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Kinetics and Mechanisms of Monolayer Interactions II: Effect of Chain Length of Alkyl Ionic Surfactants on Their Interaction with Dipalmitoyl Glycerol, Dipalmitoyl Phosphatidylethanolamine, and Dipalmitoyl Lecithin

FEDERICO A. VILALLONGA▲ and EDWARD R. GARRETT

Abstract □ The energies of activation of the interaction of alkyl trimethylammonium surfactants with dipalmitoyl glycerol, dipalmitoyl phosphatidylethanolamine, and dipalmitoyl lecithin monolayers spread at the air–water interface were estimated from the increase of the surface pressure with the concentration of the injected surfactant. The energies of activation are a linear function of the chain length of the injected surfactant for the six-, eight-, 12-, and 16-carbon chains studied. The ionic groups of the polar hydrophilic moiety of dipalmitoyl lecithin were not equivalent in the perturbation that an attached surfactant ion produces in the surface pressure of the monolayer. The energies of activation per methylene group of the hydrocarbon chain and per polar ionic head of the injected surfactant were calculated and were compared with those that correspond to the energies of adsorption of these groups at hydrocarbon–water and air–water interfaces.

Keyphrases □ Alkyl sulfates and alkyl trimethylammonium ions—interactions with dipalmitoyl glycerol, dipalmitoyl phosphatidylethanolamine, and dipalmitoyl lecithin monolayers, kinetics, mechanisms □ Monolayers, dipalmitoyl glycerol, dipalmitoyl phosphatidylethanolamine, and dipalmitoyl glycerol—interactions with alkyl sulfate and alkyl trimethylammonium ions, kinetics, mechanisms □ Phospholipid monolayers—interactions with long-chain surfactants, kinetics, mechanisms □ Surfactants, long chain—interaction with phospholipid monolayers, kinetics, mechanisms □ Chain length effect—interaction of alkyl surfactants with phospholipid monolayers

The energies of interaction of cetyl sulfate and cetrimonium ions with dipalmitoyl glycerol and dipalmitoyl lecithin monolayers spread at the air–water interface were estimated recently (1) from the variation of the equilibrium surface pressure with varying concentrations of subphase-injected surfactants on the premise that the entropy factor calculated on the basis of collision theory was constant for all species.

The energies of adsorption at the oil–water and air–water interfaces of homologous series of alkyl surfactants are provided in the literature (2–11). These data permit the estimation of the contributions of the hydrocarbon moiety per methylene group and that of the polar head to the total energy of adsorption at a "clean" interface. A clean interface is defined here as a liquid–liquid or air–liquid interface without any spread monolayer.

These studies were designed to determine the dependence of the maximum obtainable changes in surface pressure and energies of interaction of monolayers of dipalmitoyl glycerol, dipalmitoyl phosphatidylethanolamine, and dipalmitoyl lecithin with subphase-injected surfactants on the numbers of methylene groups and on the nature of the charged polar head in the injected surfactant.

EXPERIMENTAL

Reagents—Dipalmitoyl glycerol[1], dipalmitoyl lecithin[1], cetyl sodium sulfate[1], and cetrimonium bromide[2] were the same samples

[1] Schwarz/Mann Research Laboratories, Orangeburg, N. Y.
[2] Eastman Kodak, Rochester, N. Y.

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

Interaction		ψ, kcal. mole^{-1}				Energy of Activation, kcal. group^{-1} mole^{-1}			Percent of Methylene Groups of C_n Interacting[a]
		C_n							
Lipidic Monolayer	Surfactant Ion	6	8	12	16	$-CH_2-$	$-SO_4^-$	$-N^+-(CH_3)_3$	
Dipalmitoyl glycerol	$C_{n(2n+1)}\cdot SO_4^-$	−4.8	−6.1	−8.2	−10.7	−0.58	−1.3	—	58
	$C_nH_{(2n+1)}\cdot N^+(CH_3)_3$	−4.4	−5.8	−8.2	−10.8	−0.63	—	−0.64	63
Dipalmitoyl phosphatidylethanolamine	$C_nH_{(2n+1)}\cdot SO_4^-$	−5.1	−5.7	−8.6	−10.9	−0.60	−1.2	—	60
	$C_nH_{(2n+1)}\cdot N^+(CH_3)_3$	−5.1	−6.3	−9.1	−11.3	−0.63	—	−1.4	63
Dipalmitoyl lecithin	$C_nH_{(2n+1)}\cdot SO_4^-$	−4.2	−5.4	−8.4	−9.6	−0.56	−1.0	—	56
	$C_{n(2n+1)}\cdot N^+(CH_3)_3$	−4.7	−5.9	−10.6	−12.0	−0.78	—	−0.1	78

[a] Calculated on the premise that 1 kcal. mole^{-1} is the energy of interaction per pair of methylene groups in close contact (4.2 Å).

described previously (1). Dipalmitoyl phosphatidylethanolamine[1] was chromatographically homogeneous by TLC (12). Sodium lauryl sulfate[2], sodium octyl sulfate[1], sodium hexyl sulfate[1], and dodecyltrimethylammonium bromide[3] gave no minima in the curves of surface tension against the logarithm of the concentration. The octyltrimethylammonium and hexyltrimethylammonium bromides were prepared[4] by the reaction of *n*-dimethyloctylamine with methyl bromide and *n*-hexyl bromide with trimethylamine and were chromatographically homogeneous by TLC (13). The hexanes[2,5] used to prepare the lipidic spreading solutions and the distilled water used as subphase fulfilled the requirements described previously (1).

Instruments and Methods—Surface tension was measured with a Wilhelmi platinum plate attached to an electrobalance[6]. Surface potential was measured with an americium-241 air electrode and an electrometer[7]. The output of the electrobalance and the electrometer were fed into a dual-pen recorder[8].

The experimental assembly and the methods for the measurement of the increase of the surface pressure after the injection of the alkyl surfactant ion were described previously (1). The concentration of the surfactant solution to be injected was kept below the critical micelle concentration (CMC) in all cases. The average values of three different experiments for each of three different final concentrations of injected surfactant ions were used for the estimation of the slope of the plot of the reciprocal of the change of the surface pressure at equilibrium ($1/\Delta\pi_{eq}$) against the reciprocal of the number of surfactant ions per cubic centimeter ($1/n$) (1). The reproducibility of the changes of the surface pressure was within ±0.5 dyne cm.$^{-1}$. All experiments were performed at an initial surface pressure of the lipidic monolayer of 5 dynes cm.$^{-1}$ (±0.1 dyne cm.$^{-1}$) and at 22° (±1°).

RESULTS

The values of the maximum change of the surface pressure ($\Delta\pi_m$) were calculated from the intercept, $1/\Delta\pi_m$, of the plot of the reciprocal of the surface pressure change at equilibrium ($1/\Delta\pi_{eq}$) against the reciprocal of the number of subphase surfactant ions per cubic centimeter ($1/n$) on the assumption of the continuity of the straight-line relationship for surfactant concentrations above those used in the experiments in accordance with the equation: $1/\Delta\pi_{eq} = 1/\Delta\pi_m + B/\Delta_m \cdot 1/n$. They are plotted in Fig. 1 for dipalmitoyl glycerol, dipalmitoyl phosphatidylethanolamine, and dipalmitoyl lecithin monolayers against the chain length of the injected alkyl sulfate anions and alkyl trimethylammonium cations.

The maximum change of the surface pressure ($\Delta\pi_m$) is not a linear function of the chain length of the injected surfactant ion. The plots present distinct *maxima* in $\Delta\pi_m$ at the eight-carbon atom chain for the observed cases of the interaction of the positively charged alkyl trimethylammonium cations with the monolayers. The plots present *minima* at this same chain length for the interaction of the negatively charged alkyl sulfates with dipalmitoyl glycerol and dipalmitoyl lecithin monolayers. The minimum may be at 12-carbon atoms with dipalmitoyl phosphatidylethanolamine monolayers.

The energies of activation (ψ) were calculated on the premise of a collision model and a constant entropy factor (1) for the interace tion of the subphase-injected surfactant ion with the moleculer that form the monolayer at the air-water interface. They ahe plotted in Fig. 2*a* for dipalmitoyl glycerol monolayers against ts-chain length of the injected alkyl sulfate anions and alkyl trimethylammonium cations. The calculated energy of activation for a given chain length of the injected surfactant was independent of the

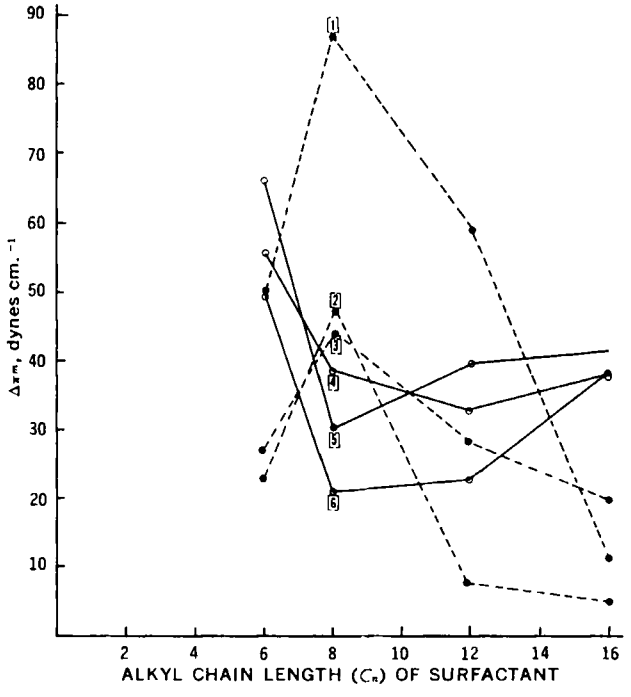

Figure 1—*Effect of chain length of alkyl surfactants* (C_n) *on the maximum surface pressure* ($\Delta\pi_m$). *Key: ---, alkyl trimethylammonium surfactants interacting with: (1) dipalmitoyl glycerol, (2) dipalmitoyl lecithin, and (3) dipalmitoyl phosphatidylethanolamine; and ——, alkyl sulfate surfactants interacting with: (4) dipalmitoyl phosphatidylethanolamine, (5) dipalmitoyl glycerol, and (6) dipalmitoyl lecithin.*

[3] Pfaltz and Bauer, Flushing, N. Y.
[4] Prepared by K. V. Rao.
[5] Spectroquality, Matheson, Coleman and Bell, East Rutherford, N. J.
[6] Cahn Division, Ventrom Instruments Corp., Paramount, Calif.
[7] Keithley Instruments (610c), Cleveland, Ohio.
[8] Speedomax w/1, Leeds and Northrup, North Wales, Pa.

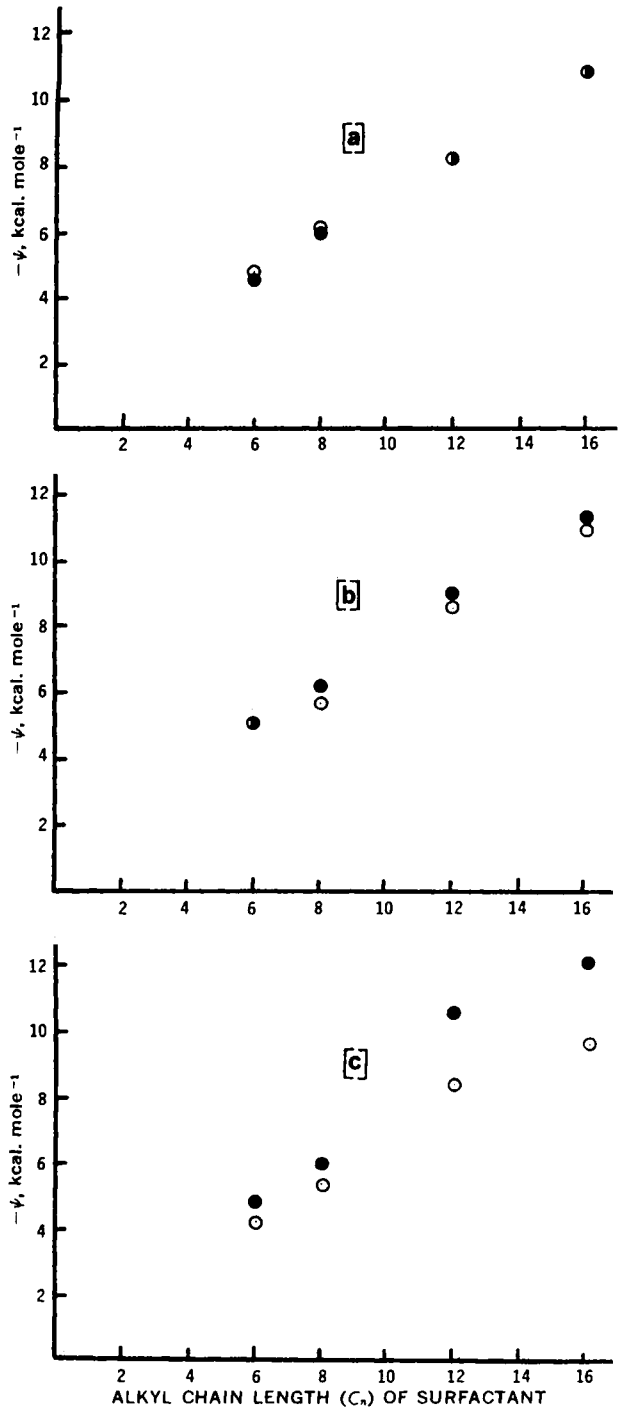

Figure 2—*Effect of chain length of alkyl sulfate* (○) *and alkyl trimethylammonium* (●) *surfactants on the energy of activation of their interactions with: (a) dipalmitoyl glycerol, (b) dipalmitoyl phosphatidylethanolamine, and (c) dipalmitoyl lecithin monolayers.*

nature of their polar heads within the limits of experimental error (±0.1 kcal. mole^{-1}) and was a linear function of the chain length between the studied six- and 16-carbon atoms.

The energies of activation (ψ) for the interaction of dipalmitoyl phosphatidylethanolamine monolayers were plotted against the chain lengths of the same series of alkyl sulfate and alkyl trimethylammonium ions in Fig. 2*b*, and the results were similar.

The energies of activation (ψ) for the interaction of dipalmitoyl lecithin monolayers with both series of surfactant ions are given in Fig. 2*c*. In these cases, there was a difference between the energy of activation of the interaction with the surfactant with the positively charged polar head compared with the interaction with the surfactant with the negatively charged head at a given chain length. This was more pronounced at the higher chain lengths. The same general increase in the energy of activation with the number of carbon atoms in the alkyl chain of the injected surfactant ion was observed as with the two other monolayers.

The energy of activation (Table I) per methylene group of the surfactant hydrocarbon chain was calculated from the regression coefficient of the best straight lines through the experimental points of Fig. 2. The energies of activation for the sulfate or trimethylammonium groups were calculated from the intercept on the assumption that the extrapolation of the straight line to $C_n = 0$ (C_n represents alkyl chain length of surfactant) gives a numerical value related to the energy of activation of the polar head group of the surfactant ion. The values of the energy of activation per methylene group were in the range of from −0.56 to −0.77 kcal. mole^{-1}, with an overall average value of −0.63 kcal. mole^{-1}. The values of the energy of activation per polar head of the injected surfactant ion were in the range of from −0.10 to −1.4 kcal. mole^{-1}.

DISCUSSION

Interfacial Phase—It has been suggested (1, 14) that the molecules that form these lipidic monolayers at a surface pressure of 5 dynes cm.$^{-1}$ are oriented with the long hydrocarbon chains perpendicular to the plane of the air-water interface. The total length of the dipalmitoyl glycerol molecule is roughly 26 Å measured from space-filling models. The length of dipalmitoyl phosphatidylethanolamine and dipalmitoyl lecithin molecules, assuming a configuration of the phosphatidylethanolamine polar group parallel to the interface (see Fig. 9, *Reference 1*), is roughly 30 Å. If the molecules are completely submerged in the water phase, the volume of the interfacial phase is determined by the length of the molecule times the total surface area of the air-water interface, which was 42.54 cm.^{2} in all of the experiments. The concentrations (in moles per liter) in this thin crust of water were calculated (Table II) for each lipid on this basis, since the total number of molecules necessary to form the monolayer was known.

The percentage of water in this interfacial phase (Table II) can be estimated from the total volume of the interfacial phase and from the volume occupied by the lipidic molecules calculated from the length of the molecule, the cross-sectional area of two hydrocarbon chains, and the total number of lipidic molecules. According to the values of Table II, the interfacial phase can be considered to be predominantly hydrocarbon.

Surfactant-Ion Hydrocarbon Chain—Van der Waals' forces between methylene groups of neighboring hydrocarbon chains can be calculated from Salem's equation (15):

$$W_{disp} = A\left(\frac{9.42}{8 \times l}\right)\left(\frac{n}{d^6}\right) \qquad \text{(Eq. 1)}$$

where W_{disp} is the energy of interaction in kcal. mole^{-1} for each pair of methylene groups of neighboring chains, A is a constant (−1.340 kcal. mole^{-1}), l is the effective distance between carbon atoms along a chain (1.253 Å), n is the number of methylene pairs, and d is the distance between the central axes of the hydrocarbon chains. The equation is valid for $d \geq 4.0$ Å. The energy of interaction becomes appreciable when d is less than 7 Å and increases exponentially with shorter distances as the hydrocarbon chains approach each other.

The minimum possible distance between the central axes of two neighboring hydrocarbon chains is 4.2 Å. From Eq. 1, the energy of interaction per pair of methylene groups will be 1.0 kcal. mole^{-1} for that distance.

The contribution to the total energy of activation that corresponds to the hydrocarbon chain for the interaction of the surfactant ions with the lipidic monolayers can be estimated by subtracting the contribution that corresponds to the polar ionic head from the total energy of activation (Table I). On the assumption that each methylene group of the surfactant ion that interacts with a methylene group of the hydrocarbon chains of the molecules that form the monolayer is in close contact (*i.e.*, at 4.2 Å with 1.0 kcal. mole^{-1} per pair of methylene groups), these values would numerically approximate the number of methylene groups of the surfactant ion that interacts with the hydrophobic moiety of the monolayer.

It was suggested (1) that the first step of the interaction process was the attachment of the polar charged head of the injected surfactant ion to the polar or the ionic attraction centers of the hydro-

Table II—Average Values of the Surface Parameters of Dipalmitoyl Glycerol, Dipalmitoyl Phosphatidylethanolamine, and Dipalmitoyl Lecithin before Injection of the Surfactant

	Dynes cm.$^{-1}$	Å^2 molecule^{-1}	ΔV, mv.	μ, mD.	Total Number of Molecules at Interface (42.54 cm.^{2})	Molarity at Interfacial Phase, mole l.$^{-1}$	Molecular Volume, Å^3	Volume of Water in Interfacial Phase, %
Dipalmitoyl glycerol	5(±0.1)	46(±2)	+340(±20)	500	9.3×10^{15}	1.4[a]	1014	14.7
Dipalmitoyl phosphatidyl-ethanolamine	5(±0.1)	40(±2)	+500(±20)	530	10.7×10^{15}	1.4[b]	1170	1.9
Dipalmitoyl Lecithin	5(±0.1)	55(±2)	+460(±20)	560	7.7×10^{15}	1.0[b]	1170	29.4

[a] Calculated from an estimated 1106 Å^3 volume of the interfacial phase. [b] Calculated from an estimated 1276 Å^3 volume of the interfacial phase.

philic moiety of the molecules that form the monolayer. The subsequent increase of the surface pressure could then be rationalized as reflecting the subsequent interactions of the hydrocarbon chains with the hydrophobic moiety.

The hydrophilic region of the dipalmitoyl glycerol molecule does not have an ionic charge. However, the two fatty acid ester linkages and an alcoholic group give a high electronic density to this region. It can be assumed that the positively charged or negatively charged surfactant-ion polar head tends to be attached by ion-dipole interactions to this region. It is noted that the percentage of methylene groups in close contact with the hydrophobic region of the monolayer apparently is independent of the charge of the surfactant ion (64 and 58%) (Table I).

The hydrophilic zwitterionic group of dipalmitoyl phosphatidylethanolamine is fully charged at the experimental conditions (pH 6.0–6.5). Its coplanarity with the plane of the interface (18–21) produces an attractive potential between vicinal molecules which keeps the monolayer closely packed (Table II). The strong P^-–N^+ electrostatic interaction jointly with the attractive potential between neighboring molecules should produce a rigid structuring of the monolayer in this case where any net charge is essentially negligible. Thus, it is understood that the calculated percentage of methylene groups in close contact with the hydrophobic region of this monolayer is similar to that of the nonionic dipalmitoyl glycerol (Table II).

The hydrophilic zwitterionic group of dipalmitoyl lecithin is similarly charged in the experimental conditions and should show a similar rigid structure. [The studies on the conformation of this group have resulted in much disagreement. The zwitterion has been considered oriented with its axis parallel to the plane of the interface (1, 14, 16–19), perpendicular (19, 20), or at some unlocalized intermediate position (23). On the basis of the apparent dipole moment measured (1, 14) on monolayers spread at the air–water interface, it will be assumed in the following discussion that the zwitterionic group of dipalmitoyl lecithin is oriented with its axis *parallel* to the plane of the interface.]

However, it can be argued that the dipalmitoyl lecithin monolayer would be less closely packed at the air–water interface than that of dipalmitoyl phosphatidylethanolamine (Table II) because of the shielding effect of the positively charged amino group by the three methyl groups that decreases the attractive potential between neighboring molecules or because of the lack of hydrogens that would promote hydrogen bonding. Accordingly, a less rigid structural organization of this monolayer can be expected as compared with the dipalmitoyl phosphatidylethanolamine and has been shown experimentally as indicated by the comparison of the Å^2 molecule^{-1} in Table II.

The calculated percentage of methylene groups of the surfactant hydrocarbon chain supposedly in close contact with the hydrophobic region of the dipalmitoyl lecithin monolayer differs markedly with the nature of the charge of the surfactant polar head. This contrasts with the other two monolayers. It can be assumed that the first step of the interaction involves the attachment of the negatively charged polar head of the surfactant to the positively charged trimethylammonium group of the P–N ionic dipole. Such an attachment could produce a change in the dipole orientation toward the perpendicular position that would displace the trimethylammonium group of the phosphatidylcholine moiety 5 Å farther below the hydrophobic region of the dipalmitoyl lecithin molecules.

The phosphate group of the P–N dipole constitutes the hinge for this change or orientation (Fig. 9 of *Reference 1*). Consequently the attachment of a positively charged polar head to the hinge should not readily produce rotation about the hinge. However, the attachment of a negatively charged polar head to the extreme end of the lever could readily cause rotation about the hinge to 5 Å below the hydrophobic portion of the dipalmitoyl lecithin molecule. The greater rigidity of the dipalmitoyl phosphatidylethanolamine molecule and its greater affinity for its neighboring molecules in the monolayer, as already stated, could rationalize the greater resistance to such a change in the steric position of its P–N axis. The fact that space-filling models show that a 5-Å lowering of the trimethylammonium group subtracts two to three pairs of methylene groups of an attached surfactant hydrocarbon chain from the hydrophobic region of the dipalmitoyl lecithin molecule is consistent with this explanation.

These phenomena strongly suggest that entropic factors associated with the configuration of the molecules that form the monolayer *do* contribute to the energies of interaction. Thus, the simplified assumption of a common entropy factor for such interactions based on collision theory on which the calculations of energies of activation were based (1) is most probably an oversimplification of a complex process.

Surfactant-Ion Polar Head—The contribution of the sulfate group to the total energy of adsorption of an alkyl sulfate surfactant has been estimated to be −1.8 kcal. mole^{-1} at a "clean" hydrocarbon–surfactant solution interface (8) and 2.4 kcal. mole^{-1} at a "clean" air–surfactant solution interface (11). The positive value for the latter interface has been attributed to the strongly hydrophilic nature of the sulfate group (11).

The values estimated for the contribution of the sulfate group to the total energy of activation of the interaction of alkyl sulfates with the three lipidic monolayers studied in this work are negative in all cases (Table I), which seems to indicate that the interaction of this group takes place at a hydrocarbon environment.

The contribution of the trimethylammonium group to the total energy of adsorption of an alkyl trimethylammonium surfactant has been estimated to be −0.95 kcal. mole^{-1} at a clean hydrocarbon–surfactant solution interface (8). From literature data (9, 10), a value of 2.9 kcal. mole^{-1} for the adsorption at a clean air–surfactant solution interface can be calculated. Again the negative estimated values of the contribution of the trimethylammonium group to the total energy of activation of the interaction of alkyl trimethylammonium surfactants suggest the interaction of this group with a hydrocarbon environment.

The adsorption of the polar heads of the surfactant ions to a hydrocarbon–water interface would be in accord with the postulated hydrocarbon nature of the interfacial phase (Table II). However, there is a discrepancy in regard to the hydrocarbon chains of the surfactant ion. The free energy of adsorption per methylene group of a hydrocarbon chain that adsorbs at a clean hydrocarbon–surfactant solution interface has been estimated to be between −0.80 and −0.82 kcal. mole^{-1} (6–8). At a clean air–surfactant solution interface, the estimated value is between −0.600 and −0.625 kcal. mole^{-1} (2, 8, 9, 11). With the exception of the value that corresponds to the alkyl trimethylammonium–dipalmitoyl lecithin

interaction (−0.77), the energy of activation per methylene group in all cases (Table I) has been found to be much closer to that which corresponds to the adsorption of the methylene group to an air-water interface.

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▲ To whom inquiries should be directed.

Absorption of Chlormadinone Acetate and Norethindrone from *In Situ* Rat Gut

KATHLEEN S. PELZMANN

Abstract □ The GI absorption of chlormadinone acetate and norethindrone was studied in a rat *in situ* preparation. The data evaluated with respect to the absorption half-life in the ligated stomach and intestine suggest that chlormadinone acetate is absorbed slightly more rapidly than norethindrone and that both steroids are absorbed to a greater extent in the intestine. The effects of bile duct cannulation, ethanol, and exogenous bile salts were investigated. The presence of bile significantly altered the absorption and metabolism of chlormadinone acetate. Ethanol, which was used as the drug vehicle, did not improve or depress the absorption of either steroid. Tissue accumulation experiments indicated that the jejunum of the intestine and the pyloric and cardiac area of the stomach accumulate the steroids to a greater extent than other GI tissues. Both compounds exhibited biexponential absorption with significant membrane accumulation.

Keyphrases □ Chlormadinone acetate and norethindrone—absorption from *in situ* rat gut □ Norethindrone and chlormadinone acetate—absorption from *in situ* rat gut □ Absorption, GI—norethindrone and chlormadinone acetate, *in situ* rat gut

Although the oral administration of steroids is common today, relatively little is known about their GI absorption characteristics. Most published studies measure absorption indirectly by plasma or urine levels of a steroid and its metabolites. The present investigations were undertaken to study the GI absorption of two progestins, chlormadinone acetate and norethindrone, and to develop an animal model for these studies.

Some investigators have used the *in vitro* rat intestine preparation (1, 2) or the everted intestinal sac method (3, 4). The absorption rates measured in these experiments are not influenced by blood and blood pressures, lymph, nerves, and GI secretions. Levine *et al.* (5) reported the loss of structural integrity and cellular death occurring in the intestinal mucosa within 10–15 min. after the preparation of an everted intestine. Consequently, the only observation that can be made is the movement of drug across a semiviable membrane.

Searching for a less traumatic intestinal preparation, Schanker *et al.* (6) used *in situ* perfusion techniques consisting of a single pass or a recycling of the drug solution through the cannulated intestine. Doluisio *et al.* (7) described an *in situ* technique with duodenal and ileal ends of the intestine cannulated with L-shaped cannulas. Stopcocks and syringes were attached to both cannulas for instilling and sampling the drug solution. Although Doluisio *et al.* reported very reproducible results, this preparation was found cumbersome by the present author.

Only a few experiments involving steroid intestinal absorption in animals have been published. Symchowicz