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Influence of Induced Dipoles, Metal Ions, and Cholesterol on the Characteristics of Phospholipid Monolayers

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The interaction of metal ions and cholesterol with various phospholipid monolayers was studied by measuring the surface pressure, potential, and rheology of mixed monolayers. The concept of intermolecular cavities has been proposed to explain the apparent condensation of mixed monolayers and the structure of liquid-expanded monolayers. The unsaturation of fatty acyl chains strikingly influences the ionic structure, binding with metal ions, association with cholesterol, and enzymic hydrolysis of lecithin monolayers. It is possible to distinguish various types of interactions—ion-ion, ion-dipole, hydrocarbon-hydrocarbon—or their combinations by measurement of surface pressure, potential, and viscosity of mixed films. The surface rheology of cholesterol-phospholipid monolayers suggests that cholesterol may act as a biological plasticizer, increasing the distensibility of the membrane.

Phospholipids, cholesterol, and metal ions are important components of the cell membrane, which is generally believed to be composed of a bimolecular lipid leaflet or, alternatively, of lipoprotein subunits (*4*). Phospholipid monolayers afford a physical system suitable for studying lipid-lipid, lipid-protein, and lipid-metal ion interactions that may be analogous to those occurring at the cell surface. Using this approach, previous workers (*1, 2, 3, 7, 9, 10, 12, 13, 14, 15, 16, 20, 23, 26, 27, 28, 34,*

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35, 36, 39, 40, 41, 42, 43, 44, 45, 48, 50, 54, 55) have obtained valuable information about physicochemical aspects of reactions occurring at a lipid–water interface. Similar studies in our laboratory showed that the unsaturation of fatty acyl chains strikingly influences the ionic structure (43), binding of metal ions (41), association with cholesterol (42), and enzymic hydrolysis of pure lecithin monolayers (40). This paper presents our studies on various phospholipid monolayers employing surface pressure and surface potential techniques.

Methods

The method of measuring surface pressure by a modified Wilhelmy plate and surface potential by an ionizing air electrode has been described (41). For surface potential measurements, the electrometer was calibrated with stearic acid monolayers on 0.01*N* HCl, for which a value of 395 to 400 mv. at 21 sq. A. per molecule is assumed to be standard (17).

Results and Discussion

Induced Dipoles in Phosphatidal Choline and Sphingomyelin. Hughes and Rideal (25) showed from surface potential measurements that a dipole is induced in a double bond if it is situated in the α-position but not if it is further away. In phosphatidal choline (plasmalogen), an induced dipole in the vinyl ether linkage strikingly reduces the surface dipole of the molecule and hence the surface potential (41). In contrast, the presence of a double bond in the 4-5 carbon position of sphingomyelin increases the surface dipole and hence the surface potential of the monolayer (44). The known difference between the surface dipole of lecithin and plasmalogen in relation to the observation that beef-heart lecithin (containing about 40% plasmalogen) is unable to form stable bilayers (24) suggests that the induced dipole may influence the stability and organization of lipid bilayers.

Phospholipid Monolayers

Lecithin Monolayers. We have shown from surface pressure–area and surface potential–area curves of various lecithins that the molecular area increases and the interaction with metal ions decreases with increasing unsaturation of the fatty acyl chains (41, 43).

On the basis of the interaction of metal ions and ΔV-pH and ΔV-log C plots (41, 43), we propose ionic structures for dioleoyl, egg, and dipalmitoyl lecithin monolayers represented in Figure 1. Schematically shown in Figure 1A is the internal salt linkage between the phosphate and trimethylammonium groups in dioleoyl lecithin, preventing the inter-

action of metal ions with the phosphate group. Increasing saturation of the fatty acyl chains progressively decreases the intermolecular spacing which weakens the internal salt linkage (because of increase in ionic repulsion between similar charges), and thereby increases the binding of calcium.

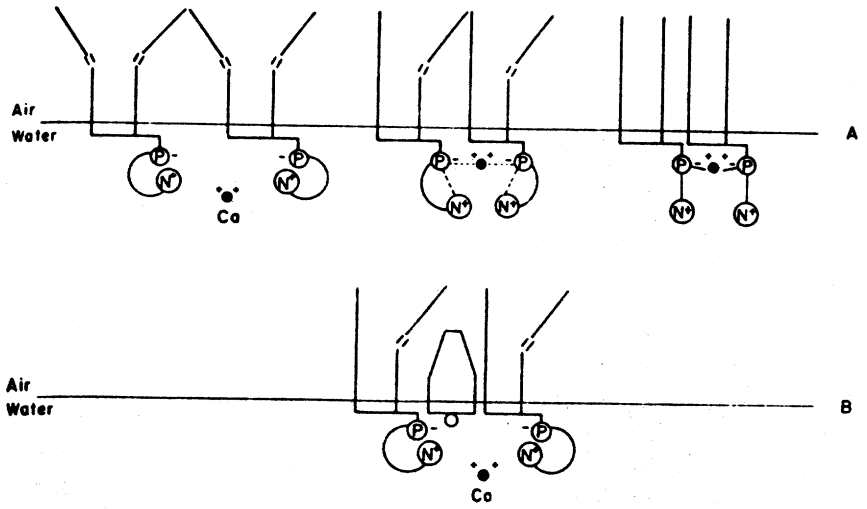


Figure 1. Schematic of interaction of calcium ion with dioleoyl, egg, and dipalmitoyl lecithins, and of egg lecithin–cholesterol monolayers

A: Dioleoyl lecithin shows internal salt linkage between phosphate and trimethylammonium groups. Broken lines in egg lecithin diagram represents weak interactions of phosphate with Ca^{++} and trimethylammonium group. Solid line between Ca^{++} and phosphate group in dipalmitoyl lecithin diagram represents strong interaction

B: Egg lecithin-cholesterol monolayers. Increased spacing between phosphate groups results in strong internal salt linkage preventing binding of Ca^{++}

We have shown (*42*) that monolayers of egg lecithin interact with Ca^{++}, whereas those of egg lecithin–cholesterol do not. A comparison of Figure 1B with the middle diagram of Figure 1A illustrates the explanation of this effect. In egg lecithin, the phosphate group interacts with a calcium ion and also with the adjacent trimethylammonium group (Figure 1A). However, the presence of cholesterol causes (statistically) a small net increase in the spacing between phosphate groups, which reduces the ionic repulsion between the polar groups, thereby strengthening the internal salt linkage (Figure 1B). Consequently, mixed monolayers of egg lecithin–cholesterol do not bind Ca^{++}. Figure 1 (A, B) is a static representation of average kinetic states of molecules at the interface and is meant to illustrate the increase in effective molecular area with increase in unsaturation of fatty acyl chains.

Phosphatidic Acid Monolayers. Phosphatidic acid, prepared from egg lecithin by the action of phospholipase D, forms considerably more expanded monolayers than egg lecithin, presumably because of ionic repulsion between the phosphate groups in the phosphatidic acid monolayers (*42*). Phosphatidic acid monolayers showed about four times more increase in surface potential when $CaCl_2$ is substituted for NaCl in the subsolution than did egg lecithin monolayers (*43*). This again supports the conclusion that the trimethylammonium group competes with Ca^{2+} for the anionic phosphate group in egg lecithin monolayers (Figure 1A).

Cardiolipin Monolayers. Among various phospholipids studied by monolayer techniques, only cardiolipin (*41*) and phosphatidylserine (*36*) monolayers show significant condensation of their surface pressure–area curves in the presence of divalent as compared with monovalent cations in the subsolution. The condensation of cardiolipin is explained by the decrease in molecular area caused by the attraction between a divalent cation and the two phosphate groups in the molecule. This condensation is eliminated when the ratio of monovalent to divalent cations is greater than 5 to 1. At high surface pressures, the difference in the compressibility of cardiolipin monolayers correlates with the ionic radii of the metal ions ($Mg^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+}$).

Sphingomyelin Monolayers. The surface pressure–area curve of sphingomyelin is similar to that of dipalmitoyl lecithin in that both have approximately the same limiting areas (42 to 44 sq. A. per molecule). However, the interaction of metal ions with sphingomyelin monolayers is considerably smaller, as shown by the increase in the surface potential (*44*). The ΔV-log C plot of sphingomyelin suggests that the monolayer has a net positive surface charge (*39*). Thus, the weak interaction with metal ions and the positive surface charge of sphingomyelin can be explained satisfactorily by an ion-dipole association between the hydroxyl group and ionic oxygen of the phosphate group of sphingomyelin (Figure 2). This association in sphingomyelin molecules reduces the unit negative charge of the oxygen of phosphate group to a partial ionic charge, $\delta-$, which consequently decreases the interaction of the phosphate group with metal ions and the trimethylammonium group, leaving a net positive surface charge.

Position of Metal Ions in Monolayer Lattice. The surface potential is an important parameter for studying the ionic structure of monolayers, including the position of metal ions in the monolayer lattice. The interaction of cations with anionic groups in a monolayer results in a formation of ionic dipoles which influence the surface potential. If the polarity of the ionic dipole is in the same direction as that of the rest of the molecule, the surface potential of the monolayer increases; if the polarities are opposite, the surface potential decreases. It is known (*21, 41, 46*)

that the surface potential of fatty acid monolayers decreases, whereas that of alkyl phosphates and sulfates increases because of interaction with metal ions—*e.g.*, Ca^{2+}, Mg^{2+}. Using the above reasoning, the position of Ca^{2+} in various monolayers is proposed as shown in Figure 3.

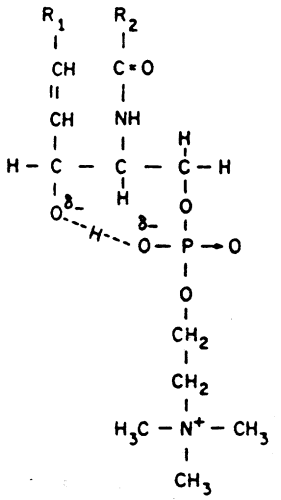


Figure 2. Ion-dipole association between hydroxyl group and ionic oxygen of phosphate group of sphingomyelin

δ−: Partial ionic charge on oxygen atoms

Influence of Intermolecular Spacing on Enzymic Hydrolysis of Lecithin Monolayers. When snake venom phospholipase A is injected under a lecithin monolayer, it splits lecithin into lysolecithin and free fatty acid. The change in polar groups of the monolayer results in a change of surface potential. However, if prior to injection of enzyme into the subsolution, a lecithin monolayer is compressed to such a surface pressure that the active site of the enzyme is unable to penetrate the monolayer, hydrolysis does not proceed. For monolayers of dipalmitoyl, egg, soybean, and dioleoyl lecithins the threshold surface pressure values at which hydrolysis does not proceed are 20, 30, 37, and 45 dynes per cm., respectively (*40*). This is also the same order for area per molecule in their surface pressure–area curves, indicating that enzymic hydrolysis of lecithin monolayers is influenced by the unsaturation of the fatty acyl chains and hence the intermolecular spacing in monolayers (*40*).

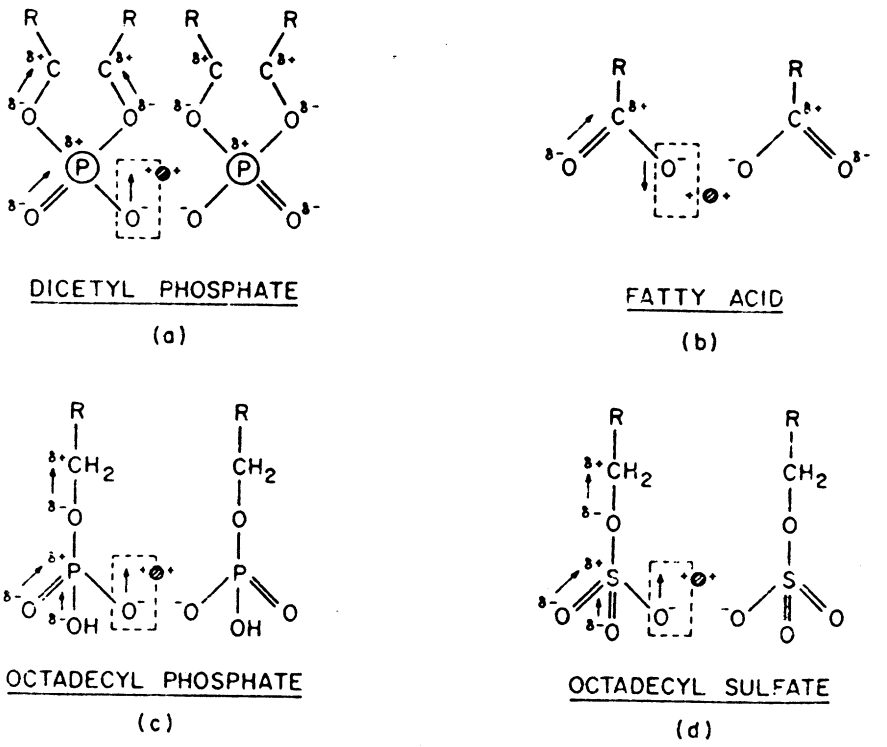


Figure 3. Position of calcium in relation to anionic oxygen in monolayers
→ Electric dipoles derived from covalent linkages
- - - Ionic dipole caused by divalent cation
δ+, δ−. Partial ionic charges on atoms

Mixed Monolayers

Concept of Intermolecular Cavities in Mixed Monolayers. In mixed monolayers a deviation in average area per molecule occurs if one component forms expanded and the other condensed monolayers. This reduction in average area per molecule has been attributed by previous workers to an interaction between components in the mixed monolayer. However, this need not be true in all cases where condensation occurs. In several instances the condensation can be explained on the basis of steric considerations in the mixed monolayers. Although the following discussion is based on lecithin-cholesterol monolayers, it is equally applicable to other mixed monolayers.

To understand the concept of intermolecular cavities we should consider the structure of an expanded monolayer. Since the hydrocarbon chains in an expanded monolayer are in the liquid state, they have greater

freedom to oscillate, rotate, and vibrate than in a condensed monolayer (*see* Figure 4a, representing a liquid-expanded monolayer of dipalmitoyl lecithin). The fatty acyl chains, because of their thermal motion, occupy an effective volume represented by a cone with its apex at the interface and base at the terminal end of the fatty acyl chains (Figure 4b). This would result in a "cavity" or vacancy between molecules. If molecules having dimensions smaller than or equal to those of the cavity are added to the lecithin monolayer, they may partially occupy these cavities to form a two-dimensional solution without causing a proportional increase in area of the monolayer (*see* Figure 4c, representing a mixed monolayer in which cholesterol partially occupies the cavity and reduces the average area per molecule). Furthermore, the liquid expanded monolayers of myristic acid, palmitic acid, and ethyl palmitate also show a reduction in the average area per molecule in the presence of cholesterol (*1*, *16*, *30*), which can be explained on the basis of intermolecular cavities. When the fatty acyl chains of lecithin are shorter—*e.g.*, C_{16}—the height of the intermolecular cavity is less than that of cholesterol (Figure 4d). Therefore, the presence of cholesterol causes a proportional increase in area; hence, such a mixed monolayer follows the additivity rule. The failure of cholesterol to condense highly expanded monolayers of lecithins containing polyunsaturated fatty acyl chains can also be explained on the basis of intermolecular cavities. For example, the monolayers of egg phosphatidic acid (*42*) or lecithins containing polyunsaturated fatty acyl chains (*48*) are more expanded than those of dipalmitoyl or egg lecithins. The greater area per molecule in these monolayers causes a larger intermolecular spacing, which results in a cavity of smaller height which does not accommodate a cholesterol molecule (Figure 4e). Therefore, such mixed monolayers follow the additivity rule of molecular areas.

Surface Pressure, Potential, and Fluidity Characteristics for Various Interactions in Mixed Monolayers. It is possible to distinguish various types of interactions which occur in mixed monolayers by measuring the surface pressure, surface potential, and surface fluidity of the monolayers. Deviation from the additivity rule of molecular areas indicates either an interaction between components or the "intermolecular cavity effect" in mixed monolayers.

Surface potential, ΔV, can be expressed (*37*) as $\Delta V = Kn\mu$ where K is a constant, n is the number of molecules per square centimeter of film, and μ is the surface dipole of the molecule. Thus $\Delta V/n = K\mu$, where the term on the left side of the equation, representing the surface potential per molecule (mv. per molecule), is proportional to the surface dipole, μ, of the molecule. When $\Delta V/n$ is plotted against mole fraction of the components, deviation from the additivity line indicates ion-ion or ion-dipole interaction between components (*42*).

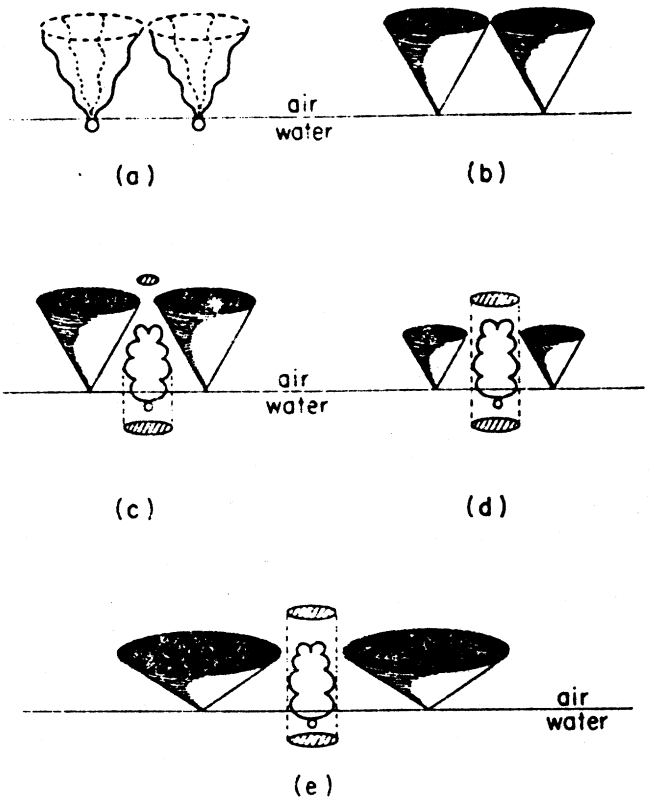


Figure 4. Schematic representation of structure of monolayers

a: Liquid-expanded monolayers. Broken lines indicate kinetic states of chains
b: Average space occupied by molecules at interface
c: Mixed monolayer—e.g., dipalmitoyl lecithin-cholesterol—in which true molecular area (cross-lined region) is shown below cholesterol and apparent increase in area above molecule
d: Mixed monolayer of short-chain lecithin, showing intermolecular cavity of smaller height. Cross-lined regions below and above cholesterol indicate true molecular area and increase caused in film area
e: Mixed monolayer of cholesterol with phosphatidic acid or lecithin having polyunsaturated fatty acyl chains whose intermolecular cavity is smaller in height. Cross-lined regions below and above cholesterol indicate true molecular area and increase in film area

Boyd and Vaslov (*6*) showed that the curve of log ϕ_s *vs.* mole fraction (where ϕ_s is the surface fluidity) for mixed monolayers exhibits additivity for mixed films of miscible components, positive deviation for immiscible components, and negative deviation for components having molecular interactions. Figure 5 shows various interactions which occur

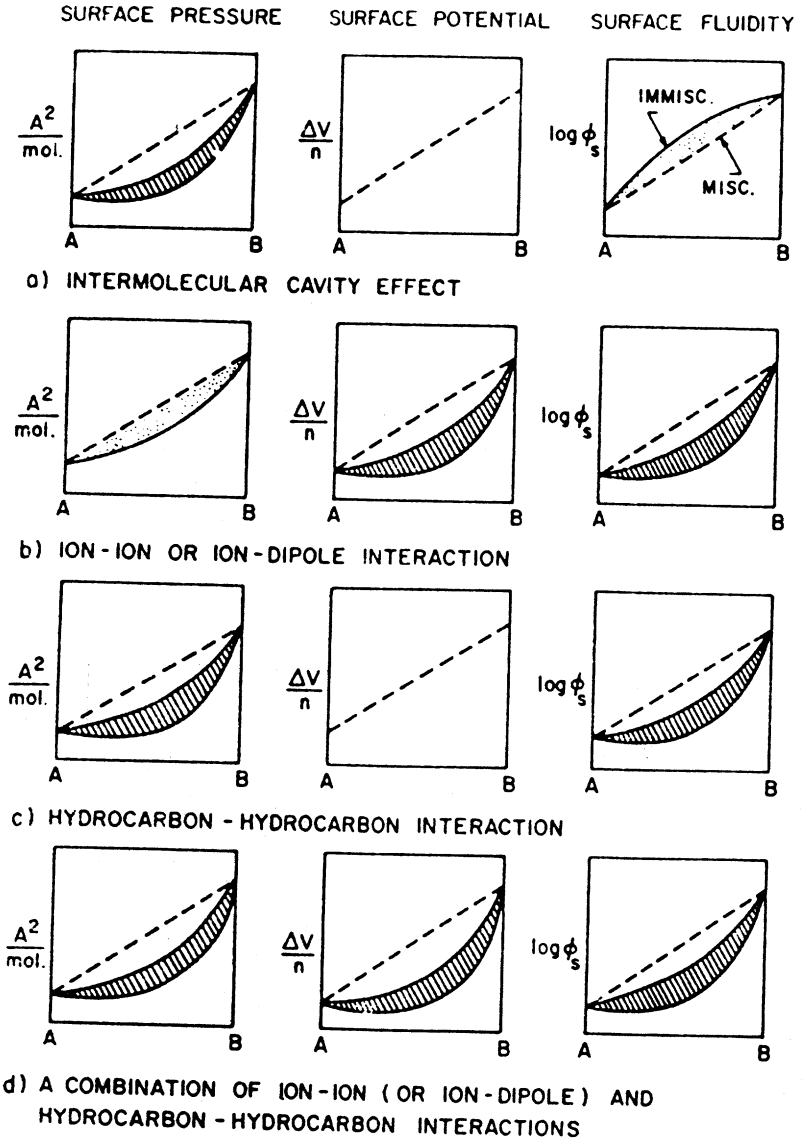


Figure 5. Interactions in mixed monolayers and their surface pressure, potential, and fluidity characteristics
--- Ideal additivity. A and B indicate pure components. Abscissa indicates mole fraction

in mixed monolayers and their characteristic changes in surface pressure, potential, and fluidity.

INTERMOLECULAR CAVITY EFFECT. Figure 5a shows the general characteristics of mixed monolayers in which the "intermolecular cavity effect"

occurs—*e.g.*, lecithin–cholesterol monolayers. The average area per molecule shows deviation, whereas surface potential per molecule ($\Delta V/n$) follows the additivity rule. Log ϕ_s can either follow the additivity rule (miscible components) or show positive deviation (immiscible components) (*6*). The cross-lined region in these diagrams represents obligatory deviation, whereas the stippled region represents optional deviation.

Ion-Ion or Ion-Dipole Interaction. Figure 5b, shows the general characteristics of mixed monolayers in which ion-ion or ion-dipole interaction takes place—*e.g.*, alkyl phosphate–alkyl trimethylammonium, or steric acid–octadecanol monolayers. The average area per molecule may or may not show a deviation from the "additivity rule" line, depending upon whether the two components form expanded or condensed monolayers. However, surface potential per molecule must show a deviation from the additivity line since ion-ion or ion-dipole interactions reduce the average surface dipole of the molecules in mixed monolayers (*31, 42*). These interactions result in a negative deviation in the plot of log ϕ_s *vs.* mole fraction (*6*).

Hydrocarbon-Hydrocarbon Interaction. Figure 5c shows the general characteristics of mixed monolayers in which hydrocarbon-hydrocarbon interaction occurs—*e.g.*, trimyristin–myristic acid monolayers (*16*). The average area per molecule shows a deviation, whereas the surface potential per molecule follows the additivity rule. Hydrocarbon-hydrocarbon interaction also increases the cohesive force in the lipid layer and therefore reduces the fluidity of the mixed monolayer. It is evident from Figures 3a and 3c that surface fluidity is the only parameter which distinguishes an intermolecular cavity effect from hydrocarbon-hydrocarbon interaction.

Ion-Ion (or Ion-Dipole) and Hydrocarbon-Hydrocarbon Interaction. Figure 5d shows the general characteristics of mixed monolayers in which ion-ion (or ion-dipole) and hydrocarbon-hydrocarbon interactions occur. The average area per molecule, the surface potential per molecule, and log ϕ_s all show deviations from the additivity rule.

Dicetyl Phosphate–Cholesterol Monolayers. Dicetyl phosphate, cholesterol, and their mixed monolayers are highly condensed—*i.e.*, steep surface pressure–area curves. However, their surface fluidity is strikingly different. Mobility of talc particles sprinkled on the monolayer indicates that dicetyl phosphate monolayers are solid, whereas cholesterol and all their mixed monolayers are liquid (*42*). The presence of 10 to 15 mole % of cholesterol is enough to liquefy the monolayers of dicetyl phosphate, indicating that cholesterol is a strong liquefying agent in these mixed monolayers. Work is in progress to determine whether these liquid films are Newtonian or non-Newtonian.

Monolayers of dicetyl phosphate–cholesterol follow the additivity rule for average area per molecule at all surface pressures, whereas surface potential per molecule shows deviation (Figures 6 and 7) (*42*). Thus, the surface potential measurements indicate that there is ion-dipole interaction between the ionic oxygen of phosphate and the hydroxyl group of cholesterol in the mixed monolayers (Figure 10a). The presence of Ca^{2+} in the subsolution neutralizes some of the phosphate groups and hence reduces the ion-dipole interaction in the mixed monolayer. Therefore, smaller deviations occur on subsolutions containing $CaCl_2$.

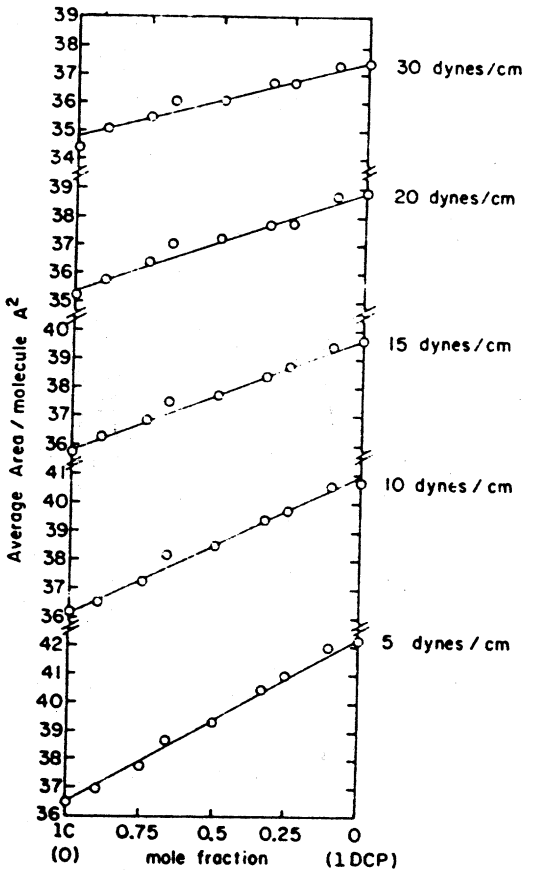


Figure 6. Average area per molecule of dicetyl phosphate–cholesterol monolayers at various surface pressures

Subsolution 0.02M NaCl or 0.01M $CaCl_2$, pH 5.6, at 25°C.

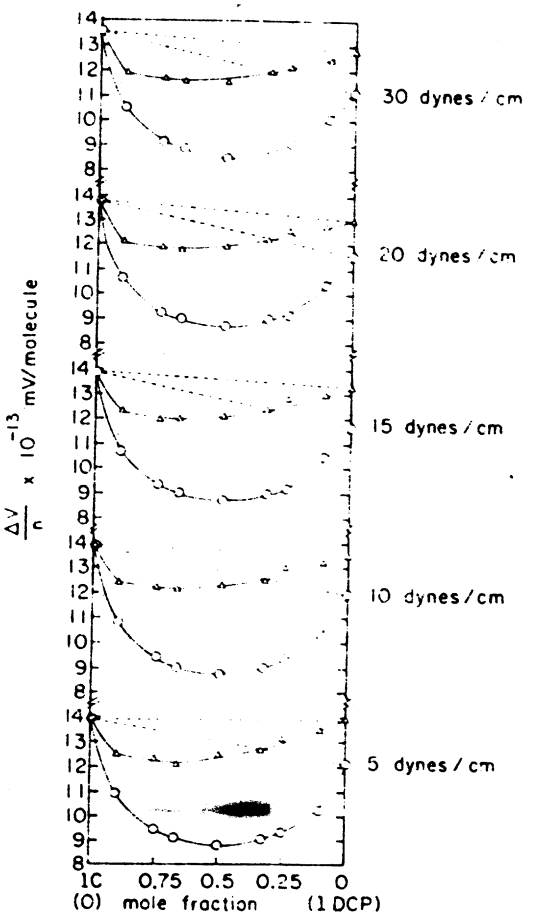


Figure 7. Average potential per molecule of dicetyl phosphate-cholesterol monolayers at various surface pressures

Subsolutions
○ *0.02M NaCl*
△ *0.01M CaCl₂*
--- *Line that would be followed if additivity rule held*

Dipalmitoyl Lecithin–Cholesterol Monolayers. The average area per molecule in dipalmitoyl lecithin–cholesterol monolayers shows deviation at low surface pressures, whereas at 30 dynes per cm. it follows the additivity rule (Figures 8 and 9) (*42*). The surface pressure–area curve of dipalmitoyl lecithin monolayers is liquid-expanded up to 30 dynes per cm., whereas above this surface pressure it is relatively incompressible (*42*). Figures 10b and c represent the structures of the dipalmitoyl

lecithin monolayer at low and high ($\sim$ 30 dynes per cm.) surface pressures. At low surface pressures, a dipalmitoyl lecithin monolayer has intermolecular cavities which can be partially occupied by cholesterol molecules (Figure 10c). At high surface pressures ($\sim$ 30 dynes per cm.) the fatty acyl chains are vertically oriented in the dipalmitoyl lecithin monolayer (Figure 10e); therefore, addition of cholesterol causes a proportional increase in area (Figure 10f).

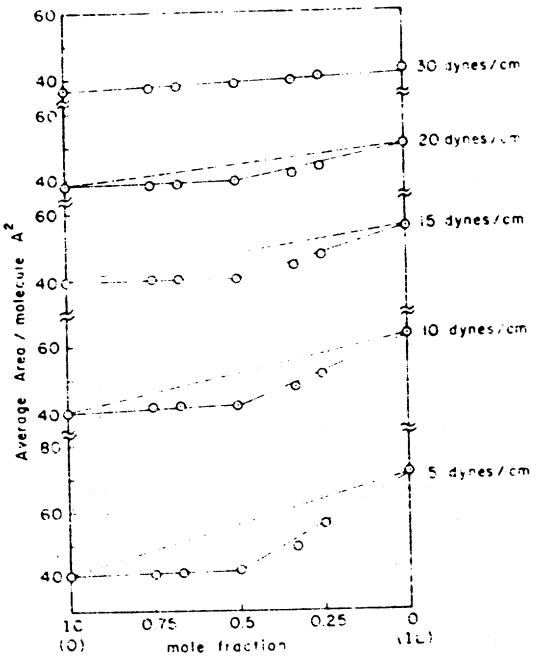


Figure 8. Average area per molecule of dipalmitoyl lecithin–cholesterol monolayers at various surface pressures

- - - Additivity rule line

Van Deenen has reported (*48*) that the mixed monolayers of didecanoyl lecithin–cholesterol follow the additivity rule of molecular areas even though this lecithin forms expanded monolayers. This can be explained similarly by an intermolecular cavity of smaller height, which cannot accommodate cholesterol (Figures 10d and 4d).

The concept of ion-dipole interaction between lecithin and cholesterol has been suggested by many workers for the packing of these lipids in myelin or in the cell membrane (*18, 19, 51*). This concept is not supported by the surface potential measurements of mixed monolayers of lecithin and cholesterol. In contrast to dicetyl phosphate–cholesterol

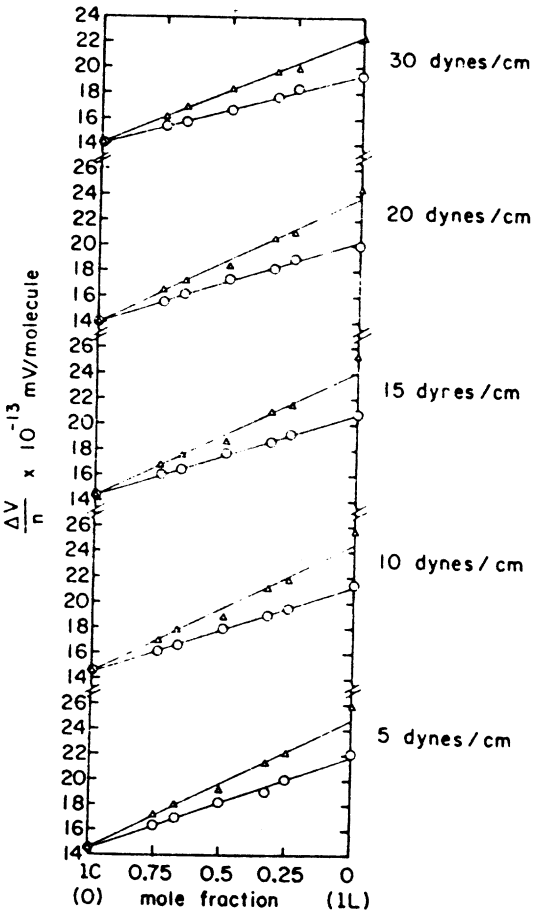


Figure 9. Average potential per molecule of dipalmitoyl lecithin–cholesterol monolayers at various surface pressures

Subsolutions
○ *0.02M NaCl*
△ *0.01M $CaCl_2$*
Points follow additivity rule

monolayers, the lecithin–cholesterol monolayers follow the additivity rule of average potential per molecule even at high surface pressures (*42*).

Egg Lecithin–Cholesterol Monolayers. The average area per molecule in egg lecithin–cholesterol monolayers shows deviation from the additivity rule at all surface pressures (*42*). The deviation in this case could be explained by the presence of molecular cavities caused by the kink in the oleoyl chain of egg lecithin, which would reduce the average area per molecule at low as well as high surface pressures (Figure 10g).

Similar reasoning accounts for the condensation of monolayers of dioleoyl lecithin by cholesterol reported by Van Deenen (*48*) (Figure 10h).

Even though 1,2-dilinoleoyl and 1-palmitoyl-2-linolenoyl lecithins form more expanded monolayers than egg lecithin, their mixed monolayers with cholesterol follow the additivity rule (*48*). This can be explained as follows. At low surface pressures, these lecithins have greater intermolecular spacing and hence form intermolecular cavities of smaller height which cannot accommodate cholesterol molecules (Figure 4e). At high surface pressure, the linoleoyl and linolenoyl chains, as opposed to oleoyl chains, do not form cavities in the monolayer (Figure 10i).

The optimum condensation at molecular ratios of 3 to 1 and 1 to 3 in egg lecithin–cholesterol monolayers and 1 to 1 in dipalmitoyl lecithin–cholesterol monolayers (*42*) do not imply complex formation between lecithin and cholesterol but rather suggest average geometrical arrangements of these molecules.

Recently Bourges, Small, and Dervichian (*5*) reported that a paracrystalline lamellar structure of egg lecithin can solubilize cholesterol up to a maximum of one molecule of cholesterol per molecule of lecithin. However, they conclude that this should not be considered as a molecular association but rather the consequence of the relative arrangement of the molecules in the lamellar structure which is a mutual (solid) solution of lecithin and cholesterol. They also reported that the state of compression in the lamellar structure corresponds to that of a highly compressed mixed monolayer of lecithin–cholesterol. The NMR results of Chapman and Penkett (*8*) also appear to indicate that solubilization of cholesterol in egg lecithin dispersions results in a highly packed structure in which fatty acyl chains possess little molecular motion. Our results from lecithin–cholesterol monolayers also suggest that these mixed monolayers are two-dimensional solutions with no specific interaction and that the apparent condensation in some instances is caused by the steric factors of the fatty acyl chains and not by the interaction or association between lecithin and cholesterol.

Phosphatidic Acid–Cholesterol Monolayers. In contrast to egg lecithin–cholesterol monolayers, the average area per molecule in phosphatidic acid–cholesterol monolayers shows a smaller deviation from the additivity rule at low surface pressures and a larger deviation at high surface pressures (*42*). The explanation for this is that at low surface pressure the large area per molecule in phosphatidic acid monolayers results in a cavity, but of a decreased height which cannot accommodate a cholesterol molecule (Figure 4e). At high surface pressures—*i.e.*, smaller area per molecule—the molecular cavity formed by the oleoyl chains of phosphatidic acid would have sufficient height to allow cholesterol to occupy partially these molecular cavities. Further in contrast

to lecithin–cholesterol monolayers, the average potential per molecule in phosphatidic acid–cholesterol monolayers shows a deviation from the additivity rule (*42*). This indicates ion-dipole interaction between the hydroxyl group of cholesterol and the ionic phosphate of phosphatidic acid.

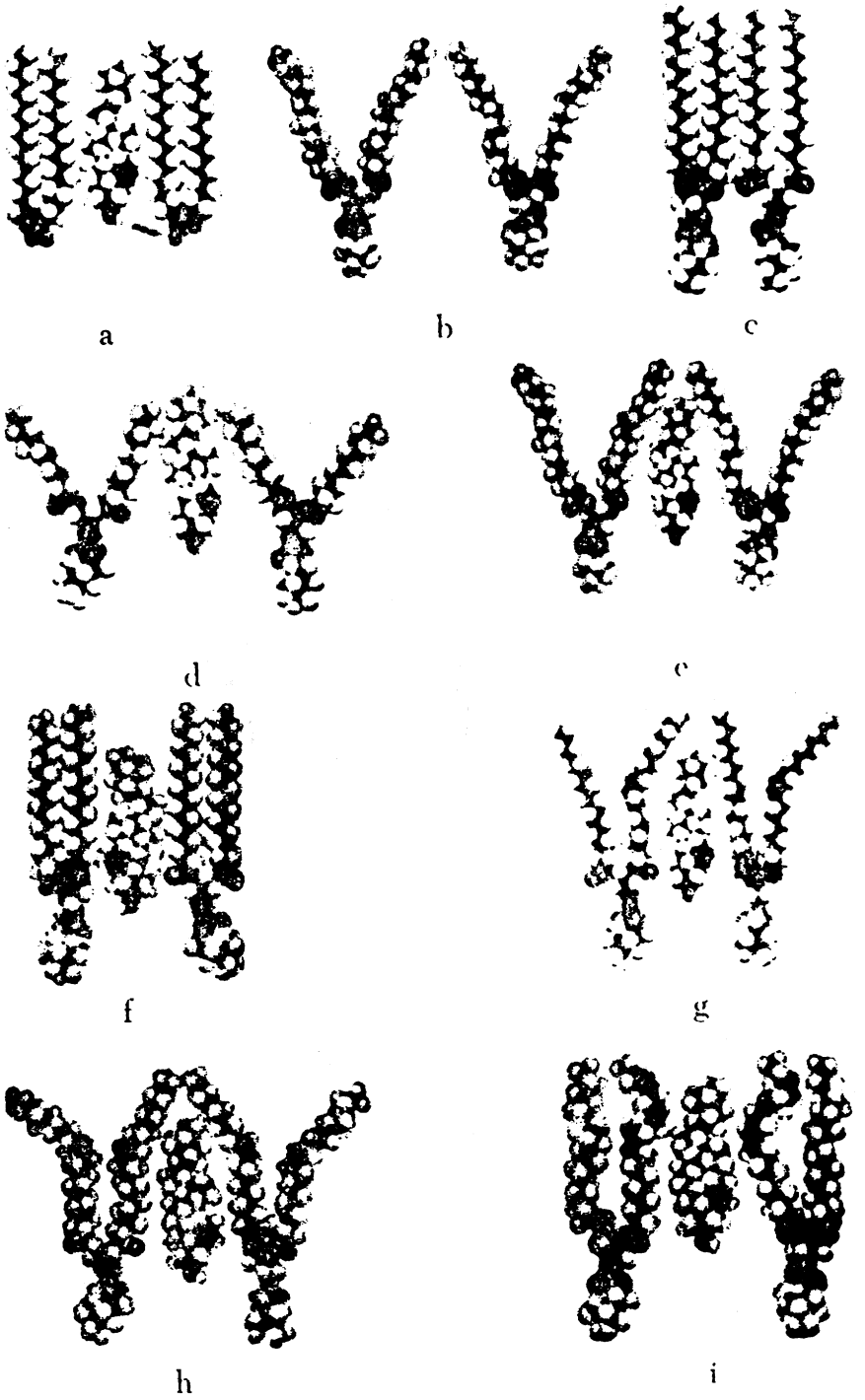
Thermodynamics of Mixed Monolayers. Goodrich (*22*) considered mixed monolayers as "two-dimensional solutions" and derived an expression for excess free energy of mixing, G_{xs}, which is given as

$$G_{xs} = \int_{\pi^*}^{\pi} (\sigma_{12} - N_1\sigma_1 - N_2\sigma_2)d\pi \qquad (1)$$

where N_1 and N_2 are mole fractions of components 1 and 2 with $N_1 + N_2 = 1$; σ_1 and σ_2 are the areas of the respective monolayers; and σ_{12} is the area of the mixed monolayer. It is evident from Equation 1 that if $\sigma_{12} = N_1\sigma_1 + N_2\sigma_2$, then G_{xs} is zero. In other words, if a mixed monolayer follows the additivity rule of molecular areas at all surface pressures, it represents ideal mixing of components with no specific interaction between them. However, Goodrich's treatment is unsatisfactory and contradictory when applied to mixed monolayers of dicetyl phosphate–cholesterol. These mixed monolayers follow the additivity rule of molecular areas at all surface pressures (Figure 6), which indicates that G_{xs} is zero and that there is no specific interaction between dicetyl phosphate and cholesterol. However, surface potential measurements (Figure 7) clearly indicate ion-dipole interaction between dicetyl phosphate and cholesterol (*42*). Similarly, negative values of G_{xs} have been reported for lecithin–cholesterol monolayers (*15, 55*) where no specific interaction is found on the basis of surface potential (*42*) and viscosity measurements (*11, 12, 13*). This contradiction of experimental results to Goodrich's theoretical treatment points out the need for further experimental data and refinement of theoretical treatment in this area.

Figure 10. Molecular arrangement in various monolayers by space filling Fischer-Hirschfelder-Taylor molecular models

a: Dicetyl phosphate and cholesterol
- - - Ion-dipole interaction between hydroxyl group of cholesterol and ionized oxygen of phosphate group
b: Dipalmitoyl lecithin at low surface pressures (< 30 dynes per cm.)
c: Dipalmitoyl lecithin at high surface pressures (> 30 dynes per cm.)
d: Didecanoyl lecithin and cholesterol
e: Dipalmitoyl lecithin and cholesterol at low surface pressures (< 30 dynes per cm.)
f: Dipalmitoyl lecithin and cholesterol at high surface pressures (> 30 dynes per cm.)
g: Egg lecithin and cholesterol monolayers
h: Dioleoyl lecithin and cholesterol monolayers
i: 1-Palmitoyl-2-linolenoyl lecithin and cholesterol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Correlation of Surface Properties of Cholesterol with Biomembranes. From the early work on condensation of lecithin monolayers in the presence of cholesterol (*13, 48*), it has been assumed generally that cholesterol increases the cohesive force in the lipid layer and thereby stabilizes the membrane. In contrast, we find that the two striking surface properties of cholesterol are its ability to liquefy lipid layers, even when present as a small fraction (10 to 15 mole %), and to occupy the molecular cavities between the fatty acyl chains. This agrees with the findings of Langmuir and Schaefer (*29*), who showed that the presence of cholesterol in C_{23} acid monolayers markedly increases the rate of evaporation through the monolayers, possibly because of an increase in the fluidity of the monolayer. In brain tissue, the high percentage of phospholipids with monounsaturated chains, and of cholesterol (*33, 53*) may lead to effects similar to those reported in the present work. Schulman, Waterhouse, and Spink (*38*) showed that addition of cholesterol to a lubricant reduces the frictional force between two metal surfaces by 80%, which can be attributed to increasing fluidity of the lipid layer. Hence the high cholesterol content of the erythrocyte membrane (*49*) suggests that cholesterol may play a role as a biological plasticizer, increasing the distensibility of the membrane.

Fluidity of the hydrocarbon chains is essential for the existence of lecithin bilayers (*24, 32, 47*). Bilayers of egg lecithin–cholesterol (molar ratio 1 to 1) are as stable as those of egg lecithin alone (stability being the time between formation and collapse of a bilayer), suggesting the liquid state for the fatty acyl chains (*47, 52*). If cholesterol had increased the cohesive force and hence the solidity of the bilayer, its stability would have been reduced considerably. Fluidity rather than rigidity is important for stability of thin films. A fluid film can adjust itself against thermal and mechanical fluctuations in the environment and thereby exhibit greater stability than rigid films. Cholesterol is unusual, in that its monolayers are highly incompressible but have low surface viscosity (*11, 12, 42*). Therefore, the liquefying property of cholesterol is presumably caused by its rigid, planar, asymmetrical molecular shape, which can act as a disordering agent, reducing the cohesive force between fatty acyl chains and thereby increasing the fluidity of the lipid layer.

Summary

The interaction of metal ions with lecithin monolayers, as measured by the increase in surface potential, decreases with increasing unsaturation of fatty acyl chains. The phosphate and trimethylammonium groups of a lecithin molecule form an internal salt linkage which dissociates upon

increasing saturation of fatty acyl chains, or upon increasing electrolyte concentration in the subsolution.

The double bond adjacent to the polar group strongly influences surface potentials of plasmalogen and sphingomyelin. In sphingomyelin, an ion-dipole association between the hydroxyl and ionic phosphate groups of the molecule results in a net positive surface charge.

The position of metal ions in a monolayer lattice has been proposed from surface potential measurements.

Enzymic hydrolysis of lecithin monolayers is strikingly influenced by the degree of unsaturation of fatty acyl chains and hence by the intermolecular spacing in monolayers.

Monolayers of dicetyl phosphate–cholesterol follow the additivity rule for average area per molecule, whereas lecithin–cholesterol monolayers deviate from it. The reverse is true for the additivity rule of average potential per molecule. Thus, the surface potential indicates that there is no interaction (or complex formation) between lecithin and cholesterol, but there is ion-dipole interaction between dicetyl phosphate and cholesterol as well as between phosphatidic acid and cholesterol.

The apparent condensation of mixed monolayers of lecithin in the presence of cholesterol is explained by a consideration of molecular cavities or vacancies caused by thermal motion of fatty acyl chains, the height of these cavities being influenced by the length, inclination, and degree of unsaturation (especially the proportion of monounsaturation) of the fatty acyl chains and the extent of compression of the monolayer. Monolayers are liquefied by the presence of unsaturated fatty acyl chains or by the addition of cholesterol.

Various types of molecular interactions which occur in mixed monolayers can be distinguished by simultaneous measurements of the surface pressure, potential, and fluidity of monolayers. Limitations of Goodrich's thermodynamic treatment of mixed monolayers are mentioned. Surface properties of cholesterol have been correlated with its function in biomembranes.

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The untimely death of Jack H. Schulman, a truly brilliant and creative scientist, is reported with profound regret. Those who worked with him share a deep sorrow in this tragic event.

Literature Cited

(1) Adam, N. K., Jessop, G., *Proc. Roy. Soc. (London)* **A120**, 473 (1928).
(2) Anderson, P. J., Pethica, B. A., "Biochemical Problems of Lipids," G. Popjak and E. Le Breton, Eds., pp. 24-29, Butterworth & Co., London, 1956.
(3) Bangham, A. D., Papahadjopoulos, D., *Biochim. Biophys. Acta* **126**, 181 (1966).
(4) Benson, A. A., *J. Am. Oil Chemists' Soc.* **43**, 265 (1966).
(5) Bourges, M., Small, D. M., Dervichian, D. G., *Biochim. Biophys. Acta* **137**, 157 (1967).
(6) Boyd, G. E., Vaslow, F., *J. Colloid Sci.* **13**, 275 (1958).
(7) Chapman, D., Owens, N. F., Walker, D. A., *Biochim. Biophys. Acta* **120**, 148 (1966).
(8) Chapman, D., Penkett, S. A., *Nature* **211**, 1304 (1966).
(9) Colacicco, G., Rapport, M. M., *J. Lipid Res.* **7**, 258 (1966).
(10) Dawson, R. M. C., *Biochem. J.* **98**, 35C (1966).
(11) Deamer, D. W., Ph.D. thesis, Ohio State University, 1965.
(12) Deamer, D. W., Cornwell, D. G., *Biochim. Biophys. Acta* **116**, 552 (1966).
(13) De Bernard, L., *Bull. Soc. Chim. Biol.* **40**, 161 (1958).
(14) Demel, R. A., Van Deenen, L. L. M., *Chem. Phys. Lipids* **1**, 68 (1966).
(15) Demel, R. A., Van Deenen, L. L. M., Pethica, B. A., *Biochim. Biophys. Acta* **135**, 11 (1967).
(16) Dervichian, D. G., "Surface Phenomena in Chemistry and Biology," J. F. Danielli, K. G. A. Pankhurst, A. C. Riddiford, Eds., pp. 70-87, Pergamon Press, New York, 1958.
(17) Dreher, K. D., Sears, D. F., *Trans. Faraday Soc.* **62**, 741 (1966).
(18) Finean, J. B., *Circulation* **26**, 1151 (1962).
(19) Finean, J. B., *Experientia* **9**, 17 (1953).
(20) Galdston, M., Shah, D. O., *Biochim. Biophys. Acta* **137**, 255 (1967).
(21) Goddard, E. D., Ackilli, J. A., *J. Colloid Sci.* **18**, 585 (1963).
(22) Goodrich, F. C., Proceedings of 2nd International Congress on Surface Activity, J. H. Schulman, ed., Vol. 1, p. 85, Butterworth & Co., London, 1957.
(23) Hauser, H., Dawson, R. M. C., *European J. Biochem.* **1**, 61 (1967).
(24) Huang, C., Wheeldon, L., Thompson, T. E., *J. Mol. Biol.* **8**, 148 (1964).
(25) Hughes, A., Rideal, E. K., *Proc. Roy. Soc. (London)* **A140**, 253 (1933).
(26) Hyono, A., Kuriyama, S., *Nature* **210**, 300 (1966).
(27) Kimizuka, H., Koketsu, K., *Nature* **196**, 995 (1962).
(28) Kimizuka, H., Nakahara, T., Uejo, H., Yamauchi, A., *Biochim. Biophys. Acta* **137**, 549 (1967).
(29) Langmuir, I., Schaefer, V. J., "Surface Chemistry," F. R. Moulton, Ed., *AAAS Publ.* **21**, 17-39 (1943).
(30) Leathes, J. B., *Lancet* **208**, 853 (1925).
(31) Marsden, J., Schulman, J. H., *Trans. Faraday Soc.* **34**, 748 (1938).
(32) Mueller, P., Rudin, D. O., Tien, H. T., Wescott, W. C., "Recent Progress in Surface Science," J. F. Danielli, K. G. A. Pankhurst, A. C. Riddiford, Eds., Vol. 1, p. 384, Academic Press, New York, 1964.
(33) O'Brien, J. S., Sampson, E. L., *J. Lipid Res.* **6**, 545 (1965).
(34) Rogeness, G. A., Krugman, L. G., Abood, L. G., *Biochim. Biophys. Acta* **125**, 319 (1966).
(35) Rojas, E., Lettvin, J. Y., Pickard, W. F., *Nature* **209**, 886 (1966).
(36) Rojas, E., Tobias, J. M., *Biochim. Biophys. Acta* **94**, 394 (1965).
(37) Schulman, J. H., Rideal, E. K., *Proc. Roy. Soc. (London)* **A130**, 284 (1931).

(38) Schulman, J. H., Waterhouse, R. B., Spink, J. A., *Kolloid Z.* **146**, 77 (1956).
(39) Shah, D. O., Schulman, J. H., *Biochim. Biophys. Acta* **135**, 184 (1967).
(40) Shah, D. O., Schulman, J. H., *J. Colloid Interface Sci.* **25**, 107 (1967).
(41) Shah, D. O., Schulman, J. H., *J. Lipid Res.* **6**, 341 (1965).
(42) *Ibid.*, **8**, 215 (1967).
(43) *Ibid.*, p. 227.
(44) Shah, D. O., Schulman, J. H., *Lipids* **2**, 21 (1967).
(45) Shimojo, T., Ohnishi, T., *J. Biochem. (Japan)* **61**, 89 (1967).
(46) Spink, J. A., Sanders, J. V., *Nature* **175**, 644 (1955).
(47) Thompson, T. E., "Cellular Membranes in Development," M. Locke, Ed., p. 87, Academic Press, New York, 1964.
(48) Van Deenen, L. L. M., "Progress in the Chemistry of Fats and Other Lipids," R. T. Tolman, Ed., Vol. 8, Part 1, pp. 14, 59, Pergamon Press, New York, 1965.
(49) Van Deenen, L. L. M., Gier, J. D., "Red Blood Cell," C. Bishop, D. M. Surgenor, Eds., pp. 243-307, Academic Press, New York, 1964.
(50) Van Deenen, L. L. M., Houtsmuller, U. M. T., de Haas, G. H., Mulder, E., *J. Pharm. Pharmacol.* **14**, 429 (1962).
(51) Vandenheuvel, F. A., *Ann. N.Y. Acad. Sci.* **122**, 57 (1965).
(52) Van Zutphen, H., Van Deenen, L. L. M., Kinsky, S. C., *Biochem. Biophys. Res. Commun.* **22**, 393 (1966).
(53) Veerkamp, J. H., Mulder, I., Van Deenen, L. L. M., *Biochim. Biophys. Acta* **57**, 299 (1962).
(54) Villallonga, F., Altschul, R., Fernandez, S., *Biochim. Biophys. Acta* **135**, 406 (1967).
(55) *Ibid.*, p. 557.

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