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# Kinetics and Mechanisms of Monolayer Interactions I: Cetyl Sulfate and Cetrimonium Ions Interacting with Dipalmitoyl Lecithin and Dipalmitoyl Glycerol

**FEDERICO A. VILALLONGA▲, EDWARD R. GARRETT, and MARCELINO CEREIJIDO***

**Abstract** □ The interaction of cetyl sulfate and cetrimonium (cetyltrimethylammonium) ions with dipalmitoyl lecithin and dipalmitoyl glycerol monolayers spread at the air/water interface was followed by the variation of the surface pressure and surface potential. The kinetics and the final equilibrium varied with the nature of the injected surfactant. An approximative method was devised to calculate the energies of activation, which are comparable with others obtained on a classical thermodynamic basis for cetyl alcohol monolayers interacting with sodium lauryl sulfate. The presence of the phosphoryltrimethylethanolamine group in the dipalmitoyl lecithin molecule decreases to some extent the interaction with cetyl sulfate and cetrimonium ions. The values of the energies of activation obtained indicate that the ionic groups of the polar moiety of dipalmitoyl lecithin are not equivalent in the perturbation that an attached hydrocarbon chain produces in the surface pressure of the monolayer. This difference may be explained by the different mobilities that a hydrocarbon chain would have when attached to one or the other ionic attraction centers because of the unrestricted movement of the positively charged trimethylammonium group around the phosphate linkage of the dipalmitoyl lecithin. Some implications of this observation to microstates of biomembranes are suggested.

**Keyphrases** □ Cetyl sulfate and cetrimonium ions—interactions with dipalmitoyl lecithin and dipalmitoyl glycerol monolayers, kinetics, mechanisms □ Monolayers, dipalmitoyl lecithin and dipalmitoyl glycerol—interactions with cetyl sulfate and cetrimonium ions, kinetics, mechanisms □ Phospholipid monolayers—interaction with long-chain surfactants, kinetics, mechanisms □ Surfactants, long chain—interaction with phospholipid monolayers, kinetics, mechanisms

The interaction of an insoluble monolayer spread at an interface with a soluble surface-active species injected in the subphase has been termed monolayer penetration (1–4). This interaction presents two aspects: the kinetics of approach to and the resultant equilibrium. The equilibrium has been studied from the two formally different aspects of the application of a modified Gibbs adsorption equation (5, 6) and the postulation of an osmotic equilibrium (7) between two presumed phases: dissolved surfactant in the bulk solution and surfactant molecules already penetrated into the monolayer.

Monolayers of dipalmitoyl lecithin at the air/water interface may be considered as suitable models to use in the study of certain properties which can be associated with the outer lipidic layer of cell membranes. For example, the discrimination in their interaction with monovalent cations such as sodium and potassium is similar to that exercised by the phospholipids that form the epithelial cell membrane (8–11).

The present study was carried out on the premise that the kinetics and the mechanisms of the interaction of phospholipid monolayers with ionic long-chain surfactants may yield useful information to further the understanding of the role of phospholipids in the cell membrane and in drug absorption.

## EXPERIMENTAL

**Reagents**—Dipalmitoyl lecithin[1] was chromatographically homogeneous by TLC (12). The dipalmitoyl glycerol[1] was known to be a mixture of 1,2- and 1,3-isomers. Sodium cetyl sulfate[1] and cetrimonium bromide[2] gave no minima in the curves of surface tension against the logarithm of concentration.

Deionized and triple-distilled water was used throughout. Its pH after air equilibration was consistently between 5.6 and 6.0, and its surface tension was always, in approximately 200 experiments, between 99.8 and 100.2% of the value calculated from the Harkins equation (13). The air/water interfacial potential was −470 mv. (± 20 mv.). The hexanes[2,3] were spread (0.2 ml.) on 42.56 cm.$^2$ of air/water interface and gave no significant variation on the surface tension of water (less than 0.02 dyne cm.$^{-1}$).

[1] Mann Research Laboratories, Orangeburg, N. Y.
[2] Eastman Kodak, Rochester, N. Y.
[3] Spectroquality, Matheson, Coleman and Bell, East Rutherford, N. J.

Table I—Average Values of the Surface Parameters of Dipalmitoyl Lecithin and Dipalmitoyl Glycerol Monolayers before the Injection of the Surfactant

| | $\pi_L$, dyne cm.$^{-1}$ | $A$, Å$^2$·molecule$^{-1}$ | $V_L$, mv. | $\Delta V_L$, mv. | $\mu$, mD. | $C_S = \frac{1}{A}$, molecule·cm.$^{-2}$ |
|---|---|---|---|---|---|---|
| Dipalmitoyl lecithin | 5(±0.1) | 55(±2) | −130 | +340 (±20) | 500 | $1.82 \cdot 10^{14}$ |
| Dipalmitoyl glycerol | 5(±0.1) | 46(±2) | −10 | +460 (±20) | 560 | $2.19 \cdot 10^{14}$ |

**Instruments**—Surface tension was measured with a Wilhelmi platinum plate (2.5 × 1.25 × 0.01 cm.) attached to an electrobalance[4]. Surface potential was measured with an air electrode[5] and an electrometer[6].

The outputs of the electrobalance and the electrometer were fed into a dual pen recorder[7].

The experimental assembly, with a 9-cm. Teflon dish as a trough, is diagrammed in Fig. 1. The final water volume was always 45 cm.$^3$, which corresponded to a depth of water of 1.58 cm. A Teflon-coated stirring bar (1.25 × 0.8 cm.) was located inside the Teflon dish. The total area of the air/water interface for that volume was calculated by measuring the diameter at the contact of the water and the Teflon and correcting this surface for the contact angle Teflon/water. The tips of two identical microburets[8] (1 ± 0.0001 ml.) were immersed in the water contained in the Teflon dish. One microburet contained the surfactant solution with an air bubble in the capillary to isolate initially this solution from the water. The other microburet was used to withdraw, prior to the injection of the surfactant solution, exactly the same volume of water from the Teflon dish to avoid the effects of variations of buoyancy on the platinum plate. A sintered-glass tube at 1.5 cm. above the air/water interface (not shown in Fig. 1) was used to pass a continuous flow of wet nitrogen over the interface during the experiments.

**Methods**—With the platinum plate, the electrodes, the microburets, and the nitrogen tube in position, water was slowly added so that it just made contact with the lower edge of the platinum plate (14). After 5 min. the surface tension ($\gamma_o$) and the potential of the interface ($V_o$) were measured and recorded. These control values were to be used for the calculation of the monolayer surface pressure ($\pi_L = \gamma_o - \gamma_L$), its increment ($\Delta\pi = \pi_i - \pi_L$), the surface potential ($\Delta V_L = V_L - V_o$), and its increment [$\Delta(\Delta V) = V_i - V_L$]. The subscript $L$ indicates the corresponding value for the monolayer before the injection, and the subscript $i$ indicates the value after the surfactant injection; $\gamma_o$ and $V_o$ represent the surface tension and the interfacial potential of the air/water interface, respectively.

The spreading solution was prepared previously by dissolving the corresponding substance (dipalmitoyl glycerol or dipalmitoyl lecithin) in water-saturated hexane. Gentle heating completed the dissolution and thus avoided the use of potential surface contaminants such as ethanol. A microsyringe (Hamilton) was filled with the spreading solution. The solution was delivered dropwise at the water surface on the dish with a simultaneous starting of the stirring and the rapid nitrogen flow. Stirring and rapid nitrogen flow were continued for 10 min. after the achievement of the desired surface pressure. In all of the experiments reported, the initial surface pressure was 5 ± 0.1 dyne cm.$^{-1}$.

After the monolayer at the desired surface pressure was thus obtained, a given volume of water equal to the volume of surfactant solution to be injected was withdrawn from the dish using the appropriate micropipet. The injection of the surfactant solution was then started simultaneously with the stirrer. The stirring was maintained for 1 min. and then stopped. At this time the recording of $\Delta\pi$ and $\Delta V$ was started. A slow flow of wet nitrogen was maintained during the experiment. At least three experiments were performed for each final surfactant concentration. The reproducibility was within ±0.5 dyne cm.$^{-1}$ for $\Delta\pi$ and ±20 mv. for $\Delta(\Delta V)$.

The experiments performed to obtain the surface tension–log of concentration curves showed that the CMC of cetrimonium bromide was $1 \cdot 10^{-3}$ $M$ and that of sodium cetyl sulfate was $5 \cdot 10^{-4}$ $M$. Accordingly, the concentration of the surfactant solution to be injected was kept at $2.5 \cdot 10^{-4}$ $M$ in order to be below the CMC. The volumes of surfactant solution injected were such as to obtain a final concentration in the 45 ml. of water in the Teflon dish of 6, 4, 1, 0.5, or $0.25 \cdot 10^{-6}$ $M$. At these concentrations, it has been shown that cetrimonium bromide as well as sodium cetyl sulfate is completely dissociated (15). The ratio of the *total* number of surfactant ions injected to the number of lipid molecules forming the monolayer varied consequently from about 1 for the lowest concentration to 16 for the highest concentration.

The experiments were performed at 22 ± 1°.

## RESULTS

The average values of the surface parameters of the monolayers before the injection of the surfactant ion are represented in Table I.

The effects of the injection of cetrimonium bromide and sodium cetyl sulfate on the surface pressure ($\Delta\pi$) and surface potential ($\Delta V$), respectively, of a dipalmitoyl lecithin monolayer at 5 dyne cm.$^{-1}$ are compared in Figs. 2 and 3. Both the kinetics and the final equilibrium varied with the nature of the injected surfactant. The kinetics of interaction are contrasted most dramatically in the case of the rate of change of surface pressure, $\Delta\pi$ (Fig. 2). The interaction of cetrimonium bromide with the dipalmitoyl lecithin monolayer is virtually completed in the first several minutes after

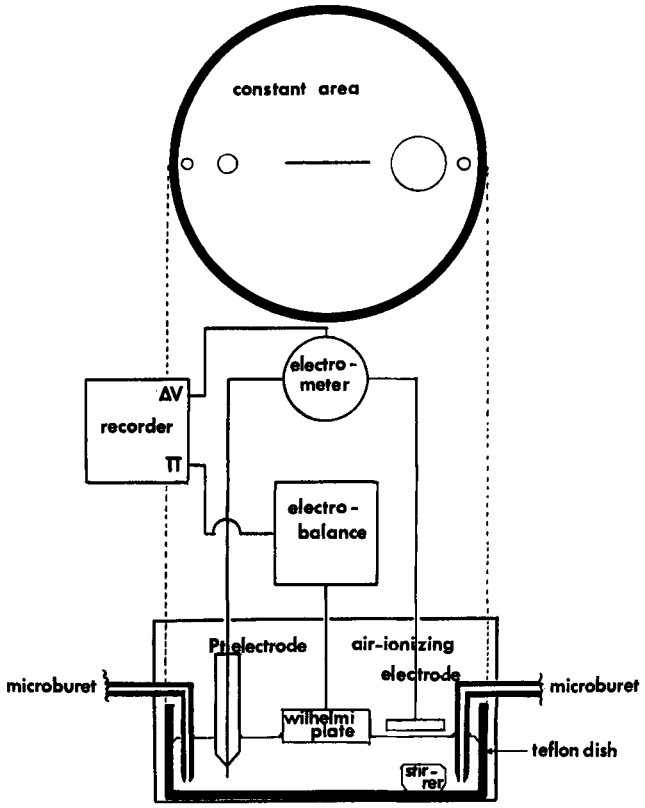


**Figure 1**—*Experimental assembly for the* $\Delta(\Delta V)$ *recording of changes in surface pressure* ($\Delta\pi$) *and surface potential.*

[4] Cahn Division, Ventrom Instruments Corp., Paramount, Calif.
[5] Americium-241.
[6] Keithley Instruments (610 c), Cleveland, Ohio.
[7] Speedomax W/L, Leeds and Northrup, North Wales, Pa.
[8] Monostat, New York, N. Y.

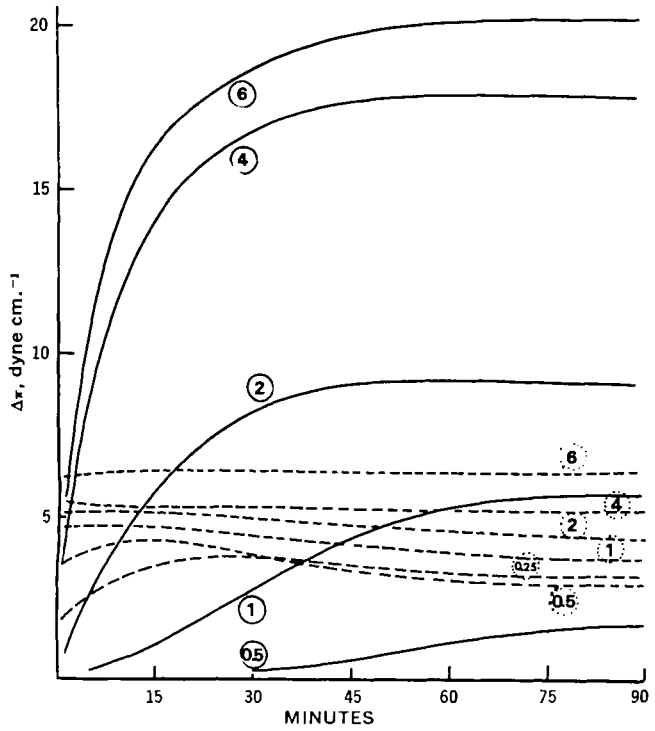


**Figure 2**—*Effect of the injection of cetrimonium bromide* (---) *or sodium cetyl sulfate* ( —) *on the surface pressure of a dipalmitoyl lecithin monolayer at 5 dyne cm.*$^{-1}$. *Curves are labeled as to micromolar final concentrations of the injected surfactant ion.*

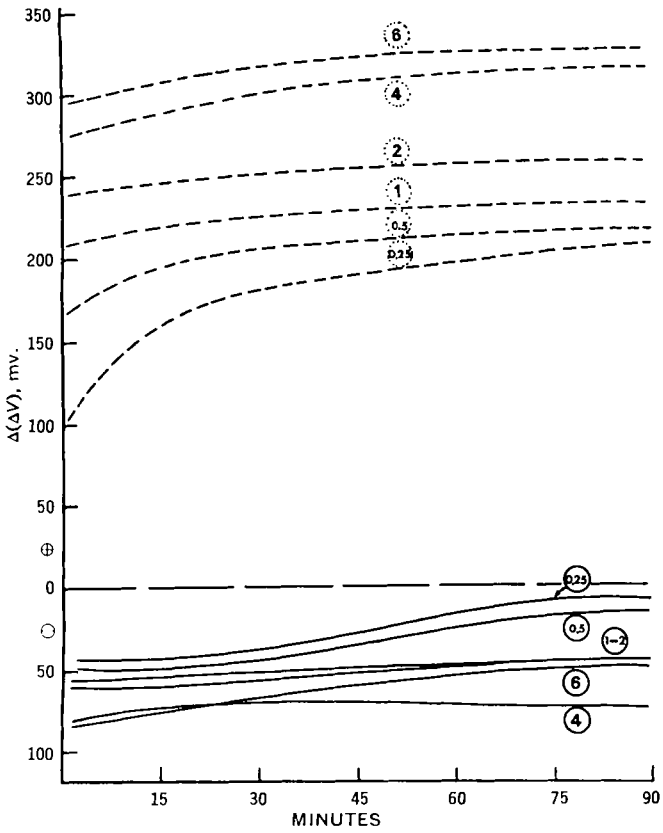


**Figure 3**—*Effect of the injection of cetrimonium bromide* (---) *or sodium cetyl sulfate* (·——) *on the surface potential* [Δ(ΔV)] *of a dipalmitoyl lecithin monolayer at 5 dyne cm.*$^{-1}$. *Curves are labeled as to micromolar final concentrations of the injected surfactant ion.*

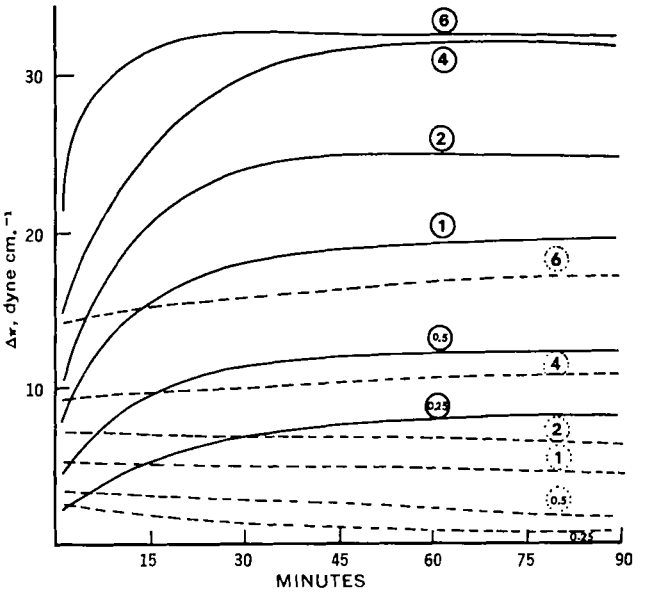


**Figure 4**—*Effect of the injection of cetrimonium bromide* (---) *or sodium cetyl sulfate* (——) *on the surface pressure* ($\Delta\pi$) *of a dipalmitoyl glycerol monolayer at 5 dyne cm.*$^{-1}$. *Curves are labeled as to micromolar final concentrations of the injected surfactant ion.*

injection, except for the lowest concentrations (0.25 and 0.5 $\mu M$) and a slight subsequent drift which does not seem pertinent to the major changes in the measured values. The interaction of sodium cetyl sulfate with the monolayer took a much longer time to achieve equilibrium with respect to $\Delta\pi$.

The effect of the negatively charged cetyl sulfate anion on the surface pressure was more than three times the effect of positively charged cetrimonium cation at the highest concentration (6 $\mu M$) used. Thus, the interaction of cetyl sulfate anion with the dipalmitoyl lecithin monolayer at 5 dyne cm.$^{-1}$ is the slower process with a larger final effect on the surface pressure than the interaction of cetrimonium cation with the dipalmitoyl lecithin monolayer.

The injection of the negatively charged cetyl sulfate ion increased (Fig. 3) the negativity of the dipalmitoyl lecithin monolayer about 40–80 mv. There was an effect on the surface potential of the monolayer at the lowest concentration (0.25 $\mu M$) in spite of the fact that the surface pressure was invariant after the 60 min. of the experiment.

The injection of the positively charged cetrimonium ion converted the negative interfacial potential of the dipalmitoyl lecithin monolayer at 5 dyne cm.$^{-1}$ into a positive value. This effect represented a total change in the surface potential of 320 mv. for the highest concentration and of 200 mv. for the lowest concentration, both at 90 min. Thus, the effects on the surface potential of the dipalmitoyl lecithin monolayer at 5 dyne cm.$^{-1}$ by the injection of cetrimonium on cetyl sulfate ions are equivalent fast processes where the positive effect of cetrimonium cation is much larger than the negative effect of an equivalent amount of cetyl sulfate anion.

The effects of the injection of cetrimonium and cetyl sulfate ions on the surface potential of a dipalmitoyl glycerol monolayer at 5 dyne cm.$^{-1}$ are compared in Figs. 4 and 5. The kinetics and the final equilibrium varied with the nature of the injected surfactant. Again, the kinetics of the interaction are contrasted most dramatically in the rate of change of surface pressure, $\Delta\pi$. The interaction of cetrimonium cation with the dipalmitoyl lecithin monolayer was completed almost instantaneously at all concentrations studied, but the complete interaction of cetyl sulfate anion with the dipalmitoyl glycerol monolayer took up to 1 hr. The effect on the equilibrium surface pressure of the dipalmitoyl glycerol monolayer was about three times larger for cetyl sulfate anion than for equivalent amounts of cetrimonium cation.

The injection of the negatively charged cetyl sulfate ion (0.25–4 $\mu M$) quickly decreased the surface potential of the dipalmitoyl glycerol monolayer (Fig. 4) to −30–−100 mv., and a light subsequent drift to lower negative values was noted.

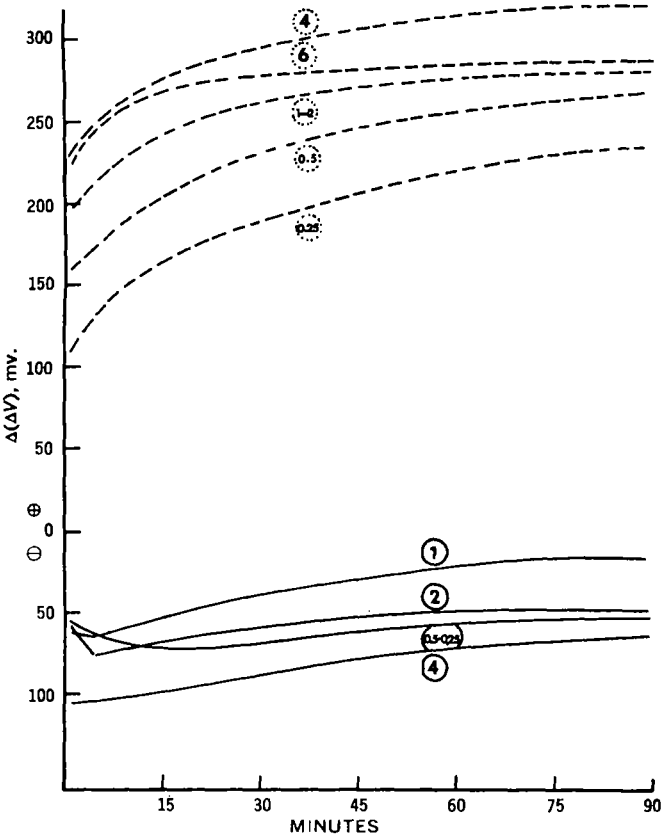


**Figure 5**—*Effect of the injection of cetrimonium bromide* (---) *or sodium cetyl sulfate* (——) *on the surface potential* [Δ(ΔV)] *of a dipalmitoyl glycerol monolayer at 5 dyne cm.*$^{-1}$. *Curves are labeled as to micromolar final concentrations of the injected surfactant ion.*

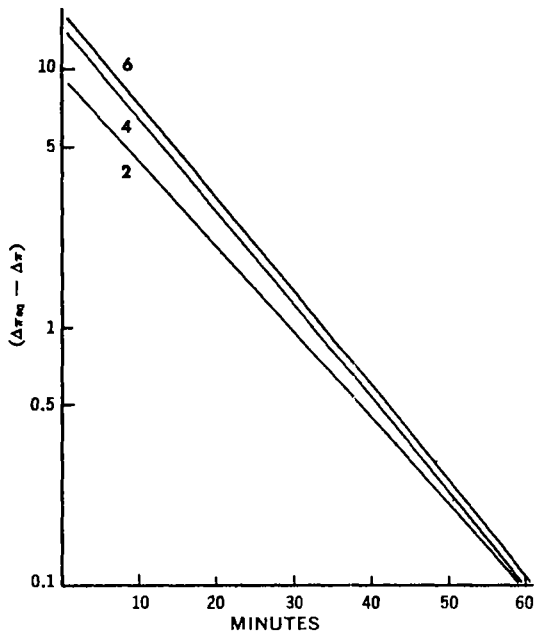


**Figure 6**—*Typical apparent first-order plots for the interaction of dipalmitoyl glycerol with cetyl sulfate ion. Curves are labeled with the final cetyl sulfate concentration.*

The injection of the positively charged cetrimonium ion produced increases in the surface potential of the dipalmitoyl glycerol monolayer to the range of +220–+320 mv. Table II summarizes the average values of $\Delta\pi$ and $\Delta(\Delta V)$ obtained.

**Kinetics of Interaction of Surfactant Ions with Monolayers**—The effective changes in the surface pressure, $\Delta\pi$, for the interaction of cetrimonium cation with dipalmitoyl glycerol and dipalmitoyl lecithin were essentially instantaneous processes under the experimental conditions. The new steady-state values were reached within a few minutes after the injection of the surfactant ions at practically all concentrations studied.

The general pattern for the kinetics of the increase of surface pressure on the interactions of cetyl sulfate anion with dipalmitoyl lecithin was an apparent first-order increase to a new steady-state value, $\Delta\pi_{eq}$. This was substantiated by the adherence of the data to the equation:

$$\log(\Delta\pi_{eq} - \Delta\pi) = -kt + \log(\Delta\pi_{eq} - \Delta\pi^0) \quad \text{(Eq. 1)}$$

as shown in Fig. 6.

The apparent first-order rate constant was 0.081 min.$^{-1}$ and did not appear to depend, between the limits of the experimental error, on the surfactant ion concentration between 2 and 6 $\mu M$. The curves at lower concentrations may have an induction period prior to the first-order attainment of their maximum value.

The kinetics of the cetyl sulfate anion interaction with dipalmitoyl glycerol was similar, and several first-order plots in accordance with Eq. 1 are given in Fig. 7. Again, the apparent first-order rate constant, $k$ = 0.075 min.$^{-1}$, for the achievement of the new steady-state value ($\Delta\pi_{eq}$) appears to be independent, between the limits of the experimental error, of the surfactant concentrations studied.

The rate constants for the interaction of cetyl sulfate anion with dipalmitoyl glycerol and dipalmitoyl lecithin were reasonably coincident.

## DISCUSSION

**Configuration of Molecules Forming Monolayers**—At the air/water interface, water molecules are almost completely oriented with their negative vertices toward the air phase. The interaction

**Table II**—Surface Parameters of Dipalmitoyl Lecithin and Dipalmitoyl Glycerol Monolayers after the Injection of Cetrimonium or Cetyl Sulfate Ions so that the Final Bulk Concentration is $C$

| | $C$, $\mu M$ | $t_{eq}$, min. | $\Delta\pi_{eq}$, dynes/cm. | $\Delta(\Delta V)$, mv. (90′) | | $C$, $\mu M$ | $t_{eq}$, min. | $\Delta\pi_{eq}$, dynes/cm. | $\Delta(\Delta V)$, mv. (90′) |
|---|---|---|---|---|---|---|---|---|---|
| Dipalmitoyl lecithin | 6 | 60 | 20.4 | −45 | Dipalmitoyl lecithin | 6 | 5 | 6.4 | +324 |
| ⇅ | 4 | 60 | 17.7 | −72 | ⇅ | 4 | 5 | 5.3 | +275 |
| | 2 | 60 | 9.1 | −47 | | 2 | 5 | 5.2 | +243 |
| | 1 | 75 | 5.7 | −56 | | 1 | 5 | 4.8 | +217 |
| Cetyl sulfate | 0.5 | 90 | 2.0 | −10 | Cetrimonium | 0.5 | 15 | 4.2 | +190 |
| | 0.25 | >180 | | | | 0.25 | 25 | 3.8 | +178 |
| Dipalmitoyl glycerol | 6 | 60 | 32.6 | −45 | Dipalmitoyl glycerol | 6 | 5 | 18.6 | +290 |
| ⇅ | 4 | 60 | 32.1 | −68 | ⇅ | 4 | 5 | 10.4 | +324 |
| | 2 | 60 | 24.5 | −44 | | 2 | 5 | 7.4 | +222 |
| | 1 | 60 | 19.5 | −20 | | 1 | 5 | 5.4 | +213 |
| Cetyl sulfate | 0.5 | 60 | 12.4 | −52 | Cetrimonium | 0.5 | 5 | 3.5 | +178 |
| | 0.25 | 90 | 8.4 | −46 | | 0.25 | 5 | 2.3 | +140 |

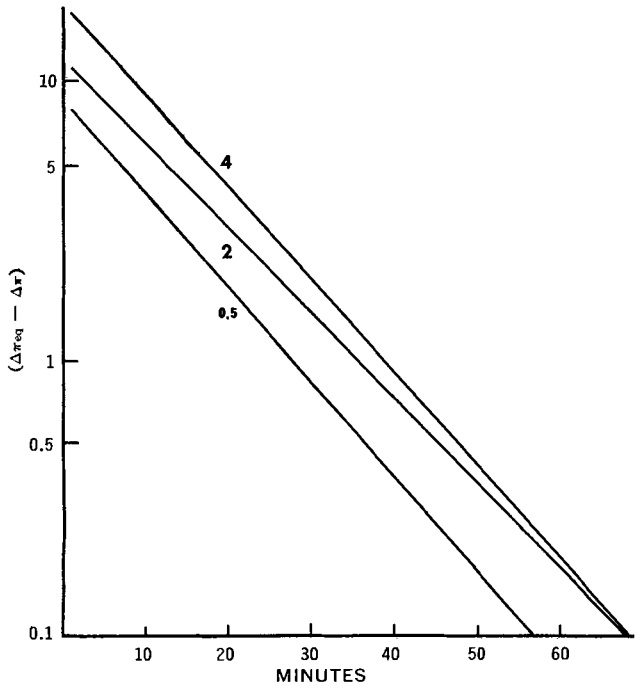


**Figure 7**—*Typical first-order plots for the interaction of dipalmitoyl glycerol with cetyl sulfate ion. Curves are labeled with the final cetyl sulfate concentration.*

energy for this orientation is supplied by the electrical asymmetry of the water molecule (16–18). Dipalmitoyl glycerol does not have ionic groups. However, its two fatty acid ester linkages and an alcoholic group give it a high electronic density in the region that, because of its hydrophilicity, tends to be oriented toward the water phase. The fact that the spreading of a dipalmitoyl glycerol monolayer at 5 dyne cm.$^{-1}$ reduces the negative air/water interfacial potential from −470 to −10 mv. (Table I) can be interpreted as the net result of the introduction at the air/water interface of a dipole with its positive end oriented to the air phase and its negative end oriented to the water phase. This fact and the 46 Å$^2$ area per molecule (Table I), roughly twice the cross-sectional area of a saturated hydrocarbon chain (~20 Å$^2$), suggest that the most probable statistical configuration of the dipalmitoyl glycerol molecule in the monolayer is that represented in Fig. 8, with the long vertical axis perpendicular to the plane of the interface.

The phosphoryltrimethylethanolamine group of dipalmitoyl lecithin has a phosphate group and a trimethylammonium group. Acid–base titrations of phospholipid monolayers followed by measuring the surface potential at constant area per molecule have shown that the lecithins have a zwitterionic structure in which both the phosphate ($PO_4^-$) group and the trimethylammonium ($N^+$) group are charged (19) between pH 3 and 7.

The spreading of a dipalmitoyl lecithin monolayer at 5 dyne cm.$^{-1}$ reduces the negative air/water interfacial potential from −470 to −130 mv. (Table I). Again, this effect can be accounted for by the introduction at the air/water interface of a dipole oriented with the positive end toward the air phase and the negative end toward the water phase. As before, this fact and the area per molecule of 55 Å$^2$ (Table I) suggest that the most probable statistical position of the dipalmitoyl lecithin molecule would be almost perpendicular to the plane of the interface with the phosphoryltrimethylethanolamine group directed toward the water phase and the hydrocarbon chains directed to the air phase.

Space-filling models show that unobstructed movements around the phosphate ($PO_4^-$) linkage permit the trimethylethanolamine group to adopt different extreme positions related to the long vertical axis of the dipalmitoyl lecithin molecule (Fig. 9). The trimethylammonium group can be extended toward the water phase below the phosphate group (Fig. 9*a*) or toward the air phase and over this group (Fig. 9*b*). In both cases, an imaginary line passing through the P and N atoms, which could represent its dipole moment, would be parallel to the long vertical axis and contribute to the total

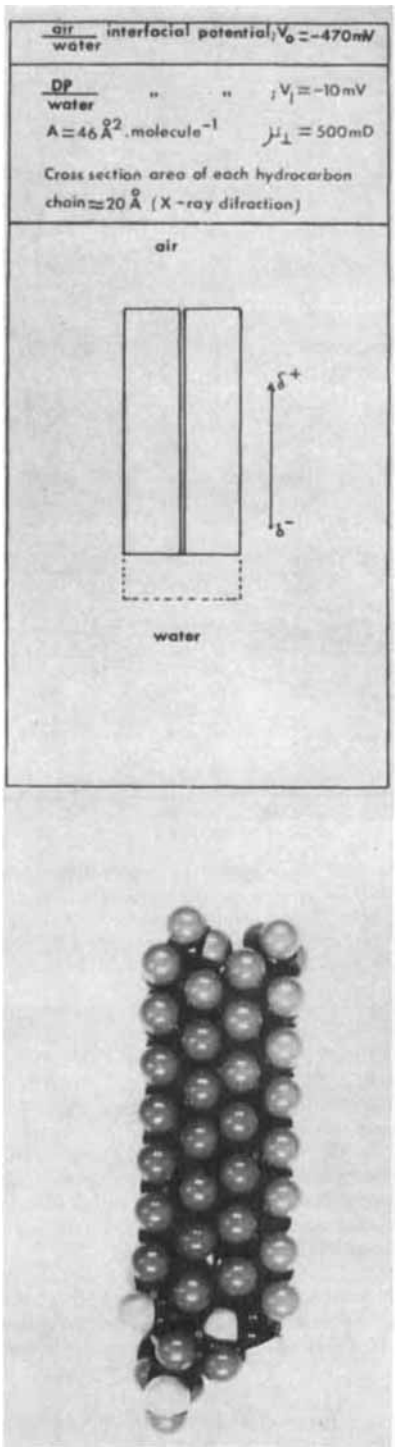


**Figure 8**—*Space-filling model of dipalmitoyl glycerol molecule.*

dipole moment of the molecule when measured along the vertical axis.

The trimethylammonium group also can adopt such a position (Fig. 9*c*) that the imaginary line is perpendicular to the long vertical axis. In this position, the P—N dipole would not contribute *per se* to the total dipole moment of the molecule when measured along the vertical axis. Some indirect contribution could be expected, however, because of induction effects of the positively charged nitrogen group on the diester linkage.

The average contribution of each molecule of the monolayer $\mu_\perp$ to the surface potential ($\Delta V$) can be calculated using the equation (9): $\mu_\perp = 2.65 \cdot 10^{-2} \cdot A \cdot \Delta V$, in which $\mu_\perp$ is the apparent operational total surface dipole moment per molecule in millidebyes

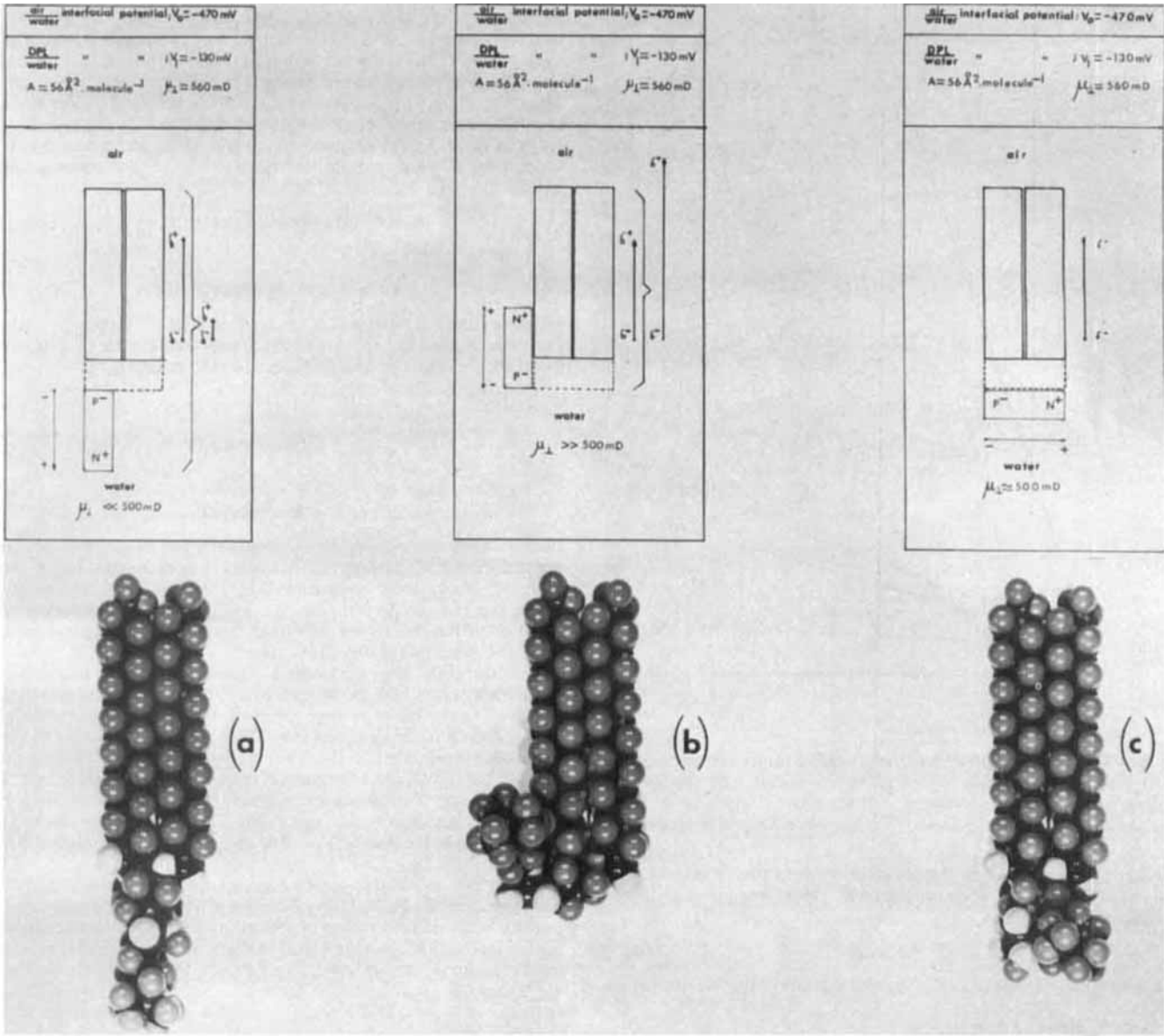


**Figure 9**— *Space-filling model of dipalmitoyl lecithin molecule showing (a, b, and c) the three different extreme positions of the phosphoryltrimethylethanolamine group.*

(1 mD. = $10^{18}$ e.s.u.), $A$ is the area per molecule in square Angstroms, and $\Delta V$ is the surface potential of the monolayer in millivolts.

The comparison of the surface dipole moments of dipalmitoyl lecithin and dipalmitoyl glycerol (Table I) shows that the phosphoryltrimethylethanolamine group slightly affects the apparent surface dipole moment of the dipalmitoyl glycerol moiety of the dipalmitoyl lecithin molecule, which in both cases could be principally attributed to the two fatty acid ester linkages alone (20–24). This suggests that the most probable statistical configuration of the phosphoryltrimethylamine group would be that represented in Fig. 9*c*. Here the perpendicularity of the P—N dipole to the long vertical axis of the dipalmitoyl lecithin molecule eliminates its contribution to the total surface dipole moment of the molecule when measured along this axis; such is the case in the measurement of the surface potential of the monolayer. Measurements of electrokinetic potentials (23, 24), surface dipole moment, and surface pK (22, 25) indicate that this configuration exists in monolayers of octadecyl lecithin, dipalmitoyl phosphatidyl ethanolamine, and natural lecithin monolayers at the air/aqueous solution interface. A parallel configuration for this dipole was previously proposed (12).

**Affinities of Interactions**—An insoluble monolayer at the air/water interface can be considered as an ordered array of active sites for the adsorption of surfactant ions approaching the monolayer. The rate of adsorption is a function of the number of free active sites and the frequency of collisions of surfactant ions against the monolayer. The rate of desorption is, in turn a function of the number of surfactant ions already adsorbed and the average probability per second that an adsorbed ion will become detached (26).

If $N_s$ is the total number of active sites, $N_a$ is the number of active sites occupied by the adsorbed ions, $Z$ is the frequency of collisions, and $W$ is the probability of detachment, at equilibrium:

$$(1 - N_a/N_s)\cdot Z = N_a \cdot W \qquad \text{(Eq. 2)}$$

and rearranging:

$$(N_s/N_a) = 1 + (N_s \cdot W/Z) \qquad \text{(Eq. 3)}$$

If ideal gas behavior is assumed, the frequency of collisions is given by:

$$Z = A \cdot n(kT/2\pi m)^{1/2} \qquad \text{(Eq. 4)}$$

in which $A$ is the total surface area, $n$ is the concentration, $k$ is the

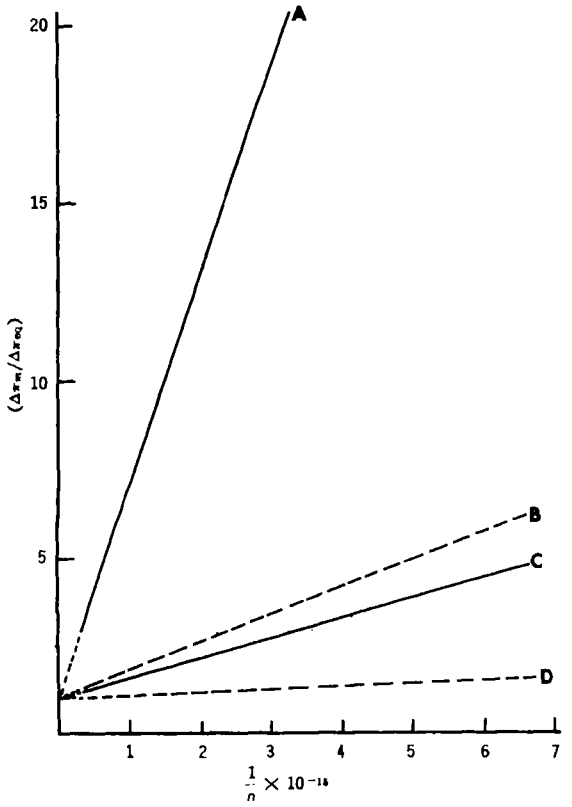


**Figure 10**—*Typical plots of* $\Delta\pi_m/\Delta\pi_{eq}$ *against* 1/n *for the interactions: (A) dipalmitoyl lecithin* ⇄ *cetyl sulfate anion, (B) dipalmitoyl glycerol* ⇄ *cetrimonium cation, (C) dipalmitoyl glycerol* ⇄ *cetyl sulfate anion, and (D) dipalmitoyl lecithin* ⇄ *cetrimonium cation.*

Boltzman constant, $T$ is the absolute temperature, $\pi$ is 3.14, and $m$ is the mass of the adsorbed particle. The average probability of detachment is given by:

$$W = \nu \cdot e^{-\psi/kT} \qquad \text{(Eq. 5)}$$

in which $\nu$ is a frequency (sec.$^{-1}$), and $\psi$ is an energy of activation (27).

Substituting and rearranging:

$$N_s/N_a = 1 + (N_s/A)(2\pi m/kT)^{1/2} \cdot \nu \cdot e^{-\psi/kT} \cdot \frac{1}{n} \qquad \text{(Eq. 6)}$$

in which $n$ is the concentration (particles/cm.$^3$).

It may be assumed that: (*a*) each molecule forming the monolayer constitutes one and only one active site for the attachment of a surfactant ion, and (*b*) the increase in the surface pressure by the injection of the surfactant solution is due to the attachment and interaction of the surfactant ion to a molecule at the monolayer. Thus, the maximum increase of the surface pressure occurs when each molecule at the monolayer has a surfactant ion attached so that the ratio $N_s/N_a$ is numerically equal to the ratio $\Delta\pi_m/\Delta\pi$, in which $\Delta\pi_m$ is the maximum increment of the surface pressure. By letting:

$$B = (N_s/A)(2\pi m/kT)^{1/2} \cdot \nu \cdot e^{-\psi/kT} \qquad \text{(Eq. 7)}$$

Eq. 6 can be rewritten:

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

It follows that from the plot of the reciprocal of the surface pressure increment against the reciprocal of the concentration at equilibrium (Fig. 10), the values of $\Delta\pi_m$ and $B$ can be calculated.

From Eq. 7:

$$\nu \cdot e^{-\psi/kT} = \frac{B}{(N_s/A)(2\pi m/kT)^{1/2}} = b \qquad \text{(Eq. 9)}$$

and:

$$\ln b = \ln \nu - \psi/kT \qquad \text{(Eq. 10)}$$

Phospholipid monolayers exhibit surface phase transitions which are temperature dependent (8). Attempts to obtain the value of the energy of activation by experimentally measuring the temperature coefficient of $b$ would introduce an ambiguity in the definition of the state of the monolayer. However, the following approximative method can be used to obtain $\psi$.

The statistical thermodynamic derivation of the adsorption isotherm assumes that the motion of a localized adsorbed molecule is that of an oscillator in three dimensions, with two of them in the plane of the interface and the third dimension perpendicular to the plane of the interface (28, 29). The corresponding partition function for this last dimension is a function only of the temperature and of the classical oscillation frequency, $\nu$, which has the order of magnitude of $10^{12}$ sec.$^{-1}$ in typical cases (30). The values of the energy of activation calculated using $\nu = 10^{12}$ sec.$^{-1}$ for each interaction are shown in Table III.

Our values for $\nu$ and $\psi$ may be compared with others obtained on a thermodynamic basis. Using Clausius–Clapeyron-type equations, integral heats of penetration of 12 kcal. mole$^{-1}$ have been reported for the interaction of sodium lauryl sulfate with cetyl alcohol monolayers spread at the air/water interface (6). If this value is used as an energy of activation, the procedure just outlined permits an estimate of $\nu = 0.8 \cdot 10^{12}$ sec.$^{-1}$ from the reported experimental data.

**Mechanism of Interaction**—It has been suggested (31) that for solutions of long-chain alcohols, the disagreement between the rates of change of surface pressure and surface potential with time may be due to the time-dependent rearrangements of the dipoles at the interface after adsorption. In the case of sodium cetyl sulfate solutions (32), with or without a cholesterol or cetyl alcohol

**Table III**—Estimated[a] Energies of Activation, $\psi$, of the Interaction of Dipalmitoyl Glycerol and Dipalmitoyl Lecithin Monolayers with Cetrimonium or Cetyl Sulfate Ions

| Interaction | $\Delta\pi_m$, dyne·cm.$^{-1}$ | $B$, molecule·cm.$^{-3}$/ dyne·cm.$^{-1}$ | $N_s/A$, molecule·cm.$^{-2}$ | $m$, g. | $\nu \cdot e^{-\psi/KT}$ | $\psi$, kcal.·mole$^{-1}$ |
|---|---|---|---|---|---|---|
| Dipalmitoyl glycerol ⇅ Cetyl sulfate | 41.6 | $5.8 \cdot 10^{14}$ | $2.19 \cdot 10^{14}$ | $53.6 \cdot 10^{-23}$ | $9.2 \cdot 10^{3}$ | 10.8 |
| Dipalmitoyl glycerol ⇅ Cetrimonium | 13.7 | $7.8 \cdot 10^{14}$ | $2.19 \cdot 10^{14}$ | $47.4 \cdot 10^{-23}$ | $13.2 \cdot 10^{3}$ | 10.6 |
| Dipalmitoyl lecithin ⇅ Cetyl sulfate | 37.6 | $59.6 \cdot 10^{14}$ | $1.82 \cdot 10^{14}$ | $53.6 \cdot 10^{-23}$ | $114.4 \cdot 10^{3}$ | 9.4 |
| Dipalmitoyl lecithin ⇅ Cetrimonium | 5.9 | $0.9 \cdot 10^{14}$ | $1.82 \cdot 10^{14}$ | $47.4 \cdot 10^{-23}$ | $1.8 \cdot 10^{3}$ | 11.8 |

[a] Estimated by use of the relations: $\frac{1}{\Delta\pi_{eq}} = \frac{1}{\Delta\pi_m} + \frac{B}{\Delta\pi_m} \cdot \frac{1}{n}$; $B = \left(\frac{N_s}{A}\right)\left(\frac{2\pi m}{kT}\right)^{\frac{1}{2}} \cdot \nu \cdot e^{-\psi/KT}$; $\nu = 10^{12}$ sec.$^{-1}$

monolayer spread at the air/solution interface, the surface pressure increases with the compression of the monolayer while the surface excess concentration of the adsorbed cetyl sulfate anion does not change appreciably.

The comparison of the curves of Figs. 2–5 suggests that the increase of the surface pressure and the increase of the surface potential may reflect two different steps of the interaction process. In spite of the differences in the rates of increase of the surface pressure by the injection of cetrimonium cation or cetyl sulfate anion, the rates of change of the surface potential increment are practically the same in all cases. More than 80% of this increment is achieved during the first 5–15 min. after the injection.

This variation of the surface potential could be mainly the result of the attachment of the injected ion to the molecules that form the monolayer. The subsequent drift observed could be attributed to the variation of the surface dipole concentration because of a progressive change in the surface pressure of the injected monolayer or because of a rearrangement of the adsorbed surfactant dipoles.

This interpretation is supported by the fact that the injection of trimethyl(2-hydroxyethyl)ammonium chloride (choline chloride) up to a maximum concentration of 0.15 *M* beneath a dipalmitoyl glycerol or a dipalmitoyl lecithin monolayer does not affect the surface pressure for as long as 120 min. but increases the surface potential by 110 mv. in the first 10 min.

If this hypothesis is correct, the increment of the surface pressure should reflect the interaction of the hydrocarbon chains of the injected surfactant ion with the hydrophobic moiety of the molecules that form the monolayer. Consequently, the value of $\Delta\pi_{eq}$ represents the maximum of this interaction in each case.

Table II shows that the $\Delta\pi_{eq}$ values for the injection of cetyl sulfate anion beneath a dipalmitoyl lecithin monolayer average about 12 dyne cm.$^{-1}$ lower than those corresponding to the injection of cetyl sulfate anion beneath a dipalmitoyl glycerol monolayer. Thus, the presence of the phosphoryltrimethylethanolamine group decreases to some extent the interaction of the cetyl sulfate ion hydrocarbon chain, presumably already attached to the trimethylammonium group of dipalmitoyl lecithin with the hydrophobic moiety of the monolayer.

A similar effect can be observed for the interaction of the cetrimonium cation, but in this case the average difference is smaller and even inverted for the lower concentration. In this case it may be presumed that this cation is attached to the phosphate group of dipalmitoyl lecithin.

The comparison of the $\Delta\pi_{eq}$ values for the interaction of cetyl sulfate anion and cetrimonium cation with dipalmitoyl glycerol monolayers shows a higher effect of the former on the surface pressure, even though both ions are of the same chain length. On the other hand, Table III shows that the energy of activation for both interaction processes have almost identical values (10.6–10.8 kcal. mole$^{-1}$). The positive cetrimonium undoubtedly interacts with the high electronic density region of the hydrophilic moiety of the dipalmitoyl glycerol molecule through ion–dipole interactions, although some steric restrictions may exist so that the interaction of the hydrocarbon chains of the surfactant with the hydrophobic moiety of the dipalmitoyl glycerol molecules may be inhibited to some extent.

A "reactivity series" has been claimed for the interaction of cholesterol monolayers with a series of dodecyl compounds with various ionic heads when the latter are injected in the subphase. Sodium lauryl sulfate (dodecyl sodium sulfate) was stated to be the "most reactive" and the dodecyltrimethylammonium iodide the "least reactive" (33). The experimental data of the original paper (Fig. 8, *Reference 33*) are expressed in terms of milligrams of injected surfactant. When these experimental data are recalculated using *surfactant ion·cm.*$^{-3}$ as the concentration units, the method outlined above gives the same value (11.2−11.4 kcal. mole$^{-1}$) for the energy of activation for either interaction.

Thus, the results obtained with nonionic dipalmitoyl glycerol monolayers and cetyl sulfate anion or cetrimonium cation injection compare with these corrected values and strongly suggest that the main forces involved in the interaction process between the hydrocarbon chain of the injected surfactant ion and the hydrophobic moiety of the molecules that form the monolayer are of the van der Waals type.

The comparison of the cetyl sulfate anion and cetrimonium cation interactions with dipalmitoyl lecithin monolayers shows higher $\Delta\pi_{eq}$ values for cetyl sulfate at 6, 4, 2, and 1 $\mu M$ concentrations. At 0.5 $\mu M$, the value is higher for the cetrimonium interaction (Table II). The energies of activation are significantly different for the interaction of cetyl sulfate anion (9.4 kcal. mole$^{-1}$) and cetrimonium cation (11.8 kcal. mole$^{-1}$) (Table III). However, the almost identical energies of activation for the interaction of these ions with dipalmitoyl glycerol monolayers permit the conclusion that the observed difference with dipalmitoyl lecithin monolayers is due to the presence of the phosphoryltrimethylethanolamine group.

It can be safely assumed in the case of dipalmitoyl lecithin that the cetyl sulfate anion tends to be attached electrostatically to the trimethylammonium group while the cetrimonium cation tends to be similarly attached to the phosphate group. The difference in the energies of interaction indicates that the ionic groups of dipalmitoyl lecithin are not equivalent in the perturbation that they introduce in the state of an attached hydrocarbon chain, as reflected by the differences in the surface pressures of the monolayers.

This difference may be explained by the different mobilities that a hydrocarbon chain would have when attached to one or the other ionic attraction centers of the phosphoryltrimethylethanolamine group. The attachment of the cetrimonium cation to the phosphate group would be equivalent to the attachment of this ion to the region of high electronic density of dipalmitoyl glycerol because of the position of the phosphate group in the dipalmitoyl lecithin molecule. However, when the unrestricted movement of the trimethylammonium group around the phosphate linkage is taken into account, the attachment of the cetyl sulfate anion to the trimethylammonium group would necessarily produce a completely different situation for the attached hydrocarbon chain.

**Biomembrane Implications**—It has been proposed that transitions between "open" and "closed" configurations on biomembranes produced by the geometrical changes of the micellar form of the lipidic micelles of the membrane could provide a basis to explain protoplasmic streaming, ameboidal locomotion, and active transport (34). This was based on the assumption that one principal regulating factor is the *local* charge distribution in the thin crust of water, which is oriented and compressed or expanded to varying degrees at the membrane interfaces.

The large movement of flexible dipoles at one end of the long hydrocarbon chain of phospholipids has been rationalized to control the ion flow in biomembranes by acting as a gate operated by changes in the electric field (35). This model proposes that the gate mechanism is highly sensitive to the nature of the approaching ion.

The selective expansion effect of sodium ions on dipalmitoyl lecithin monolayers (11) has been used jointly with the measurements of unidirectional flux of sodium ions from the outer bathing solution into the epithelium to conclude that the penetration of sodium ions does not occur by simple diffusion but depends on a specific interaction of the sodium ions with the polar groups of the outer leaflet of the plasma membrane (36).

All these views have the common characteristic that the lipids of the cell membranes react differently according to the nature of the approaching ion or molecular species.

The observations reported here that identical hydrocarbon chains attached to the positive or to the negative attraction centers of dipalmitoyl lecithin molecules produce different perturbations in the state of the monolayer with different energies of activation gives some insight into the molecular characteristics of these interactions and on how the approaching molecule could affect the microstates of biomembranes.

Studies are in progress on the effect of chain length on the energy of activation and the variations of the monolayer properties. These studies should give further insight into the dynamics of biomembrane interactions with pharmacologically important molecules.

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# Electron Impact Fragmentation Studies of β-Blocking Drugs and Their Metabolites by GC–Mass Spectroscopy

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**Abstract** □ This work describes the mass fragmentation patterns of trifluoroacetylated derivatives of five *aryloxy* β-blocking drugs as well as five metabolites of this chemical and therapeutic class. This chemical class is characterized by intense ions at *m/e* 308, 266, and 43 and by a strong metastable ion at *m/e* 229.2. The mechanism of fragmentation was confirmed with the hexadeuterated ($d_6$) analogs of these compounds. In addition, mass spectral features are described for three *arylalkyl* β-blocking drugs. These mass spectral data should facilitate the rapid and accurate determination of the metabolic fate of these and other β-blocking drugs of this chemical class.

**Keyphrases** □ β-Adrenergic blocking agents and metabolites—mass fragmentation patterns of trifluoroacetyl derivatives □ Metabolites of β-adrenergic blocking agents—mass fragmentation patterns of trifluoroacetyl derivatives □ Electron impact fragmentation patterns—β-adrenergic blocking agents and metabolites, GC–mass spectroscopy □ GC–mass spectroscopy—electron impact fragmentation patterns, β-adrenergic blocking agents and metabolites

Rigorous qualitative or quantitative studies in drug metabolism ultimately require proof of the molecular structures under investigation. These structure determinations must frequently be carried out on submicrogram quantities of drugs and drug metabolites in complex chemical mixtures of biological origin and can become difficult, time consuming, and expensive.

The combined gas chromatograph–mass spectrometer has provided a powerful tool to facilitate structure elucidation of compounds eluting from a GC column. Proof of structure by this technique still requires a comparison of recorded mass spectra to those of pure reference compounds, or a thorough knowledge of rigorously established fragmentation mechanisms of the chemical class under investigation may suffice.

This report describes electron impact fragmentation patterns and associated mechanisms for trifluoroacetylated derivatives of a pharmacologically complex and