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THE BIOLOGY OF SURFACES

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ABSTRACT

The principles of chemistry and physics of surfaces which are relevant to biological systems are briefly reviewed. The increase in surface concentration of surface-active molecules due to adsorption is emphasized. It is shown that the degree of unsaturation of fatty acid chains of lipids determines the average area per molecule and hence the intermolecular spacing between lipid molecules. The change of 1 to 2 Å in the intermolecular spacing between lipid molecules strikingly influences their enzymic hydrolysis, interaction with cations or anions, and association with cholesterol. The mechanism of hysteresis of films is explained in terms of exchange of soluble surface-active molecules between the film and subsolution during the expansion and compression of the film. From the bubble stability measurements, it is shown that the conformation of polypeptides strikingly influences the hydration, bubble stability, and the rate of drainage in thin aqueous films. The discrepancy between the melting points of cholesterol in three-dimensional and two-dimensional forms clearly indicates the need to understand the rheology and phase transitions in surfaces. It is pointed out that there is a high degree of cooperativity between molecules at surfaces. In a lamellar liquid-crystalline model system, it is shown that 2 calcium ions for every 100 lipid molecules are needed to cause a phase transition from birefringent lamellar structure to an isotropic spherical one.

INTRODUCTION

The need for understanding the physicochemical aspects of biological surfaces can hardly be overemphasized. It has been long recognized that cells and cell organelles are surrounded by membranes which exhibit selective permeability. Many enzymes are known to be associated with these membranes. Besides ion transport and enzymic activities, membranes are known to be involved in electrical excitability, cell-cell interaction, locomotion, cell-surface interaction, and in determining immunological specificity. The elucidation of the structure and function of membranes, at a molecular level, appears to be one of today's most challenging problems.

The present article briefly reviews the principles of chemistry and physics of surfaces in relation to biological surfaces. In order to elucidate and establish the major concepts, relevant experimental results are presented in the text.

1. Adsorption at Surfaces:

In general, a separation between the hydrophilic and hydrophobic groups in a molecule causes the molecule to orientate at the air-water or oil-water interface. The hydrophilic group tends to dissolve in the aqueous phase, whereas the hydrophobic group tends to stay out of the aqueous phase. This type of molecule is generally referred to as surface-active because it tends to adsorb or accumulate at the interface and decrease the interfacial tension. It has been shown (1) that membrane lipids and proteins also possess such hydrophilic and hydrophobic groups and hence are expected to orientate at the interface. There are many drugs, local anesthetics and other pharmacological agents which are surface-active and hence are expected to adsorb at the interface. When adsorption occurs at the interface, there is a greater concentration of molecules at the interface than the concentration in the bulk solution. It should be emphasized that adsorption is an equilibrium phenomenon which will always tend to maintain a greater surface concentration than bulk concentration for any surface-active compound.

At low concentrations, a soluble surface active compound is molecularly dispersed (or soluble as monomers) in the aqueous solution. However, if the concentration is further increased,

the molecules begin to aggregate into micelles as shown in Figure 1. The concentration at which the formation of micelles begins is called critical micelle concentration (or CMC). The interior of the micelle has essentially the properties of a liquid hydrocarbon and hence solubilizes many oil soluble compounds (Fig. 1A, B). Absorbed films or micelles can also interact with proteins or polypeptides in aqueous solutions by ionic and Van der Waals forces (Fig. 1C, D).

The following calculations indicate the ratio of concentration of surface-active molecules in the surface to that in the bulk solution. The critical micelle concentration of sodium dodecyl sulfate at 25°C is reported to be 8.3×10^{-3} moles/liter (2). At the CMC, the surface is saturated with the surfactant molecules. The average area per molecule is about 30 \AA^2 . Therefore, the number of molecules per cm^2 (n_s) at the surface is equal to $10^6 / 30 = 3.33 \times 10^{14}$. Since the length of the sodium dodecyl sulfate molecule is about 20 Å, the volume occupied by the surfactant film at the interface is $1 \times 1 \times 20 \times 10^{-8} \text{ cm}^3$. Now, if we take a volume element in the bulk solution of the dimension $1 \times 1 \times 2 \times 10^{-8} \text{ cm}^3$, the number of molecules in this volume element, n_b is equal to,

$$n_b = 8.3 \times 10^{-14} \times 6.025 \times 10^{23} \times 20$$

$$\text{or } n_b = 1 \times 10^{12}.$$

Therefore, the ratio

$$\frac{n_s}{n_b} = \frac{3.3 \times 10^{14}}{1.0 \times 10^{12}} = 330 .$$

These calculations suggest that the concentration of dodecyl sulfate molecules at the surface is 330 times greater than the concentration in the bulk. The surface to bulk concentration ratio would increase with two factors, namely, the presence of opposite surface charge at the interface and the increase in the chain length of the surfactant molecules. In the presence of a positively

charged monolayer of an insoluble surfactant at the interface, the surface concentration to bulk concentration ratio would be considerably greater than 330. For any homologous series of normal hydrocarbon-chain surfactants, the CMC value decrease by one-half for an increase of one carbon atom in the hydrocarbon chain. The CMC values at 25°C of dodecyl, tetradecyl, and hexadecyl sulfates are known to be respectively 8.3×10^{-3} , 2.05×10^{-3} and 0.42×10^{-3} moles per liter. Similar calculations as mentioned above would suggest that n_g/n_b ratio for dodecyl, tetradecyl and hexadecyl sulfates would be respectively 330, 1320, and 5280, indicating that surface concentration of surface-active molecules can be several thousand times greater than their bulk concentration. Since many drugs, anesthetics and pharmacological agents exhibit surface activity, it is important to realize that surface concentration of these molecules at the biological surfaces can be considerably greater than their bulk concentration.

2. Surface pH vs. Bulk pH:

In general, biological surfaces possess fixed charges due to various ionic groups on lipids and proteins. Red blood cells are known to exhibit a net negative charge on the membrane surface, as measured by their electrophoretic mobility. Figure 2 schematically shows that for a negatively charged surface, the concentration of H^+ ions near the surface may be greater than the concentration far away from the surface because of the Coulombic attraction between the oppositely charged ions near the surface. The negative charges on the surface attract H^+ ions near the surface. If one assumes that the distribution of H^+ ions in the diffuse layer near the charged surface is governed by the Maxwell-Boltzmann distribution, then the surface concentration

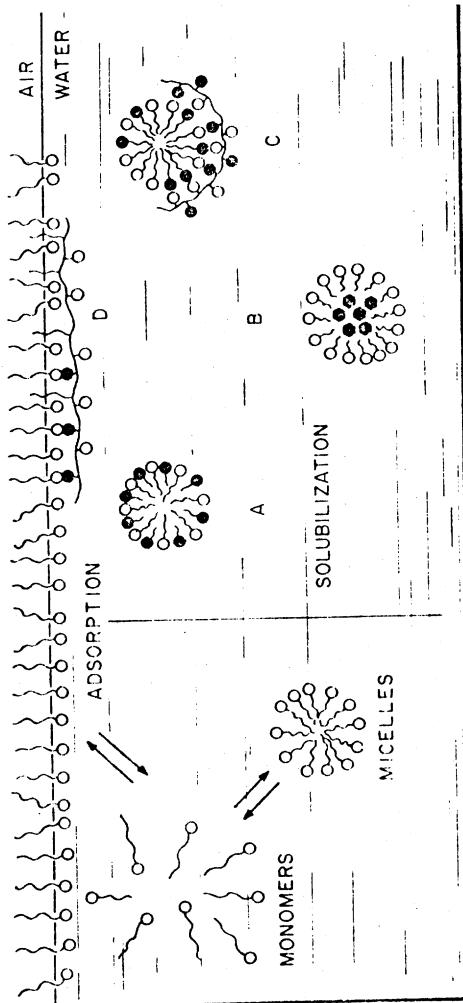


Figure 1: A schematic representation for adsorption of soluble surface-active molecules at the air-water interface, and for micelle formation. (A) represents solubilization of polar molecules in a micelle; (B) represents solubilization of non-polar molecules within a micelle; (C) represents adsorption of a polymer at the micellar surface; (D) represents the interaction of a polymer with the adsorbed film of surface active molecules. O and \ominus represent ionic and nonionic polar groups, respectively.

Upon integration and taking logarithms of both sides, this equation becomes,

$$H_S^+ = (H^+)_b \times e^{-e\psi/kT}$$

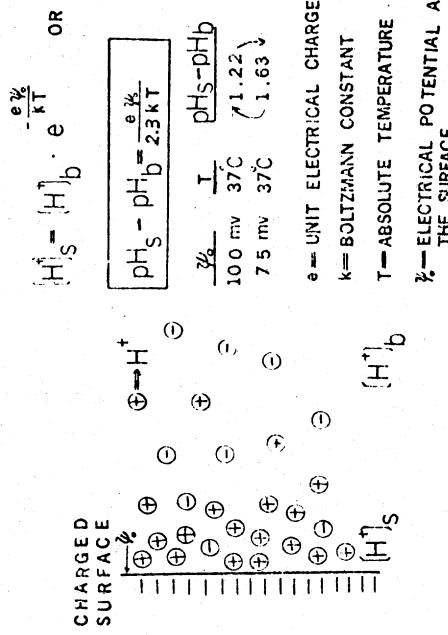


Figure 2: The pH near a charged surface in relation to the bulk pH of the solution. $[\text{H}^+]_S$ and $[\text{H}^+]_b$ represent the surface and bulk concentrations of H^+ ions, respectively. ψ_o = electrical potential at the surface, e = unit electron charge, k = Boltzmann constant, and T = absolute temperature.

$$\text{pH}_S - \text{pH}_b = \frac{e\psi_o}{2.3kT}$$

For ψ_o of 75 mv the difference between the $\text{pH}_S - \text{pH}_b = 1.22 \text{ pH}$ units. Often there has been considerable controversy on the ionic vs. nonionic form being the active form of drugs; however, it is important to remember that the precise ionic structure of a drug molecule will be determined by the pH near the site of action or near the membrane surface rather than the pH of the bulk solution.

3. Intermolecular Spacing and Unsaturation of Lipids:

Shah and Schutman (3-5) investigated the influence of unsaturation of fatty acid chains on the interaction of calcium ions and phospholipase A with various phospholipid monolayers. The surface pressure-area curves of various lecithins are shown in Figure 3, which clearly indicate that at the same surface pressure, the area per molecule and consequently the intermolecular spacing in the monolayers are in the order: dipalmitoyl lecithin < egg lecithin < soybean lecithin < dioleoyl lecithin. These differences were attributed to the fatty acid composition of the lecithins as determined by gas chromatography. From the surface pressure-area curves shown in Figure 3, these lecithins can be schematically represented as shown in Figure 4. The order of these molecular areas and intermolecular spacings can be confirmed independently from enzymic hydrolysis of these monolayers. If the enzymic hydrolysis is studied as a function of surface pressure, it is expected that, above a specific surface pressure, the hydrolysis may not occur if the intermolecular spacing is smaller than the dimensions of the active-site of the enzyme molecule. Figure 5 illustrates the initial rate of hydrolysis of various lecithin monolayers as measured by a decrease in the surface potential. It is evident that hydrolysis does not occur above surface pressures of 20, 30, 37 dynes/cm for dipalmitoyl, egg, and soybean lecithins, respectively. In contrast, hydrolysis of dioleoyl lecithin monolayers occurs even at a surface pressure of 40 dynes/cm. The critical surface pressure values to prevent hydrolysis of lecithin monolayers are in the same order as their

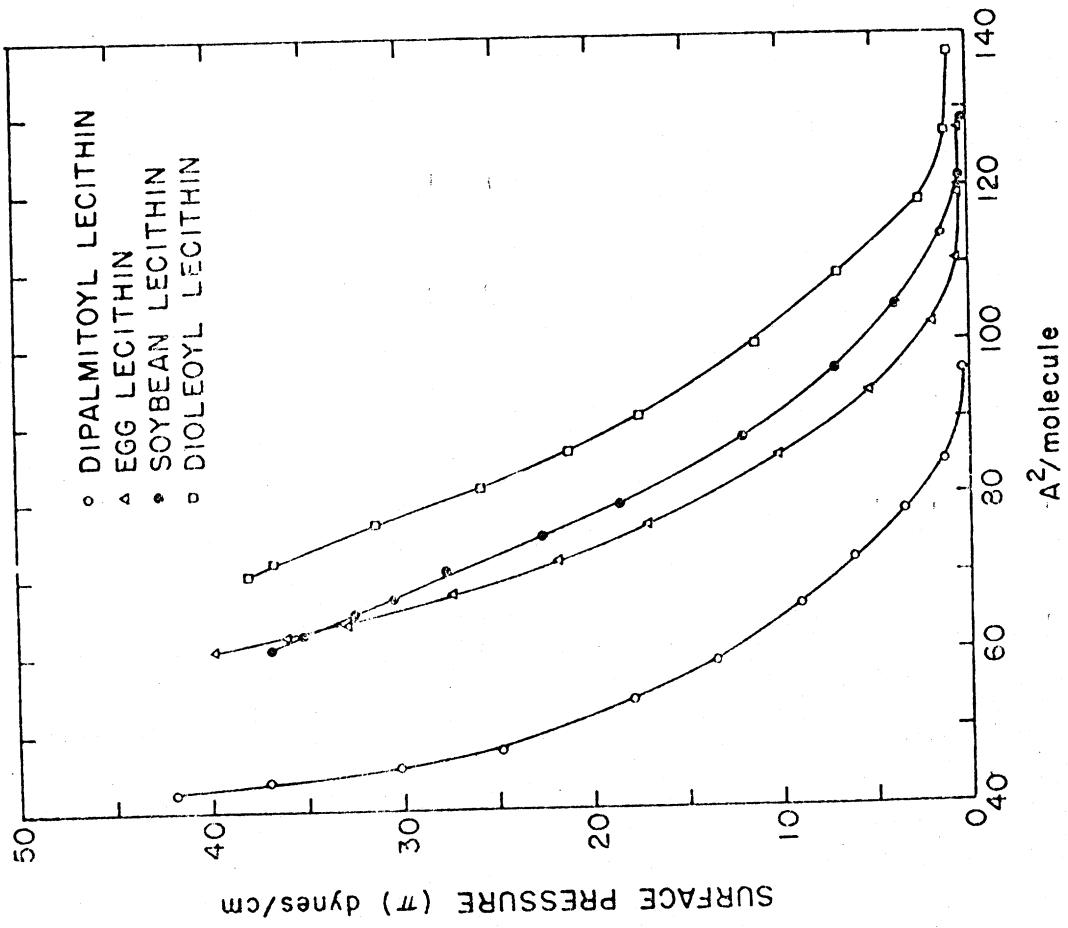


Figure 3: Surface pressure-area curves of dipalmitoyl, egg, soybean, and dioleoyl lecithins on 0.02 M NaCl sub-solution, pH 5.6, at 25°C.

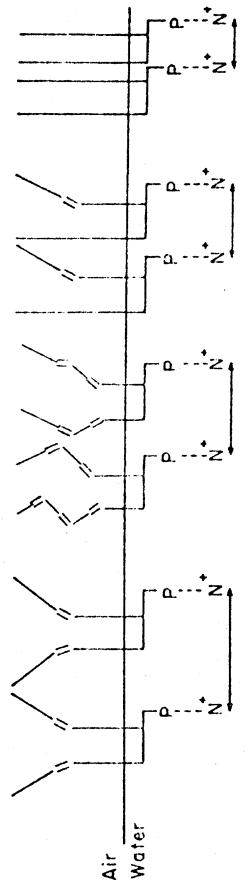


Figure 4: A schematic representation of the influence of unsaturation on the intermolecular spacing in lecithin monolayers. The lecithin molecules are shown in the order from left to right: dioleoyl lecithin, soybean lecithin, egg lecithin, dipalmitoyl lecithin.

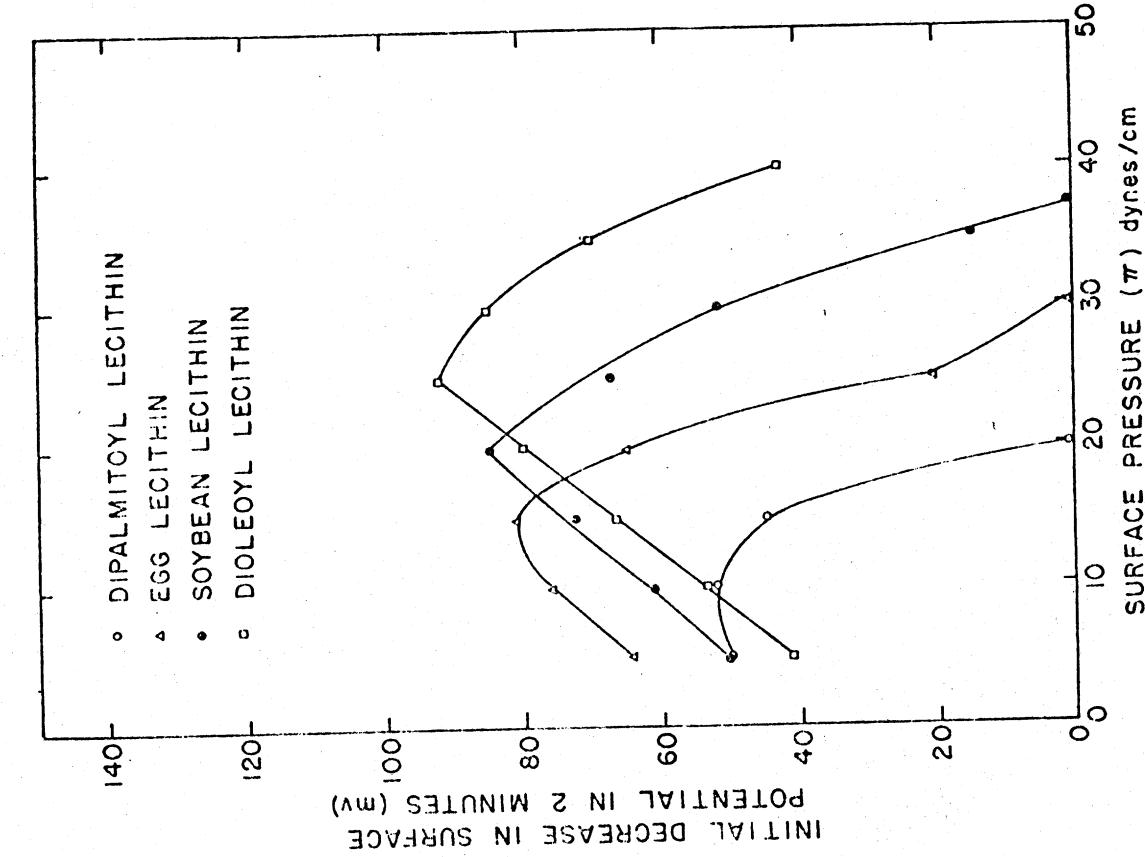


Figure 5: The effect of surface pressure on initial rate of hydrolysis, as measured by the initial decrease of surface potential in the first 2 minutes, for various lecithin monolayers. The sub-solution consists of tris (0.05 M) + NaCl (0.02 M) + CaCl (0.01 M), pH 7.2, at 25°C.

intermolecular spacing or area per molecule. Hence, the study of enzymic hydrolysis by this method provides an independent measure of the order of intermolecular spacing in lecithin monolayers.

The intermolecular spacing (i.e., average distance between two adjacent phosphate groups) in lecithin monolayers can be calculated approximately assuming the "average area per molecule" to be the area of a circle with a radius, r ; in this case, the intermolecular spacing is $2r$. If we consider areas of 51 A^2 , 73 A^2 , 76 A^2 and 87 A^2 for dipalmitoyl, egg, soybean and dioleoyl lecithins, respectively, at the surface pressure of 20 dynes/cm, the corresponding intermolecular spacings are 8.1 \AA , 9.6 \AA , 9.8 \AA , and 10.5 \AA , indicating that a change of 1 to 2 \AA in the intermolecular spacing strikingly influences the hydrolysis of lecithin monolayers. Similarly, from surface potential measurements of these lecithins on sub-solutions containing divalent cations, Shah and Schullman (4, 5) have shown that the increasing unsaturation of fatty acid chains increases the average area per molecule and hence the intermolecular spacing, and decreases the interaction of divalent cations with adjacent phosphate groups in the lecithin monolayers.

The area per molecule of the lipids in a membrane is a very important membrane parameter. In this connection the observations of Meyer and Bloch (6) on the fatty acid composition of the phospholipids from yeast cells which were grown anaerobically are highly interesting. Under these conditions, the unsaturated fatty acid chains which are common for aerobically grown cells are replaced by saturated shorter fatty acid chains. It has been shown by Van Deenen (7) that both lecithins having either longer unsaturated or shorter saturated fatty acid chains have the same area per molecule and, hence, the same intermolecular spacing in monolayers. This implies that yeast cells maintain the same area per lipid molecule in both aerobic and anaerobic conditions in order to maintain the structural and functional integrity of the membranes. The studies on the effect of structural modifications of drugs or substrates on pharmacologic and enzymic reactions, respectively, also provide the inference that changes of 1-2 \AA in distances have profound effects on biological systems.

4. Hysteresis of Biological Surfaces: The pressure-volume plot of lungs, the surface pressure-area curves of lung extract films, or the films of mucin-lipids from meibomian glands in eyelids exhibit hysteresis during repeated compression and expansion of these systems. It is important to understand the molecular mechanism of the hysteresis phenomenon. From our investigation on various surface chemical systems, it appears (8-10) that there are two possible mechanisms for hysteresis in the film. First, the reduction in the surface pressure of a film upon re-expansion could be due to aggregation of molecules in the form of "islands." A strong cohesion between the molecules may prevent them from a rapid dissociation upon re-expansion and, hence, the number of individual molecules contributing to the surface pressure would be less during re-expansion of a previously compressed film. The second mechanism is shown to be the "penetration" and "squeezing out" of molecules upon expansion and compression of the film, as explained below.

We were able to isolate a surface-active lipoprotein from rabbit lung lavage by repeated centrifugation at 50,000 G. As shown by disc gel electrophoresis in the presence of sodium deoxycholate, this surface-active lipoprotein consisted of a single protein band and a lipid band (8). The gas chromatographic analysis of the fatty acid composition of the lipids of this lipoprotein showed that about 42% of the total fatty acids were unsaturated whereas 58% were saturated fatty acids. The insoluble monolayers of this lung lipoprotein did not exhibit any hysteresis during compression and re-expansion as shown in Figure 6. This clearly indicates that the molecules in a compressed film were able to resorb spontaneously upon expansion of the monolayer. However, when crude lung extract was injected under this insoluble monolayer, the surface pressure rose to about 26 dynes/cm due to penetration of soluble surfactants such as lipids and proteins into the lung lipoprotein monolayer. This mixed film made up of insoluble monolayer of lung lipoprotein and penetrated soluble lipids and proteins exhibited hysteresis upon compression and re-expansion. This observation suggested that the hysteresis is related to the "squeezing out" of the molecules from the film during the compression and their sub-

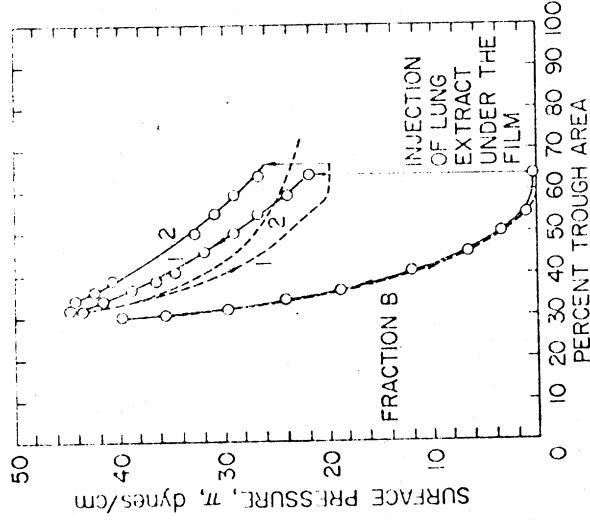


Figure 6: Hysteresis of monolayers of rabbit lung lipoprotein in the surface pressure area curves, before and after injection of 10 ml of soluble components of 0.9% saline minced lung extract (600 mg) in 400 ml of subsolution of 0.9% NaCl, pH 6.0 and 25 C. Solid lines indicate compression and broken lines expansion of the film.

sequent reentry into the film during expansion of the film. Therefore, the number of molecules in the film at the intermediate film area during expansion is always less than the number of molecules present at the same area of the film during compression of the film. Therefore, surface tension is higher during expansion as compared to that during compression of such penetrated monolayers.

5. Hydration of Surfaces and Protein Conformation:

If an air bubble is produced under the water surface, it bursts almost immediately as soon as it reaches the surface. However, when the surface is covered with a monolayer of lipids or proteins or polypeptides, the bubble will not burst instantly but will be stabilized by the film for a few seconds. At a first approximation, the stability or the survival time of the bubble depends upon the rate of drainage in the thin aqueous film at the interface as shown in Figure 7. The rate of drainage in the film depends upon the hydration of the film. As shown in Figure 7, if the polypeptide monolayer interacts strongly with water molecules, it will retard or decrease the rate of drainage of water in the thin layer. This would enhance the bubble stability and increase the survival time of bubble at the interface.

Figure 8A shows the data of Applequist and Doty (11), which indicates that random coil to helix transition in poly-L-lysine solution occurs in the pH range 10 to 11. Figure 8 shows our data on the bubble stability of stearic acid monolayers in the presence of poly-L-lysine in the subsolution. The bubble stability for stearic acid monolayers alone, which is not shown in Figure 8B, did not exceed 10-15 seconds over the whole pH range. It is evident from Figure 8B that at pH 11 the conformation of poly-L-lysine molecules, which is nearly helical, presumably affords maximum stability to the bubble lamellae. Figure 8C shows the poly-L-lysine solutions exhibit surface activity (or surface pressure) in the pH range 10-11. Although the surface pressure of poly-L-lysine is very low (approximately 3 dynes/cm²), it strikingly influences the bubble stability. The results shown in Figure 8B suggest that helical conformation affords surface activity to poly-L-lysine at pH 11, decreases the rate of drainage of water in the bubble lamellae and, hence, increases the bubble

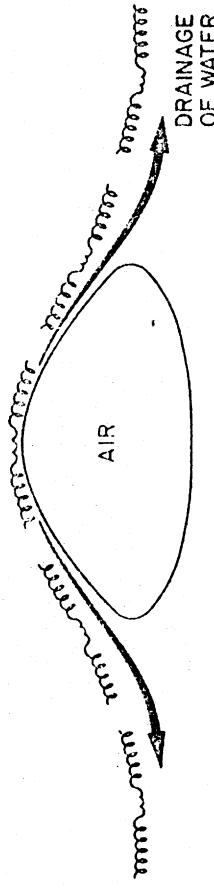


Figure 7: Schematic representations of the mechanism governing the stability of an air bubble covered with a film. A faster rate of drainage of water in the lamellae decreases the bubble stability.

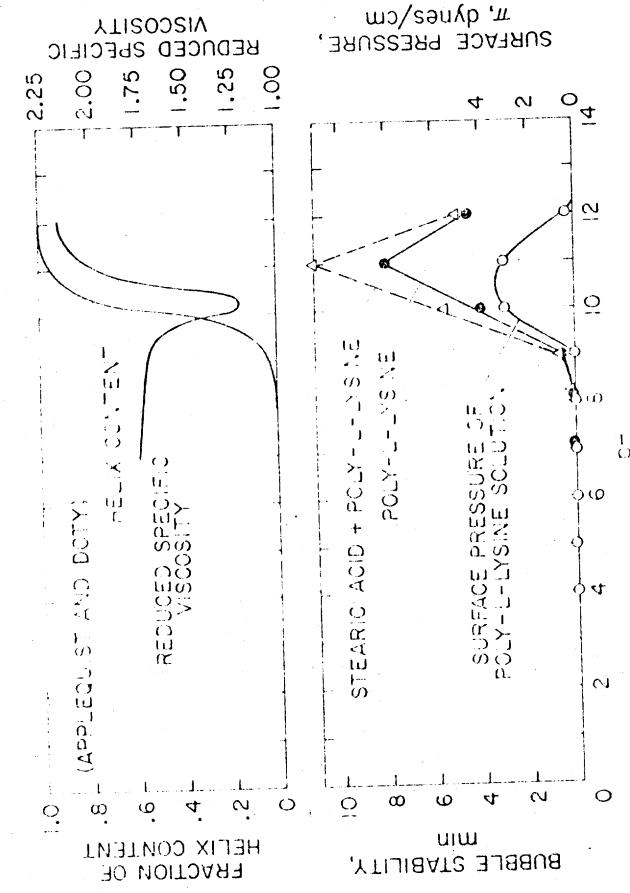


Figure 8: Data of Applequist and Doty on helix content and reduced specific viscosity of poly-L-lysine solutions (A). Surface pressure (or surface activity), and bubble stability of poly-L-lysine solutions with or without stearic acid monolayers (B). The bubble stability of stearic acid monolayers in the presence of poly-L-lysine is shown by a broken line, whereas that of stearic acid alone (not shown in the diagram) was 10-15 sec. in the whole pH range.

stability (12).

Figure 9 shows the survival time of air bubbles covered with the monolayer of the surface-active lipoprotein from lung at various surface pressures in the presence of 0.02 molar NaCl or 0.01 molar CaCl_2 . It is evident that the presence of calcium ions in the subsolution decreases the survival time by 75% as compared to that in the presence of Na^+ ions. This indicates that the presence of calcium presumably decreases the degree of hydration of lung lipoprotein monolayer. Analogous to this effect, it has been shown (13) that the presence of calcium in the lamellar liquid crystalline structure of phosphatidyl-water system exhibits a striking decrease in the thickness of water layers in the presence of calcium ions. Therefore, it appears that the valency and concentration of electrolytes are expected to influence the hydration of biological surfaces.

6. Rheology and Phase Transition of Surfaces:

It should be emphasized that analogous to the state of matter in three dimensions, the matter in two dimensions, such as monolayers, can also exist as two-dimensional solids, liquids, and gases. The monolayers also exhibit the phase transitions from one state to another depending upon the temperature, the state of compression, and the structure of the film forming molecules. It is very interesting to compare the melting point of materials in three-dimensional crystalline state vs. two-dimensional monolayers. For many compounds, such as fatty acids, the melting point in monolayers and crystalline state are approximately the same. However, for compounds such as cholesterol, the melting point in crystalline form is 148.5°C whereas its melting point in monolayer is below 0°C, indicating that for temperatures above 0°C cholesterol monolayers always exist as a two-dimensional liquid. The biological role of cholesterol presumably can be attributed to the two-dimensional state of cholesterol and it is only in the disease conditions that one observes the deposition of crystalline form of cholesterol in tissues, e.g. atherosclerosis.

Shah and Schulman (14) have shown that in mixed monolayers of cholesterol-lecithin or cholesterol-diethyl phosphate, the cholesterol acts as a liquifying agent. A minimum of 10-15 mole percent of cholesterol liquifies the solid monolayers of other

lipids. This is presumably due to a planar, asymmetric structure of cholesterol molecules.

It has been known (15) that the presence of calcium ions in the subclotting strikingly increases the surface viscosity of fatty acid monolayers. The changes in local surface viscosity and phase transitions of membranes may occur as a result of changes in the local concentration of calcium ions or pH, which may be associated with cellular processes, such as cell locomotion, guidance, and electrical excitability.

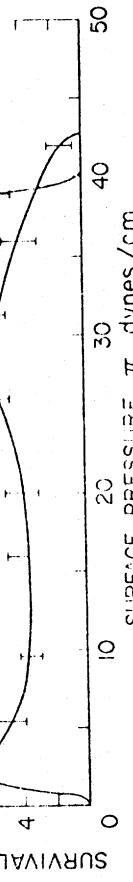


Figure 9: The effect of 0.01 M CaCl_2 and 0.02 M NaCl on the survival time of bubbles stabilized by the lung lipoprotein monolayer at various surface pressures, $T = 25^\circ\text{C}$.

7. Cooperativity Between Molecules at Surfaces:

The difference between the surface pH and bulk pH as discussed above can be considered as a typical example of cooperativity among charges on the surface which results in an attraction or repulsion of H^+ ions from the surface. Another example of such cooperativity between the molecules in the surface is given by the pK values of fatty acids of increasing chain length. It is known that the pK of acetic acid is 4.8. Hence, at this pH value, 50% of the molecules are in the ionized form and the other 50% are in the nonionized form. However, as the chain length of these fatty acids increases, such as hexanoic, octanoic, decanoic, lauric acid, etc., the pK value also increases. It has been shown that the pK of stearic acid monolayers is 9.3 (16, 17).

The change of pK from 4.8 to 9.3 cannot be explained by the "surface pH effect" alone, since a change of 4.5 pH units in pK value would require ψ_0 of 276 mV which is too high for stearic acid monolayers. The increasing pK values with increase in the chain length rather suggests the cooperativity between fatty acid molecules. As the chain length increases, the tendency for the association also increases due to Van der Waals attraction between the hydrocarbon chains. As the chain length increases, the average area per molecule decreases and hence the carboxyl groups approach one another closely. Once the initial ionization occurs, the further ionization is prevented because the attraction of negatively charged oxygen on the remaining hydrogen atoms in the plane of the carboxyl groups. Therefore, at a considerably high pH or high OH ion concentration, the dissociation of remaining carboxyl groups occurs. In other words, when molecules are arranged with specific orientation in a

plane, their effect on any molecule or ion in the vicinity is a collective action of all of the molecules present in the plane. This cooperativity is of great importance in relation to membrane phenomena, because the molecules in membranes can exhibit similar cooperativity in their properties.

In order to understand the structure of water at interfaces, we have been studying extensively the structure of microemulsions in recent years using electrical, birefringence and high resolution NMR techniques (18, 19). We found that when water-in-oil microemulsions are converted to oil-in-water type, it forms a lamellar liquid-crystalline structure in the phase-inversion region. We use this lamellar liquid crystalline structure as a model system to investigate the effect of local anesthetic agents shown in Table I and calcium ions on the structure of this system. It was observed that as the drug was added to the birefringent lamellar liquid-crystalline structure, at a certain concentration of the drug or calcium, the birefringence was completely destroyed and the system became isotropic. Surprisingly, although the drug molecules or calcium ions are charged species, the electrical resistance increased upon addition of these agents. It was concluded from the experimental results that these drugs or calcium ions converted the lamellar liquid crystalline structure into a water-in-oil microemulsion (20). Table I shows the number of calcium ions or drug molecules per 100 lipid molecules in the lamellar structure to cause 50% change in the electrical resistance of this system. It is clear from Table I that for every 100 lipid molecules in the lamellar structure only about 2 calcium ions are needed to cause the phase transition from the lamellar structure to spherical one (Fig. 10). The number of drug molecules needed for this phase transition are between 5 and 10 molecules for every 100 lipid molecules. These results clearly show the cooperativity between molecules at surfaces. The number of drug molecules needed for various drugs are in the same order as their clinical potency. The most potent drug requires the smallest number of molecules to cause this phase-transition. Since on the average one calcium ion influences 50 lipid molecules, this is not an example of stoichiometric association but rather a valid example of long range interaction

TABLE I
Molar ratio of drug to Potassium Oleate

Drug	Dose at 50% increase in electrical resistance (mM drugs/ml L.C.)	Potassium Oleate	Molar ratio of drug to Potassium Oleate
Ca ⁺⁺	0.00430	0.1325	5.74/100
Tetraacaine	0.01457	0.31/100	8.75/100
Cocaine	0.02020	0.75/100	10.2/100
Procaine	0.02355		

The Concentration and the Molar Ratio of Drug to Potassium Oleate Required to Cause a 50% Increase in the Electrical Resistance of the Lamellar Liquid-Crystalline Structures.

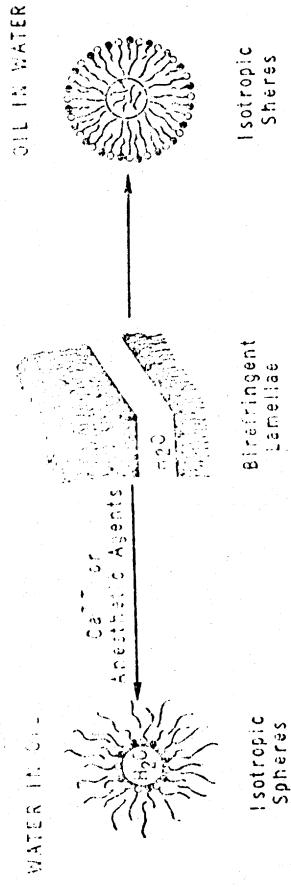


Figure 10: The effect of addition of CaCl_2 or local anesthetic agents on the lamellar liquid-crystalline structures, composed of potassium oleate, hexanol, hexadecane and water. The system becomes oil-continuous upon addition of these agents.

or cooperativity among molecules at surfaces. It should be pointed out that it has been reported (21) that for the state of anesthetic to occur, the concentration of anesthetic agents in the brain tissue has to correspond to about 6 molecules of anesthetic agent for every 100 lipid molecules in the brain. Therefore, this lamellar liquid-crystalline model system exhibits the same sensitivity toward drugs for its phase transition as that shown by brain tissue to anesthetic agent for the induction of state of anesthesia.

Recently, Rothen (22) has done considerable work on immunological reactions and enzymatic hydrolysis of adsorbed films of protein when the antigen and antibody or enzyme and substrate are separated by 200–300 Å thick formvar film. He has suggested that cooperativity among the surface atoms is believed to induce a cooperativity among protein molecules, which results in long-range interaction in these systems. The stability of α -helix of proteins and double helix of DNA can be attributed to cooperativity of a large number of hydrogen bonds and stacking interaction of nucleotides in these polymers. From the theoretical and experimental considerations, it appears that cooperativity among molecules at surfaces is probably one of the most important characteristics of biological surfaces and yet, it is a very poorly understood area of scientific research.

SUMMARY

The major points discussed in this article can be summarized as follows:

1. Membrane lipids and proteins, as well as many drugs and pharmacological agents, are surface-active. The surface-active molecules adsorb at the interface resulting in a considerably higher concentration at the interface than in the bulk solution.
2. The pH near a charged surface is different from the pH in the bulk. The pH near the negatively charged surface is less and for positively charged surfaces greater than the bulk pH. For the electrical potential of 100 mv at the surface, the difference in the bulk and surface pH values is about 1.63 pH units.
3. The unsaturation of fatty acid chains of phospholipids increases the average area per molecule, which subsequently increases the intermolecular spacing in monolayers. This strikingly

- influences the enzymatic hydrolysis, the interaction of divalent cations, and the association with cholesterol of phospholipid monolayers.
4. The hysteresis of lung surfactant films is associated with the penetration and squeezing out of the soluble surface-active molecules, such as lipids and proteins, during the expansion and compression of the surface film.
 5. The helical conformation of a polypeptide has a greater hydration and, hence, affords a greater bubble stability as compared to the random-coil conformation.
 6. The biological properties of lipid-molecules are attributable to two-dimensional properties of lipids. The phase transition of lipids, e.g., cholesterol, in two-dimensional form are most relevant to their biological function.
 7. The cooperativity among molecules at the surface is responsible for greater sensitivity of biological surfaces to changes in the adjacent region.
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DISCUSSION

KARNOVSKY: I was first stimulated to take cognizance of the tremendous importance that surface phenomena have in biology by Dr. Schulman's remarks at a meeting in Italy some years ago, to which we had come to discuss the structure and function of biological membranes. He, in a very characteristic way, urged us to begin thinking about the sort of things you so beautifully summarized this morning. The message that comes out of your seven points is the complexity that must come to mind when we consider that phenomena in each of those seven listed categories affect phenomena in some or all of the other categories. With this realization in mind, we must attack the very complex particular biological situation that we're here to discuss.

I have one or two specific questions. First I think it is intriguing to speculate teleologically on the question of why it is that nature has selected, apparently, as a surfactant molecule in the lung, this particular lecithin, that indeed has the very smallest intermolecular spacing. Is it because it is more stable? I would gather from your pictures (although I cannot interpret the pressure on the absciss in terms of lung alveolar pressure) that this lecithin, in addition to whatever other qualities it has, is going to be the most stable species of lecithin that one could design for the specific purpose? Secondly, I would go back to the previous paper and wonder whether we're not, because of our sophistication in metabolic and enzymatic fields, looking into questions of the biosynthesis of the lecithins and its control in a way that is over complicated in relation to the disease per se. I wonder (and this is going back really to your talk, Dr. Weinhold) whether anybody has made adequate studies of the exchange phenomena, and perhaps the enzymes that might be present (acyl exchange enzymes). Wouldn't this particular most stable lecithin in the lung become predominant over a period of time? In other words, do we really have to look for specific biosynthetic mechanisms to give us this molecule; are there not phenomena that arise from the sort of things that you have described that might give us these molecules?

Finally I shall say that I was very intrigued by your comments on the simple titration of oleic acid because I recollect that about 15 years ago we reported the titrations of a whole bunch of phosphatides and fatty acids. Because of unsophistication of phosphatides and fatty acids. Before we took pains to titrate only the relevant group, that is the carboxyl, and of course we found that all the carboxyls from acetate up to oleic had virtually the same pK under those conditions. So I'm very struck by what you have pointed out, i.e. that when one has a set of molecules lined up in these kinds of surface arrangements, one has to recognize that the pK of the carboxyl group is going to be a reflection of the total structure.

WEINHOLD: In response to your question, I don't know of any study of the exchange phenomenon, but there are enzymes known that can remodel lecithins to give a certain fatty acid composition, hence acyl transferases may in fact be very important. If you look at the total phospholipid content of the fetal lung versus the total phospholipid content of the adult - the total phosphatidyl choline - this increases. There is a net increase in the bulk of the phospholipid and so you can't just change the fatty acids - you've got to make more of the total molecule.

KARNOVSKY: Yes, but it's the level of a particular phospholipid in this disease that concerns us at the moment and I wonder whether we're looking too far back in the biosynthetic process.

THOMAS MORGAN: In thinking of the problems of presenting the enzymes, the lipids, the organization of the lung and the surfaces and surface phenomenon, and getting them all together, I am reminded of the famous Gluyas Williams cartoon showing the swimming pool at Proctor and Gamble on the day that the bar of Ivory soap sank (laughter). I have a strange feeling that one of the things that ought to be said here is that the lung is the bar of soap to some extent and the problems Dr. Weinhold has talked about and the detergent effect, the problems that Dinesh has talked about, the seven rules, all add up really to a simple question in my mind -- How are the enzymes and lipids arranged in lung? Do the lipids flocc in enzymes? The majority of in vitro effects have to be looked at all together. We have to look at the effects of the lipid at the surface, the effect of the lipids on the enzymes, and so on.

SHAH: I would like to reply to Dr. Karnovsky's question about dipalmitoyl lecithin -- why did nature pick out this particular lecithin as a surfactant? If one of the major surface active species is in fact a lipoprotein, then from the viewpoint of lipid-protein association it is important that the amino acids in the protein have predominantly non-polar side chain residues so that the protein and lipid could interact by hydrophobic forces. A lecithin with two saturated fatty acid residues would interact better by such hydrophobic forces with a protein than would a lecithin with one saturated and one un-

saturated fatty acid. Furthermore, if we examine this just from the viewpoint of the phospholipid and examine its two dimensional surface elasticity as was done by Dr. Schechter at Austin, Texas. In such experiments a tension particle is sitting on top of the film and you observe when you measure the ratio of surface oscillation to bulk oscillation, which is a measure of the surface elasticity of the film, that there is a certain inherent frequency at which the film shows maximal elasticity. For dipalmitoyl phosphatidic acid optimal elasticity is shown at the same rhythmic frequency of breathing, that is about six per minute. This suggests there may be yet another way in which this particular phospholipid has optimal qualities to serve as a surface active material in the lung. This is, this particular kind of substance shows its maximal elasticity at the same frequency at which normal breathing occurs.

BLOCH: I was very much impressed by the analytical data on the slide that you showed, Dr. Masoro, relative to the fatty acid content of the phospholipid in fetal lung, which is largely C-14, is that correct?

MASORO: Yes, those were the data from Dr. Gluck's analyses.

BLOCH: Now has the "elasticity" of this particular lecithin been compared with that of the dipalmitoyl compound? I think there is a very sharp discontinuity between the C-14 and C-16 compounds.

SHAH: Yes, the elasticity of the compound is due in large part to the thermal motion of the chain, the C-14 compound with its shorter chain would have greater elasticity than the C-16.

BLOCH: I wondered about that because it was my impression that fetal liver, indeed all fetal tissues, are unable to make unsaturated fatty acids. Perhaps they substitute for their shorter chain saturated fatty acids which would have properties similar to the longer chain unsaturated fatty acids.

KARNOVSKY: Is the C-14 chain mainly in the beta position of the lecithin?

WEINHOLD: Yes, it is.

KARNOVSKY: Then that would fit in well with your comment.

MASORO: Let me ask you a broader question. I had the understanding from reading Dr. Small's work that when dealing

with diacyl lecithins free of any other component that this lecithin makes a surface monolayer which does not interact with the bulk liquid beneath it either as a monomer or a micelle. Is this true?

SHAH: I think so. If you are going to keep the film at zero surface pressure, then there is no interaction of the film with the bulk. However, if you maintain that film at a high pressure, then the molecules have a very high tendency for aggregation. You have all sorts of molecular aggregations within the film and as a result some of these micelles may enter into the bulk solution beneath the film.

CLEMENTS: May I ask a question? You mentioned the relationship between pH and the configuration of the film. It appears that in respiratory distress syndrome there are changes in pH in the lung. However, the changes are a few tenths of a pH unit. In your figures I noticed that you used a very wide pH range and that the changes in conformation occurred over this wide range. Do these changes with pH become more subtle with increasing chain length?

SHAH: Well, I think you are all familiar with the fact that the pK value of the carboxyl group of the fatty acid represents the pH around which there is the greatest change in the ratio of ionized to unionized group as pH changes. Hence, a small change in pH may be significant if it occurs right around the pK of the fatty acid itself. A change of 0.1 pH unit may result in a large change in the ionized form.

CLEMENTS: Thus, if you are dealing with C-16 fatty acids the greatest sensitivity would be observed between pH 7 and pH 8.

SHAH: That's right.

CLEMENTS: That seems to fit very nicely physiologically and pathophysiologically.

VILLEE: I think we are rapidly coming to the realization that even at this molecular level evolution has resulted in a peculiarly exact adaptation of optimal molecular structure for molecular function.