rate and may be attributed to decreased effectiveness of ultrasonic vibration. As the temperature increases, the difference in viscosity between 5 and 50% samples becomes less and the slope of the degradation approaches that of the control. In the ethanol and diethyl ether solvent systems, there are not significant enough differences in viscosity to produce this effect. Since the differences in viscosity in these two systems are very small, the slope of the ultrasonically effected degradation approaches that of the control throughout the temperature range.

SUMMARY AND CONCLUSIONS

1. Under the conditions stated in this study, the application of ultrasonic energy to a system undergoing degradation will cause an increase in kinetic rate in ethanol–water, diethyl ether–water, and diethylene glycol–water systems.

2. The lowering of the heat of activation is apparently due to the mechanical vibrations of ultrasonic energy applied to the degrading system, since the thermal energy is kept constant.

3. The ultrasonic vibration appears to increase the effect that the movement of the molecules toward each other and the movement of the products away from each other have on the overall rate.

4. As the concentration ratio is increased in an ethylene glycol–water system, the subsequent increase in viscosity apparently reduces the effect on the movement of molecules caused by ultrasonic vibration.

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Photoinduced Interaction of Phenothiazine Drugs with a Lecithin Monomolecular Film

A. FELMEISTER and R. SCHAUBMAN

Abstract □ Monomolecular films of dipalmitoyl lecithin (DPL) were spread onto an aqueous phase into which a potential photosensitizing drug had been dissolved. Chlorpromazine, promazine, triflupromazine, prochlorperazine, and trifluoperazine were the drugs used. The drug-film system was exposed to ultraviolet irradiation and resultant changes in the drug-film interaction determined. The interaction of chlorpromazine and prochlorperazine with the DPL film was found to increase after irradiation. The film interaction of trifluoperazine showed an initial decrease, while that of promazine and triflupromazine was not affected by the irradiation. Thus the substituent in the 2-position of the phenothiazine nucleus appears to be critical in the photosensitized interaction. A phototoxic index was calculated and related to *in vivo* data.

Keyphrases □ Phenothiazine compounds—photosensitivity □ Lecithin monomolecular films-phenothiazines—irradiation □ UV light—film-drug irradiation □ Phototoxic index—determination □ Photoreaction—halogen substitution

The cutaneous edema and erythema that develops in mammals, exposed to sunlight subsequent to treatment with a photosensitizing drug, is indicative of increased cell-membrane permeability. It appears likely then that, at least in some instances, photosensitized reactions and their consequent symptoms are the result of an interaction of a photoproduced species with one or more of the structural elements which maintain membrane integrity. On the basis of this postulation recently proposed was the use of monomolecular films of phospholipids and other cell membrane constituents as a model system for the investigation of photosensitized reactions (1).

In this paper the interaction of a series of UV-irradiated phenothiazine drugs with a monomolecular film of dipalmitoyl lecithin (DPL) is reported. A "phototoxic index" is calculated and related qualitatively to some limited clinical data from the literature.

EXPERIMENTAL

Materials—The *l*-α-dipalmitoyl lecithin (DPL) was chromatographically pure.[1] The following phenothiazine derivatives were used without further purification: chlorpromazine hydrochloride, prochlorperazine hydrochloride, and trifluoperazine dihydrochloride[2]; promazine hydrochloride[3]; and triflupromazine hydrochlo-

[1] Mann Chemical Co., New York, N. Y.
[2] Smith Kline & French, Philadelphia, Pa.
[3] Wyeth Laboratories, Philadelphia, Pa.

ride.[4] The water was prepared by fractional distillation of deionized water using all glass equipment. All other chemicals were reagent grade.

Apparatus and General Methods—A 0.1 *M* sodium acetate–acetic acid buffer, adjusted to pH 5.9, was used as the subphase in all experiments. The DPL was dissolved in hexane–absolute ethanol (90–10% v/v) and spread from a micrometer syringe[5] onto the subphase contained in a Langmuir-type trough. A movable Teflon barrier was used to change the trough area. The temperature of the trough and the subphase was maintained constant at 25 ± 0.1° by circulating water from a constant-temperature bath. Surface pressure, π (the difference between the surface tension of the subphase and that of the film-covered subphase) was measured by the Wilhelmy plate method (2). A thin platinum plate, roughened to ensure complete wetting, was used. UV irradiation of the films was accomplished by means of a lamp[6] fitted with a filter to screen out the radiation below 2800 Å. The lamp was positioned about 50 mm. above the subphase, and its output was continuously monitored by means of a UV meter.[6]

Film Studies—The surface pressure of the films was determined at various areas per DPL molecule on a series of subphases containing buffer alone or buffer plus 1×10^{-5} *M* of the phenothiazine derivative. The trough area was decreased by 2.5-cm.² increments and surface pressure readings were taken immediately after each area change. Approximately 30 sec. elapsed between each reading. Irradiation was initiated at full trough area after the DPL was spread on the subphase. A 2-min. irradiation period was used for all experiments. The surface pressure–surface area (π–*A*) curves were determined immediately after irradiation. A pure DPL film, *i.e.*, in the absence of any phenothiazine drug, was irradiated for 30 min. to determine its UV stability. The subphase containing the phenothiazine drug (1×10^{-5} *M*) was irradiated for 30 min. to determine whether any surface activity developed in the absence of the film.

RESULTS AND DISCUSSION

Irradiation for 30 min. of a pure DPL film produced no detectable change in surface pressure, demonstrating the stability of the film to the radiation. Similarly, irradiation of 1×10^{-5} *M* solutions of the phenothiazine compound in the absence of the film showed no surface pressure changes, with the exception of prochlorperazine. Irradiation of the buffered solution of prochlorperazine produced a slight surface pressure of about 1.5 dynes/cm. when the surface was compressed to the smallest allowable trough area. At larger trough areas no surface pressure was detected. No significant temperature change occurred at the film surface over the period of irradiation.

DPL was found to form a mixed film with each of the phenothiazines, with the exception of promazine, as evidenced by an increase in area/molecule of DPL in the presence of these drugs (Figs. 1–5). This apparently is the result of penetration of the phenothiazine molecule into the DPL film (3). On compression of each of the mixed films, the π–*A* curves gradually approach that of the pure DPL film. At high pressures ($\approx$30 dynes/cm.) the mixed film curves coincide with the pure DPL curve, indicating ejection of the phenothiazine molecule from the film (3).

Irradiation modified the π–*A* characteristics of most of the drug-film systems investigated. In the case of the chlorpromazine-DPL mixed film, an increase in surface pressure at all areas per molecule developed on irradiation (Fig. 1). This expansion suggests an increased film interaction and penetration by the photoproduced species.

No attempt was made at this point to separate or identify the reacting species. However, it is known that a variety of compounds are formed on irradiation of chlorpromazine. Huang *et al.* (4, 5) reported that UV photolysis of chlorpromazine yields the sulfoxide, *N*-oxide, a dimer, and a polymer along with at least 20 unidentified compounds. It is unlikely that the sulfoxide is responsible for the increase in surface pressure, since it has been shown to be more polar and less surface-active than chlorpromazine (3). One would expect that the *N*-oxide, which is also more polar than chlor-

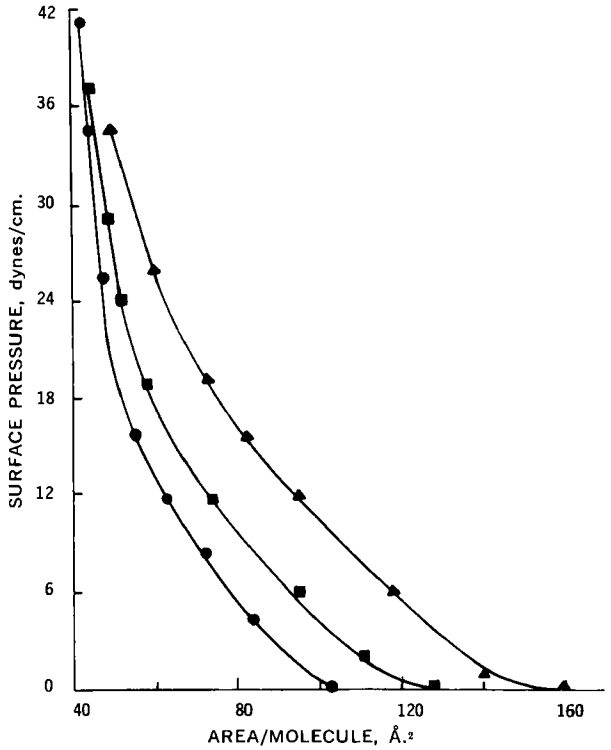


Figure 1—*Surface pressure* versus *area per molecule for* l-α-*dipalmitoyl lecithin on an acetic acid–sodium acetate buffer (pH 5.9) at 25° and ionic strength 0.1. Key:* ●, *zero concentration of chlorpromazine HCl, irradiated and nonirradiated;* ■, 1×10^{-5} M *chlorpromazine HCl, nonirradiated;* ▲, 1×10^{-5} M *chlorpromazine HCl, irradiated.*

promazine, would behave in a similar manner. The decrease in polar properties that no doubt result on dimerization or polymerization of chlorpromazine suggest that such species may be involved in the observed interaction. However, since a large number of unidentified compounds are also produced on irradiation, any discussion as to the compound or compounds involved in this photoinduced reaction must be considered speculative at this time.

Promazine had no influence on the π–*A* curve of DPL film (Fig. 2), indicating no drug-film interaction. Irradiation of this system also had no effect on the π–*A* characteristics. Thus it appears that any photospecies produced as a result of the irradiation possesses approximately the same degree of surface activity and reactivity toward the DPL film as does promazine itself.

Trifluromazine interacts with the DPL to form a mixed film (Fig. 3). The π–*A* characteristics of this mixed film are similar to that observed with the chlorpromazine-DPL system. However, in contrast to the latter, irradiation of the trifluromazine-DPL system produced no detectable change. Again as with promazine, it may be concluded that any photospecies produced apparently interacts with the film to the same extent as the starting compound.

The π–*A* curve of the prochlorperazine-DPL film is slightly more expanded than that of either chlorpromazine or trifluromazine. This may be a reflection of the difference in pKa between the perazine and promazine derivatives, approximately 8.1 and 9.3, respectively (6), as well a difference in the polar properties of the R_{10} substituents.

Irradiation of the prochlorperazine-DPL film resulted in an additional increase in area/molecule (Fig. 4), similar to that observed with chlorpromazine, indicating increased drug-film interaction.

Trifluoperazine penetrates the film to a greater degree than any of the other compounds, exhibiting this effect even at maximum trough area ($\approx$160 Å.²/DPL molecule). None of the other phenothiazine derivatives showed any surface pressure at such large areas. In contrast to the effect observed with the other compounds, irradiation of the trifluoperazine-DPL system resulted in an initial decrease in surface pressure. On compression of the irradiated film the π–*A* curve gradually approached that of the nonirradiated film (Fig. 5). By checking the surface pressure at periodic intervals it was

[4] Squibb Institute for Medical Research, New Brunswick, N. J.
[5] Agla, Burroughs Wellcome Corp., Tuckahoe, N. Y.
[6] Mineralite model V-41 UV lamp, and a Blak-Ray UV meter, Ultraviolet Products, Inc., San Gabriel, Calif.

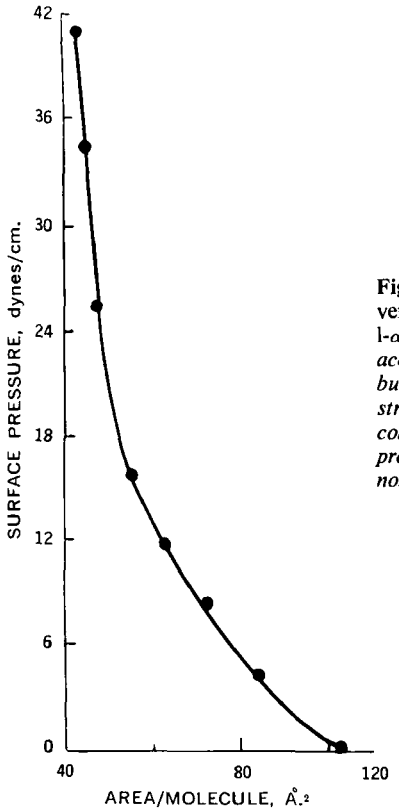


Figure 2—*Surface pressure* versus *area per molecule for* l-α-*dipalmitoyl lecithin on an acetic acid–sodium acetate buffer (pH 5.9) at 25° and ionic strength 0.1. Key:* ●, *zero concentration and* 1×10^{-5} M *promazine HCl, irradiated and nonirradiated.*

determined that this latter effect was a function of elapsed time after irradiation rather than the increasing pressure. While the surface pressure of the irradiated film decreased initially, about 15 min. after the irradiation was stopped the surface pressure began to increase until it finally reached a value approximately equal to that of the nonirradiated film. Thus it appears that the photospecies produced during irradiation is less surface active than trifluoperazine itself and probably desorbs from the surface. After the irradiation is stopped unchanged trifluoperazine molecules diffuse from the bulk, penetrate the DPL film, and restore the surface pressure to the original value. This time effect was not observed with any of the other systems studied.

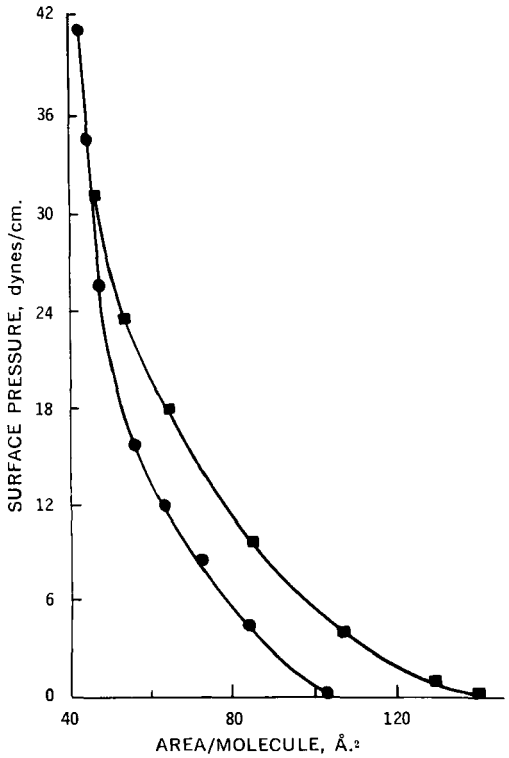


Figure 3—*Surface pressure* versus *area per molecule for* l-α-*dipalmitoyl lecithin on an acetic acid–sodium acetate buffer (pH 5.9) at 25° and ionic strength 0.1. Key:* ●, *zero concentration of trifluopromazine HCl, irradiated and nonirradiated;* ■, 1×10^{-5} M *trifluopromazine HCl, irradiated and nonirradiated.*

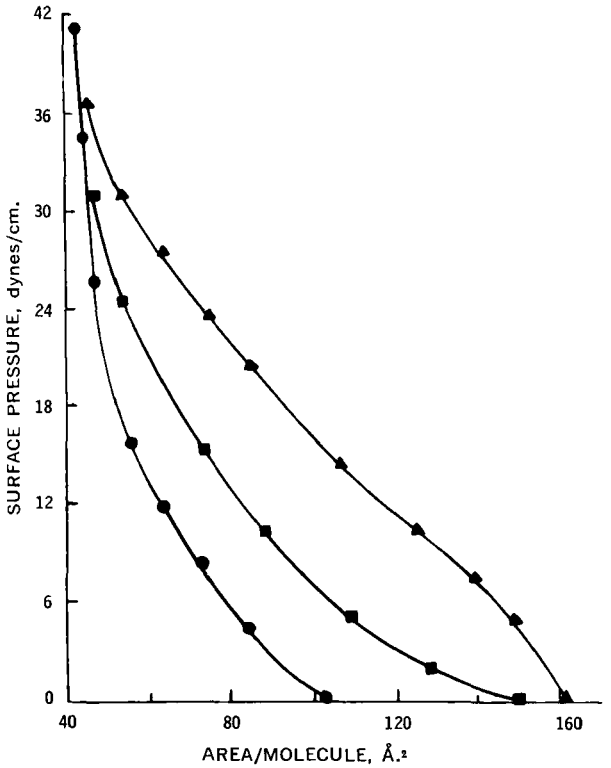


Figure 4—*Surface pressure* versus *area per molecule for* l-α-*dipalmitoyl lecithin on an acetic acid–sodium acetate buffer (pH 5.9) at 25° and ionic strength 0.1. Key:* ●, *zero concentration of prochlorperazine dihydrochloride, irradiated and nonirradiated;* ■, 1×10^{-5} M *prochlorperazine dihydrochloride, nonirradiated;* ▲, 1×10^{-5} M *prochlorperazine dihydrochloride, irradiated.*

CONCLUSION

It can be seen that both the R_2 and R_{10} substituents influence DPL film penetration. As for the R_2 substitution, the degree of penetration increases in the order H < Cl < CF_3. An equivalent increase in penetration results when the methylpiperazinylpropyl group is substituted for the dimethylaminopropyl group on the 10-position. In contrast only the R_2 substituent seems to have any significant influence on the irradiated drug-DPL interactions. Of the five compounds studied only the two compounds with chlorine in the 2-position interacted more strongly with the DPL film after irradiation, though the R_{10} substituent differed in these two compounds.

It has been reported that chlorpromazine photopolymerizes *via* a free radical formed by elimination of the chlorine from the 2-position (4). It can be postulated that prochlorperazine will polymerize *via* a similar mechanism. The fact that only the chlorine-containing compounds exhibit increased film penetration on irradiation, and that only these compounds would be likely to polymerize on exposure to UV irradiation, suggests that a photopolymer is the reactive species in this study. Similarly, a photopolymer may conceivably be involved in the *in vivo* photosensitized reactions which have been observed in the presence of chlorpromazine and prochlorperazine. Harber *et al.* (7) suggested a similar free radical mechanism to explain the photosensitization properties of halogen-substituted salicylanilides. Though in this latter study a free radical-protein complex was assumed to occur rather than a direct free radical polymerization.

To permit comparison of the five phenothiazine derivatives, a phototoxic index (PI) was calculated using the relationship below. The PI values listed in Table I were calculated at an arbitrarily

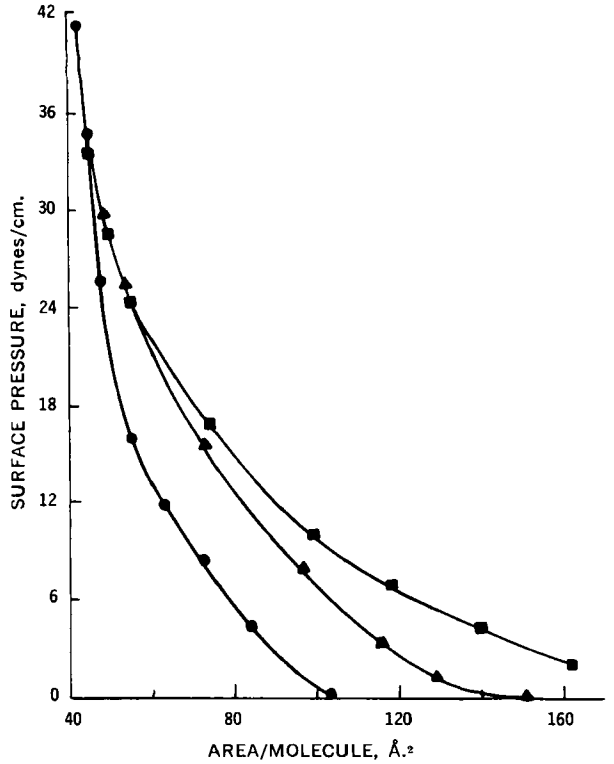


Figure 5—*Surface pressure* versus *area per molecule for* l-α-*dipalmitoyl lecithin on an acetic acid–sodium acetate buffer (pH 5.9) at 25° and ionic strength 0.1. Key:* ●, *zero concentration of trifluoperazine dihydrochloride, irradiated and nonirradiated;* ■, 1×10^{-5} M *trifluoperazine dihydrochloride, nonirradiated;* ▲, 1×10^{-5} M *trifluoperazine dihydrochloride, irradiated.*

selected area/molecule of 75 Å.². However, the values were not significantly different when calculated from data selected within the range of 60 to 90 Å.².

$$PI \equiv (\Delta\pi_i/\Delta\pi_t) \times 100$$

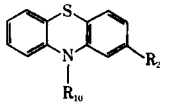
where $\Delta\pi_i$ is the difference between the surface pressure of the irradiated drug-DPL film and that of the nonirradiated drug-DPL film, and $\Delta\pi_t$ is the difference between the surface pressure of the irradiated drug-DPL film and that of the pure lecithin film.

Thus PI represents the percentage of the total increase in surface pressure which is due to irradiation of the drug-DPL film.

From Table I it can been seen that 60% of the total increase in surface pressure that develops in a DPL film when spread over a subphase containing 1×10^{-5} *M* chlorpromazine and irradiated is due directly to the effect of the irradiation. In the case of prochlorperazine, 50% of the total increase is due to this effect. Irradiation of the other three drug-DPL systems did not produce any increase in surface pressure. In fact, in the case of trifluoperazine the initial value of PI was negative. The significance of this, if any, cannot be determined from the data presently available.

Drug photosensitization reactions have been shown to increase permeability of a variety of cells, including red blood cells, lysozomes, and mast cells (8). In addition, the observed clinical symptoms are indicative of increased cell membrane permeability. Therefore, it appears reasonable to postulate that the increase in film pressure observed on irradiation of chlorpromazine and prochlorperazine is a measure of a photoproduced cell-membrane expansion and subsequent increased permeability. Such increases in film pressure should then be related to phototoxic activity, at least within a series of congeners. Chlorpromazine and prochlorperazine then presumably are phototoxic, while the other three compounds should not be to any significant degree.

Table I—Phototoxic Index (PI)[a] of Five Phenothiazine Derivatives



Compound	R_{10}	R_2	PI
Promazine	$-(CH_2)_3N(CH_3)_2$	H	0
Chlorpromazine	$-(CH_2)_3N(CH_3)_2$	Cl	60
Triflupromazine	$-(CH_2)_3N(CH_3)_2$	CF_3	0
Prochlorperazine	$-(CH_2)_3-N\langle\rangle N-CH_3$	Cl	50
Trifluoperazine	$-(CH_2)_3-N\langle\rangle N-CH_3$	CF_3	−10[b] 0[c]

[a] See text for definition and method of calculation. [b] Value recorded initially. [c] Value recorded after about 15 min. No further change was noted in this value.

Qualitatively, the literature supports this contention. The bulk of the reports dealing with photosensitization by phenothiazine drugs almost always implicate either chlorpromazine or prochlorperazine, and only rarely other derivatives. Ison and Blank (9) recently ranked chlorpromazine and prochlorperazine based on phototoxicity toward mice. These workers determined the PDR_{50} (*i.e.*, the minimum dose which produces a phototoxic reaction in 50% of a group of test animals) of these compounds to be 20 and 46 mg./kg., respectively. No other phenothiazine derivatives were investigated in their study. These data correlate with the authors' PI values as shown in Table I.

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