Surface properties of binary mixtures of some pulmonary surfactant components

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Abstract The dynamic surface pressure-area properties of pure and binary mixed films of rac-1,2-dipalmitoyl-glycero-3-phosphocholine, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine and cholesterol were investigated at 23°C and 37°C. The pure films and binary mixtures of rac-1,2-dipalmitoyl-glycero-3-phosphocholine:1,2-dioleoyl-*sn*-glycero-3-phosphocholine and rac-1,2-dipalmitoyl-glycero-3-phosphocholine:cholesterol were characterized both for dilute surface initial concentrations of 150 Å²/molecule and for surface excess initial values of 15–50 Å²/molecule. The results show that the addition of1,2-dioleoyl-*sn*-glycero-3-phosphocholine or cholesterol in a binary film with rac-1,2 dipalmitoyl-glycero-3-phosphocholine acts to improve the surface re-entry and respreading properties over those of pure rac-1,2-dipalmitoyl-glycero-3-phosphocholine films upon dynamic compression past collapse. This effect is even more pronounced at 37°C than at 23°C, as demonstrated by the application of a collapse plateau ratio criterion. The enhanced dynamic respreading correlates with the effect of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine and cholesterol in decreasing the gel to liquid crystal transition temperature of rac-1,2-dipalmitoyl-glycero-3-phosphocholine water dispersions, as well as with their facilitation of post-collapse dynamic surface pressure relaxation in binary films with rac-1,2-dipalmitoyl-glycero-3-phosphocholine.—**Notter, R. H., S. A. Tabak, and R. D. Mavis.** Surface properties of binary mixtures of some pulmonary surfactant components. *J. Lipid Res.* 1980. **21:** 10–22.

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Because of the physiologic importance of the complex, multicomponent pulmonary surfactant system (e.g., 1), a good deal of research effort has been directed toward an elucidation of the surface properties of films of its major phospholipid component DPL. It is now well known that pure DPL films are able to achieve extremely high dynamic surface pressure (π)[2] of the order of 70 dynes/cm when compressed to monolayer collapse on a water or saline subphase in a surface balance (2–8). Consequently, it is widely accepted that saturated phospholipids (primarily DPL) are responsible for the high degree of surface tension lowering achieved by lung extract or lung alveolar wash films during similar in vitro dynamic cycling experiments (e.g., 9, 10).

In addition to high dynamic π values, another consistent characteristic of the surface pressure-area (π-A) curves of lung extracts or washings is the large reproducible hysteresis observed in cycling experiments. The fact that this hysteresis is relatively reproducible for lung extract films on successive cycles of compression and expansion of the surface appears to indicate that surfactant molecules which are expelled from the interface during compression are able to re-enter the surface film during the following expansion. This re-entry facility of pulmonary surfactant should have physiologic importance, because otherwise surfactant would be continuously depleted at the alveolar liquid–air interface as surfactant is compressed and expanded during breathing. The surface entry property is also relevant in terms of fresh surfactant being able to penetrate and enter the interface after being produced by alveolar cells, or to spread from an aerosol after alveolar deposition (7).

In contrast to the case of extracted natural lung surfactant, films of pure DPL do not exhibit reproducible hysteresis on dynamic cycling. While pure DPL films do exhibit an initially large π-A hysteresis, it decreases continuously with successive cycles of compression past film collapse followed by expansion. This is found to be true both at room temperature (2–6) and at body temperature (4–6) and indicates that molecules of DPL will apparently not easily respread once they are expelled from the air–water interface. Moreover, the poor spreading properties of pure

Abbreviations: DOL, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DPL, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (or its racemic mixture); DSC, differential scanning calorimetry; π, surface pressure; π-A, surface pressure–area; GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

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[2] Defined as the surface tension of the pure subphase minus the surface tension when the surfactant film is present.

DPL vis à vis lung surfactant are also pointed out by the fact that in order to obtain high surface pressures at an air–water interface, pure DPL must be spread initially with the aid of a spreading solvent (e.g., hexane–ethanol 9:1 (v/v)) while lung extract films adsorb spontaneously from an aqueous phase. Because of the relatively poor dynamic spreading and re-penetrating properties of DPL, it has been postulated that the role of some of the other components of pulmonary surfactant is to aid the DPL molecules in re-entry of the surface when they are expelled during film compression (5, 7). Identification of those components of pulmonary surfactant which are functional in enhancing the respreading of DPL is of obvious importance not only for characterizing the underlying mechanics of the system, but also in developing effective surfactant replacement therapy for neonatal respiratory distress syndrome (7, 11–15).

In the present work, we have studied the dynamic surface tension lowering and respreading properties of pure films of DPL, DOL and cholesterol, and of binary mixed films of DPL:DOL and DPL:cholesterol. It has been suggested that cholesterol or unsaturated phospholipids may be of functional import in pulmonary surfactant (7, 16), particularly because of their ability to lower the bulk phase gel to liquid crystal transition temperature (T_c) of DPL (17–21). Consequently, a primary aim of this study is to assess the effect of these components on dynamic surface properties when combined in mixed films with DPL.

MATERIALS AND METHODS

Materials

rac-1,2 Dipalmitoyl-glycero-3-phosphocholine (DPL) and cholesterol were obtained from the Sigma Chemical Company, St. Louis, MO. 1,2 Dioleoyl-*sn*-glycero-3-phosphocholine (DOL) was purchased from Applied Science Laboratories, State College, PA. The DPL and DOL were analyzed by thin-layer chromatography and by gas–liquid chromatography, and were found to give single spots on TLC with >99% fatty acid chain purity by GLC. The solvent system used for TLC was chloroform–methanol–water–saturated ammonium hydroxide 70:30:4:1 (by volume). Cholesterol was supplied with a purity analysis from Sigma which showed one major spot and one unidentified minor spot (0.1%) by TLC. All three compounds, DPL, DOL, and cholesterol, were used without further purification.

Other chemicals used in the experiments here were Nanograde hexane (Mallinckrodt, St. Louis, MO) and 200 proof ethyl alcohol (Publicker Industries, Linfield, PA) which were used to form 90:10 or 95:5 (v/v) hexane–ethanol spreading solvent mixtures. Triply distilled water from all-glass stills (3) was used to form all film subphases and in all surface balance cleaning procedures.

Surface pressure—area (π-A) measurements

All dynamic π-A measurements were made on a custom designed Wilhelmy surface balance described in detail previously (22). This surface balance permitted the use of a ribbon barrier, as well as a more conventional "dam" type barrier, in the dynamic cycling experiments. The use of the ribbon barrier allowed the minimization of film leakage effects that are particularly bothersome for in vitro studies of films of phospholipids such as DPL at or near body temperature (22). However, the dam type compression barrier was also used in some of the experiments reported here, particularly those at room temperature. All body temperature measurements are for relative humidities from 75 to 100%.

Differential scanning calorimetry measurements

Differential scanning calorimetry was performed with a Dupont 990 Thermal Analyzer. Lipid samples of 1–2 mg were deposited in the sample pans by evaporation of a chloroform solution under a stream of nitrogen gas. The dried film was redissolved in diethyl ether, and the ether was then evaporated under nitrogen. The sample was further dried under vacuum for 1 hour, and 10 μl of 40% ethylene glycol in water was added. The pan was then sealed and heated in boiling water to hydrate the lipids. Reference pans contained 10 μl of 40% ethylene glycol. Heating scans were performed at a rate of 5 degrees per minute.

RESULTS AND DISCUSSION

π-A Behavior of the pure component films

The dynamic surface pressure-area (π-A) behavior of pure films of DPL, DOL, and cholesterol was investigated at both room (23°C) and body (37°C) temperature. At each temperature, and for each pure component film, dynamic π-A data were measured as a function of two surface initial conditions: *1*) low initial surface concentration such that no effect on surface tension was apparent at maximum film area; *2*) high initial surface concentration where the amount of surfactant spread from hexane–ethanol solutions was greater than that required for monolayer coverage of the surface. The first of these surface initial conditions is termed the "lift-off" initial condition because

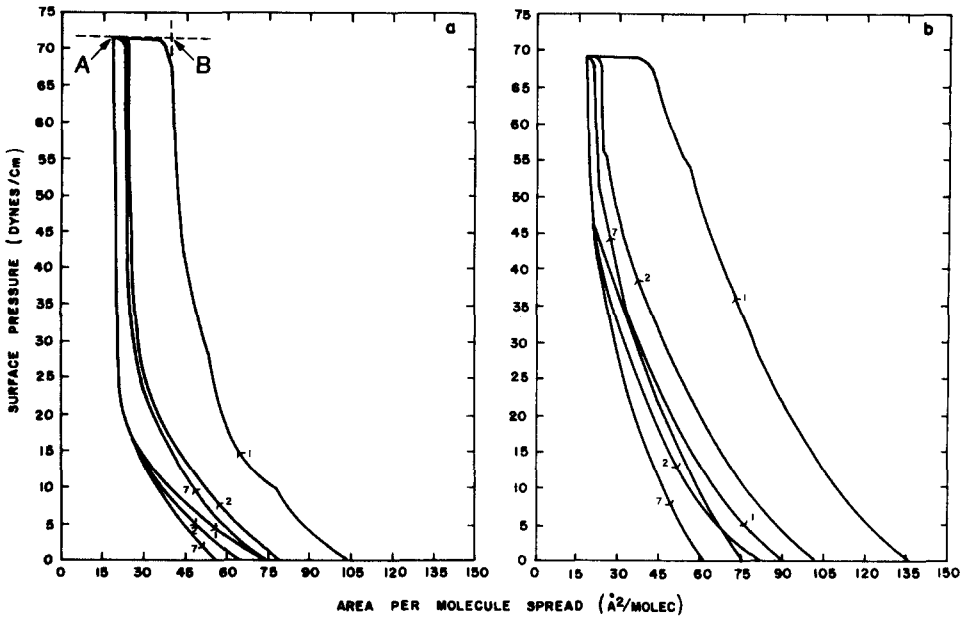

Fig. 1. Dynamic compression expansion behavior of pure DPL surface films under "lift-off" conditions. a, 23°C; b, 37°C. Cycles 1, 2 and 7 are shown as designated. Initial concentration was 150 Å²/molecule. Cycling rate was 53 Å²/molecule/minute. The dashed lines on Fig. 1a illustrate the analysis used to determine the collapse plateau length (line AB) of a given compression curve as described in text.

it permits the determination of the concentration (area/molecule) where the surfactant film first exerts a measurable surface tension lowering, i.e., a non-zero surface pressure (5). The second initial condition is called the "surface excess" initial condition, and its primary utility is that it permits compression–expansion experiments which display prominently the respreading behavior of a given surfactant film after dynamic compression past collapse.

The dynamic π-A results for pure component films of DPL, DOL, and cholesterol at 23°C and 37°C are shown in **Figs. 1–3** for the lift-off initial condition. In each case, DPL, DOL, or cholesterol was spread to an initial concentration of 150 Å²/molecule and compressed at a rate of 53 Å²/molecule/min with a compression ratio (maximum area to minimum area) of 8:1. For all experiments the given surfactant was spread from hexane–ethanol 9:1 or 95:5 (v/v) on a triply distilled water subphase at pH 6.5. The effect of subphase ions was not determined in either the pure or mixed film experiments, although there is evidence that such effects should be small.[3]

The first, second and seventh compression–expansion cycle π-A results for DPL at 23°C and 37°C on Fig. 1 are essentially identical to those found previously in our laboratory (3–5). As discussed elsewhere, these results and those of other workers (6, 8, 25) show that pure DPL films are able to generate maximum surface pressures corresponding to minimum surface tensions of <1 dyne/cm when compressed dynamically past monolayer collapse. This high maximum surface pressure is a dynamic compression effect for DPL films as shown by Tabak et al. (3) and by Notter et al. (21). By contrast, if DPL is spread statically from a spreading solvent at a given surface area with no dynamic compression, values of π do not exceed 50 dynes/cm even at surface concentrations greater than monolayer coverage (3, 21). Moreover, although DPL reaches high maximal surface pressures on dynamic compression past collapse, it is clear from Fig. 1a at 23°C and Fig. 1b at 37°C that at both temperatures the DPL molecules ejected from the surface during compression do not re-enter the surface very effectively upon expansion (2, 4–7, 25). Thus, the second and subsequent compression cycles for pure DPL are displaced to the left, showing an effective loss of molecules from the surface film on successive cycles past collapse. This point is made somewhat more

[3] For zwitterionic surfactants such as DPL and DOL, or uncharged molecules such as cholesterol, dynamic π-A behavior is not found to be greatly affected by subphase ionic composition in the physiologically relevant range. For example, Shah and Schulman (23) found little effect of Na^+, K^+, Mg^{2+}, or Ca^{2+} ions, among others, on DPL π-A behavior at 25°C. Similarly, Simon et al. (24) have shown that Na^+, K^+, Ca^{2+} and other mono- and di-valent ions in concentrations less than or equal to 0.1 M have a negligible effect on the gel to liquid-crystal transition temperature of DPL dispersions. Thus, the conclusions drawn from the pure water subphase π-A data presented in this paper should not be substantially modified by the inclusion of subphase ions, particularly in the range of physiologic ionic strengths.

quantitative later on in this paper by the application of a collapse plateau ratio criterion (5).

The π-A curves of Figs. 2 and 3 are the first and second dynamic compression–expansion cycles for lift-off films of DOL and cholesterol respectively. Two major differences between these results and those just discussed for DPL are as follows. *1*) Both cholesterol and DOL films exhibit dynamic surface pressures below 50 dynes/cm at monolayer collapse at 23°C and 37°C, and thus do not yield minimal surface tensions of less than one dyne/cm as is the case for DPL. *2*) Both DOL and cholesterol lift-off films exhibit better respreading after compression past collapse than is the case for corresponding films of DPL, as shown below.

One measure of respreading ability for a given surfactant film is to consider the length of the dynamic collapse plateau on successive cycles of the surface. As discussed in detail by Turcotte et al. (5), the ratio of successive cycle collapse plateau lengths for films compressed to the same minimum surface area can be looked upon, very roughly, as a measure of the fraction of molecules ejected from the surface during the first compression past monolayer collapse that have re-entered the surface in time to participate in lowering surface tension during the subsequent compression.

A major difficulty in applying a respreading criterion involving collapse plateau lengths is the determination of the point on a π-A curve that actually represents the onset of collapse. As discussed elsewhere (e.g., 5, 25) no single method of determining a meaningful collapse point is agreed upon by surface chemistry researchers, particularly in terms of dynamic compression–expansion π-A data. For the results here, we have adopted the collapse plateau criterion suggested by Turcotte et al. (5) to allow at least a semi-quantitative comparison of surfactant respreading following dynamic compression past collapse.[4] On a given π-A compression, a nearly vertical line is drawn tangent to the point of steepest slope and a second line is drawn tangent to the nearly horizontal post-collapse region. Examples of these lines are shown as dashed lines in Fig. 1a. The distance from the intersection of these lines (point B on Fig. 1a) to the end of the compression stroke (point A on Fig. 1a) is then defined as the collapse plateau length for the given compression. Collapse plateau ratios listed in **Table 1** are simply the ratio of the collapse plateau lengths calculated for the specific compression cycles indicated.

An important point to note is that this arbitrary criterion will be more meaningful for some surfactant π-A isotherms than for others. Specifically, if π-A compression curves are smoothly varying with very gradual slope changes with area, the collapse plateau determination above can be subject to considerable uncertainty. Also the criterion will be less accurate if the length of the first cycle collapse plateau is short, i.e., if only a small fraction of the first compression is in the collapse regime as is the case in "lift-off" experiments.

The results of analyzing pure DPL π-A behavior for the "lift-off" films of Fig. 1 in terms of collapse plateau ratios are shown in the first two rows of Table 1. As noted above, the numerical values given should be taken only as approximate. For example, for room temperature DPL films spread initially to 150 Å²/molecule (row 1 on Table 1), the cycle 2/cycle 1 and cycle 7/cycle 1 ratios of 0.27 and 0.24, respectively, are calculated from the given π-A curve of Fig. 1a. However, for similar initial concentrations and temperature, other π-A experiments in our laboratory have resulted in cycle 2/cycle 1 ratios up to 0.4–0.5 for some DPL lift-off films. In spite of this variability, it is significant that all of these ratios lead to the same general conclusion, i.e., that a majority of the DPL molecules ejected during collapse in cycle 1 fail to re-enter the interface during subsequent film expansion. Similar analysis of the respreading behavior of DOL lift-off films on **Fig. 2** shows that although some molecules are lost between the first and second compressions, the amount lost was not as great as found for DPL. As shown in rows 3 and 4 of Table 1, the ratio of second to first cycle collapse plateau lengths for the DOL lift-off films of Fig. 2 are 0.73 (23°C) and 0.79 (37°C) compared to 0.27 (23°C) and 0.24 (37°C) for DPL.

The enhanced respreading of DOL films compared to DPL at 23°C and 37°C is at least partly attributable to its lower gel to liquid crystalline transition temperature (T_c = −22°C) which is 63°C lower than that of DPL (T_c = 41°C) (20). Thus, at 23°C or 37°C, DOL is many degrees above its transition, and displays relatively high mobility of its hydrocarbon chains. DOL is therefore not suited at these temperatures to the formation of a closely packed, condensed monolayer of high surface pressure and undergoes monolayer collapse at a lower surface pressure than DPL, which is below its T_c even at 37°C.

The enhanced dynamic respreading of films of DOL as opposed to those of DPL is also found to be true for cholesterol. The π-A behavior of lift-off films of cholesterol at 23°C and 37°C is shown in **Fig. 3,** and collapse plateau ratios are summarized in rows 5 and 6 of Table 1. Cholesterol forms a relatively

[4] A closely related method of characterizing molecular loss at film collapse has recently been presented by Snik et al. (25).

TABLE 1. Characteristics of dynamic π-A isotherms of DPL, DOL and cholesterol

Film	Initial Conc.	Temp.	Collapse Plateau Ratio			Maximum Surface Pressure[b]		
			Cycle 2/ Cycle 1	Cycle 7/ Cycle 1	Cycle 7/ Cycle 2	Cycle 1	Cycle 2	Cycle 7
	Å²/molec.	*°C*						
DPL	150	23	0.27	0.24		72	72	72
DPL	150	37	0.24	0.11		69	69	69
DOL	150	23	0.73			47	47	
DOL	150	37	0.79			46	46	
CHOL	150	23	0.56			52	52	
CHOL	150	37	0.69			52	52	
DPL	25	23	0.30 (0.38)[a]	0.16 (0.24)		72	72	72
DPL	25	37	0.10 (0.17)	0.05 (0)		69	69	63
DPL	15	23	0.40	0.17		72	72	72
DPL	15	37	0.10	0.02		69	69	59
DOL	25	23			0.69		48	48
DOL	25	37			0.64		47	47
DOL	15	23			1.00		48	48
DOL	15	37			1.00		47	47
CHOL	50	23			0.87		58	58
CHOL	50	37			0.85		50	50
CHOL	25	23			1.0		50	50
CHOL	25	37			1.0		50	50
DPL:CHOL (9:1)	15	23	0.44	0.12		72	72	72
DPL:CHOL (9:1)	15	37	0.23	0.17		55	55	54
DPL:DOL (9:1)	15	23	0.66	0.44		72	72	72
DPL:DOL (9:1)	15	37	0.4	0.2		69	69	69
DPL:DOL (5:5)	15	23	0.9	0.3		72	72	72
DPL:DOL (5:5)	15	37	0.6	0.4		55	55	64

[a] Values in parentheses are previously published (5) results for 25 Å²/molecule DPL surface excess films to display the variability of the collapse plateau ratio test (see text for details). The poor respreading of DPL surface excess films, however, is clear from both sets of ratios.

[b] A surface pressure of 72 dynes/cm at 23°C or one of 69 dynes/cm at 37°C corresponds to minimum surface tensions ≤1 dyne/cm.

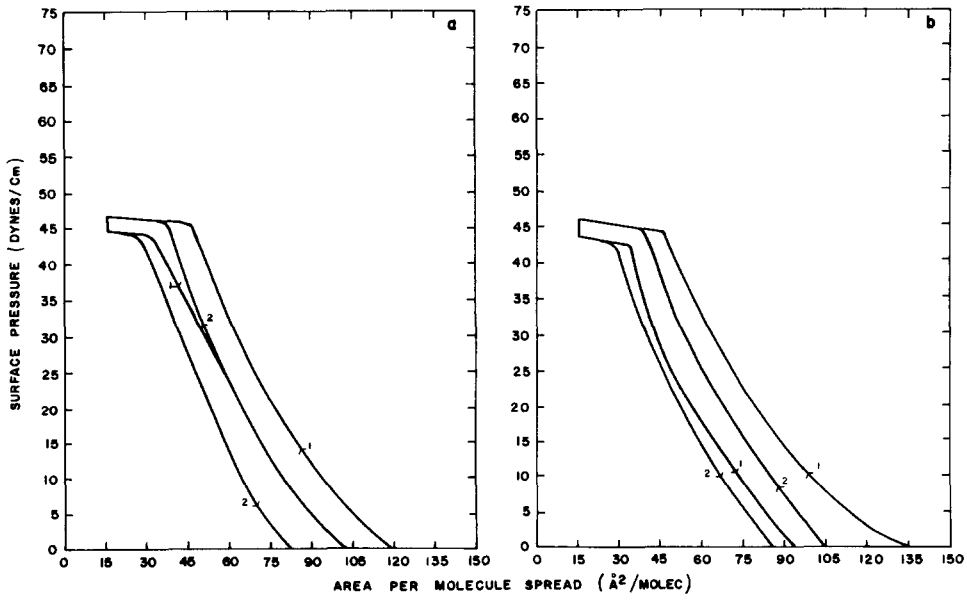

Fig. 2. Dynamic compression expansion behavior of pure DOL surface films under "lift-off" conditions. Details as in Fig. 1.

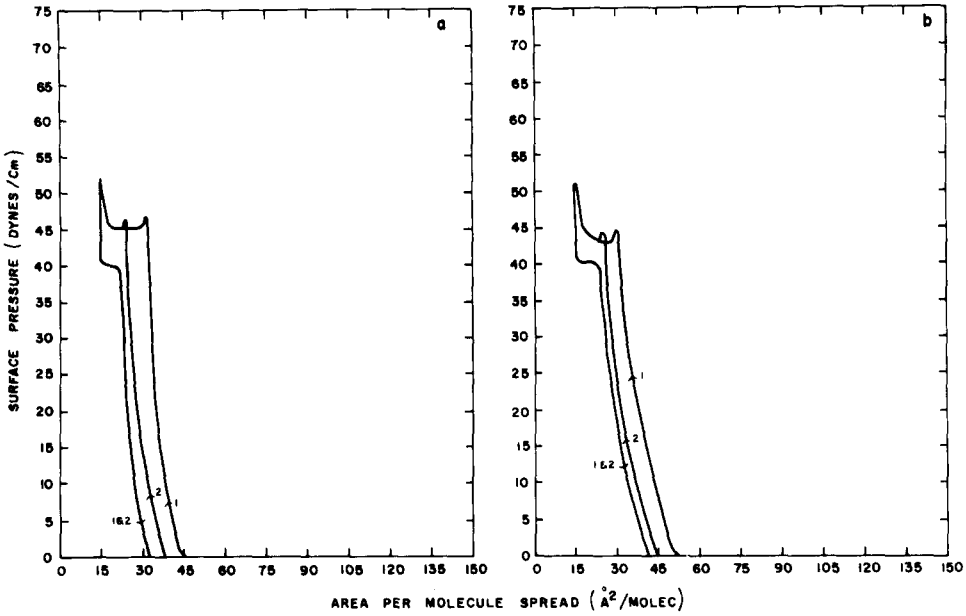

Fig. 3. Dynamic compression expansion behavior of pure cholesterol surface films under "lift-off" conditions. Details as in Fig. 1.

incompressible monolayer lifting-off at 45 Å²/molecule at 23°C and 50 Å²/molecule at 37°C, while dynamic collapse occurs at initial surface pressures of about 45 dynes/cm at 23°C and 43 dynes/cm at 37°C. As shown in Table 1, cholesterol is a moderately good respreader with a cycle 2 to cycle 1 collapse length ratio of 0.56 at 23°C and 0.69 at 37°C.

The dynamic collapse behavior of cholesterol in Fig. 3 is slightly different from that previously discussed for DPL and DOL. Specifically, during dynamic collapse cholesterol is seen first to generate a peak surface pressure, then to drop a few dynes/cm, and finally to rise by 5–7 dynes/cm at end compression. This form of collapse behavior also occurs during dynamic collapse of some fatty acid monolayers (27). The collapse pressures for lift-off cholesterol films found here are above the solvent spread (21) or crystal spread (18) equilibrium pressure of about 36 dynes/cm for cholesterol at 23°C and 37°C, showing that a dynamic to static surface pressure difference exists for cholesterol as it does for DPL (3). However, a significant dynamic to static π difference is not found in the collapse regime for the DOL films of Fig. 2, which show maximal dynamic values close to the known equilibrium pressures of about 46–48 dynes/cm for DOL (18, 21).

Fig. 4 shows the first, second and seventh cycles of DPL surface excess films at 23°C and 37°C, for an initial spread concentration of 15 Å²/molecule.[5] The collapse plateau ratios and maximum surface pressures of these films, and also of 25 Å²/molecule surface excess DPL films, are summarized in rows 7 to 10 of Table 1. These results show that DPL respreading for the surface excess initial condition is not enhanced over the corresponding lift-off isotherms of Fig. 1. In fact, the body temperature π-A curve of Fig. 4b shows that by the seventh surface cycle essentially all of the excess DPL originally spread to 15 Å²/molecule has been irrevocably lost through collapse ejection and the DPL film can no longer attain the maximal dynamic collapse pressure reached on earlier compressions.

Interestingly, the π-A curves of Figs. 1 and 4 and the corresponding collapse plateau length ratios of Table 1 show that for both lift-off and surface excess initial conditions, DPL tends to respread even more poorly at body temperature than it does at room temperature. Although we cannot give a complete explanation for this result, the effect of increasing temperature is to increase the mobility of the palmitic acid chains in DPL and to expand the film (Fig. 1). Similarly, monolayer fluidity is increased as the gel to liquid crystalline transition temperature is approached (20). Thus, as temperature is increased, it

[5] The initial concentrations of all surface excess isotherms are given in units of Å²/molecule spread, even though the surface concentrations are generally of such a magnitude that surfactant cannot all spread to form a monolayer. These units are retained for initial concentration to facilitate comparison among the various kinds of experiments presented in this paper, although the actual relevance of the Å²/molecule unit is questionable beyond monolayer collapse. Thus, for all surface excess π-A curves the more qualitative units of percent trough area are used to define the area axis, with the exception of the initial concentration value. Cycling rate was 300 sec/cycle.

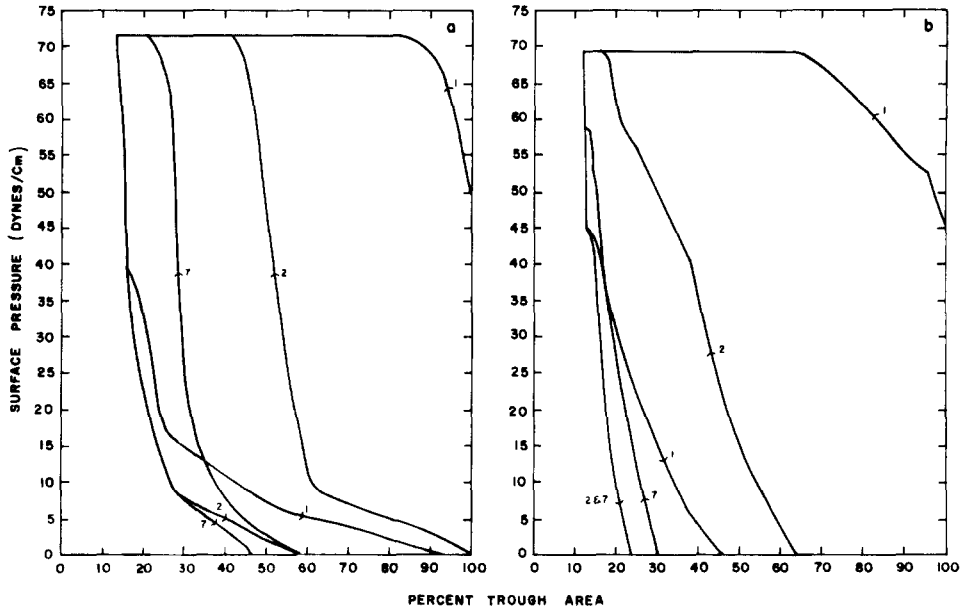

Fig. 4. Dynamic compression expansion behavior of pure DPL surface films under "surface excess" conditions. Initial concentration was 15 Å²/molecule. Cycling rate was 300 sec/cycle. Other details as in Fig. 1.

would seem that enhanced hydrocarbon chain motion should facilitate both molecular ejection from the surface during collapse as well as molecular re-entry during subsequent expansion. If the ejection process were increased more than the subsequent re-entry process, then the net result would be more molecules lost from the interface in a given compression past collapse at 37°C than at 23°C.

A final point regarding the 37°C results of Figs. 1b and 4b is that they show pure DPL films able to reach surface pressures corresponding to minimum surface tensions of ~1 dyne/cm. These measurements were done at relative humidities varying from 75–100%, and are thus at odds with those reported by Colacicco, Basu, and Scarpelli (6), who were able to achieve minimum surface tensions of only 18 or more dynes/cm in DPL surface excess films at 37°C under conditions of "humidity", whereas minimum values of "zero surface tension" were found in a nonhumidified environment at 37°C. This point is of obvious relevance for extrapolation of in vitro π-A results to the mammalian lung, which presumably is at humidities approaching 100%. Although a definitive explanation for the discrepancy between our results and those of Colacicco et al. (6) is not available, it is clear that many error sources such as film leakage in DPL π-A measurements at low surface area and high surface pressure tend to give values of surface tension that are too high rather than too low. Moreover, our low minimum surface tensions for DPL at 37°C appear to be in agreement with those found by Slama, Schoedel, and Hansen (28) and by Reifenrath and Zimmerman (29) using an oscillating bubble technique which requires complete saturation of the bubble atmosphere with water vapor. Regardless of this point about humidity effects, however, the lack of respreading in pure DPL films compressed past collapse is clearly shown at body temperature by the results found here (Figs. 1b and 4b) and by those of previous investigators (4, 6, 28, 29).

The second and seventh cycle surface excess behavior of DOL at 23°C and 37°C is shown on **Fig. 5** for initially spread concentrations of 25 and 15 Å²/molecule. These data, and the collapse plateau ratios on lines 11–14 of Table 1 show surface excess DOL films to be extremely respreadable on successive surface cycles past collapse. Note that the essentially uniform surface pressure observed for the 15 Å²/molecule DOL surface excess film is very close to its equilibrium spreading pressures of about 46–48 dynes/cm at 23°C and 37°C (18). This seems to indicate that an accessible reservoir of DOL has been established which is in continuous equilibrium with the monolayer during dynamic compression and expansion at the cycling rate of 300 sec/cycle used here.

The surface excess behavior of cholesterol at 23°C and 37°C is shown in **Fig. 6** for initial concentrations of 50 and 25 Å²/molecule; it is similar but not exactly equal to that just discussed for surface excess films of DOL. Two differences are that cholesterol reaches what appears to be complete respreading at a lower initial concentration than DOL, 25 versus 15 Å²/molecule, and that it exhibits several dynamic compression effects. These compression effects are the rise in surface pressure at end compression seen in Fig. 6, and the

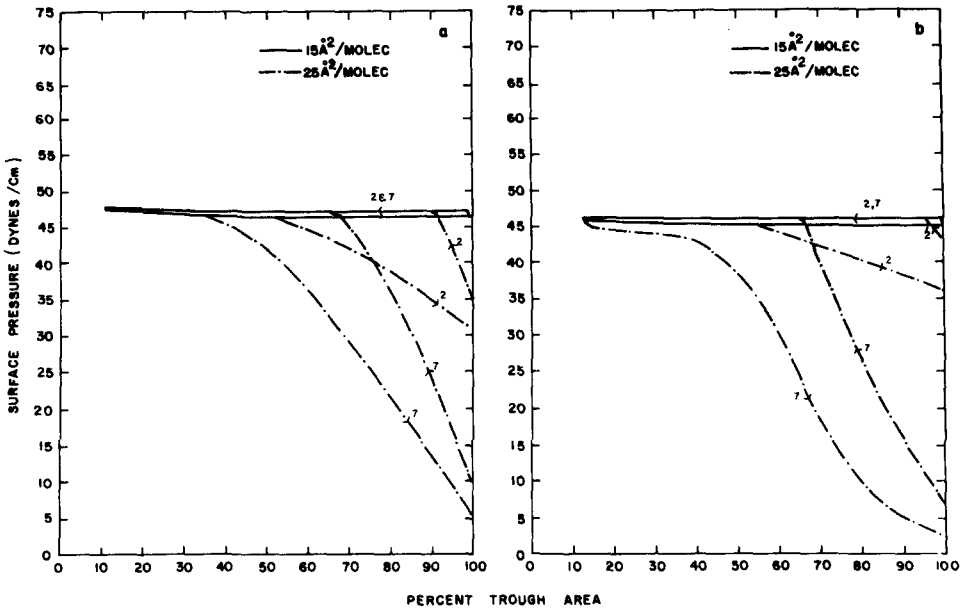

Fig. 5. Dynamic compression expansion behavior of pure DOL surface films under "surface excess" conditions. Initial concentrations were 15 Å²/molecule, ——; and 25 Å²/molecule, ——·——. Other details as in Fig. 4.

difference between dynamic and equilibrium π-A behavior for cholesterol. Since the equilibrium collapse pressure of cholesterol is ~36 dynes/cm at both 23°C and 37°C, the collapse surface pressure values during compression on Fig. 6 represent a dynamic effect, just as was noted earlier for the cholesterol lift-off film results of Fig. 3. Such a dynamic to static π difference is not found for DOL surface excess films on Fig. 5. Regardless, the surface excess results for DOL and cholesterol films in Figs. 5 and 6 show that both these surfactants can generate π-A isotherms exhibiting essentially complete respreading if a high enough initial surface concentration is used at either 23°C or 37°C.

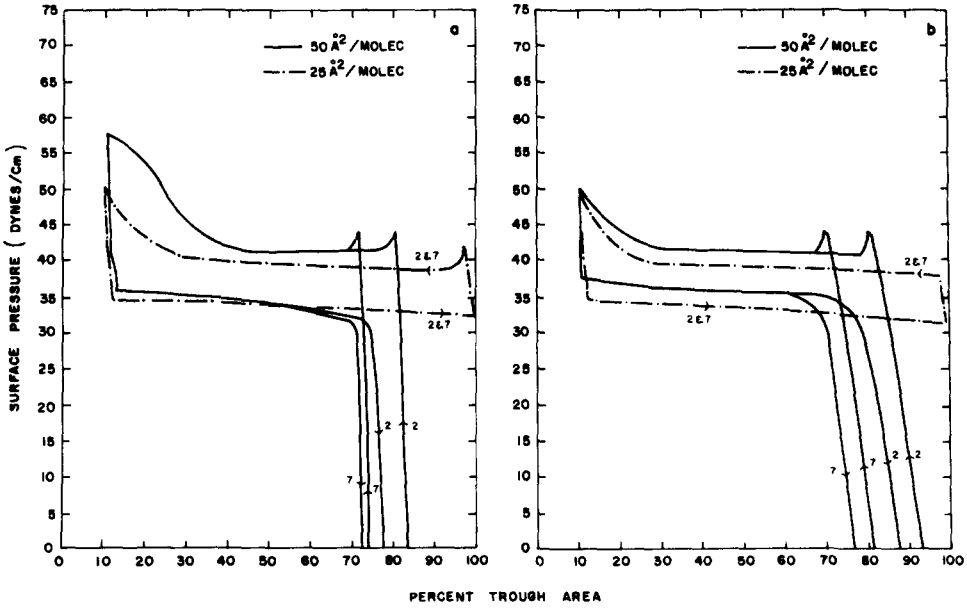

Fig. 6. Dynamic compression expansion behavior of pure cholesterol surface films under "surface excess" conditions. Initial concentrations were 50 Å²/molecule, ——; 25 Å²/molecule, ——·——. Other details as in Fig. 4.

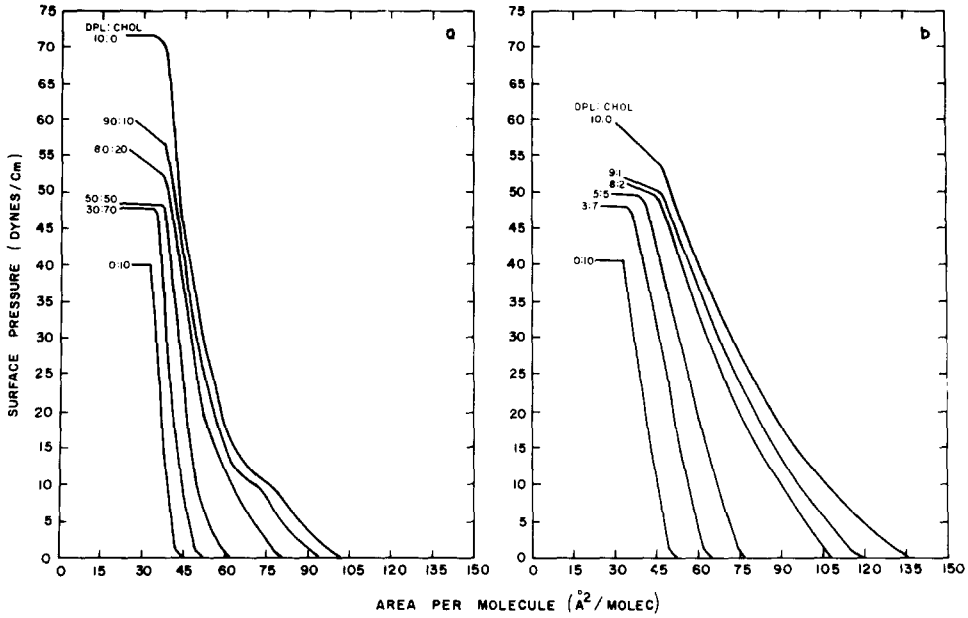

Fig. 7. First compression behavior of binary surface films of DPL and cholesterol under "lift-off" conditions at a slow compression rate. DPL:cholesterol molar ratios are shown for each curve. Initial concentration was 150 Å²/molecule. Compression rate was 9 Å²/molecule/minute. a, 23°C; b, 37°C.

Behavior of mixed films of DPL with cholesterol and DOL

Because of the relevance to mixed film miscibility, we begin this section with a consideration of the bulk phase gel to liquid-crystalline transition temperature (T_c) of mixtures of DPL:DOL and DPL:cholesterol. Phillips, Ladbrooke, and Chapman (20) have shown that the effect of DOL in equimolar mixtures with DPL is to lower the T_c of the DPL, while a separate DOL melting peak occurs at an unchanged value of −22°C. We did DSC measurements of the melting profiles of mixtures of DPL:DOL in concentrations of 50:50, 90:10, 95:5, and 98:2. The results in general support the conclusions of Phillips, et al., (20) that DPL and DOL form only a partially miscible system even at low DOL concentration. Specifically, all four mixtures gave a clear DOL melting peak at −22°C, and increasing amounts of DOL gave an increasing shift of the onset of melting of DPL to lower values (melting onset at 40°C for pure DPL, at 35°C for DPL:DOL 98:2 and 95:5, at 34°C for 90:10, at <30°C with a widely broadened peak for 50:50).

In terms of DPL:cholesterol mixtures, a rather complete description of bilayer transition temperatures and heats found by DSC has been given by Ladbrooke, Williams, and Chapman (17), and such data were not repeated here. The major points for the present work are that DPL and cholesterol form a miscible system and that cholesterol acts to make fluid the DPL molecules in an aqueous dispersion.

Mixed films of DPL:cholesterol and DPL:DOL were investigated for both the lift-off and surface excess initial conditions discussed earlier. For the mixed film lift-off first compression curves of **Fig. 7,** a slow rather than fast cycling rate was used, i.e., 9 Å²/molecule/min as opposed to the 53 Å²/molecule/min cycling rate used in the pure film lift-off π-A data of Figs. 1–3. However, some surface pressure relaxation was apparent even for the slow cycling speed of 9 Å²/molecule/min if barrier movement was interrupted during compression (not shown on Fig. 7). Thus, the data of Fig. 7 must be taken as representing dynamic compression and they are included here primarily because they help to demonstrate that mixed monolayer collapse pressure is a function of temperature, monolayer composition and surface cycling rate. Moreover, as discussed by Notter, et al. (21), this sensitive parameter is also dependent upon surface initial condition.

The dependence of mixture collapse pressure on DPL:cholesterol mixed film composition for an initial spreading concentration of 150 Å²/molecule is shown at 23°C in Fig. 7a and at 37°C in Fig. 7b. The two extreme curves at each temperature represent pure cholesterol and pure DPL films. Note that although some of the films collapse with a flat collapse plateau, this is not always true at the slow cycling rate used, and some of the films on Fig. 7 have a maximum surface pressure that depends upon the extent of compression past collapse.

The dependence of monolayer collapse pressure

on cycling rate is best shown by the DPL pure component film at 37°C on Fig. 7b. At 9 Å²/molecule/min compression, and for the given initial condition of 150 Å²/molecule spread, the DPL film is seen to begin to exhibit collapse behavior at a surface pressure of below 60 dynes/cm. By comparison, the pure DPL film of Fig. 1b spread to the same initial 150 Å²/molecule at 37°C, but compressed at the rate of 53 Å²/molecule/min, is able to achieve a collapse surface pressure of 69 dynes/cm. Since any DPL collapse surface pressure value in excess of 50 dynes/cm represents a dynamic compression effect, as the compression rate is decreased it would be expected that slow enough rates should eventually result in a lowering of the dynamic DPL surface pressure toward its static value. It is apparently this effect which is shown for DPL films by comparing Figs. 1b and 7b. A similar effect is seen by comparing the pure cholesterol film fast compression rate data of Fig. 3 with the corresponding slow rate data of Fig. 7.

Dynamic π-A results are now given for a series of mixed films investigated under the surface excess initial condition. One potential application of such experiments is the identification of specific mixtures which, if delivered to the lung alveoli in sufficient initial concentrations, would have the respreading properties to remain effective without continuous exogenous replacement. The three specific mixed films studied are DPL:cholesterol (90:10), DPL:DOL (90:10), and DPL:DOL (50:50). The behavior of these three films at 23°C and 37°C for an initial concentration of 15 Å²/molec is shown in Figs. 8–10. The collapse plateau lengths and maximum surface pressure of these films are summarized in the last six rows of Table 1.

The effect of 10% cholesterol on DPL surface excess behavior is shown in **Fig. 8** for 23° and 37°C. Essentially, the respreadability found for this 23°C DPL:cholesterol (90:10) film (Fig. 8a) during successive cycles is equivalent to that observed earlier for pure DPL at the same surface excess concentration (Fig. 4a). As shown in Table 1, the cycle 2 to cycle 1 and cycle 7 to cycle 1 collapse plateau lengths were 0.44 and 0.12, respectively, for the DPL:cholesterol (90:10) and 0.40 and 0.17 for pure DPL. This similarity between the respreadability of DPL and DPL:cholesterol (90:10) films at 23°C is not surprising, since the cholesterol is relatively dilute in the DPL (10%), and the DPL:cholesterol mixture is still significantly below its T_c. Aside from respreading, the DPL:cholesterol film attains the same high surface pressure (72 dynes/cm) on compression at 23°C as does pure DPL. This high maximal surface pressure agrees with the results of Colacicco and Basu (30), who reported maximum surface pressures of 72 dynes/cm for surface excess DPL:cholesterol films at 23°C for cholesterol concentrations less than 20 mol%. Note, however, that for the case of DPL:cholesterol films spread to lift-off rather than surface excess initial concentrations, this maximum collapse π value may be lowered at room temperature (21).

At 37°C (Fig. 8b), the DPL:cholesterol (90:10) surface excess film exhibits a lower collapse pressure but greater respreadability on successive cycles than

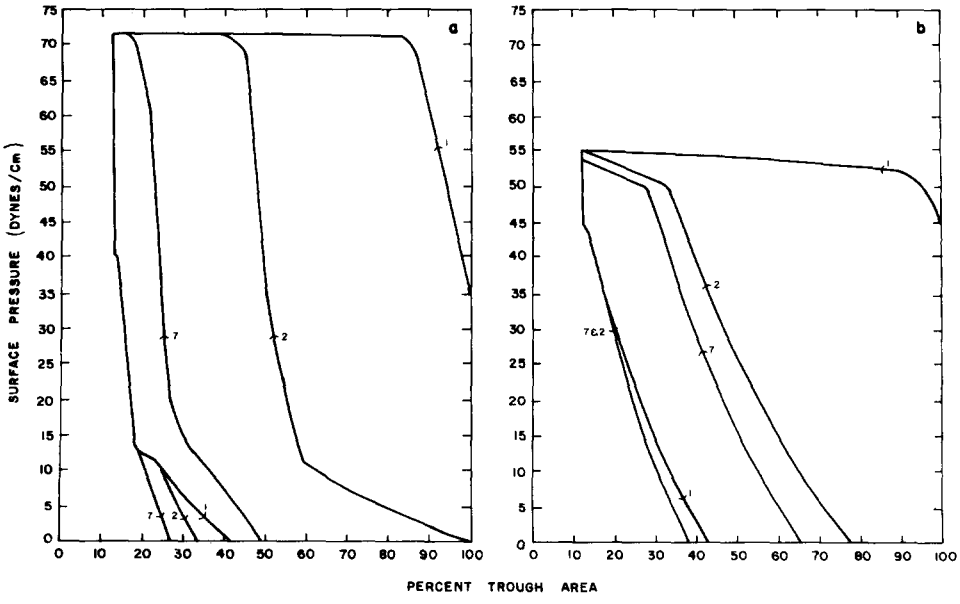

Fig. 8. Dynamic compression-expansion behavior of binary surface films of 90:10 DPL:cholesterol under "surface excess" conditions. Details as in Fig. 4.

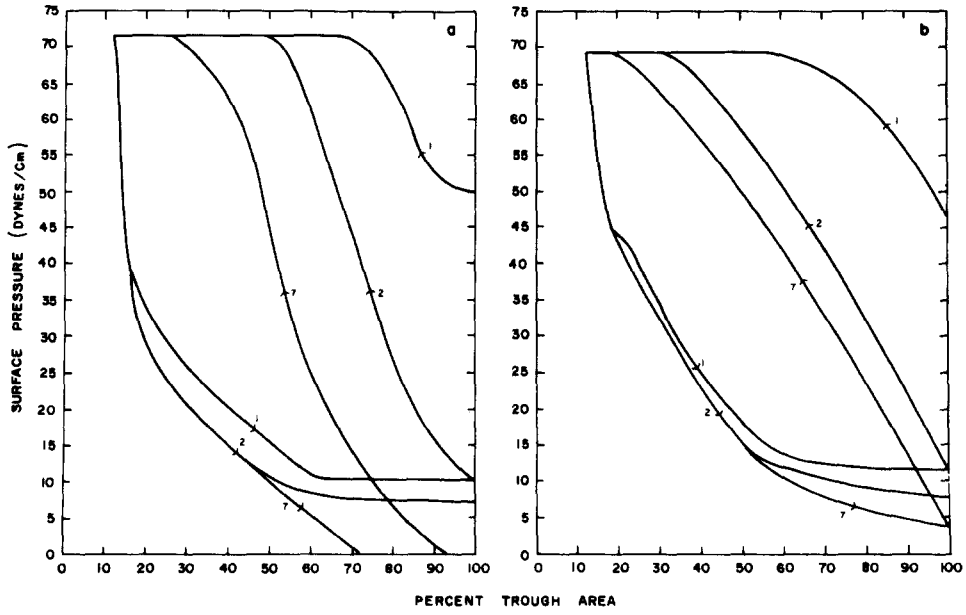

Fig. 9. Dynamic compression-expansion behavior of binary surface films of 90:10 DPL:DOL under surface excess conditions. Details as in Fig. 4.

the corresponding pure DPL film. In Table 1, the cycle 7 to cycle 1 collapse plateau length ratios are shown as 0.17 for the DPL:cholesterol film at 37°C and 0.02 for the equivalent DPL film. Significantly, the experimental temperature of 37°C is now very close to the 38°C T_c for this mixture (17), and this apparently contributes to increased respreading but lowered dynamic collapse pressure. The low collapse surface pressure found here for DPL:cholesterol (90:10) at 37°C agrees with the observations of Colacicco and Basu (30). It is also consistent with the data of Reifenrath and Zimmerman (29) who found a maximum surface pressure of approximately 49 dynes/cm for DPL:cholesterol (92:8) cycled at 37°C on the surface of an oscillating bubble.

The surface excess behavior of DPL:DOL (90:10) at 23°C and 37°C is shown in **Fig. 9.** From these data, the DPL:DOL system is seen to exhibit enhanced respreadability over pure DPL at both 23°C and 37°C. Moreover, the system reaches the same maxi-

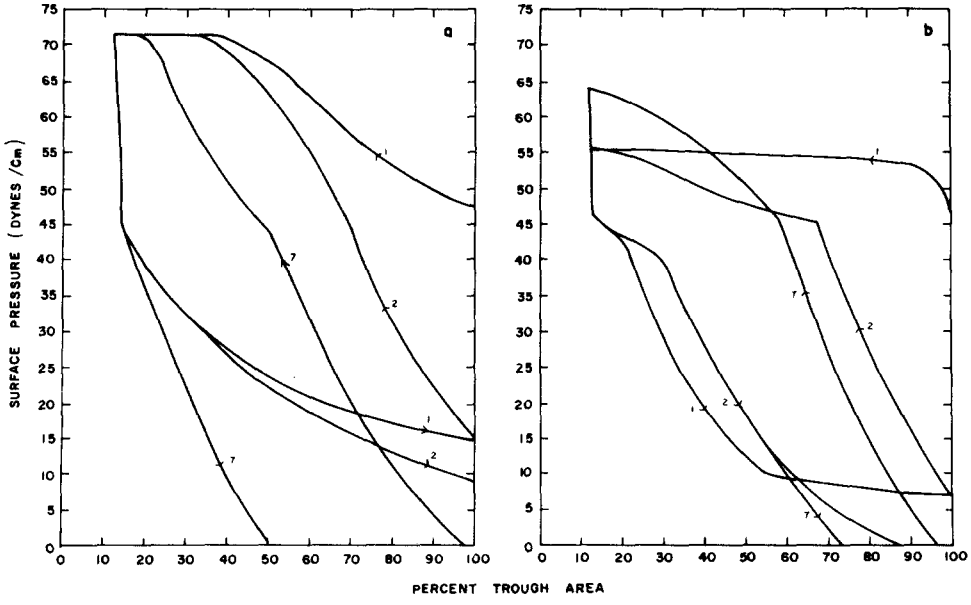

Fig. 10. Dynamic compression-expansion behavior of binary surface films of 50:50 DPL:DOL under surface excess conditions. Details as in Fig. 4.

mum surface pressures as DPL, 72 dynes/cm at 23°C and 69 dynes/cm at 37°C (Table 1). The cycle 7 to cycle 1 collapse length ratios for DPL:DOL (90:10) shown in Table 1 are 0.44 at 23°C and 0.2 at 37°C, significantly improved over the corresponding values of 0.17 and 0.02 for a pure DPL film at the same conditions. This increased respreadability imparted by DOL correlates not only with T_c effects, but also with the finding that DOL enhances the relaxation of post-collapse dynamic π in mixed films with DPL as discussed by Notter et al. (21).

The result of an increased DOL concentration is shown in **Fig. 10** for a DPL:DOL (50:50) surface excess film. At 23°C, the DPL:DOL (50:50) π-A behavior is generally similar to that observed for DPL:DOL (90:10) at the same temperature. Note, however, that the π-A compression curves for the 37°C DPL:DOL (90:10) films in Fig. 9b and for the DPL:DOL (50:50) films in Fig. 10 are more compressible than pure DPL, with a varying π-A slope throughout the compression cycle. For this kind of relatively compressible π-A behavior it is more difficult to define collapse plateau lengths by the method adopted throughout this discussion. With this caution, the 23°C DPL:DOL (50:50) isotherms of Fig. 10a give cycle 2 to cycle 1 and cycle 7 to cycle 1 ratios of approximately 0.9 and 0.3, respectively. This can be contrasted with the DPL:DOL (90:10) results at the same temperature which give corresponding ratios of 0.66 and 0.44. Thus, respreading at 23°C is enhanced for cycle 2/1 but not for cycle 7/1 by an increased DOL concentration from 10% to 50%. Moreover, the maximum attainable surface pressure is not affected by this increased DOL concentration at 23°C, and the data of Fig. 10a show the DPL:DOL (50:50) binary capable of reaching 72 dynes/cm at collapse at this temperature, initial concentration and cycling rate.

At 37°C, the DPL:DOL (50:50) binary in Fig. 10b was found to exhibit unusual behavior, with a cycle 1 and cycle 2 maximum surface pressure of 55 dynes/cm followed by an increase to 64 dynes/cm on cycle 7. This increasing surface pressure on successive cycles may be due in part to the preferential expulsion of DOL from the surface film during compression to surface pressures in excess of those generated by pure DOL. Thus, the monolayer becomes enriched with DPL during successive surface cycles and the collapse pressure correspondingly increases. In spite of this apparent loss of DOL, the DPL:DOL (50:50) surface excess system at 37°C shows cycle 2 to cycle 1 and cycle 7 to cycle 1 collapse plateau length ratios of 0.6 and 0.4, respectively, although it is stressed again that the numerical values are subject to some uncertainty. These 2/1 and 7/1 ratios may be compared to corresponding values of 0.4 and 0.2 for DPL:DOL (90:10), and 0.10 and 0.02 for pure DPL at the same temperature.

In summary, the dynamic π-A behavior of surface excess films of DPL with cholesterol or DOL shows that these added components enhance respreading after dynamic compression past collapse as compared to pure DPL. At room temperature, all three binaries investigated (DPL:cholesterol 90:10 and DPL:DOL 90:10 and 50:50) were able to generate minimum surface tensions ≤ 1 dynes/cm. However, at body temperature, only the surface excess DPL:DOL 90:10 binary gave rise to such low surface tensions. Thus it appears at the present time that the DPL:DOL binary is promising for possible use as an aerosol to treat states of surfactant insufficiency in vivo. However, it is clearly not an optimal mixture, and further work on other mixed films such as DPL with phosphatidylglycerol is presently continuing. Moreover, in addition to dynamic π-A determinations, the property of surfactant adsorption after subphase deposition needs to be investigated for all of these mixed systems.

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