ces regarding the interactions of polypeptides and nucleic acids. The coplanar interaction of lysine side chains and adenine rings suggests that exposed adenine rings in adenine–thymine-rich regions of nucleic acids may serve as important binding sites for regions of polypeptide chains having amino acid residues of appropriate structure.

Finally, these studies have important implications for the synthesis of drugs intended to interact with specific regions of nucleic acids. Such drugs should be designed to take advantage of the specificity that can be achieved by cooperative self-association once initial attachment is made to the nucleic acid.

REFERENCES

(1) G. Zubay and P. Doty, Biochim. Biophys. Acta, 29, 47(1958).

(2) M. Leng and G. Felsenfeld, Proc. Nat. Acad. Sci. USA, 56, 1325(1966).

(3) J. T. Shapiro, M. Leng, and G. Felsenfeld, Biochemistry, 8, 3219(1969).

(4) K. Mueller, Mol. Gen. Genet., 111, 273(1971).

(5) P. H. von Hippel and J. D. McGhee, Ann. Rev. Biochem., 41, 231(1972).

(6) H. W. Schaup, M. Green, and C. G. Kurland, Mol. Gen. Genet., 109, 193(1970).

(7) J. Applequist and P. Doty, in "Polyamino Acids, Polypeptides and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p. 161.

(8) J. M. Rifkind, Biopolymers, 8, 685(1969).

(9) M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, J. Amer. Chem. Soc., 90, 1042(1968).

(10) C. E. Bugg, J. M. Thomas, Sundaralingam, and S. T. Rao, Biopolymers, 10, 175(1971).

(11) C. R. Woese, Proc. Nat. Acad. Sci. USA, 59, 110(1968).

(12) J. C. Lacey and K. M. Pruitt, Nature, 223, 799(1969).

(13) K. G. Wagner and R. Arav, Biochemistry, 7, 1771(1968).

(14) K. G. Wagner, Eur. J. Biochem., 10, 261(1969).

(15) K. Wulff, H. Wolf, and K. G. Wagner, Biochem. Biophys. Res. Commun., 39, 870(1970).

(16) J. M. Rifkind and G. L. Eichorn, Biochemistry, 9, 1753(1970).

(17) P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, J. Amer. Chem. Soc., 85, 1289(1963).

(18) R. Arav and K. G. Wagner, Eur. J. Biochem., 13, 267(1970).

(19) K. G. Wagner and K. Wulff, Biochem. Biophys. Res. Commun., 41, 813(1970).

(20) K. Wagner, in "Proceedings of the First European Biophysics Congress," E. Broda, A. Locka, and H. Springer-Leder, Eds., Verlag der Wiener Medizinischen Akademie, 1972, p. 359.

(21) J. C. Lacey, Ph.D. thesis, University of Alabama, Birmingham, Ala., 1969.

(22) M. Razka and M. Mandel, Proc. Nat. Acad. Sci. USA, 68, 1190(1971).

(23) T. Montenay-Garestier and C. Hélene, Biochemistry, 10, 300(1971).

Drug–Biomolecule Interactions:
Mechanisms and Kinetics of Interactions of
Biomolecules at Interfaces

FEDERICO A. VILALLONGA

Abstract □ At the air–water interface, the area occupied by the molecules of each component of mixed monomolecular layers of cholesterol with hexadecyl alcohol, hexadecylic acid, or hexadecylamine was independent of the presence of the other component. The values of the free energy of mixing of these lipids were within the range of the entropic factor of the free energy even at high surface pressures. Mixed monolayers of cholesterol and bovine serum albumin showed a similar independence of the area per molecule and of the free energy of mixing values when the concentration of the protein was expressed in terms of amino acid residues per molecule of protein forming the mixed monolayer. Higher values of the free energy of mixing were obtained for mixed monolayers of cholesterol with dipalmitoyl lecithin and dipalmitoyl phosphatidylethanolamine than were expected from an entropic factor. The interaction between monomolecular layers of lipidic biomolecules with bulk subphase components, the energy of activation, interaction kinetics, and effects of added electrolytes were also studied. The implications of these data to a mechanism of action are discussed.

Keyphrases □ Phospholipid monolayer interactions—mechanisms and kinetics at interfaces □ Interfaces—mechanisms and kinetics of interactions, surface pressure and surface potential □ Monolayers, phospholipid—mechanisms and kinetics of interactions of biomolecules of interfaces □ Drug–biomolecule interactions—mechanisms and kinetics of interactions of biomolecules at interfaces □ Interactions—drugs with biomolecules, symposium

Drug molecules incorporated into body fluids are distributed and directed to receptors located in tissue cells. If the receptors form part of the cell membrane, as seems to be the case for some local anesthetics (1) and bactericides (2), the interaction with the receptor alters the permeability of the cell membrane and thus completely defines the pharmacological activity of the drug. If the receptors are located in subcellular
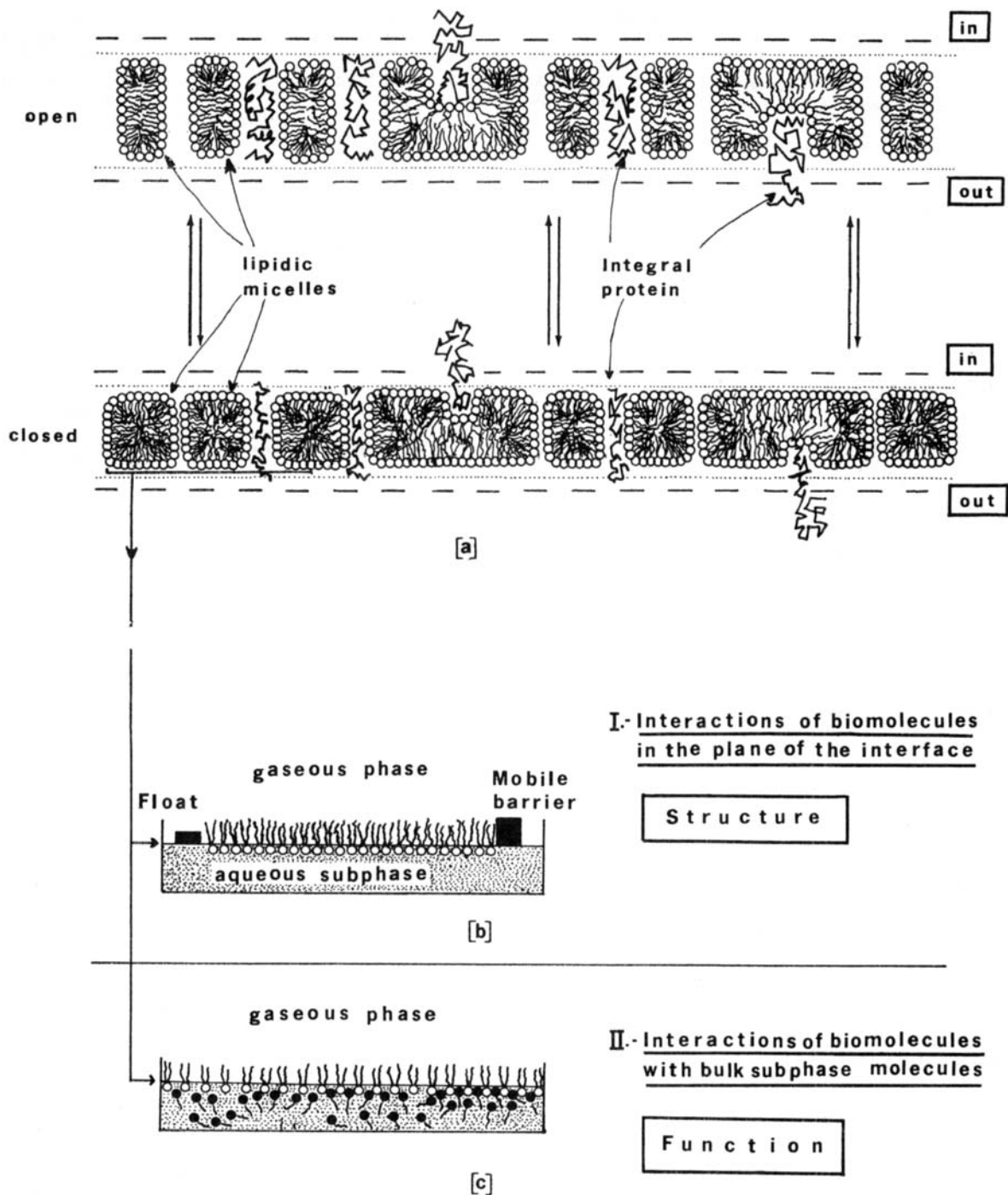
Figure 1—*Speculative diagrammatic representation of the changes of micellar form and the reversible transformation from the open to the closed configuration of biomembrane. Dashed lines represent the limits of the membrane; dotted lines represent the oriented layer of water.*

organelles, as seems to be the case for many antibiotics (2), the drug molecule must penetrate through the cell membrane to find its way to the receptor.

It follows then that, in all cases, the first stage in the exercise of the pharmacological action of a drug must be the interaction of the drug molecule with the cell membrane components.

DISCUSSION

It is not possible here to discuss the many different models of membrane structure that have been proposed since the Danielli–

Dawson model (3). The reader is referred to Ref. 4 for this purpose. Biological membranes are composed mainly of lipids and proteins and maintain their integrity because of the interactions between their components. Figure 1a represents a speculation that brings together the "open–closed" hypothesis (5) with the existence of lipid bilayers between repeating "integral protein" units (6).

Changes in the local charge distribution in the thin crust of water that is oriented at the membrane interface provoke reversible variations of the micellar forms. In turn, they produce the reversible transformation of an "open" state to a "closed" state of biomembranes (5). Drug molecules approaching the cell interface can produce these changes.

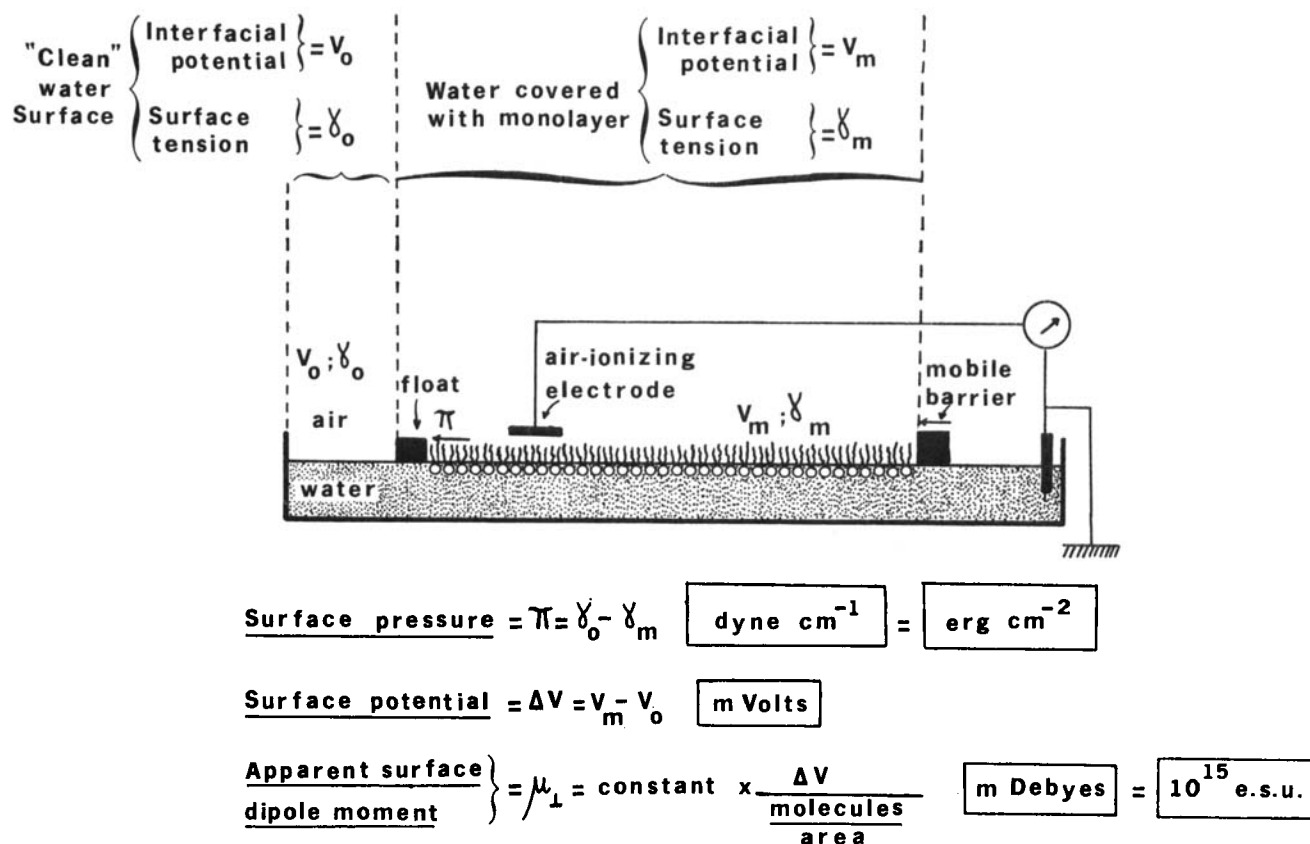The study of the interactions in the plane of the interface be-

Figure 2—*Schematic representation of the surface pressure, surface potential, and apparent surface dipole moment.*

tween biomolecules of the type found in biomembranes (Fig. 1b) may yield useful information on the orgin and strength of the forces involved in the maintenance of the integrity of biomembranes.

The study of the interaction between monomolecular layers of biomolecules similar to those that form the cell membranes with molecules that approach from the bulk subphase (Fig. 1c) can further the understanding of the role of specific components of biomembranes in the interaction and of how the approaching molecule can affect the microstates of biomembranes.

To define the state of the molecules forming a monomolecular layer (7), three primary parameters are used: *(a)* the area per molecule, *(b)* the surface pressure, and *(c)* the surface potential. The area per molecule is simply obtained by dividing the total surface area of the interface by the number of molecules forming the monomolecular layer.

The surface pressure, π (Fig. 2), a force acting in a plane parallel to the interface, is defined as the difference between the surface tension of the "clean" air–water interface and the surface tension of the water covered with the monomolecular layer. The surface potential, ΔV, which corresponds to the summation of the contributions of each molecule to the interfacial potential of the monomolecular layer, is defined by the difference between the interfacial potential of the monomolecular layer and the interfacial potential of the clean air–water interface. The apparent surface dipole moment per molecule, μ, which is the contribution of each molecule to the interfacial potential of the monomolecular layer, is defined as proportional to the ratio of the surface potential to the number of molecules per unit area of the monomolecular layer.

Interactions between Biomolecules in Plane of Interface—
Pure, single lipids and proteins, when spread at an interface, form monomolecular layers which can be studied and characterized by the measurement of their surface parameters. Binary mixtures of these substances spread at the air–water interface may show values of their surface parameters that adhere to or depart from those expected from the addition of the values corresponding to each single, isolated component. Analysis of the experimental data obtained can give information about interactions between the components.

Figure 3 represents the plot of the mean area per molecule, A_{ab}, obtained by dividing the total surface area by the *total* number of molecules that form the monolayer (Component a + Component b) against the molar fraction of the binary mixed monolayer (N_a or N_b) (8). The broken line (Fig. 3, line I) is obtained with the experimental points when either nonmixing or ideal mixing of the components occurs. In both cases the area occupied by each molecule of Components a and b is identical to the area that these molecules occupy when they are in a single, pure monomolecular layer of each component. The additivity rule is obeyed and for any composition of the mixed monolayer the mean surface area per molecule is given by:

$$A_{ab} = N_a A_a + N_b A_b \qquad \text{(Eq. 1)}$$

in which A_{ab} is the mean area per molecule in the binary mixed monomolecular layer; and N_a, N_b, A_a, and A_b are the molar fractions of Components a and b and the area per molecule of each of these components, respectively, when they are in a single, pure monolayer at the same conditions of surface pressure and temperature.

The enthalpy of mixing for an ideal mixing process, $\Delta H_M{}^I$, is equal to zero and from Eq. 2:

$$\Delta G_M{}^I = \Delta H_M{}^I - T \Delta S_M{}^I \qquad \text{(Eq. 2)}$$
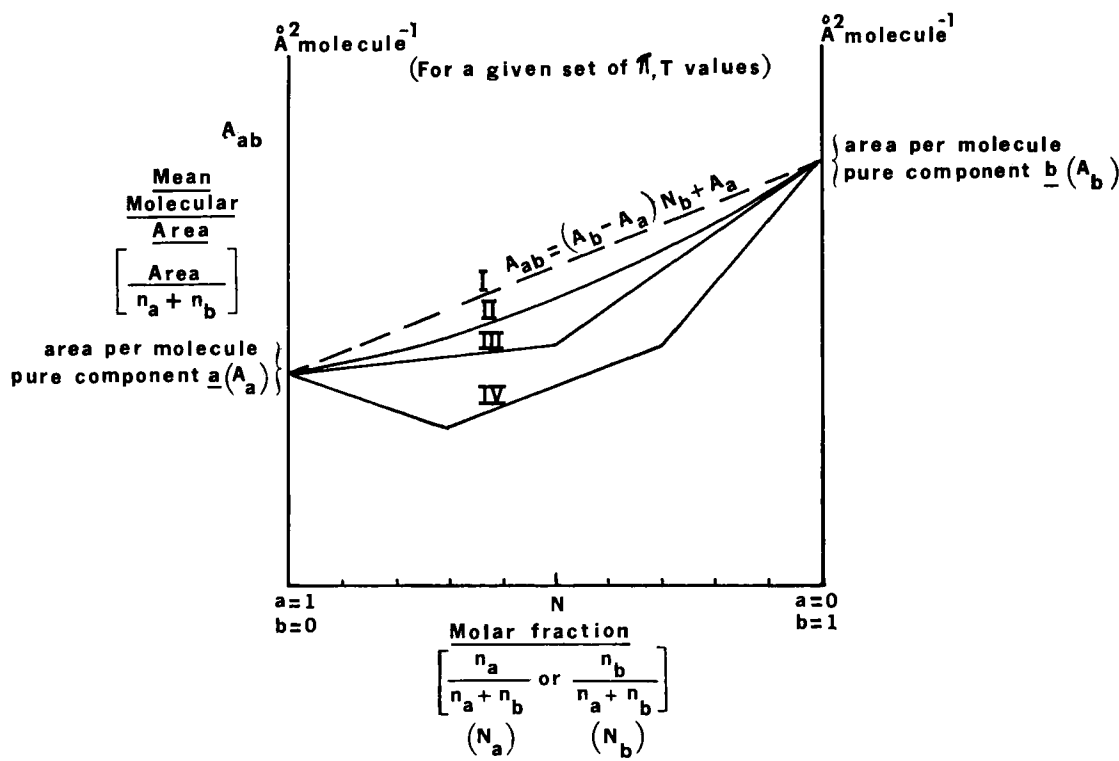
it follows that:

$$\Delta G_M{}^I = -T \Delta S_M{}^I \qquad \text{(Eq. 3)}$$

which states that the free energy of mixing, $\Delta G_M{}^I$, or the "driving force" of the mixing process is entirely of entropic origin, $\Delta S_M{}^I$, and is a function only of the molar fraction:

$$\Delta G_M{}^I = RT N_a \ln N_a + RT N_b \ln N_b \qquad \text{(Eq. 4)}$$

in which R is the universal gas constant, T is the temperature (°K), and N_a and N_b are the molar fractions of Components a and b in the binary mixed monomolecular layer, respectively.

The full lines II, III, and IV (Fig. 3) are obtained when conden-

$\overset{\circ}{A}{}^2 molecule^{-1}$ $\overset{\circ}{A}{}^2 molecule^{-1}$

(For a given set of π,T values)

A_{ab}

Mean
Molecular
Area

$\left[\dfrac{Area}{n_a + n_b}\right]$

$A_{ab} = (A_b - A_a) N_b + A_a$

{area per molecule
{pure component $\underline{b}$ (A_b)

area per molecule }
pure component $\underline{a}(A_a)$ }

I

II

III

IV

a=1   N   a=0
b=0   b=1

Molar fraction

$\left[\dfrac{n_a}{n_a + n_b} \text{ or } \dfrac{n_b}{n_a + n_b}\right]$

(N_a) (N_b)

I.- Non mixing or ideal mixing; $A_{ab} = N_a A_a + N_b A_b$ }
                                      } **Additivity rule**
$\Delta V_{ab} = N_a \Delta V_a + N_b \Delta V_b$ }

II.- Partial condensation of a fully expanded component   }
                                          } (Cadenhead
III.- Complete condensation of a fully expanded component   } and Phillips)
                                          }
IV.- Condensation of expanded component with intermediate region }

Figure 3—*Plot of the mean molecular area against molar fraction in mixed monolayers.*

sation effects occur on an expanded Component b because of the presence of Component a in the mixed monomolecular layer. Plots of the III and IV type are typically obtained at different surface pressures for mixed monomolecular layers of cholesterol and lecithin (9–13). The presence of sharp changes of slope at a given composition was taken to indicate (9) the formation of molecular complexes. This concept involves the existence of some energy of interaction between the cholesterol and lecithin molecules forming the complex.

The negative departure from the additivity rule can be explained in this particular case without referring to the postulation of complex formation (14, 15). In the expanded liquid state, adjacent lecithin molecules with their fatty acyl chains could generate an empty volume similar to an inverted cone that constitutes a "cavity" which can be "filled" with cholesterol molecules without causing an increase in the monomolecular layer.

A thermodynamic treatment (16) can be used with some restrictions (8, 12, 16–18) for the experimental evaluation of the free energy of mixing, ΔG_M, of the components of a mixed monolayer. However, evidence was presented recently (19) which indicates that the thermodynamic interpretations in the case of dipalmitoyl lecithin systems are tenuous and probably incorrect.

The excess free energy of mixing, $\Delta G_M{}^E$, is given by:

$$\Delta G_M{}^E = \int_{\pi^*}^{\pi} (\delta_{ab} - N_a \delta_a - N_b \delta_b) \, d\pi \qquad \text{(Eq. 5)}$$

where δ_{ab}, δ_a, and δ_b represent the area of the mixed monolayer

and the areas of the pure, single monolayers of Components a and b, respectively; N_a and N_b represent the respective molar fractions, π is the surface pressure, and π^* is usually taken as zero.

The integrals $\int_{\pi^*}^{\pi} \delta_{ab} \, d\pi$, $\int_{\pi^*}^{\pi} \delta_a \, d\pi$, and $\int_{\pi^*}^{\pi} \delta_b \, d\pi$ can be separately evaluated by weight integration of the compression curve of the mixed monolayer, the monolayer of pure Component a, and the monolayer of pure Component b.

The free energy of mixing can be calculated (20) using:

$$\Delta G_M = \Delta G_M{}^E + \Delta G_M{}^I \qquad \text{(Eq. 6)}$$

in which $\Delta G_M{}^I$ (see Eq. 4), being independent of the chemical nature of the components, can be numerically calculated for each value of the molar fraction.

Table I represents the results obtained by the application of this method of mixed monolayers of cholesterol and dipalmitoyl lecithin, dipalmitoyl cephalin, hexadecylamine, hexadecanol, and palmitic acid (17).

The significance of cholesterol–phospholipid interactions in the maintenance of the integrity of some biological membranes has been emphasized (21, 22). The free energy of mixing for a binary system is a measure of the tendency of its components to undergo a dissolution process. From Table I, it can be seen that the most negative value for all mixtures studied corresponds to the systems of cholesterol–dipalmitoyl lecithin and cholesterol–dipalmitoyl cephalin, which are roughly 200 cal/mole more negative than those corresponding to the ideal system or to cholesterol–hexadecanol and cholesterol–palmitic acid. The greater tendency for dissolution

Table I—Free Energy of Mixing (ΔG_M, cal/mole) at Air–Water Interface for Mixtures of Cholesterol with Phospholipid or C_{16} Hydrocarbon Chain Compounds[a]

	Molar Fraction				
	0.20	0.33	0.50	0.67	0.80
β,γ-Dipalmitoyl-DL-α-lecithin	−330	−480	−630	−510	−300
	(−210)	(−450)	(−460)	(−460)	(−300)
β,γ-Dipalmitoyl-DL-α-cephalin	−430	−440	−640	−430	−340
	(−300)	(−250)	(−430)	(−310)	(−220)
Hexadecylamine	(−240)	(−350)	(−390)	(−340)	(−280)
1-Hexadecanol	−180	−260	−370	−250	−200
	(−220)	(−290)	(−380)	(−280)	(−230)
Palmitic acid	−380	−460	−450	−350	−280
	(−330)	(−430)	(−430)	(−350)	(−280)
Ideal system, $\Delta G_M^I =$ $RT(N_1 \ln N_1 + N_2 \ln N_2)$	−290	−370	−400	−370	−290

[a] Temperature, 20°; subphase, phosphate buffer (pH 7.2, ionic strength 0.15). Integration limits are 0 and 40 dynes/cm. Figures in parentheses correspond to the integrals between 0 and 27.5 dynes/cm.

of the phospholipids in equimolecular amounts of cholesterol at the air–water interface could originate from some interaction between cholesterol and these phospholipids.

The difference between the values of the free energy of mixing for both pairs of integration limits in the cholesterol–phospholipid systems seems to indicate that a closed, packed configuration is necessary for enhancement of mutual dissolution. Since this effect is not observed for systems containing cholesterol and hexadecanol or palmitic acid, it may be concluded that some geometrical molecular arrangement of the phospholipid molecules is necessary to increase the mutual dissolution between these molecules.

This type of deviation from the additivity rule has been observed in many mixed monomolecular layers involving lipids known to form biomembranes. From the results reported (8, 12, 13, 17, 23), it seems that specific interactions between groups cannot explain all of the experimental facts. Cavity effects and van der Waals forces between the methylene groups of the hydrocarbon

chains probably are involved in this interaction. The overall picture obtained with the results points out that dispersive or van der Waals forces may constitute a main factor in the maintenance of the structure of the lipid micelles of biomembranes.

Biomembrane models suggest that proteins could be structural as well as functional components of biomembranes and that lipid–protein interactions could play an important role in the structure of biomembranes. It was suggested (24) that cholesterol monomolecular layers at the air–water interface interact with proteins injected into the subphase because of specific forces that involve the hydroxy group of cholesterol and the peptide bond of bovine serum albumin. However, the compression curves of mixed monomolecular layers of cholesterol and bovine serum albumin show no significant departure from the additivity rule in the plot of the mean molecular area as a function of the molar fraction (Fig. 4) (25).

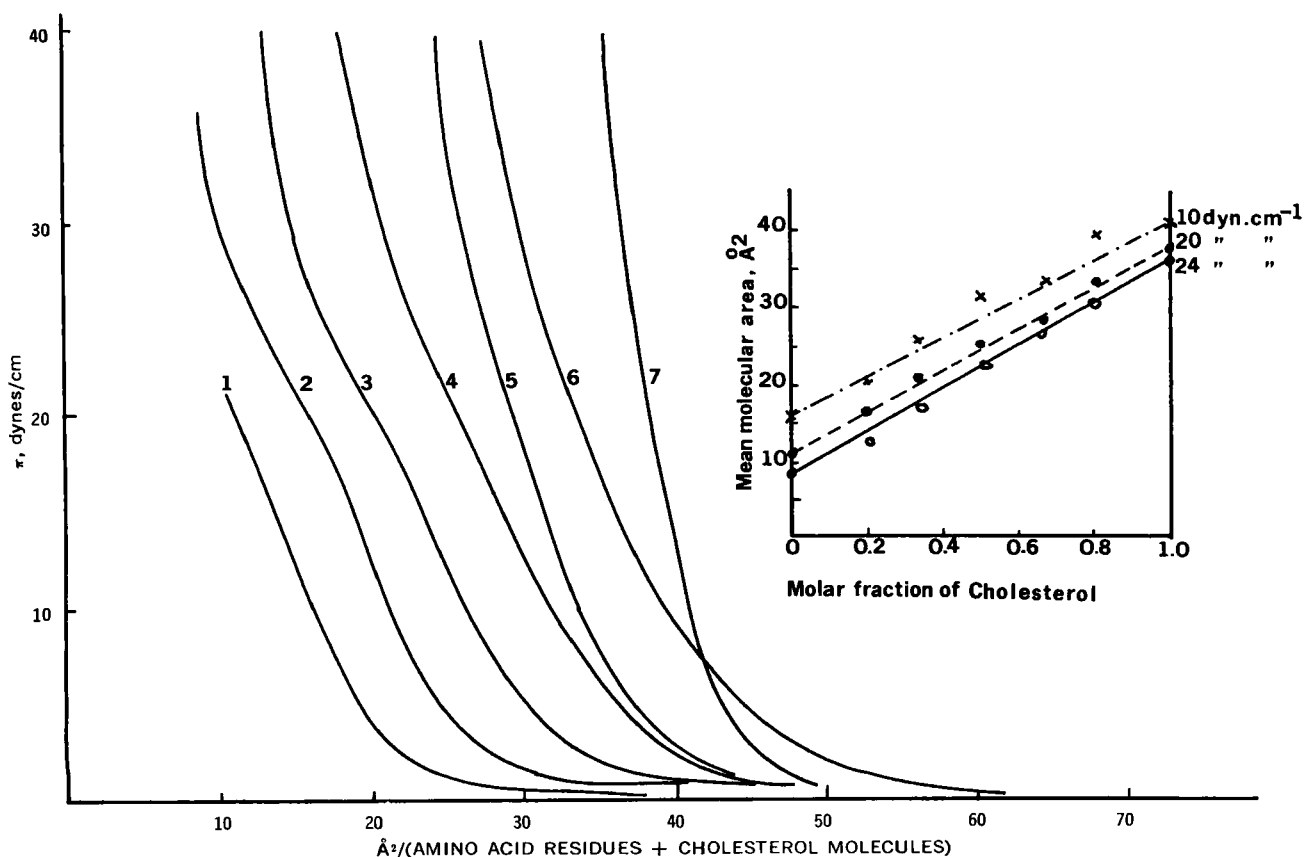In this case, the protein molecules were spread directly at the

Figure 4—Surface pressure–area curves of mixed monolayers of bovine serum albumin and cholesterol. Key: 1, 100% albumin; 2, 20% cholesterol; 3, 33% cholesterol; 4, 50% cholesterol; 5, 67% cholesterol; 6, 80% cholesterol; and 7, 100% cholesterol.
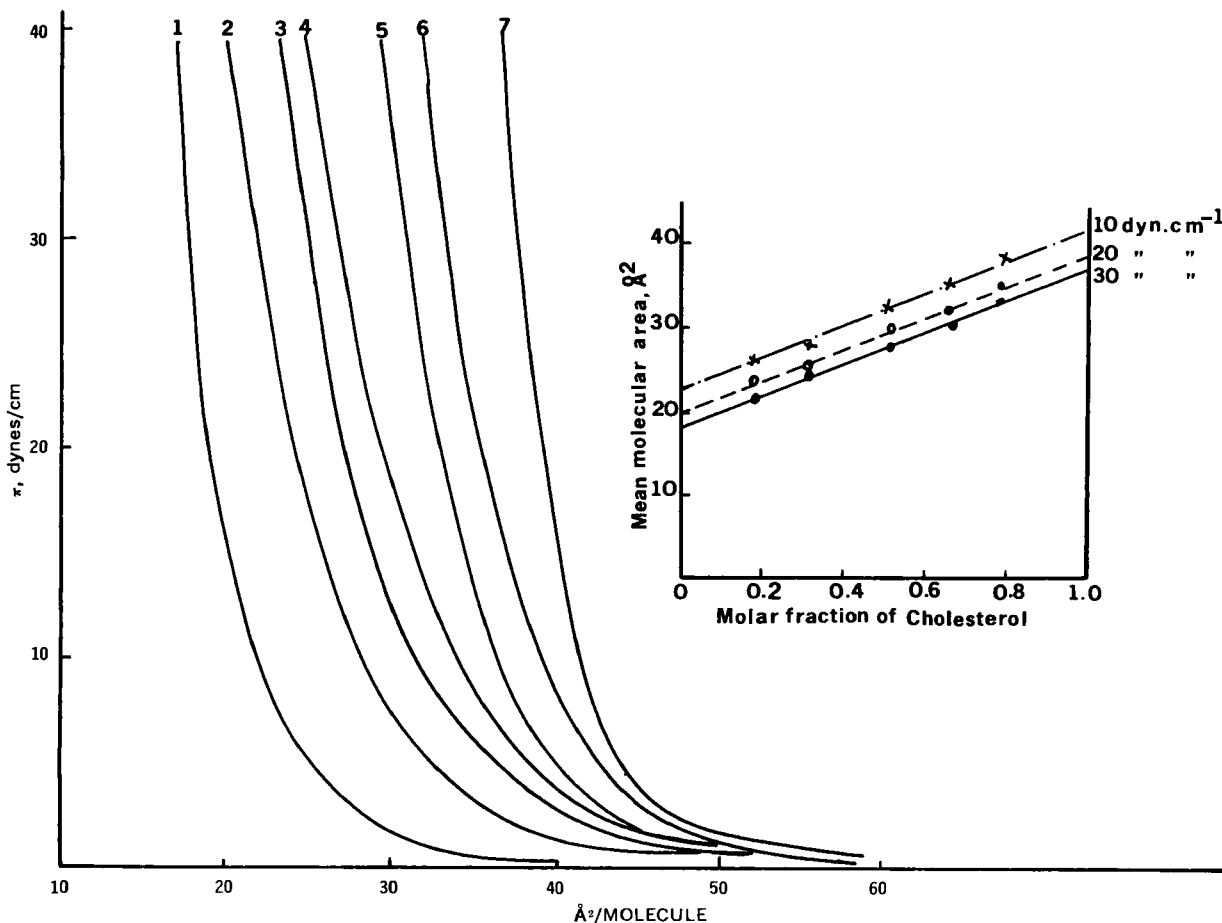
Figure 5—*Surface pressure–area curves of mixed monolayers of cetyl alcohol and cholesterol. Key: 1, 100% cetyl alcohol; 2, 20% cholesterol; 3, 33% cholesterol; 4, 50% cholesterol; 5, 67% cholesterol; 6, 80% cholesterol; and 7, 100% cholesterol*

plane of the cholesterol monomolecular layer and the mean molecular area was calculated using the number of amino acid residues per molecule of bovine serum albumin. Similar plots are obtained for mixed monomolecular layers of cholesterol and cetyl alcohol (Fig. 5). In both cases (Fig. 6), the calculation of the free energy of mixing shows that the values obtained are those to be expected from an entropic factor only, without any contribution of an excess of free energy of mixing.

Furthermore, when bovine serum albumin is added at the plane of the interface in which a cholesterol monolayer was previously spread, the plot of the increment of the surface pressure against the number of bovine serum albumin molecules is of the Langmuir type (Fig. 7). If the concentration is expressed in molar fraction calculated with the number of amino acid residues per molecule of bovine serum albumin, the plot is then a straight line (Fig. 8). The same type of linear plot of the increment of surface pressure against concentration is obtained for the addition (in the plane of the interface) of sodium hexadecyl sulfate, sodium lauryl sulfate, bovine growth hormone, insulin, or 5-valineangiotensin when (in the last three cases) the concentration is expressed as molar fractions of amino acid residues (Fig. 9).

Within the limits of the experimental error, all cases studied adhered to:

$$\pi_{tot} = \pi^0_{chol} + mN_R \qquad \text{(Eq. 7)}$$

in which π_{tot} represents the total surface pressure after the spreading of the protein, polypeptide, or surfactant in the plane of the cholesterol monolayer; π^0_{chol} represents the surface pressure of the cholesterol monomolecular layer before the spreading of the other substances; and N_R is the molar fraction in the monomolecular layer of the added substance calculated, in the case of proteins and peptides, with the number of amino acid residues.

The adherence to this equation, which represents the total vapor

pressure of a tridimensional system as a function of the molar fraction, strongly suggests that the process is simply a bidimensional, nonspecific mutual dissolution between the amino acid residues (or the long hydrocarbon chains of the surfactants) and the cholesterol monomolecular layer.

Here, again, the experimental evidence seems to indicate that nonspecific forces of the van der Waals type are likely involved in the interaction of proteins with monomolecular layers of cholester-
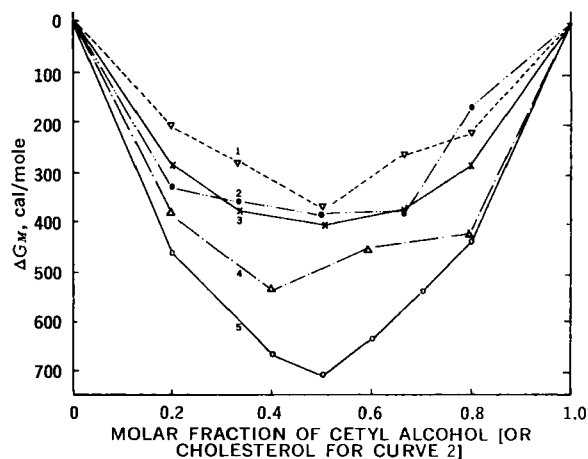

Figure 6—*Free energy of mixing, ΔG_M, as a function of molar fraction. Key: 1, △, cetyl alcohol–cholesterol; 2, ●, cholesterol–bovine serum albumin; 3, ×, ideal system, calculated from Eq. 4; 4, △, cetyl alcohol–$C_{14}H_{29} \cdot CH(CH_3)OSO_3Na$ calculated from Ref. 1; and 5, ○, cetyl alcohol–sodium hexadecyl sulfate calculated from the data of Ref. 10.*
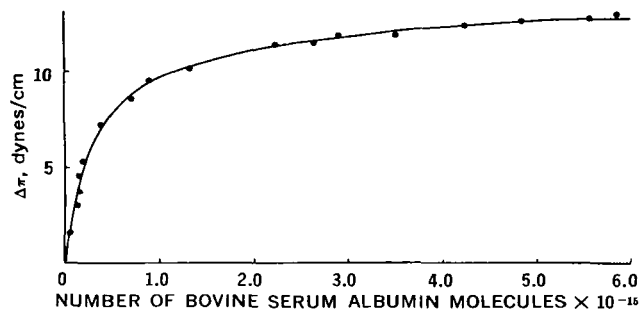
Figure 7—*Increment of surface pressure, $\Delta\pi$, in a cholesterol monolayer at 10 dynes/cm initial surface pressure with the number of bovine serum albumin molecules added at the plane of the interface at constant area.*
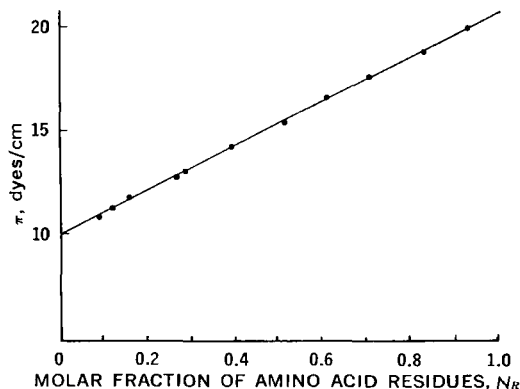

Figure 8—*Total surface pressure, π, as a function of molar fraction of amino acid residues of bovine serum albumin, N_R, at constant area. Initial surface pressure of cholesterol monolayer = 10 dynes/cm.*

ol and, consequently, that these forces could play an important role in the maintenance of the integrity of biomembranes.

Interactions of Biolipid Molecules with Subphase Components—Studies of this type have been used since the 1930's with biological processes. A review on this subject is not intended here, and the reader is referred to Refs. 26–28.

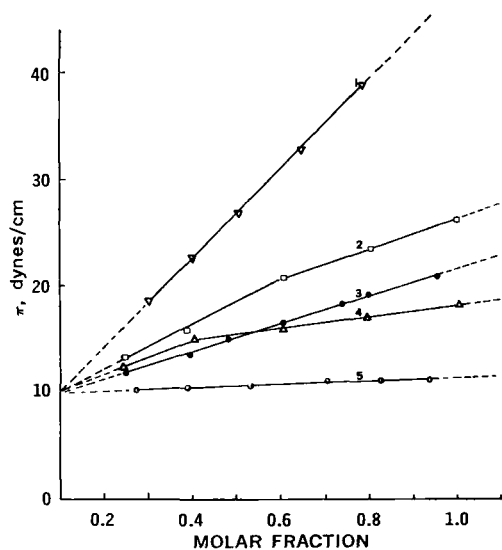The process of the interaction of a molecule approaching to a

Figure 9—*Total surface pressure as a function of molar fraction for: 1, sodium hexadecyl sulfate; 2, bovine growth hormone, amino acid residues; 3, sodium lauryl sulfate; 4, insulin, amino acid residues; and 5, 5-valineangiotensin, amino acid residues. Initial surface pressure of cholesterol monolayer = 10 dynes/cm.*
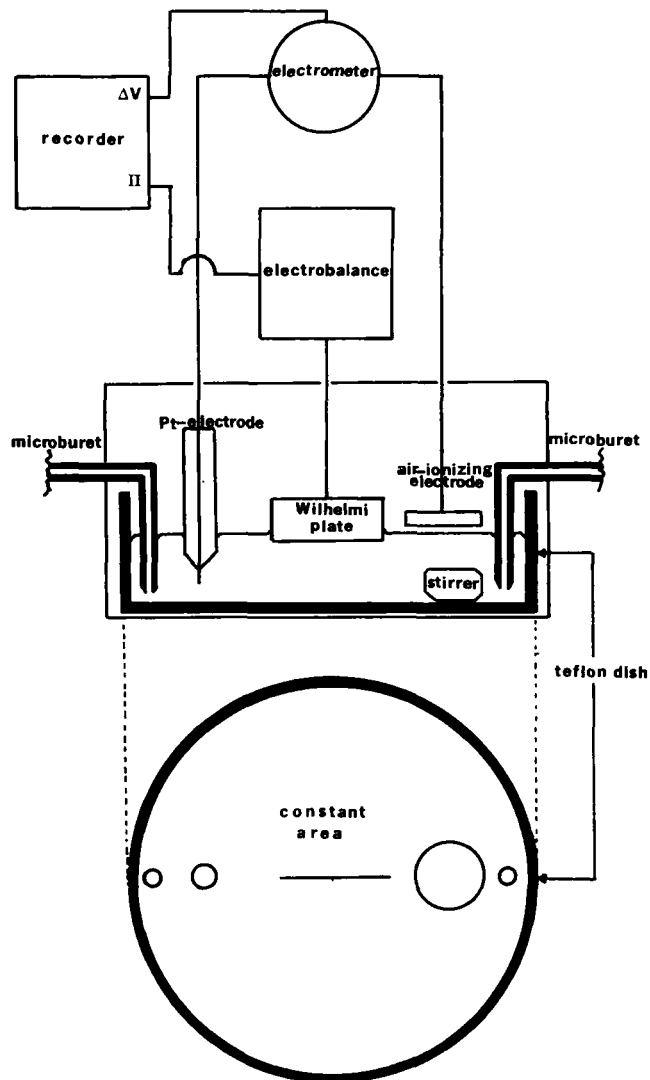

Figure 10—*Experimental assembly for the recording of changes in surface pressure and surface potential.*

monomolecular layer from the subphase can be followed by the measurement of the variations of the surface parameters as a function of time. The study can be performed at constant surface pressure or at constant surface area. When energetic values are sought, the parameter of choice is the variation of surface pressure at constant surface area after the injection of the desired molecule in the subphase beneath the monomolecular layer. This variation is a function of the excess surface concentration and the changes in the monolayer properties that could affect its surface pressure such as conformational changes and phase transitions.

Figure 10 shows a simple experimental device (29). A Teflon dish containing a final and constant volume of water provided with a Teflon-coated magnetic stirring bar is used as a trough. The tips of two identical microburets are immersed in the water. One microburet contains the solution of the substance to be injected. The other microburet is used to withdraw, prior to the injection, exactly the same volume of water to avoid effects of the variations of buoyancy on the platinum plate.

The Wilhelmy platinum plate for the measurement of the surface pressure is attached to an electrobalance. The [241]Am, air-ionizing electrode for the measurement of the surface potential is attached to an electrometer. The outputs of both instruments are fed into a dual-pen recorder, allowing measurement of the variation of the surface pressure and surface potential as a function of time at constant surface area and temperature.

The study of the interaction concerns two aspects: the kinetics of approach to equilibrium and the resultant equilibrium. The equilibrium has been studied by the application of a modified
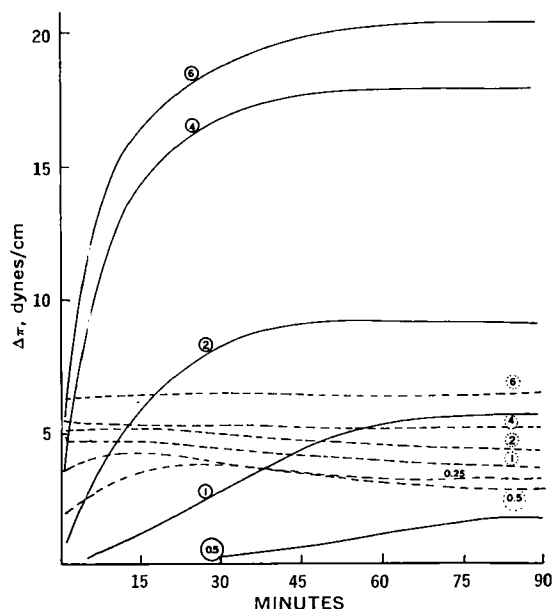
Figure 11—*Plots of the increment of surface pressure against time after the injection of sodium cetyl sulfate (———) or cetrimonium bromide (– – –) beneath a dipalmitoyl lecithin monolayer at 5 dynes/cm. Curves are labeled as to micromolar final concentrations of the injected surfactant.*

Gibbs adsorption equation (30, 31) and by the postulation of an osmotic equilibrium (32) between two presumed phases. A systematic study of the kinetics of these processes was performed only recently (29).

Figure 11 shows typical plots of the increment of surface pressure, $\Delta\pi$, against time after the injection of sodium cetyl sulfate or cetrimonium bromide beneath a dipalmitoyl lecithin monolayer at 5 dynes/cm (29). Both the kinetics and the final equilibrium vary with the nature of the polar head of the injected molecule. The interaction of cetrimonium bromide is virtually completed in the first few minutes after the injection and the effect on the surface pressure is relatively small. The interaction of sodium cetyl sulfate takes a much longer time to achieve equilibrium, and the effect on the surface pressure is more than three times greater than the effect of the cetrimonium bromide.

Energetics—The final value of the increment of the surface pressure ($\Delta\pi$, dynes/cm) as a function of the concentration in the bulk subphase of the injected molecules (n, molecules/cm^3) permits the estimation of the energy of activation of the process on the premise of a collision model and a constant entropy factor (29, 33) from:

$$1/\Delta\pi = 1/\Delta\pi_m + (B/\Delta\pi_m)(1/n) \qquad \text{(Eq. 8)}$$

$$B = (Ns/A)(2\pi_m/kT)^{1/2}(\nu)(e^{-\varphi/kT}) \qquad \text{(Eq. 9)}$$

in which *(Ns/A)* is the number of molecules per unit area that form the lipidic monolayer; $\pi = 3.14 \ldots$; m is the mass of the approaching molecule (or ion); k and T are the Boltzman constant and the absolute temperature, respectively; and φ is the energy of activation. The frequency factor, ν, that corresponds to the vibration of an oscillator perpendicular to the plane of the interface can be assumed to be 10^{-12} sec (34).

Figure 12 shows the values of the energies of activation for the interaction of cetyl sulfate and cetyl sulfate–cetrimonium ions with several lipid and phospholipid monolayers (40).

Taking as a reference the dipalmitoyl glycerol molecule, it can be observed that the attachment of a complex phosphoryl group with a net negative charge produces a significant difference between the energies of activation of the interaction of the molecules that form the monolayer with the positive or the negative long chain hydrocarbon ion injected. The zwitterionic dipalmitoyl lecithin and dipalmitoyl phosphatidylethanolamine, with a net charge equal to zero in the conditions of the experiments, differentiate, however, through the value of the energy of activation the charge

Figure 12—*Energies of activation for the interaction of cetyl sulfate and cetrimonium ions with different lipids. Initial surface pressure of the monolayers = 5 dynes/cm.*

of the injected ion. This seems to indicate that even for the zwitterionic phospholipids the ionic groups of the polar hydrophilic moiety are not equivalent in the perturbation that an attached hydrocarbon chain produces in the surface pressure of the phospholipid monolayer.

The energy of activation is a function of the chain length of the injected molecule (33). Figures 13–15 show the plots of the energies of activation against the chain length for the interaction of series of alkyl sulfate and alkyl trimethylammonium ions with dipalmitoyl glycerol, dipalmitoyl phosphatidylethanolamine, and dipalmitoyl lecithin. The slope of the best straight line passing through the experimental points is related to the energy of activation per methylene group of the hydrocarbon chain of the injected ion, and the intercept could be interpreted as related to the contribution of the polar head to the total energy of interaction.

Table II shows the value of the energy of activation per methylene group and per polar group for the interaction of alkyl sulfate and alkyl trimethylammonium ions with dipalmitoyl glycerol, di-
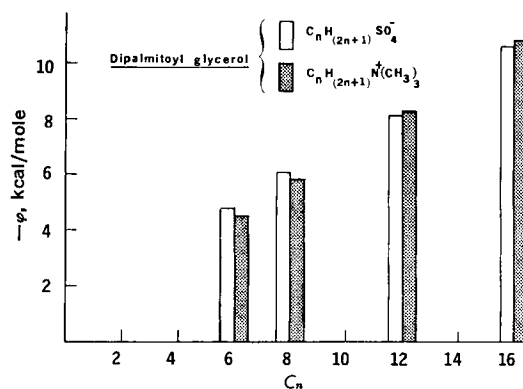

Figure 13—*Energies of activation as a function of the chain length of alkyl sulfate and alkyl trimethylammonium ions for the interaction with dipalmitoyl glycerol monolayers at 5 dynes/cm initial surface pressure.*

Table II—Energies of Activation of the Interaction of Alkyl Sulfate and Alkyl Trimethylammonium Ions with Dipalmitoyl Glycerol, Dipalmitoyl Phosphatidylethanolamine, and Dipalmitoyl Lecithin

Interaction		φ, kcal/mole $n =$				Energy of Activation, kcal/Group mole		
Lipidic Monolayer	Surfactant Ion	6	8	12	16	SO_4^-	$N^+(CH_3)$	CH_2
Dipalmitoyl glycerol	$C_nH_{(2n+1)} \cdot SO_4^-$	−4.8	−6.0	−8.3	−10.6	−1.30	—	−0.58
	$C_nH_{(2n+1)} \cdot N^+(CH_3)_3$	−4.4	−5.8	−8.4	−10.8	—	−0.60	−0.64
Dipalmitoyl phosphatidyl-ethanolamine	$C_nH_{(2n+1)} \cdot SO_4^-$	−4.9	−6.1	−8.5	−10.8	−1.4	—	−0.62
	$C_nH_{(2n+1)} \cdot N^+(CH_3)_3$	−5.1	−6.4	−8.8	−10.3	—	−1.4	−0.62
Dipalmitoyl lecithin	$C_nH_{(2n+1)} \cdot SO_4^-$	−4.4	−5.5	−7.6	−10.0	−1.0	—	−0.56
	$C_nH_{(2n+1)} \cdot N^+(CH_3)_3$	−4.7	−6.3	−9.3	−12.4	—	−0.10	−0.77

palmitoyl phosphatidylethanolamine, and dipalmitoyl lecithin. As it can be observed, the energy of activation per methylene group is in the range of from −0.56 to −0.77 kcal/mole, and the contribution to the energy of activation of the sulfate or the trimethylammonium group always has a negative value.

The energy of adsorption at the air–clean water interface of long chain hydrocarbon ions has been measured by different investigators. Table III represents the numerical values obtained for the energy of adsorption per methylene group and per polar head calculated using literature data (36–38).

The energy of activation per methylene group in the interaction process of the long chain hydrocarbon ion with the lipidic monolayers is energetically equivalent to that of the adsorption of meth-

Figure 14—*Energies of activation as a function of the chain length of alkyl sulfate and alkyl trimethylammonium ions for the interaction with dipalmitoyl phosphatidylethanolamine monolayers at 5 dynes/cm initial surface pressure.*

Figure 15—*Energies of activation as a function of the chain length of alkyl sulfate and alkyl trimethylammonium ions for the interaction with dipalmitoyl lecithin monolayers at 5 dynes/cm initial surface pressure.*

ylene groups at an air–clean water interface in all cases studied, with the exception perhaps of the interaction of alkyl trimethylammonium ions with dipalmitoyl lecithin monolayers.

The energy of activation per polar head of the injected long chain hydrocarbon ion, because of its numerical value and the negative sign, is closer to the values obtained for the energy of adsorption of sulfate and trimethylammonium polar heads at a hydrocarbon–clean water interface.

The picture that emerges from these energetic findings is by far more complex than that associated with the mechanical effects suggested by the semantics of "monolayer penetration" coined by Schulman and Rideal (39). The comparison of the values obtained in the interaction processes with those obtained for the adsorption at clean interfaces (without any monolayer) suggests that the polar head group of the injected long chain hydrocarbon ion could be located near to a hydrocarbon environment with its hydrocarbon chain more likely near to an aqueous environment.

This suggestion and the differences observed in the energies of activation of the interaction of alkyl sulfate and alkyl trimethylammonium ions with the zwitterionic phospholipids point out that both the polar hydrophilic moiety of the phospholipid and the polar head of the long hydrocarbon chain determine the different perturbation that identical hydrocarbon chains can produce in the state of the monolayer. The presence of this type of phospholipids is almost constant in biomembranes and, consequently, these facts strongly suggest that the microstates of the lipid micelles of biomembranes could be differently affected according to the nature of the approaching molecule.

Kinetics—The effective change in surface pressure for the interaction of cetrimonium ions with dipalmitoyl lecithin, dipalmitoyl phosphatidylethanolamine, and dipalmitoyl glycerol monolayers is essentially an extremely fast process under the experi-
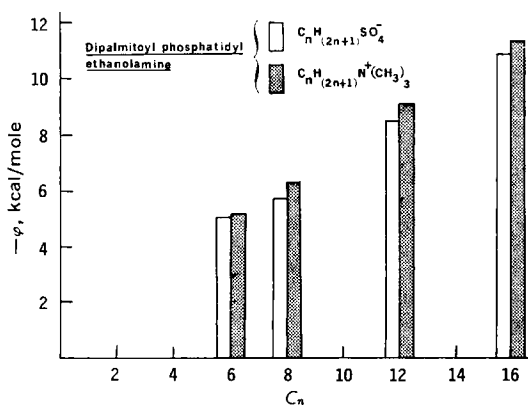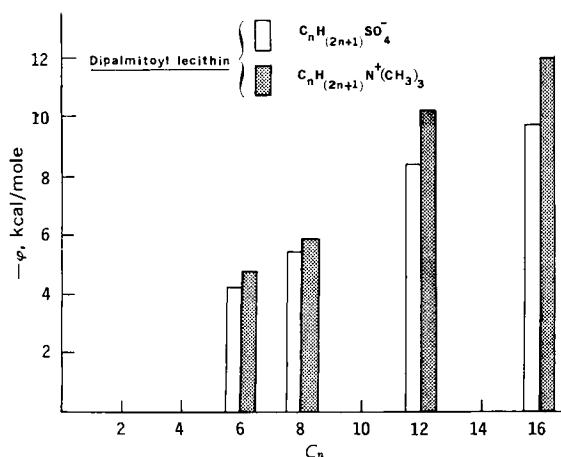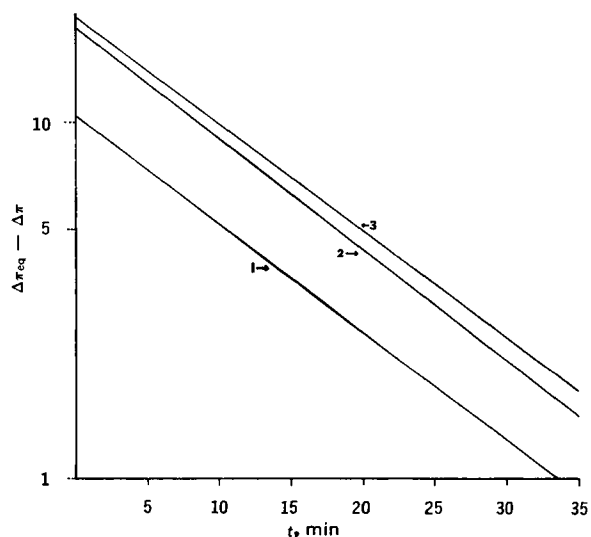
Figure 16—*Typical apparent first-order plots for the interaction of 4×10^{-6} M cetyl sulfate ion with: 1, dipalmitoyl lecithin; 2, dipalmitoyl glycerol; and 3, dipalmitoyl phosphatidylethanolamine monolayers.*

Table III—Energies of Adsorption per Group of Alkyl Sulfate and Alkyl Trimethylammonium Ion at the Hydrocarbon–Water and Air–Water Interfaces[a]

Energy of Adsorption, kcal/Group mole					
Hydrocarbon–Water			Air–Water		
SO_4^-	$N^+(CH_3)_3$	CH_2	SO_4^-	$N^+(CH_3)_3$	CH_2
-1.2	-0.70	-0.81	$+2.4$	$+2.7$	-0.63

[a] From Refs. 34 and 36–38.

mental conditions. The new steady-state values are reached within a few minutes after the injection.

The interaction of cetyl sulfate ions with these three lipids is comparatively a slower process. Figure 16 shows the plots of the logarithm of the difference between the value of the increment of the surface pressure at equilibrium, $\Delta\pi_{eq}$, and the increment at a time t, $\Delta\pi$, as a function of time for the interaction of cetyl sulfate at a final concentration of 4×10^{-6} M with dipalmitoyl glycerol, dipalmitoyl lecithin, and dipalmitoyl phosphatidylethanolamine (40). The linear plot suggests an apparent first-order increase of the surface pressure to a new steady-state value with almost identical values of the apparent first-order rate constant.

The presence of an inert electrolyte affects the kinetics of these processes and permits the evaluation of the apparent first-order rate constants even in the cetrimonium-ion interaction. Figure 17 shows the decrease of the apparent first-order rate constant in the presence of an increasing concentration of sodium chloride, from 0.15 to 0.45 M for the interaction of cetrimonium ion at a final concentration of 4×10^{-6} M with dipalmitoyl lecithin monolayers[1]. This same effect can be observed using potassium chloride as the added electrolyte (Fig. 18).

The plot of the logarithm of the apparent first-order constant against the square root of the ionic strength gave straight lines in both cases, with an identical negative slope of approximately unity (Fig. 18) but with a different intercept. The adherence to the straight-line equation, the negativity, and the numerical value of the slope indicate that the interaction between cetrimonium ions
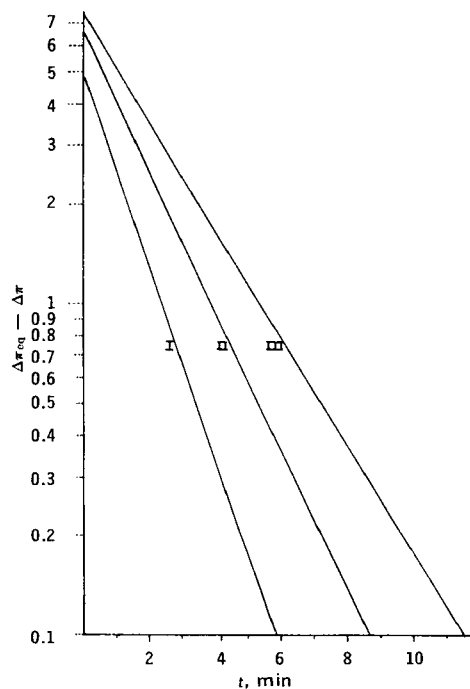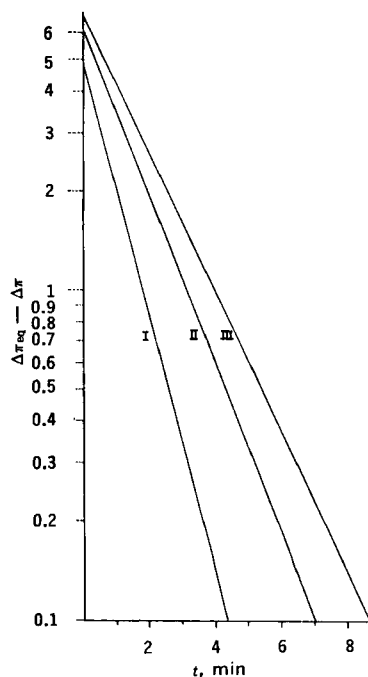

Figure 18—*Typical apparent first-order plots for the interaction of cetrimonium ions with dipalmitoyl lecithin monolayers. Key: I, 0.15 M KCl; II, 0.30 M KCl; and III, 0.45 M KCl.*

with the dipalmitoyl lecithin monolayer could be described as an interaction between ions of opposite charge (40) and suggests that the first event in the process is the attachment of the polar head of the cetrimonium ion, possibly to the phosphate group of the zwitterionic polar hydrophilic moiety of dipalmitoyl lecithin.

The difference observed between the plots of the logarithm of $\Delta\pi_{eq} - \Delta\pi$ as a function of time for subphases containing sodium chloride or potassium chloride indicates that for a given ionic strength the interaction of cetrimonium ions with dipalmitoyl lecithin monolayers is a faster process in the presence of potassium ions than in the presence of sodium ions. This experimental finding could be related to selective effects observed in the compression curves of dipalmitoyl lecithin monolayers obtained on subphases containing sodium chloride, potassium chloride, or lithium chloride (41).

The selective effect of sodium ions on dipalmitoyl lecithin monolayers (42), which has been also observed on dipalmitoyl phosphatidylethanolamine monolayers (43), has been used jointly with
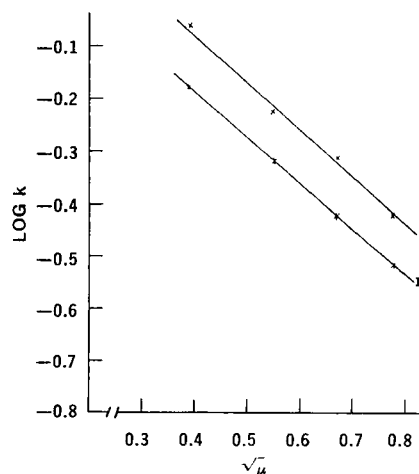

Figure 17—*Typical apparent first-order plots for the interaction of 4×10^{-6} M cetrimonium ions with dipalmitoyl lecithin monolayers. Key: I, 0.15 M NaCl; II, 0.30 M NaCl; and III, 0.45 M NaCl.*

Figure 19—*Plot of the logarithm of the apparent first-order rate constant for the interaction of cetrimonium ions with dipalmitoyl lecithin monolayers against the square root of the ionic strength. Key: I, KCl; and II, NaCl.*

[1] E. R. Garrett and F. A. Vilallonga, unpublished results.

the measurement of unidirectional flux of sodium ions to conclude that the penetration of sodium ions through the epithelium of frog skin does not occur by simple diffusion but depends on a specific interaction with the polar group of the outer leaflet of the plasma membrane (44).

It has been suggested (45) that, if a cell membrane has a water channel whose walls are covered with phospholipids of the dipalmitoyl lecithin type, the discriminative properties of these phospholipids, when spread in monolayers (41), would cause the radius and the length of such a pore to vary according to which ion is flowing through its lumen.

It has been established that the nerve cell membrane can distinguish between sodium and potassium ions and that the permeability to these two ions is variable (46). The effect of quaternary ammonium ions injected into the axoplasm on the potassium conductance of squid axons strongly suggests that the postassium ions traverse the nerve membrane by way of pores which sodium ions cannot enter (47).

In accord with the findings on monolayer experiments, it is conceivable that the potassium ion generates *per se* the selective channel in absence of quaternary ammonium ions and that, because of its accelerating effect on the kinetics of the interaction of the quaternary ammonium ion with the phospholipids that cover the walls of the channel, it assists the closing of the activation gates when the quaternary ammonium ion is injected into the axoplasm.

CONCLUSION

A better knowledge of the interactions of biomolecules at the plane of an interface is necessary to understand the complexity of the structure of the cell interfaces and the order of magnitude of the energy required to produce nondisruptive structural changes that, in turn, can produce functional modifications of biomembranes. If these functional modifications could be produced by the approach of a selected drug molecule to the cell interface, the better knowledge of the kinetics and energetics of this type of interaction will be invaluable in the design of new and better drugs.

REFERENCES

(1) M. S. Fernandez and J. Cerbon, *Biochim. Biophys. Acta,* 298, 8(1973); J. Cerbon, *ibid.,* 290, 51(1972).

(2) T. J. Franklin and G. A. Snow, "Biochemistry of Antimicrobial Action," Academic, New York, N.Y., 1971, p. 47.

(3) J. R. Danielli and H. Dawson, *J. Cell. Physiol.,* 5, 495(1935).

(4) "Conference on Membrane Structure and Its Biological Applications," D. E. Green, Ed., *Ann. N.Y. Acad. Sci.,* 195, 1972.

(5) J. L. Kavanau, "Structure and Function of Biological Membranes," vol. 1, Holden-Day, San Francisco, Calif., 1965, p. 66.

(6) S. J. Singer and G. L. Nicolson, *Science,* 175, 720(1972).

(7) G. L. Gaines, "Insoluble Monolayers at Liquid Gas Interfaces," Interscience, New York, N.Y., 1966, pp. 44, 73; J. T. Davies and E. K. Rideal, "Interfacial Phenomena," Academic, New York, N.Y., 1963, p. 217.

(8) D. A. Cadenhead, in "Recent Progress in Surface Science," vol. 3, Academic, New York, N.Y., 1970, p. 169.

(9) L. DeBernard, *Bull. Soc. Chim. Biol.,* 40, 161(1958).

(10) D. B. Dervichian, "Surface Science in Chemistry and Biology," Pergamon, London, England, 1958, p. 70.

(11) L. L. M. van Deenen, V. M. T. Houtsmuller, C. H. de Haas, and E. Mulder, *J. Pharm. Pharmacol.,* 14, 429(1962).

(12) R. A. Demel, L. L. M. van Deenen, and B. A. Pethica, *Biochim. Biophys. Acta,* 135, 11(1967).

(13) D. A. Cadenhead and M. C. Phillips, *Advan. Chem. Ser.,* 84, 131(1968).

(14) D. O. Shah and J. Schulman, *J. Lipid Res.,* 8, 215(1967).

(15) D. O. Shah and J. Schulman, *Advan. Chem. Ser.,* 84, 189(1968).

(16) F. C. Goodrich, *Proc. Int. Congr. Surface Activity, 2nd,* 1, 85(1957).

(17) F. Vilallonga, R. Altschul, and M. S. Fernandez, *Biochim. Biophys. Acta,* 135, 552(1967).

(18) D. O. Shah and R. W. Capps, *J. Colloid Interface Sci.,* 27, 319(1968).

(19) N. L. Gershfeld and R. E. Pagano, *J. Phys. Chem.,* 76, 1244(1972).

(20) E. A. Guggenheim, "Thermodynamics," 3rd ed., North Holland Publishing Co., Amsterdam, The Netherlands, 1957, p. 241.

(21) F. A. Vandenheuvel, *J. Amer. Oil Chem. Soc.,* 40, 455(1963).

(22) F. A. Vandenheuvel, *Ann. N.Y. Acad. Sci.,* 122, 57(1965).

(23) R. A. Demel, K. R. Bruckdorfer, and L. L. M. van Deenen, *Biochim. Biophys. Acta,* 255, 311(1972).

(24) D. D. Eley and D. G. Hedge, *Discuss. Faraday Soc.,* 21, 221(1956).

(25) F. Vilallonga, R. Altschul, and M. S. Fernandez, *Biochim. Biophys. Acta,* 135, 406(1967).

(26) J. L. Kavanau, "Structure and Function of Biological Membranes," vol. 1, Holden-Day, San Francisco, Calif., 1965, p. 111.

(27) H. T. Tien and K. J. Leylin, in "Chemistry of the Cell Interface," part B, H. D. Brown, Ed., Academic, New York, N.Y., 1971, p. 205.

(28) J. T. Davies and E. K. Rideal, "Interfacial Phenomena," Academic, New York, N.Y., 1973.

(29) F. Vilallonga, E. R. Garrett, and M. Cereijido, *J. Pharm. Sci.,* 61, 1720(1972).

(30) B. A. Pethica, *Trans. Faraday Soc.,* 51, 1402(1955).

(31) P. J. Anderson and B. A. Pethica, *ibid.,* 52, 1080(1956).

(32) F. M. Fowkes, *J. Phys. Chem.,* 65, 355(1961).

(33) F. Vilallonga and E. R. Garrett, *J. Pharm. Sci.,* 62, 1605(1973).

(34) D. A. Haydon and F. H. Taylor, *Phil. Trans. Roy. Soc.,* A252, 225(1960).

(35) T. L. Hill, "Introduction to Statistical Thermodynamics," Addison-Wesley, Reading, Mass., 1960, p. 89.

(36) N. D. Weiner and G. Zografi, *J. Pharm. Sci.,* 54, 436(1965).

(37) J. Llopis and P. Artalejo, *An. Fis. Quim. (Madrid),* 58, 367(1962).

(38) H. Kimizuka, L. G. Abood, T. Tahara, and K. Kaibara, *J. Colloid Interface Sci.,* 40, 27(1972).

(39) J. H. Schulman and E. K. Rideal, *Proc. Prog. Soc. G,* 122, 29, 46(1937).

(40) S. W. Benson, "The Foundations of Chemical Kinetics," McGraw-Hill, New York, N.Y., 1960, p. 525.

(41) F. A. Vilallonga, M. S. Fernandez, C. Rotunno, and M. Cereijido, *Biochim. Biophys. Acta,* 183, 98(1969).

(42) M. Cereijido, M. Fernandez, C. Rotunno, and F. A. Vilallonga, *ibid.,* 203, 586(1970).

(43) M. M. Standish and B. A. Pethica, *Trans. Faraday Soc.,* 64, 1113(1968).

(44) C. A. Rotunno, F. A. Vilallonga, M. Fernandez, and M. Cereijido, *J. Gen. Physiol.,* 55, 716(1970).

(45) M. Cereijido, F. A. Vilallonga, M. Fernandez, and C. Rotunno, in "The Molecular Basis of Membrane Transport," D. C. Tosteson, Ed., Prentice Hall, Englewood Cliffs, N.J., 1969, p. 161.

(46) A. L. Hodgkin, and A. F. Huxley, *J. Physiol. (London),* 117, 500(1952).

(47) C. M. Armstrong, *J. Gen. Physiol.,* 58, 413(1971).

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