction above was purified as follows. This material was extracted with chloroform and the chloroform extract was condensed to a small volume and precipitated with *n*-heptane. The yellowish-brown precipitate was filtered and dried. This material was slightly soluble in acetone, ethyl acetate, and ethanol. Both the sodium fusion and Beilstein's flame tests gave a negative reaction for chlorine. It had a bluish-purple color reaction with 50% sulfuric acid, and a green color with 1% ferric chloride reagent. The R_f value on a thin-layer chromatogram was 0–0.01. This compound was found to be extremely resistant to oxidation by hydrogen peroxide. Attempts to degradate this compound with acid or base at elevated temperatures and pressures failed to produce any product. This material is a greenish-brown powder, with m.p. > 300° (loses color reaction with 50% sulfuric acid at 235°). Its picrate has a m.p. 144–145°. The infrared absorption spectrum is shown in Fig. 2. This compound does not rotate polarized light. The proposed structure of this compound is illustrated in Scheme I.

Anal.—Calcd. for $(C_{17}H_{18}N_2S \cdot {}^1/_2H_2O)n$: C, 70.10; H, 6.24; N, 9.63; S, 11.00. Found: C, 70.47; H, 5.90; N, 9.22; S, 10.91; mol. wt., 1990.

A quaternary ammonium salt of the polymer was prepared by refluxing methanol solution of this compound (1 mole) with methyliodide (1 mole) for 15 min. After cooling, ether was added into the mixture to precipitate the quaternary ammonium salt. This compound is a yellowish-brown powder (m.p. 239–245°) with ultraviolet absorption peaks at 220 and 262 mμ.

Anal.—Calcd. for $(C_{18}H_{21}N_2SI)n$: C, 50.95; H, 4.99; N, 6.60; S, 7.56. Found: C, 50.87; H, 5.07; N, 6.36; S, 7.79.

A Preliminary Test on Skin.—Ten milligrams of the polymer was suspended in 10 ml. of normal saline solution, and 0.1 ml. each of the suspension was injected intracutaneously into the inner side of forearms of two volunteers. It produced a bluish-purple stain resembling that of the skin discoloration observed in patients (1–16). The irritating effects of this compound on the injected site was almost negligible. The color of the injected site faded and the skin returned to its normal color within 3 days.

CONCLUSION AND DISCUSSION

Liberation of hydrogen chloride and the subsequent formation of a polymer of chlorpromazine in the tissues has been suggested as a possible cause of the photosensitivity and skin discoloration of patients receiving chlorpromazine medication for a prolonged period.

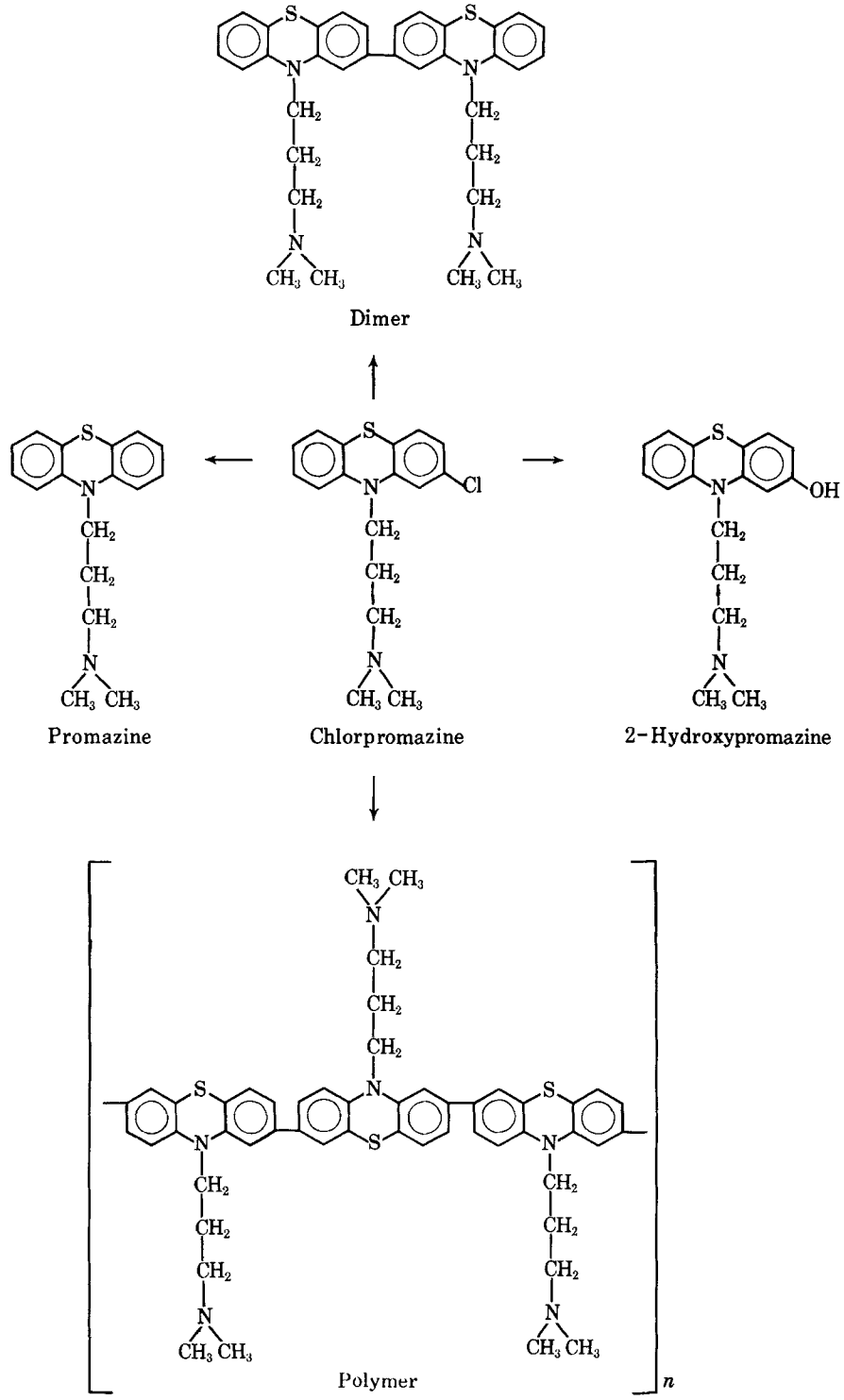
The hydrogen chloride in the initial phase of photochemical reaction could be responsible for the skin rash (7) observed in patients who were exposed to sunlight. The skin irritability of the patients subsided after they took cover from the sunlight; however, the inflammatory signs remained for hours. It could be due to the residual effects of the hydrogen chloride on the tissues. The chloride function at position 2 of the chlorpromazine molecule seems to be cleaved easily under these conditions; this is evidenced by a shift of the pH toward an acidic side (33) and an increase in the concentration of hydrogen chloride in the irradiated medium after 15 min. Similar findings, namely, cleavage of the $SO_2N(CH_3)_2$ group from thioproperazine to form sulfuric acid has been reported (32).

As the results of the cleavage of the chloride function (31), a carbonium ion is created at position 2. On the other hand, there exists in the chlorpromazine molecule a carbon at position 7 which acquires a relatively high electron density due to the greater *o,p*-directing force of the N at position 10. Thus, it has been speculated that a free radical attack on position 7 triggered by the carbonium ion at position 2 takes place, and a polymer with 2–7 linkage would be formed. Also the formation of a dimer from chlorpromazine with a possible 2–2 linkage has been found in this study (32, 34). Possible roles this compound might have in connection with the ocular and skin changes are currently under investigation.

The polymer isolated from the reaction medium is a greenish-brown powder, insoluble in almost all organic solvents except hot methanol, and resistant to acidic or basic hydrolysis at elevated pressures and temperatures. The intracutaneous injection of this polymer produced a purple color in the injected area of skin resembling that of the photosensitivity observed in patients. Therefore, the polymer formed in tissues as the results of the photo-induced polymerization reaction may be responsible for the skin discoloration.

This photochemical reaction takes place quite rapidly in reference to the observations in two patients (who were receiving 600 mg. three times a day of chlorpromazine medication); 15–30 min. of exposure to a bright sunlight was enough to produce a skin discoloration preceded by a condition

of sunburn. This indicated that photo-induced reaction of chlorpromazine took place at the site of exposure. It appears that chlorpromazine or its metabolites are in a bound state with melanin in tissues, since the drug has been demonstrated to be capable of forming complexes with melanin *in vitro*



Illustrated Structures of Ultraviolet Light-Induced Reaction Products of Chlorpromazine Under Anaerobic Condition

Scheme I

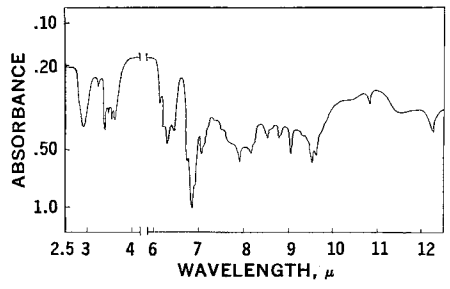


Fig. 2.—Infrared absorption spectrum of the polymer taken on a Perkin-Elmer model 237 spectrophotometer.

(35) and *in vivo* (36–39); however, skin discoloration would not occur without photo energy to trigger the polymerization reaction of chlorpromazine. Since chlorpromazine and its polymer react readily with silver, iron, and other metal ions to produce a color or a precipitate, the reagents currently applied to stain melanin (7) in tissues are expected to react with this drug or its derivatives to produce a melanin-like stain (7, 38).

The skin discoloration produced by the polymer injected intracutaneously to two volunteers faded, and the skin resumed its normal color within 3 days. These findings indicated that the polymer is transported from the site of administration or metabolized. Accordingly, a polymer of the same nature was isolated from a large amount of urine specimens from patients receiving a long-term medication of chlorpromazine (40). This finding suggested that the polymer formed in the skin by a photo-induced reaction is metabolized and excreted in urine. It is speculated that part of the polymer might be deposited or picked up by other organs during the course of transportation; this may be responsible for the appearance of a melanin-like material in the organs not being exposed to sunlight (41). Organs such as hairs and nails which are less affected by the normal process of metabolism would be expected to have a relatively high concentration of the drug. As the results of the accumulation of the drug, the hair might alter its shade because of a possible formation of the polymer, though the rate of formation would be lower than that occurring in the skin. It would be of interest to see further observations on this subject. The polymer of chlorpromazine may be bound to protein or deposited in fat-rich tissues; however, attempts were not made to clarify this matter in this study.

The polymers isolated from the U.V. irradiated medium showed a bluish-purple color reaction with 50% sulfuric acid; however, the reaction is rather sluggish—namely, the color reaction takes 30–60 sec. to develop, and the color density reaches its peak after 2–3 hr. The U.V. absorption peaks were found at 208 and 263 mμ; this indicated a shift toward the longer side of wavelength. There is no evidence of a 310-mμ peak when compared with chlorpromazine (207, 256, and 310 mμ).

The quaternary ammonium salt of this polymer is less soluble in organic solvents than the free base. In the U.V. irradiated medium under the anaerobic condition, 12 fractions (Table II) were detected on a thin-layer chromatogram; however, the sulfoxide could not be demonstrated. As the reaction proceeded and the starting material, chlorpromazine, was consumed, the concentration of the polymer increased in an almost parallel relationship to the irradiation time. The relationship of the appearance of various fractions *versus* irradiation time is illustrated in Fig. 1. The isolated polymer reacted readily with methyliodide to form a quaternary ammonium salt; this indicated the presence of an intact dimethylamino side chain.

Various organic solvents were used in the preparation of chlorpromazine solution for irradiation under anaerobic condition. Chloroform is the only sol-

TABLE II.—PHYSICAL PROPERTIES OF PHOTODEGRADATION PRODUCTS OF CHLORPROMAZINE UNDER ANAEROBIC CONDITION

Fraction	R_f (TLC[a])	Color Reactions: 50% H_2SO_4	Color Reactions: 1% $FeCl_3$	Nature	U.V. (QAS[b]) mμ	M.p., °C. (QAS[b])
1	0.78	Reddish-purple	Brownish-green	...	...	...
2	0.73	Brownish-purple	Brownish-green	...	...	...
3	0.69	Orange	Brown	...	...	...
4	0.66	Purple	Blue (changes to reddish-purple)	...	220, 265, 320	198–200°
5	0.48	Reddish-purple	Brownish-purple	2-PZOH[c]	212, 254	...
6	0.46	Orange	Orange	PZ[d]	208, 256, 300	190–194 (Picrate, 143–144)
7	0.30	Purple	Purple	...	219, 263, 316	195–199
8	0.28	Purple	Reddish-purple	...	219, 262, 312	192–198
9	0.26	Bluish-purple	Purple	...	218, 262, 313	193–198
10	0.20	Bluish-purple	Purple	...	218, 262, 313	190–197 (Picrate, 167–171)
11	0.14	Reddish-purple	Reddish-purple	CP-dimer[e]	262, 313	(Picrate, 136–142)
12	0	Bluish-purple	Green	Polymer	208, 263	235–239 (Picrate, 149–155)

[a] Thin-layer chromatography. [b] Quaternary ammonium salt. [c] 2-Hydroxypromazine. [d] Promazine. [e] Chlorpromazine dimer.

vent which prevented the formation of the polymer, and other solvents, such as benzene, ethylene dichloride, ethyl acetate, and carbon tetrachloride, failed to suppress the polymerization reaction. The chromatographic pattern of the ethylene dichloride solution of chlorpromazine resembles that of the deoxygenated aqueous solution. A deoxygenated chlorpromazine solution in phosphate buffer solution (pH 7.8), treated with U.V. energy under the same conditions, failed to prevent the polymer formation. It was of interest to observe that ascorbic acid added to the aqueous solution of chlorpromazine prevented the formation of the polymer, whereas sodium bisulfite did not inhibit the polymerization reaction under the same conditions. The aqueous medium containing maleic acid produced only a trace of the polymer. Free radical (24–27) formation elicited by the short wavelength photon is apparently the key step toward the formation of the polymer. However, these free radicals are detectable only in the solvent media, and merely a trace or none of them exists in a solid state.

It appears that the intact chlorpromazine molecules are deposited in the tissues or organs either in the free or bound form (41). When these tissues or organs are exposed to ultraviolet energy, photochemical reactions take place to cleave the chloride function on the chlorpromazine molecules to form hydrogen chloride and, on the other hand, creating free radicals at the site of irradiation. Immediately, a polymerization reaction of these free radicals proceeds to form a polymer which deposits at the site of irradiation along with melanin (7, 38) to cause the skin discoloration. Although hydrogen chloride liberation and the polymer formation are proposed as the main cause of the skin irritation and the abnormal skin discolorations, caution must be taken to differentiate between the photosensitivity of this nature and that of a photo allergy or drug allergy developed in individuals who are sensitive to even a low dose of phenothiazines.

REFERENCES

(1) Schwank, R., and Jirasek, L., *Cesk. Dermatol.*, **36**, 4(1961).
(2) Knox, J. M., *Ann. Allergy*, **16**, 749(1961).
(3) Hayes, G. B., Lyle, C. B., Jr., and Wheeler, C. E., Jr., *Arch. Dermatol.*, **90**, 471(1964).
(4) Zelickson, A. S., and Zeller, H. C , *J. Am. Med. Assoc.*, **188**, 394(1964).
(5) Ban, T. A., and Lehman, H. E., *Can. Psychiat. Assoc. J.*, **10**, 112(1965).
(6) Zelickson, A. S., *J. Am. Med. Assoc.*, **194**, 670(1965).
(7) Satanove, A., *ibid.*, **191**, 263(1965).
(8) Greiner, A. C., and Nicolson, G. A., *Can. Med. Assoc. J.*, **91**, 627(1964).
(9) Perry, T. C., Culling, C. F. A., Berry, K., and Hansen, S., *Science*, **146**, 81(1964).
(10) Feldman, P. E., and Frierman, B. D., *Am. J. Psychiat.*, **121**, 187(1964).
(11) Tridici, L. M., Schiele, B. C., and McClanahan, W. S., *Minn. Med.*, **48**, 569(1965).
(12) Brill, H., Schiele, H. G., and Delong, S. L., *Am. J. Psychiat.*, **122**, 326(1965).
(13) Cairns, R. J., Capoose, H. S., and Gregory, I. D. R., *Lancet*, **I**, 239(1965).
(14) Richards, F., *ibid.*, **II**, 437(1965).
(15) Nicolson, G. A., Greiner, A. C., McFarlane, W. J. G., and Baker, G. A., *ibid.*, **I**, 344(1965).
(16) Massey, L. W. C., *Can. Med. Assoc. J.*, **92**, 186(1965).
(17) Barsa, J. A., and Saunders, J. C., *Psychopharmacologia*, **7**, 138(1965)
(18) Rutschmann, J., Kalberer, F., Schalch, W., and Stähelin, H., *Psychopharm. Serv. Cent. Bull.*, **2**, 73(1962).
(19) Ellis, P. P., *Arch. Ophthamol.*, **74**, 96(1965).
(20) Mathalone, M. B. R., *Lancet*, **II** (7403), 111(1965).
(21) Delong, S. L., Poley, B. J., and McFarlane, J. R., Jr., *Arch. Ophthamol.*, **73**, 611(1965).
(22) Barsa, J. A., Newton, J. D., and Saunders, J. C., *J. Am. Med. Assoc.*, **193**, 10(1965).
(23) Siddall, J. R., *Arch. Ophthalmol.*, **74**, 460(1965).
(24) Huang, C. L., and Sands, F. L., *J. Chromatog.*, **13**, 246(1964).
(25) Forrest, I. S., Forrest, F. M., and Berger, M., *Biochim. Biophys. Acta*, **29**, 441(1958).
(26) Lagercrantz, C., *Acta Chem. Scand.*, **15**, 1545(1961).
(27) Lagercrantz, C., and Yhland, M., *ibid.*, **16**, 508(1962).
(28) Wollmann, M., and Keleti, T., *Arzneimittel-Forsch.*, **12**, 360(1962).
(29) Borg, D. C., and Cotzias, G. C., *Proc. Natl. Acad. Sci.*, **48**, 643(1962).
(30) Martin, H. F., Price, S., and Gudzinowicz, B. J., *Arch. Biochem. Biophys.*, **103**, 196(1963).
(31) Beckett, A. H., Beaven, M. A., and Robinson, A. E., *Biochem. Pharmacol.*, **12**, 779(1963).
(32) Yamamoto, R., and Fujisawa, S., *Yakugaku Zasshi*, **82**, 1396(1962).
(33) Felmeister, A., and Discher, C. A., *J. Pharm. Sci.*, **53**, 756(1964).
(34) Jung, E. G., Schwarz-Speck, M., and Kormany, G., *Schweiz. Med. Wochschr.*, **93**, 249(1963).
(35) Forrest, I. S., Gutmann, F., and Keyzer, H., *Rev. Aggressol.*, **7**, 147(1966).
(36) Potts, A. M., *Invest. Ophthalmol.*, **3**, 405(1964).
(37) Blois, M. S., Jr., *J. Invest. Dermatol.*, **44**, 475(1965).
(38) Zelickson, A. S., *J. Am. Med. Assoc.*, **194**, 670(1965).
(39) Meier-Ruge, W., Kalberer, F., and Cerletti, A., *Experientia*, **22**, 153(1966).
(40) Huang, C. L., *Intern. J. Neuropharmacol.*, to be published.
(41) Forrest, F. M., Forrest, I. S., and Roizin, L., *Rev. Aggressol.*, **4**, 259(1963).