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# Systematic study of phospholipids linked to a steroid derivative, spread into a monolayer at the air/water interface

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**Isothermal pressure-area curves of different phospholipids linked to a cortisol derivative, spread into monolayers at the air/water interface are studied. It is shown that derivatives containing saturated lipid chains and those with unsaturated chains present quite different behaviours. With saturated derivatives, the main phase transition plateau and the stability of the fluid phase are very sensitive to the length of the lipid chains, the presence of a spacer between the lipid and the steroid moieties, the temperature and the presence of di- and trivalent cations in the aqueous subphase; the calcium ion shows an especially high effect, compared to the other ions studied. The presence of the steroid on the lipid modifies the specific area of the molecules of unsaturated lipids, which is not the case with saturated lipids, probably due to differences in the lipophilic cohesion.**

## Introduction

For a number of years, the lipid monolayer technique has been the subject of extensive studies in different areas such as the prevention of evaporation in storage tanks, the elaboration of new surfactants for industry or the modelization of biological systems for studies of the membrane structure or the biological activities associated with membranes [1,2].

Very recently, lipid monolayers were successfully used as a support for the two-dimensional crystallization of biological macromolecules (proteins), thus allowing the determination of their structure by means of electron microscopy and image processing, at a resolution not yet reached using more classical approaches such as X-ray diffraction [3–6]. Once developed, this technique has the advantage of being fast and efficient for low resolution requiring only micrograms of purified material, whereas X-ray crystallography requires several milligrams.

The two-dimensional crystallization of macromolecules specifically bound to a ligand linked to a lipid monolayer is possible due to the following characteristics of the system: (i) the fluidity of the lipids in the monolayer (facilitates the crystallographic organization of the macromolecules); (ii) the accessibility of the ligand by the macromolecule to be bound; (iii) the high local concentration of the material of interest at the surface of the monolayer; (iv) the stability of the monolayer.

Abbreviations: 16:0, dipalmitoyl; 18:0, distearoyl; 18:1, dioleoyl; 22:0, dibehenoyl; PBS, phosphate-buffered saline (8 g/l NaCl, 0.2 g/l KCl, 1.15 g/l $NaH_2PO_4$, 0.2 g/l $KH_2PO_4$).

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To develop this technique for the glucocorticoid and progesterone receptors, we have started with the characterization of glycerophospholipids linked to a steroid hormone, cortisol, and spread into a monolayer state on a hypophase. To determine experimental conditions, it appeared necessary to undertake a systematic study of some parameters modulating the physical properties of the monolayer. The investigation of the isothermal pressure-area curves of lipid derivatives (i.e. the surface tension $\pi$ as a function of the area $A$ available per lipid molecule) enabled us to study the film properties as a function of the nature of the lipid chains (length and degree of unsaturation of the carbon chains), presence of a spacer between the lipid and the corticoid derivative, temperature, presence of cations in the hypophase and dilution with other lipids not linked to a ligand.

## Material and Methods

Pressure-area curves of lipid derivatives spread at the air/water interface were obtained using a teflon through (165 × 270 × 10 mm) and a compression barrier made of kevlar (hydrophilous material). The surface of the aqueous solution was swept clean by means of an aspirator. The surface tension was measured with an electrobalance (KSV 2200 surface barostat, KSV Finland).

L-α-Diacylphosphatidylcholines used (16 : 0 PC, 18 : 0 PC, 18 : 1 PC, 22 : 0 PC) were purchased from Avanti Polar Lipids and Sigma. L-α-Diacylphosphatidylethanolamines (16 : 0 PE, 18 : 0 PE, 18 : 1 PE, 22 : 0 PE) were synthesized in our laboratory as well as L-α-diacylcaproylphosphatidylethanolamines (16 : 0 Cap PE, 18 : 1 Cap PE) [7].

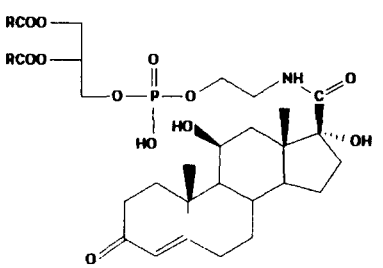



Fig. 1. General structure of PEC. 16 : 0 PEC, R = $n C_{15}H_{31}$-; 18 : 0 PEC, R = $n C_{17}H_{35}$-; 22 : 0 PEC, R = $n C_{21}H_{43}$-; 18 : 1 PEC, R = *cis*-$n C_8H_{17}CH{=}CH\text{-}C_7H_{14}$-.

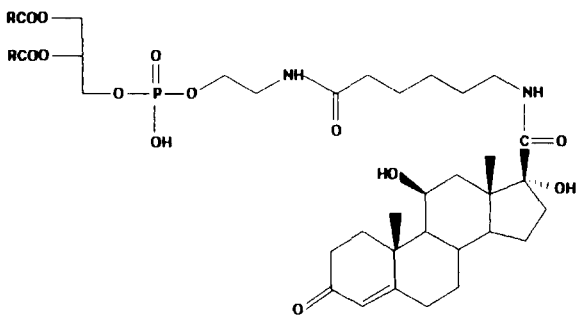



Fig. 2. General structure of Cap PEC. 16 : 0 Cap PEC, R = $n C_{15}H_{31}$-; 18 : 1 Cap PEC, R = *cis*-$n C_8H_{17}CH{=}CH\text{-}C_7H_{14}$-.

Cortisol was bound to PE and Cap PE after oxidation with periodic acid and a peptide junction formed to give L-α-diacylphosphatidyl(17β-cortisol)ethanolamides (PEC) (Fig. 1) and L-α-diacylphosphatidyl(17β-cortisol)(6-amidocapro)ethanolamides (Cap PEC) (Fig. 2).

PEC and Cap PEC were purified by high performance liquid chromatography (HPLC) (column: Waters microporasil 5 μm, 25 × 0.76 cm; flow rate: 6 ml/min; elution: chloroform/methanol from 98 : 2 to 70 : 30 (v/v) within 30 minutes; Waters gradient controller M680, curve No. 7).

Lipid derivatives were solubilized in chloroform (3 mg/ml) and spread (typically 15 μl using a microsyringe) at the thermo-controlled and dust sheltered air/water interface. Lipid solutions were stored under argon at −20 °C; when used, they were maintained on ice. Concentrations were checked regularly by determination of the dry weight.

## Results

The arrangement of the lipid derivatives into the monolayer formed at the air/water interface was characterized by recording the variations of the surface pressure as a function of the surface available per lipid molecule. For this purpose, 30 to 40 μg of lipid were applied to a 400 $cm^2$ clean water surface. The lipid monolayer formed was then compressed with a mobile barrier displaced at 60 mm/min. These conditions correspond to

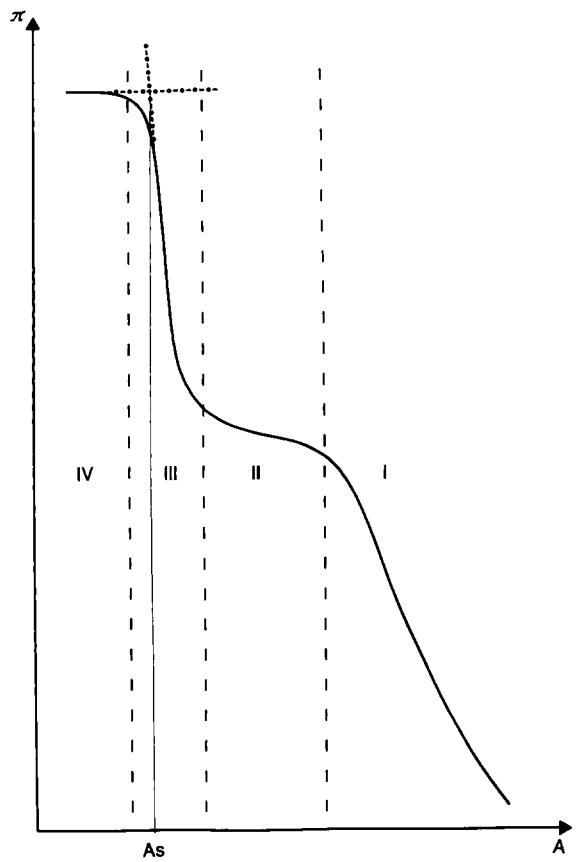



Fig. 3. Typical pressure-area curve obtained by isothermal compression of a phospholipid monolayer. The specific area $A_s$ is extrapolated from the intersection between the tangents to the rapid increase (region III) and the collapse (region IV) parts.

the reduction of the surface by 0.2 $nm^2$ per lipid molecule and per minute. In order to verify that the velocity of the barrier did not affect the pressure values recorded, we tried different speeds (15 to 300 mm/min) and observed no significant change in the pressure-area curves. We noted that the total volume of solvent applied to the surface should not be too large. In our case, a lipid concentration of more than 1 mg/ml was used routinely. When a large volume of solvent and low concentration of lipids were used, the recorded area per lipid molecule was abnormally small, suggesting that all the lipid molecules did not participate in the monolayer organization.

The general appearance of the isothermal pressure-area curves is depicted in Fig. 3. The precise identification of the different domains I, II and III was reported previously [8]. Region I corresponds to the fluid or expanded state and is characterized by a slow and progressive increase of the pressure when the area available per molecule decreases. Region II is referred to as the transition between the fluid and the organized states and is called the main phase transition. Region III, showing a rapid increase of the pressure is characteristic of the most compact configuration of the lipid monolayer. Region IV, or collapse, is representative of the monolayer transformation into a multilayer organization.

The specific area ($A_s$ on Fig. 3) corresponds to the minimum surface necessary per lipid molecule in the very condensed state of the monolayer and is obtained through an extrapolation.

All assays were repeated more than three times. The different parts of the curves were reproducibly observed in all cases, showing that the different parts can be considered as representative of the different possible organizations of the lipid monolayer. Unless otherwise stated, all experiments were realized on a buffered physiological ionic strength hypophase (PBS, pH 7.3) and at 16 °C. This medium represents a standard condition compatible with most biological activities.

### *A. Influence of the nature of the lipid chain*

#### *(1) Length of the chain*

Different polycarbon chain lengths (16, 18 and 22 carbon atoms) were checked. This range was selected so as to obtain a fluid film between 4 and 20 °C [9], a condition imposed a priori by the necessity to preserve the macromolecular structures and activities. When the length of the carbon chains increases from 16 to 22 carbon atoms, one

TABLE I

VALUES OF SPECIFIC AREA $A_s$ OBTAINED UNDER STANDARD CONDITIONS

| | $A_s$ ($nm^2$/mol) | | |
|---|---|---|---|
| | PE | PC | PEC |
| 16:0 | 0.38–0.39 | 0.41 | 0.42 |
| 18:0 | 0.33 | 0.34 | 0.34 |
| 22:0 | – | 0.32 | 0.31 |

observes a decrease in the specific area $A_s$ (Table I). This effect could be due to a higher lypophilic cohesion, compensating the electrostatic repulsion forces between the polar groups. Under this hypothesis, one should expect the presence of a limit value for $A_s$ beyond a certain chain length. Our observations would support the hypothesis that the specific area decreases from 16 : 0 to 18 : 0 and 22 : 0 thus approaching the value corresponding to a close packing of the phospholipids with lipid chains of about twenty carbon atoms in length. The abnormally low [10,11] but reproducible values obtained for 18 : 0 and 22 : 0 phospholipids should result from a loss of material participating in the formation of the monolayer. At present we are unable to explain this apparent loss that seems to be more important for the more stable monolayer which is also the less fluid (see Section D3).

For PE and PC, the plateau corresponding to the main phase transition occurs at a very low pressure and when the temperature is at or below 16 °C, this plateau can not longer be resolved

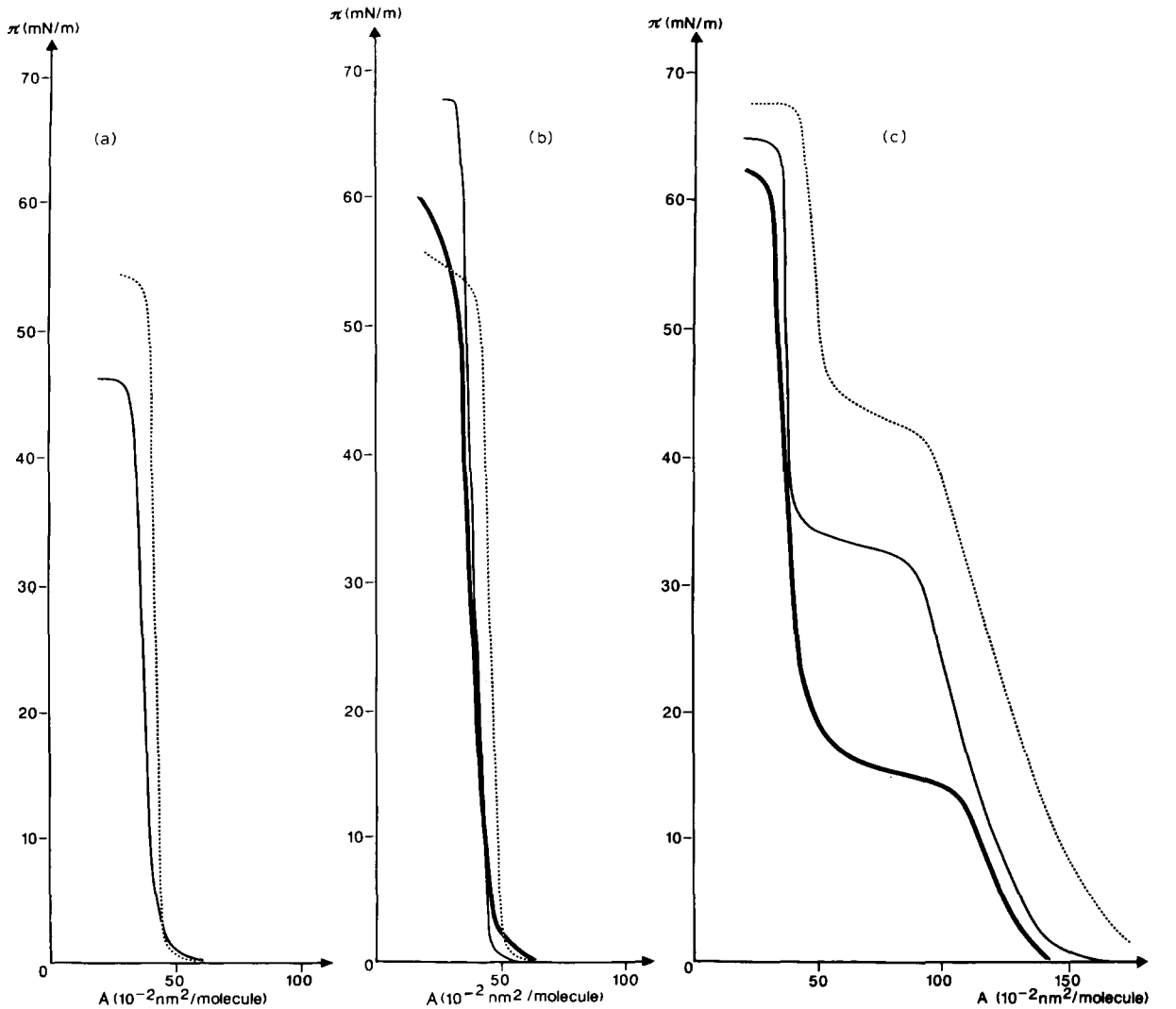



Fig. 4. Isothermal pressure-area curve obtained with PE (a), PC (b) and PEC (c) (standard conditions). ·····, 16 : 0; ———, 18 : 0; ▬▬, 22 : 0.

under our experimental conditions (Figs. 4a, b). In contrast, for PEC, the main phase transition is always clearly prominent and occurs at a higher pressure (Fig. 4c). The presence on the lipid molecule of cortisol, a poorly soluble molecule in aqueous solutions, seems to interfer with the normal condensation of the lipid chains, which require a higher pressure to reach the main phase transition; this phenomenon is even more important when the lipid chains are short. By the increase in the chain molecule the specific area is not significantly changed. This confirms that chain length favors the lipid cohesion and partially counterbalances the presence of the cortisol as shown by the drastic lowering of the main phase transition pressure. Unlike the main phase transition, that is greatly modified by the presence of a cortisol despite its hydrophobic character, the steroid points towards the aqueous phase when the crystalline state of the film is reached.

*(2) Influence of an unsaturation in the lipid chains*

The introduction of an unsaturation in the lipid chains modifies the physical properties of the monolayer, especially its fluidity. The comparison of the 18 : 1 derivatives, linked or not to cortisol, is shown Fig. 5. In all cases (PE, PC and PEC), we observed a fluid phase very similar to that of saturated PEC (fig. 4c). The first plateau, observed at a pressure close to 44–47 mN/m, is representative of the collapsed or multilamellar state and does not correspond to the main phase transition plateau. In further support of this interpretation, the pressure does not increase further irrespective of the reduction in surface that is realized. The most condensed state obtained (before the collapse plateau), still corresponds to a fluid phase. The $A_s$ values obtained are significantly higher than the corresponding values for saturated derivatives (compare Fig. 4c to Fig. 5). Note that in the case of the cortisol linked derivatives, when the surface pressure increases, the first phase is identical to that of the corresponding saturated derivative (Fig. 4c), but at higher pressure the curve continues and increases until the collapse state is reached, due to the fact that the phospholipid is above its $T_c$ for the main phase transition. In this case, for the unsaturated cortisol derivatives 18 : 1 PEC and 18 : 1 Cap PEC, the

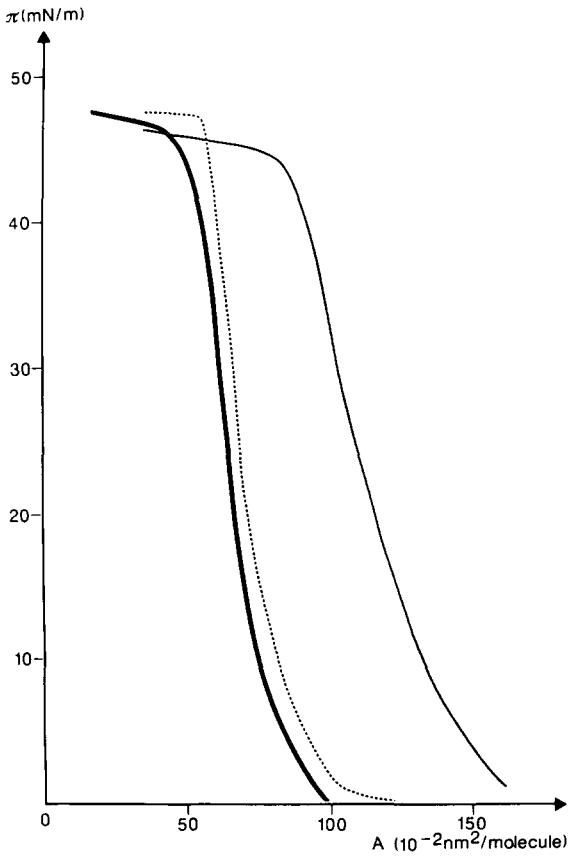



Fig. 5. Influence of an unsaturation in the lipid chain (standard conditions). ·····, 18:1 PC; ———, 18:1 PEC; ▬▬, 18:1 PE.

measured specific areas are about 40% higher than for 18 : 0 PE, 18 : 0 PC or 18 : 0 Cap PE. These results show clearly that the cohesion of the lipid moieties is not as good in the presence of a double bond in the chains, as previously reported in the literature [12,13]. The presence of unsaturated lipid chains does not permit the formation of an organized monolayer before a collapse state is reached. As a consequence, the significant difference between the areas per molecule at a given pressure for the PE, PC and PEC derivatives is due to the fact that the lipophilic cohesion is too week to stimulate the complete exclusion of the cortisol portion into the aqueous phase, in contrast to that which is observed with saturated lipids. It is likely that the steroid interacts in the polar region with itself or the polar headgroup.

*B. Influence of a caproyl spacer*

In a systematic study on the optimized conditions for the purification of steroid receptors on affinity columns, it was demonstrated that a 9 carbon atom spacer, between the steroid hormone and the chromatography support, was necessary to obtain the optimal fixation of the receptor [14].

With the intent to eventually crystallize steroid receptors, we incorporated a linker between the phospholipid and the corticoid to improve the accessibility of the hormone to the protein. We thus compared the phospholipids PE, PEC, Cap PE and Cap PEC (Fig. 6). (In Cap PEC the cortisol is a caproyl spacer group linked to the phospholipid (Figs. 1 and 2).) In the case of unsaturated lipids, the presence of a caproyl moiety does not induce any significant change in the pressure-area curves. When the lipid chains are saturated, the caproyl group leads to the lowering of the main phase transition plateau and to a

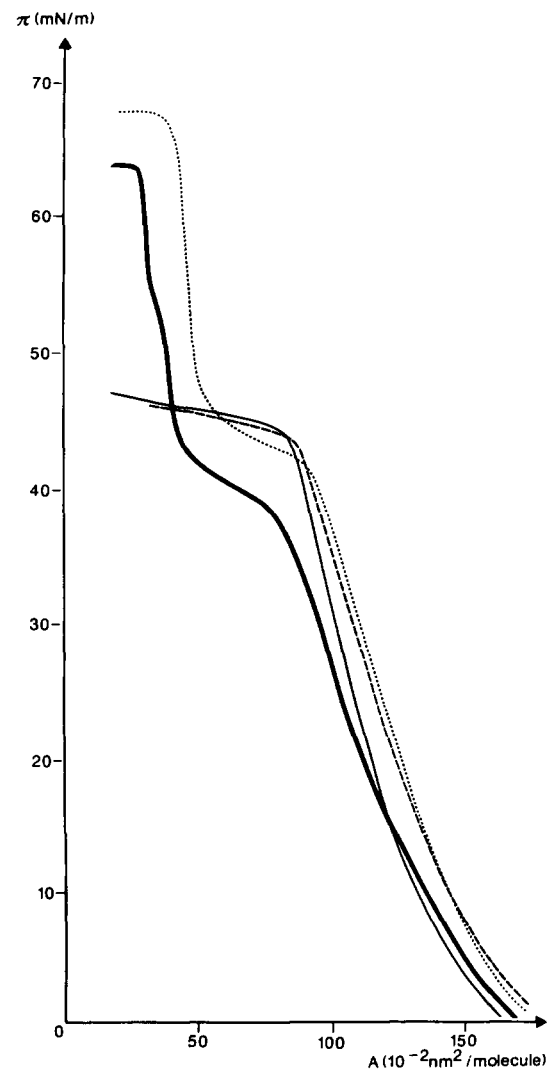



Fig. 6. Influence of a caproyl spacer (standard conditions). ———, 18:1 PEC; - - - - - -, 18:1 Cap PEC; ·····, 16:0 PEC; ▬▬, 16:0 Cap PEC.

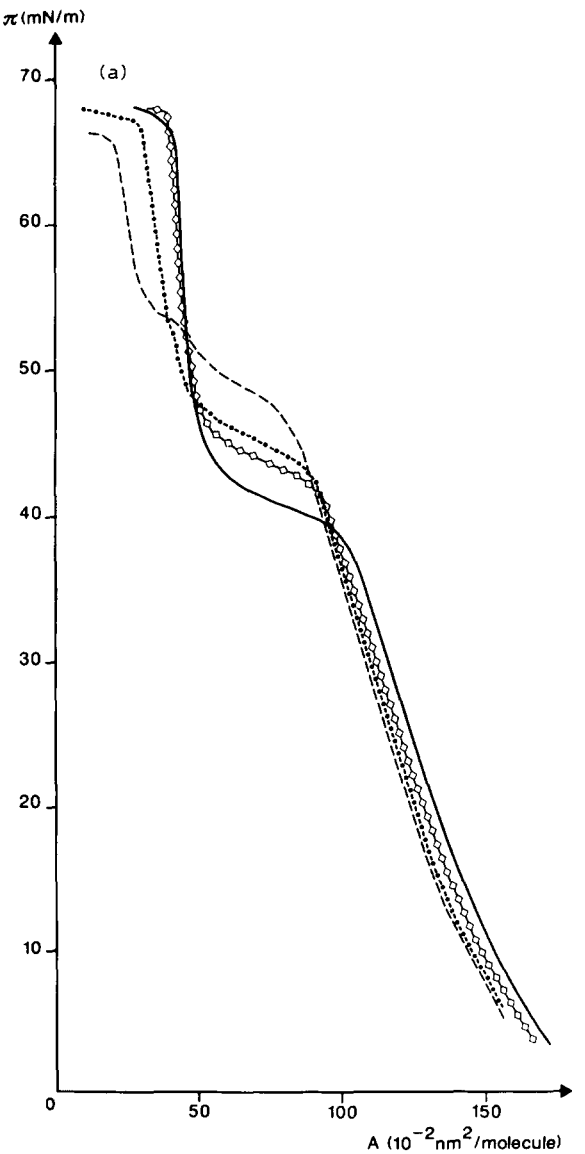



Fig. 7 (a).

decrease of the specific area, indicating a better cohesion of the system. The low value of $A_s$ clearly shows that we are dealing with a very compact organization of the lipid derivatives in the monolayer, a structure that can be interpreted as a 'linear' conformation of the phosphatidyl-ethanolamine-caproyl-cortisol entities and an extra lipophilic interaction between the caproyl groups.

*C. Effect of temperature*

When the temperature increases, as reported in previous studies [8,10,15], the main phase transition occurs at a higher pressure (Fig. 7). In the case of short chains (16 : 0 PEC, 16 : 0 Cap PEC), specific areas below 0.3 $nm^2$ per molecule are obtained. These values can be explained easily by the increase in solubility of these compounds when

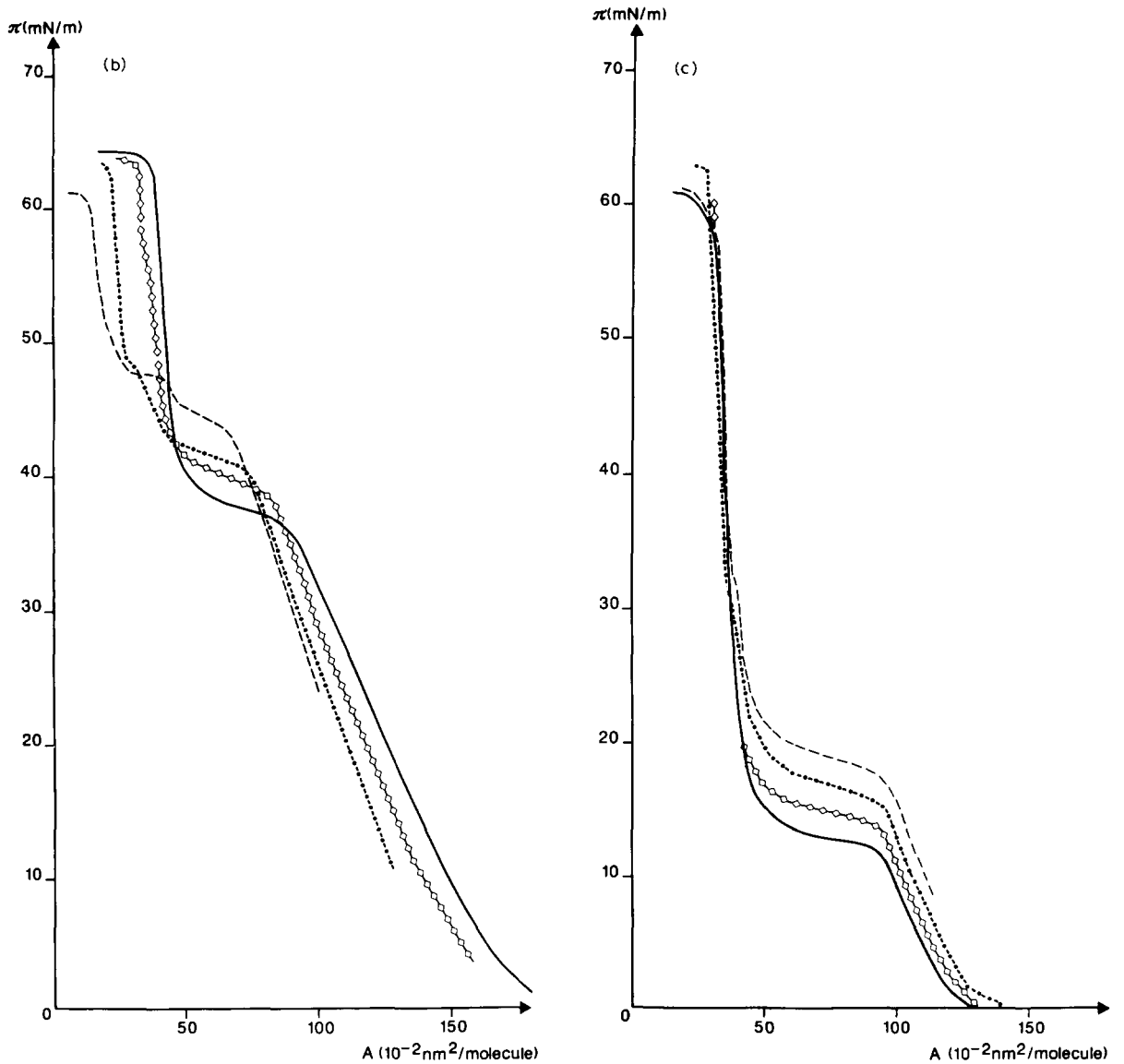



Fig. 7. Influence of temperature over a film of 16 : 0 PEC (a), 16 : 0 Cap PEC (b) and 22 : 0 PEC (c) (PBS, pH 7.3). ———, 11° C; ◇◇◇◇, 15° C; – · – · –, 19° C; - - - - - -, 24° C.

the temperature rises. With short chains, one can observe a second plateau, which becomes more and more important as the temperature increases. This phenomenon is also apparent in the case of 22 : 0 PEC but only at high temperature (24° C). These observations were reported previously by others and attributed to the transition from a tilted crystalline state to a non-tilted one [8].

*D. Influence of ions in solution over the stability of the films*

The stability of the protein structure and the specific binding on the steroid ligand require the use of a buffered medium and the presence of certain ions, as well as sufficient stability of the monolayer during the incubation. The stability of the monolayer in its different phases, in our experimental conditions, was tested by stopping, for one minute, the progression of the compression barrier, at several points of the pressure area curve. The variation of pressure recorded during that time ($\Delta\pi$) gives a good indication of the film stability. Each time an instability was noticed, we observed a rapid decrease of the pressure during the first minute (60 to 80% of the phenomenon) which was much slower later on. Fig. 8 shows a typical example of such variations of pressure recorded at the different monomolecular film states. A film was considered as stable in the conditions where the variation of pressure was lower than 1 mN/m during one minute. The influence of different salt conditions were tested. Di- and trivalent cations were systematically studied, using their chloride salts.

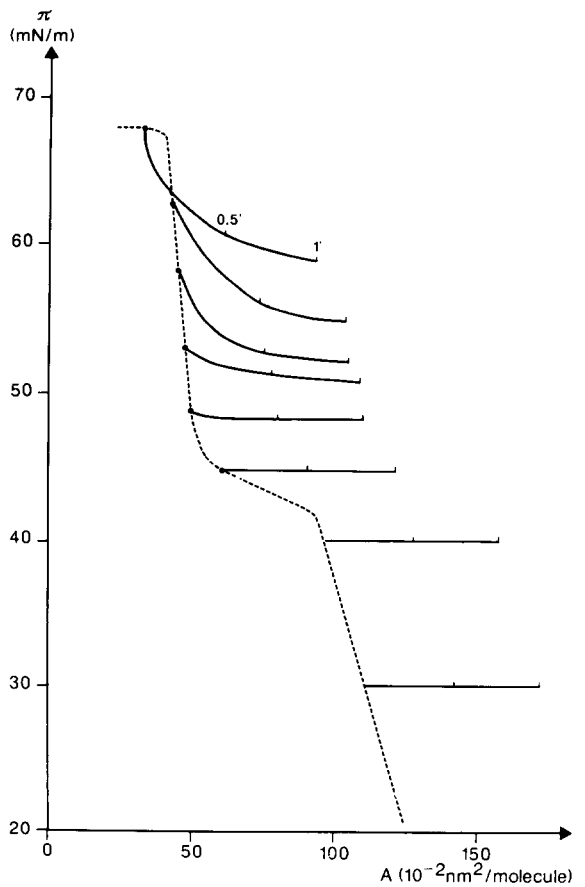



Fig. 8. Instability of the monolayers, ------, pressure-area curve; ———, ($\Delta\pi$) decrease of the pressure during the first minute at constant area (16 : 0 PEC; standard conditions).

*(1) Influence of the concentration of magnesium*

The presence of increasing magnesium chloride concentration in the hypophase, on which is formed a 16 : 0 PEC monolayer, induces a progressive lowering of the pressure at which the main phase transition occurs (Fig. 9). This pressure tends toward a limit at about 10 mM $MgCl_2$. The specific area is not significantly affected. The absence of magnesium, together with the addition of chelating agents (EDTA and EGTA), induces a slight elevation of the transition plateau. This is interpreted as the result of complexation of traces of divalent cations present in the solution. In the following experiments, we used cations at a 0.1 mM concentration, a condition producing an important effect on the isotherm curves.

*(2) Influence of the nature of the di- or trivalent cations on the pressure-area curves*

The different divalent cations used ($Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$) do not induce any significant difference in the specific area values, but clearly alter the pressure-area curves, lowering the pressure of the main phase transition. This effect is present whatever the length of the saturated chains is, in the presence or absence of a caproyl group (Table II). We found consistantly that among all the cations tested, calcium presents a far stronger effect compared to the others.

The pressure-area curves of unsaturated deriva-

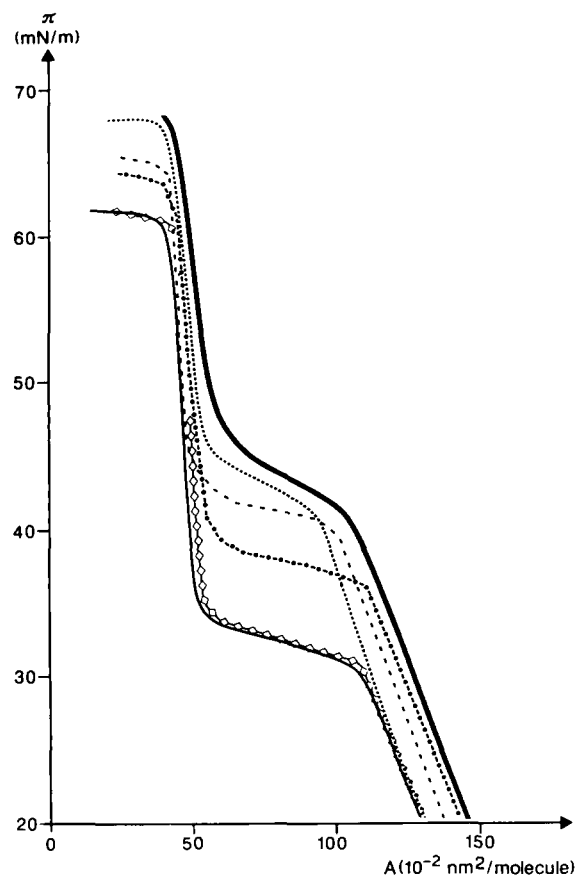



Fig. 9. Influence of various concentration of $Mg^{2+}$ on monolayers (16:0 PEC; standard conditions). ·····, 0 mM; - - - - -, 0.1 mM; -·-·-, 1 mM; ◇◇◇◇, 10 mM; ———, 100 mM; ▬▬, 10 mM EDTA + 10 mM EGTA.

TABLE II

MEAN PRESSURE OF THE PHASE TRANSITION PLATEAU OBSERVED IN ISOTHERMAL COMPRESSION IN PRESENCE OF DI- OR TRIVALENT CATIONS, 0.1 mM IN PBS (16 °C, pH 7.3)

| | Mean pressure (mN/m) | | | |
|---|---|---|---|---|
| | 16:0 PEC | 18:0 PEC | 22:0 PEC | 18:0 Cap PEC |
| – | 44.0 | 33.6 | 15.6 | 40.8 |
| $Mg^{2+}$ | 41.3 | 30.6 | 14.0 | 38.7 |
| $Ca^{2+}$ | 36.4 | 27.7 | 11.6 | 34.0 |
| $Sr^{2+}$ | 43.0 | 31.2 | 14.5 | 39.8 |
| $Ba^{2+}$ | 41.3 | 30.6 | 14.5 | 39.4 |
| $Al^{3+}$ | 44.4 | 34.2 | 16.0 | 40.8 |
| $Fe^{3+}$ | 44.0 | – | – | – |
| $La^{3+}$ | 44.4 | 34.0 | – | – |

tives (18:1 PEC and 18:1 Cap PEC) are not modified by the presence of divalent cations (unpublished results). These observations would support the idea that the divalent cations act by simultaneous complexation of several phospholipids, so decreasing the energy necessary to bring the molecules closer to each other and to compact the system. This stabilizing effect upon the polar regions can be related to the stabilization observed when lipid derivatives with longer carbon chains are tested. As in the absence of divalent cations, the specific areas tend to a limit close to 0.3 $nm^2$ per molecule.

The trivalent cations ($Al^{3+}$, $La^{3+}$, $Fe^{3+}$) by themselves do not alter the pressure-area curves in a significant way, when compared to the curves obtained on PBS alone.

*(3) Influence of different cations on the monolayer stability*

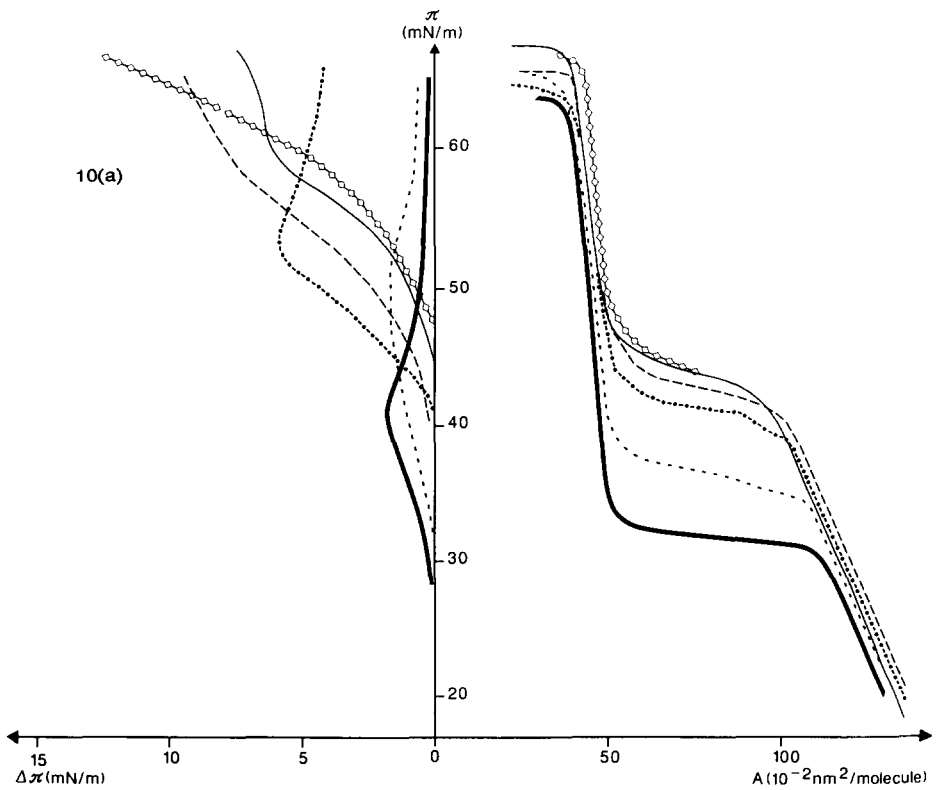
The lack of stability of a monolayer, when observed, was always seen in the crystalline phase (see Fig. 8), that is to say at pressures higher than that of the mean phase transition. In the case of 16:0 PEC and 16:0 Cap PEC, the instability of the film is reduced in the presence of $Ca^{2+}$ but is increased by the presence of $Sr^{2+}$ or $Ba^{2+}$ (Figs. 10a, b). The effects on stability decrease when the chain length increases and the stability of the 22:0 PEC monolayer is not altered in the presence of di- or trivalent cations (Figs. 10c, d).

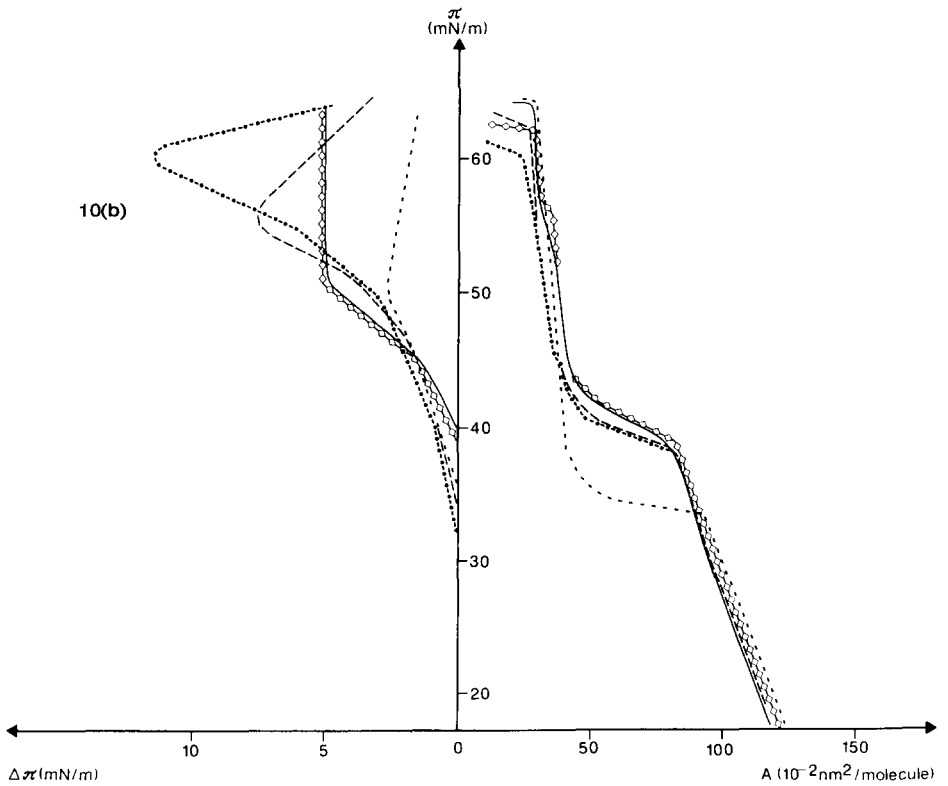
Unsaturated phospholipids 18:1 PEC and 18:1 Cap PEC, which do not present any organized or crystalline state, are very stable until the surface pressure reaches values close to the collapse pressure and in all cases higher than 40 mN/m (unpublished results).

*E. Effect of the dilution with other lipids*

To increase the accessibility of the steroid ligand towards the receptor on the monolayer, as well as in the affinity chromatography technique, it may be helpfull to scatter the lipids bearing the hormonal derivatives. This was realized by the use of dilutions with the corresponding lipids not linked to a ligand. Two kinds of pressure-area curves were obtained for such mixtures (Figs. 11, 12).

With the saturated derivatives (16:0 PEC, 16:0


π
(mN/m)
10(a)
60
50
40
30
20
15
10
5
0
50
100
Δπ(mN/m)
A(10⁻²nm²/molecule)


π
(mN/m)
10(b)
60
50
40
30
20
10
5
0
50
100
150
Δπ(mN/m)
A(10⁻²nm²/molecule)

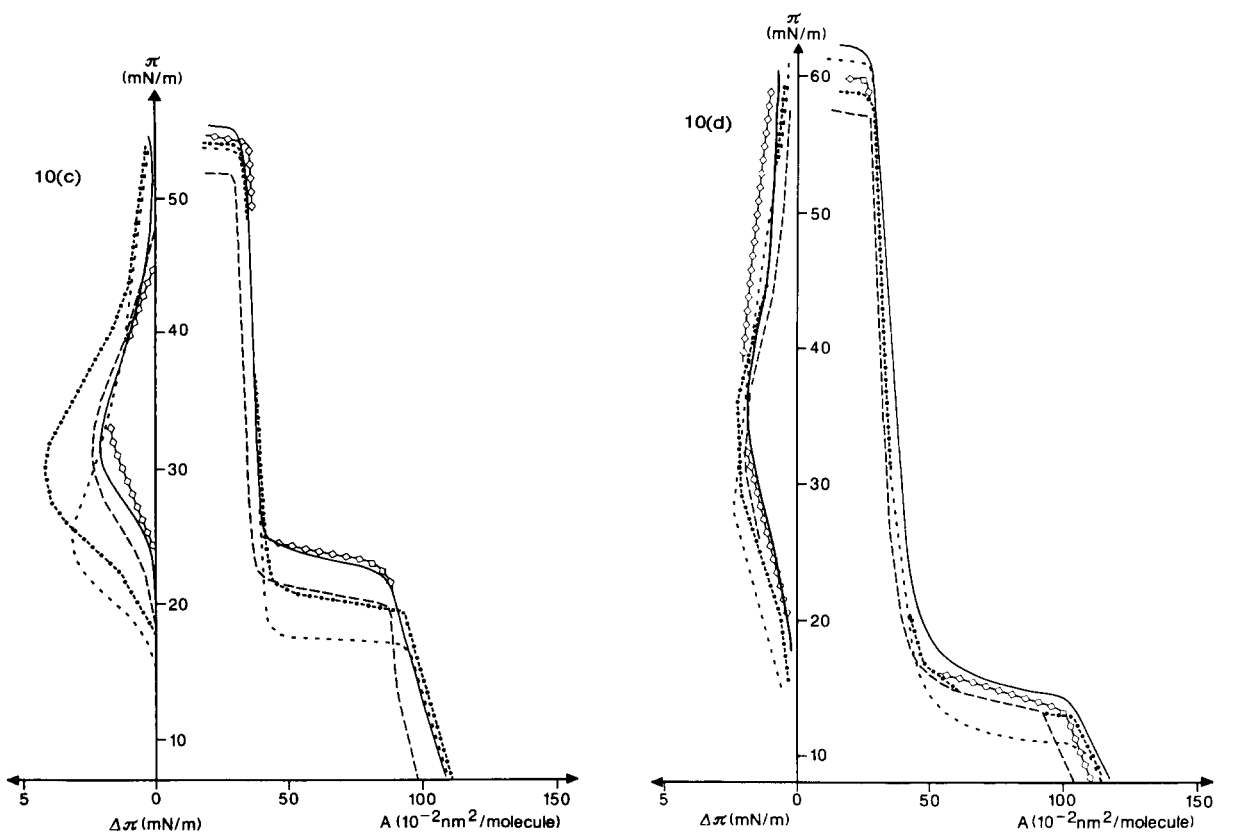



Fig. 10. Influence of cations in solution on the monolayer stability: 16:0 PEC (a), 16:0 Cap PEC (b), 18:0 PEC (c) and 22:0 PEC (d). ———, PBS alone; - - - - - -, 0.1 mM $Ca^{2+}$; ▬▬▬, 1 mM $Ca^{2+}$; – – –, 0.1 mM $Sr^{2+}$; –·–·–, 0.1 mM $Ba^{2+}$; ◇◇◇◇, 0.1 mM $Al^{3+}$. The right part of each diagram represents the pressure-area curve, the left one shows the stability of the monolayer under different conditions.

Cap PEC, 18:0 PEC, 22:0 PEC), all the curves have the same appearance; the plateau becomes less and less visible when the dilution increases (Fig. 11). The short chain derivatives (16:0 PEC, 16:0 Cap PEC) when diluted with 16:0 PC are clearly more stable than when mixed with 16:0 PE. This stability is still enhanced by the addition of calcium (unpublished results).

When an unsaturation is introduced in the carbon chain, the mixtures have a behaviour similar to that of pure PEC or Cap PEC (Fig. 12) and the monolayer stability is high, comparable to that of saturated compounds in their fluid phase (where they are the most stable).

In all cases, in a 1:1 mixture, the specific area (calculated per lipid bearing a ligand) is inferior by about 10 to 15% to the one we can estimate by the simple addition of the respective areas. This difference becomes less and less important when the dilution increases, suggesting the presence of a compacting effect probably due to the decrease in repulsive interactions between lipid derivatives bearing the ligand.

Mixtures realized with fatty acids were also analysed. The recorded stabilities in our experimental conditions (pH 7.3) were extremely poor, even with behenic acid, and these mixtures were not studied further.

## Conclusion

Our present study accounts for the quite different behaviour of the monolayers obtained with saturated and unsaturated lipids of similar chain lengths.

In the case of saturated lipid chains, we observe

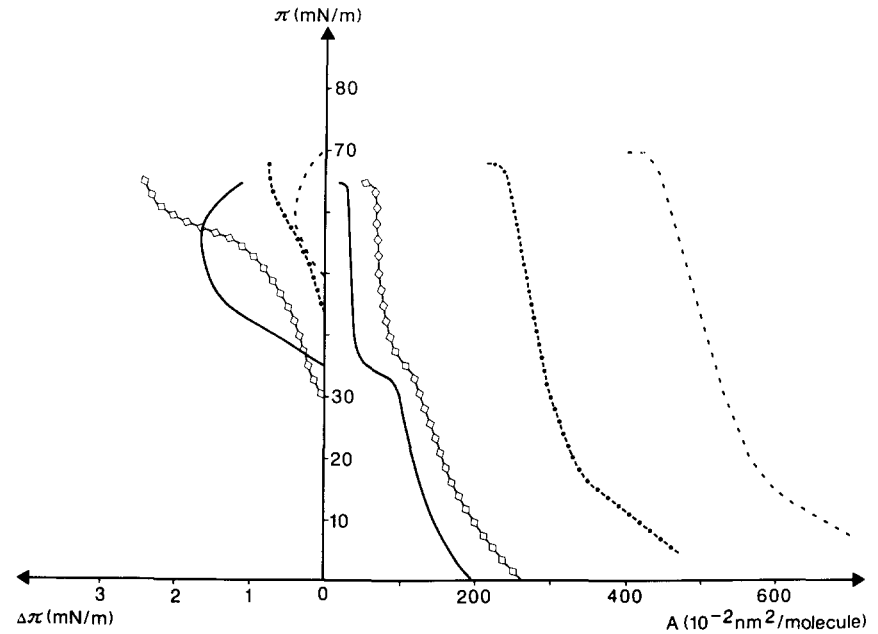



Fig. 11. Influence of the dilution of 16:0 PEC with 16:0 PC in various ratios. ———, 1:0; ◇◇◇◇, 1:1; –·–·–, 1:5; ------, 1:10 (PBS, $Ca^{2+}$ 0.1 mM, pH 7.3, $T$ 16 °C).

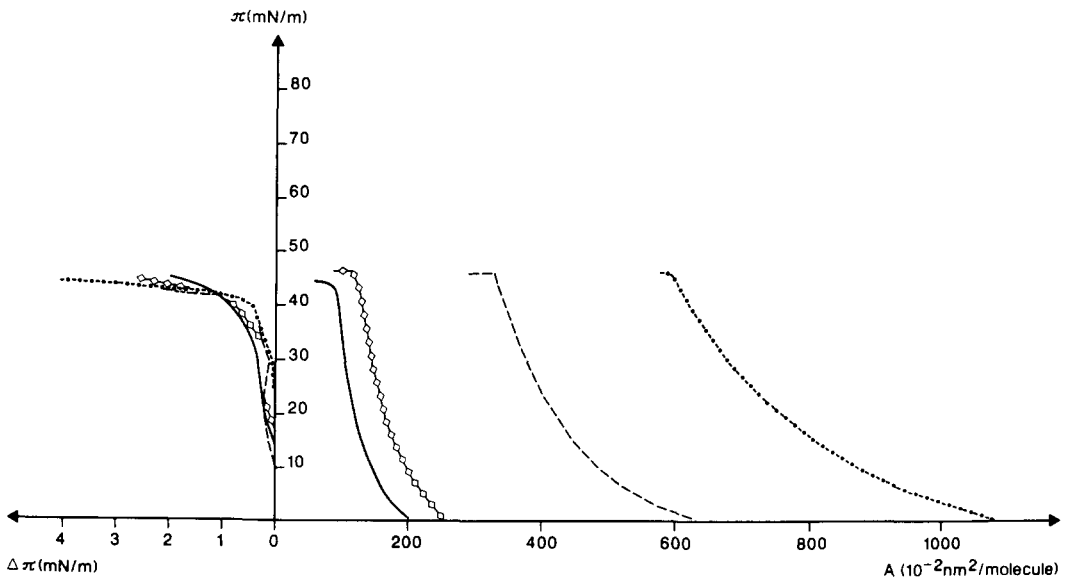



Fig. 12. Influence of the dilution of 18:1 Cap PEC with 18:1 PE in various ratios (standard conditions). ———, 1:0; ◇◇◇◇, 1:0.85; – – –, 1:4.25; –·–·–, 1:8.5.

phase transitions. The pressures at which the phase transitions occur, as well as the stability of the crystalline phase, are quite dependant on the chain length, on the temperature and on the presence of a spacer between the lipid and the steroid moieties. Low concentrations of di- and trivalent cations, in the same range as those necessary for the biological activities, affect both the phase transi-

tion and the stability of the monolayer. Calcium ions, in our experimental conditions, seem to have a particularly important effect, as yet unexplained, when compared to the other ions studied.

The monolayers constituted of phospholipids with unsaturated chains present a pressure-area curve profile quite different and do not show any phase transition. Irrespective of the pressure imposed to the monolayer, it ever stays in a fluid phase and is largely insensitive to the parameters affecting the saturated derivatives in their crystalline phase.

The presence of cortisol on a saturated lipid does not modify the specific area of the molecule (or rather weakly), in contrast to the unsaturated derivatives where one can observe a 40% increase of the minimum surface occupied per phospholipid.

With all derivatives linked to cortisol, we obtained a specific binding of anticortisol antibodies to the surface of the monolayers, even with unsaturated phospholipids, with which the cortisol is not completely exposed in the aqueous phase.

The successful bidimensional crystallizations of proteins reported in the literature [4–6] were obtained in conditions corresponding most probably to a multilayer configuration when compared to the results of our analysis. The systematic characterization of the lipid monolayer during the bidimensional crystallization of proteins will allow us to obtain a rational approach for the determination of optimal crystallization conditions. We are presently appliing the results obtained here to the crystallization of different biological systems.

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## References

1 Gorter, E. and Grendel, F. (1925) J. Exp. Med. 41, 439.
2 Danielli, J.F. and Davson, H. (1935) J. Cell Comp. Physiol. 5, 495.
3 Fromhertz, P. (1971) Nature 231, 267–268.
4 Uzgiris, E.E. and Kornberg, R.D. (1983) Nature 301, 125–129.
5 Ludwig, D.S. Ribi, H.O. Schoolnik, G.K. and Kornberg, R.D. (1986) Proc. Natl. Acad. Sci. USA 83, 8585–8588.
6 Uzgiris, E.E. (1986) Biochem. Biophys. Res. Commun. 134, 819–826.
7 Lebeau, L., Mioskowski, C. and Oudet, P. (1988) Chem. Phys. Lipids 46, 57–62.
8 Albrecht, O., Gruler, H. and Sackmann, E. (1978) J. Phys. (Paris) 39, 301–313.
9 Keough, K.M.W. and Taesch, H.W., Jr. (1986) J. Colloid Interface Sci. 109, 364–375.
10 Dörfler, H.D. and Rettig, W. (1980) Colloid Polymer Sci. 258, 839–848.
11 Abrahamsson, S., Dahlen, B., Löfgren, H. and Pascher, I. (1978) Prog. Chem. Fats other Lipids 16, 125–143.
12 De Gier, J., Mandersloot, J.G. and Van Deenen, J.L.M. (1968) Biophys. Biochim. Acta 150, 666–675.
13 Van Zoelen, E.J.J., De Jesus, C.H., De Jonge, E., Mulder, M., Blok, M.C. and De Gier, J. (1978) Biophys. Biochim. Acta 511, 335–347.
14 Lustenberger, P., Formstecher, P. and Dautrevaux, M. (1981) J. Steroid Biochem. 14, 697–703.
15 Phillips, M.C. and Hauser, H. (1974) J. Colloid Interface Sci. 49, 31–39.