Surface Activity of Chlorpromazine and Chlorpromazine Sulfoxide in the Presence of Insoluble Monomolecular Films

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The surface activity of chlorpromazine and its sulfoxide has been investigated at interfaces covered with insoluble monomolecular films which simulate the oriented structure of biological membranes. These compounds have been observed to form mixed monomolecular films with cholesterol, L-α-dipalmitoyl phosphatidylethanolamine, and L-α-dipalmitoyl phosphatidylcholine at concentrations where the drugs alone exhibit no surface activity. In all cases chlorpromazine was more surface active than its sulfoxide. The penetration of the drugs into the films and their subsequent ejection at higher surface pressures were found to depend on the surface characteristics of the lipid, the pH, and ionic strength of the underlying solution.

IN RECENT years it has become increasingly apparent that biological membranes make up a large part of the cell and that many physiological processes take place at or near membrane surfaces. It is apparent also that membranes are not just static lipoprotein barriers around cells and cell organelles. Instead, they are dynamic structures capable of changing size and shape and capable of active participation in many processes such as oxidative phosphorylation (1), fat digestion (2), active transport (3), and cell locomotion (4). It would appear that the activity of drugs on such systems might be related to their interfacial activity in the presence of membranes. Once they have accumulated at the interface, drug molecules could compete directly with some normal process or alter the steric and electrical nature of the membrane involved in the process.

Recent work has indicated that chlorpromazine and other phenothiazine derivatives accumulate at biological membranes, and that this might be a factor in their pharmacological activity. Spirtes and Guth (5) have shown that mitochondrial swelling, ordinarily caused by a variety of swelling agents, is inhibited by chlorpromazine, but not by its sulfoxide. Freeman and Spirtes (6) have shown that chlorpromazine and trifluoperazine inhibit hemolysis of human erythrocytes, whereas the sulfoxide does not. The order of inhibition was related to the amount of each drug adsorbed by the erythrocyte. Axelrod *et al.* (7) have demonstrated that chlorpromazine prevents the uptake of circulating norepinephrine by various tissues. They felt that this might be due to interference with binding at the cell membrane. Gey and Pletscher (8) observed that the reserpine-induced release of 5-hydroxytryptamine, norepinephrine, and dopamine in rat brain is partially inhibited by chlorpromazine. They concluded that chlorpromazine is able to decrease the membrane permeability of the monamine storage granules. In view of the diverse action of phenothiazines as tranquilizers, local anesthetics, and antihistamines, it would appear that, at least in part, some common mechanism such as membrane action may be operating. The importance of action at membranes has been demonstrated for local anesthetics (9), and membranes are known to enclose mast cell granules which contain histamine (10).

The surface activity of various phenothiazines has been demonstrated at solid interfaces (11) and at air–liquid interfaces containing either 0.1 *N* HCl (12) or a modified Ringer's solution at pH 7 (13). A preliminary communication by the present authors (14) is in general agreement with these studies, but it appears that the concentrations of drug reported to be surface active by Seeman and Bialy (13) are too low by a factor of ten.

This study was initiated to determine the factors which influence the surface activity of phenothiazine derivatives in the presence of insoluble

Received March 29, 1965, from the College of Pharmacy, University of Michigan, Ann Arbor, and the College of Pharmacy, Columbia University, New York, N. Y.

Accepted for publication June 22, 1965.

Presented to the Scientific Section, A.PH.A., Detroit meeting, March 1965.

Abstracted in part from a thesis submitted by David E. Auslander to Columbia University, New York, N. Y., in partial fulfillment of Master of Science degree requirements.

This investigation was supported in part by grant GM 12886-01 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

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After the preparation of this paper, a preliminary communication [Van Deenan, L. L. M., and Demel, R. A., *Biochim. Biophys. Acta*, **94**, 314(1965)] related to the present study was reported. A series of psychoactive drugs, including chlorpromazine was found to penetrate a variety of lipid monomolecular films similar to those used in this study. Marked penetration of natural lipids such as cerebrosides, sphingomyelin, and gangliosides was observed at high degrees of film compression, these substances exhibiting a much less condensed film structure than synthetic lipids, which showed less penetration. In general, these results are in agreement with those presented here, and they tend to strengthen the conclusion that drug specificity may be related to the film characteristics of membrane components.

lipid monomolecular films. A preliminary report demonstrating such activity was presented earlier (14). The lipid films were chosen to simulate the oriented structure of membranes. Particular emphasis was placed on the effect that two-dimensional structure of the film has on the surface activity of dissolved drugs.

EXPERIMENTAL

Chemicals.—Chlorpromazine hydrochloride (CPZ) and chlorpromazine sulfoxide hydrochloride (CPZ-O) were obtained from Smith Kline & French Laboratories and were used as received. The substances spread as insoluble films were: dipalmitoyl phosphatidylethanolamine (cephalin), dipalmitoyl phosphatidylcholine (lecithin), and cholesterol. They were all obtained chromatographically pure from the Mann Chemical Co. All inorganic chemicals used in this study were of reagent grade. The water used was prepared by slowly passing it through a mixed resin bed cartridge of a Bantam demineralizer.

General Methods.—Surface pressures (surface tension of solvent minus surface tension of solvent plus film) were measured by means of the Wilhemy plate method (15); a roughened platinum plate was used to insure complete wetting. A microtorsion[1] balance placed on a movable platform was used to measure the force on the plate. The film balance was constructed[2] so that films could be compressed to fixed areas of 0.01 mm. intervals. The entire film balance was enclosed in a Plexiglas dry box to exclude dust and other airborne impurities. The trough was maintained at a constant temperature of 25 ± 0.1° by circulating water from a constant-temperature bath. The edges of the trough and the barrier, which came in contact with the aqueous surface, were lightly coated with paraffin to prevent wetting of the trough and barrier by the monolayer at high surface pressures. The trough had a capacity of 900 ml. Before spreading any films, the surface was cleaned by means of a capillary tube attached to a water aspirator.

The various lipids were dissolved in a suitable spreading solvent and placed on the surface from an Agla micrometer syringe[3] which is capable of accurately delivering 0.001 ml. Chloroform (Baker reagent) was used to dissolve cephalin, and petroleum ether was used to dissolve cholesterol. The latter solvent was purified by mixing with fuming sulfuric acid (18%), separating, drying over anhydrous sodium sulfate, and then distilling at 30–40°. A mixture of normal hexane (64% v/v), petroleum ether (20% v/v), chloroform (10% v/v), and methyl alcohol (6% v/v) was used to dissolve lecithin. All solvents were checked for the presence of surface-active impurities by compressing the surface after the addition of a small amount of solvent. No surface pressure was obtained for any solvent used.

All films were measured a number of times and found to be reproducible to 0.5 dynes. To ensure equilibrium after each area change, a period of at least 5 min. was required between measurements of the pure film. In the penetration studies, 10 to 15 min. were required for establishing equilibrium, depending on the degree of compression of the film.

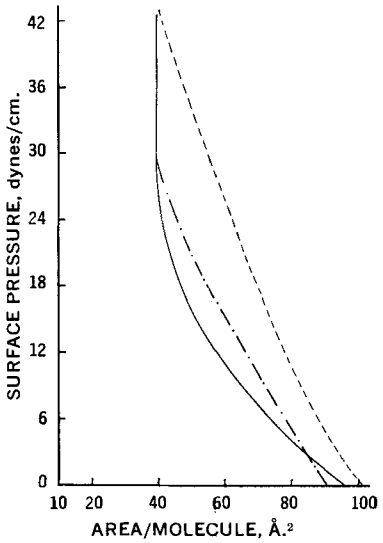

Fig. 1.—Surface pressure *vs.* area per molecule for L-α-dipalmitoyl phosphatidylcholine spread at 25° and: pH 6.9, ionic strength 0.04 (—); pH 6.9, ionic strength 0.1 (—·—); pH 2.3, ionic strength 0.1 (- -).

Lipid Film in the Absence of Drug.—The first series of experiments were conducted in order to determine the surface pressure of the various films at different areas per molecule in the absence of CPZ and CPZ-O. A Sörenson phosphate buffer (0.01 *M*) was used to obtain a pH of 6.9, while 0.01 *M* HCl was used for a pH of 2.3. The ionic

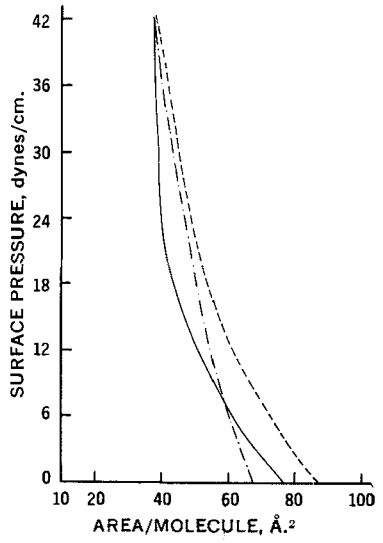

Fig. 2.—Surface pressure *vs.* area per molecule for L-α-dipalmitoyl phosphatidylethanolamine at 25° and: pH 6.9, ionic strength 0.04 (—); pH 6.9, ionic strength 0.1 (—·—); pH 2.3, ionic strength 0.1 (- -).

[1] Bethlehem Instrument Corp.
[2] Frater Instrument Corp., Corona, N. Y.
[3] Burroughs Wellcome Corp., Tuckahoe, N. Y.

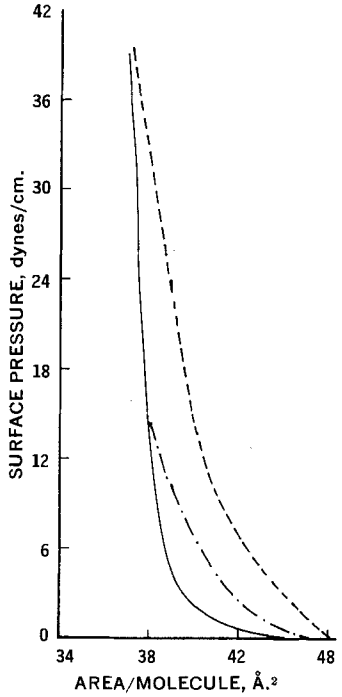

Fig. 3.—Surface pressure *vs.* area per molecule for cholesterol at 25° and: pH 6.9, ionic strength 0.04 (—); pH 6.9, ionic strength 0.1 (—·—); pH 2.3, ionic strength 0.1 (- -).

strength was adjusted with sodium chloride. All pH measurements were made with a Beckman Zeromatic pH meter.

Lipid Films in the Presence of Drug.—The lipids were each spread on a 1×10^{-5} *M* solution of CPZ and CPZ-O at the various pH values and ionic strengths. This concentration of drug was chosen because of lack of air–water surface activity, regardless of the pH values and ionic strengths used in this study. To demonstrate that the change in surface activity is an equilibrium process, films were spread on a buffer to a fixed surface pressure. The drug was then introduced into the solution beneath the film. Although the same change in surface pressure occurred as in the first method, the time required to reach equilibrium was much longer, particularly at higher surface pressures. In view of this, the first method was used throughout the study.

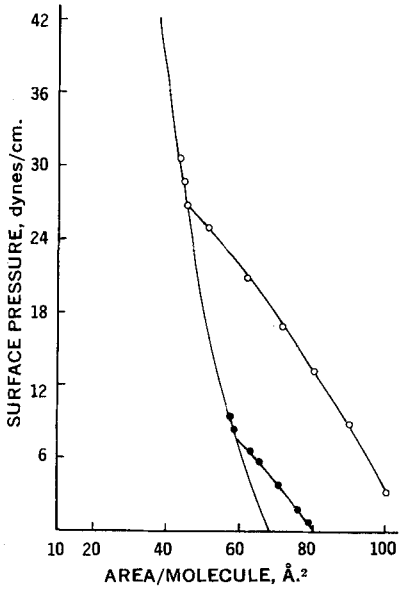

Fig. 4.—Surface pressure *vs.* area per molecule for L-α-dipalmitoyl phosphatidylethanolamine spread on: buffer, pH 6.9, ionic strength 0.1(—); buffer plus 10^{-5} *M* chlorpromazine (○); buffer plus 10^{-5} *M* chlorpromazine sulfoxide (●) at 25°.

RESULTS AND DISCUSSION

Pure Films.—The surface pressure *versus* area curves (referred to as π–*A* curves) for the various lipids, spread in the absence of drug, are shown in Figs. 1–3. Such films will be referred to as "pure films" throughout this report. The results at pH 6.9 are in general agreement with previous studies utilizing synthetic phospholipids (16) and cholesterol (17). At a pH of 6.9 and an ionic strength of 0.04, lecithin and cephalin exhibit an expanded film behavior before reaching the limiting area, whereas cholesterol develops little or no surface pressure before becoming a highly condensed film. It is interesting to note that merely increasing the ionic strength to 0.1 with NaCl has some effect on the film characteristics of the three lipids. Lecithin and cephalin become more condensed or exhibit decreased compressibility in what previously was a more expanded region; the greater the rate of surface pressure development with changing area per molecule, the less the compressibility. Since the two-dimensional state of the film depends on attractive and repulsive forces between film molecules, one would expect the presence of electrolytes to be significant, particularly with charged films. At pH 6.9 lecithin is zwitterionic due to the presence of the quaternary choline nitrogen and the anionic phosphate groups; cephalin under these conditions is negatively charged since the protonated nitrogen of the ethanolamine portion is quite acidic (18). Although it is difficult to know exactly, it appears that electrolyte alters the balance of charges on lecithin and cephalin, reduces the repulsive forces between the molecules, and allows them to pack more closely. The fact that the effect of electrolyte is not confined to charged groups is indicated by the slight but significant change in the π–*A* curve of cholesterol at the higher ionic strength.

By reducing the pH to 2.3 and maintaining the ionic strength at 0.1, very marked changes in the film behavior of lecithin and cephalin were noted. Large increases in surface pressure, at a given area per molecule, for lecithin and cephalin would appear to be due to a reduction in the dissociation of the phosphate groups. This results in a change from zwitterionic to predominantly positive charge for lecithin and from negative to positive charge for cephalin. In view of the similar nonpolar portions of cephalin and lecithin and similar charge, differences between these films must be related to differences between the choline and ethanolamine nitrogen groups. The cholesterol film shows only a slight change due to pH, as would be expected

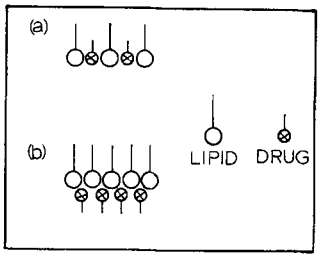

Fig. 5.—Schematic diagram of: (*a*) penetration of drug molecules into lipid monomolecular films at high areas per molecule; (*b*) complete ejection of drug molecules from the lipid film at lower areas per molecule.

for an unchanged monolayer. The slight change may be due to the presence of phosphate ion at pH 6.9 compared to chloride ion at pH 2.3. The various film characteristics will be discussed later in connection with the penetration of CPZ and CPZ-O into these films.

Effects of CPZ and CPZ-O on Various Lipid Films.—As can be seen in the typical plot shown in Fig. 4, when the various films are spread on solution of 1×10^{-5} *M* CPZ and CPZ-O, surface pressure develops at an area per molecule of lipid where the pure film exhibits no surface pressure. Similar plots may be observed in an earlier communication by the present authors (14). Since the drugs (CPZ and CPZ-O) are not ordinarily surface active at the concentration utilized, it may be assumed that they form mixed films with the lipids, as illustrated in Fig. 5*a*. Upon compression, the surface pressure increases until at lower areas per molecule it becomes identical to the corresponding surface pressure of the pure film. This would indicate that the CPZ and CPZ-O molecules are being ejected from the film as illustrated in Fig. 5*b*. Similar patterns of surface pressure change have been observed for lipid films spread on solutions of local anesthetics, such as procaine (9). If ejection did not occur, but rather a stable interfacial complex was being formed, the surface pressure would increase greatly at much higher areas per molecule, and the film would collapse with both molecules being ejected as a unit. This has been observed for films containing long-chain surfactants such as cholesterol and sodium lauryl sulfate, where a strong interaction occurs between the respective polar and nonpolar portions of the molecules involved (19). Chlorpromazine and its sulfoxide would appear to have the polar portion capable of interacting with that of cholesterol and the phospholipids; however, this must be offset by the nonpolar interactions between the long chains of the lipids which are greater than that between the nonpolar portions of drugs and lipids.

In order to examine drug–surface film interactions more closely, surface pressure changes in two regions of area per molecule will be considered: first, at areas per molecule before the pure film ordinarily develops surface pressure; and second, at areas per molecule after which surface pressure of pure film ordinarily begins to develop.

Table I.—Constants for Drug Penetration into Lipid Films at High Areas per Molecule

Lipid and Drug	A_0[a]	m[b]
pH 6.9 and Ionic Strength 0.04		
Lecithin–CPZ	245	−0.209
Cephalin–CPZ	139	−0.252
Cholesterol–CPZ	56	−0.600
Lecithin–CPZ-O	127	−0.250
Cephalin–CPZ-O	89	−0.209
pH 6.9 and Ionic Strength 0.1		
Lecithin–CPZ	145	−0.292
Cephalin–CPZ	109	−0.366
Cholesterol–CPZ	62	−0.861
Lecithin–CPZ-O	118	−0.377
Cephalin–CPZ-O	79	−0.363
pH 2.3 and Ionic Strength 0.1		
Lecithin–CPZ	124	−0.344
Cephalin–CPZ	105	−0.380
Cholesterol–CPZ	56	−1.25
Lecithin–CPZ-O	112	−0.208
Cephalin–CPZ-O	...	...

[a] Units of Å.^{2} per molecule. [b] Slope of plot of $\Delta\pi$ *vs.* *A*, the area per molecule.

In the region before surface pressure develops ordinarily any surface pressure developed should be the result of the combination of lipid and drug at the surface, since neither alone is surface active. Typical plots of surface pressure, π, *versus* *A*, the area per molecule of lipid, are given in Fig. 6. In all cases the increase in surface pressure upon compression is linear with area per molecule, up to the area where the pure film would ordinarily develop surface pressure. Extrapolation of the plots to π equal to zero yields the area per molecule of lipid required to first develop surface pressure in the presence of drug. These areas are designated as A_0 and are given in Table I.

In general, it appears that a critical area per molecule of lipid is required before any surface pressure will develop in the presence of drug, and that this area is different for each film under a given set of conditions. The order of A_0 values is lecithin > cephalin > cholesterol. This is the same order for the area of initial surface pressure development of the pure films. The effect of increased ionic strength on A_0 also parallels its effect on the pure

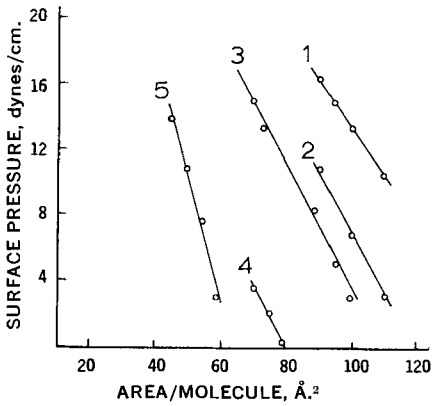

Fig. 6.—Surface pressure *vs.* area per molecule of lipid film, at pH 6.9, ionic strength 0.1, 25°, in the region where lipid and drug alone exhibit no surface pressure. Key: *1*, CPZ plus lecithin; *2*, CPZ-O plus lecithin; *3*, CPZ plus cephalin; *4*, CPZ-O plus cephalin; *5*, CPZ plus cholesterol.

TABLE II.—SURFACE PRESSURE CHANGES FOR DRUGS AT VARIOUS INITIAL LIPID FILM SURFACE PRESSURES[a]

Lipid and Drug	Surface Pressure Change at Various Initial Pressures 5	10	15	30	Ejection Pressure
		pH 6.9 and Ionic Strength 0.04			
Lecithin–CPZ	17.2	15.8	15.0	3.3	33.6
Cephalin–CPZ	13.9	13.4	11.7	2.2	32.5
Cholesterol–CPZ	9.6	6.5	1.5	...	16.5
Lecithin–CPZ-O	7.9	6.8	5.3	...	27.9
Cephalin–CPZ-O	2.2	...	...	...	9.1
Cholesterol–CPZ-O	1.1	...	...	...	6.6
		pH 6.9 and Ionic Strength 0.1			
Lecithin–CPZ	13.7	11.9	11.1	6.9	36.9
Cephalin–CPZ	14.2	11.2	8.7	...	25.5
Cholesterol–CPZ	10.7	5.9	...	...	15.9
Lecithin–CPZ-O	9.1	7.4	5.0	...	27.9
Cephalin–CPZ-O	2.0	...	...	...	7.0
Cholesterol–CPZ-O	...	...	...	...	...
		pH 2.3 and Ionic Strength 0.1			
Lecithin–CPZ	6.1	4.1	2.1	...	18.0
Cephalin–CPZ	7.3	5.6	3.3	...	19.8
Cholesterol–CPZ	10.0	5.3	0.6	...	15.6
Lecithin–CPZ-O	...	...	...	...	4.0
Cephalin–CPZ-O	...	...	...	...	...
Cholesterol–CPZ-O	2.4	...	...	...	3.3

[a] Units of surface pressure are dynes per cm.

lipid. This is particularly apparent for cholesterol, where increasing ionic strength has much less effect on the initial surface pressure of the pure film and the mixed film, as compared to lecithin and cephalin. The A_0 values for all films with CPZ and for lecithin with CPZ-O do not change appreciably when the pH is reduced to 2.3, even though such a change increases the surface pressure of the pure film at higher areas, an effect which would tend to increase A_0. The lack of change in A_0 at lower pH values appears to be due to factors which offset the expected increase in A_0. The most probable factors are: (*a*) the predominant positive charge of the film at low pH values which repels the positively charged drug molecules, and (*b*) the apparent reduced tendency of dissociated molecules, as compared to undissociated molecules, to leave the bulk solution and go to the interface. The importance of the second factor is apparent from a number of additional observations. First, increasing the concentration of CPZ and CPZ-O gives greater surface pressures (22) and a higher A_0; and second, CPZ-O, which is much more water soluble than CPZ, cannot penetrate cholesterol under any set of conditions studied, or cephalin at pH 2.3. These latter effects are probably due to a reduction in bulk thermodynamic activity, which is too great to overcome the close packing of cholesterol and cephalin. The effects of pH on the results with CPZ-O and cephalin, and CPZ and cephalin, particularly indicate the critical relationship that exists between the state of the lipid film and the tendency of the solute to go to an interface.

The requirement that there be a close proximity between drug and film molecules for maximum surface pressure development, and consequently penetration of the surface film, is apparent from the increasing surface pressure as the lipid film is compressed. The rate of change of surface pressure with the change in area per molecule, therefore, would be expected to depend primarily on the state of the lipid film. This seems to be the case when one observes the slopes for plots of π *versus* A labeled m in Table I. In general for CPZ, the more condensed the film originally, the greater the absolute value of the slope; the order is cholesterol > cephalin > lecithin. The effect of pH and ionic strength on the slopes parallels their effect on film condensation. For CPZ-O, as mentioned earlier, there is no penetration into cholesterol at all, and none into cephalin at pH 2.3. It is interesting to note, however, that the slope for CPZ-O into lecithin at pH 6.9 is greater than that for cephalin, which is just the opposite observed for CPZ. In view of the marked differences in surface activity of CPZ and CPZ-O (π for CPZ-O less than 2 dynes at 10^{-3} M at pH 6.9) and the lack of penetration into cholesterol at pH 6.9, and cholesterol and cephalin at pH 2.3, it appears that ejection of CPZ-O from the surface begins even at these higher areas per molecule. Thus, since cephalin is more condensed than lecithin, the rate of surface pressure development is greater for lecithin.

The factors influencing the ejection of molecules are observable in the region where the surface pressure of the pure film ordinarily develops. Table II gives the surface pressure change, $\Delta\pi$ (π of mixed film minus π of pure film), for the various systems. Values of $\Delta\pi$ are reported at areas per molecule where pure film ordinarily develops surface pressures of 5, 10, 15, and 30 dynes/cm. The surface pressure at which pure film and mixed film coincide is designated the ejection pressure. In general, the values of $\Delta\pi$ decrease with increased film compression. The effect of pH and ionic strength parallels their effect at higher areas per molecule. Greater condensation of film behavior promotes surface pressure changes, but beyond an initial surface pressure of 5 dynes, the $\Delta\pi$ values decrease to a greater extent for the more condensed films.

In general, therefore, it would appear that films tending ordinarily to develop surface pressure will assist the penetration of lipid films by molecules dissolved in the underlying solution at high areas

per molecule if the molecules have some tendency to adsorb at interfaces. Once the solute enters the surface region, its tendency to remain will depend on the nature of the lipid film. If the film is in a moderately condensed state, penetration may be enhanced, but as the film becomes highly condensed the possibility of ejection increases greatly. It is quite conceivable that the local interfacial changes in the state of biological membranes, due to environmental changes, could control the surface activity of drugs at the membrane surface in a similar manner.

CONCLUSIONS

A number of interesting conclusions of possible pharmacological importance may be drawn from the preceding experiments. It is apparent that the phenothiazines under consideration are surface active in the presence of lipid monomolecular films at bulk concentrations which are much lower than those ordinarily required to lower surface tension at the air–water interface and much closer to the range of concentrations required for biological activity. This study has demonstrated the significant role that the physical and chemical nature of the lipid film plays in the penetration process. When one realizes the variation of lipid composition in all biological membranes, and consequently, the various two-dimensional states of these membranes, possible reasons for drug specificity or potency are apparent. Van Deenan *et al.* (16) have shown that varying fatty acid content in phospholipids markedly alters the film characteristics of these substances. He and his co-workers have detected different amounts of the various phospholipids in the red blood cells of sheep, ox, pig, man, rabbit, and rat (20). They have also demonstrated tissue specificity, with respect to phospholipid content, in a particular animal (21). These interfaces along with adsorbed protein films could become quite specific for a particular drug under a given set of conditions. The widespread activity of phenothiazines as tranquilizers, antihistamines, and local anesthetics could be due in part to the variation in the membranes involved.

Finally, the marked reduction in surface activity when chlorpromazine is converted to its sulfoxide indicates how chemical modification of a drug may markedly affect its pharmacological activity at biological membranes. Preliminary studies have shown that other phenothiazines of very similar structure also have greatly differing surface activities (22).

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