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TIME-DEPENDENT SURFACE BEHAVIOR OF DIPALMITOYLLECITHIN AND LUNG ALVEOLAR SURFACTANT MONOLAYERS

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SUMMARY

The time-dependent behavior of dipalmitoyllecithin and lung alveolar surfactant monolayers have been examined with an automated, recording film balance of conventional design. Surface pressure relaxation and recycling effects were observed. The results are interpreted in terms of molecular expulsion and solution processes peculiar to each of the films examined. These observations and explanations provide a clearer understanding of the phenomenon of surface hysteresis in monolayers. The surface films of lung alveolar surfactant consistently demonstrate a greater stability than those of dipalmitoyllecithin, even though the latter is a major component of lung alveolar surfactant. The surface phenomena reported support the proposal that lung alveolar surfactant exerts a stabilizing effect in the alveoli.

INTRODUCTION

The isolation of a highly surface active material, referred to as lung alveolar surfactant, from lung washings had led to investigations concerning the role which surface forces play in normal lung mechanics[1,2]. Current theory regarding the function of the lung surfactant is based largely on its surface active behavior when rapidly compressed and expanded as a spread film on a aqueous substrate. Since most of this work to date has employed specifically modified film balances involving various film restraining devices, it was felt desirable to characterize the dynamic behavior of lung alveolar surfactant and its major lipid component, dipalmitoyllecithin by means of conventional monolayer techniques and apparatus in order that such behavior might be explained in terms of the larger body of monolayer data obtained using conventional approaches.

As a result of these studies with both lung alveolar surfactant and dipalmitoyllecithin monolayers, the authors wish to present their observations on surface pressure relaxation following compression to preselected film pressures, and surfaces hysteresis as affected by repeated cycling and minimum area of compression.

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EXPERIMENTAL

Materials

L-α-Lecithin dipalmitoyl (synthetic), reportedly homogeneous as shown by paper chromatography, was used as received from the supplier (Mann Research Labs, New York, N.Y.). The dipalmitoyllecithin was dissolved in absolute alcohol–*n*-hexane (1:9, v/v) for spreading. A suspension of lung alveolar surfactant, kindly supplied by Dr R. M. Mendenhall, Public Health Service, Division of Occupational Health, Cincinnati, Ohio, and which represented the combined, centrifuged, foam washings of three hog lungs, was prepared in 0.9% NaCl solution and the volume adjusted to 100 ml. From this concentrated suspension, 1:10 (by vol.) dilutions in normal saline were prepared for direct application to the subphase. When not in use the concentrated suspension was stored frozen under nitrogen. Subphases of doubly distilled water and 0.9% NaCl solution prepared from doubly distilled water were employed throughout the experiments.

Film balance

The automatic recording Wilhelmy-type film balance has been described previously[3]. All surface pressure–area data were obtained at 25 °C.

Procedure

Calibration of the balance, cleaning, and calculations of surface pressure and area were carried out as reported previously[3]. Following the spreading of dipalmitoyllecithin, compression was begun after a 10-min equilibration period. With lung alveolar surfactant a procedure similar to that of Galdston *et al.*[4] was used, approximately 6 ml of the 1:10 lung alveolar surfactant suspension being required to produce a 2–3 dynes/cm rise in surface pressure. 15 min were allowed to elapse after spreading before compression was initiated.

Surface pressure–area isotherms were obtained with the movable barrier operated at 2.54 cm/min, corresponding to a compression rate of 24.2 Å^2/molecule per min (dipalmitoyllecithin) and 16.9% trough area/min (lung alveolar surfactant). In all studies, at least three monolayers were studied under each set of experimental conditions.

Surface pressure relaxation studies were conducted as described previously[3]. The equilibrium surface pressure, π_{eq} was assumed to have been reached when the rate of decrease in surface pressure was less than 0.1 dyne/cm per min, indicative of a stable state[5]. This normally occurred within 10 min.

Five-cycle compression–expansion isotherms were obtained for both dipalmitoyllecithin and lung alveolar surfactant. The dipalmitoyllecithin monolayers were compressed and expanded between 152.7 and 28.6 Å^2/molecule, while lung alveolar surfactant films were cycled between 100 and 23.3% trough area. No film collapse at the minimum areas, as evidenced by spillage of material over the edges of the trough was observed. One-cycle compression–expansion studies were also conducted to successively smaller areas.

The absence of any film leakage past the barriers was checked in all studies undertaken in a manner described previously[3].

RESULTS AND DISCUSSION

The surface pressure isotherm for dipalmitoyllecithin monolayers (Fig. 1) is typical of that found in the literature. Extrapolation to zero film pressure gave areas lying within the range of 50–65 Å²/molecule reported by others[6–11].

Films of lung alveolar surfactant are considered "normal" if they show (i) surface pressures of more than 52 dynes/cm, and (ii) marked hysteresis upon repeated compression and expansion. As seen from Fig. 2, maximum surface pressures in the "normal" range were obtained. Hysteresis of a marked nature was observed, as discussed later. The plateau observed at approximately 41 dynes/cm in the present study is felt to be a notable feature also of the lung alveolar surfactant isotherm. Watkins[9] has concluded that the plateau was due to the rapid squeeze out of material, perhaps protein, and its solution in the aqueous subphase. The fact that higher surface pressures are obtainable with lung alveolar surfactant than with pure dipalmitoyllecithin suggests that the other constituents in lung alveolar surfactant are essential if lung alveolar surfactant is to exhibit its characteristic behaviour.

Surface pressure relaxation

The decreases from preselected initial surface pressures as a function of time are shown in Figs 3 and 4 for dipalmitoyllecithin and lung alveolar surfactant, respectively. The equilibrium π values are also included in Figs 1 and 2.

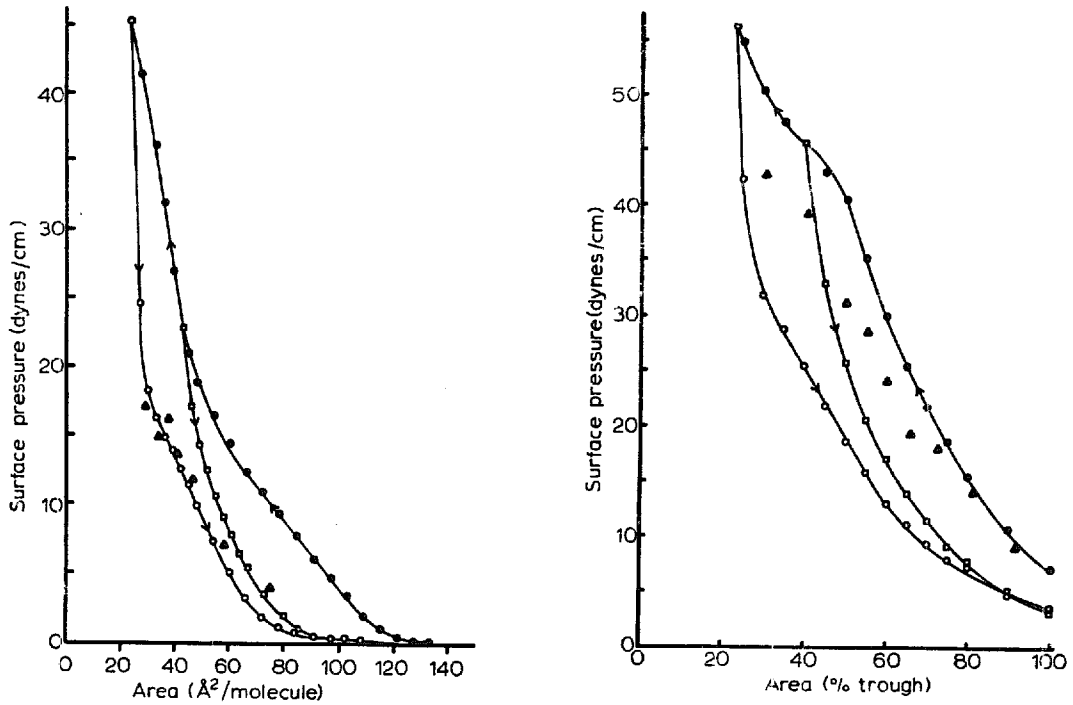

Fig. 1. Comparison of equilibrium surface pressures following relaxation with continuous compression of dipalmitoyllecithin and first-cycle expansion isotherms. ▲, equilibrium surface pressure; ●, compression isotherm obtained at 24.2 Å²/molecule per min: ○, expansion isotherm from 23.8 Å²/molecule; □, isotherm from 42.9 Å²/molecule.

Fig. 2. Comparison of equilibrium surface pressures following relaxation with continuous compression of lung alveolor surfactant and first-cycle expansion isotherms. ▲, equilibrium surface pressure; ●, compression isotherm obtained at 16.9% trough area/min; ○, expansion isotherm from 23.3% trough area; □, expansion isotherm from 40% trough area.

Compared to stearic acid[3], the relaxations shown for dipalmitoyllecithin are generally slower throughout the range of initial surface pressures studied. Even so, it is felt that the mechanism of relaxation is similar to that for stearic acid. Thus, at larger areas per molecule relaxation arises in part due to the reorientation and redistribution of unequally distributed molecules, a state generated by the disturbing effects of compression[3]. Unequal distribution of molecules in spread films has been demonstrated by Blank and Lee[12].

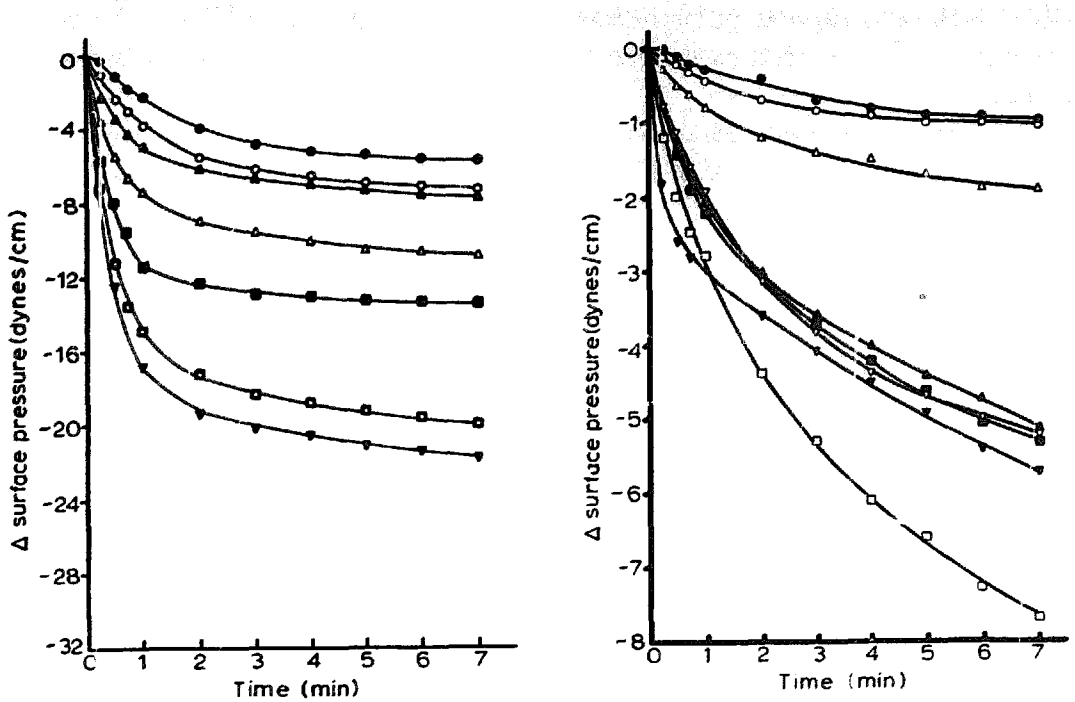

Fig. 3. Surface pressure relaxation of dipalmitoyllecithin films from various surface pressures. Relaxation from: ●, 10 dynes/cm; ○, 15 dynes/cm; ▲, 20 dynes/cm; △, 25 dynes/cm; ■, 30 dynes/cm; □, 35 dynes/cm; ▼, 40 dynes/cm.

Fig. 4. Surface pressure relaxation of lung alveolor surfactant films from various surface pressures. Relaxation from: ●, 10 dynes/cm; ○, 15 dynes/cm; △, 20 dynes/cm; ▲, 25 dynes/cm; ■, 35 dynes/cm; □, 40 dynes/cm; ▽, 45 dynes/cm; ▼, 50 dynes/cm.

At low areas per molecule, dipalmitoyllecithin molecules are sufficiently packed such that only a small reduction in area is possible before molecules begin to be expelled from the monolayer. Except at very low rates of compression, it can reasonably be expected that this expulsion process lags behind the decrease in area. Once compression is stopped at the lower areas per molecule, rapid initial decreases in surface pressure appear, due to expulsion of residual excess molecules. This process, when complete, is followed by the slower relaxation observed at larger areas, which is governed by reorientation and redistribution. The magnitude of the initial decrease in surface pressure increases with increasing initial surface pressure since there are more molecules in excess at the lower areas required to attain the high pressures.

While a similar mechanism for relaxation of dipalmitoyllecithin and stearic acid is proposed, difference do exist because of the molecular characteristics of these two species. Thus, Phillips and Chapman[13] have shown from entropy calculations that dipalmitoyllecithin molecules possess less configurational freedom than straight chain fatty acid molecules. The dipalmitoyllecithin molecules might therefore be expected

to reach stable molecular arrangements quicker than stearic acid. The disruptive effects of compression would be lessened and less relaxation would occur, since less reorientation would be required.

The greater stability of dipalmitoyllecithin monolayers at high surface pressures, as evidenced by their slower relaxation rates compared to stearic acid, may also be due to increased van der Waals forces. As a result, the energy required to expel a dipalmitoyllecithin molecule would be greater than that to expel a single fatty acid chain and the process of expulsion should occur less readily. In addition, the strong water–choline interaction that exists[13] might also be expected to offer some resistance to expulsion.

The relatively limited relaxation exhibited by lung alveolar surfactant films indicates that these are more stable than dipalmitoyllecithin films. Recycling results support this contention (see later). In addition to the forces contributed by the dipalmitoyllecithin content, lung alveolar surfactant film must also receive a contribution from its other components, notably protein. These other materials appear to hold the lung alveolar surfactant film together, limiting both expulsion and reorientation. As a result, higher surface pressures are attainable and less relaxation occurs in comparison to the single component dipalmitoyllecithin and stearic acid films.

The relaxation curves for lung alveolar surfactant show two groupings based upon the total change in surface pressure over the observed time, a break occurring between 20 and 25 dynes/cm initial pressure. It appears that when a lung alveolar surfactant film is held at a constant area that would initially generate a surface pressure equal to or greater than 25 dynes/cm, certain soluble components leave the interface; at areas greater than those generating 25 dynes/cm, these materials are not strained sufficiently to leave. Continuous compression evidently obscures this point, since molecules are not leaving rapidly enough to register this squeeze-out.

Compression–expansion studies

The effect of repeated cycling is shown in Table I, which contains data for the

TABLE I

EFFECT OF RECYCLING ON DIPALMITOYLLECITHIN AND LUNG ALVEOLAR SURFACTANT FILMS

Data were obtained at a compression rate of 24.2 Å²/ molecule per min for dipalmitoyllecithin and 16.9% trough area/min for lung alveolar surfactant.

Parameter	*System*	*Cycle No.*				
		1	*2*	*3*	*4*	*5*
Extrapolated area (Å²molecule)	Dipalmitoyllecithin	62	53	50.5	50.5	50.0
Area of hysteresis (% of first cycle)	Dipalmitoyllecithin	100	52	43	40	35
	Lung alveolar surfactant	100	65	57	52	46
Maximum surface pressure (dynes/cm)	Dipalmitoyllecithin	40.8	35.3	32.3	29.1	27.3
	Lung alveolar surfactant	55.6	53.8	53.4	52.4	51.6

first five continuous compression–expansien cycles of dipalmitoyllecithin and lung alveolar surfactant monolayers. The effect of the minimum area of compression with one-cycle studies on the areas of hysteresis of dipalmitoyllecithin and lung alveolar surfactant isotherms is shown in Fig. 5. Qualitatively, these were similar to those for stearic acid[3].

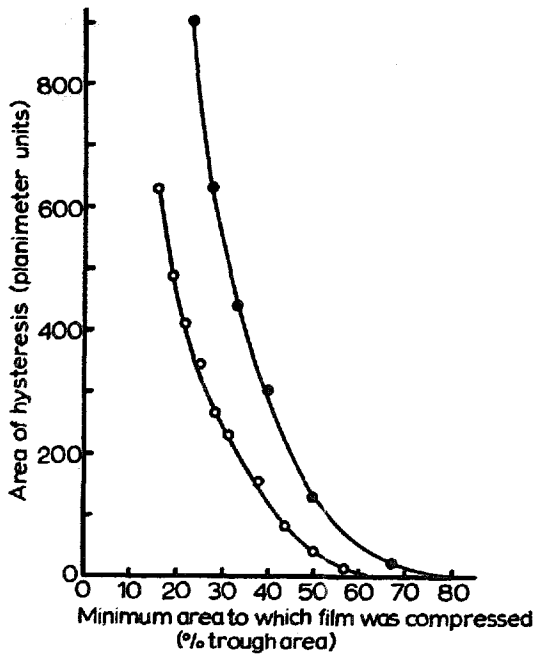

Fig. 5. Effect of minimum area of compression on area of hysteresis for dipalmitoyllecithin monolayers and lung alveolar surfactant films on 0.9% NaCl. ●, lung alveolar surfactant; ○, dipalmitoyllecithin.

As shown in Table I, the extrapolated area per molecule of dipalmitoyllecithin is reduced with successive compression. Since no leakage past the barriers was detected, it would appear that, upon repeated compression, the monolayer becomes, in part at least, a multilayer due to the expulsion and overlap of dipalmitoyllecithin molecules. The reductions in the film parameters studied were smaller than those for stearic acid[3], again reflecting the greater resistance to expulsion of dipalmitoyllecithin monolayers.

An examination of the dipalmitoyllecithin expansion isotherms and the equilibrium surface pressures following relaxation (Fig. 1) aids in understanding the hysteresis phenomenon. It can be seen that the expansion isotherms almost coincide with the equilibrium values. Apparently, on expansion, dipalmitoyllecithin rapidly readjusts to its equilibrium configuration. Even when compressed to and expanded from areas per molecule which are greater than those which initiate expulsion dipalmitoyllecithin adjusts rapidly upon expansion giving surface pressure values that are near to equilibirum. This results presumably from the lack of configurational freedom mentioned earlier.

Repeated cycling of lung alveolar surfactant films shows decreases in measured film parameters which are less than with dipalmitoyllecithin, indicating that material which leaves the film during compression is better able to re-enter the film on expansion. The surface hysteresis seen with lung alveolar surfactant films has been attri-

buted to expulsion and subsequent re-entry[4,9] or the formation of a surface network[14]. The present data would support the hypothesis that both types of mechanism may be operative. A comparison of the expansion isotherms and the equilibrium surface pressures following relaxation (Fig. 2) reveals that the latter fall consistently above the expansion curve. This behaviour is quite different from that observed with dipalmitoyllecithin. Thus, it would appear that soluble components of lung alveolar surfactant are squeezed out into the subphase during compression; however, on expansion of the film, this material does not re-enter as rapidly as it left. The rate of expansion adds further to the observed hysteresis by negating any small increases in surface pressure that might occur due to slow re-adsorption of the expelled soluble material. This effect has been demonstrated by stopping expansion at mid-area[15], whereupon the surface pressure of lung alveolar increases. Network formation resulting in high surface viscosity probably contributes to the observed hysteresis indirectly by limiting the ease (and hence the rate) with which squeezed-out material can re-enter the film.

The observed stability of lung alveolar surfactant monolayers in the present study appears significant in explaining normal lung mechanics. Due to the very stable nature of lung alveolar surfactant, the alveoli are ultimately stabilized. This would be particularly important at the minimum alveolar size where surface pressure should be highest. Since there is normally a lag time between exhalation and inhalation the fact that lung alveolar surfactant shows limited relaxation is highly important in maintaining alveolar stability through the maintenance of a relatively high surface pressure.

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