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ON THE CHEMISTRY OF ION EXCHANGE IN MONOMOLECULAR LAYERS OF LIPIDS

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SUMMARY

The main purpose of this work has been to study further the association of cations and anions with cell membrane lipids, in particular with phosphatidylcholine.

The adsorption of Ca^{2+} or SO_4^{2-} to phosphatidylcholine monolayers (measured with isotopes) shows that there is no uptake of these ions under various experimental conditions including different bulk pH values and concentrations.

The presence or absence of charges in monomolecular films of lipids can be inferred by utilizing a method of analysis proposed here; it depends on the study of the cation and anion exchange properties of each functional group found in lipids. To mimic the structure of the polar head of phosphatidylserine and phosphatidylcholine, surface-active molecules with phosphoric, carboxyl and trimethylammonium groups were combined in various proportions and used to form a mixed monolayer. The uptake of Ca^{2+} or SO_4^{2-} by mixed monolayers of octadecyltrimethylammonium and octadecyl phosphate (both groups present in phosphatidylcholine) indicate that when the molar fraction of octadecyltrimethylammonium is about 0.4 the mixed monolayers behaves as neutral.

INTRODUCTION

Monomolecular films have been extensively used as structural models of the cell membrane[1–8]. This structural approximation provides a system to study the interactions among different membrane molecules[6–8] and between membrane phospholipids and the ions usually present in the cellular environment[9–17]. We are interested in the latter because there are many observations which suggest that the cell membrane is an ion exchanger[18–21].

In the context of interactions between membrane phospholipids and ions, ALEXANDER *et al.*[9] showed that Ca^{2+} produces a small reduction in area per molecule of "cephalin" spread at the air–water interface at a given surface pressure. These results suggested that Ca^{2+} was adsorbed by cephalin. ANDERSON AND PETHICA[10], working with synthetic lecithin, have shown indirectly that this phospholipid does not bind Na^+, K^+ or Li^+ from dilute solutions (100 mM). Mg^{2+}, however, seems to be

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Abbreviation: OTA, octadecyltrimethylammonium.

adsorbed from dilute solutions. More recent experiments indicate that, among the phospholipid components of the cell membrane, phosphatidylserine and phosphatidylethanolamine generate negatively-charged monolayers[12,14,16,17].

With respect to phosphatidylcholine, another major constituent of cell membranes, even though previous reports indicate the presence of a net charge in monolayers of this molecule, there is no agreement regarding the sign of this charge; thus, monolayers of this lipid behave either as negatively[11] or positively charged[12]. The phosphatidylcholine experiments presented in this communication show that this phospholipid generates monolayers which do not adsorb Ca^{2+} or SO_4^{2-}. This lack of adsorption of divalent ions from the hypophase is not modified by changes in the degree of packing of the monolayer from 50 to 70 Å^2, by changes in the pH of the hypophase from 1.1 to 8.9 or by changes in the concentration of divalent ions from 0.025 to 2 mM.

Under these experimental conditions, there seems to be internal neutralization of the phosphate and the trimethylammonium group present in the polar head of this molecule. The phosphate and the trimethylammonium group seem to form a stable salt.

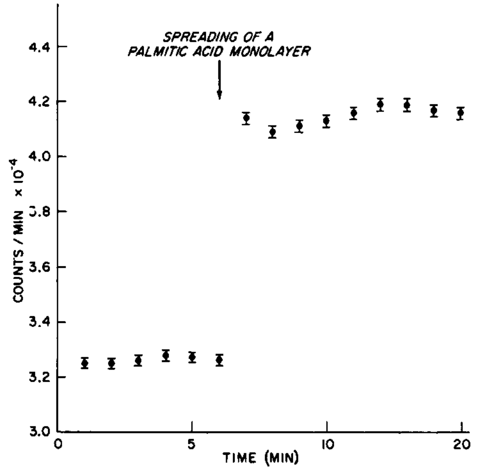
METHODS

Ca^{2+} and SO_4^{2-} adsorption measurements

Uptake of Ca^{2+} and SO_4^{2-} by the monolayer was measured by counting the number of β particles emanating from a hypophase containing $^{45}Ca^{2+}$ or $^{35}SO_4^{2-}$ before and after spreading of the monolayer. This technique has been described in detail in previous publications from this laboratory[12,14]. However, there are some relevant aspects of the experimental procedure which need to be described here.

The number of Ca^{2+} or SO_4^{2-} adsorbed per molecule in the monolayer were determined by modification of the method used by ANIANSSON[23] as follows: a counter tube (gas-flow detector D-47 with Micromil window, Nuclear Chicago) was suspended over a Petri dish (approx. 50 mm in radius) placed on a vertically adjustable platform so that the distance between the surface and the window could be controlled. The number of Ca^{2+} or SO_4^{2-} adsorbed per molecule in the monolayer were computed with two values of counts/min obtained from each experiment. The first, obtained in the absence of the monolayer, was used as background and was subtracted from the other obtained after spreading of the monolayer. Fig. 1 illustrates a typical experiment. This is a plot of counts/min emanating from the surface of the solution in the Petri dish (25 ml of solution, 0.04 mM $CaCl_2$, specific activity 25 mC/mole) before and after spreading of a palmitic acid monolayer as a function of time. After six determinations of counts/min, 10 μl of a solution of palmitic acid in chloroform were added to the surface of the solution. The difference, 8827 ± 104 counts/min is interpreted as being due to adsorption to the monolayer. The standard error of this difference was calculated as the standard error of the difference between two means. This difference was transformed to number of Ca^{2+} or SO_4^{2-} by the use of a factor, the evaluation of which involves knowing the counting efficiency. The counting efficiency at various points under the window was evaluated by moving a point source of $^{45}CaCl_2$ or $K_2{}^{35}SO_4$ along a straight line going through the projected center of the counter window circle, 10 mm below the window (where the surface of the hypophase was placed), and tabulating the counts/min as a function of position.

A circle of 50 mm in radius was taken as the area from which radiation emanating from a plane 10 mm below the window was measured. $^{45}CaCl_2$ or $K_2{}^{35}SO_4$ solutions of known concentration were then evaporated as discs 50 mm in radius on plexiglass plates. These were centered 10 mm below the window and were counted. Fig. 2 shows a graph of counts/min as a function of the number of moles in the evaporated solution. The slopes of these lines were used to convert counts/min to number of ions.



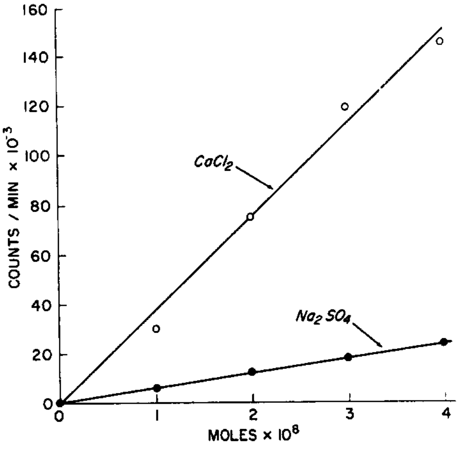


Fig. 1. Effect of spreading of a palmitic acid monolayer on the counting rate. Vertical axis represents counts/min emanating from the surface of the solution. Horizontal axis represents time. Counts were determined every minute. Vertical bars represent 2 times the standard error of the counting. The area per palmitic acid molecule was 35 Å². The pH of the hypophase was adjusted to 9 with KOH.

Fig. 2. Determination of a factor to transform counts/min to number of Ca^{2+} or $SO_4{}^{2-}$. This is a plot of counts/min emanating from $^{45}CaCl_2$ or $K_2{}^{35}SO_4$ deposited on plexiglass discs as a function of concentration of $CaCl_2$ or K_2SO_4.

Surface pressure–area diagrams

To determine the surface pressure–area diagrams, the Whilhelmy method was used. (The area of the glass plate used was 25 cm², and the area of the trough was 600 cm².) The relation between surface pressure and the amount of surface-active molecules delivered in a single dose applied to a fixed surface area (75 cm²) of hypophase was examined. It was found that for computed areas greater than 30 Å² per molecule (in the case of a single hydrocarbon chain per molecule and using chloroform as a solvent), the pressure recorded after single dose delivery to a fixed area was the same as that obtained after gradual compression to the same area. This made it possible to do the experiment in a more convenient manner, since the need for a movable barrier was eliminated. Single doses of surface-active agents were delivered to a fixed area and used without further manipulation.

Reagents

Radioactive isotopes used during the present research were obtained from New England Nuclear Corp. (Boston, Mass.). Surface-active molecules (synthetic, grade-A type) were obtained from Lachat Chemicals (Chicago, Ill.). Their purity was checked by us by ascending chromatography by the method of MARINETTI AND

STOTZ[24]. Silicic acid-impregnated paper was prepared from rectangular pieces of Whatman paper (20 cm × 10 cm) which were immersed in a solution of sodium silicate during 5 min. After dripping off the excess sodium silicate, the paper was immersed in 6 M HCl for 30 min. Finally, each piece of paper was rinsed in distilled water for 60 min, dried at room temperature and placed in a heater at 70° for 10 min before use. Each chromatography was run in a glass chamber using a mixture of diisobutylacetone–acetic acid–water (40:30:7, by vol.) as the solvent. A detailed description of the technique used can be found in MARINETTI AND STOTZ[24].

The following standard reagents were used to develop the chromatograms: ninhydrin (amino N), $KMnO_4$ (unsaturated bonds), $HClO_4$ (phosphate), rothamine (phospholipid), phosphomolybdate (choline). Only those samples that showed homogeneous chromatograms were used.

The literature on the ion-exchange properties of phosphatidylcholine[11–15] shows considerable discrepancies which may be due to the source or purity of the molecule. During the experiments with phosphatidylcholine, we regularly determined the purity and condition of our samples[12]. The average R_F of 27 determinations in four lots received was 0.192 ± 0.003.

Our samples did not stain with ninhydrin or permanganate and did stain with phosphomolybdic acid and fluoresced under ultraviolet light with rodamine. No tails were apparent on the spots.

We also performed some control experiments with samples of synthetic phosphatidylcholine from Mann Research Laboratories, with no observed change in the results. Furthermore, we have studied the adsorption of Ca^{2+} on monolayers of natural phosphatidylcholine which was obtained from crude animal lecithin which was repetitively purified following MARINETTI AND STOTZ[24]. Even though natural phosphatidylcholine gave a slightly higher R_F (0.25) than the samples from Lachat Chemicals, there was no adsorption of either $^{45}Ca^{2+}$ or $^{35}SO_4^{2-}$ from a hypophase containing 0.1 mM of either $CaCl_2$ or K_2SO_4 at pH 7.

All the surface-active reagents were stored in the dark at −20° in a desiccator under partial vacuum.

RESULTS

Lack of cation and anion adsorption by phospatidylcholine monolayers

Previous reports have indicated the presence of a net charge in phosphatidylcholine monomolecular films even though there is no agreement regarding the sign of this charge. For instance, ROJAS AND TOBIAS[12] have shown that the polar group of this lipid acts at pH 7 as if it might be slightly positive. On the other hand, KIMISUKA AND KOKETSU[11] have reported that phosphatidylcholine binds Ca^{2+} at neutral pH suggesting that it is negatively charged. Some of these previous results could be explained on the basis of the spatial configuration of the polar head, which in the case of phosphatidylcholine is formed by a phosphate and a trimethylammonium group separated by two methylene groups. If the space availabe to each phosphatidylcholine molecule in the monomolecular layer is smaller than the size of the hydrated trimethylammonium, the separation between these two charged groups is maximal; the electrical field around the trimethylammonium group, which is positively charged, would repel cations in the hypophase and monolayers of phosphatidylcholine will behave as positively charged. On the other hand, when the space available to each hydrated polar head is greater than the size of the hydrated trimethylammonium, the structure of the polar head of phosphatidylcholine may allow internal neutralization of the phosphate and trimethylammonium groups; monolayers of phosphatidylcholine will behave as negatively charged or neutral depending on the degree of ionization and neutralization of these two groups.

TABLE I

LACK OF Ca^{2+} ADSORPTION BY PHOSPHATIDYLCHOLINE MONOLAYERS

Synthetic phosphatidylcholine (di-stearoyl) dissolved in chloroform (this sample was insoluble in acetone, ethanol–methanol or ethyl ether). pH of the hypophase was measured immediately after the experiment. The pH of the hypophase was adjusted with HCl or KOH for Part A and with H_2SO_4 or KOH for Part B. Area per molecule 50 Å^2.

Number of experiments	*Ca Cl$_2$ concn. in the hypophase (mM)*	*pH*	*counts/min$_{after}$ minus counts/min$_{before}$*
(A) Adsorption of $^{45}Ca^{2+}$			
3	0.025	1.1	159 ± 76
3	0.025	6.7	412 ± 387
3	0.060	8.5	158 ± 551
3	0.080	8.9	3 ± 514
(B) Adsorption of $^{35}SO_4^{2-}$			
3	0.050	9.0	114 ± 89
3	0.080	6.7	75 ± 197

To test these possibilities, the association of Ca^{2+} or SO_4^{2-} to phosphatidylcholine and to lysophosphatidylcholine monolayers was studied. Table I summarizes the results on adsorption of Ca^{2+} or SO_4^{2-} by phosphatidylcholine monolayers at 50 Å^2 per molecule. In METHODS a typical experiment was described and showed that the difference counts/min$_{after}$ − counts/min$_{before}$ spreading of a palmitic acid monolayer is of the order of 10000 counts/min. It is immediately apparent that the differences shown in Table I are very small. Statistical analysis of the data shown in Table I led us to conclude that there is no adsorption or repulsion of either Ca^{2+} or SO_4^{2-} by monomolecular layers of phosphatidylcholine under these various conditions. Thus, spreading phosphatidylcholine at 50 Å^2 per molecule evidently generates a neutral monolayer even when the pH of the hypophase is varied over a wide range. Increasing the area available to each molecule in the monolayer did not affect these results. No adsorption was seen in four experiments working at 70 Å^2 and a 2 mM $CaCl_2$ hypophase.

We have also studied the adsorption of Ca^{2+} and SO_4^{2-} by lysophosphatidylcholine monolayers, which can be compressed sufficiently to reduce the area per molecule to 30 Å^2. It was found that lysophosphatidylcholine does not adsorb Ca^{2+} or SO_4^{2-} from solutions of concentrations ranging from 0.1 to 1 mM. No systematic studies on pH effects were carried out with this molecule. These results do not support the notion that the spatial configuration of the phosphoric and trimethylammonium groups determines the behavior of the phosphatidylcholine monolayers.

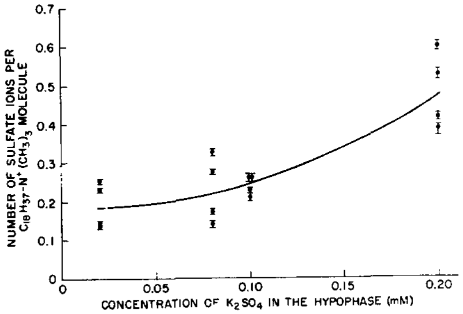
It has been suggested that there exists an internal neutralization in the polar head of phosphatidylcholine which is related to the spatial configuration of the groups[22]. The experimental results which follow were designed to analyze this alternative. Each functional group present in the polar head of phosphatidylcholine or phosphatidylserine was studied separately. Three molecules were used for this purpose: octadecyltrimethylammonium chloride (OTA), palmitic acid and octadecyl phosphate.

Properties of a single functional group

In order to characterize ion-exchange properties of monomolecular films it is necessary to study three aspects: (i) ion adsorption isotherm, (ii) pH dependance of the ion adsorption and (iii) selectivity. These three aspects were studied working with monolayers made of either OTA, palmitic acid or octadecyl phosphate.

Monolayers of octadecyltrimethylammonium ($R\text{-}N^+(CH_3)_3$)

Adsorption isotherm of SO_4^{2-}. To determine the adsorption isotherm of SO_4^{2-} by OTA, monolayers were spread on hypophases in which the concentration of K_2SO_4 was varied between 0.02 and 0.20 mM. Fig. 3 shows the adsorption isotherm obtained. It can be seen that when the concentration of the hypophase is 0.2 mM, the number of SO_4^{2-} absorbed to the monolayer per OTA molecule is close to 0.5, thus achieving electroneutrality. The effect of dilution in the hypophase from 0.2 to 0.02 mM on the apparent absorption of SO_4^{2-} to the monolayer is to reduce the number of ions per OTA molecule from 0.5 to 0.2.



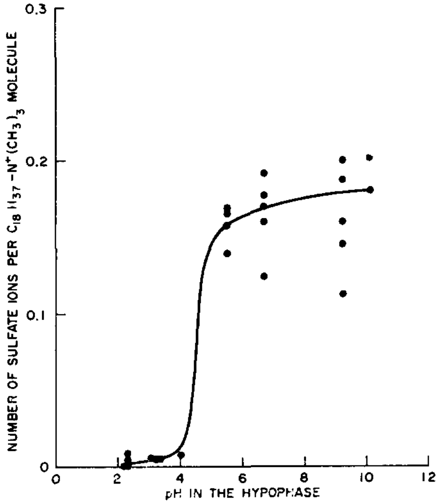


Fig. 3. Sulfate adsorption by OTA monolayers. Each point represents one experiment. Vertical bars represent 2 times the standard error of the counting. The pH of the hypophase was 6.5 ± 0.2 (unbuffered solution). Experiments performed at room temperature of about 25°. The area per OTA molecule was 50 Å^2.

Fig. 4. pH dependance of SO_4^{2-} adsorption by OTA. Each point represents one experiment. To change the hypophase pH, small amounts of either H_2SO_4 or KOH were added to the hypophase. The SO_4^{2-} concentration in the hypophase was kept constant at 0.05 mM for pH values above 6. For pH values smaller than 6 care was taken to avoid changes in the specific activity of the $^{35}SO_4^{2-}$. Area per OTA molecule was 50 Å^2.

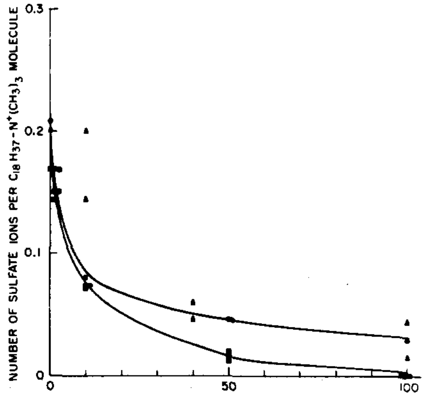
As the double-layer thickness is increasing with dilution (and on the order of 1000 Å) it is quite probable that the soft β radiation from $^{35}SO_4^{2-}$ may be absorbed by such a water layer. The energy of the β radiation from ^{35}S is 0.169 MeV, almost half that of ^{45}Ca, (0.26 MeV) for which the effect was not noticed.

More elaborate analysis would require accurate information about the penetration of the β radiation through water. The experiments which follow were performed with hypophase concentration at 0.1 mM; thus, the pH dependance and selectivity experiments should be considered only qualitatively.

pH dependance of SO_4^{2-} adsorption. The SO_4^{2-} adsorption of OTA varies with the pH of hypophase. This pH dependance is shown in Fig. 4 which is a plot of the number of SO_4^{2-} per OTA molecule as a function of the pH in the hypophase. Below pH 4 there is no adsorption of SO_4^{2-} apparent. Adsorption increases sharply between pH 4 and 6 and for pH values greater than 6 the adsorption remains fairly constant.

In these experiments the pH of the hypophase was lowered from 6 to 2 by adding H_2SO_4 to the solution. For pH 2 the concentration of SO_4^{2-} was approx. 5 mM. This concentration is 25 times greater than the concentration for which the ratio SO_4^{2-}: OTA molecule was maximal, namely 0.2 mM and yet below pH 4 there was no SO_4^{2-} adsorption. The inflection at pH 4.5 and the lack of absorption below that would reflect the dissociation constants of the protons of H_2SO_4. However, for pH values above the pH of distilled water (pH 5.5–6.5), adsorption of SO_4^{2-} seems to be independent of the pH of the hypophase.

SO_4^{2-} displacement from OTA monolayers by F^-, Cl^- and $H_2PO_4^-$. The experiments of displacement of SO_4^{2-} from OTA monolayers by F^-, Cl^- and $H_2PO_4^-$ were designed to test whether these monolayers would discriminate between F^-, Cl^-



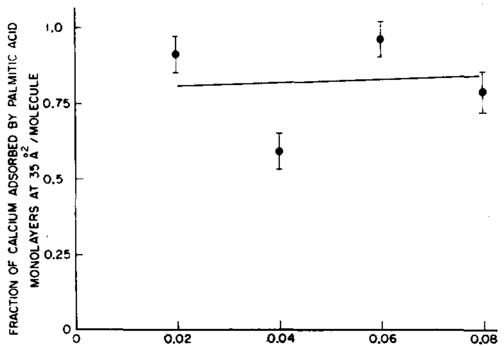


Fig. 5. Selectivity in OTA monolayers. Each point represents one experiment. Monolayers were spread on different solutions of the same specific activity of $^{35}SO_4^{2-}$ but with increasing concentration of either Cl^-, F^- or $H_2PO_4^-$. In this last case the pH of the hypophase was controlled and was 7.0. Area per OTA molecule was 50 Å². Experiments performed at room temperature of about 25°. ■—■, F^-; ●—●, Cl^-; ▲—▲, $H_2PO_4^{2-}$. The SO_4^{2-} concentration was 0.05 mM.

Fig. 6. Adsorption of Ca^{2+} by palmitic acid monolayers. Vertical axis represents the fraction of Ca^{2+} adsorbed by palmitic acid monolayers. Horizontal axis represents the concentration in the hypophase. Each point represents the average of several experiments. Vertical bars represent 2 times the standard error. The pH of the hypophase was 8.5 (The ratio of Ca^{2+} per palmitic acid molecule remained constant at 0.45 for all concentrations of $CaCl_2$ used.)

and $H_2PO_4^-$. The amount of $^{35}SO_4^{2-}$ adsorbed by the film in the presence of different concentrations of NaF, KCl or KH_2PO_4 in the hypophase was taken as a measure of efficiency of displacement of SO_4^{2-} by F^-, Cl^- or $H_2PO_4^{2-}$. Fig. 5 shows that Cl^- and $H_2PO_4^-$ displace about the same amount of SO_4^{2-} from the monolayer. There is a slight difference between F^- and Cl^-, F^- being more effective than Cl^-.

Monolayers of palmitic acid (R–COOH)

Adsorption of Ca^{2+} as a function of concentration. Fig. 6 shows the fraction of Ca^{2+} adsorbed by the palmitic acid monomolecular films when the concentration of $CaCl_2$ in the hypophase is increased from 0.02 to 0.08 mM at pH 9. It can be seen that Ca^{2+} adsorption is nearly constant for all concentrations used, unlike the results obtained with OTA and SO_4^{2-}. The number of Ca^{2+} per palmitic acid molecule is about 0.45. Palmitic acid monolayers were spread allowing 35 Å^2 of area for each molecule.

pH dependance of calcium adsorption. Fig. 7 is a plot of two experiments. Fig. 7A shows the results obtained when the pH in the hypophase was 4.9; the arrow indicates the time at which 25 μl of palmitic acid dissolved in chloroform were delivered to the surface. It can be seen that there is no variation in the rate of counts from the surface. However, when the hypophase pH was 8.9 there was an increase in the counting rate after spreading for the palmitic acid monolayer. This is seen in Fig. 7B.

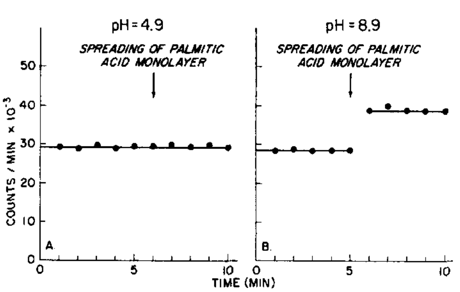


Fig. 7. Protocol of experiments on adsorption of Ca^{2+} by palmitic acid monolayers as a function of hypophase pH.

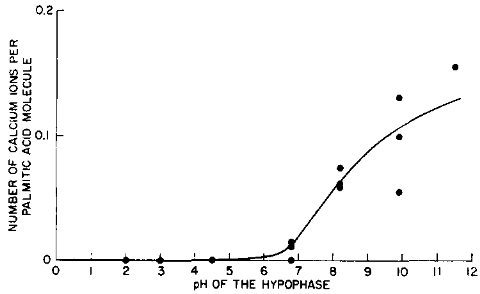


Fig. 8. pH dependance of the adsorption of Ca^{2+} by palmitic acid monolayers. Each point represents one experiment. The pH in the hypophase was modified by adding small amounts of HCl or KOH. The final concentration of KOH was about 10 mM.

This difference was used to calculate the number of Ca^{2+} adsorbed as described in METHODS. Ca^{2+} adsorption to the palmitic acid monolayer as a function of pH is summarized in Fig. 8. Each point represents an experiment like those described in Fig. 7. Ca^{2+} adsorption starts when the pH in the hypophase is above 6. The $CaCl_2$ concentration in these experiments was lowered to 0.02 mM to improve the sensitivity of the method. To raise the pH, KOH was added to the hypophase, and thus at pH 12 the number of Ca^{2+} per palmitic and molecule is lower than expected (0.15 instead of of 0.5) due to the competition with K^+ (see Table II). If this curve is interpreted as a titration curve, the apparent pK_a is 7 which seems to be higher than the pK_a of other water-soluble organic acids. This apparent pK_a may include an electrostatic

TABLE II

DISPLACEMENT OF Ca^{2+} FROM PALMITIC ACID MONOLAYERS BY K^+

All experiments were performed at room temperature of about 25°. Palmitic acid was dissolved in chloroform and was delivered to the surface in a single dose to form a monomolecular layer of a packing equal to 35 $Å^2$ per molecule.

	KCl concn. in the hypophase (mM)			
	0.08	*1.0*	*25*	*100*
Number of Ca^{2+} per palmitic acid molecule	0.41 ± 0.04	0.39 ± 0.1	0.34 ± 0.09	0.14 ± 0.04

TABLE III

ADSORPTION OF $^{45}Ca^{2+}$ BY OCTADECYL PHOSPHATE AS A FUNCTION OF THE CONCENTRATION OF $CaCl_2$ IN THE HYPOPHASE

All experiments were performed at room temperature of about 25°. The hypophase pH was measured in each experiment and ranged from 6.4 to 6.7. Specific activity of solutions ranged from 20 to 20.4 C/mole. Octadecyl phosphate was dissolved in ethyl ether–chloroform (1:2, v/v) and was delivered to the surface in a dose of 25 µl to form a monolayer of a packing equal to 35 $Å^2$ per molecule.

$CaCl_2$ concn. in the hypophase (mM)	*counts/min$_{after}$ minus counts/min$_{before}$*	*Number of Ca^{2+} per octadecyl phosphate molecule*
0.02	10649	0.087
	16279	0.133
	12974	0.104
	17993	0.147
		0.118 ± 0.23*
0.05	18815	0.153
	15457	0.124
	17666	0.140
	12806	0.102
		0.129 ± 0.019*
0.1	11677	0.095
	9165	0.075
	14687	0.120
	16646	0.136
		0.106 ± 0.017*

* Average ± S.D.

term, which takes into consideration the effects of a highly charged monolayer surface (see DISCUSSION).

Ca^{2+} displacement from the monolayer by K^+ in the hypophase. Table II summarizes the results obtained when studying the displacement of Ca^{2+} by K^+. The pH of the hypophase was maintained at pH 8 with 1 mM Tris buffer and the concentration of $CaCl_2$ was 0.2 mM.

Monolayers of octadecyl phosphate ($R–PO_2(OH)_2$)

Adsorption of $^{45}Ca^{2+}$ as a function of the $CaCl_2$ concentration in the hypophase. Table III shows the results obtained at three different concentrations in the hypophase.

It can be seen that the number of Ca^{2+} adsorbed per octadecyl phosphate molecule was almost constant.

pH dependance of Ca^{2+} adsorption. Fig. 9 shows the pH dependance of Ca^{2+} adsorption by octadecyl phosphate monolayers. Ca^{2+} adsorption starts when the pH in the hypophase is above 2. It can be seen that Ca^{2+} adsorption is maximal when the pH in the hypophase is greater than 9.5.

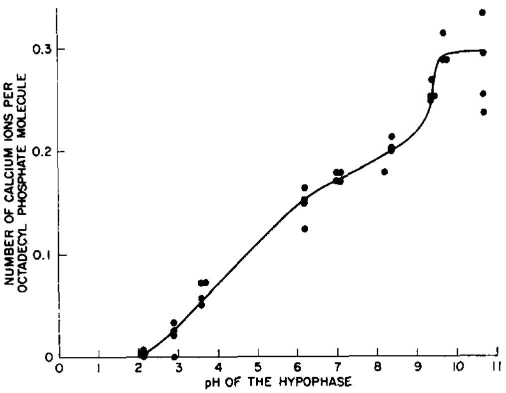


Fig. 9. pH dependance of the Ca^{2+} adsorption by octadecyl phosphate monolayer. Each point represents one experiment. The pH in the hypophase was changed as before (see Fig. 8).

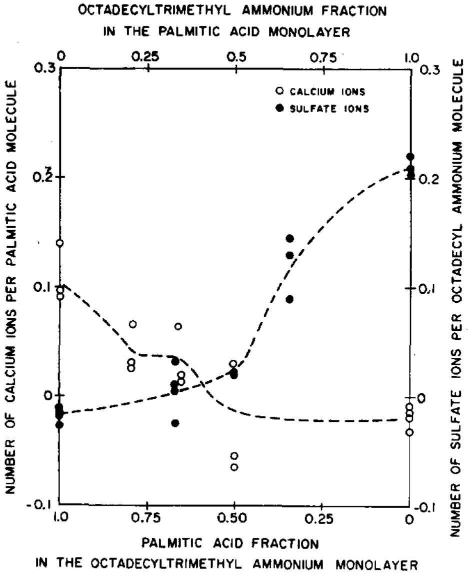
Anion and cation exchange properties of mixed monomolecular layers

The experiments which will be desribed in this section were designed to characterize the ionic exchange properties of monomolecular layers made of mixtures of two molecules described in the previous section. It was thought that once the ionic exchange properties of each functional group were known it would be possible to predict the ideal behavior of various mixtures. Thus, mixed monolayers were prepared using mixtures of either palmitic acid and OTA or octadecyl phosphate and OTA.

Comparison between the adsorption of Ca^{2+} and SO_4^{2-} in mixed monolayers of palmitic acid and OTA

Fig. 10 summarizes the results obtained. Vertical axis represents either the number of Ca^{2+} per palmitic acid molecule (left vertical axis) or the number of SO_4^{2-} per OTA molecule (right vertical axis). Horizontal axis represents either the fraction of palmitic acid in the OTA monolayer (lower axis) or the fraction of OTA in the palmitic acid monolayer (upper axis). It can be seen that: (i) the decrease in Ca^{2+} adsorption induced by increasing the proportions of OTA in the mixed monolayer is not linear. This is also true in the case of the adsorption of SO_4^{2-}. (ii) Although the concentration of either $CaCl_2$ or K_2SO_4 for both curves (adsorption of Ca^{2+} or adsorption of SO_4^{2-}) was the same, the number of SO_4^{2-} per OTA molecule is 2 times the number of Ca^{2+} per palmitic acid molecule. This indicates that the association between SO_4^{2-} and OTA polar group is stronger than the association between Ca^{2+} and the palmitic acid polar group. This conclusion is also evident when comparing the adsorption isotherms of Ca^{2+} and SO_4^{2-} by either palmitic acid or OTA shown in the previous section. An alternative explanation is that the carboxyl group is less dissociated than the trimethylammonium group in OTA. (iii) Mixed monolayers of palmitic acid and

OTA behave as neutral monolayer (there is no adsorption or repulsion of either Ca^{2+} or SO_4^{2-} when the ratio plamitic acid/OTA is 0.55).



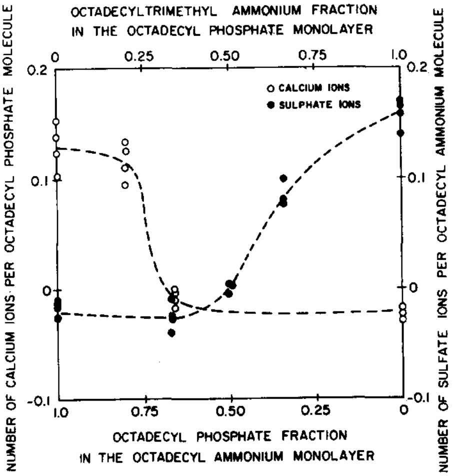


Fig. 10. Ca^{2+} or SO_4^{2-} adsorption by mixed monolayers: palmitic acid and OTA. The pH in the hypophase was adjusted to 9 by adding KOH to the hypophase. Area per molecule 35 Å^2.

Fig. 11. Ca^{2+} or SO_4^{2-} adsorption by mixed monolayers: octadecyl phosphate and OTA. The pH of the hypophase was 6.5. Area per molecule 35 Å^2.

Comparison between the adsorption of Ca^{2+} and SO_4^{2-} by mixed monolayers of octadecyl phosphate and OTA

Mixtures of octadecyl phosphate and OTA were used to form mixed monolayers and to study the adsorption of Ca^{2+} or SO_4^{2-}. The results obtained are shown in Fig. 11 which is organized as Fig. 10. The results obtained are essentially similar to those obtained with mixtures of palmitic acid and OTA. However, there are some differences. For example, it can be seen that Ca^{2+} adsorption is hardly altered by adding OTA up to 20 % in the mixed monolayer. Further, the fraction of OTA which generates a neutral mixed monolayer is 0.4. For larger fractions of OTA in the mixed monolayer there is no deadsorption of Ca^{2+}.

DISCUSSION

Previous reports indicate the presence of a net charge in phosphatidylcholine monomolecular films even though there is no agreement regarding the nature of this charge[11,12,22]. The present work has shown that synthetic phosphatidylcholine and lysosphosphatidylcholine do not adsorb cations or anions under various experimental conditions suggesting that phosphatidylcholine generates neutral monolayers. These results can be tentatively interpreted as follows. The structure of the polar head of phosphatidylcholine (phosphate and trimethylammonium groups separated by two methylene groups) may allow internal neutralization (formation of an internal salt) of the phosphate and the trimethylammonium groups. SHAH AND SCHULMAN[22] suggested that this internal salt linkage is dissociated upon increasing of the electrolyte concentration of the hypophase, Ca^{2+} being the most effective ion in dissociating this linkage. Thus, for concentrations of Ca^{2+} in the hypophase greater than 10 mM, a small adsorption of this ion has been measured[22], presumably due to the dissociation of the internal salt in the polar group of phosphatidylcholine and the association of Ca^{2+} with the phosphate group. As all the experiments presented in this communication were performed with concentrations of $CaCl_2$ smaller than 2 mM, an internal salt linkage could explain the neutral behavior of phosphatidylcholine. The experiments on Ca^{2+} or SO_4^{2-} adsorption by phosphatidylcholine were carried out at different degrees of packing of the monolayer. It was thought that the orientation of the trimethylammonium groups of the phosphatidylcholine with respect to the fatty acyl chains could determine the ion-exchange properties of phosphatidylcholine. Similar changes in orientation of dipoles in the surface of the cell membrane are often suggested. However, no adsorption of either Ca^{2+} or So_4^{2-} was detected at different degrees of packing of the monolayer. Thus, the experimental results, with phosphatidylcholine and with lysophosphatidylcholine do not support the notion that changes in the position of the dipoles in the polar heads of lipids may induce changes in the ion-exchange properties of cell surfaces. The results presented in this paper do not discard the possibility of a charged polar head. In fact, another explanation of the results can be obtained by considering the distribution of Ca^{2+} (or SO_4^{2-}) in the polar group region of phosphatidylcholine. If the phosphate and trimethylammonium group are approximately parallel to the fatty acyl chains, then there are two positions available to Ca^{2+} in the polar group phase. The first position is near the phosphate group closer to the air–water interphase. The second position is below the positively charged trimethylammonium group which repels Ca^{2+}. Thus, Ca^{2+} will be more concentrated in the upper position and more diluted in the lower position. The technique used in the present work allows us to detect a net increase in surface concentrations; therefore, it would not resolve this spatial distribution of Ca^{2+}.

Ion-exchange properties of palmitic acid, OTA and octadecyl phosphate monolayers

The polar groups of membrane phospholipids contain different ionogenic groups with different pK values. The pH-dependance experiments were designed to determine the relation between ionization of these groups and their ion-exchange properties.

The pH-dependance experiment with palmitic acid has shown an inflection at a pH values close to 7.5. The pH-dependance experiment with octadecyl phosphate has shown inflection at pH values close to 3.5 and 9.5. In these two cases, palmitic acid and octadecyl phosphate, the pK_a values obtained are shifted with respect to the pK_a values described in the literature for carboxyl and phosphoric groups. The pH dependance of the surface potential of monolayers of weak acids or bases has been studied by a number of investigators[25,26]. The relation between surface potential and hypophase pH resembles a titration curve. The inflections on these curves give apparent pK_a values which differ markedly from the pK_a values of soluble related acids or bases. Several of these authors explain these differences as due to unmeasurable differences between the pH of the bulk solution and the charged monolayer surface. However, CRAWFORD *et al.*[27] have indicated that "surface pH" cannot be operationally defined. BETTS AND PETHICA[28] have reexamined this problem and have developed a method (valid for condensed monolayer at the air–water interface) to obtain the pK_a of weak acids and bases measuring surface potentials as a function of pH of the hypophase. These authors have calculated the intrinsic pK of stearic acid to be equal to 5.55 which is shifted 0.7 units towards neutral pH with respect to soluble fatty acids. The pK_a determined by us is shifted about 2.5 units. This shift must include an electrostatic term to account for the effect of the highly charged palmitic acid monolayer[28].

The experiments on SO_4^{2-} displacement by monovalent anions were designed to show whether OTA monolayers would discriminate between Cl^-, F^- and $H_2PO_4^-$. The degree of packing of the films was kept constant at 50 Å². It was found that F^- is more effective than Cl^- in displacing SO_4^{2-} from the trimethylammonium group. This difference, however, is small. Presumably if the space available to the polar end of the molecule were decreased, the less hydrated ion might penetrate it more easily. A decrease in the space available will increase the difference observed. This phenomenon is probably due to the fact that at high-packing density there is less room available for ion intrusion, and monovalent anions have less easy access to the SO_4^{2-} adsorption sites[29].

Adsorption of Ca^{2+} or SO_4^{2-} by mixed monomolecular layers

This communication presents data on the properties of mixed monolayers of palmitic acid–OTA and octadecyl phosphate–OTA and the interactions of the monolayers, with Ca^{2+} and SO_4^{2-}. These experiments were designed to determine whether mixed monolayers would behave as amphoteric ion exchangers, *i.e.* monolayers which contain acidic and basic groups which will exchange cations and anions[29].

Plots of adsorption of SO_4^{2-} or Ca^{2+} as a function of the mole fraction of OTA in the mixed monolayer (Figs. 10 and 11) have shown a large deviation from the additivity rule which predicts a linear relationship between adsorption of SO_4^{2-} and OTA mole fraction. These results are readily interpreted considering the properties of each functional group involved in the mixed monolayer. For example, Fig. 10 shows the effects of the addition of OTA upon the adsorption of Ca^{2+} by octadecyl phosphate monolayers. The addition of OTA to octadecyl phosphate monolayers not only changes the molar fraction of positive charges in the mixed monolayer but also changes concentration of H^+ in the neighborhood of the polar groups. We have seen that OTA adsorbs anions (for example OH^-); thus, the pH in the neighborhood of the polar groups will become "more alkaline", and this in turn will increase the dissociation of the octadecyl phosphate polar group (see Fig. 9). Therefore, octadecyl phosphate will adsorb more Ca^{2+}.

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