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REVIEW ARTICLE

Relationships between Surface Activity and Biological Activity of Drugs

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Keyphrases ☐ Surface activity–biological activity relationships—at air/water or monolayer/water interfaces, review ☐ Drug interactions at air/water or monolayer/water interfaces—correlated with biological activity, review ☐ Interfacial drug accumulation—correlation between surface and biological activities, review

A wide variety of biologically active agents have been shown to exhibit the ability to accumulate at an air/water or monolayer/water interface. Furthermore, in a number of instances, a definite correlation between this surface activity and biological effect has been demonstrated. However, while it is tempting to accept such correlations as an indication of the mechanism of action of these agents, this effect often is nonspecific and a measure only of the hydrophobic nature of the molecule. Nevertheless, although this surface activity *per se* is not likely to be solely responsible for a particular pharmacologic or physiologic effect, it does influence availability and probably reactivity of an agent at a site of action. Thus, studies of this property can provide useful information relative to the *in vivo* action of many membrane-active agents. The research articles surveyed in this review, particularly the classic studies of Schulman and Rideal (1), Skou (2–6), and, more recently, Demel and van Deenen (7), clearly demonstrate this fact.

Other membrane model systems, such as the lipid bilayers and spherules, also have served effectively for exploring the tendency of drugs and other biologically active materials to accumulate at an interface in this attempt to gain a better understanding of the behavior of such materials at the surface of cell membranes (8–10).

However, to limit the scope of this review, it has mainly been restricted to studies of drugs and some potentially toxic components of the environment in which a correlation between the interaction at an air/water or monolayer/water interface and biological activity has been developed. Studies of the interaction of simple cations (*e.g.*, Na^+, K^+, and Ca^{+2}), proteins, lipids, and polysaccharides with monolayers have not been included because: (*a*) they normally are not employed as drugs, and (*b*) they constitute such a large body of literature that they could be the subject of a separate review.

LOCAL ANESTHETICS

Excellent correlations have been found between the ability of a number of local anesthetics to block nerve impulse conduction and their interaction with lipid monolayers (11). Skou, in a series of papers (2–6), showed that local anesthetics increased the surface pressure of lipid monolayers, although the area at which film collapse occurred remained unchanged. This indicated that the drug molecules penetrated the lipid monolayer but were expelled at high surface pressures. In the studies using lipids extracted from nerve tissue, the penetration of the monolayers was well correlated with the blocking potency of cocaine, tropocaine, tetracaine, dibucaine, and other local anesthetics (4). Alkyl and aryl alcohol local anesthetics, such as butanol, pentanol, thymol, and β-naphthol, gave similar results (5). Fur-

thermore, both the film penetration and blocking activity of cocaine increased with increasing pH (4), indicating that the uncharged species is the active form. Investigations using other models, however, suggested that both the uncharged and the cationic form of tertiary amine anesthetics may be involved in the total process of nerve conduction blockage (12).

Shanes (13), on the basis of monolayer studies, postulated that local anesthetics block nerve conduction by increasing the lateral pressure within the lipid membrane of nerve cells, with a resultant blocking of the pores through which ions normally move. Bangham (14), however, considered the effect was more likely due to a modification of the compositional lipid mosaic of the membrane.

Comparison of the effects of procaine and veratrum alkaloids on lipid monolayers also correlated with their effects on nerve fiber membranes (15). The latter drugs, which increase membrane permeability to Na^+ and K^+ in contrast to the effect of local anesthetics, reduced the area per molecule of a stearic acid monolayer (16). This apparently was due to dissolution of the lipid–alkaloid complex in the aqueous subphase (15). This effect was antagonized by procaine, Ca^{+2}, and low pH. Gershfeld (17) observed a relationship between the biological effects of veratrine and procaine and their ability to influence the desorption of a monolayer of monooctadecyl phosphate. This latter material slowly desorbs from an air/water interface. At concentrations above 10^{-4} *M*, procaine (a compound which decreases membrane ionic permeability) prevents desorption apparently by adsorbing to the undersurface of the monolayer. Veratrine, which increases membrane ionic permeability, in contrast, increased the rate of film desorption. Cuthbert (9) suggested that the K^+ exchange and repetitive activity seen in nerves treated with veratrine alkaloids and the effect that these alkaloids have on monolayers probably have a common causation.

Sekerba and Vrba[1] developed a relationship between local anesthetic activity and surface tension of 30 diethylaminoethyl esters of substituted carbamic acids. Inactive derivatives, in addition, were found to exhibit little surface activity.

Hersh (18) used monolayers of a synthetic dipalmitoyl lecithin to study the surface interaction of eight drugs with local anesthetic activity. The eight drugs were dibucaine, ephedrine, β-naphthol, procaine, phenyltoloxamine, quinine, tetracaine, and thymol. He observed that the minimum blocking concentration of each of these anesthetics lowered the surface tension of the lecithin/water interface by approximately the same amount. A linear relationship was developed between the log of the rate of change of the surface pressure with concentration and the product of the mole refraction and ionization potential. This relationship was interpreted as an indication of a relationship between the distribution of these drugs between the interface and the subsolution and London interaction energies. Thus, additional support was given to the postulations that the site of activity of local anesthetics is at the cell membrane and that the interaction between nonpolar groups is of primary importance.

PSYCHOTHERAPEUTIC AND SEDATIVE-HYPNOTIC AGENTS

A number of *in vivo* and *in vitro* studies have led to the postulation that the biochemical and pharmacological actions of phenothiazine derivatives and other psychoactive drugs are related to the ability of these compounds to accumulate at biological membranes and, in particular, to modify the permeability characteristics of these membranes (19). This postulation has been supported by studies that relate biological activity of psychoactive drugs with their tendency to accumulate at the air/water or monolayer/water interfaces.

Villalonga *et al.* (20) reported surface activity of promazine, promethazine, diethazine, and imipramine in a 0.1 *N* HCl solution. While no definite relationships were developed in this study between surface activity and clinical potency, differences in the area per molecule at maximal adsorption of promethazine (46 $Å^2$) and promazine (66 $Å^2$) were offered as a basis for their differing pharmacological action. Correlation, however, between surface activity and the clinical potencies of a series of nine "antipsychotic neuroleptic" phenothiazine derivatives and reserpine was demonstrated by Seeman and Bialy (21) (Fig. 1). In contrast, structurally similar drugs, which were categorized as nonneuroleptics (imipramine, trimeprazine, promethazine, and promazine) as a group did not show any correlation with clinical effects. In addition, chlorpromazine sulfoxide, a relatively inactive antipsychotic agent, was in-

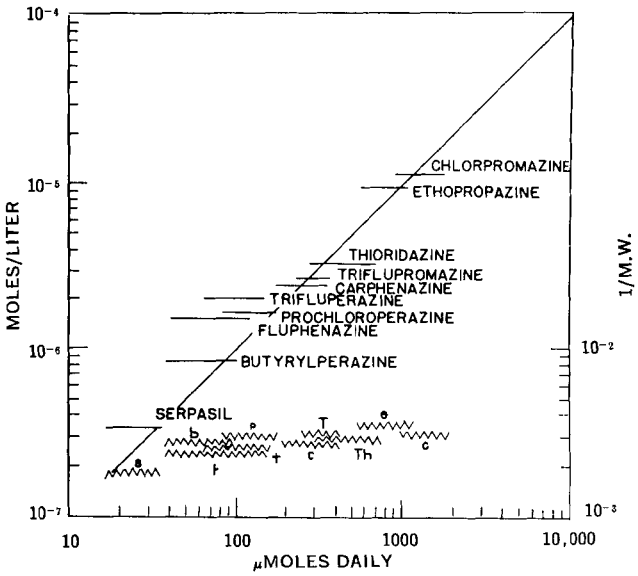


Figure 1—*Demonstrating a correlation between surface activity and clinical activity. The concentration of drug that lowers the surface tension by 5 dynes/cm. is on the ordinate. The abscissa represents the average dose range used to control acute paranoid schizophrenia by one group of physicians on one hospital ward. The solid black bars indicate the surface activity, and the wavy lines represent the inverse of the molecular weight. The straight line is the theoretical line for an exact 1:1 correlation. It is seen that a 100-fold increase in surface activity is approximately associated with a 100-fold decrease in the daily oral dose in micromoles.* [*Reprinted, with permission, from P. M. Seeman and H. S. Baily*, Biochem. Pharmacol., **12**, *1181(1963)*.]

[1] *Added in proof:* A. Sekerba and C. Vrba, *J. Amer. Pharm. Ass., Sci. Ed.*, **49**, 394(1960).

effective in lowering the surface tension of an HCl solution (21).

The interaction of chlorpromazine, chlorpromazine sulfoxide, and trifluoperazine with various lipid monolayers also was found to correlate with biological activity (22, 23). Drug–film interaction was observed in these studies at drug concentrations (10^{-5} *M*) that exhibited no surface activity at the air/water interface. While the data indicated that the drugs penetrated into the lipid film, forming a mixed film, at high surface pressures the drugs appeared to be ejected from the film.

Zografi and Zarenda (24) measured the surface pressure (surface tension of the solvent minus surface tension of the drug solution) of five phenothiazine drugs at the air/water interface under a variety of conditions. At pH 2.0, where the drugs existed essentially only in the salt form, the ability of the various derivatives to increase surface pressure correlated with their relative nonpolarities, with trifluprομazine being the most effective and chlorpromazine the least. This study clearly demonstrated the hydrophobic nature of the phenothiazine drugs and their tendency to transfer from an aqueous to a more nonpolar environment. The addition of phthalate, citrate, and succinate buffers all increased the surface activity of these drugs, while acetate buffer decreased surface activity. From these data, it was postulated that ion-pair formation may play a role in transport to, and accumulation of, these compounds at membrane and other receptor surfaces.

In another study, the effect of a number of inorganic and organic ions on the surface activity of chlorpromazine was investigated (25). Significant decreases in surface activity were observed in the presence of short-chain quaternary ammonium ions and methanesulfonate ion. This effect was attributed to changes in the water structure induced by the alkyl chains of these ions. The addition of bromide, iodide, propanesulfonate, benzenesulfonate, and naphthalenesulfonate ions caused an increase in surface activity beyond what might be expected from ionic strength changes alone. This latter effect was apparently due to interfacial ion-pair formation. Chemical modification of the phenothiazine derivatives also was found to influence behavior at an air/water interface (26). This study was conducted under conditions in which all of the drugs existed essentially as the protonated form in order to eliminate the effect of difference in degree of ionization. Substitution on the phenothiazine ring was found to enhance surface activity in the order of $CF_3 \gg Cl > H$. Changes in the position of the chloro group on the ring significantly influenced surface activity in the order of 3Cl > 2Cl > 1Cl. An increase in the chain length of the alkylamino group on the 10-position resulted in an expected increase in surface activity because of the increase in hydrophobicity of the molecule. The addition of a piperazine ring to the alkylamino group gave a similar effect, while branching of the alkyl group reduced both hydrophobicity and surface activity.

The surface tension of chlorpromazine was found to be markedly reduced in the presence of adenosine triphosphate, apparently as the result of salt formation (27). The author postulated that the *in vivo* action of phenothiazines may, in some situations, involve the formation of such a surface-active complex.

Van Deenen and Demel (28) studied the interaction of orphenadrine hydrochloride, chlorpromazine hydrochloride, and reserpine with monomolecular films of cholesterol, synthetic phosphoglycerides, natural sphingomyelin, and preparations of cerebrosides and gangliosides from beef brain. Orphenadrine exhibited a slight interaction with cholesterol and cerebroside films and a very strong interaction with the ganglioside films. Chlorpromazine and reserpine revealed a similar specificity pattern, with the latter compound showing the most dramatic effect on ganglioside films. (A maximum increase of 30 dynes/cm. was observed.)

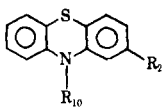
The interaction of additional psychoactive drugs was compared using the ganglioside film. Reserpine gave the greatest effect by far, but interactions to varying degrees were observed for other drugs of differing structural type including the phenothiazines, diphenylmethanes, and benzodiazepines. In addition, meprobamate and sodium pentobarbital were studied and found to give little or no interaction with the ganglioside film.

It was speculated that those psychoactive drugs which interact with gangliosides may act *in vivo* by affecting the transfer or release of acetylcholine from synaptic vesicles known to be rich in gangliosides. An additional study, in which interactions between psychoactive drugs and anionic lipid monolayers were observed, supported the view that a coulombic interaction between cationic drugs and negatively charged lipids may also be involved (29).

Sears and Brandes (30) studied the interaction of four phenothiazine derivatives (10^{-4} *M* concentration) at lecithin and cholesterol monolayers by the use of surface pressure, surface potential, and surface viscosity measurements. These researchers concluded that the phenothiazines acted immediately below the lipid monolayers and that there is no evidence of coplanar orientation of the drugs with the molecules which form the monolayer. These conclusions contrast with the postulation of Zografi and Auslander (23) which pictures the phenothiazines penetrating into the lipid monolayer. The observed changes in area per molecule of the monolayer produced by the phenothiazines were attributed to induced changes in the association of the lipids with the water molecules at the interface (30). It was further postulated that the phenothiazines may act *in vivo* by increasing the intermolecular spacings in membranes and thereby decreasing the binding of Ca^{+2}. Since Ca^{+2} plays an important role in releasing a transmitter from the synaptic vesicles (31), the presence of a phenothiazine at the synaptic vesicle membrane may block the action of Ca^{+2} on the vesicle membrane.

Felmeister and Schaubman (32, 33) studied the effect of UV irradiation on the interaction of a series of phenothiazine drugs (10^{-5} *M*) with dipalmitoyl lecithin monomolecular films. Only the chlorine-substituted phenothiazines, chlorpromazine and prochlorperazine, exhibited an immediate increased interaction with the film following irradiation. In contrast, trifluoperazine exhibited a decrease and trifluprομazine and promazine exhibited no change in reactivity after irradiation (33). Since, of the drugs studied, only chlorpromazine and

Table I[a]—Phototoxic Index (PI)[b] 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
Trifluprom azine	$-(CH_2)_3N(CH_3)_2$	CF_3	0
Prochlorperazine		Cl	50
Trifluoperazine		CF_3	-10^c, 0^d

[a] Reprinted, with permission, from A. Felmeister and R. Schaubman, *J. Pharm. Sci.*, **58**, 64(1969). [b] See text [original article] for definition and method of calculation. [c] Value recorded initially. [d] Value recorded after about 15 min. No further change was noted in this value.

prochlorperazine have been reported to be significantly phototoxic, it was postulated that the ability of UV-irradiated phenothiazine drugs to interact with a lecithin monolayer may be a measure of their *in vivo* membrane-penetrating and phototoxic properties. A "Phototoxic Index" was reported for five phenothiazines (Table I). In another study, similar results were obtained following the irradiation of higher concentrations (10^{-4} *M*) of phenothiazine drugs in the absence of a lipid film (34). The authors suggested that the photoactivated phenothiazines do not produce photosensitized reactions by interacting directly with cellular components but rather through the formation of new, stable, more surface-active compounds. These latter compounds presumably induce a cutaneous reaction *via* changes in membrane permeability.

While correlations were reported between tranquilizing potency and surface activity (21–26), Green (35) showed that when the ring substituent is transferred to a position other than the 2-position, or when the alkyl side chain is altered in length, a marked fall in tranquilizer activity is noted which is not accompanied by a corresponding decrease in surface activity. This failure to discriminate between potent tranquilizers and closely related but much less active compounds casts some doubt on the significance of these activity correlations in terms of being evidence for a particular mechanism of action (35). However, as noted by Domino *et al.* (36), the physicochemical behavior of the various substituted phenothiazine derivatives plays an important role in their CNS activity and must at least influence their transport to a site of action.

Several other classes of CNS-acting drugs have been shown to exhibit surface activity, although generally the relationship between surface activity and biological activity has not been well established. Kuffner *et al.* (37) reported the surface tension of a 0.0025 *M* solution of butylbarbituric acid to be 56 dynes/cm. Lewis (38) reported two short-acting barbiturates, pentobarbital and quinalbarbital, to be surface active, while barbital and phenobarbital, both longer acting compounds, showed little or no surface activity. The ability of codeindioine and oxycodone to lower the surface tension of water was reported by Sliwa (39). These reports led Florence (40) to speculate that the surface activity of centrally acting hypnotics is an important feature of their action.

Abood and his coworkers (41–44) utilized surface pressure, potential, and viscosity to study the nature of the interaction of some psychotomimetic glycolate esters with lipid and lipoprotein monolayers. *N*-Methyl-2-pyrrolidylmethyl cyclopentylphenyl glycolate (10^{-5}–10^{-4} *M*) was found to increase the rigidity of stearic acid and phospholipid monolayers in a manner similar to Ca^{+2}. At higher concentrations (10^{-3} *M*), a reduction in surface pressure was observed indicating interfacial dissolution. It was noted that the concentration range in which this drug acted on the monolayer in the same manner as Ca^{+2} was in the range at which it restored the resting potential of muscle fibers (41). *N*-Methyl-3-piperidyl glycolate was found to exhibit surface activity in water at pH 7.6 at concentrations above 10^{-5} *M*. Condensations of stearic acid films along with increased viscosity were observed with this drug that were similar to those produced by Ca^{+2}, suggesting a similarity in the mode of interaction with the film (42, 43). Glycolate esters were also found to interact with adenosine triphosphate which had been previously interacted with a lipid film (44). The authors concluded that the results of surface film studies give support to the postulation that these drugs affect excitatory membranes by substituting for Ca^{+2} or by interfering with the action of adenosine triphosphate on the membrane. However, they cautioned that since compounds without psychotomimetic activity can produce similar effects on monomolecular films, additional explanations must be sought for the action of agents on the CNS (42).

ANTIBIOTICS AND ANTIBACTERIAL AGENTS

The surface activity of salts of penicillin G was studied more than 20 years ago (45–47), although correlation with biological activity was not established. Hauser and Marlowe (47) reported that aqueous solutions of sodium and potassium penicillin G were highly surface active, but Kumler and Alpen (48) reported only slight decreases in surface tension of water in the presence of these salts. Later work, however, demonstrated that much of the surface activity observed in the early studies (45–47) was due to impurities in the penicillin. Few and Schulman (49), using purified samples of sodium penicillin G, observed very little surface activity except at pH values below 4.1. Marked changes, however, were

observed in the surface pressure and surface potential of lecithin, cephalin, and cardiolipin monolayers in the presence of sodium salts of penicillin G (49). These effects were found to increase as the pH of the penicillin solution was decreased.

Surface properties of cyclic decapeptide antibiotics have been reported (50–52). A relationship between the surface properties of the polymyxins A, B, D, and E spread as monomolecular films at the air/salt solution interface and their nephrotoxic actions was developed (50). Few (51) compared the surface tension at the air/water interface of aqueous solutions of the cyclic decapeptide antibiotics: tyrocidine A, gramicidin SA, and polymyxin E. The latter compound exhibited only slight surface activity over the concentration range of 1×10^{-5} to 5×10^{-5} *M*. In contrast, tyrocidine A showed marked surface activity, exhibiting a 30-dyne decrease in surface tension at 2×10^{-5} *M*. Gramicidin SA was intermediate in its surface activity. These properties were related to the basicity of the decapeptides, but it was concluded that surface activity *per se* was not the major factor in their antibacterial action. It was proposed that an electrostatic interaction between the amino group of the antibiotics and the phosphate groups of the bacterial membrane lipids was involved in the biological activity and selectivity of these antibiotics.

Excellent correlation has been developed between the interaction of a group of polyene antibiotics with lipid monolayers and their ability to produce membrane damage (7, 53). Filipin, etruscomycin, amphotericin B, pimaricin, and nystatin all interacted strongly with cholesterol and only slightly or not at all with lecithin monolayers (Fig. 2). The degree of interaction was in the same order in which these antibiotics caused lysis of erythrocytes (53). The interaction of derivatives of filipin of varying degrees of potency with cholesterol films was also investigated. The results correlated reasonably well with the hemolytic potencies of these derivatives. When mixed films of lecithin and cholesterol were used, a reduction in the film–antibiotic interaction was observed, suggesting that the phospholipid–sterol ratio was a factor in the interaction. It was observed that the antibiotic–lipid ratio was a factor. At high ratios, the interaction was nonspecific and filipin interacted with phospholipids in the absence of cholesterol. This latter effect was consistent with the results of Sessa and Weissman (54). It was also concluded that a free hydroxyl is necessary for the interaction with the polyenes since esterification of cholesterol significantly reduced the extent of interaction while monolayers of cetyl alcohol at low molar ratios of antibiotic–lipid showed considerable interaction. The addition of urea to the subphase also inhibited the sterol–antibiotic interaction, suggesting that hydrogen bonding was an important factor.

Kinsky (10) noted that while monolayer studies confirm the hypothesis that low concentrations of polyenes are selectively toxic for organisms that contain sterol in their cell membrane, they cannot in themselves establish the mechanism by which the polyene–sterol interaction produces membrane damage. Whether the observed increase in film pressure is caused by actual penetration of the antibiotics into the monolayer, or by the accumulation beneath the monolayer with a subsequent spatial reorientation of the sterol molecules, or by both has not been determined.

A relationship between the rate of absorption of tetracycline and the surface tension of isotonic solutions buffered at pH 2.0 by a variety of buffering agents was reported by Perrin and Vallner (55). The surface tension of the solution at constant tetracycline concentration was dependent on the nature of the anionic species of the buffer system apparently through ion-pair formation. However, while absorption of the tetracycline increased as surface activity of the buffered solution decreased, absorption of the ion pair appeared not to be the dominant factor in the absorption process. Rather, increased surface activity led to a higher concentration of the positively charged tetracycline at the solution/membrane interface, which in turn resulted in the increased absorption rate (55).

In an effort to develop "dermophilic prophylactic agents with perdurable efficacy," Quintana *et al.* (56–60) used the monolayer technique to study the interaction of antibiotics and antibacterials with representative dermal constituents. In an initial study, griseofulvin was shown to increase the surface pressure of a stearic acid monolayer at low film pressures. At high pressures, the antibiotic was ejected from the monolayer. In contrast, cholesterol films exhibited a loss of surface pressure in the presence of griseofulvin, suggesting an interfacial

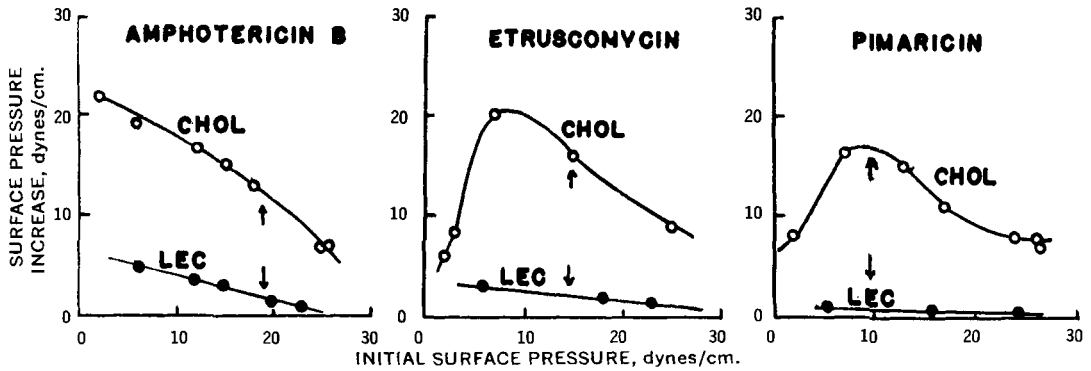


Figure 2—*Interaction of amphotericin B, etruscomycin, and pimaricin with cholesterol and lecithin monolayers. Monolayers were prepared with 0.064 μmole of cholesterol and 0.038 μmole of synthetic lecithin (18:0/18:1 phosphatidyl choline) and compressed to the initial surface pressures shown on the abscissa. The antibiotics (6.38 nmoles) were then injected underneath, and the increase in surface pressure was determined.* [*Reprinted, with permission, from R. A. Demel, F. J. L. Crombag, L. L. M. van Deenen, and S. C. Kinsky*, Biochim. Biophys. Acta, **150**, *1(1968).*]

dissolution effect. The authors indicated that both of these effects could represent an affinity between griseofulvin and the lipid molecules (56). It was suggested that these effects might be related to the postulation that lipids present in the skin are involved in griseofulvin uptake (61).

Two synthetic grisan derivatives were spread alone as monomolecular films and in the presence of stearic acid, lecithin, and cholesterol (57, 58). The surface pressure–surface area isotherms of the pure films of the grisans were interpreted on the basis of their possible orientation at the surface. In the mixed films, generally, the grisan derivatives exhibited interaction at low pressure. At intermediate and high pressure, they were ejected from the film. Nonadditive behavior was observed with both derivatives in the presence of a lecithin monolayer.

Quintana and Owens (59) studied the interaction of three phenolic esters at the air/water and lipid/water interfaces. The effect of structure on monolayer stability was reported. Esters of hexachlorophene (mono- and diundecanate and monostearate) were studied alone or as components of mixed monolayers with a variety of lipids (60). The two monoesters exhibited expanded monomolecular films which collapsed at relatively low pressures ($\approx$8.0 dynes/cm.), indicating little cohesive force. The diester, as might be expected, collapsed at a still lower pressure (2.5 dynes/cm.), indicating the importance of the phenolic —OH group for film stability. Interaction was observed between both monoesters and all the lipid films studied (the diester did not yield stable mixed films). This interaction was related to the reduced antibacterial potency reported when hexachlorophene interacts with skin lipids (62).

Albert *et al.* (63) and Mason (64) determined the surface activity of a group of acridine antibacterials and antimalarials but were unable to establish a correlation with biological activity. Mason (64), however, suggested that the physicochemical properties such as surface activity might explain the order of activities of these compounds.

While a correspondence between surface properties and antibacterial action of numerous cationic surfactants has been observed, surface activity *per se* does not determine bactericidal action. Many anionic and nonionic surfactants with equivalent surface activity are inactive or only weakly active against bacteria.

Schulman and Rideal (65) and Pethica and Schulman (66) postulated a mechanism of action of lytic surface-active agents from data based on interactions with lipid monomolecular films. Long-chain ionic surfactants showed a correlation between penetration into cholesterol monolayers and cell lysis. No such relationship was observed with nonionic surfactants. It was proposed that a critical collapse pressure (34 dynes/cm.) exists for the membranes of erythrocytes (or bacterial cells). Cell lysis could be achieved then by one of two mechanisms:

1. By simple detergency, *i.e.*, when the concentration of the lysin is sufficient to attain a surface pressure of 34 dynes/cm. Nonionic surfactants were assumed to produce cell lysis by this mechanism.

2. By the development of a surface pressure of 34 dynes/cm. by the penetration of the lysin into a cholesterol monomolecular film. This would occur at concentrations of the lysin that would result in surface pressures in the absence of the film of less than the critical collapse pressure. This type of behavior was exhibited by the long-chain ionic surfactants and saponin.

Both of these mechanisms would lead to the collapse of the cholesterol–phospholipid–protein membrane complex and to subsequent cell lysis. Salton (67), however, pointed out that while these mechanisms of lysis are compelling, the integrity of the cell membrane likely involves protein–protein and protein–lipid interactions, and these factors should also be considered in any mechanism describing lytic action.

Later work using models other than monolayers demonstrated that anionic agents in general fit the mechanism postulated by Schulman and Rideal (65) and Pethica and Schulman (66). No correlation, however, was observed between penetration into cholesterol films and lytic activity of cationic surfactants. This was particularly true in the case of the shorter-chain members of a series which retained their lytic activity but showed little tendency to penetrate lipid films. It was suggested that the lytic activity of such compounds may be related to the release of phospholipid from the membrane, while an interaction with cholesterol and protein also may be involved for higher members of a cationic series. The lytic phenomenon in the case of the higher (active) members of an anionic series is likely related to an interaction with both cholesterol and protein (68).

Glazer and Dogan (69, 70) investigated the interaction of cetyltrimethylammonium bromide with monolayers of bovine serum albumin. The degree of interaction increased with increasing pH. This effect was attributed to an increase in the ionization of carboxylic acid groups on the albumin. Substantial binding of the cetyltrimethylammonium bromide was observed also at low pH values ($<$4.0), suggesting that the cetyltrimethylammonium bromide, in addition to reacting with the carboxylate ion as observed at high pH values, was able to replace the proton from the unionized acid. The data were related to the differences in antibacterial activity of cationic and anionic surfactants, the latter being relatively poor germicides, which appear to penetrate bacterial cells to a much lesser degree than cationic agents. It was determined that washing of bacterial cells following exposure to anionic surfactants reversed their inhibitory effect on bacterial growth, while inhibition of growth resulting from exposure to cationic materials could not be reversed by this technique. This finding suggested that a strong ionic bond, as observed in the monolayer studies, was involved in the mechanism of action of the cationic compounds.

Thomas and Clough (71) determined the surface tension of a series of 4-alkyl-1,1′-spirobipiperidinium bromides. While no correlation with biological activity was established, they noted that the surface activity of quaternary ammonium ions results in a higher concentration of these ions at the bacterial "receptors" than in the bulk of the solution.

Weiner *et al.* (72) found that the surface activities of three quaternary ammonium compounds (dodecyltrimethylammonium chloride, dodecyldimethylethylam-

monium chloride, and dodecylpyridinium chloride) were in the same order as their activities against the microorganisms studied. Furthermore, solutions of the different compounds with equal antimicrobial activities against a specific organism all had surface concentrations of the same order of magnitude. This finding was essentially in agreement with the findings of Zissman (73) that solutions of quaternary ammonium compounds having equal antimicrobial activity have surface tensions of the same order of magnitude.

The interactions of a series of bactericidal alkylbenzyldimethylammonium chlorides with monolayers spread at the air/water interface were investigated to elucidate the manner in which these compounds initiate bacteriolysis in Gram-positive and Gram-negative organisms. A monolayer of the protein gliadin was selected to represent the Gram-positive bacterial wall, while a gliadin–cephalin film was used to simulate the Gram-negative wall. Interactions were monitored by measuring changes in surface pressure and surface potential. The data suggest that with the Gram-positive organisms the bactericide first becomes associated with the protein in the cell wall; subsequent reorientation leads to cell wall disruption. In the Gram-negative wall, the phospholipid associated with the protein affords the latter some protection. The addition of protamine into the subphase beneath the monolayer appears to eliminate this protection (74).

Joos and Ruyssen (75) reported an increase in the mean molecular area of mixed monolayers of cholesterol and senegin, a saponin, indicating an interaction. No such interaction was observed between cholesterol and seneginen, a weak hemolytic agent. The authors concluded that this result was further confirmation of the postulation that hemolysis by saponins is due to their reaction with cell membrane cholesterol.

GASEOUS AGENTS

While relatively little work has been reported on the interaction of gases or vapors with monomolecular films, it is clear from an examination of the literature that considerable information can be obtained by such investigations. In an early study, Dean *et al.* (76) demonstrated that the vapors of the anesthetic agents (chloroform, divinyl ether, and diethyl ether) significantly increased the surface pressure (10–30 dynes) of lipid monolayers. In the case of the gaseous anesthetics (nitrous oxide and ethylene), only very slight increases (<2 dynes) were observed. Because of the uncertainty of these data, the authors did not feel justified in extrapolating to concentrations equivalent to actual anesthetic levels.

Clements and Wilson (77) studied the interaction of a series of inert gaseous anesthetics with lipid and lipoprotein monolayers. A systematic relationship was obtained between anesthetic potency and affinity for a lipid film, leading the authors to conclude that these inert gases "interact significantly with the interfacial lipoprotein of living cells." Such a sorption into the cellular interfaces could result in changes in cell permeability, excitability, and metabolic activity. The data also indicated, because of a lack of interaction in the absence of a film, that the interaction does not occur solely with water. A film appears to be necessary either as the site of interaction or to stabilize a water–anesthetic complex of the type postulated by Pauling (78) and Miller (79).

In a later study, Evans *et al.* (80) were unable to observe any interaction between the inert anesthetic gas, halothane, and a lecithin monolayer. However, as noted by Felmeister *et al.* (81), this apparent contradiction of the previous work (77) was the result of differences in procedures and techniques.

Blank (82) used monomolecular films to study the transport of carbon dioxide, oxygen, and other gases and related his data to the transport of these gases through biological membranes.

Mehard *et al.* (83) examined the interaction of ethylene on a variety of unsaturated lipid and protein monomolecular films to help elucidate the plant growth-regulating mechanism of this compound. With the exception of protein-containing films, ethylene caused no significant changes in surface tension. Protein and protein–lipid films showed surface tension decreases of 1.3 and 2.7 dynes/cm., respectively. Comparison with other aliphatic gases showed interactions that appeared to be related to the molecular size of the gases. Because of the lack of specificity of the film–ethylene interaction, the authors concluded that the mechanism of ethylene interaction cannot be explained as a simple physical effect on membranes.

A number of studies have utilized monolayers of lipids and proteins to obtain information relative to the interaction of noxious gaseous air pollutants with all membrane components. Since such materials produce effects that appear to modify membrane structure and function much as drugs do, they have been included in this review.

Cigarette smoke was observed to decrease the surface tension of a monomolecular film of material extracted from lung tissue (84), indicating a smoke component–film interaction. Since lung stability is dependent on surface-tension effects, it was postulated that if a similar interaction occurred *in vivo*, overdistention of the lung alveoli might be the consequence. Ozone was also found to decrease the surface tension of films of lung extracts (85). Inclusion of sulfhydryl-containing compounds in the subphase minimized the effect of ozone, suggesting a protective role for such compounds. Kahana and Arnovitch (86) reported a reduction in the surface tension of lung extracts from rats as the result of exposure to sulfur dioxide, indicating that this pollutant may lead to increased production of lung surfactant.

A series of investigations was reported in which monomolecular films of lipids and proteins were used as a model system to study the interaction of the olefin gases, oxygen, and nitrogen dioxide on cell membrane components (87–98). In these studies, the gases were permitted to flow over the monolayers and the effect on the surface area–surface pressure isotherm was determined. The olefins did not exhibit an interaction with any of the phospholipid films studied, while nitrogen dioxide caused an increase in film pressure of the unsaturated phospholipids (egg lecithin and animal cephalin) but not of the dipalmitoyl lecithin, a saturated phospholipid (87–89). It was concluded that a

chemical interaction between the nitrogen dioxide and the double bonds of the unsaturated phospholipids led to the observed increase in pressure. *In vivo* studies of the interaction of nitrogen dioxide with unsaturated fatty acids (99, 100) appear to support this contention. The monolayer data also led to the postulation that nitro compounds might form as a result of the nitrogen dioxide–unsaturated fatty acid interaction (87). The work of Estefan *et al.* (101) supports this conclusion.

The fact that the olefins (ethylene and *trans*-2-butene) did not exhibit any significant interaction with the phospholipid or lipid–protein films (87, 89, 90) suggested that these gases do not interact with these particular cell membrane components. *In vivo* studies gave corresponding results. Low molecular weight olefin gases were shown to have no effect on the growth of microorganisms, while nitrogen dioxide inhibited their growth (102).

Films of bovine albumin, in contrast to the phospholipid films, exhibited a decrease in pressure on exposure to nitrogen dioxide (90), indicating either a loss of the protein through desorption or a contraction as the result of an increased protein–protein interaction (*e.g.*, cross-linking). This latter effect was observed when the protein from lung tissue was exposed to oxidizing pollutant gases (103). When phospholipids were interacted with the albumin monolayer, the protein appeared to be protected against attack by the nitrogen dioxide, and the lipid–protein film assumed the characteristics only of the lipid fraction (90).

Monolayers of phospholipids extracted from *Escherichia coli* grown at 15 and 37° exhibited behavior toward nitrogen dioxide that was indicative of differences in degree of unsaturation, leading to the conclusion that changes in environmental conditions may affect the degree of interaction of pollutants such as nitrogen dioxide with cell membranes (91).

Monomolecular films of cholesterol were found to be extremely sensitive to nitrogen dioxide. These films, on exposure to this pollutant gas, showed a significant loss of pressure (Fig. 3), which was attributed to a desorption of the cholesterol from the interface (92). A relationship between the production of pulmonary edema and vascular congestion and the rate of loss of cholesterol from the interface as a function of nitrogen dioxide concentration was proposed. The addition of dipalmitoyl lecithin and egg lecithin to the cholesterol film inhibited the nitrogen dioxide-induced loss of cholesterol (93). Furthermore, the data indicated that the sensitivity of cell membranes to nitrogen dioxide may be dependent on the membrane cholesterol–phospholipid ratio and the degree of unsaturation of the latter. Kamel *et al.* (94) compared the interaction of nitrogen dioxide with monolayers of cholesterol with that of monolayers of dihydrocholesterol and cholesteryl acetate to determine the influence of the 3-hydroxy group and the 5,6-double bond on this reaction. They concluded that different, and relatively independent, reactions occur at these two reactive sites of cholesterol, which lead to products with differing surface properties. The products that result from the reaction of nitrogen dioxide with the 3-hydroxy group are desorbed from the interface. The reaction involving the 5,6-double bond

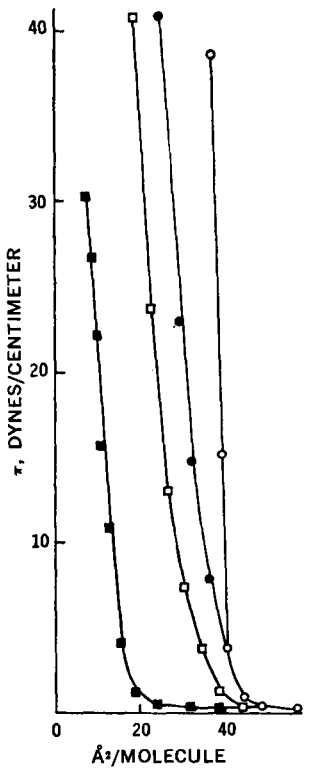


Figure 3—π–A *curves of cholesterol films exposed to 175 ± 25 p.p.m. of* NO_2 *at an initial surface pressure of 0 dyne/cm. for various time periods. Key:* ○, *control;* ●, *20 min.;* □, *35 min.; and* ■, *60 and 90 min.* [*Reprinted, with permission, from A. M. Kamel, A. Felmeister, and N. D. Weiner,* J. Pharm. Sci., **59**, *1807(1970).*]

leads to products that remain at the surface and that occupy a larger area per molecule than cholesterol.

In later studies, it was established that the formation of cholesteryl nitrate, which apparently involves the reaction of nitrogen dioxide and the 3-hydroxyl group, was responsible for the observed loss in pressure of cholesterol monolayers (95). Simultaneous air oxidation occurred, although at a slower rate than the esterification reaction. Films of steroids that did not have a 3-hydroxy group, *e.g.*, 5-cholesten-3-one, did not undergo esterification and showed an increase in pressure on exposure to nitrogen dioxide indicative of oxidation (95).

Cholesteryl nitrate could not be spread as a monolayer; when spread in the presence of cholesterol, it desorbed rapidly from the interface, forming a separate solid phase (95) similar to that observed by Snart (104) with polycyclic hydrocarbons. It was postulated that should such a reaction occur *in vivo*, marked changes in cell membrane permeability would be expected with subsequent consequences (95). Cholesteryl nitrate was found to inhibit its own formation at a cholesteryl nitrate–cholesterol ratio of 3:1, apparently *via* the formation of a "probability" complex with the unreacted cholesterol (96).

Exposure of cholesterol monolayers to air for 45 min. or longer resulted in an increase in surface pressure (97). This effect was determined to be the result of autoxidation of the cholesterol. It was concluded, however, that in the presence of other lipids normally found in cell membranes, oxidation of cholesterol would not likely

alter significantly the permeability characteristics of a biological membrane (98).

HORMONES

Since the proposal by Willmer (105) that there may be a correlation between physiological action of steroid hormones and their packing into a lipoprotein membrane, a number of studies have dealt with the interaction of hormones with monomolecular films. Gershfeld and Heftmann (106) were able to demonstrate only weak interactions between condensed stearic acid and cholesterol monolayers and various steroid hormones. Progesterone was reported to penetrate monolayers of cholesterol and dipalmitoyl lecithin in amounts of 1–5% of the total membrane lipid (107). The orientation of the hormone with respect to the monolayer, however, could not be determined; while the results were not inconsistent with Willmer's hypothesis of hormone action (105), they did not constitute a significant test of this hypothesis.

Pak and Gershfeld (108) studied the interaction of four biologically active steroids with a stearyl alcohol monomolecular film. At concentrations of less than 5×10^{-6} *M*, no film–steroid interaction was observed. An increase in film area was observed at initial pressures of less than 12 dynes/cm. when the concentration was increased to 2×10^{-5} *M*. The influence of these steroids on the viscosity of the water just below the film was determined at film pressures >12 dynes/cm. in order to eliminate the effect of film penetration. Desoxycorticosterone and aldosterone were found to reduce the viscosity of this water layer much more in the presence of K^+ than of Na^+, whereas the effects of androsterone and etiocholanolone on viscosity were not influenced by these ions. The authors postulated that the ability to distinguish between K^+ and Na^+ by one pair of steroids and not the other indicates a difference in their mode of interaction with water.

Cadenhead and Phillips (109) examined monomolecular films of some estrogens and other naturally occurring polycyclic compounds. Their work in general confirmed earlier work reported by Adam *et al.* (110) that these molecules essentially lie flat on the surface at low pressures if the hydrophilic groups are sufficiently separated and at higher pressures they lift up from the surface and orient perpendicular to the surface. No correlation with biological activity was offered in this study.

The effects of three steroids, desoxycorticosterone, androsterone, and aldosterone (4×10^{-6} *M*), on the rate of aggregation (polymerization) of monolayers of monooctadecyl phosphate were studied by the measurement of surface viscosity (111). The steroids all caused a marked increase (30–40 times) in the rate of increase of surface viscosity, indicating that the steroids accelerate the formation of aggregates in the film. It was postulated that the steroids either form crosslinks *via* an interaction with the phosphate groups or alter the monolayer configuration so that formation of intermolecular hydrogen bonds among the phosphate groups is favored. These effects suggested that steroids may affect physiological responses by inducing structural changes in cellular systems (111).

Snart and Sanyal (112) investigated the interaction between lipid monolayers and three polypeptide hormones (oxytocin, 8-arginine-vasotocin, and 1-asparagine-5-valine-angiotensin II) injected into the subphase. All of these polypeptides interacted with the lipid monolayers, as evidenced by increases in surface pressure. It was assumed that the polar groups of the lipid associate with the peptide linkages of the proteins, leading to an arrangement that would create "pores" to facilitate the movement of water and other small molecules. This postulation is consistent with the biological effects reported for these compounds. The presence of urea in the subphase increased adsorption of the polypeptides but did not help distinguish between the different hormones.

Intact insulin in low concentrations was shown to influence the interaction of Ca^{+2} with a monooctadecyl phosphate monolayer (113); it inhibited the uptake of Ca^{+2} by the monolayer and facilitated the release of Ca^{+2} already adsorbed on the monolayer. Evidence was presented that insulin competes with Ca^{+2} for the free phosphate groups of the monolayer. Albumin showed a slight inhibitory action on Ca^{+2} uptake. Parathyroid hormone did not influence either the uptake or release of Ca^{+2} by the monolayer.

The effect of insulin analogs (vasopressin, oxytocin, thyrocalcitonin, adrenocorticotropin, and adenosine 3′,5′-monophosphate) on the uptake of Ca^{+2} by monolayers of monooctadecyl phosphate was also studied (114). Inhibition was observed but, in all cases, was less than that observed with intact insulin. Facilitation of the release of previously adsorbed Ca^{+2} was only observed in the presence of vasopressin and the intact insulin. The authors suggested that inhibition of Ca^{+2} uptake at the monooctadecyl phosphate monolayer by a peptide hormone may serve as a model for the inhibition of Ca^{+2} adsorption at the outer surface of the plasma membrane of cells. Since Ca^{+2} is known to reduce the permeability of some cells to a variety of substances, peptide hormones that diminish Ca^{+2} adsorption might thereby increase permeability of sensitive cells to water and water-soluble substances. For example, the antidiuretic action of vasopressin could be explained on the basis of this monolayer work. The presence of Ca^{+2} at the plasma membrane is assumed to impede the reabsorption of water in the renal tubules. Vasopressin might, as was observed with the monolayer, both inhibit Ca^{+2} adsorption and facilitate the release of previously bound Ca^{+2} at the plasma membrane of renal tubule cells, thereby increasing water reabsorption. Thorn and Schwartz (115) found that a release of Ca^{+2} accompanies both the antidiuretic action of vasopressin in the mammalian kidney and its action which leads to an increased permeability to water by the toad bladder. The inhibitory action of thyrocalcitonin on Ca^{+2} uptake by the monolayer was contrasted with the lack of effect by parathyroid hormone (113). A relationship was suggested between the opposing *in vivo* effects of these two compounds on bone resorption and the Ca^{+2} concentration in extracellular fluids.

FAT-SOLUBLE VITAMINS

While the role of vitamin A in the formation of retinol pigments is known, its other biological functions remain

obscure, although it is well established that its presence is required for normal cell growth. Some evidence suggests that this vitamin, as well as other fat-soluble vitamins, do regulate membrane structure and permeability through their ability to act at a water/lipid interface (116).

Bangham *et al.* (117) demonstrated the ability of vitamin A to penetrate lecithin–cholesterol monomolecular films. A greater interaction was observed between vitamin A and lecithin films than cholesterol films, suggesting that the interaction of this vitamin and its hemolytic effect on red blood cells are due to a vitamin A–lecithin interaction. It was further postulated that membrane penetration may be the initial step in some biological actions of this vitamin.

Dreher *et al.* (118) noted that the interaction of all-*trans*-retinol and *d*-α-tocopherol with egg lecithin monolayers protected the egg lecithin against oxidative attack by OsO_4 present in the subphase. This finding suggested that the presence of these compounds in the cell membranes may provide protection against oxidation of unsaturated fatty acids and also may play a role in regulating membrane stability.

Studies were conducted on the surface properties of mixed monolayers containing retinol, dipalmitoyl lecithin, and egg lecithin in an attempt to define the role of retinol in the lipid–lipid associations in membranes (119). It was observed that the polarity of the retinol hydroxyl group resulted in an ion–dipole interaction with the phospholipids in the monolayers which did not occur with the hydroxyl group of cholesterol. The authors proposed that the combination of retinol with other lipids in membranes might enhance the polarity of certain areas of the membranes, thereby increasing the possibility of lipid–protein interactions. Moreover, such areas also may be of particular importance in membrane transport processes as well as in membrane structure and stability.

Bonting (120) and Bonting and Bangham (121) used monolayers of rhodopsin at an air/water interface as a model for the structure of the rod-sac membrane. Illumination of the film caused a small increase in film pressure. This effect was believed due to the penetration into the monolayer of retinaldehyde liberated during the photolysis of rhodopsin. However, when retinaldehyde was added to the subphase and allowed to penetrate into rhodopsin and phospholipid monomolecular films, it was noted that the increase in pressure caused by this penetration decreased as the initial film pressure was increased. At high pressures close to film collapse (40 dynes/cm.), lecithin–cholesterol films were impenetrable to retinaldehyde, while a residual penetration occurred in films of rhodopsin and cephalin (phosphatidylethanolamine). It was postulated that the residual penetration was due to a Schiff base formation between the aldehyde group of retinaldehyde and the amino group of the phospholipid cephalin. The absence of an amino group in lecithin and cholesterol explains why no residual penetration was observed with monolayers of these lipids. A further test of this hypothesis was reported by DePont *et al.* (122) by a comparison of the penetration of retinol and retinaldehyde into phospholipid monolayers. While a much higher pressure was observed when retinaldehyde was allowed to penetrate into cephalin films than into lecithin films, no such difference was observed with all-*trans*-retinol. Furthermore, the higher pressure produced by retinaldehyde with the cephalin monolayer occurred only when the pH of the subphase was sufficiently high (>7) for the amino group to exist in the nonprotonated form, *i.e.*, conditions under which a Schiff base will form. This increase in pressure also was accompanied by a shift in the absorption spectrum of the film to shorter wavelengths, a further indication of Schiff base formation. The monolayer studies were advanced as evidence in support of the hypothesis that Schiff base formation may be involved in the mechanism of visual excitation.

Gaines *et al.* (123, 124) reported the monolayer characteristics of several biologically important quinones (vitamin K_1, ubiquinone 6, and α-tocopherolquinone).

MISCELLANEOUS COMPOUNDS

Chemical Carcinogens—Clowes *et al.* (125, 126) studied the interaction of a variety of polycyclic hydrocarbons with monomolecular films of sterols. Most of the hydrocarbons, in spite of their inability to spread on water, displayed sufficient reactivity toward the sterol molecules to form stable mixed films. The hydrocarbon–film interactions were interpreted in terms of either a two-dimensional solution or an association complex consisting of one hydrocarbon molecule and two appropriately oriented sterol molecules. It was suggested that this type of interaction may have significance in the *in vivo* transport of hydrocarbons and in the modification of biological structures.

Snart (104) described the interaction of six polycyclic hydrocarbons with cholesterol and lecithin monolayers. Association between the hydrocarbons and the lipid films was observed to a limited extent, after which additional hydrocarbon did not contribute to the area of the film but formed an excess solid phase. In the case of lecithin, up to a 3:1 molecular ratio of hydrocarbon–lipid could be obtained and up to a 1:1 ratio could be obtained in the case of cholesterol, after which additional hydrocarbon could not be retained in the film. The data indicated that the interaction of polycyclic hydrocarbons with cell membranes and their biological effects may be dependent on the membrane lipid composition.

The interaction of a carcinogenic hydrocarbon, 3-methylcholanthrene, with monolayers of various mole fractions of cholesterol and lecithin was reported by Weiner *et al.* (127). The extent of interaction between the hydrocarbon and the cholesterol in the film was found to be influenced by the competitive interaction between cholesterol and lecithin. At a 50:50 cholesterol–lecithin molar ratio, where the lipids interact to the greatest extent, the 3-methylcholanthrene interaction was the weakest. This competitive interaction was offered as: (*a*) an explanation for the observation that phospholipids retard, whereas cholesterol promotes, the formation and growth of tumors when injected simultaneously with a chemical carcinogen (128); and (*b*) support for the postulation that the phospholipid–cholesterol ratio influences interactions of hydrocarbon carcinogens with cells (129).

Acetylcholine—Hyono and Kuriyma (130) observed a plateau in the surface pressure–surface area curves when egg lecithin was spread on a subphase containing acetylcholine (1 m*M*). This plateau occurred at about 2 dynes/cm. and at molecular areas of the lecithin greater than 100 Å^2. Blank and Essandoh (131), however, were unable to reproduce these results, possibly because of differences in the source of the lecithins or in the selection of the spreading solvents. They did note changes though in the surface potential of lecithin monolayers in the presence of 0.1 *M* acetylcholine or 0.01 *M* acetylcholine plus 0.1 *M* Na^+. This change in potential was also observed when NH_4^+ or $(CH_3)_4N^+$ (but not K^+) was added to the subphase, implying a rather nonspecific type of interaction between fairly large positive ions and the monolayer. Thus, it appears that depolarizing agents can affect the interfacial charge distribution or potential without altering the packing of the monolayer. Such changes in phase boundary potentials may influence the distribution and flow of other charged species and thus may be involved indirectly in the mechanism of action of many substances on natural membranes (131).

Concentrations of 2 m*M* of acetylcholine were also reported to influence the surface potential, but not the surface pressure, of egg lecithin films (132). Houri (133, 134), using higher concentrations of acetylcholine (0.1 and 0.25 *M*), observed significant increases in the surface pressure of both egg lecithin and dipalmitoyl lecithin. However, as Cuthbert (9) pointed out, these monolayer studies in themselves cannot be taken as being of biological significance without at least a comparison between inactive isomers.

Antihistamines—Bangham *et al.* (135) utilized both surface-tension-lowering properties at the air/water interface and the effect on surface pressure of lecithin–cholesterol monolayers to study a wide variety of compounds known to prevent liver necrosis in rats from carbon tetrachloride poisoning. These compounds, including quaternary ammonium salts, local anesthetics, and the antihistamines diphenhydramine hydrochloride, promazine hydrochloride, and promethazine hydrochloride, do not seem to prevent the primary attack of the carbon tetrachloride on the cells but rather appear to prevent loss of intracellular potassium and soluble proteins (*i.e.*, inhibit permeability changes). A correlation between protective activity of a given compound and its interaction at both the air/water and lipid/water interfaces was observed. However, while the three quaternary ammonium compounds studied were very effective in inhibiting leakage of β-glucuronidase from hepatic cells, anionic compounds of equal surface activity were ineffective. Thus, it appeared that both surface affinity and a plus-charged group were essential prerequisites for protective action.

Additional Compounds—The adsorption of alkyl betaines was studied at the air/water interface (136). The standard free energies of adsorption were calculated and resolved into separate contributions from the polar head group and the methylene groups in the alkyl chain. Evans and Pilpel (137) studied the effects of pH and temperature on insoluble monolayers of two alkyl betaines. Enthalpies and entropies of spreading were reported. It was noted that drug adsorption at the membrane surface may be a factor in biological activity and that such effects require a knowledge of molecular adsorption at an interface (136); however, no specific correlations with biological activity were developed.

Good correlation was reported between the surface activity of solutions of dextromethorphan in different buffer systems and the *in vitro* rate of absorption of the drug (138). The surface tension of the solutions was found to be dependent on the nature of the anionic species of the buffer system. The ability of the anion to lower the surface tension was in the order of: chloride < trichloroacetate < nitrate < perfluoropropionate. However, while ion-pair formation appeared to influence surface activity and absorption rate, the drug apparently was not absorbed as the lipid-soluble ion pair. Instead, the data indicated that the protonated dextromethorphan is the surface-active species and that the reduction in surface tension of the solution leads to an accumulation of the dextromethorphan at the interface (membrane). This local increase in drug concentration results in the observed increase in rate of absorption.

The surface pressure–surface area properties of di- and monoundecanoyloxy derivatives of hydroxyacetone alone and with stearic acid monolayers were reported in connection with studies designed to develop long-lasting substantive insect repellents (139).

Surface-active materials have been shown to influence enzyme activity. Quintana (140) reported a correlation between the surface tension of aqueous solutions of a series of mono[3-(*N*,*N*′-diethylcarbamoyl)piperidino] alkanes and their cholinesterase inhibitory properties. The cholinesterase activity of a homologous series of surface-active trimethylphenylalkylammonium salts, however, could not be explained on their surface-tension-lowering properties alone (141). Cholinesterase activity was found to be inhibited in direct relationship with increasing concentration of the surfactants sodium cholate and sodium lauryl sulfate, apparently because of a competitive adsorption effect (142). Fluorophosphate anticholinesterases in concentrations of 1% were found to reduce the surface tension of water by as much as 23 dynes/cm., depending on the size of the nonpolar portion of the molecule (143).

The surface activity of urea was determined at an air/water interface and a stearic acid monolayer/water interface and found to be dependent on the nature of the interface (144). Urea was desorbed from the air/water interface and adsorbed at the stearic acid/water interface. This latter effect, evidenced by a slight expansion of the monolayer, apparently was the result of an interaction of urea with the —COOH groups, or with water, or with both. This study led the author to speculate that the mechanism by which urea causes lysis of erythrocytes involves: (*a*) penetration into the interfacial region of the cell membrane, (*b*) expansion of the membrane and separation of neighboring phospholipids sufficiently to alter their association with Ca^{+2}, and (*c*) release of Ca^{+2} from the membrane and subsequent changes in permeability to ions and water (144).

Smith *et al.* (145) were unable to obtain a positive relationship between the antibacterial activity of a series of seven synthetic azasteroids and their surface-tension-lowering properties. However, since such a relationship has been reported with quaternary ammonium antibacterial agents, it was postulated that these steroids act by a different mechanism.

Davies (146), in support of his "penetration and puncturing" theory of odor, reported a correlation between the rates of desorption of a group of volatile organic compounds from the air/water interface into the air and the intensity of their musklike odor. All strong musks had desorption rates in the range of 0.4–1.7 dynes cm.$^{-1}$/30 sec. This apparently represents a necessary condition for their odor to be musky. However, this range of desorption rates is not a sufficient condition for the musk odor.

CONCLUSION

Studies of the interaction of drug and other agents at the air/water or monolayer/water interface have provided data that contribute to a better understanding of the site of action and mechanism of interaction of such components at biological membranes. In many instances, a remarkable correlation between surface activity and biological function was observed. Furthermore, investigations of the interaction of physiologically important endogenous materials with monolayers of lipids and proteins have provided information on the biological function of these materials. While, as noted previously, these latter studies have not been included in this review, a brief consideration of a few representative studies affords a more complete view of the potential of the monolayer model.

For example, investigations of the interaction of Na^+, K^+, Li^+, Ca^{+2}, and Mg^{+2} with a variety of lipid monolayers have provided interesting information related to the effect of these cations on membrane structure, permeability, and transport (147–150).

Protein–lipid monolayer interactions also have received considerable attention in attempts to establish the lipoprotein membrane structure and function. Colacicco (151) and Arnold and Pak (152) reviewed some of these studies. A most interesting aspect of protein–lipid interactions in monomolecular films was recently reported by Romeo *et al.* (153); a functioning galactosyl transferase system was formed in a mixed monolayer of lipopolysaccharide, cephalin, and protein (enzyme).

Thus, these models offer a relatively simple, easily modified system of known composition by which an almost unlimited number of interactions can be easily and rapidly studied. But, as noted by Schulman (154), "these systems are not models of biological processes, but a means of studying molecular function which must apply to processes in biology wherever these molecules are found in cell structures or interfaces." An understanding of the interfacial behavior of these membrane molecules and their interaction with ions, water, and other agents must then be considered a prerequisite to an understanding of the membrane itself. Progress toward the attainment of this goal certainly has been provided by the studies reported here.

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RESEARCH ARTICLES

Elimination of 4-*n*-Butoxyphenylacethydroxamic Acid (Bufexamac) in Man

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Abstract □ A GLC determination showed that about 80% of a dose of bufexamac (125–500 mg.) can be recovered from urine after acid hydrolysis as 4-*n*-butoxyphenylacetic acid. Excretion is apparently complete within 24 hr. Not more than 1% of the dose is excreted as free bufexamac or 4-butoxyphenylacetic acid. Enzymic hydrolysis indicated that about 75% of the dose is excreted with the hydroxamic function intact and that the elimination of bufexamac is mainly by conjugation, probably with glucuronic acid. About 6% of the dose was recovered from urine as 4-butoxyphenylacetic acid glucuronide. Bufexamac is fairly rapidly absorbed and eliminated, the peak rate of excretion of total 4-butoxyphenylacetic acid occurring 3–6 hr. after dosage. 4-Butoxyphenylacetic acid glucuronide is less rapidly excreted, and the ratio of bufexamac conjugate to 4-butoxyphenylacetic acid glucuronide decreases steadily with time after dosage. The rate of excretion of total 4-butoxyphenylacetic acid could not be interpreted as log-linear during the period (16 hr.) of the kinetic studies.

Keyphrases □ 4-*n*-Butoxyphenylacethydroxamic acid (bufexamac) —absorption, metabolism, urinary excretion rates, man □ Bufexamac—absorption, metabolism, urinary excretion rates, man □ Excretion rates, urinary—4-*n*-butoxyphenylacethydroxamic acid (bufexamac), man

4-*n*-Butoxyphenylacethydroxamic acid (bufexamac)[1] is an anti-inflammatory drug which has been used clinically in doses up to 2.0 g. daily (1, 2). The metabolism of the ^{14}C-labeled compound has been studied in both animals and man (3, 4). In man, about 80% of the dose was recovered in the urine as radioactive material within 72 hr. From results obtained using ion-exchange chromatography, Roncucci *et al.* (5) concluded that the hydroxamic function was largely degraded *in vivo*, leading possibly to the formation of the corresponding amide (4-butoxyphenylacetamide) or carboxylic acid (4-butoxyphenylacetic acid).

The present report describes studies undertaken to gain further information on the absorption and metabolism of bufexamac in man.

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