

# Kinetics and Mechanisms of Monolayer Interactions IV: Surface Activity of Alkanols and Energies of Their Interaction with Dipalmitoyllecithin and Dipalmitoylphosphatidylethanolamine

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**Abstract** □ The free energies of adsorption of the C<sub>1</sub> to C<sub>14</sub> alkanols at the air–water interface, estimated from plots of the surface pressure ( $\pi \leq 5$  dynes/cm) against the bulk concentration, were a linear function of the chain length for 1-alkanols. From C<sub>3</sub> to C<sub>8</sub>, the 2- and 3-isomers showed lower values than the 1-isomers. The energies of interaction of the C<sub>1</sub> to C<sub>14</sub> alkanols with dipalmitoylphosphatidylethanolamine and dipalmitoyllecithin monolayers, previously spread at the air–water interface, were estimated from the increase of the surface pressure with increasing concentrations of the injected alkanol. The energies of interaction of the C<sub>1</sub> to C<sub>5</sub> 1-alkanols were linear functions of the chain length. The energy of interaction per methylene group of the alkyl chain suggests that the phospholipid monolayers behaved as ultrathin “oil” phases. The 2- and 3-isomers presented marked departures from linearity. The literature data for reflection coefficients in biomembranes and for partition coefficients between olive oil and water, red cell membranes and water, and phospholipid liposomes and water for the C<sub>1</sub> to C<sub>5</sub> alkanols show similar linearities with the chain length for the 1-isomers and comparable departures for the 2- and 3-isomers.

**Keyphrases** □ Monolayer interactions—various aliphatic alcohols, free energies of adsorption and energies of interaction with phospholipids at air–water interface □ Alcohols, various aliphatic—free energies of adsorption and energies of interaction with phospholipid monolayers at air–water interface □ Phospholipid monolayers—energies of interaction with various aliphatic alcohols at air–water interface □ Surface activity—various aliphatic alcohols, energies of interaction with phospholipid monolayers at air–water interface

The process of permeation of a solute across the lipid regions of biomembranes can be separated (1) into three successive events that contribute to the total permeation rate: (a) adsorption of the hydrated solute at the lipid–water interface, (b) dehydration, and (c) diffusion of the solute through the lipid region. Monolayers of phospholipids spread at the air–water interface are considered to be suitable models for the study of the adsorption of solutes to ordered arrays of lipid molecules (2, 3).

The effect of chain length and the nature of the polar group of homologous series of *n*-alkyl sulfates and *n*-alkylammonium ions on the energy of their interaction with dipalmitoylglycerol, dipalmitoyllecithin, and dipalmitoylphosphatidylethanolamine monolayers were estimated recently (2, 3) from the variation of the equilibrium surface pressure with varying concentrations of subphase-injected *n*-alkyl derivatives on the premise of a collision model and a constant entropy factor.

The present work compares the energies of adsorption of alkanols at a “clean” air–aqueous interface with the energies of their interaction with dipalmitoyllecithin (I) and dipalmitoylphosphatidylethanolamine (II) monolayers. The implications of these relations on the permeability of alkanols through biomembranes are discussed. A clean interface is defined here as an air–aqueous interface without any previously spread monolayer.

## EXPERIMENTAL

**Reagents**—Dipalmitoyllecithin<sup>1</sup> and dipalmitoylphosphatidylethanolamine<sup>1</sup> were chromatographically homogeneous by TLC (4, 5). The purity of the reagent grade alcohols used, methanol<sup>2</sup>, ethanol<sup>3</sup>, 1-propanol<sup>2</sup>, 2-propanol<sup>4</sup>, 1-butanol<sup>5</sup>, 2-butanol<sup>6</sup>, 1-pentanol<sup>6</sup>, 2-pentanol<sup>6</sup>, 3-pentanol<sup>6</sup>, 1-hexanol<sup>7</sup>, 2-hexanol<sup>6</sup>, 3-hexanol<sup>6</sup>, 1-octanol<sup>4</sup>, 2-octanol<sup>7</sup>, 1-decanol<sup>8</sup>, 1-dodecanol<sup>9</sup>, and 1-tetradecanol<sup>10</sup>, was better than 99.5% as tested by GLC. The hexane<sup>10</sup>, used to prepare the spreading solutions and the distilled water used to prepare the alkanol solutions and as a subphase for the monolayers fulfilled the requirements described previously (2, 3).

**Instruments**—A 9-cm diameter polytef dish, provided with two identical microburets<sup>11</sup> and a polytef-coated stirring bar (1.25 × 0.8 cm), was used as a trough in a closed system described previously (2, 3). Surface tension was measured with a Wilhelmy platinum plate (2.5 × 1.25 × 0.01 cm) attached to an electrobalance<sup>12</sup>, and the output was fed into a dual-pen recorder<sup>13</sup>.

**Surface Tensions of Alkanol Solutions**—The aqueous alkanol solution was slowly added to the polytef dish so that it just contacted the lower end of the positioned platinum plate (6). The surface tension,  $\gamma$ , was continuously recorded after 1 min, and the equilibrium values,  $\gamma_{eq}$ , reported here were determined from values that remained constant for 30 min. The time necessary to reach equilibrium varied with the alcohol concentration. The reproducibility of the surface tension measurements was within  $\pm 0.2$  dyne/cm. All experiments were performed at  $21 \pm 1^\circ$ .

The surface pressure,  $\pi$ , of the alcohol solutions is the difference between the previously determined surface tension of the pure water,  $\gamma_0$ , and the equilibrium surface tension,  $\gamma_{eq}$ . The surface pressure was fitted to a function of the concentration,  $C$  (moles per liter), of the alkanol by digital computerized nonlinear regression to exponential equations of the form:

$$\pi = \gamma_0 - \gamma_{eq} = B_1 e^{[B_2(\log C)^2 + B_3 \log C]} \quad (\text{Eq. 1})$$

where the  $B_i$  values were adjustable parameters. This equation was chosen because the computer fittings of  $\pi$  to polynomial functions of concentration were significantly aberrant for experimental points at low surface pressures.

At constant temperature and pressure, the Gibbs adsorption equation may be written (7):

$$d\pi = \sum_i \Gamma_i d\mu_i = \sum_i \Gamma_i^1 d\mu_i \quad (\text{Eq. 2})$$

where  $\pi$  is the surface pressure, and  $\Gamma_i$ ,  $\mu_i$ , and  $\Gamma_i^1$  are the number of moles per unit area, the chemical potential, and the surface excess of the  $i$ th species, respectively. The surface excess,  $\Gamma_i^1$ , of an  $i$ th species is defined by (8):

$$\Gamma_i^1 = \Gamma_i - \frac{X_i}{X_1} \Gamma_1 \quad (\text{Eq. 3})$$

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**Table I—Surface Tensions,  $\gamma$ , Energies of Adsorption,  $\Delta G$ , and Areas per Molecule,  $A_0$ , at the Air–Water Interface**

Alkanol	$\gamma$ (Alkanol/Air), dynes/cm	From Plot of $\pi$ versus $X_2$			From Plot of Reciprocal $\pi$ versus $A$	
		$r$	Slope $\pi/X_2$	$\Delta G$ , kcal/mole	$r$	$A_0$ , $\text{\AA}^2/\text{molecule}$
Methanol	23.5	0.9972	$4.65 \times 10^2$	3.59	0.9995	9.7
Ethanol	22.4	0.9983	$1.27 \times 10^3$	4.18	0.9961	18.5
1-Propanol	23.8	0.9993	$3.96 \times 10^3$	4.84	0.9992	19.6
2-Propanol	20.7	0.9986	$3.41 \times 10^3$	4.75	0.9999	18.5
1-Butanol	24.5	0.9994	$1.09 \times 10^4$	5.43	0.9999	16.1
2-Butanol	23.0	0.9986	$1.00 \times 10^4$	5.38	0.9989	26.1
1,1-Dimethyl-1-ethanol	—	0.9985	$7.33 \times 10^3$	5.20	0.9999	20.2
2-Methyl-1-propanol	22.1	0.9999	$1.14 \times 10^4$	5.46	0.9999	17.7
1-Pentanol	24.9	0.9998	$3.98 \times 10^4$	6.19	0.9996	18.7
2-Pentanol	23.8	0.9977	$3.39 \times 10^4$	6.10	0.9992	21.9
3-Pentanol	24.4	0.9981	$2.34 \times 10^4$	5.88	0.9996	21.3
1-Hexanol	25.8	0.9922	$1.13 \times 10^5$	6.80	0.9999	15.7
2-Hexanol	24.7	0.9984	$9.45 \times 10^4$	6.70	0.9999	19.2
3-Hexanol	24.0	0.9997	$9.00 \times 10^4$	6.60	0.9999	9.5
1-Octanol	27.0	0.9917	$1.23 \times 10^6$	8.20	0.9998	9.5
2-Octanol	25.5	0.9997	$9.51 \times 10^5$	8.05	0.9999	14.8
1-Decanol	—	0.9882	$1.12 \times 10^7$	9.49	—	—
1-Dodecanol	—	0.9837	$1.32 \times 10^8$	10.93	—	—

where  $X_i$  is the mole fraction of the  $i$ th component, and the solvent is denoted by  $i = 1$ . Thus, for a two-component mixture:

$$\Gamma_2^1 = \Gamma_2 - \frac{X_2}{X_1} \Gamma_1 \quad (\text{Eq. 4})$$

where  $X_2$  is the mole fraction of the solute.

For a dilute aqueous solution with a single solute ( $X_2/X_1 \leq 0.01$ ), it can be reasonably assumed that  $\Gamma_2^1 \approx \Gamma_2$ .

Since the chemical potential is defined as:

$$d\mu_2 = RT d \ln a_2 \approx RT d \ln C_2 \quad (\text{Eq. 5})$$

where  $a_2$  is the activity and  $C_2$  is the solute concentration, substitution into Eq. 2 gives:

$$\Gamma_2 = \frac{d\pi}{d \ln C_2} \frac{1}{kT} \quad (\text{Eq. 6})$$

where  $\Gamma_2$  is the surface concentration (molecules per square centimeter) of the solute,  $k$  is the Boltzmann constant (ergs per molecule degree),  $T$  is the absolute temperature ( $^\circ\text{K}$ ),  $\pi$  is the surface pressure (dynes per centimeter), and  $C_2$  is the solute concentration (moles per liter) in the bulk solution.

The derivative ( $d\pi/d \ln C_2$ ) of the exponential equation (Eq. 1) that characterized the dependence of the surface pressure on concentration was computed with respect to the logarithm of the solute concentration. Substitution of these values into Eq. 5 permitted calculation of the surface concentration,  $\Gamma_2$ , at any bulk concentration,  $C_2$ . The reciprocal  $1/\Gamma_2 = A$  is the apparent area occupied by a molecule in square Angstroms per molecule and is the area within which one molecule of solute can be found at the interface.

Densities of the alcohol solutions for the calculation of mole fractions were determined, using 10-ml specific gravity bottles previously calibrated with water. The precision of the weighing was  $\pm 0.1$  mg. Densities of methanol, ethanol, 1-propanol, 1-butanol, and 1-pentanol solutions were obtained from the literature (9).

**Injection beneath Phospholipid Monolayer**—With the platinum plate and the microburets in position in the previously described apparatus (2), water was added slowly just to make contact with the lower edge of the platinum plate. After recording the surface tension for 1 min, the volume was adjusted to  $45 \text{ cm}^3$ . The hexane spreading solution of I or II was delivered dropwise at the air–water interface with a microsyringe<sup>14</sup> with simultaneous stirring and rapid nitrogen flow to facilitate rapid hexane elimination until the desired surface pressure was achieved. In all reported experiments, the initial surface pressure of the monolayer was  $5 \pm 0.1$  dynes/cm.

A given volume of water, equal to the volume of alcohol solution to be injected, was withdrawn from the dish by the appropriate microburet. The alcohol solution was injected within 30 sec from the other microburet, with simultaneous magnetic stirring, into the bulk phase below the surface. Since no significant differences were observed in the changes in

surface pressure after stirring 1, 3, 5, or 10 min for a given final alcohol concentration, the stirring was halted after 1 min and the surface tension was continuously recorded. The criterion of equilibrium was the constancy ( $\pm 0.1$  dyne/cm) of the surface pressure increment,  $\Delta\pi$ , during 30 min. The experiments were performed at  $21 \pm 1^\circ$ .

## RESULTS

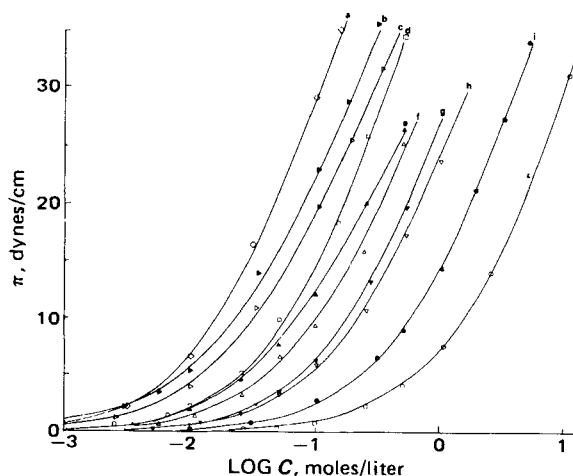
**Adsorption of Alkanols at a Clean Air–Aqueous Interface**—The surface tensions of the alkanols (Table I) in the pure state against air agreed with the literature (10–14).

Typical plots of the surface pressure,  $\pi$ , against the logarithm of the concentration,  $C$  (moles per liter), for some studied alkanols are given in Fig. 1.

The apparent linear slope of the plot of the surface tension,  $\gamma$ , against concentration for very dilute solutions increased by a factor of three for each additional methylene group in the alkyl chain of a homologous series of aliphatic compounds (15). A simple expression for the free energy change was derived (7) from thermodynamic and molecular kinetic considerations:

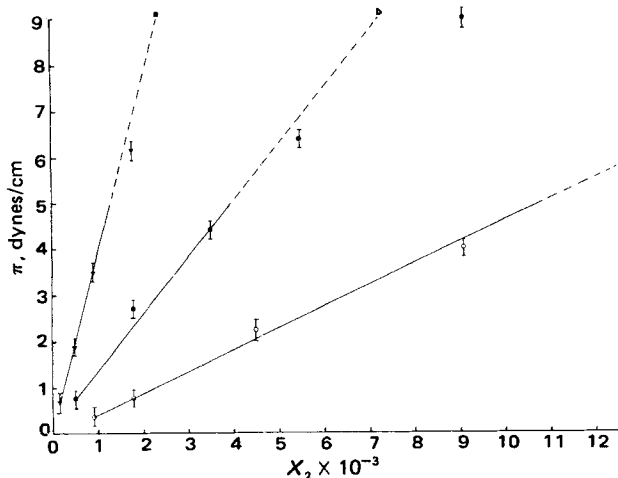
$$\Delta G_{ss} = -RT \ln \frac{\pi}{X^*2} \quad (\text{Eq. 7})$$

where  $\Delta G_{ss}$  is the change in standard free energy associated with the adsorption process at a clean air–aqueous solution interface,  $\pi$  is the surface pressure, and  $X^*2$  is the activity of the solute. Thus, the numerical



**Figure 1**—Plots of the surface pressure,  $\pi$ , against the logarithm of the bulk concentration,  $C$ , for alkanols from  $C_1$  to  $C_5$ . Key: a, 1-pentanol; b, 2-pentanol; c, 3-pentanol; d, 1-butanol; e, 2-butanol; f, 1,1-dimethyl-1-ethanol; g, 1-propanol; h, 2-propanol, i, ethanol; and j, methanol. The lines drawn through the experimental points were the best fit to Eq. 1 obtained from the computer.

<sup>14</sup> Hamilton.



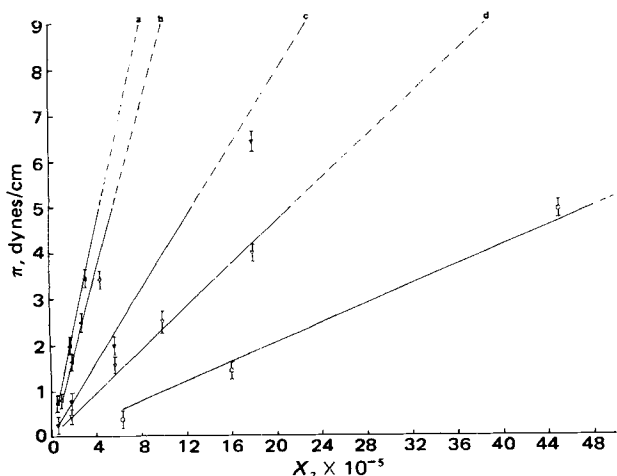
**Figure 2**—Plots of the surface pressure,  $\pi$  ( $\leq 5$  dynes/cm), against the mole fraction,  $X_2$ , for methanol (a), ethanol (b), and 1-propanol (c). The lines drawn through the experimental points were the best fit to Eq. 1 obtained from the computer.

value of  $\Delta G_{ds}$  can be calculated from the slope ( $\pi/X_2$ ) of a linear plot of the surface pressure against the mole fraction,  $X_2$ , of the solute in bulk solution when  $X_2 \rightarrow 0$  and  $X_2 \rightarrow X_2^*$  at low mole fractions.

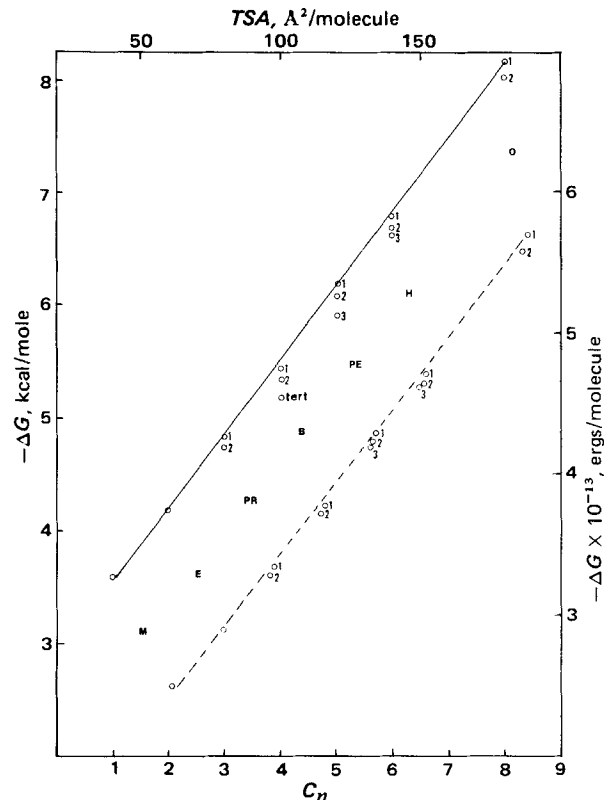
For all alkanols, plots of the surface pressure against the concentration of the alcohol in the bulk solution were reasonably linear in the region  $\pi \leq 5$  dynes/cm under the experimental conditions. Typical plots for some alkanols are given in Figs. 2 and 3. The correlation coefficients,  $r$ , the slopes, and the estimated values of  $\Delta G$  obtained from such plots are given in Table I. The plot was also linear in this range for 1-hexanol in contrast to a previous report (7).

Plots of the calculated free energies of adsorption,  $\Delta G_{ds}$  (Eq. 7), against the number of carbon atoms in the alkyl chain,  $C_n$ , are given in Fig. 4. The experimental points that corresponded to the primary or 1-alkanols determined a straight line within the limits of the experimental error ( $\pm 0.2$  kcal/mole). The numerical values of  $\Delta G_{ds}$  (Table I) for the 1-alkanols,  $C_1$  to  $C_5$ , were in excellent agreement with the literature (7). The change of the position of the hydroxyl group along the hydrocarbon chain produced a small, but significant, decrease in the  $\Delta G$  value.

The slope of the plot  $\Delta G$  versus  $C_n$  for the 1-alkanols from  $C_1$  to  $C_{12}$  was  $-0.66$  kcal/mole and agreed with the literature values of from  $-0.600$  to  $-0.625$  kcal/mole reported for the free energy of adsorption per methylene group of a hydrocarbon chain that adsorbs at the air-aqueous solution interface (16–20). The free energy of adsorption of the hydroxyl group can be estimated from the intercept on the assumption that the extrapolation of the straight line to  $C_n = 0$  gives a numerical value related to the free energy of adsorption of the polar moiety of the alcohol molecule and is  $-2.87$  kcal/mole.



**Figure 3**—Plots of the surface pressure,  $\pi$  ( $\leq 5$  dynes/cm), against the mole fraction,  $X_2$ , for: 1-hexanol (a); 3-hexanol (b); 1-pentanol (c); 3-pentanol (d); and 1-butanol (e). The lines drawn through the experimental points were the best fit to Eq. 1 obtained from the computer.



**Figure 4**—Left and lower coordinates: plot of the free energy of adsorption at the air-aqueous solution interface,  $\Delta G$  (kcal/mole), against the number of carbon atoms,  $C_n$ , of alkanols from  $C_1$  to  $C_8$ . Right and upper coordinates: plot of the free energy of adsorption at the air-water interface,  $\Delta G$  (ergs per molecule), against the total molecular surface area, TSA (square Angstroms per molecule). Key: M, methanol; E, ethanol; PR, propanols; B, butanols; PE, pentanols; H, hexanols; O, octanols; and tert, 1,1-dimethyl-1-ethanol. Numbers represent the position of the hydroxyl group.

**Interaction of 1-Alkanols with Phospholipid Monolayers**—The energies of interactions,  $\Psi$ , can be estimated from the slopes of the reciprocals of the equilibrium surface pressures,  $\Delta\pi_{eq}$ , after injection against the reciprocals of the final concentrations,  $n$  (molecules per cubic centimeter), of the subphase injected alcohol (2, 3). Such energies are plotted in Fig. 5 against the number of carbon atoms,  $C_n$ , of the 1-alkanol chain.

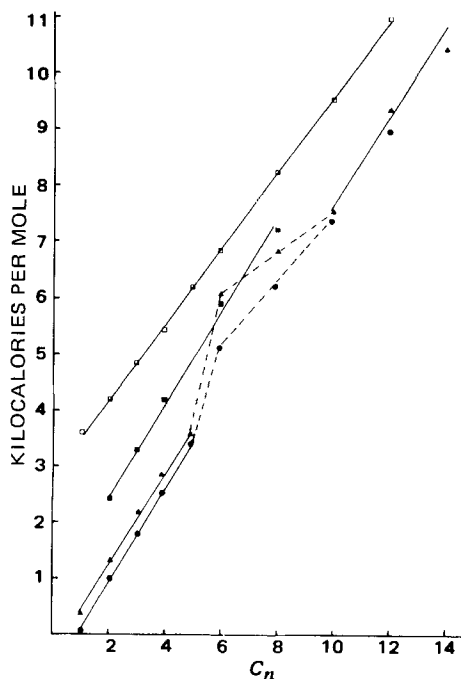
The plot was linear from  $C_1$  to  $C_5$  for both I and II. Departures from linearity were observed for the energy of interaction of 1-hexanol with the two phospholipids and for 1-octanol with II. In the  $C_{10}$  to  $C_{14}$  region, the experimental points obtained for both phospholipids were again on the straight line determined by the five first members of the 1-alkanol series.

The energies of interaction per methylene group of the injected 1-alkanol with the molecules that form the monolayer were calculated from the regression coefficient of the best straight line through the experimental points from  $C_1$  to  $C_5$ . They were 0.81 and 0.83 kcal/mole/methylene for II and I monolayers, respectively. The energy of interaction of the hydroxyl group was calculated from the intercepts of the straight lines based on the previously stated assumption. The values were 0.73 and 0.42 kcal/mole/methylene for II and I, respectively.

## DISCUSSION

**Adsorption of Alkanols at Clean Air-Water Interface**—The surface activity of alkanols arises from the presence in the same molecule of a soluble hydroxyl group and an insoluble hydrocarbon chain (21). The ratios of the instantaneous slopes of  $\pi$  against  $\ln C_2$  at a given  $C_2$  and temperature for different alkanols are the ratios of the adsorptions,  $\Gamma_2$ , of the solutes at the liquid interface (Eq. 5).

The relatively parallel slopes for higher concentrations of alkanols (Fig. 1) indicate that the same number of molecules of any of the alkanols is adsorbed per unit area of the interface. The relative linearity of the curves in this region indicates that the interfacial concentration is not signifi-



**Figure 5**—Plots of energies of adsorption and energies of interactions against the number of carbon atoms of 1-alkanols from  $C_1$  to  $C_{14}$ . Key:  $\square$ , free energy of adsorption at the air-aqueous solution interface;  $\blacksquare$ , free energy of adsorption at the petroleum ether-aqueous solution interface (33);  $\blacktriangle$ , energy of interaction with II monolayers; and  $\bullet$ , energy of interaction with I monolayers.

cantly increased when the solution concentration is increased and that the adsorbed molecules are too closely packed on the interface to admit others after a certain bulk concentration.

The equation of state (22, 23):

$$\pi = \frac{kT}{(A - A_0)} \quad (\text{Eq. 8})$$

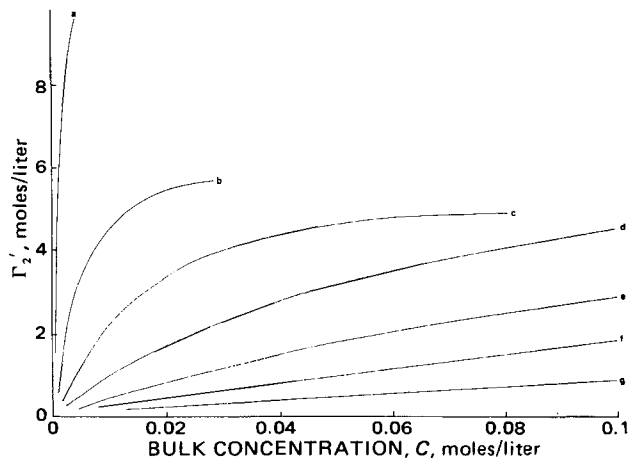
where  $\pi$  is the surface pressure (dynes per centimeter or ergs per square centimeter),  $k$  is the Boltzmann constant ( $1.3805 \times 10^{-16}$  erg/molecule),  $T$  is the absolute temperature ( $^\circ\text{K}$ ),  $A$  is the area per molecule (square Angstroms per molecule) at  $\pi$ , and  $A_0$  is the limiting area (square Angstroms per molecule), was tested for all alkanols studied. Table I lists the values of the limiting areas,  $A_0$ , and the correlation coefficients estimated from plots of  $1/\pi$  against  $A$  from 30 to 100  $\text{\AA}^2/\text{molecule}$ , where  $A = 1/\Gamma_2$  was calculated from Eq. 6. With the exception of methanol, 3-hexanol, and 1-octanol, the values of  $A_0$  were between 15 and 25  $\text{\AA}^2/\text{molecule}$ . Values of 18.5  $\text{\AA}^2/\text{molecule}$  were reported previously for ethanol at the air-water interface (24, 25) and for ethanol, 1-propanol, and 1-butanol at the petroleum ether-water interface (22).

These values roughly correspond to the cross-sectional area of an aliphatic hydrocarbon chain. This fact can be taken to mean that, under conditions of quasisaturation of the interface, the adsorbed molecules are arranged parallel to one another and at a steep angle to the interface.

The values of  $A_0$  found for methanol, 3-hexanol, and 1-octanol were roughly one-half of the average  $A_0$  values of the other alkanols and may suggest an association of those molecules that takes place at the air-water interface (7).

On the assumptions that the alkanol molecules are oriented quasi-perpendicular to the plane of the interface and that the stretched hydrocarbon chains are immersed completely in the aqueous phase, the volume,  $V_s$ , of this interfacial region containing the number of molecules per square centimeter that corresponds to the surface concentration,  $\Gamma_2$  (Eq. 5), can be approximately estimated from the length of the alkanol molecule.

The plots of the concentration of such a thin interfacial region [ $\Gamma_2' = (\Gamma_2/V_s)$  in moles per liter] estimated from these volumes and from the surface concentration ( $\Gamma_2$  in molecules per square centimeter) against the bulk concentration ( $C$  in moles per liter) are given in Fig. 6 for some 1-alkanols and indicate that the concentration of the adsorbed alkanols in the thin interfacial region ( $<30 \text{\AA}$ ) could be up to  $10^3$  times greater than that of the bulk solutions when equilibrium is attained.



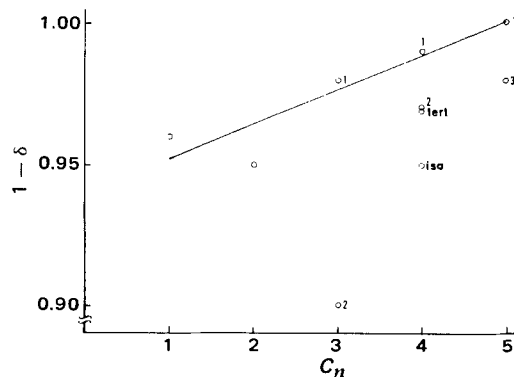
**Figure 6**—Plots of the concentration at the interfacial region,  $\Gamma_2'$  (moles per liter), against the bulk concentration,  $C$  (moles per liter), of 1-alkanols from  $C_1$  to  $C_8$ . Key: a, octanol; b, hexanol; c, pentanol; d, butanol; e, propanol; f, ethanol; and g, methanol.

The surface activity of alkanols is the result of the hydrophilic-hydrophobic balance between the hydroxyl and the methylene groups, and the free energy of adsorption is linearly related to the number of carbon atoms,  $C_n$ , in their hydrocarbon chain (Fig. 4). However, the values for 2- and 3-alkanols deviate from the linearity of their corresponding 1-alkanols and indicate that free energy is also affected by the position of the hydroxyl group along the hydrocarbon chain.

Recent studies (26, 27) showed a striking correlation between the solubility of alkanols in water and their molecular surface areas, *i.e.*, the surface area of the alkanol molecule in the bulk solution around which the water molecules can be packed. The group surface area corresponding to the hydroxyl group decreased from an average value of 20.2  $\text{\AA}^2$  in the 1-position to 17.3  $\text{\AA}^2$  for the 3-position of the alkanols (27).

The plot of the free energy of adsorption (ergs per molecule) against the total molecular surface area (square Angstroms per molecule) of some 1-alkanols and their 2- and 3-isomers is compared in Fig. 4 (right and upper coordinates) with that of the free energy of adsorption (kilocalories per mole) as a function of the number of carbon atoms in the hydrocarbon chain. The excellent linearity for all alkanols independent of the hydroxyl position indicates that the simultaneous effect of chain length and of position of the hydroxyl group on the surface activity of alkanols is best described by the total molecular surface area of the alkanol molecule.

**Interaction of Alkanols with Phospholipid Monolayers**—Compound II forms a condensed liquid monolayer at the air-water interface. The surface area that corresponds to each molecule that forms the monolayer was  $40 \pm 2 \text{\AA}^2$  at  $5 \pm 0.1$  dynes/cm and  $22 \pm 1^\circ$  (3). On the assumption that the stretched molecules were submerged completely in the water phase with the phosphatidylethanolamine group parallel to the interface, the concentration in this thin ( $\sim 30 \text{\AA}$ ) crust of water was estimated to be 1.4 moles/liter (3). The percentage of water in this interfacial region estimated from its volume and the volume occupied by the phospholipid molecules was 1.9%. It follows that this interfacial region



**Figure 7**—Plot of  $1 - \delta$  obtained from Ref. 29 against the number of carbon atoms of alkanols from  $C_1$  to  $C_5$ . Numbers represent the position of the hydroxyl group. Key: tert, 1,1-dimethyl-1-ethanol; and iso, 2-methyl-1-propanol.

may be considered to be a predominantly hydrocarbon phase approximately 30 Å thick.

Compound I forms a relatively less condensed liquid monolayer at the air-water interface. The surface area that corresponds to each molecule at 5 dynes/cm and 22° was  $55 \pm 2 \text{ \AA}^2$ . On the same assumptions, its concentration in the 30-Å crust of water was estimated to be 1.0 mole/liter and the percentage of water at the interfacial region was estimated to be 29.4% (3).

Plots of the energies of interaction of the injected 1-alkanols with each phospholipid monolayer against the chain length are compared in Fig. 5 with those of the free energies of adsorption at the clean air-water interface obtained in the present work and at the clean petroleum ether-water interface obtained from the literature (23).

Up to pentanol, the energy of interaction per methylene group with the phospholipid monolayers compares with the free energy of adsorption per methylene group at the clean petroleum ether-water interface (0.82 kcal/mole/methylene) within the limits of the experimental error; *i.e.*, the slopes in Fig. 5 are parallel up to  $C_n = 5$ . This finding seems to indicate that the phospholipid monolayers behave as ultrathin simple hydrocarbon phases in their interaction with the injected 1-alkanols and also that the polar moiety of the phospholipid does not have a major influence on the adsorption of the hydrocarbon chain of the 1-alkanols. The energies of interaction per hydroxyl group of the 1-alkanols, on the other hand, seem to be influenced by the nature of the nonaqueous phase, as indicated by the numerical values of the corresponding intercepts: air, 2.84; petroleum ether, 0.84; II, 0.73; and I, 0.42.

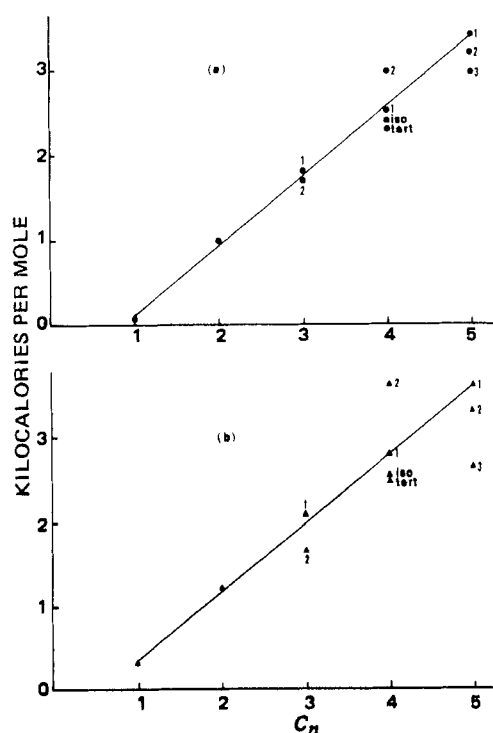
The origin of the sudden discontinuity observed for the values of the energies of interactions of 1-hexanol and 1-octanol with II and of 1-hexanol with I is as yet unexplained. It was suggested (3) that entropic factors associated with the configuration of the molecules at the interfacial region do contribute to the energy of interaction. Thus, the simplified assumption of a common entropy factor (2, 3) for such interactions on which the calculations of energies were based may be an oversimplification of a more complex process. The difference between the experimental value of the energy of interaction of 1-hexanol with II and the value expected from the extrapolation of the straight line through the first five terms was 1.65 kcal/mole. If this difference were totally an entropic contribution, it would correspond to 5.5 entropic units, which is not an unreasonable value for entropic changes associated with adsorption and desorption processes at oil-water interfaces (28).

The experimental points corresponding to the interaction of 1-decanol, 1-dodecanol, and 1-tetradecanol with the phospholipid monolayers were again on the extrapolation of the straight line determined by the first five numbers of the 1-alkanol series. Perhaps the increased chain length of the  $C_{10}$  to  $C_{14}$  1-alkanols permits the adoption of configurations to compensate for the entropic deviation of the  $C_6$  to  $C_8$  alkanols. The latter's lengths are optimum for cyclization.

**Correlations with Permeability Processes**—"The permeability of biological membranes to nonelectrolytes depends intimately upon molecular structure, so that a small change in the structure of a solute often causes a profound change in its permeating power" (29). Reflection coefficients,  $\delta$ , of alkanols for epithelial cells of rabbit gallbladder were measured (29). The reflection coefficient is 1 for an impermeant solute and 0 for a solute as permeant as the solvent itself. In general, it decreases from 1.0 to 0 with increasing lipid-water partition coefficients, which seems to indicate that the intermolecular forces governing the permeability of nonelectrolytes are similar to those governing partition between a bulk lipid phase and water. For most solutes, the values of  $(1 - \delta)$  give the same permeability sequences as the solute permeability coefficient in *Nitella* (30) and rabbit gallbladder (31) and the partition coefficients and reflection coefficients in goldfish bladder, bullfrog gallbladder, bullfrog intestine, and guinea pig intestine (32) are well correlated, indicating that the  $(1 - \delta)$  parameter is a good measure of permeability for nonelectrolyte solutes permeating *via* membrane lipids.

The values of  $(1 - \delta)$  obtained from Ref. 29 are linearly related to the chain length of the primary alkanols from  $C_1$  to  $C_5$  (Fig. 7). The position of the hydroxyl group does affect the permeability of these alkanols. The deviation of methanol has been explained by the premise that small polar solutes, which should interact the least with the hydrocarbon moiety of membrane lipids, must have an alternative route of permeation through the localized concentration of membrane polar groups associated with "frozen" water molecules (29). The deviation of ethanol, if experimentally significant, is difficult to explain on the premise of the linear relation assumed for Fig. 5.

Plots of the energies of interaction of these alkanols with I and II monolayers are represented in Fig. 8. No deviation was observed for the lowest members of the series as compared with the plots of  $(1 - \delta)$  against



**Figure 8**—Plot of the energy of interaction of alkanols from  $C_1$  to  $C_5$  with I(a) and II(b) monolayers. Numbers represent the position of the hydroxyl group. Key: *tert*, 1,1-dimethyl-1-ethanol; and *iso*, 2-methyl-1-propanol.

the chain length. The nonexistence of "localized" concentrations of polar groups associated with frozen water molecules for the phospholipid monolayers may explain the absence of anomalies in these plots for methanol. Thus, for the 1-alkanols, plots of their energies of interaction with the lipid monolayer against the chain length show an acceptable correlation with the permeation of these substances across biological membranes.

The different permeation behavior of the 1- and 2-propanols is not reflected, within the limits of experimental error, in their energies of interaction with the I monolayers. However, with II, a small significant difference between the energies of interaction can be observed.

2-Butanol and 1,1-dimethyl-1-ethanol have less permeability than 1-butanol (Fig. 7), and both have higher permeability (*i.e.*,  $1 - \delta$ ) than 2-methyl-1-propanol. The directions of the deviations of these alkanols from the energy of interaction of 2-butanol with the phospholipid monolayers (Fig. 8) do not correlate with the direction of these permeability differences.

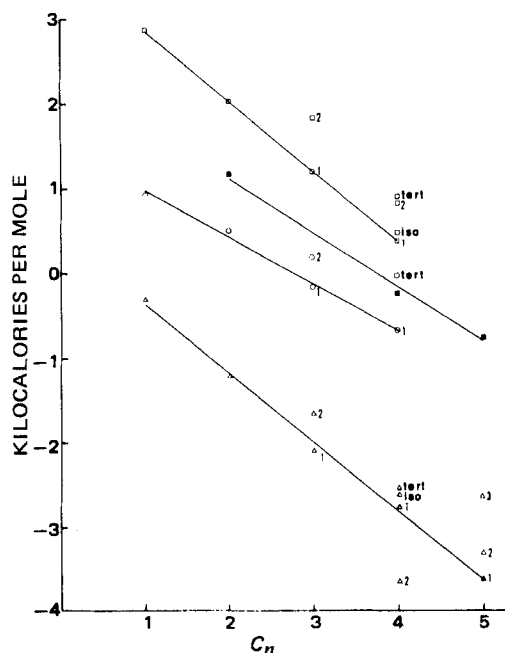
3-Pentanol has a lower permeability than 1-pentanol (Fig. 7), consistent with the fact that the energies of interaction of the pentanols with I and II monolayers decreased in the sequence  $1 \rightarrow 2 \rightarrow 3$  for the three isomers (Fig. 8).

Presently accepted membrane models (33) postulate the existence of lipid regions, formed mainly by phospholipids with a bilayer configuration, in cell membranes. The experimental model used in the present work could represent an external monolayer of the lipid regions of cell membranes. Thus, it seems plausible that correlations exist between the energies of interaction of the 1-alkanols with the phospholipid monolayers and their permeability across cell membranes. The fact that even the effect of the hydroxyl group position of the isomers is reflected in their energies of interaction indicates that this experimental model may present interesting perspectives for future work with more complex molecules.

Partition coefficients were measured previously between olive oil-aqueous solution (30), erythrocyte membrane-aqueous solution (34), and dimyristoyllecithin liposomes-aqueous solution (35) for some of the  $C_1$  to  $C_5$  alkanols. For dimyristoyllecithin liposomes, the equation:

$$\Delta F = -RT \ln K \quad (\text{Eq. 9})$$

in which  $R$  is the universal gas constant,  $T$  is the absolute temperature, and  $K$  is the experimentally measured partition coefficient, was used to estimate a  $\Delta F$  with the physical meaning (35) of the change in free energy



**Figure 9**—Plot of the free energy of transfer from an aqueous phase to a nonaqueous phase against the number of carbon atoms of alkanols from  $C_1$  to  $C_5$ . Key:  $\square$ , olive oil-aqueous solution, calculated from Ref. 30;  $\blacksquare$ , red blood cell membrane-aqueous solution, calculated from Ref. 34;  $\circ$ , dimyristoyllecithin liposomes-aqueous solution (35);  $\blacktriangle$ , II monolayer-aqueous solution; tert, 1,1-dimethyl-1-ethanol; and iso, 2-methyl-1-propanol. Numbers represent the position of the hydroxyl group.

of the transfer of 1 mole of solute from a hypothetical aqueous solution to a hypothetical lipid solution.

On the assumptions that the given numerical values of the energies of interaction of the  $C_1$  to  $C_5$  alkanols with the phospholipid monolayer were valid (i.e., entropies were invariant among the alkanols) and that the same energies were operative under the conditions of the studied partition coefficients, the changes in free energies corresponding to the partition coefficients of Refs. 30, 34, and 35 were calculated (Eq. 9).

Acceptable correlations were observed between the free energy values that correspond to these four different "lipid phases" and the number of carbon atoms of alkanols from  $C_1$  to  $C_5$  (Fig. 9). The slopes that correspond to the olive oil-aqueous solution and II monolayer-aqueous solution interfaces were the same (0.83 and 0.81 kcal/mole/methylene, respectively) within the limits of experimental error. The slope for the red blood cell membrane-aqueous solution interface was lower (0.65 kcal/mole/methylene) and may be due to the nonhomogeneous distribution of the lipids of the cell membrane which permits alternative routes of permeation. The slope for the dimyristoyllecithin liposome-aqueous solution interface (0.55 kcal/mole/methylene) may be due to the lax structure of the phospholipid monolayer of the liposome.

These results and correlations may give further insight into the interaction of biomembranes with approaching molecules. They indicate that the energies of interaction of alkanols from  $C_1$  to  $C_5$  with phospholipid monolayers spread at the air-water interface may be taken as a criterion to predict their relative behavior in the permeation process across cell membranes.

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