Studies on Surface Films of Soybean Lecithin III: The Effect of Lysolecithin on Lecithin Monolayers at the Air-Water Interface

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Abstract □ The influence of lysolecithin on lecithin monolayers has been studied. Evidence that lysolecithin, when in the subsolution, appears to expand lecithin films to a greater extent than expected is presented. It has been postulated that the increased expansion over the amount expected is due to the enhancement of migration of the lysolecithin to the air-water interface in the presence of the lecithin film. Compression of such films results in what appears to be the loss of material from the lecithin-lysolecithin film. Studies in which the lysolecithin concentration in the subsolution was varied while the lecithin concentration layered at the surface was held constant indicate that an optimum lysolecithin concentration exists at which the initial film pressure generated reaches a maximum. Time dependent behavior of compressed films at lysolecithin subsolution concentrations exceeding the critical concentration indicate that one, both, or possibly some combination of the two components may be leaving the interface.

Keyphrases □ Lecithin monolayers, air-water interface—lysolecithin effect □ Lysolecithin—lecithin film expansion □ Compression effect—lecithin-lysolecithin films □ Time effect—compressed lecithin film, lysolecithin subsolution

Most authors agree that at the present stage of knowledge, the cell wall is considered to be a lipid-protein-cholesterol complex. DeGier *et al.* (1) have found that of the total phospholipid content in erythrocytes lecithin comprised 36% and phosphatidyl ethanolamine 30%. The remaining phospholipids consisted of lysolecithin 2%, and a mixture containing 32% sphingomyelin and lysophosphatidyl ethanolamine. These figures indicate that the largest single component in the erythrocyte cell wall is lecithin; therefore with some justification, investigators have used lecithin monolayers in penetration studies involving various drugs (2–4) as a first approximation to what may be occurring at the cell wall with these same agents. However, since lysolecithin has been found to be a normal constituent of the erythrocyte cell wall, and because it is produced in separation and purification procedures while attempting to isolate lecithin from natural sources, it would seem pertinent to investigate the effect of lysolecithin on lecithin monolayers. It is the results of such an investigation that are reported here.

EXPERIMENTAL

Materials—The lecithin used in this study was prepared by the method of Saunders and Perrin (5, 6) from a soybean concentrate (Azolectin, Associated Concentrates, Inc., Woodside, N. Y.). Lysolecithin was prepared from the isolated lecithin after the method of Saunders (7) using lyophilized viper venom. The homogeneity of these materials was determined by TLC after the method of Skipski *et al.* (8). All phospholipid materials were found to be homogeneous.

Apparatus and Experimental Procedure—The film balance employed in these studies has been described by Poulsen and Lemberger (9).

The compression of lecithin films on subsolutions with varying concentrations of lysolecithin was performed in the following manner. The lysolecithin was dissolved in double distilled water and the solution was placed in the trough. A solution of lecithin in benzene was then layered on the surface of the lysolecithin solution and compressed manually. Readings were made at 1-min. intervals.

The procedure varied slightly for the experiments in which the changes in film pressure with time were observed. Lecithin was layered from benzene solutions onto subsolutions with various concentrations of lysolecithin and the film was compressed to a predetermined constant trough area. The change in the film pressure was then followed for 1 hr.

Temperature was held constant throughout a particular experiment and all runs were conducted at 25 ± 0.5°.

RESULTS AND DISCUSSION

Expansion of Lecithin Films by Lysolecithin—The expansion of lecithin films, apparently due to the presence of lysolecithin from the subsolution in the film, is shown in Fig. 1. A lecithin film layered on a subsolution containing 1.7×10^{-7} g./ml. of lysolecithin is shown to expand to higher areas. A further increase in lysolecithin concentration in the subsolution to 3.3×10^{-7} g./ml., causes an even greater expansion of the lecithin film. In fact, the lower portion of the isotherm at this lysolecithin concentration is unobtainable since at these concentrations the available area is such as to cause the first film pressure reading to be in the vicinity of 16.3 dynes/cm. It can further be seen that compression of the film at this lysolecithin subsolution concentration failed to yield the usual compression isotherm. Failure to obtain a stable lecithin film on more highly concentrated lysolecithin subsolutions is further shown by the experiment in which the subsolution contained 3.3×10^{-6} g./ml. of lysolecithin.

Change of Film Pressure with Time—A series of experiments was conducted in which a constant amount of lecithin was layered on subsolutions with various concentrations of lysolecithin. After the lecithin was layered on the subsolution, the trough area was reduced to a predetermined value and the film pressure followed at this constant area for 1 hr. Figure 2 shows the change in film pressure with time for lecithin films layered on subsolutions containing different concentrations of lysolecithin up to 3.3×10^{-7} g./ml. and compressed to a fixed, constant trough area. Comparison of the several plots shows the increase in lecithin film pressure obtained at constant trough area as the lysolecithin concentration was increased over this range. Figure 3 shows that with further

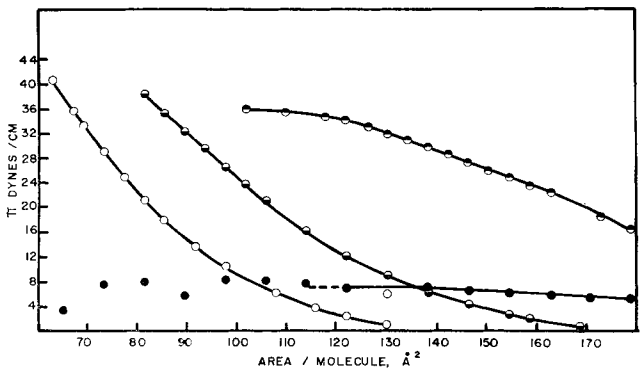

Figure 1—*Compression isotherms for lecithin films layered on subsolutions containing various concentrations of lysolecithin. The area/molecule axis is based on the total number of lecithin molecules. Key: Lecithin on:* ○, *distilled water;* ⊖, *1.7×10^{-7} g./ml. lysolecithin;* ◓, *3.3×10^{-7} g./ml. lysolecithin;* ●, *3.3×10^{-6} g./ml. lysolecithin*

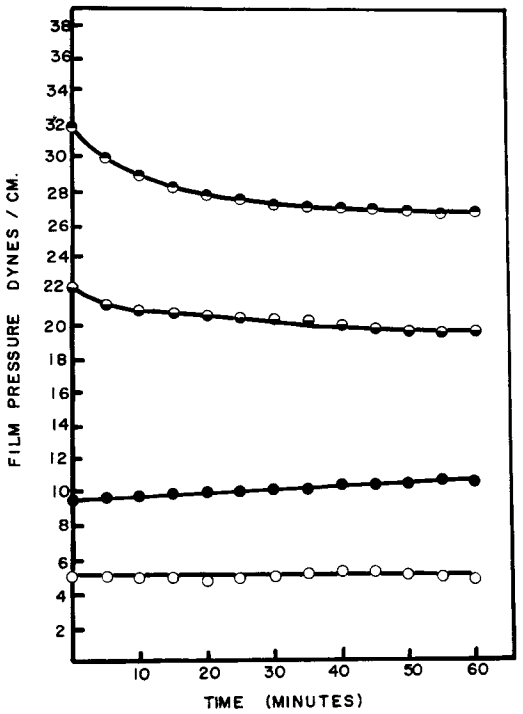

Figure 2—*The change with time of lecithin film pressure at constant trough area for films spread on subsolutions containing lysolecithin. Points represent the mean for two or more determinations. Key: Lecithin on:* ○, *distilled water;* ●, *6.6 × 10⁻⁸ g./ml. lysolecithin;* ◒, *1.7 × 10⁻⁷ g./ml. lysolecithin,* ◓, *3.3 × 10⁻⁷ g./ml. lysolecithin.*

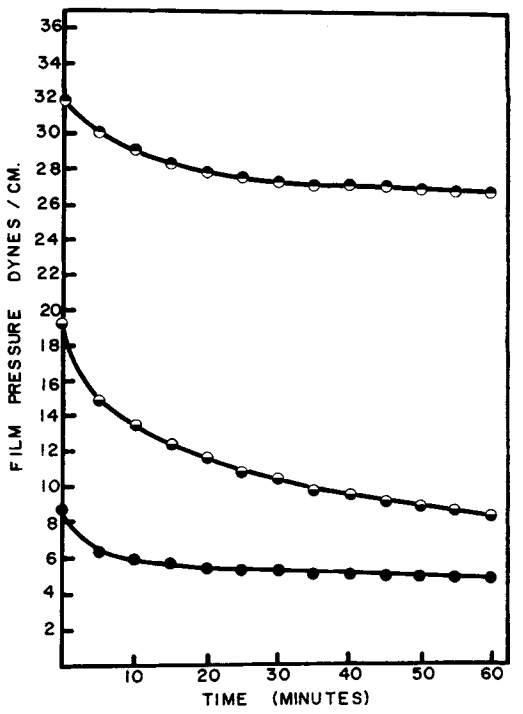

Figure 3—*The change with time of lecithin film pressure at constant trough area for films spread on subsolutions containing lysolecithin. Points represent the mean for two or more determinations. Key: lecithin on:* ◓, *3.3 × 10⁻⁷ g./ml. lysolecithin;* ◒, *6.6 × 10⁻⁷ g./ml. lysolecithin;* ●, *3.3 × 10⁻⁶ g./ml. lysolecithin.*

increases in lysolecithin concentration in the subsolution, initial film pressures at constant trough area are reduced. Table I summarizes the film pressures obtained with various lysolecithin concentrations. It can be seen that a concentration of 3.3×10^{-7} g./ml. produces an initial film pressure of 32 dynes/cm., while at double this concentration the initial film pressure is only 19.9 dynes/cm., indicating that a maximum initial film pressure is generated at an optimum lecithin-lysolecithin ratio. At a lysolecithin concentration of 3.3×10^{-6} g./ml., the initial film pressure falls to an even lower value.

DISCUSSION

The Gibbs equation for the surface excess concentration, $\Gamma^2 = -(c/RT)(d\gamma/dc)$, where Γ_2 = surface excess concentration, c = concentration, R = gas constant, T = absolute temperature, γ = surface tension, predicts that a proportional amount of a surface-active substance will migrate to the interface. Thus, the expansion of the lecithin films shown in Fig. 1 may be explained on the basis that there are more molecules at the interface than simply those of lecithin.

Robinson and Saunders (10) have reported on the surface activities of lysolecithins. If the assumption is made that the Gibbs equation holds, that lysolecithin equilibrates throughout the air/water interface regardless of the barrier position and that the areas occupied by lecithin and lysolecithin molecules in the film are additive, an approximate calculation for lysolecithin molecular areas at various surface pressures in our films is possible. Calculated lysolecithin molecular areas for subsolutions containing 1.7×10^{-7} and 3.3×10^{-7} g./ml. lysolecithin are given in Table II. The value obtained for lysolecithin alone from the data of Robinson and Saunders (10) is 90Å.²/molecule at zero surface pressure. This value and calculated values from this study at high film pressures are in good agreement offering some support to the tentative conclusion that at very low concentrations of lysolecithin, stable mixed films are produced which demonstrate an additive behavior.

Lysolecithin molecular areas calculated for the mixed film on the subsolution containing 3.3×10^{-7} g./ml. of lysolecithin are larger initially but approach the same limiting value near the collapse point. This would indicate that either the lecithin and lysolecithin are so oriented in this film that they occupy a greater area per molecule or that more lysolecithin than is predicted by the Gibbs equation enters the mixed film from subsolutions of higher concentration. This implies that lecithin may be enhancing the adsorption of lysolecithin molecules at the interface possibly through some interaction between lecithin and lysolecithin. This migration of lysolecithin to the interface of a lecithin film can be seen to occur at low pressure values to a slight extent in Fig. 2 at a lysolecithin

Table I—Film Pressures of Lecithin Films on Subsolutions Containing Lysolecithin[a]

Lysolecithin, g./ml.	Film Pressure Initially,[b] dynes/cm.	Film Pressure after 1 hr. dynes/cm.
6.6×10^{-8}	9.64	10.7
1.7×10^{-7}	22.4	19.7
3.3×10^{-7}	31.9	26.8
6.6×10^{-7}	19.3	8.4
3.3×10^{-6}	8.7	4.9

[a] Trough area and lecithin content constant. [b] Averages of 2 or more values.

Table II—Calculated Molecular Areas of Lysolecithin in Mixed Lecithin-Lysolecithin Film

Film Pressure (dyne cm.⁻¹)	Molecular Areas, (Å.²)		
		Lysolecithin	
	Lecithin[a]	$c = 1.7 \times 10^{-7}$ g./ml.	$c = 3.3 \times 10^{-7}$ g./ml.
	115	103	—
8	103	103	—
16	88	104	188
24	78	103	182
32	70	97	155
36	67	91	95

[a] Obtained from Fig. 1.

concentration of 6.6×10^{-8} g./ml. The increase of the film pressure with time may be the result of migration of lysolecithin into the lecithin monolayer.

At higher lysolecithin concentrations, stable mixed films are formed but the initial film pressure developed in compressing the film to the predetermined trough area falls off to some lower value after a time. This characteristic behavior was observed to a minor degree in the systems containing lysolecithin at 1.7×10^{-7} g./ml. and to a much greater extent in the system containing 3.3×10^{-7} g./ml. This behavior is thought to be due to the loss of one or more of the components from the interface under the influence of the higher film pressure which is developed in these systems. The inability of lecithin films to develop an initial film pressure over 20 dynes/cm. at a lysolecithin concentration of 6.6×10^{-7} g./ml. and the marked decrease in film pressure with time is shown in Fig. 3. At lysolecithin concentrations of 3.3×10^{-6} g./ml., the initial film pressure is seen to be even lower than that produced with the previous lysolecithin concentration and after 1 hr. decreases to the pressure level of lecithin itself. This is consistent with the observation of Fig. 1 showing a failure to form a stable film.

SUMMARY AND CONCLUSIONS

It is clear from these studies that small concentrations of lysolecithin as an impurity in lecithin may result in the distribution of lysolecithin between the bulk solution and the air-water interface. Small amounts of lysolecithin have been shown to cause the expansion of lecithin films. At higher concentrations of lysolecithin, the initial film pressures generated decrease with time, indicating that the molecules of lysolecithin, lecithin, and/or combinations of them leave the air-water interface for the bulk solution.

These observations imply that small amounts of lysolecithin may lead to erroneous results concerning the determination of molecular cross-sectional areas for lecithin. More importantly, a change in the character of the isotherm occurs, indicating that the nature of the film has been altered and studies of the behavior of other compounds at this artificial lipid membrane may be misleading if traces of lysolecithin are present.

Biological implications may also be drawn. According to deGier *et al.* (1), lysolecithin is normally present at low concentrations in erythrocytes. The authors' observations are consistent with the concept that membrane stability is maintained in the presence of low concentrations of lysolecithin, and they would also support the concept that at higher lysolecithin concentrations interfacial interactions between lecithin, lysolecithin, and perhaps other materials may occur in cell membranes altering their strength and/or permeability.

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