INFLUENCE OF ACYL CHAIN MOBILITY ON THE PROPERTIES OF MONOLAYERS OF SOME PHOSPHOLIPIDS

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INFLUENCE OF ACYL CHAIN MOBILITY ON THE PROPERTIES OF MONOLAYERS OF SOME PHOSPHOLIPIDS

by

© Michael W. Hawco, B.Sc.

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ABSTRACT

Surface properties of various phospholipids and their simple mixtures have been studied to investigate the requirements of lipid phase in lung surfactant. A series of saturated and unsaturated lecithins have been examined using a modified Wilhelmy surface balance for their ability to reach low surface tension (γ). Both monolayers of unsaturated and saturated lecithins were capable of achieving near zero γ as long as they were compressed below their respective bulk phase transition temperatures (t_c). When monolayers were formed at 37°C using mixtures of dipalmitoyl phosphatidylcholine (DPPC) and other lower melting lecithins the ability to reach low surface tension was dependent on: (1) the relative amounts of the two components (2) the t_c of the lower melting lecithin and (3) the rate at which the monolayer was compressed. It has been suggested that compression of binary monolayers may result in a "squeeze-out" of the more fluid lecithin producing a DPPC-enriched monolayer capable of reaching low γ.

Surface tension-area isotherms of monolayers of dipalmitoyl phosphatidyl ethanolamine (DPPE) and its methylated analogues, N-methyl dipalmitoyl phosphatidylethanolamine (N-me DPPE) and N,N-dimethyl dipalmitoyl phosphatidyl-ethanolamine (N,N-dime DPPE) have been examined. The minimum surface tensions achieved were $\gamma_{DPPC} < \gamma_{N,N\text{-dimeDPPE}} < \gamma_{N\text{-MeDPPE}} < \gamma_{DPPE}$. Monolayer mixtures of DPPE and DPPC showed that lower

tensions were achieved with increasing proportions of DPPC.

Results from monolayer studies suggested that the ability of lung surfactant to reach low γ may be dependent on lipid phase and hence its composition. Furthermore, infants born with Respiratory Distress Syndrome (RDS) may contain the "wrong mixture" of lipids in their surfactant so that low γ can not be achieved to stabilize the lung. The results presented here, using simple systems, may offer some insight in the consideration of potential mixtures to be used in artificial replacement surfactant.

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LIST OF ABBREVIATIONS

egg PC	1,2-diacyl-sn-glycerol-3-phosphocholine from egg yolk
egg PG	rac-1,2-diacyl-sn-glycero-3-phospho-sn-glycerol phosphatidylglycerol made by transphosphatidylation of egg PC
DMPC	1,2-dimyristoyl-sn-glycero-3-phosphocholine
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
N,N-diMe DPPE	1,2-dipalmitoyl-sn-glycero-3-phospho-N,N-dimethylethanolamine
DPPE	1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine
N-Me DPPE	1,2-dipalmitoyl-sn-glycero-3-phospho-N-methylethanolamine
DSPE	1,2-distearoyl-sn-glycero-3-phosphoethanolamine
$dyne \cdot cm^{-1}$	dynes per centimeter
γ_{eq}	equilibrium surface tension
HMD	Hyaline Membrane Disease
μmole	micromole
$mN \cdot m^{-1}$	milliNewtons per meter
γ_{min}	minimum surface tension attained during compression of a monolayer
lyso oleoyl PC	1-oleoyl-sn-glycero-3-phosphocholine
OSPC	1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
PC	phosphatidylcholine
PG	phosphatidylglycerol
PE	phosphatidylethanolamine
PV	pressure-volume

LIST OF ABBREVIATIONS CONT'D

RDS	Respiratory Distress Syndrome
SLPC	1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine
SAM	surface active material
t_c	transition temperature

INTRODUCTION

The mammalian lung is an intricate network of airways leading from the trachea through the bronchi reaching to the terminal air exchange vessels, the alveoli. The alveolus, the Latin word for "little hollow", is a cup-shaped structure measuring from 0.2 to 0.3 mm in diameter depending on the degree of inflation. In the mature human lung there are approximately 3×10^8 alveoli whose surface would cover an area of 70-80 m^2 (for general discussion see Avery et al., 1973). Each of these alveoli is surrounded by a complex vasculature to enable efficient respiratory gas exchange.

A newborn infant has one-tenth the number of alveoli as an adult and each alveoli is one-half the size of a mature structure (Avery et al., 1973). Because a newborn infant has smaller airways and has to overcome an initial surface tension resistance of a fluid filled lung, the first breath may have to overcome some 80 cm water pressure, about 10 times the pressure needed for breathing once the lung is fully aerated. The problem of overcoming high surface tension is alleviated in the mature lung by the presence of a surface active material called pulmonary surfactant at the air-alveolar interface. In some premature infants, especially those who are born after only three-quarters of the normal gestation period, a deficiency of pulmonary surfactant results in the condition known as Respiratory Distress Syndrome (RDS) or Hydraline Membrane Disease (HMD)

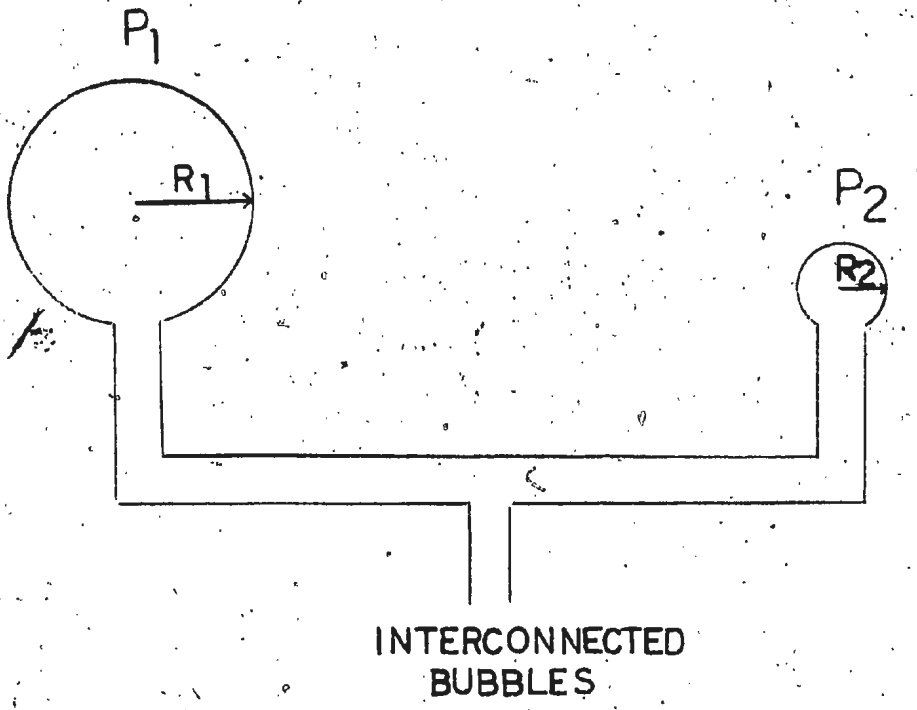
(Avery and Mead, 1959).

The presence of surfactant was proposed as early as 1929 when a German scientist, von Neergaard (1929) compared pressure-volume relationships of exised lungs filled with air and with isotonic gum arabic. The gum arabic solution eliminated the air-alveolar interface, so that retractive forces were due only to tissue elasticity. There was however a large retractive force seen in the air-filled lung when the air-alveolus interface was left intact. He intrepreted his results to mean that the lung must contain another component besides tissue elasticity to provide pulmonary retractive forces. He calculated that this component imparts between two-thirds and three-quarters of the total retractive force. This component was suggested to be due to the surface tension in the lung. Material which can accomplish such a reduction in surface tension is now known to be present at the air-alveolar interface and is called pulmonary or lung surfactant.

A simple model

The interconnecting alveolae of the pulmonary system have been described by simple model which involves the union of two bubbles as shown in Fig. 1. The alveoli in the lung at any given time are not all the same size; some are completely open while others are simultaneously closed. The pressure across any such bubble may be described by the equation of LaPlace:

Figure 1. Simple model of interconnected bubbles to describe the alveolar system. The collapsing pressure is $P = \frac{2\gamma}{r}$ where γ = the surface tension and r = the radius. If r_2 becomes very small so must γ_2 or the increase in pressure will tend to collapse the smaller bubble into the larger one. Lung surfactant has the ability to reduce surface tension with a decrease in surface area (or r in the case of a bubble) and stabilize the system.


P1
R1
P2
R2
INTERCONNECTED
BUBBLES

$$P = \frac{2\gamma}{R}$$

where P = transbubble pressure, γ = surface tension ($mN \cdot m^{-1}$) and r = bubble radius. If one considers the model in Fig. 1 and γ is constant for both bubbles, it is clear that the pressure across 2 would be greater than that across 1 so that 1 would collapse into 2. If the surface tension was the same in all the alveoli in the lung the smaller alveoli would similarly collapse into larger areas. Eventually there would be areas of the lung with very little gas exchange so that overall respiration would be less efficient. If however the surface tension decreased as the size of the bubble diminished, the pressure across the small bubble, according to the LaPlace equation, could decrease. If the pressure is the same in both bubbles then no collapse will occur, and the system remains stable. The substance called pulmonary surfactant when placed at the air-water interface is capable of varying the surface tension as the surface area is changed.

It has been suggested that this model of interconnected bubbles may be too simple and that a system of structures associated by adjacent septa might be a more appropriate model. This type of model as described by Hildebrandt (1978) maintains that the surface area of the alveolus decreases anisotropically, or without any decrease in perimeter. Figure 2, redrawn from (Hildebrandt 1978), pictures each individual alveolus hexagonally (a) and upon collapse (b) it becomes elongated to reduce surface area in

Figure 2. Anisotropic model describes the alveolar system as one in which changes in surface area are brought about without changing the perimeter of the alveoli such as extending a hexagon. System allows for the whole system of alveoli joined by adjacent septa but doesn't allow for shortening and stretching of alveolar walls. (After Hildebrandt, 1978.)

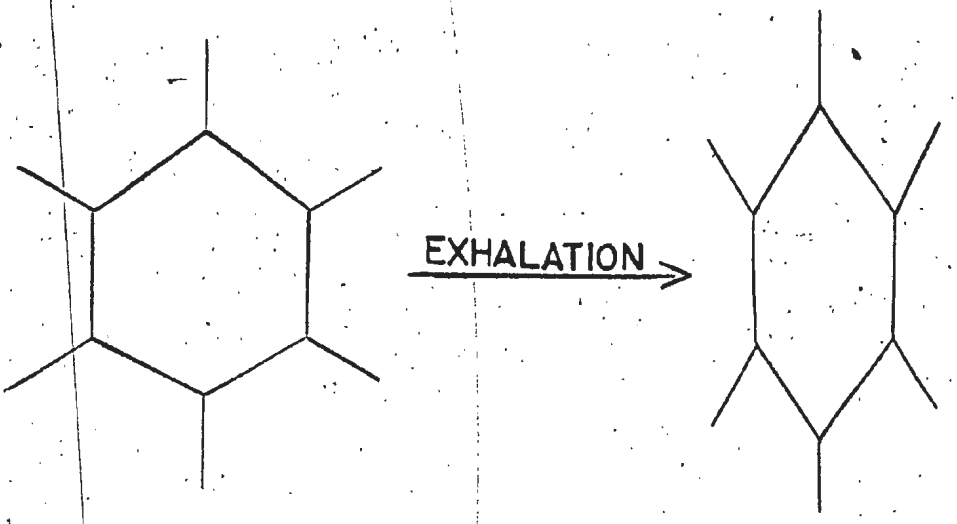
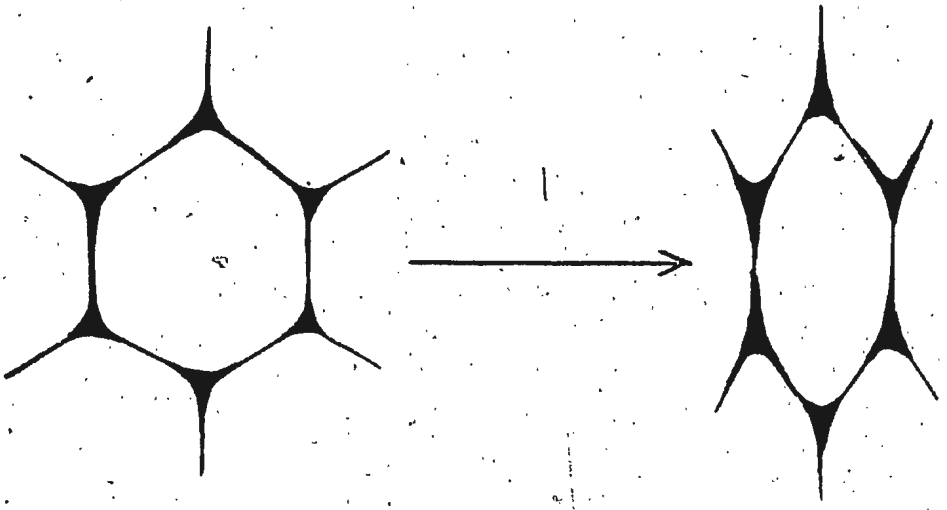

EXHALATION

Figure 3. Possible alveolar model which employs characteristics of the bubble and anisotropic model. Alveoli are joined by adjacent septa but are permitted to stretch, shorten and fold.



an "accordion" fashion. According to this model, alveolar walls form pleats and folds without shortening them. The isotropic bubble model must allow for considerable elasticity in the alveolar walls to permit large changes in surface area and hence lung volume. The anisotropic consideration however, contends that changes in surface tension are effected by the folding of the walls and exert no retractive forces. It is known however that the lung, does exert a retractive force (von Neergaard, 1929) and is facilitated by the elasticity of the alveolar walls.

It may be the case however, that alveoli might be more realistically represented by a compromise between the "bubble" and the "accordion" model. The hexagon in Fig. 3 may better represent the inter-relationship between adjacent alveoli via a common septum. The model involving interconnecting bubbles however, accounts for the elasticity exemplified by the mammalian lung. A possible pictorial representation of such a model is shown in Fig. 3.

This model is essentially the same as that shown in Fig. 2 but the alveolar walls are shortened slightly upon exhalation. In the deflated state though, one might expect the alveolar walls to be folded and wrinkled as their resting length is surpassed.

Whichever model, if any, represents a true picture of the alveolar system, the ability to reach low surface tension and resist collapse upon deflation is contingent on the efficiency of the surfactant system. In

Fig. 3, as the walls come closer together, the surface tension must decrease to near zero values to resist collapse and allow re-expansion.

The two-dimensional structure shown here is also likely an oversimplification. Even if the alveoli have flat surfaces, they probably have a large number of them and the more appropriate model will probably be polyhedral in nature. The more faces in the polyhedron, the more it will approach the shape of a sphere used in the simple, bubble model.

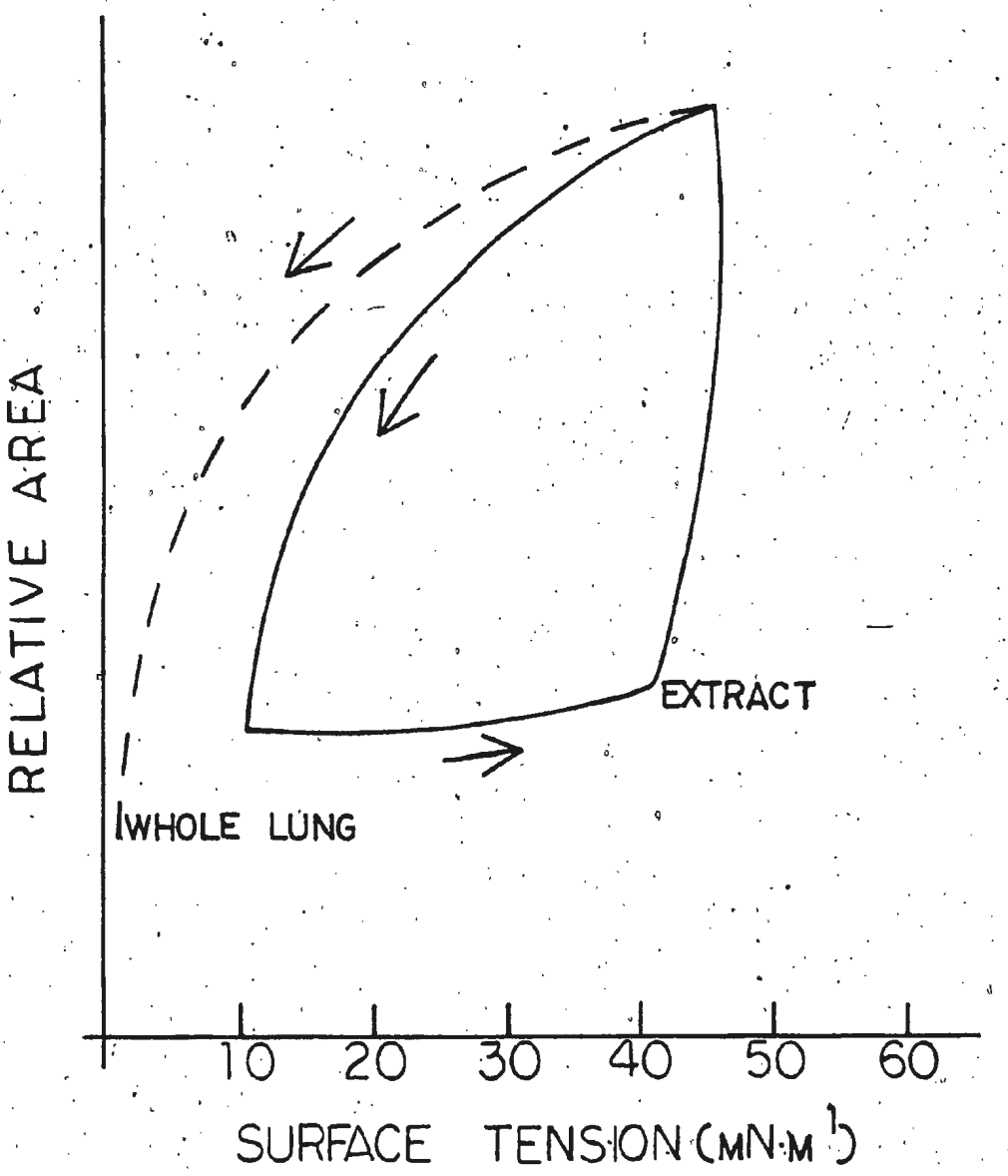
A brief history

A significant breakthrough in the understanding of respiratory mechanics came in 1955 when Pattle examined tracheal foam from rabbits with lung edema. He was surprised to notice the stability of the bubbles which arose from the foam. He attributed the stability of the foam to the insoluble surface layer inside the bubbles. These bubbles remained unchanged in size for periods of up to 60 seconds and were remarkably resistant to antifoaming agents. Their resistance was very noticeably greater than that of serum and other foams which seemed to dissolve and disappear due to what he called "internal pressures". Pattle considered the insoluble layer on the inside of the tracheal foam to be a kind of mucoprotein. He reasoned that the surface tension of the lung bubbles was zero for "if it were the same as that of an ordinary liquid, enough suction would be exerted to fill the alveoli with a transudate from the capillaries".

He also realized that the ability to reach low surface tension must be part of the design of the lung. To explain Pattle's findings one must again return to the LaPlace equation. In the stable bubble, pressures do not increase to a collapse point because the surface tension decreases appropriately; and likewise in bubbles formed from serum, the inner surface of the bubble is such that it will not sustain low surface tension and collapse occures.

Clements (1957), using a Langmuir-Wilhelmy balance, examined the surface properties of extracts of lungs from cats, rats and dogs (Fig. 4). The large loop represents lung extracts analyzed using the Wilhelmy balance and the narrow loop represents measurements of serum. The dashed curve is based on recalculation of lung pressure-volume measurements by Brown (1957). The lung extract data is clearly different from that of serum; as the surface area is decreased the surface tension drops to about 10 $mN \cdot m^{-1}$ while the serum achieved a minimum γ of about 28 $mN \cdot m^{-1}$. These results also showed that the compression portion of each curve is markedly different from the relaxation portion. Clearly, the overall process is a reversible process but possibly the mechanism of compression is different from that of relaxation. Recalculation of Brown's pressure-volume data (Brown, 1957) showed that the lung achieved a lower surface tension than the lung extract but the slopes of the curves from the pressure-volume data and surface tension data are similar during compression. The minimum surface

Figure 4. Surface tension-area isotherm based on recalculation of pressure-volume data of Brown (Whole Lung) and surface balance measurements of lung extracts (Extract). (After Clements, 1957.)



Redrawn from
Clements (1957)

tension achieved and the overall shape of the curve are similar for the PV data and lung extract but these measures were both different from serum.

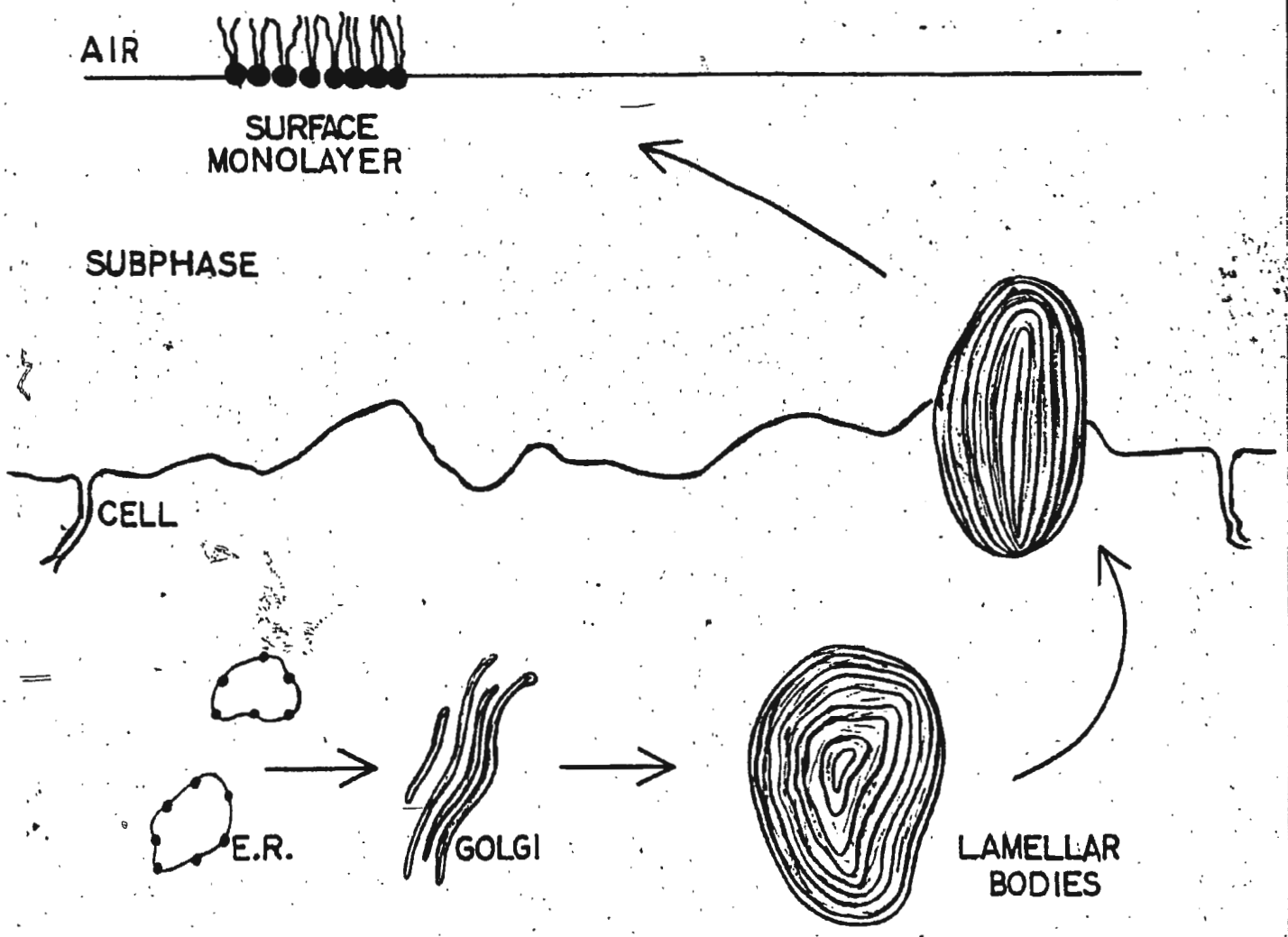
In 1959, Brown studied pulmonary preparations using a variety of techniques: i) blowing and aging of a bubble at the end of a T-tube fitted with a force transducer; ii) a modified Langmuir-Wilhelmy surface balance; and iii) pressure-volume relationships of exised lungs. With preparations made from sliced lung to expose the inner surfaces of whole lung extracts, nasal mucus, alveolar foam and blood serum, Brown found similar characteristics for all materials taken from the lung. Under compression the surface tension decreased to 10-15 $mN \cdot m^{-1}$ and on re-expansion rose to 40-50 $mN \cdot m^{-1}$. Furthermore it was found that trypsin destroyed some of the surface properties of lung extracts indicating that the effective material was proteinacious in nature. Two years later, Klaus et al., (1961) combined lyso-lecithin, sphingomyelin and dipalmitoyl phosphatidyl-choline to produce a surface tension-area diagram which closely resembled that of a whole lung extract. Both reached a minimum surface tension of 5-10 dynes cm^{-1} and had remarkably similar hysteresis loops. This was one of the first studies which directly compared a known mixture of materials with lung surfactant in an attempt to characterize the relationship between its chemical composition and its surface properties.

Brown (1964) found that surfactant was primarily

dipalmitoyl lecithin. Much research has been done to fully characterize the molecular nature of surfactant and a very thorough analysis was performed by King et al., (1972 II) on a purified fraction of canine surface active material. It was found that this material contained 11-12% protein and 87-88% lipid and very small amounts of sugar and nucleic acids. Of the 88% lipid, it was also found to contain 78% phosphatidylcholine (PC) and 63% of the PC was disaturated containing primarily palmitic acid. Similar analyses have been obtained by other researchers using different preparations (eg., Toshima et al., 1972; Traüble et al., 1974). King et al., (1973) isolated a 34-35,000 dalton protein from pulmonary surfactant which constituted 85-90% of the total surfactant protein. They also found a 10-11,000 dalton protein with a high percentage of hydrophobic residues and a great affinity for phospholipids. It may be possible that the smaller protein is a breakdown fragment of the 34,000 dalton protein. It has been observed that this larger surfactant protein may be formed late in fetal development (Gikas et al., 1977) which coincides with the appearance of disaturated lecithin and both may be determining factors in the prevention of RDS.

It has been found that alveolar lung surfactant is initially produced in the Type II cells (Macklin, 1954) and stored in the cells in the form of lamellar bodies (Askin et al., 1971). Tritiated precursors of lipid and/or protein were followed and demonstrated a rapid progression of radio-

Figure 5. Possible mechanism of formation, transport and monolayer insertion of lung surfactant. Surfactant is made on rough endoplasmic reticulum of type II pneumocytes, travels through the golgi, packaged into lamellar bodies, extruded into the subphase and inserted into a monolayer at the air-water interface. (Adapted from Goerke, 1974.)



Redrawn from GOERKE (1974)

activity from the rough endoplasmic reticulum to the golgi apparatus and into the extracellular space (bulk phase). Figure 5, based upon those by Goerke (1974) and King (1972 III), indicates current ideas about the formation and movement of surfactant to the air water interface. The surfactant which is formed on the rough endoplasmic reticulum is packaged, passes via the golgi apparatus, is stored in lamellar bodies and secreted into extracellular fluid. To achieve low surface tension, the surfactant must be inserted into a monolayer at the air-water interface. The mechanism of insertion of the monolayer has not been resolved.

The surfactant system is a complex mixture of protein and phospholipids. The remainder of this discussion will be concerned mainly with the physical properties of the phospholipids of surfactant and the ways in which the behaviour of phospholipids at the air-water interface is influenced by their thermotropic properties.

Solid pure lipids (Phillips and Hauser, 1974) and lipid mixtures (Bangham et al., 1979) when placed on a saline surface in a dry state spread only if the temperature is above the t_c of the hydrated lipid so that the lipid is fluid. Gershfeld et al., (1977, 1979) have studied the spreading of dimyristoyl phosphatidylcholine (DMPC) from bulk phase dispersions to the monolayers as a function of temperature. They found that significant increases in the amount of lipid in the monolayer only occur above the t_c of the hydrated lipid.

It has been established that dipalmitoyl phosphatidylcholine (DPPC) is responsible for the surface tension lowering ability of lung surfactant (Clements, 1967). Monolayer studies have shown that when DPPC below its t_c, is compressed in a monolayer to low areas per molecule, the surface tension is lowered to almost 0 $mN \cdot m^{-1}$ (eg. Watkins, 1968, Villalonga, 1968). As was previously discussed there are many components present in lung surfactant (eg. King et al., 1972 I: Trauble et al., 1974). Although DPPC is responsible for reaching low surface tension, it is very slow to form a monolayer from bulk phase at 37°C (eg, Tierney et al., 1965; King and Clements, 1972 III; Bangham et al., 1979). It has been proposed to be the function of the remaining phospholipids and protein in lung surfactant to aid in the insertion of DPPC into a monolayer (eg. Goerke, 1974; King et al., 1979, Clements, 1977). It is further suggested that the fluid lipid monolayer so formed undergoes refinement to leave behind rigid lipid capable of reaching low surface tension (eg. Tierney et al., 1965; Watkins, 1968; King and Clements, 1972; Hildebran et al., 1979; Bangham et al., 1979).

Phase and structure of phospholipids

Lung surfactant is found in both bilayers and monolayers and their inter-relationship is important in understanding lung function. Early monolayer studies (van Deenen et al., 1962; Standish et al., 1968) have been

applied to the elucidation of bilayer structure but the converse has not been universally true. Since lung surfactant exists as a bilayer arrangement in the cell the study of its bilayer structure can lead to a better understanding of its functional role in this state.

Phospholipids adopt a number of phases in an aqueous environment (eg., Luzzati et al., 1974). There are two main categories of these phases. The first of these is a lamellar phase which may consist of: (1) planar sheets of a one dimensional lattice (2) long ribbons of finite width organized in a two dimensional lattice and (3) discs of finite width organized in a three dimensional lattice. The second category consists of extended cylinders packed in hexagonal arrays which include Hexagonal I and Hexagonal II phases. Other phases, T and R consist of finite-length rods joined three by three or four by four forming a two dimensional hexagonal structure stacked on a three dimensional lattice.

The most predominant phases found in biological systems are the lamellar phases. Unsaturated phosphatidylethanolamines (PE's) can, however, exist in hexagonal II form (Cullis, 1979). Within the lamellar structure, there exist two biologically significant phases. The gel phase represents a phase in which the intra molecular movement of the fatty acyl chains is highly restricted. The liquid crystalline phase is characterized by a high degree of intra- and inter-molecular movement. A hydrated pure

phospholipid undergoes a transition from a gel to a liquid crystal phase at a characteristic temperature called the transition temperature, t_c (Chapman, 1975). Complex mixtures of phospholipids have transition temperatures occurring over a wide range of temperatures depending on the nature of the mixture. Gel to liquid crystal phase transitions have been demonstrated for aqueous dispersions of PC's, PE's, phosphatidic acids (PA's), phosphatidylglycerols (PG's), phosphatidylserines (PS's) phosphatidylinositiols (PI's), cardiolipins, and sphingomyelins.

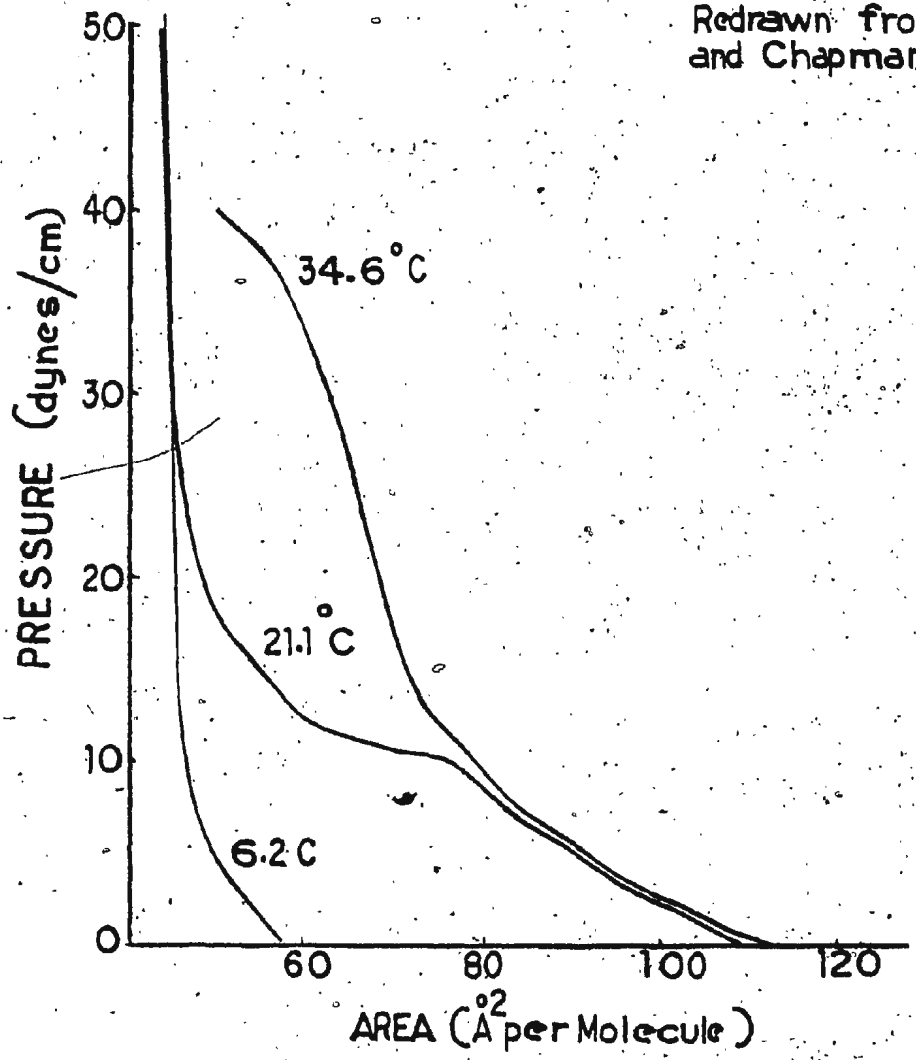
In most biological systems, the majority of phosphatidylcholines have an unsaturated chain and exist as a fluid, liquid-crystalline species at the temperature of the organism. PC in lung surfactant has a high proportion of DPPC giving it an unusually rigid character. DPPC has a t_c of 41°C, meaning that it would be in a rigid, gel phase at body temperature. It has been suggested that the high proportion of DPPC is responsible for lung stability at end expiration (Clements, 1967).

Some of the first systematic studies on the thermotropic properties of phospholipid monolayers were performed by Phillips and Chapman (1968). They obtained pressure-area (π-A) curves of various saturated, single-acid phosphatidylcholines and phosphatidylethanolamines at various temperatures. These experiments were done using a modified Langmuir surface balance. They found

that DPPC undergoes a transition from a liquid expanded to liquid condensed phase at 22°C; a similar transition was seen at 22°C for dimyristoyl PE. This transition was suggested to be similar to a gel to liquid crystal transition in bilayers. It was noteworthy that the expanded-condensed transition occurred in the two molecules with different headgroups but was not seen in dimyristoyl PC which is certainly more similar to DPPC than the PE. The key to this observation was the relationship between the temperature of the isotherm to the transition temperature of the lipids. This transition is a discontinuity in the π-A curve which results in the plateau region of the isotherm. The point at which the discontinuity occurs changes with temperature as can be seen in Fig. 6. For example the isotherm of DPPC at 6.2°C is liquid condensed (or solid) at zero surface pressure while the isotherm at 29.5°C only became condensed at 40 dyne·cm^{-1}. This was confirmed and extended in a recent study by Blume (1980) who showed for these and other lipids that the monolayer phase transition from fluid condensed to fluid expanded is shifted to higher temperature as the pressure is increased. If the temperature of a DPPC monolayer is raised above its t_c, films cannot sustain high pressures (Träuble et al., 1974).

Watkins (1968) reported that using a compression rate of 0.3 cm^2·sec^{-1}, a temperature of 25°C, there was a deflection in the compression curve of DPPC at a pressure near 45 dyne cm^{-1}; this is at a higher pressure than that

Figure 6. Pressure-area isotherm of DPPC at three temperatures: 6.2°C, 21.1°C and 34.6°C. DPPC goes through a liquid expanded to liquid condensed phase transition at 21.1°C as denoted by the discontinuity in the curve. (Adapted from Phillips et al., 1968.)


Redrawn from. Phillips and Chapman (1968)
PRESSURE (dynes/cm)
50
40
30
20
10
0
34.6°C
21.1°C
6.2 C
60
80
100
120
AREA (Å² per Molecule)

noted by Phillips and Chapman (1968). This plateau, according to Watkins denoted a loss of surface material as a result of over compression past its limiting area and represents the collapse point on the balance. This is in agreement with Gladston and Shah (1967) who described DPPC as being in a solid state above pressures of 40 dynes cm^{-1} accompanied by a loss in surface active material denoted by a reduction in hysteresis area. Walkins also noted that DPPC under high compression squeezed to a pressure of 71 $mN \cdot m^{-1}$. DMPC on the other hand reached surface pressures of only 50 $mN \cdot m^{-1}$ at which point the film collapsed. Since these experiments were performed at 25°C DMPC was already past its transition temperature (t_c = 23°).

It is apparent that above t_c when fatty acid chains are mobile there is a reduction of the maximum surface attainable by compression. In lung surfactant there are significant amounts of unsaturated lipids. It has been shown that unsaturated lecithins have a tendency to broaden and lower the transition temperature of DPPC (eg. Phillips et al., 1970; Davis et al., 1980). As a result surfactant at body temperature is in mixed fluid and rigid state. This raises the possibility of there being some other functional role for the fluid lipids rather than lowering surface tension. It has been found that DPPC is capable of spreading from a bulk crystal placed on a clean surface. This, however, may be accomplished only when the temperature is past 41°C when the lipid is in a fluid, liquid crystalline

phase (Phillips et al., 1974). Above its t_c however, DPPC is incapable of reaching near-zero surface tensions. It may be the function of the other surfactant substituents to fluidize the DPPC so that it will spread to the surface (King, 1974; Notter et al., 1975, Clements, 1977). When lower melting lecithins are mixed with DPPC in bulk phase the resultant t_c is lowered accordingly (Keough et al., 1979). This "mixed-component" surfactant has a broad phase transition from 15 to 40°C (Clements, 1977) so that it is conceivable that spreading of surface active material to the air-alveolar interface is facilitated at 37°C. It has been suggested that this type of fluidization of surfactant occurs (Bangham et al., 1979; Clements, 1977). A mechanism has been suggested by which this fluid surfactant lipid becomes rigid (Steim, 1968, Watkins, 1968; Clements, 1977; Bangham et al., 1979; Hildenbran, 1979). This mechanism involves the exclusion of the fluid lipid, leaving behind a DPPC-enriched surface capable of lowering surface tension to less than 9 $mN \cdot m^{-1}$, the value measured by Schürch et al., (1976) in situ at functional residual volume.

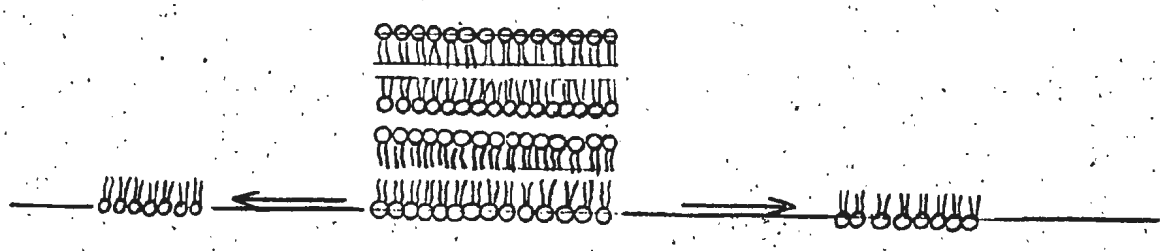
This concept of insertion and exclusion of surfactant goes back to 1965 when Tierney et al., (1965) hypothesized about the physical properties of the pulmonary surfactant monolayer. It was proposed that the composition is dependent on the solubility of the molecules in the sub-phase. They also proposed that under high compression of the surface of a monolayer, molecules must leave the surface.

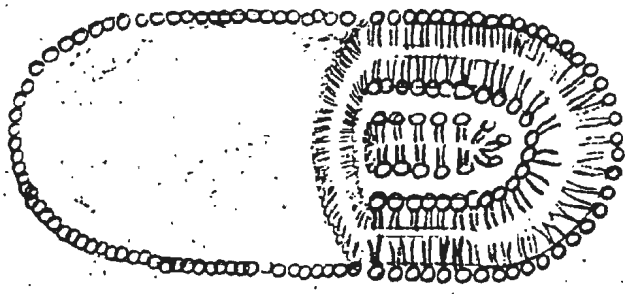
and enter the subphase. It was also speculated that some molecules are of different size and shape and have relatively weak associations which could result in one or more components being selectively squeezed from the surface. Furthermore they also suggested that surface active materials may re-enter the surface quickly when re-expanded.

A mechanism for the insertion of lipid into the monolayer in lung has been proposed by Morley et al., (1978). It involves a relatively "dry" surfactant complex (less than 15 molecules of water per molecule of phospholipid) which moves through the subphase to the air-water interface. It is suggested that spreading from the dry form is similar to spreading from a crystal (Fig. 7). This occurs however, only when the lipid is in the fluid, liquid crystalline state (Phillips et al., 1974). In support of this concept, Grathwhohl et al., (1979) have found that lamellar body structures from porcine lung have only small quantities of free water.

Gershfeld et al., (1977, 1979) examined in detail the ability of dimyristoyl phosphatidylcholine to spread to a monolayer from a bulk phase dispersion. It was observed that below the bulk phase transition temperature spreading occurred at an extremely slow rate. They explained that there exists an equilibrium between bulk-phase gel lipid and a gaseous monolayer a very low concentration of surface material. As t_c is surpassed and the lecithin becomes liquid crystal, another equilibrium is established between

Figure 7. Mechanism of insertion of surfactant from bulk phase into a monolayer at the air-water interface. Subphase lipid must be very "dry" (15 molecules of water per molecule of lipid) for insertion to be facilitated. (After Morley et al., 1978.)





the gaseous monolayer and a liquid-expanded state. This is shown by an apparent decrease in surface tension. It is noteworthy that Gershfeld et al., (1979) have found evidence that at approximately six degrees above the t_c of DMPC the surface structure may form a bilayer. Sufficient lipid is present in the surface at that point to create such a bilayer. Whether or not such a bilayer could exist in the air-water interface in alveoli and what its consequence for pulmonary function might be, are totally unknown at this time. It is interesting however, that Bangham et al., (1979) have suggested that a complex surface structure is responsible for the insertion of the monolayer but the surface structure may not be related to the surface bilayer postulated by Gershfeld et al., (1979).

It is apparent that monolayers may be formed easily from the bulk-phase lipid only when it is in a fluid state. There was reason to believe, however, that lecithins above their respective t_c's are incapable of reaching near-zero surface tension. Clements (1967) observed monolayers of egg yolk lecithin collapsed at 18 $mN.m^{-1}$ when compressed at room temperature. It is now known that these would be above their bulk-phase transition temperature. Watkins (1968) showed that DMPC, a saturated lecithin, could reach only 24 $mN.m^{-1}$ when highly compressed. The subphase temperature was reported to be poorly controlled but was about 20°C. The apparent elevated minimum surface tension indicates that the run temperature was probably nearer the t_c of DMPC which is 23°C. Trauble et al., (1974) presented isotherms of DPPC at 43°C but did

not continue the compression past approximately 20 $mN \cdot m^{-1}$. At this point however, it was evident that the shape of the curve was different than isotherms below 41°C and the monolayer could not sustain surface pressures beyond about 55 $mN \cdot m^{-1}$. If fluidized DPPC cannot maintain low surface tensions, then there must exist a mechanism by which lung lipid DPPC may become rigid. Since body temperature in the mammalian lung remains stable at 37°C, then a phase change must be effected by some means other than temperature change. It has been suggested that compression of fluid surfactant monolayer results in the exclusion of the fluid lipid leaving behind a surface enriched in DPPC (eg. Watkins, 1968, Clements, 1977, Hildebran et al., 1979). At body temperature DPPC is in a rigid gel state which is capable of lowering surface tension and stabilizing the lung at end expiration.

The fate of squeezed-out material from pulmonary surfactant is another aspect which is unresolved. There is a possibility of the formation of bilayers or micellar structures in the subphase. Another suggestion has been made regarding a more sophisticated surface structure in the form of multifolds or ridges (Ries, 1979). Notter et al., (1980) have found that some reinsertion can occur from excluded structures but it is not known if this process ia a major mechanism for providing material in the monolayer in vivo.

Objectives of the study

As early as 1963, Clements studied the effect of increasing temperature on the pressure volume relationship of mammalian lungs. He calculated that the minimum surface tension at end expiration at 27°C was 1.5 $mN \cdot m^{-1}$ while at 47°C the minimum surface tension was 18 $mN \cdot m^{-1}$. The reason for the increase in surface tension as we have discussed was because of a change of phase to a liquid crystal which is incapable of sustaining high surface pressures. A year later a similar result was seen at 48°C as well as in pneumothorax lungs (Gruenwald, 1964). Gruenwald explained the results by explaining that at 48°C "the surfactant was still present but had somehow change its state".

The mammalian lung requires rigid, gel phase lipid to sustain high surface pressures and stabilize the lung. Rigid lipid however, would not make an efficient surfactant since gel phase lipid is incapable of spreading from a bulk bilayer structure to a surface monolayer to utilize its surface activity. It has been suggested that there must exist a delicate balance of DPPC and fluidizing lipid in lung surfactant so that efficient surface spreading may be facilitated but also that the fluid lipid can be expelled on full compression (exhalation) to achieve a rigid surface monolayer (eg. Watkins, 1968; Clements, 1977; Hildebran et al., 1979; Bangham et al., 1979). This surface monolayer would be capable of stabilizing the lung. If however, the mixture of DPPC and other lipids was too fluid then

exclusion may not allow for a rigid enough surface to reach the necessary low surface tension.

To fully understand the balance of lipids needed for an efficient lung surfactant, one must have adequate knowledge of the surface and bulk phase behaviour of the lipids involved. It was the intention of this study to examine the surface characteristics of a series of pure lecithins of various types as a function of temperature to see if saturation was a *sine quo non* for achieving low surface tension.

The second objective was to study monolayers of some binary mixes of DPPC and well-defined pure fluid lipids to provide insight into potential mechanisms of surface refining. Some preliminary studies of mixtures of these pure lipids in bulk phase were also to be done.

MATERIALS AND METHODS

Materials:

Dipalmitoyl phosphatidylcholine (DPPC), dimyristoyl phosphatidylcholine (DMPC), dilauryl phosphatidylcholine (DLPC), 2-lyso-1-oleoyl phosphatidylcholine, egg phosphatidylglycerol (egg PG), and dipalmitoyl phosphatidylethanolamine (DPPE) were all purchased from Sigma Chemical Company, St. Louis, Mo. N;N-dimethyl dipalmitoyl phosphatidylethanolamine (N,N-diMeDPPE) and N-methyldipalmitoyl phosphatidylethanolamine (N-Me-DPPE) were obtained from Calbiochem. One batch of 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (POPC) was purchased from Applied Science Laboratories, State College, PA. 2-oleoyl-1-stearoyl-sn-glycero-3-phosphocholine (SOPC) and its positional isomer 1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine were prepared by the method described by Keough and Davis (1979) and of Davis et al., (1980). A second batch of POPC, used in mixed monolayer experiments, and 2-linoleoyl-1-stearoyl-sn-glycero-3-phosphocholine (SLPC) were prepared by the methods described by Cubero Robles et al., (1967) and Roseman et al., (1978). All lipids showed one spot on thin layer chromatography although a small amount of 1,3-isomers was detected on occasion. Positional analysis, as described by Keough and Davis, (1979) showed that of the two batches of SOPC prepared one contained 6% of its opposite isomer while the other contained 18% OSPC. Analysis of OSPC showed that it contained 17% SOPC while the

POPC made in our lab contained 12% of the opposite isomer; POPC from Applied Science proved to be 12% acyl migrated and contained trace amounts of stearate and palmitoleate (0.5-1.5%) DPPC was purified by thin layer chromatography and stored at -20°C in chloroform:methanol, 1:1. All syntheses of the mixed acid lecithins were carried out by Dr. K.P. Coolbear and Mr. P.J. Davis. The structures and abbreviations used for all phospholipids are given in Appendix A.

Solvents were purchased from Fisher Scientific, Dartmouth, Nova Scotia. Hexane used was pesticide grade while the methanol was 99.9 mole percent pure. Acetone and chloroform were purchased as A.C.S. grade and the chloroform was redistilled in glass. Diethyl ether was anhydrous, A.C.S. grade purchased from Fisher Scientific.

Water used to form subphase solutions was deionized and doubly distilled in glass, the second time from alkaline permanganate. The subphase used in monolayer experiments was 0.15 M sodium chloride. The pH of the subphase was monitored periodically and found to be between 5 and 6.

Methodology:

Surface tension measurements were accomplished using a modified Langmuir-Wilhelmy balance bought from Kimray Medical Associates, Oklahoma City, Oklahoma. The balance employs a teflon trough (5.0 cm x 11.5 cm) and a motor-driven teflon barrier whose speed may be varied from 0.17 to 3.6 $cm^2 \cdot sec^{-1}$ by changing the combination of the

drive pulleys. The pool size may be reduced from 100 percent pool size (53.4 cm^3) to 15 percent pool size at compression rates varying from 9 minutes to 30 seconds per cycle.

Some troughs and wipers were made to the same specifications by the university shops.

The surface tension was measured using a platinum dipping flag (5 cm in perimeter) connected to a simple force transducer and a recording scriber. The flag was roughened with fine carbonundum paper and flamed prior to use. The teflon dam and trough were cleaned by soaking them overnight in acetone, followed by three acetone washes and one ether wash, and allowed to air dry. Before forming monolayers, the surface of the saline subphase was aspirated until no reduction in surface tension occurred at maximum compression. This indicated that all surface material had been removed. With excessive wear, dams and troughs had to be replaced. This was evident by the inability of DPPC to reach near zero $mN \cdot m^{-1}$. Monolayers of DPPC were checked on a regular basis for this purpose and to maintain good quality control.

Surface tension measurements were made in an environmental chamber built by the University shops. The chamber, which was thermostatically controlled, could maintain the air temperature from 0-50°C with an accuracy of $\pm$ 1°C. Air temperature was monitored continuously and the temperature of the subphase was measured subsequent to each experiment by dipping the thermometer directly into the subphase. It is the subphase temperatures that are given in the results. Air temperatures

were generally 1-2°C higher than the subphase temperature.

Spreading of monolayers

Lipids were stored in chloroform-methanol (1:1) (v/v) at -20°C at a known phosphorous concentration as determined by the method of Fiske and Subarrow (1925). To form a monolayer an appropriate concentration of lipid was blown to dryness under nitrogen and redissolved in a known volume (1×10^{-5} to 5×10^{-5} L) of hexane methanol (98/2, v/v). The hexane solution was applied dropwise to a clean saline surface and the solvent was allowed to evaporate for five minutes before compression was begun. Calculations and other determinations were generally done on the first compression unless otherwise stated. This was to ensure that surface concentrations were accurately known; compression to low surface areas generally result in loss of material from the monolayers.

Bulk-phase dispersions

Bulk phase dispersions were prepared to achieve a final lipid concentration of 0.5 mg of lipid per ml of saline. In each case, DPPC was weighed accurately (to the nearest 0.1 mg) and dissolved in a minimum of chloroform in a large test tube. In binary and tertiary mixtures, known amounts of other constituents dissolved in chloroform-methanol (1:1) were added to the DPPC solution. The solvent was evaporated to dryness using a gentle stream of nitrogen and evacuated under reduced pressure for 2 hours. Fonty mls

of 0.15 M saline was added to the test tube containing the mixture of lipids and was then heated to 50°C. The dispersion was vortexed to achieve a homogeneous suspension and allowed to equilibrate at 37°C. The dispersion was then poured into the surface balance apparatus which had been pre-cleaned and zeroed. Various compression rates and mixture were employed and are described in the results section.

Pig lung surfactant preparation

Pure porcine surface active material was prepared according to the method of King & Clements (1972 I). Certain modifications to the procedure were necessary to achieve appropriate centrifugation forces and volumes. The pig lung was attained from the Provincial Abbatoir and put on ice immediately. Solutions used in centrifugation procedure were prepared according to the published method except in the case of solution 2 in which 0.6 M sodium bromide was used instead of 0.06 M NaBr. These solutions are given in Appendix B.

With a 50 ml plastic syringe and a piece of tapered Nalgene tubing 600 ml of solution 1 was infused endotracheally. The whole lung was massaged gently by hand and the previously infused material was withdrawn. This procedure was repeated twice. 1450 ml of lung wash was collected and spun at 900 rpm (160 x g_{av}) for 5 min in a GSA rotor in a Sorvall RC-3 centrifuge. The combined supernatants were then centrifuged at 40,000 rpm

(125,000 x g_{av}) for 48 min in a Beckman 42.1 rotor. The supernatant was then discarded and the resultant pellet was suspended in 152 mls of solution 4 (King and Clements 1972 I) and spun at 24,000 rpm (75,000 x g_{av}) for 15 hours in a Beckman SW 27 rotor. A small, brown pellet and clear supernatant were discarded while the white floating pellicle was resuspended in 70 mls of solution 1 and spun at 28,000 rpm (62,000 x g_{av}) for 2 hrs in a Beckman 42.1 rotor. The resultant supernatant was discarded and the pellet was resuspended in 78 mls of solution 3. A continuous gradient was made with solution 2 and spun 15 hrs at 24,000 rpm in a SW 27 rotor. Surface active material appeared as a white band approximately one-half way up the tube. This material was collected by aspiration, suspended in solution 1 and spun at 29,500 rpm (65,900 x g_{av}) for 2 hrs in a Beckman 42.1 rotor. The pellet was resuspended in solution 3 and spun 15 hrs (81,500 x g_{av}) in the 42.1 rotor. The floating pellicle was aspirated, suspended in distilled water and centrifuged for 2 hrs at 65,900xg in a Beckman 42.1 rotor. The pellet was suspended in distilled water and dialyzed with distilled water for 48 hrs at 4°C. The non-diffusable material was considered to be the surface active material. The final concentration of the dialysate was 1.0 µmole P per ml in a final volume of 31.5 mls.

Assuming it had a phosphate content of 2.9% by weight, the value found for purified dog lung SAM by King and Clements (1972 II), our dialyzed dispersion would have contained approximately 1.1 mg of total solids per ml.

Rabbit lung surfactant preparation

A preliminary experiment was carried out to compare the surface properties of lung lavage material to those of purified surfactant and synthetic phospholipids. Lavage material was obtained from the lungs of an adult, female, New Zealand White Rabbit. A tracheostomy tube fitted with a 50 ml syringe was inserted into the trachea and the lung was gently lavaged with 40 ml of physiological saline (0.9% (w/v NaCl). The material was collected and the procedure was repeated with two 30 ml aliquots of saline. The three washings were combined and centrifuged for 5 minutes at 500 x g in a SS34 rotor in a Sorval RC2B centrifuge. It was the combined supernatants which were examined in this experiment.

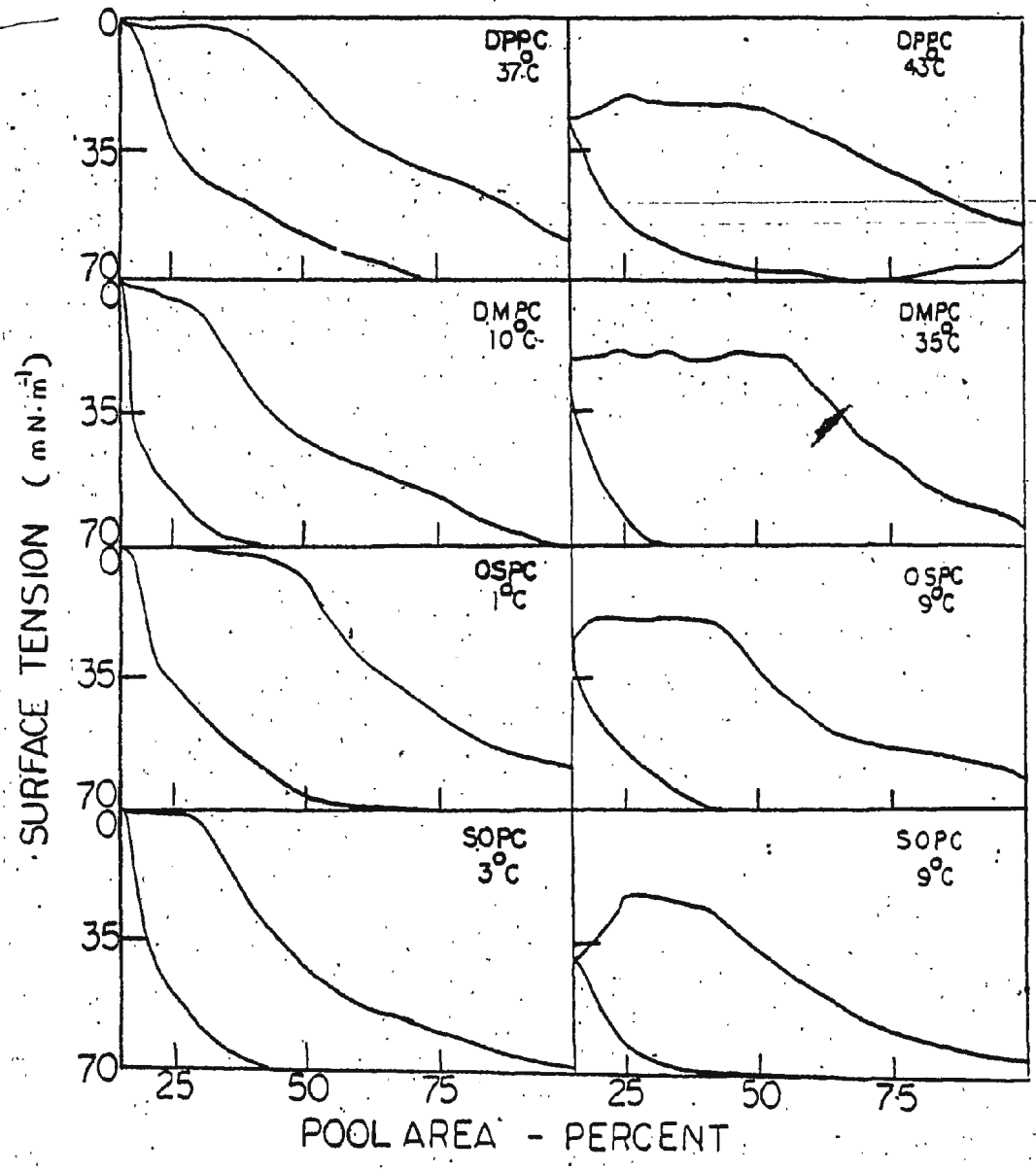
RESULTS

Simple lecithin monolayers

The lung surfactant system requires other lecithins besides DPPC to attain lung stability. As a result this study investigated a series of saturated and unsaturated lecithins. It had generally been assumed that monolayers of unsaturated lecithins would not sustain high surface pressures on high compression (eg. Clements 1963) and it had been stated that they were being compressed above their t_c's. While some reports supported this idea there were no systematic studies to sort out the relationship between lipid fluidity, lipid unsaturation and the ability of the appropriate lipids to reach very low surface tension when compressed. Using a surface balance and a temperature-controlled chamber, we undertook an investigation of monolayers of both saturated and unsaturated lecithins with known t_c's to determine whether the conditions for achieving low surface tension were either rigid chains or saturated chains or both.

Single component monolayers of various disaturated and mono-unsaturated lecithins were examined using a modified Langmuir-Wilhelmy type surface balance. Typical isotherms are outlined in Fig. 8. The structures and abbreviations for all phospholipids are given in Appendix A. In these examples compression of a fully expanded monolayer was effected at a rate of 0.172 $cm^2 \cdot sec^{-1}$ using a surface load of approximately 0.008 micromoles (μmole). Such a compression

Figure 8. Typical surface tension-area isotherms of various lecithins above and below their gel to liquid crystalline transition temperature. Surface lipid concentration was 0.008 μmole. Compression rate was 0.17 $cm^2 \cdot sec^{-1}$.


DPPC 37°C
DPPC 43°C
DMPC 10°C
DMPC 35°C
OSPC 1°C
OSPC 9°C
SOPC 3°C
SOPC 9°C
0
35
70
25
50
75
SURFACE TENSION (mN·m⁻¹)
POOL AREA - PERCENT

of dipalmitoyl phosphatidylcholine (DPPC) at 37°C results in a minimum surface tension (γ_{min}) of about 1 $mN \cdot m^{-1}$. This result is similar to those obtained elsewhere (Watkins, 1968; Hildebran et al., 1979). If however, the experimental temperature was increased to 43°C, past the transition temperature of DPPC (Chapman, 1975), the isotherm which results is in frame b of Fig. 8. As can be seen the minimum surface tension for DPPC past its bulk phase transition temperature is 20 $mN \cdot m^{-1}$.

To ensure that this effect was not peculiar to only DPPC, a lower melting disaturated lecithin, DMPC, was examined under similar conditions below and above its phase transition temperature of 23°C. At 10°C, below its t_c, DMPC can easily approach 0 $mN \cdot m^{-1}$, but can only achieve 20 $mN \cdot m^{-1}$ above its t_c. Figure 8 also shows isotherms for OSPC and SOPC which had t_c's of 8°C and 6°C respectively. For both lecithins, monolayers compressed below t_c achieved 0 $mN \cdot m^{-1}$ while above t_c, γ of only 20 mN $mN \cdot m^{-1}$ could be reached. POPC, DLPC and SLPC, because their t_c's are below 0°C could only be examined above t_c. Table 1 shows that the results of these isotherms indicate that above t_c, these lecithins are incapable of reaching near zero surface tension.

The preceeding results indicate that for a lecithin monolayer to reach low surface tension, a rigid or gel-phase lipid is necessary. These results were extended by performing isotherms at a wider variety of temperatures. These results are summarized in Table 1. From the work of Clements (1967)

Table 1. Minimum surface tension achieved by a series of monolayers of lecithins at different temperatures. T_c = gel to liquid crystalline transition temperature; T_m = measurement temperature; γmin = minimum surface tension achieved at maximum compression; F = fast compression at 1.02 $cm^2 \cdot sec^{-1}$; S = slow compression at 0.17 $cm^2 \cdot sec^{-1}$.

Lipid	T_c, °C	T_m, °C	$T_m - T_c$, °C	Compression Rate	min $mN \cdot m^{-1}$
DPPC	41 (Ref.3)	14	-27	S	0
		23	-18	S	0
		33	-8	S	1
		37	-4	S	0-1
		37	-4	F	0-1
		44	+3	S	14-16
DMPC	23 (Ref.3)	2	-21	S	0
		10	-13	S	0
		14	-9	S	2
		33	+10	S	19
		36	+13	F	16
		36	+13	S	17
DLPC	-2	0	+2	S	17
		10	+12	S	15
SOPC	6	0	-6	F	0
		0	-6	S	0
		3	-3	S	0
		9	+3	S	23
		36	+30	F	15-18
		36	+30	S	20-22
OSPC	8	1	-7	S	0
		9	+1	S	23
POPC	-6*	4	+10	S	17
		13	+19	S	20
		42	+48	S	20

* POPC from Applied Science

on DPPC and PC's containing unsaturated chains it had been thought that PC's with saturated chains were necessary to achieve very low surface tension during compression. The data in Table 1 show that this is not the case. Unsaturated lecithins when compressed below their t_c (eg. SOPC and OSPC at 0^oC) are capable of achieving surface tensions near 0 $mN \cdot m^{-1}$. This indicates that the ability to reach low surface tension is related to phase rather than the structure alone and that lecithins with "rigid" acyl chains are required.

Monolayers containing binary mixtures of lecithins

Lung surfactant is a complex, multicomponent system in which the ability to provide pulmonary stability requires a fine balance between DPPC and its other constituents. This study has investigated simple binary model systems of DPPC and unsaturated lecithins to aid in understanding their basic interactions. Figure 9 shows a series of isotherms of mixtures of DPPC and SOPC at 37^oC. The dashed line represents the first compression which was at a rate of 0.172 $cm^2 \cdot sec^{-1}$ and solid line is the second compression at a rate of 1.02 $cm^2 \cdot sec^{-1}$. Both isotherms were a result of the same initially spread monolayer and similar results were attained for the opposite compression rate sequence (ie 1.02 $cm^2 \cdot sec^{-1}$ first and 0.172 $cm^2 \cdot sec^{-1}$ second). On the faster compression each monolayer could reach a lower surface tension than that achieved at the slower rate. Mixtures containing less than 50 percent SOPC are capable of achieving less than 10 $mN \cdot m^{-1}$

for the compression at 1.02 $cm^2 \cdot sec^{-1}$ but only 12-20 $mN \cdot m^{-1}$ for the corresponding mixture at the slower rate. The mixtures containing 70% SOPC, on the other hand, did not achieve γmin of 12 $mN \cdot m^{-1}$ at either compression rate.

To see if this effect of lowering of surface tension was related to lipid phase and not lipid structure a number of controls were examined (see Fig. 10). At 0°C both DPPC and SOPC as well as mixtures of DPPC:SOPC (65:35 and 30:70) were capable of reaching nearly 0 $mN \cdot m^{-1}$, since the measurement was done below both of their t_c's. If however, mixtures of DMPC and SOPC were measured at 37°C neither the one containing 65% nor 30% DMPC was able to reach 0 $mN \cdot m^{-1}$. At 37° both components were above their respective transitions temperatures resulting in a fluid monolayer.

To check that this effect of the lowering of surface tension in mixtures of DPPC and SOPC was not a result of the continued cyclic compressions, separate monolayers were checked at both compression rates. Figure 11 shows a series of isotherms of separate monolayers of DPPC/SOPC mixtures at three different surface concentrations and two rates 0.17 cm^2/sec and 1.02 cm^2/sec. Previous isotherms were obtained using a surface concentration capable of undergoing at least two compressions. The effect of surface concentration was examined by applying individual monolayers of 0.0075, 0.015 and 0.025 µmole of each mixture. Monolayers compressed at 1.02 $cm^2 \cdot sec^{-1}$ reached a lower surface tension than comparable mixtures compressed at

Figure 9. Surface tension area isotherms of monolayers of mixtures of DPPC and SOPC at 37°C.
-·-· First compression at 0.17 $cm^2 \cdot sec^{-1}$.
—— Second compression at 1.02 $cm^2 \cdot sec^{-1}$.

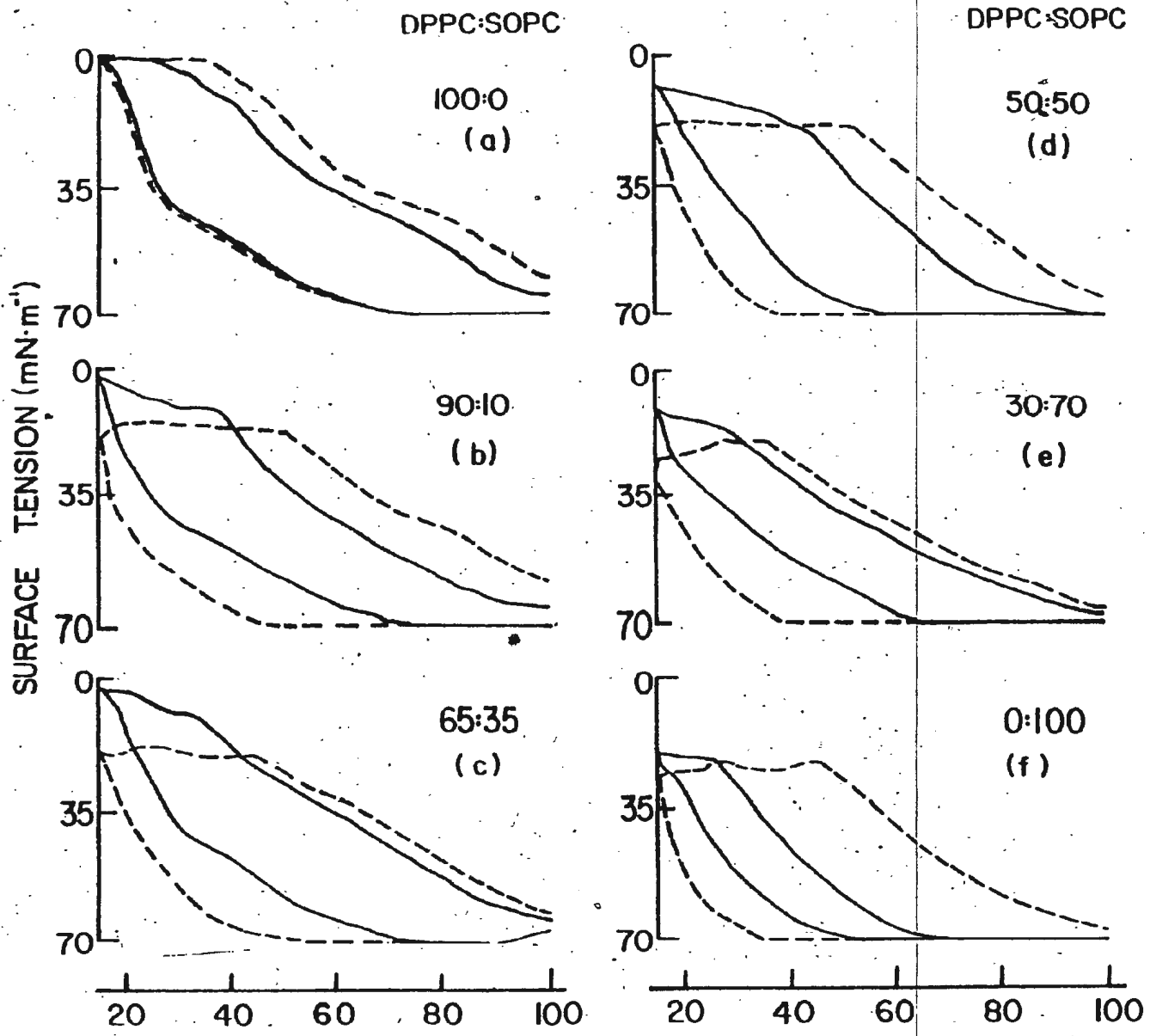

DPPC:SOPC
100:0
(a)
DPPC:SOPC
50:50
(d)
90:10
(b)
30:70
(e)
65:35
(c)
0:100
(f)
0
35
70
SURFACE TENSION (mN·m⁻¹)
20
40
60
80
100
POOL AREA - PERCENT

Figure 10. Surface tension - area isotherms of various mixtures of lipids above and below their bulk phase transition temperatures.
--- First compression at 0.17 $cm^2 \cdot sec^{-1}$.
—— Second compression at 1.02 $cm^2 \cdot sec^{-1}$

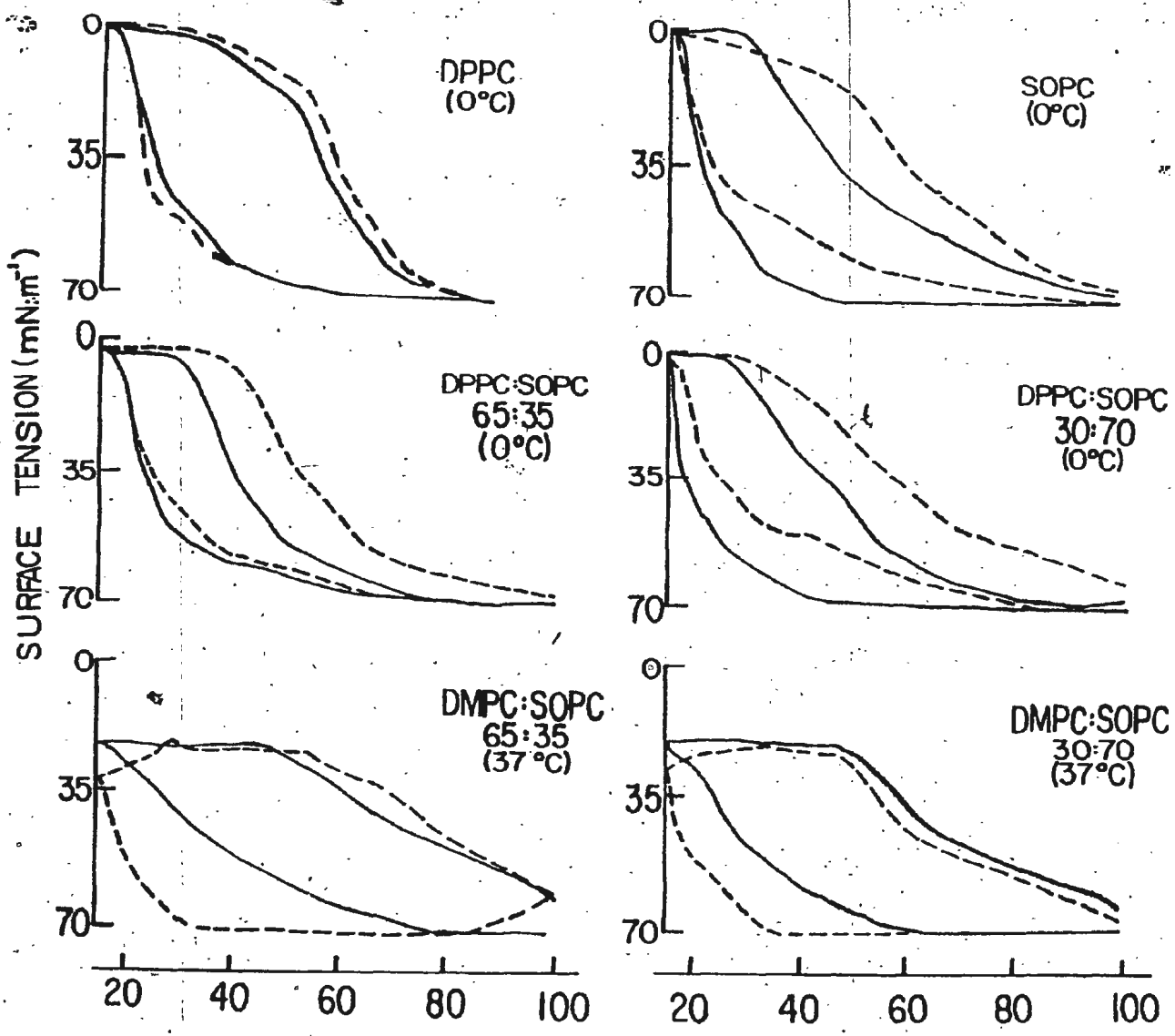
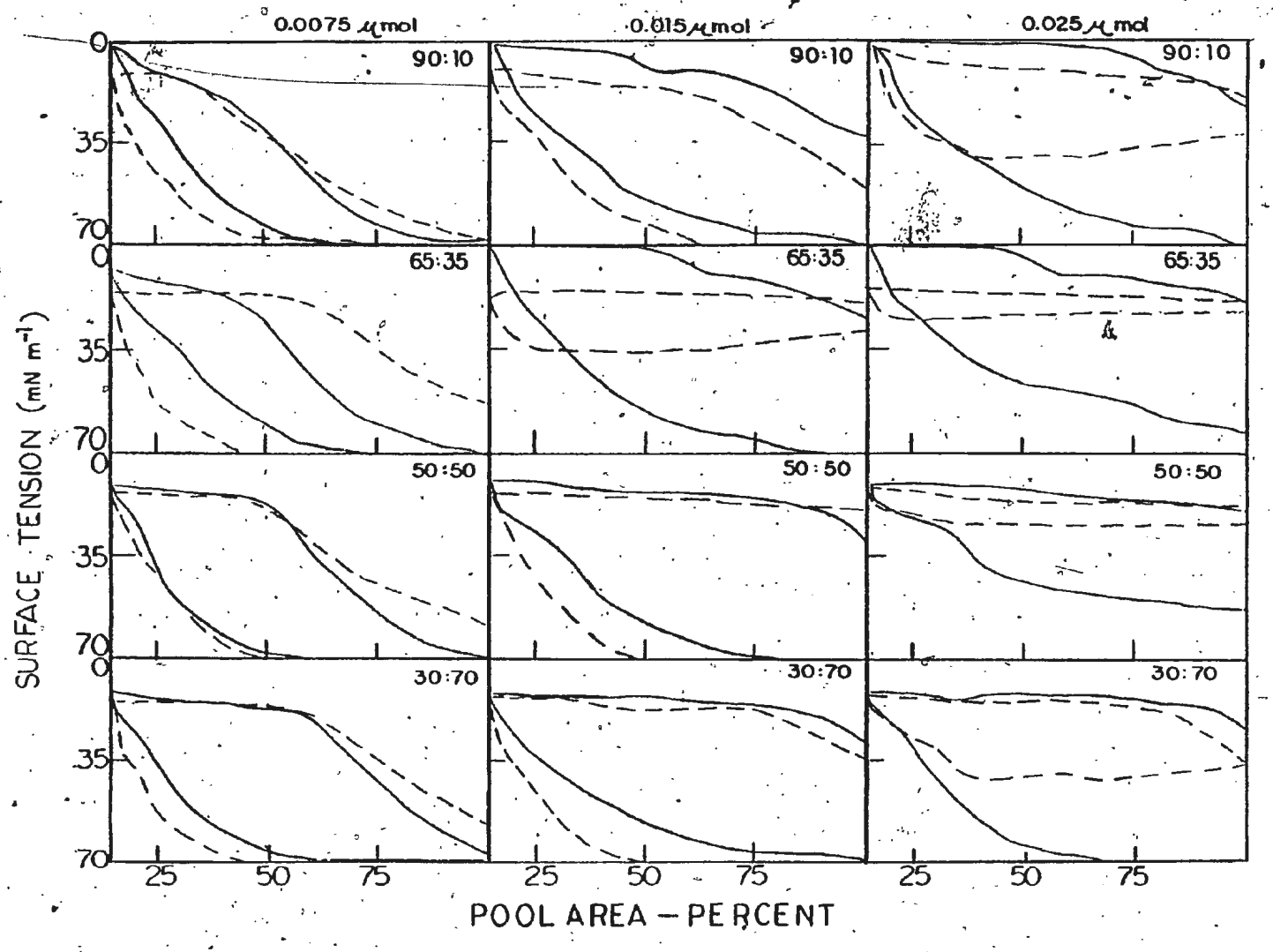

SURFACE TENSION (mN·m⁻¹)
0
35
70
DPPC
(0°C)
SOPC
(0°C)
DPPC:SOPC
65:35
(0°C)
DPPC:SOPC
30:70
(0°C)
DMPC:SOPC
65:35
(37°C)
DMPC:SOPC
30:70
(37°C)
20
40
60
80
100
POOL AREA - PERCENT

Figure 11. Surface tension - area separate isotherms of DPPC/SOPC mixtures at 3 surface concentrations, (0.0075, 0.015, and 0.025 μmoles) compressed at 37°C. Compression rates of 0.17 $cm^2 \cdot sec^{-1}$ (---), and 1.02 $cm^2 \cdot sec^{-1}$ (——).


0.0075 μmol
0.015 μmol
0.025 μmol
90:10
65:35
50:50
30:70
0
35
70
SURFACE TENSION (mN m⁻¹)
25
50
75
POOL AREA – PERCENT

0.172 $cm^2 \cdot sec^{-1}$. For some mixtures the ability to reach low surface tension was also somewhat dependent on the lipid surface concentration. As the surface concentration was increased the minimum surface tension achieveable was decreased accordingly. For example, mixtures containing 65/35 DPPC/SOPC reached lower surface tensions when initiated from a surface load of 0.025 µmole lipid than a monolayer containing 0.0074 µmole lipid when compressed at 1.02 $cm^2 \cdot sec^{-1}$. These results are expressed graphically in Figure 12. Compressions at 1.02 $cm^2 \cdot sec^{-1}$ of the 0.015 µmole nd 0.025 µmole per surface were very similar in their ability to reach low surface tension. The two highest concentrations reached lower surface tensions than the 0.0075 µmole monolayer especially at the 65/35 molar ratio. For the 0.172 $cm^2 \cdot sec^{-1}$ compression rate, a lower surface tension was achieved for the monolayer containing 0.025 µmole than either of those having 0.0075 or 0.015 µmole especially for the 90/10, 65/35 and the 50/50 molar mixtures.

The surface balance was modified to obtain cycling rates up to 3.6 $cm^2 \cdot sec^{-1}$ and monolayers were compressed at this rate. The results are shown in Fig. 13. The compressions at 3.6 $cm^2 \cdot sec^{-1}$ reached lower surface tension than either the corresponding isotherms obtained at rates of 0.172 or 1.02 $cm^2 \cdot sec^{-1}$. For example, for a 30/70 mixture of DPPC/SOPC, the minimum surface tension for the fastest rate was 11 $mN \cdot m^{-1}$ while it was 15 $mN \cdot m^{-1}$ and 14 $mN \cdot m^{-1}$ for the 0.172 and 1.02 $cm^2 \cdot sec^{-1}$ rates respectively. It is

Figure 12. Effect of surface concentration of DPPC/SOPC monolayers on their ability to reach low surface tension at 37°C. Surfaces were compressed at 0,17 $cm^2 \cdot sec^{-1}$ and 1.02 $cm^2 \cdot sec^{-1}$.

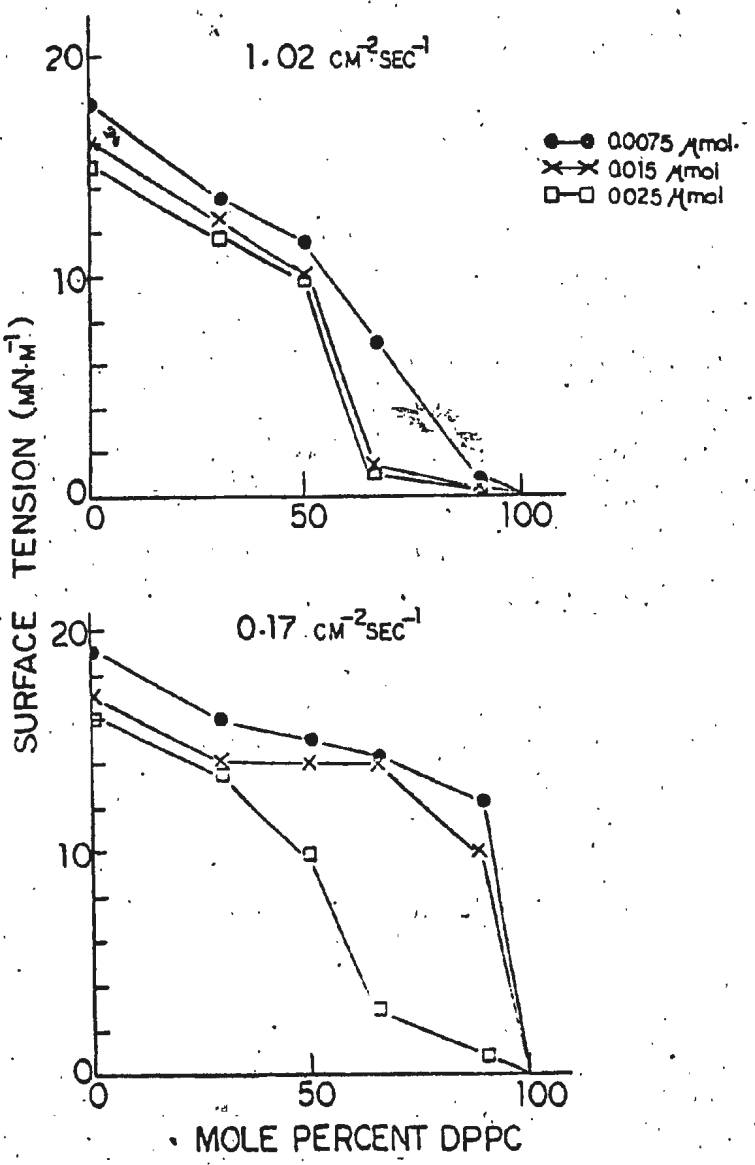
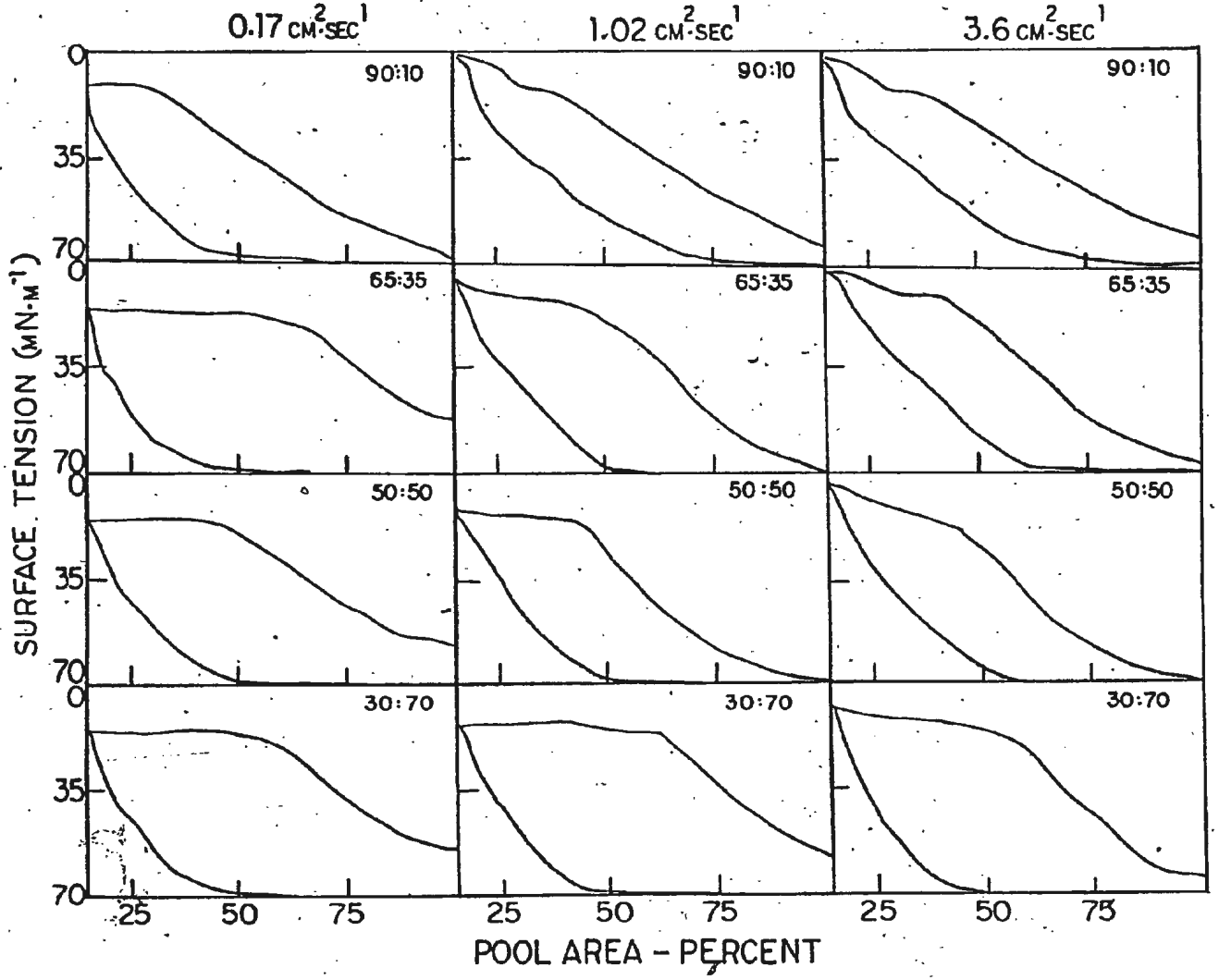

1.02 cm⁻²sec⁻¹
0.0075 μmol
0.015 μmol
0.025 μmol
0.17 cm⁻²sec⁻¹
SURFACE TENSION (mN·m⁻¹)
20
10
0
0
50
100
MOLE PERCENT DPPC

Figure 13. Surface tension - area isotherms of mixtures of DPPC/SOPC at 37°C at a surface concentration of 0.0075 μmole. Isotherms compressed at 0.17, 1.02 and 3.6 $cm^2 \cdot sec^{-1}$. The molar ratio of DPPC to SOPC is given in each panel.


0.17 CM²·SEC⁻¹
1.02 CM²·SEC⁻¹
3.6 CM²·SEC⁻¹
90:10
65:35
50:50
30:70
0
35
70
SURFACE TENSION (MN·M⁻¹)
25
50
75
POOL AREA - PERCENT

noteworthy however, that whatever the compression rate there existed a graduation in γmin which depended on DPPC content.

To make sure that this effect of surface tension lowering was not peculiar to SOPC and DPPC a series of binary monolayers were examined using other lecithins mixed with DPPC. Figure 14 shows isotherms of DPPC and POPC at a surface concentration of 0.0075 µmole at three different compression rates. It is obvious that the same effect of a stepwise variation in minimum surface tension is obtained where an increasing concentration of POPC results in an elevated γmin. It is also apparent that the minimum surface tension is dependent on compression rate. Invariably the minimum surface tension is lower for a compression at 3.6 $cm^2 \cdot sec^{-1}$ than 1.02 or 0.172 $cm^2 \cdot sec^{-1}$ for a comparable molar mixture. Even at the fastest rate however, a 30/70 mixture of DPPC and POPC was incapable of reaching surface tensions below 12-13 $mN \cdot m^{-1}$.

In an attempt to investigate whether the achievement of low γ min in mixed lecithin monolayers was a function of phase, structure, or both, a similar set of isotherms were examined using dilauroyl phosphatidylcholine (DLPC) as the fluid lipid. DLPC is a disaturated lecithin, like DPPC, but has a $t_c = -2^oC$, very near that of POPC ($t_c = -3^oC$). Figure 15 shows the results of such experiments using DPPC and DLPC at a surface concentration of 0.0075 µmole compressed at the three different rates. The same general effect of the lowering of surface tension is seen

Figure 14. Surface tension - area isotherms of mixtures of DPPC and POPC at 37°C at a surface concentration of 0.0075 μmole. Isotherms compressed at 0.17, 1.02 and 3.6 $cm^2 \cdot sec^{-1}$. The molar ratio of DPPC to POPC is given in each panel.

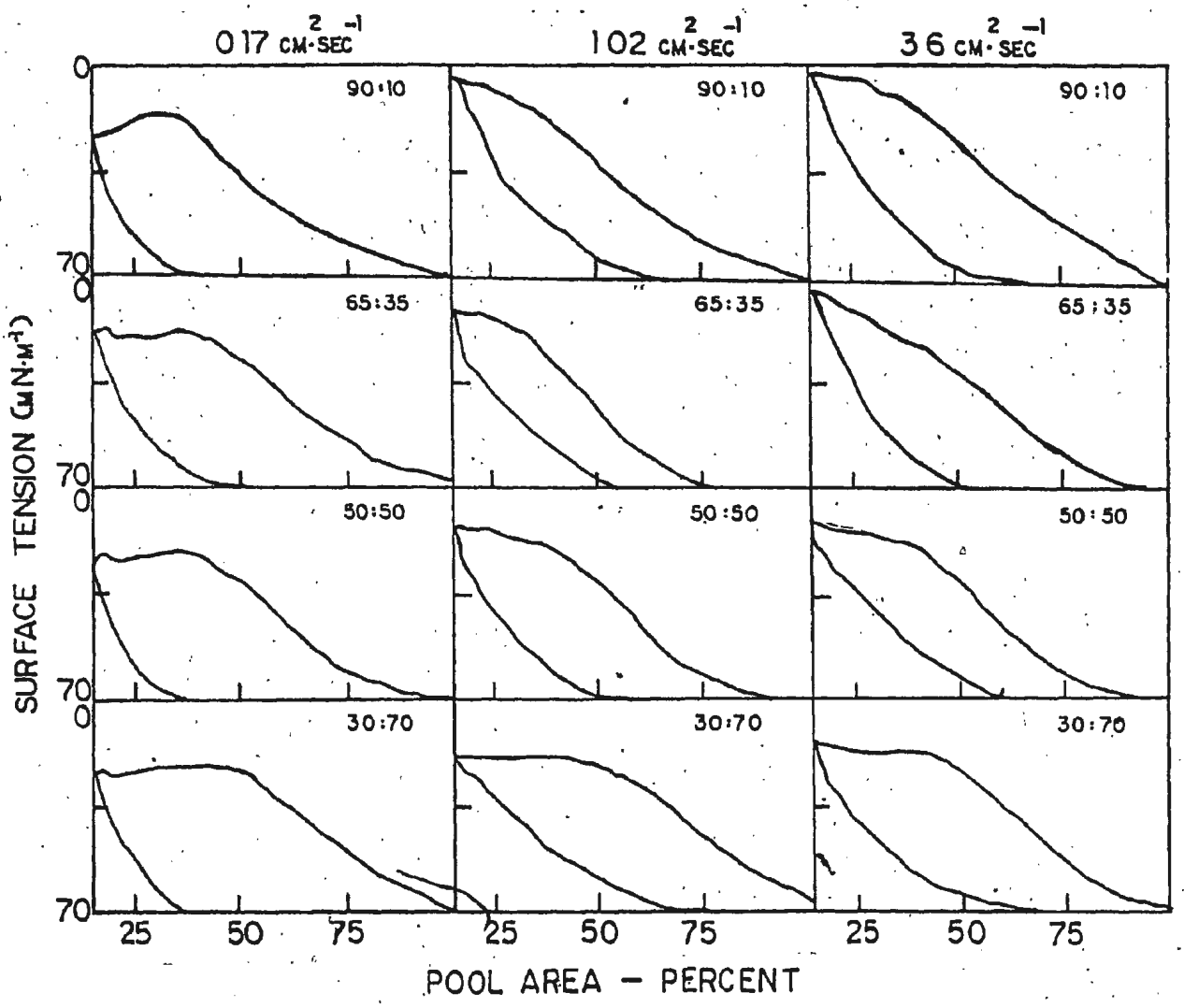
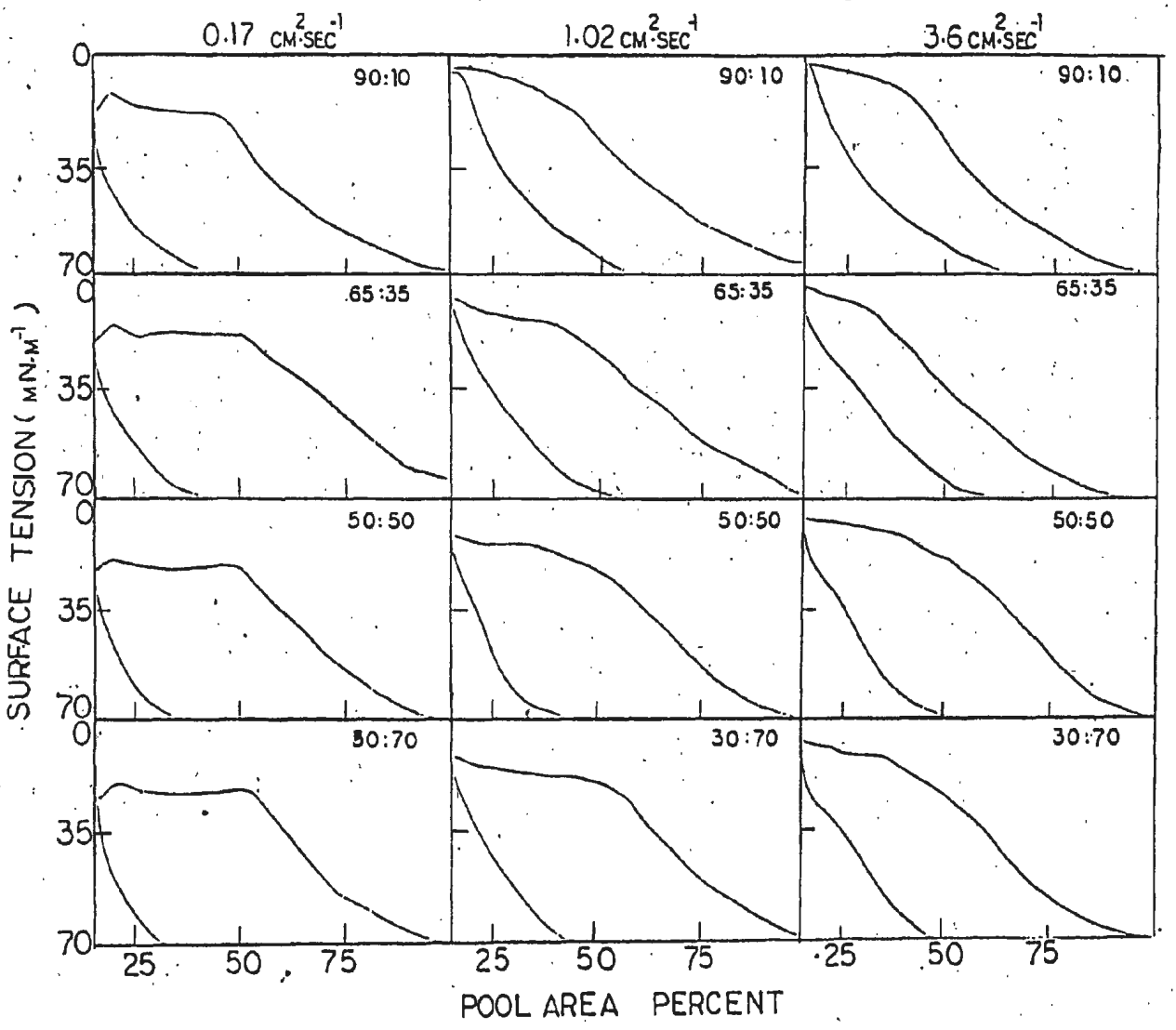

0.17 CM·SEC⁻²
1.02 CM·SEC⁻²
3.6 CM·SEC⁻²
90:10
65:35
50:50
30:70
0
70
SURFACE TENSION (mN·m⁻¹)
25
50
75
POOL AREA – PERCENT

Figure 15. Surface tension - area isotherms of mixtures of DPPC and DLPC at 37°C at a surface concentration of 0.0075 μmole. Isotherms compressed at 0.17, 1.02 and 3.6 $cm^2 \cdot sec^{-1}$. The molar ratio of DPPC to DLPC is given in each panel.


0.17 CM²·SEC⁻¹
1.02 CM²·SEC⁻¹
3.6 CM²·SEC⁻¹
90:10
65:35
50:50
30:70
0
35
70
SURFACE TENSION (MN·M⁻¹)
25
50
75
POOL AREA PERCENT

and is similarly dependent on the relative concentrations of the two components and the rate of compression.

Stearoyl linoleoyl phosphatidylcholine (SLPC) (t_c = -18°C), a more fluid lecithin, was also tested for its ability to affect DPPC's surface activity. 37°C isotherms at the four concentrations and three compression rates are shown in Fig. 16. As can be seen a similar pattern of results as the three previous mixtures is obtained.

Figure 17 shows a graphic comparison of the minimum surface tension obtained by all four lipid mixture of monolayers compressed at three different rates. The ability of monolayers to achieve γmin was dependent on the type of fluid lecithins used and their proportions with DPPC. The difference was greatest between mixtures of POPC/DPPC in comparison to SOPC/DPPC. In most cases mixtures of SOPC reached lower surface tensions than comparable mixtures of the other three lipids. For compression rates of 1.02 $cm^2 \cdot sec^{-1}$ and 3.6 $cm^2 \cdot sec^{-1}$, mixed monolayers of DLPC and DPPC behaved similarly to those of SOPC plus DPPC. The γmins, however, of all pure lipid crystalline lecithins were similar for equivalent compression rates.

It is evident that reaching low surface tensions is dependent upon compression rate because invariably a faster compression reached to lower γmin than a slower one. Also, it is very clear that γmin is dependent on the relative concentrations of the two lipids. In all cases, the mixtures with the lowest percentage of fluid lecithin reached a

Figure 16. Surface tension - area isotherms of mixtures of DPPC and SLPC at 37°C at a surface concentration of 0.0075 μmole. Isotherms compressed at 0.17, 1.02 and 3.6 $cm^2 \cdot sec^{-1}$. The molar ratio of DPPC to SLPC is given in each panel.

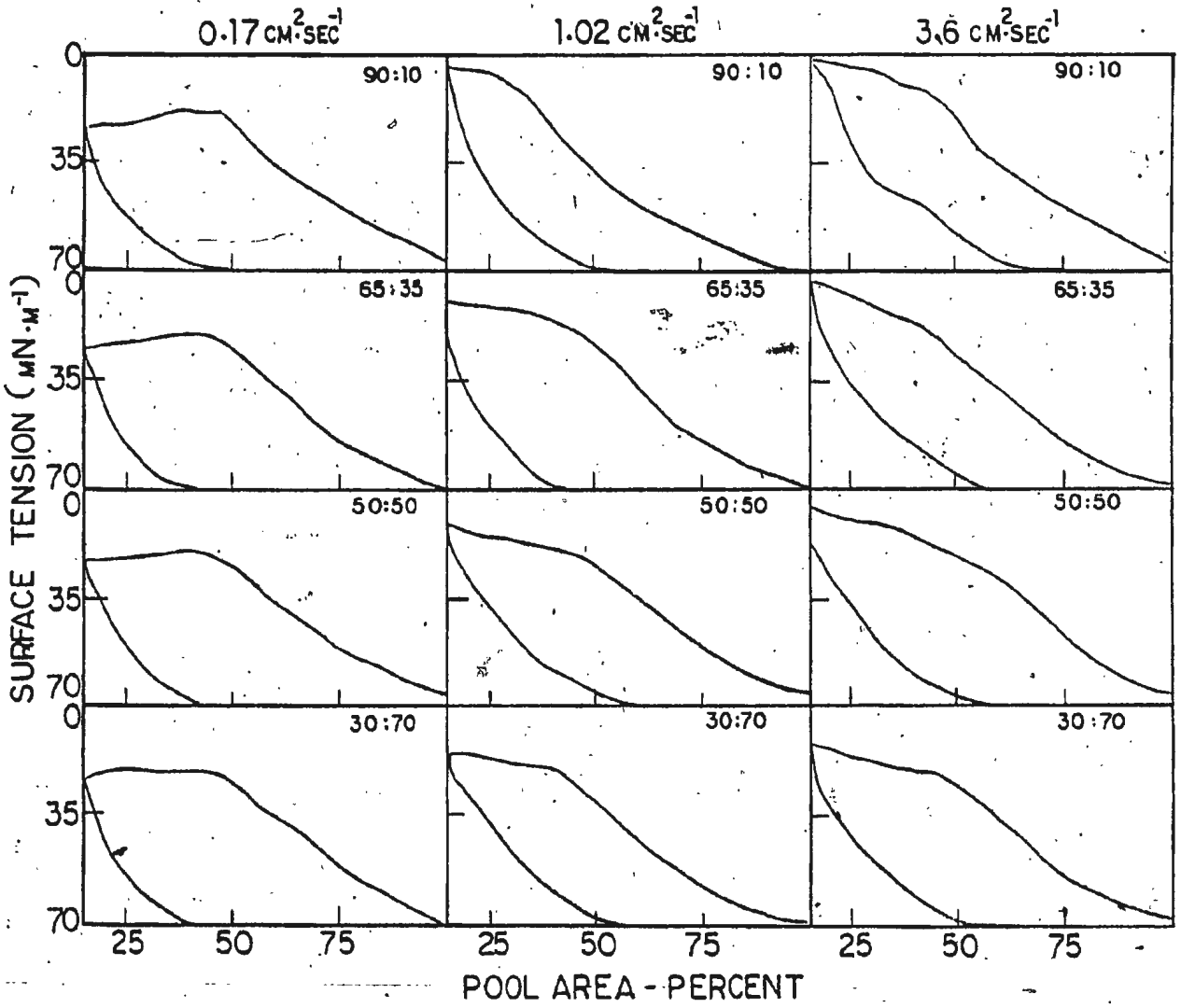
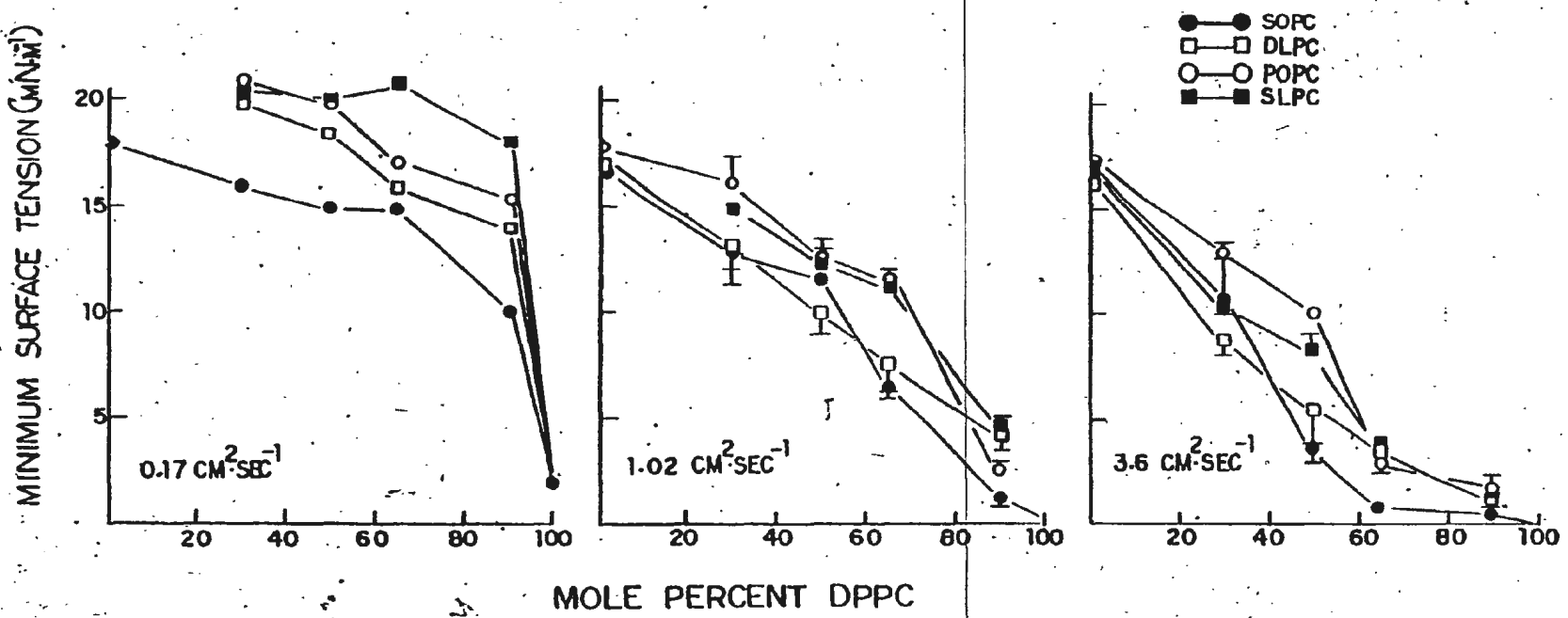

0.17 CM²·SEC⁻¹
1.02 CM²·SEC⁻¹
3.6 CM²·SEC⁻¹
90:10
65:35
50:50
30:70
0
35
70
SURFACE TENSION (MN·M⁻¹)
25
50
75
POOL AREA - PERCENT

Figure 17. Effect of various fluid lecithins mixed with DPPC on the minimum surface tension of surface tension-area isotherms compressed at 37°C. Monolayers compressed at rates of 0.17, 1.02 and ~~3.5~~, $cm^2 \cdot sec^{-1}$. Values represent mean ± ½ range (n = 2) except for DPPC/SOPC at 1.02 $cm^2 \cdot sec^{-1}$ where values are mean ± S.D. (n = 4). Values on graph entitled 0.17 $cm^2 \cdot sec^{-1}$ represent single values; otherwise where range is not given it is within the size of the symbol. For clarity, the error bars are given on one side of the symbol, i.e. they represent one-half the range.


MINIMUM SURFACE TENSION (mN·m⁻¹)
20
15
10
5
0.17 CM²·SEC⁻¹
1.02 CM²·SEC⁻¹
3.6 CM²·SEC⁻¹
20
40
60
80
100
MOLE PERCENT DPPC
SOPC
DLPC
POPC
SLPC

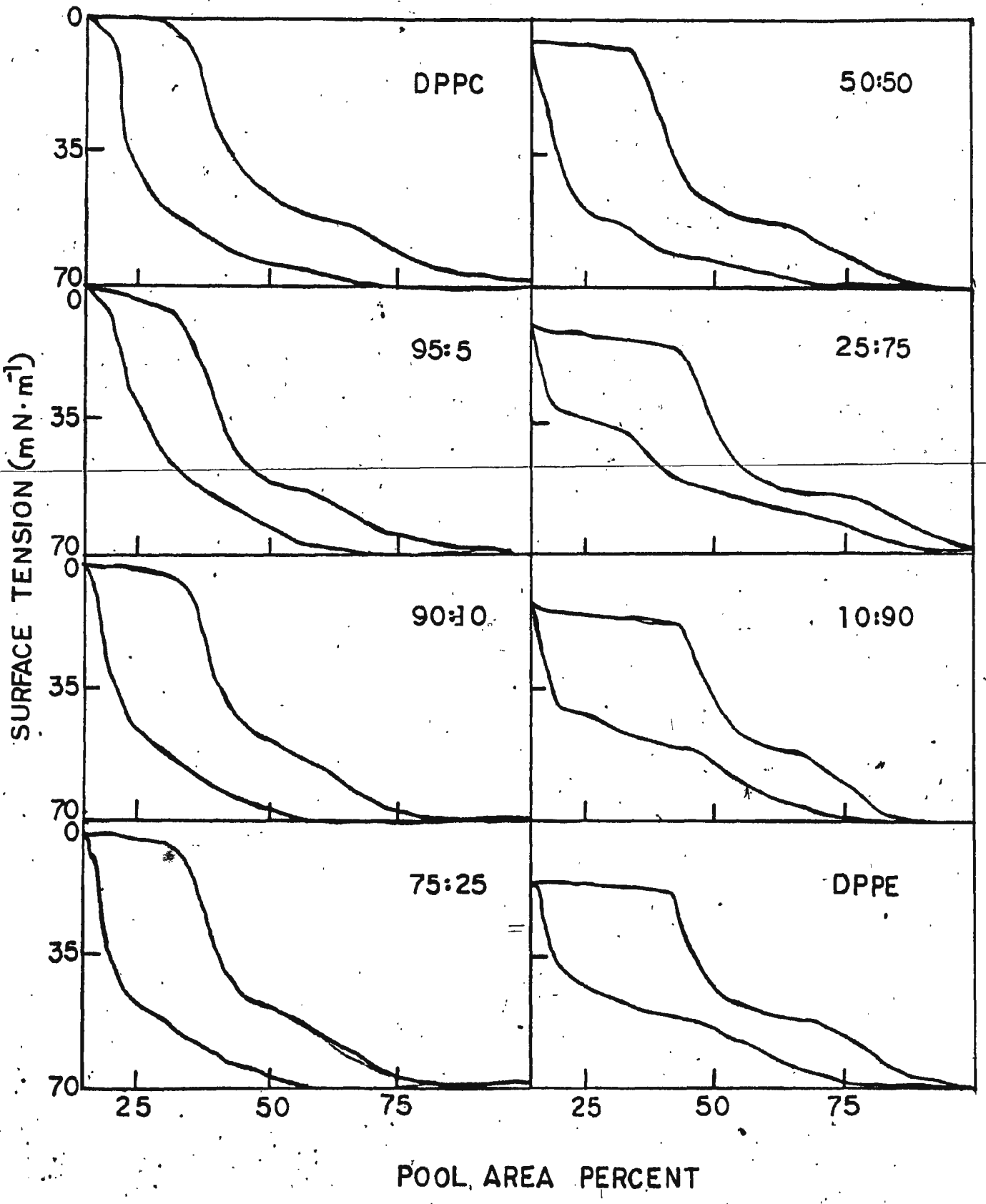
lower γ_{min} than mixtures of that particular lecithin with higher fluid lecithin concentration at the same compression rate.

Phosphatidylcholines vs phosphatidylethanolamines

The preceding studies have investigated the ability of mixed lecithin monolayers to reach low surface tension. It is also interesting to study the surface activities of mixtures of another phospholipid, dipalmitoyl phosphatidyl-ethanolamine (DPPE) with DPPC. PE has been found only in small amounts in mammalian lung surfactant (eg. King and Clements, 1972; Trauble, 1974). It is, however, a zwitterionic lipid like lecithin and would perhaps behave in a similar fashion. Indeed earlier studies at low surface pressures (eg. Phillips and Chapman, 1968; Trauble et al., 1974) would tend to confirm this. Very little work has been done with surface properties of PE monolayers. DPPE monolayers have been studied below t_c (Notter and Morrow, 1975) and they only attained a collapse pressure of 55 $mN \cdot m^{-1}$. Furthermore, it was also of interest to examine the monolayer characteristics of mono- and dimethylated PE's. Since these preliminary studies were undertaken, a report by Evans et al., (1980) has appeared where the compression of the N-methylated derivatives of DPPE have been examined. Full hysteresis loops however, were not given in the study.

Figure 18 shows a range of isotherms at 23°C

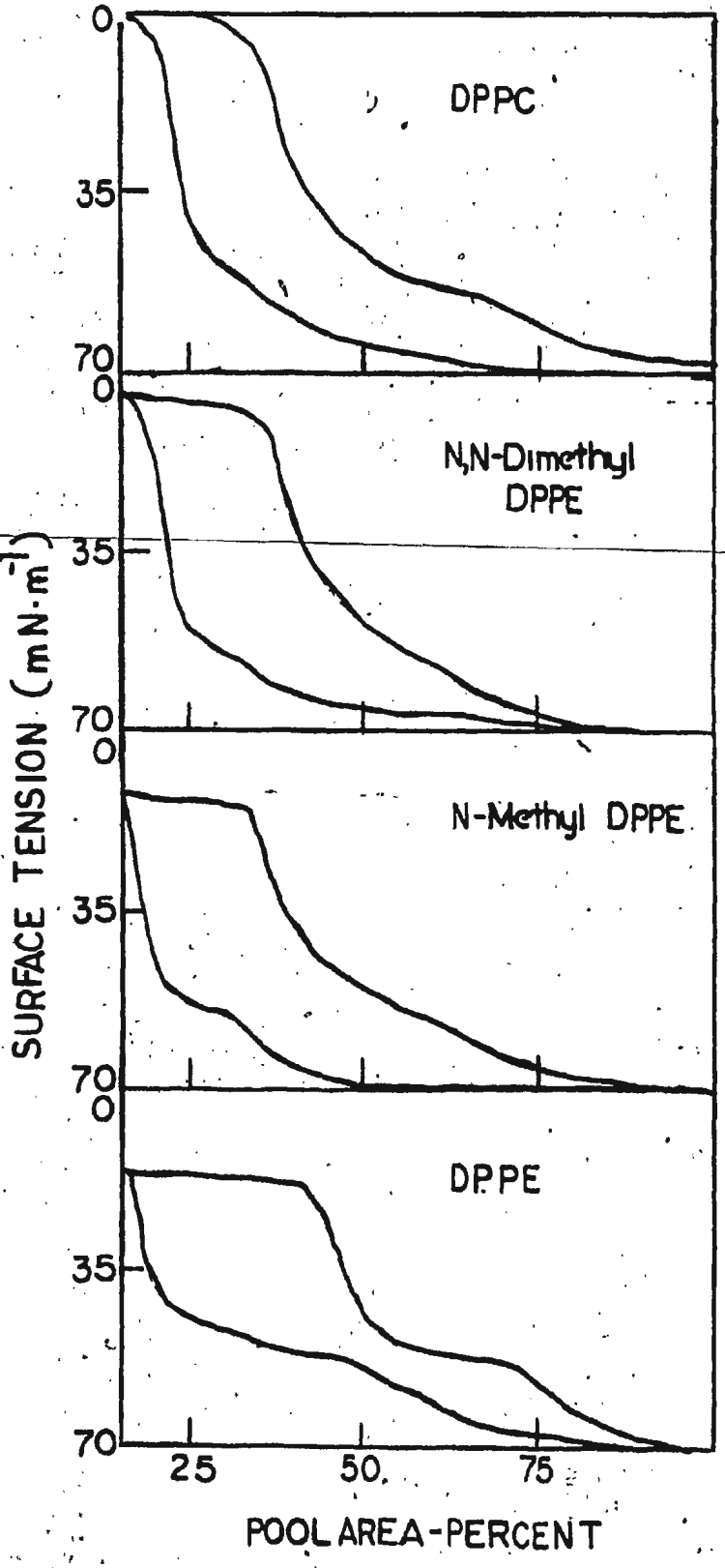
Figure 18. Surface tension - area isotherms of mixtures of DPPC and DPPE at 23°C. Initial surface concentration of 0.008 μmole. Compression rate of .17 $cm^2 \cdot sec^{-1}$.


DPPC
50:50
95:5
25:75
90:10
10:90
75:25
DPPE
0
35
70
25
50
75
SURFACE TENSION (mN·m⁻¹)
POOL, AREA PERCENT

obtained from mixtures of DPPE and DPPC. Amounts of 0.008 μmole were applied to each surface. DPPE monolayers reached only 18 $mN \cdot m^{-1}$ at a compression rate of 0.17 $cm^2 \cdot sec^{-1}$, although it is below its phase transition temperature ($t_{c\ DPPE} = 64^{\circ}C$, Vaughan and Keough, 1974). There was a progressive decrease in γ min, however, as seen in Fig. 18 which depended on the relative concentrations of the two lipids. As the concentration of DPPE was increased there were also changes in the shapes of the hysteresis loops tending toward that of DPPE itself. The DPPE isotherm on relaxation is substantially different from that of DPPC. On relaxation the surface tension drops in a similar way as DPPC until it reaches about 25 $mN \cdot m^{-1}$ at which time further relaxation results in a slow increase in surface tension to 70 $mN \cdot m^{-1}$. As the concentration of DPPE is increased, the minimum surface tension attainable tends toward that of DPPE itself. It should be noted that these are preliminary experiments and that some of these changes in hystereses shapes may be caused by changes in dipping plate contact angles (Notter and Morrow, 1975). Sandblasting of the platinum flags may be useful in further experiments of this type to attempt to reduce contact angle changes.

Figure 19 shows isotherms of N-Methyl dipalmitoyl phosphatidylethanolamine (N-Me-DPPE) and N,N-dimethyl dipalmitoyl phosphatidylethanolamine (N, N-diMe-DPPE) as well as DPPE and DPPC. Again 0.008 μmole of lipid was applied to a clean surface. The dimethyl species reached a minimum

Figure 19. Surface tension - area isotherms of DPPE, N-methyl DPPE, N,N-dimethyl DPPE and DPPC at 23°C. Surface concentration of 0.008 μmole and compression rate 0.17 $cm^2 \cdot sec^{-1}$.


0
35
70
DPPC
0
35
70
N,N-Dimethyl
DPPE
0
35
70
N-Methyl DPPE
0
35
70
DPPE
SURFACE TENSION (mN·m⁻¹)
25
50
75
POOL AREA-PERCENT

surface tension of 4 $mN \cdot m^{-1}$ while the monomethylated DPPE could be compressed to only 11 $mN \cdot m^{-1}$. It seems then that the structure of the head group is an influencing factor in the achievement of low surface tension.

Bulk phase dispersions

The production of surfactant has been suggested to occur from a secretion of material from the type II pneumocytes, through a more complex bulk phase structure and onto a surface monolayer (Goerke, 1974). The nature of the mechanism of the insertion of the monolayer into the air-water interface is unknown. It has been suggested that lamellar bodies with minimal hydration may insert at the interface and that spreading of relatively dry material may occur at the surface (Morley et al., 1978). It could be, however, that the monolayer may spread from material in the subphase which is somewhat more like traditional liposomes. Thus the surfactant monolayer may behave as a monolayer over a bulk phase dispersion. Certainly many animal models and at least one study with humans (Fugiwara, 1980) have employed bulk phase lipid dispersions as a replacement surfactant. To gain a better insight into surfactant function and to aid in the design of such "wet" replacement surfactants, studies of the properties of monolayers over bulk phase were undertaken. This would allow us to determine if the properties of solvent-spread and absorbed monolayers were similar.

Dispersions of lipid mixtures containing 0.5 mg

lipid per ml of saline were examined at 37°C and the results are shown in Figs. 20 and 21. Figure 20 shows 37°C isotherms of dispersions of DPPC and SOPC. A 65/35 molar mixture of DPPC/SOPC (Fig. 20A) showed an initial surface tension of 53 $mN \cdot m^{-1}$ which compressed to 13 $mN \cdot m^{-1}$ at a rate of 1.02 $cm^2 \cdot sec^{-1}$. When the surface was relaxed the surface tension went to 70 $mN \cdot m^{-1}$, but if the trough was left undisturbed at 100 per cent pool size, the surface tension falls without compression. After thirty minutes the surface tension dropped to 41 $mN \cdot m^{-1}$. Compression of this surface at a rate of 3.6 cm^2/sec results in a γ min of 2 $mN \cdot m^{-1}$. Relaxation to 100 percent pool size resulted in a surface tension of 70 $mN \cdot m^{-1}$. It was then decided to leave this surface undisturbed to see how low the surface would fall without compression. After sixteen hours, surface tension had fallen to 20 $mN \cdot m^{-1}$ which compressed to 0 $mN \cdot m^{-1}$ at 3.6 $cm^2 \cdot sec^{-1}$.

When a known amount of lyso-oleoyl phosphatidyl choline was added to the mixture. The resulting bulk phase molar concentration was calculated to be DPPC/SOPC/lyso-oleoyl PC, 61.5/34.0/5.5. This mixture was prepared by evaporating a known quantity of lyso-oleoyl PC/chloroform solution to dryness using nitrogen. The DPPC/SOPC dispersion was added to the dry lyso PC heated to 50°C and vortexed until an even dispersion was attained. The dispersion was poured into a clean trough at 37°C. Initially, the surface tension of the dispersion was 30 $mN \cdot m^{-1}$ and dropped to 27 $mN \cdot m^{-1}$

Figure 20. Surface tension - area isotherms of 0.5 mg lipid/ml dispersions of DPPC and SOPC at 37°C.

Fig. A. shows 3 compressions of the DPPC/SOPC (65/35) dispersion. Compression 1 (——) was at a rate of 1.02 $cm^2 \cdot sec^{-1}$ and compression 2 (·---) and 3 (---) were at 3.6 $cm^2 \cdot sec^{-1}$.

Fig. B. shows compression of a dispersion of DPPC/SOPC/lyso oleoyl PC (61.5/34.0/5.5) at 1.02 $cm^2 \cdot sec^{-1}$.

Time periods on panels represent the time required for the indicated fall in surface tension when the surface was left fully expanded.

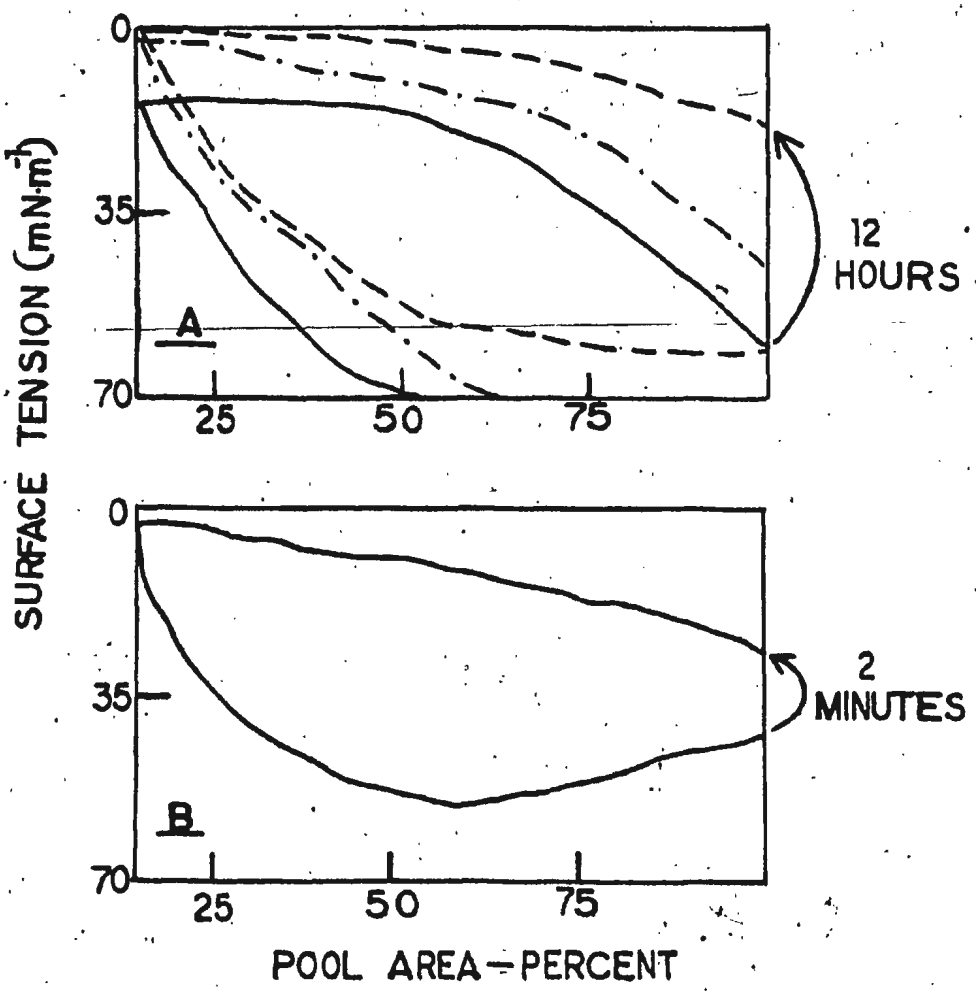
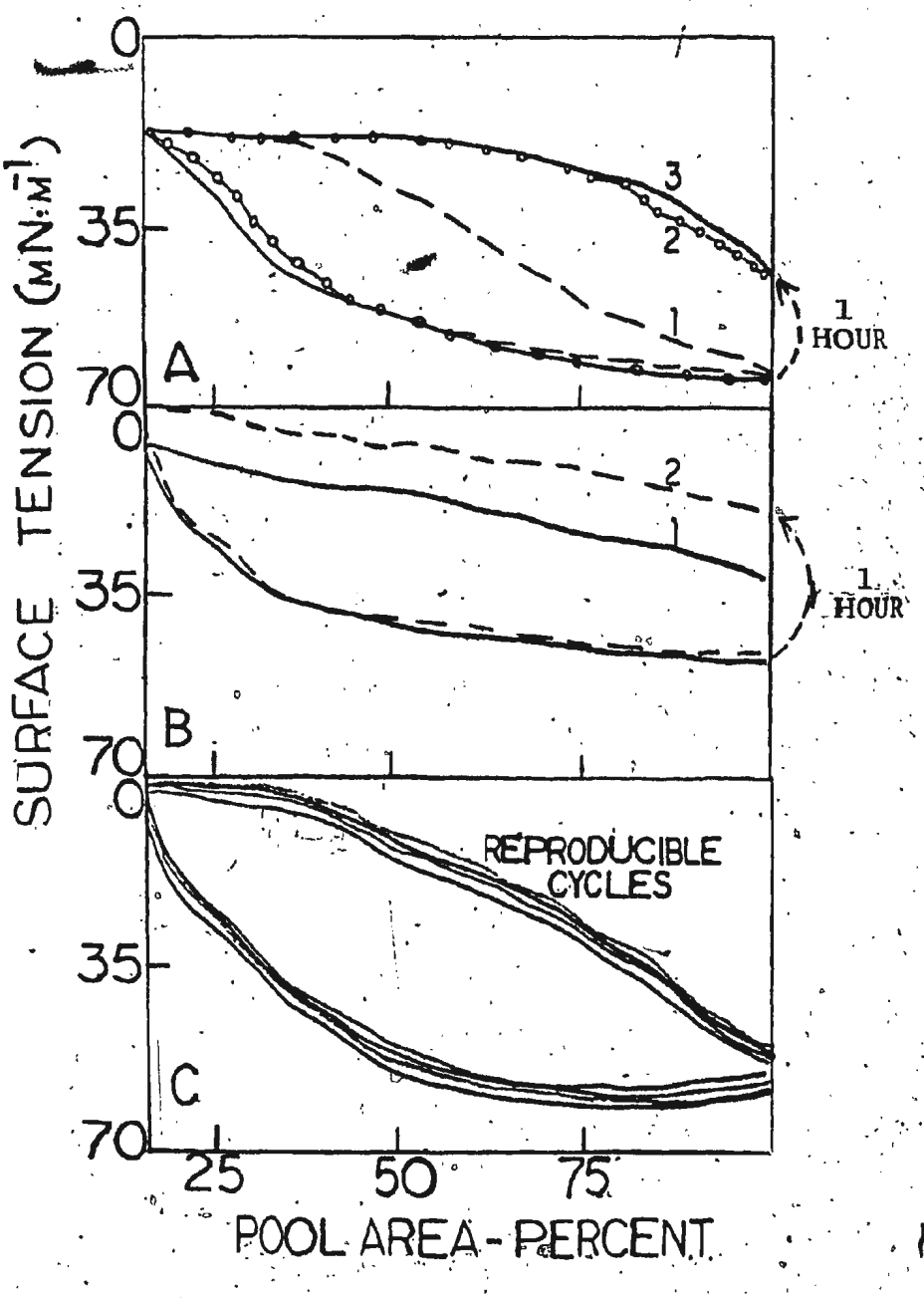

0
35
70
A
25
50
75
12
HOURS
SURFACE TENSION (mN·m⁻¹)
0
35
70
B
25
50
75
2
MINUTES
POOL AREA – PERCENT

Figure 21. Surface tension - area isotherms of: A - DPPC/egg PG/lyso oleoyl PC (65/20/15), B - DPPC/OSPC/lyso oleoyl PC (67/24/9) and C - DPPC/OSPC/lyso oleoyl PC (65/30/5). In Fig. A, compression was at 3.6 $cm^2 \cdot sec^{-1}$ while in B and C compressions were at 1.02 $cm^2 \cdot sec^{-1}$. Times shown on panels A and B represent the amount of time the surface was left fully expanded and the extent of the drop in surface tension.


SURFACE TENSION (mN·m⁻¹)
0
35
70
A
3
2
1
1 HOUR
B
REPRODUCIBLE CYCLES
C
25
50
75
POOL AREA - PERCENT

in twelve minutes (Fig. 20B). Compression of the surface at 1.02 $cm^2 \cdot sec^{-1}$ lowered the surface tension to 2.5 $mN \cdot m^{-1}$ at 15 percent pool size. When fully expanded the surface tension increased to 35 $mN \cdot m^{-1}$ but dropped to 27 $mN \cdot m^{-1}$ within two minutes. Subsequent compression again reached 2.5 $mN \cdot m^{-1}$ and re-expanded to 35 $mN \cdot m^{-1}$. Addition of more lyso-oleoyl PC by resuspension, giving a final molar concentration of 60/32/8, DPPC/SOPC/lyso, PC resulted in a minimum attainable surface tension of 10 $mN \cdot m^{-1}$. It is obvious that there is a fine balance of fluid and rigid lipid to give optimum spreading ability as well as the lowering of γ to near-zero values.

Figure 21A shows the results of isotherms obtained at 37°C with suspensions of DPPC/egg phosphatidylcholine/lyso oleoyl phosphatidylcholine, (65/20/15 molar ratio). The mixture was poured at 37°C giving an initial surface tension of 63 $mN \cdot m^{-1}$ and compressing to only 18 $mN \cdot m^{-1}$ and re-expanded to 65 $mN \cdot m^{-1}$ at 100 percent pool size. After one hour the initial surface tension dropped to 45 $mN \cdot m^{-1}$. Compression of this surface at the fastest rate of 3.6 $cm^2 \cdot sec^{-1}$ still reached only 18 $mN \cdot m^{-1}$. A second such compression again achieved 18 $mN \cdot m^{-1}$.

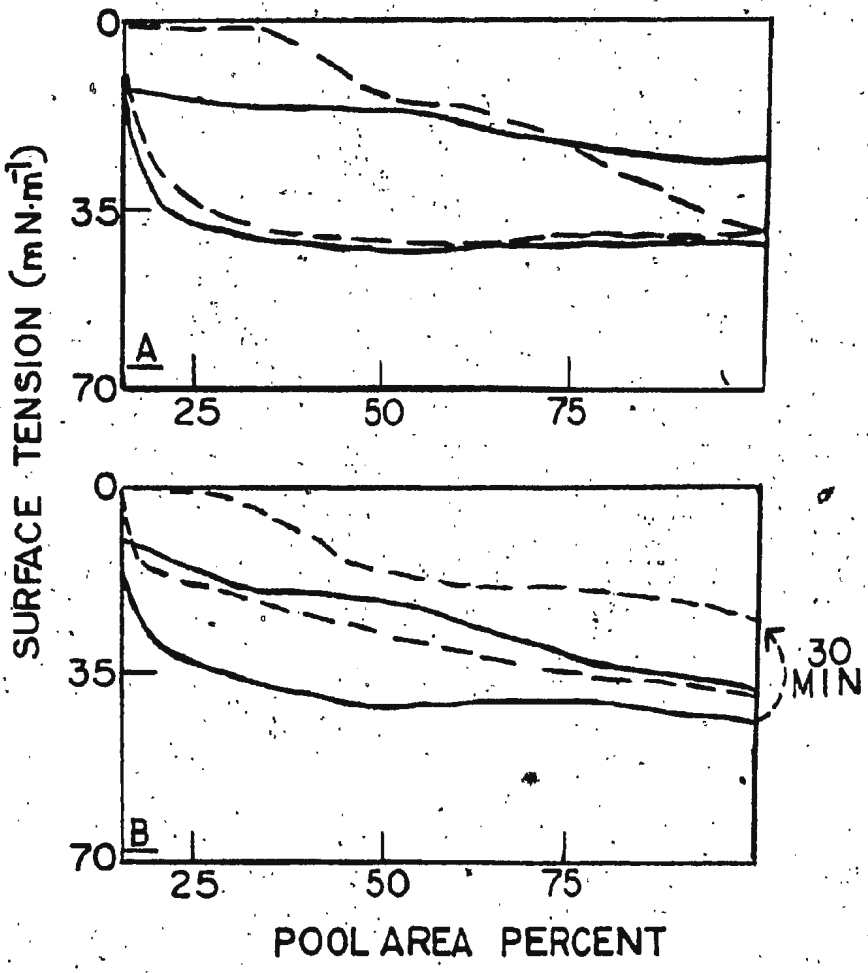
Oleoyl-stearoyl phosphatidyl choline (OSPC) was used in some dispersion preparations to determine its effect on the surface properties of DPPC. A 0.5 mg lipid per ml dispersion of DPPC/OSPC/lyso-oleoyl PC in a molar ratio of 67/24/9 was run on the surface balance (Fig. 21B). The

mixture had an initial surface tnesion of 35 $mN \cdot m^{-1}$, and compressed at a rate of 1.02 $cm^2 \cdot sec^{-1}$ to 7 $mN \cdot m^{-1}$. Re-expansion to 100 percent pool size resulted in a surface tension of 48 $mN \cdot m^{-1}$. When this surface was left at 37°C for one hour the surface tension dropped to 21 $mN \cdot m^{-1}$. Compression of this surface resulted in a γmin of 0 $mN \cdot m^{-1}$. Two subsequent compressions were done in succession, both of which started at 45 $mN \cdot m^{-1}$ and ultimately reached near zero surface tension. The second compression loop however, was smaller than the first indicating a loss of surface active material. A similar mixture containing 65% DPPC, 30% OSPC and 5% lyso oleoyl PC was examined and the results are shown in Fig. 21C. Compressions went from 50-55 $mN \cdot m^{-1}$ to 1 $mN \cdot m^{-1}$ at a rate of 1.02 $cm^2 \cdot sec^{-1}$ over at least eight cycles.

As a comparison, bulk phase dispersions of biological systems were also prepared. Figure 22A shows a 37°C isotherm of a saline wash from an adult rabbit. An initial compression at the 1.02 $cm^2 \cdot sec^{-1}$ rate reached only 12 $mN \cdot m^{-1}$ but subsequent compressions easily attained near zero surface tension. This cycling was repeated over 20 cycles but the hysteresis loop became marginally smaller on every compression.

Purified surface active material (SAM) from pig lung was prepared according to the method of King & Clements (1972 I). The isotherm of a dispersion of the SAM at a concentration of 1 µmole of phosphorus per ml of saline is shown in Fig. 22 B. It's properties were very similar to those obtained from the

Figure 22. Surface tension - area isotherms of: A - adult rabbit saline lung wash and B - purified surface active material extracted from porcine lung wash. Isotherms were compressed at 1.02 $cm^2 \cdot sec^{-1}$ at 37°C.


0
35
70
A
25
50
75
SURFACE TENSION (mN·m⁻¹)
0
35
70
B
25
50
75
30 MIN
POOL AREA PERCENT

rabbit saline wash material. When poured at 37°C the mixture had a surface tension of 37 $mN \cdot m^{-1}$ and compressed to 7 $mN \cdot m^{-1}$ at a rate of 1.02 $cm^2 \cdot sec^{-1}$. Fully expanded, the surface tension was 43 $mN \cdot m^{-1}$ but dropped to 23 $mN \cdot m^{-1}$ in just 30 minutes. Compression from 23 $mN \cdot m^{-1}$ achieved zero surface tension but subsequent compressions showed loss of surface material as indicated by a reduction in the size of the hysteresis loop.

DISCUSSION

Simple monolayers

To grasp a more effective understanding of the events which occur at the air/water interface in the surfactant system one must first discern the behaviour of it's individual components. It has been well established that lecithin, especially dipalmitoyl lecithin, is found in lung extracts (Clements, 1962; King et al., 1972 II) and is mainly responsible for the lungs reaching low surface tension values at 37°C. For almost thirty years the surface activity of various phosphatidyl cholines and phosphatidylethanolamines has been studied using modified Langmuir-Wilhelmy balances. However, many of these studies were done at 23°C and were not compressed to high surface pressures and re-expanded in a cyclic fashion. Since surfactant is comprised of saturated phospholipids, such as DPPC, as well as unsaturated lipids, (King et al., 1972 II) it is apparent that there exists in the total lipid, a mixture of liquid crystal and gel phase lipid (Träuble et al., 1974; Steim, 1976; Clements, 1977). If this is so, then the question might arise as to what are the consequences of fluid lipid on the surface 'properties'. Figure 5 shows the surface behaviour of DPPC at two different temperatures. Above the transition temperature of DPPC, the 43°C isotherm reaches only a minimum surface tension of about 20 $mN \cdot m^{-1}$. Below the t_c, at 37°C the monolayer reaches near zero surface

tension. It had been previously suggested that the determining factor in lowering surface tension was the presence of saturated chains (Clements, 1967) although it had been suggested (Steim, 1976) that chain rigidity was the important factor. If one looks at the results of the dimyristoyl phosphatidyl choline (DMPC) isotherms in Fig. 8 it becomes more apparent that the lowering of surface tension to near-zero values is related to the physical state of the phospholipid's fatty acyl chains rather than the chemical nature of the fatty acid. Below its t_c, DMPC was capable of reaching low γ but in a fluid form at 37°C it achieved only about 20 $mN \cdot m^{-1}$. The behaviour of OSPC and SOPC as shown on the table proved to be very interesting. At 0°C both are in a rigid state and easily reached near 0 $mN \cdot m^{-1}$ but when above their respective t_c's, they too only reached 20 $mN \cdot m^{-1}$. Van Deenen et al., (1962) compressed SOPC on a film balance but they applied a surface pressure of only about 36 $dyne \cdot cm^{-1}$ at room temperature. Dilauroyl PC is another disaturated lecithin which when compressed in a monolayer above its t_c cannot reach low minimum surface tension. POPC and SLPC, because their t_c's are below 0°C can only be compressed in their fluid state and did not reach values of γ near zero.

A possible mechanism for the differences in surface tension lowering ability of lecithins is shown in Fig. 23. In a rigid state, the fatty acyl chains, when highly compressed may pack very closely together. This may have the effect of excluding all the aqueous subphase from

Figure 23. Possible representation of the compression of rigid and fluid lecithin in a monolayer. The increased fatty acid chain movement in fluid lecithin may not allow tight molecular packing which may be necessary to achieve very low surface tension.

RIGID LECITHIN

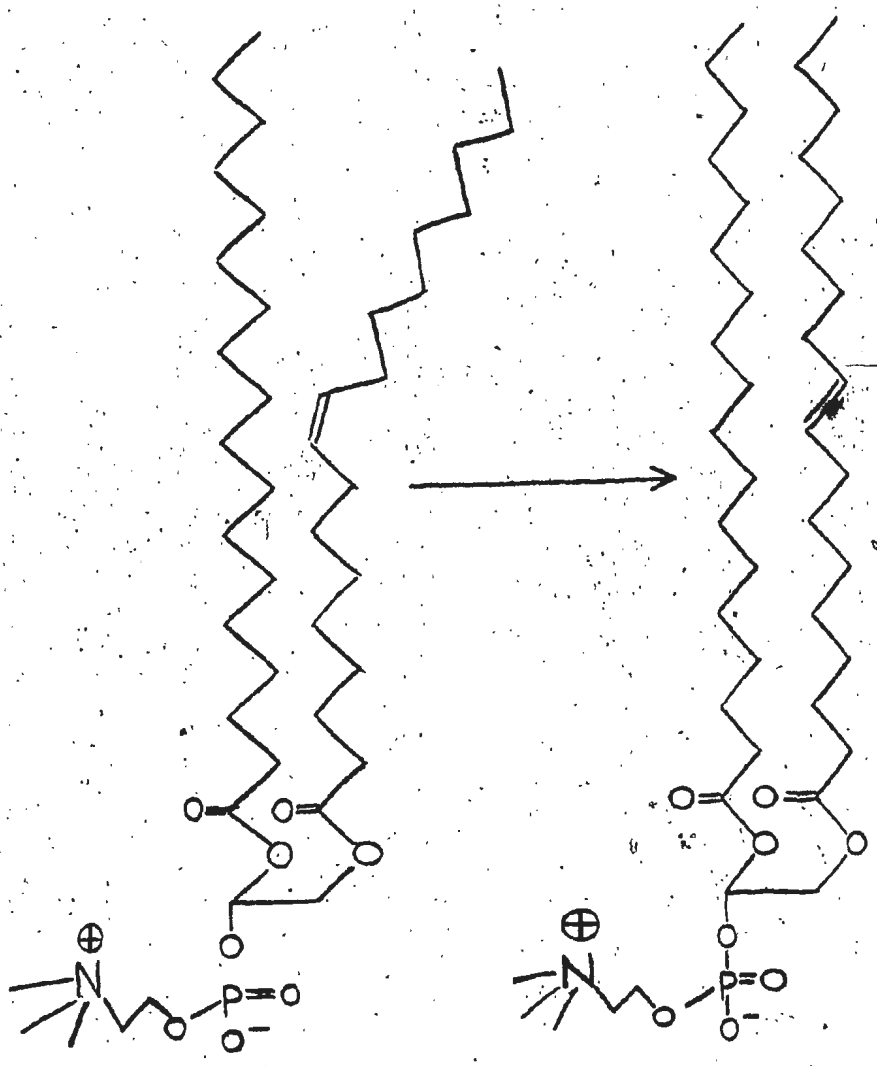
FLUID LECITHIN

the surface, thus lowering surface tension to very low values. If however, there is a high degree of chain movement as is the case with fluid lecithin surface molecules cannot be compressed to such an extent. When this is the case, the monolayer cannot sustain such a high surface pressure, leaving some aqueous material in the surface and resulting in an elevated minimum obtainable surface tension.

The use of the term "surface contractile force" has been employed by Bangham et al., (1979) instead of surface tension since the dynamic behaviour of such films past the collapse point cannot be discussed in simple thermodynamic terms. We think that the simple view presented above is not inconsistent with the loss of surface contractile force. The result of over-compressing such a monolayer shall be discussed in a later section.

It is quite conceivable that rigid disaturated lecithin might easily compress to a low area per molecule just by the nature of the shape of the molecule. Because it has long, uniform, fatty acyl chains that are identical it may pack very tightly with its neighbours. It had been widely thought however, that unsaturated fatty acids contained a kink at the point of the double bond. This would have an effect of spreading the fatty acids on a lecithin molecule to occupy a much larger area per molecule determined by the arc subtended by the fatty acid chain (Fig. 24). Huang (1977) suggested that a twist of the chain about the double bond might result in a much straighter

Figure 24. Representation of the chain "kink" in fluid created by the presence of a double bond. Rotation around the double bond permits tighter chain packing in the rigid phase.



fatty acid residue such as in Fig. 24b. This would result in only a small increase in the area per molecule especially below t_c and one might expect packing of the unsaturated lipids to be not too dissimilar from that of saturated ones below t_c. We have calculated the areas per molecule at 20 $mN \cdot m^{-1}$ for DPPC and SOPC both in the rigid and fluid state. Below its t_c, DPPC has a limiting area per molecule of 44 $Å^2$ per molecule while at 43°C in its liquid crystalline phase it has an area per molecule of 56$Å^2$ per molecule. SOPC can be compressed to 49 $Å^2$ per molecule at 1°C while above its t_c the area per molecule is 60 $Å^2$ per molecule. From these results it is evident that when in the rigid state the unsaturated lecithin can be compressed to occupy an area similar to that of the disaturated species. The increase in area per molecule in going from gel to liquid crystal is about 11-12 $Å^2$ for both molecules. The increase in the areas per molecule may come as a result of the increased chain movement in the fluid state. The fact that unsaturated phospholipids can reach low minimum surface tensions may be important in the case of poikilothermic animals. It has been suggested that turtles living at 4°C may not require as much DPPC (Lau and Keough, 1981) in their surfactant system. These unsaturated lecithins may be able to contribute lung stability at end expiration at low temperatures.

The results on the pure monolayer studies can be summarized by indicating that the ability of lecithins to

achieve very low surface tension under high film compression is a function of lipid phase and not of structure alone.

Binary monolayers

Since surfactant is a complex composition of rigid and fluid lipid, there must be numerous interactions and mechanisms involved at the air-water interface. The normal surfactant system is one in which there exists a mixture of both fluid and rigid lipid. Some questions arise about such a complex system: 1) If the lowering of surface tension requires rigid phase lipid, why is the fluid lipid present? 2) If there is fluid lipid, how does the lung reach very low surface tension? To help answer the second question, monolayers of simple binary mixtures were made with DPPC, the rigid lipid, plus a series of lower melting lecithins.

Information on mixing of two of the low melting lecithins (POPC and SOPC) with DPPC in liposomes has been provided by Davis et al., (1980). The overall effect of mixing lower melting lecithins with DPPC is that there is a net lowering of the transition temperature of DPPC, the extent of which is determined by the molar percentage of the fluid lipid. Mixtures of DPPC and SOPC were compressed in a monolayer at 37°C and the results are shown in Fig. 9. For each particular isotherm it is obvious that the faster rate is capable of reaching a lower surface tension than the slower one. It is also seen that the minimum surface tension is dependent on the ratio of SOPC and DPPC concentrations. This is consistent with a recent study showing isotherms of

DPPC and egg PC (Wildeboer-Venema, 1978).

There has been some evidence presented that in the lung and in mixed monolayers that low surface tension is achieved by a type of surface refining which involves the exclusion of all or part of the fluid moiety (Clements, 1977; Hildebran et al., 1979; Morley et al., 1979). The idea of exclusion or "squeeze out" goes back to 1968 when Watkins proposed that this phenomenon occurred in films of DPPC and egg PC (Watkins, 1968). Our results indicate that the lowering of γ in mixed monolayers may occur as a result of exclusion of SOPC. Monolayers containing greater than 50% DPPC, achieve surface tensions lower than 10 $mN \cdot m^{-1}$ although SOPC monolayers only reached 15-20 $mN \cdot m^{-1}$. Monolayers combining DPPC/SOPC (30/70) reached only 13 $mN \cdot m^{-1}$. These observations are consistent with the exclusion of the fluid component during compression. This exclusion is dependent on the rate at which the monolayer is compressed because monolayers compressed at faster rates reach lower γ than those at slower rates for the same molar mix. There are a number of potential explanations for these observations. During slow compression an exclusion-reinsertion cycle may be fast enough to yield a relatively fluid monolayer at all times during compression. Secondly, a certain maximum compression may be necessary for squeeze-out to occur. A third possibility is that the balance of forces governing lipid exclusion may be kinetically dependent. Finally, it may be that selective exclusion does not occur at all and is a

result of the kinetic responses of the mixed monolayers to applied pressure.

To ensure that the changing minimum attainable surface tension for the various mixtures was related to lipid phase, and not lipid structure alone, mixes of 65/35 and 30/70 of DPPC/SOPC were compressed at 0°C. At this temperature both components were in a rigid phase. Isotherms, as seen in Fig. 10, reached near zero $mN \cdot m^{-1}$ for both mixtures and both rates. When DMPC was substituted for DPPC to give monolayers at 37°C in which both lipids were in the fluid phase, low surface tensions could not be achieved even for 65/35 DMPC/SOPC at the faster rate. Thus, either squeeze-out did not occur at all, or if it did, only a fluid monolayer was left remaining at the surface.

Surfactant, because of its complex composition of phospholipids is comprised of rigid and fluid lipid at 37°C (King and Clements, 1972 II; Trauble et al., 1974). According to the previous discussion low surface tension may be achieved when the lipid is in a rigid state. It has been suggested that this would necessitate the exclusion of fluid lecithin on exhalation (decrease in alveolar surface area) to attain a refined surface rich in rigid phase lipid ie. DPPC (Clements, 1977; Bangham et al., 1979). In our simple monolayer studies it has been noted that repeated compression of pure DPPC results in a decrease in the area of the compression-relaxation hysterisis loop. This is indicative of a loss of DPPC from the monolayer to the aqueous phase.

It has been discussed previously that lecithin below its t_c is very slow to form a monolayer from a dispersion (Bangham, et al. 1979). If on high compression, lipid (including DPPC) is excluded from the surface there must also exist a coincidental system in which lipid might be reinserted into the surface. It is conceivable that this movement in and out of the alveolar air interface is influenced by the nature of the fluid lipid so that the efficiency of the overall process is influenced by the quality of the surfactant.

Recently tracheal and pharyngeal aspirates of normal term infants, normal premature infants as well as infants with RDS have been examined to ascertain the fatty acid profile in the phosphatidylcholine moiety (Shelley et al. 1979). A summary of this data, shown in Table 2, shows an obvious deficiency in the amount of palmitate in RDS infants as compared to normal term infants and even premature normal babies. Approximately 75 percent of surfactant lecithin fatty acid was palmitic acid in the normal term infants while this component made up only 55 percent of RDS surfactant lecithin. Lecithin from premature infants without RDS contained about 70 percent palmitic acid. From the preceding discussion it is obvious that a decrease in the amount of DPPC will reduce the overall amount of rigid lipid and hence the surface tension-lowering ability of the surfactant. A closer look at the fatty acid profiles reveals that RDS surfactant lecithin contains elevated quantities of stearate, oleate, linoleate and arachidonate. These values

Table 2. Surfactant phosphatidylcholine fatty acid profiles of pharyngeal aspirates from 1 day old premature infants with and without Respiratory Distress Syndrome (RDS). Premature infants without RDS were designated control infants. Also shown are the precent fatty acids in surfactant from normal, full term infants. (Adapted from Shelley et al., 1979.)

	WT. PERCENT OF PHOSPHATIDYLCHOLINE FATTY ACIDS		
	INFANTS WITH RDS	CONTROL	FULL TERM
MYRISTIC ACID (14:0)	1.5	2.5	3.5
PALMITIC ACID (16:0)	55.0	69.0	74.0
PALMITOLEIC ACID (16:1)	3.5	5.0	5.0
STEARIC ACID (18:0)	9.0	5.0	4.5
OLEIC ACID (18:1)	18.5	11.0	9.0
LINOLEIC ACID (18:2)	5.0	3.0	2.5
ARACHIDONIC ACID (20:4)	8.5	4.5	2.0

tend toward those values of normal infants after about eighteen days. Notter (1979) suggested that the apparent lack of palmitate in RDS surfactant could be compensated by the increased quantities of longer chain fatty acids especially stearic. As can be seen from our study described above, this would only occur if the resulting lecithins had a $t_c < 37^oC$. The increased amounts of unsaturated lecithin would tend to fluidize the overall surfactant mixture even in the presence of higher stearate content.

The fatty acid values of Shelley et al., (1979) for term, normal premature and RDS infants were recalculated to get an estimate of the relative amounts of disaturated and saturated lecithin (Table 3). If one assumes that there are no diunsaturated species and that each unsaturated fatty acid is paired with a saturated fatty acid, the ratio of saturated to unsaturated lecithin for normal, term infants is 65 percent to 35 percent. A similar calculation reveals that this ratio is 30 percent to 70 percent for RDS-infants and 53 percent to 47 percent for normal premature infants. This calculation emphasizes the difference between the "quality" of the lecithins from normal and RDS infants. Assumptions of the presence of two unsaturated chains in some lecithins could lead to a higher estimate of disaturated PC, but there would be a countervailing effect of lower fluidity for the diunsaturated (two unsaturated fatty acids) lecithins.

An investigation of the properties of very simple

Table 3. Ratio of disaturated PC to unsaturated PC from pharyngeal aspirates of premature infants with Respiratory Distress Syndrome and without Respiratory Distress Syndrome (control infants). Also given is the ratio from aspirates of normal, full term infants. (Recalculated from the data of Shelley et al., 1979.)

	RATIO OF DISATURATED TO UNSATURATED PC
NORMAL TERM INFANTS	64:36
CONTROL INFANTS	52:48
INFANTS WITH RDS	30:70

model monolayers containing one saturated (DPPC) and one unsaturated lecithin (SOPC) in ratios similar to those calculated from the data of Shelley et al., (1979) were made. The use of SOPC would be expected to give a conservative estimate of the effect of the fluid component since it's t_c is relatively high compared to other potential unsaturated lecithins in surfactant.

As can be seen from Fig. 6 compression of a mixture of DPPC/SOPC (65/35) (ie. the model mixture for the normal term infant) reached 7 $mN \cdot M^{-1}$ while the 30/70 mixture (or the RDS mimicing model) achieved only 13 $mN \cdot M^{-1}$. The 50/50, DPPC/SOPC monolayer (or that for the premature infant without RDS) could be compressed to 9 $mN \cdot M^{-1}$, the same value obtained for an in situ measurement by Schürch et al., (1976). Although these mixtures of DPPC and SOPC serve only as simple models for the surfactant lecithin compositions, the results show that the minimum surface tension achievable is dependent on the relative properties of fluid and rigid lipid in the surfactant and that the "efficiency" of the system is related to the "quality" or composition of the surfactant.

Some isotherms were studied in two sequences, (fast compression first, slow second; or slow first, fast second). It was observed that the order of compression was not important, as long as there was sufficient lipid on the surface. Fast compressions always achieved lower surface tensions than slower ones. Also, separate monolayers of

DPPC and SOPC were investigated at three different surface concentrations and at three different compression rates (Fig. 13). The γ min was a function of the ratio of the fluid and rigid lipid for a fixed surface concentration and compression rate. While monolayers at higher concentration, or those compressed at higher rates, reached lower γmins than those of lower concentrations or compression rates, the dependency of γ min on composition was maintained throughout. It is apparent that the putative process of selective exclusion is a dynamic phenomenon because for a specific molar ratio the minimum surface tension decreases with increasing compression rate.

It could be argued that the reason that the monolayers containing DPPC/SOPC (30/70) did not reach low surface tension is because there was not enough DPPC present originally to lower surface tension. To investigate this possibility 25 nmoles of DPPC/SOPC (30/70) was applied to a clean surface. This mixture contained approximately 10 nmoles of DPPC. It has been shown previously in this study that 7 nmoles of DPPC is more than sufficient to lower surface tension to near zero. This mixture, even at this high surface load, could not reach a γ min of less than 12 $mN \cdot m^{-1}$ at 3.6 $cm^2 \cdot sec^{-1}$. Thus the inability to reach low γ in these mixtures was not due to a limiting amount of DPPC but due to the nature of the monolayer itself.

Other binary monolayers

The results of the DPPC-SOPC monolayers have

suggested that low surface tension is achieved by the exclusion of SOPC and that the ultimate γ min is dependent on the relative concentrations of both components and the rate at which they are compressed. To ensure that these surface properties are not unique to SOPC, a series of other low-melting lecithins were also examined. Monolayers of binary mixtures were studied using DPPC mixed with POPC, DLPC and SLPC. As was shown with DPPC-SOPC mixtures, the minimum surface tension achieved depended on the relative molar amounts of the two components for the other three lecithin mixtures. An interesting consequence of the study was that for a particular molar ratio, the minimum surface tension depended on the fluid lecithin used. As was discussed previously the extent of exclusion of fluid lipid and hence the minimum surface tension is phase-dependent but according to the results it may also be related to the nature of the fluid lipid itself and the way in which it interacts with DPPC.

Figure 17 shows a graphic comparison of the ability of all four sets of mixtures to reach low surface tension. As can be seen, mixtures of DPPC-DLPC behave more like DPPC-SOPC mixtures than the others in this respect. If the ability to undergo squeeze-out was totally phase dependent, one might expect similar results for mixtures of DPPC-POPC and DPPC-DLPC since the fluid components have similar phase transition temperatures ($3^{o}C$ and $-2^{0}C$ respectively). However, mixtures of DPPC/POPC were least effective at attaining low

γ min. Mixtures containing SLPC (t_c = -18°C) achieved values of γ min intermediate between those of DPPC-POPC and the other mixes.

The reason that there are differences in the ability of the four mixtures to attain low surface tension on high compression might be explained in two different ways. First of all, it is possible that the amount of fluid lipid excluded is the same for all four mixtures but the fluidity of the resultant compressed monolayers varies depending upon which low-melting lecithin is present. The bulk phase transition temperatures for the four lecithins are in the order SOPC > DLPC > POPC > SLPC with the fluidity at 37°C being roughly in the opposite order. The second explanation may be that the amount of fluid lipid excluded for a given molar mixture varies from fluid lecithin to fluid lecithin.

It has been shown that there is a difference in the ability of certain low melting lecithins to mix in bilayers (Davis et al., 1980) and is reasonable to think that this applies to monolayers. In this study it was shown that POPC in the liquid crystal mixes more ideally with DPPC than does SOPC and that this maybe related to differences in chain lengths. It is conceivable that for totally fluid monolayers SOPC would be excluded more readily than POPC in a highly compressed monolayer because it mixes less well with DPPC. Similarly, mixtures of DLPC with DPPC reach lower surface tension than does a comparable mixture of POPC/DPPC. Although POPC and DLPC have similar transition temperatures,

the shorter chains of DLPC may not allow as efficient mixing as with POPC and exclusion of DLPC during compression may be promoted more. However, it may be that the exclusion of fluid lipid on high compression is a more complex process and cannot be described in such a simple way. It is apparent though that the structure and phase of the fluid lipid may be important in achieving low surface tension through exclusion.

Phosphatidylethanolamines

Previous work on monolayers at low surface pressures has shown that the surface properties of PE's are only slightly different than PC's (van Deenen et al., 1962; Phillips and Chapman, 1968; Watkins, 1968; Notter et al., 1975; Phillips and Hauser, 1974). An experiment has been carried out to determine the effect that DPPE would have on the ability of DPPC to reach low surface tension, and to study properties of various mixed monolayers of DPPE and DPPC under cyclic, high compression-expansion conditions (Fig. 18). The inability of a highly compressed monolayer of DPPE to reach low surface tension complies with observations by Watkins (1968) who found that distearoyl phosphatidylethanolamine (DSPE) reached only 20 $mN \cdot m^{-1}$. Tabak and Notter (1975) found that DPPE was capable of achieving only 17 $mN \cdot m^{-1}$. The reason why PC's are capable of reaching low surface tensions and PE's are not is probably a function of the packing of the surface monolayer. It has been suggested that PC's may have an

average headgroup orientation normal to the surface while PE's lie flat on the surface (Phillips et al., 1974; Seelig et al., 1976). This may relate to hydrogen bonding between the PE head groups. Also, Watkins (1968) suggested that the PE molecules were more readily excluded from the surface.

It is apparent from these preliminary results that the inability of PE to reach low surface tensions is not a phase dependent phenomenon since these PE's were below their phase transition temperature (Vaughan & Keough, 1974). It is interesting that the results showed that by successively adding methyl groups to DPPE forming dipalmitoyl N-methyl PE, dipalmitoyl N·N-dimethyl PE and finally DPPC, the ability to reach low surface tension is enhanced. As the monolayer achieved a higher degree of lecithin-like headgroups, lower surface tensions could be attained upon high compression. This observation is consistant with the results of binary monolayers of DPPC and DPPE (Fig. 16). As more DPPE is added to the monolayer the ability to reach low surface tension is lost. The mixtures however, reach a lower surface tension than a comparable DPPC/SOPC mixture. This upholds Watkins' (1968) contention that PE's, because of the nature of the headgroup, are easily excluded from the surface on high compression. The results described here agree with those of Watkins (1968) who showed that compression of a suspension of DPPC/DSPE (7/3) added to a saline subphase reached near zero surface tension. Compression rate and isotherm temperature were not given.

It has been shown that canine lung surfactant contains some 5-6 wt percent phosphatidyl ethanolamine (King et al., 1972II). There have not been enough experiments with PE's to elucidate their physiological role although one might speculate that they may be involved structurally or possibly to promote movement of the surfactant to the interface. Cullis (1979) has shown that unsaturated PE's form Hex II phase. Such structures may have a role in the insertion of surfactant into the monolayer at the air-water interface of the lung.

Natural surfactants

To provide a basis of comparison of synthetic studies to biological studies some experiments were initiated with crude and purified extracts of animal surfactants. Figure 22A shows surface isotherm of a suspension of a saline lung wash of an adult rabbit. After an initial compression of the surface to 12 $mN \cdot m^{-1}$, a series of subsequent repetitive compressions reached surface tensions near zero $mN \cdot m^{-1}$. There is a noticable region of high compressibility from 10-12 $mN \cdot m^{-1}$ displayed in this isotherm. Regions of high compressibility are those regions which show a slight lowering of surface tension over a relatively large change in surface area. This region has been described by Clements (1977) and Hildebran et al., (1979) as being important with respect to the exclusion of fluid material from the surface. A very similar result was found

for a purified fraction of porcine surfactant (Fig. 22B). After reaching a γ min of 11 $mN \cdot m^{-1}$ on the first compression and a return to 44 $mN \cdot m^{-1}$ at full expansion. A thirty minute waiting period decreased the γ eq to 23 $mN \cdot m^{-1}$ and the subsequent γ min to 0 $mN \cdot m^{-1}$. It, too, had a region of high compressibility similar to the rabbit saline wash.

Another factor which may play a prominent role in surfactant behaviour is the apolipoprotein described by King et al., (1973). In a recent article by King and MacBeth (1979) it was suggested that the 34,000 dalton protein may play a part in insertion of DPPC into the monolayer. It is noteworthy that the successful replacement surfactant used by Fugiwara et al., (1979) contained 2 percent bovine protein, but that employed by Morley et al., (1981) had no protein present.

Synthetic bulk phase studies

The results from experiments using known surface concentrations of phospholipids spread from solvents established a background for the understanding of the more complex bulk dispersions. Although monolayer studies relate much information concerning surface activity, they do not explain the phenomena associated with surface monolayer formation from a bulk phase. This may be of importance in the design of artificial replacement surfactants for infants born with Respiratory Distress Syndrome. A useful artificial surfactant should be capable of lowering the surface tension

in the alveoli of infants when infused endotracheally. This would necessitate the infused solution spreading to the alveoli and forming its own monolayer which, upon exhalation, would be capable of effectively lowering surface tension. Recently, Fugiwara et al., (1980) successfully used a "semi-artificial" replacement surfactant on premature infants with RDS. This mixture was comprised of a surface active fraction from bovine lung enriched with DPPC and egg yolk phosphotidylglycerol (PG). The final composition of the mixture was 56% DPPC, 21% unsaturated lipids, 10% PG, 6% other lipids normally found in lung surfactants, 5% neutral lipids and 2% protein. Out of ten RDS eight survived after endotracheal infusion and the remaining two died of unrelated causes. The mixture did however, contain 2 percent bovine protein. Since replacement surfactants from natural sources may contain protein which would have a deleterious effect, the ideal replacement surfactant would be a completely synthetic mix. Recently Morley et al., (1980) have had success in the treatment of severly distressed infants with one such mixture (DPPC/egg PG, 7/3) administered as a dry powder.

Two routes for administration of artificial surfactants have proven to be useful. The dispersal of a dry powder (Morley et al., 1980) or the infusion of a lipid mixture in bulk phase dispersion (Fugiwara et al., 1980) have been employed. With this in mind we undertook a preliminary study of the surface properties of some mixtures of unsaturated lecithins and DPPC when they were dispersed in

the bulk phase. Certain characteristics of the mixtures were especially scrutinized. Firstly the initial surface tension of the dispersion was noted. This measurement, prior to any compression gave some indication of the ability of the material to reach a static surface tension, the equilibrium surface tension (γeq). The surface tension of canine lung at total lung capacity has been measured by Schürch et al., (1976) and found to be approximately 29 $mN \cdot m^{-1}$. Tierney and Johnson (1965) measured the equilibrium surface tension of saline extracts of rabbit lung lavage and found it to be near 24 $mN \cdot m^{-1}$.

When looking at the quality of bulk phase dispersions as artificial replacement surfactant one must also look at the minimum surface tension achievable on high compression. The main attribute of surfactant is the ability to reach low-surface tension thereby to stabilize the lung. Minimum surface tensions measured in situ were found to be less than 9 $mN \cdot m^{-1}$ (Schürch, et al., 1976).

Another parameter of the surface behaviour of the bulk phase dispersions which may aid in the understanding of lung surfactant is the ability to reach the equilibrium surface tension after compression to minimum area. If material is excluded upon high compression then the rate of return of material must be sufficient to lower surface tension on the next compression. When material is excluded from the surface, there is a certain amount of lipid lost on every compression (Watkins, 1968) so that the surface

concentration would decrease to a value incapable of reaching low, lung stabilizing surface tensions. Since the surface tension of a monolayer at 100 percent pool size is dependent upon the concentration of the surface components, the return of excluded material maybe monitored by a change in the surface tension. Eventually this surface tension will reach a stable position, γeq. Measurements of readsorption of surface active material have been described previously (King & Clements, 1972III; Notter et al., 1975; Phillips et al., 1974). Purified canine surfactant studied by King et al., (1972I), was stirred in a dish and the surface tension was monitored using a Wilhelmy dipping plate. The surface of the suspension was aspirated by suction to remove any surface active material present. Readsorption rates were measured by observing the time required to reach γeq, the lowest surface tension achievable without any compression. The time required to reach γeq gave an indication of efficiency of the surfactant to form a surface monolayer. This is particularly important when considering an artificial replacement surfactant for infants with RDS. The artificial material should be capable of achieving γeq very quickly so that subsequent exhalations may be sustained at low surface tensions.

Based on the results attained in the binary monolayer study it was decided that the 65/35 percent molar mixture of DPPC and SOPC in bulk suspension would be worth further investigation. The results in Fig. 20a suggest that

this mixture can achieve low enough surface tension to stabilize the lung (Schurch et al., 1976) but that after each compression, it takes a long time to reach a low γ eq. It was noticed that if the trough was agitated gently the surface tension dropped more quickly and it conceivable that in a system where the subphase was stirred, the lowering of surface tension to equilibrium values would be facilitated. It appears from the results that high compression of the surface removed a large amount of surface active material and this material is very slow to return. This indicated that the material which had been squeezed to the subphase formed a relatively stable structure.

Since the return to a surface monolayer is an important event in surfactant function it was decided to investigate ways by which this return might be facilitated. It has been discussed previously that stirring of the subphase might aid in surface readsorption but this property is difficult to relate to a physiological parameter. However, it is conceivable that respiratory movements may play a significant role in moving surfactant to the air-water interface.

DPPC forms a very stable bilayer in bulk phase (Tierney & Johnson, 1965; King & Clements, 1972 III; Villalonga, 1972) and its movement to a surface monolayer is very slow. The addition of a third component which was capable of destabilizing the bilayer improved readsorptive properties. A mixture of DPPC, SOPC and lyso-oleoyl

phosphatidylcholine was prepared in suspension and surface isotherms are shown in Fig. 20b. Fully expanded the surface tension of the monolayer over the suspension was approximately 28 $mN \cdot m^{-1}$ and it could be compressed to 2 $mN \cdot m^{-1}$. Schürch et al., (1976) measured, in situ, values of 29 $mN \cdot m^{-1}$ and <9 $mN \cdot m^{-1}$ at total lung capacity and functional residual volume respectively in canine lung. After the initial compression, the surface tension of the expanded monolayer was 35 $mN \cdot m^{-1}$ but dropped to 27 $mN \cdot m^{-1}$ within 2 minutes. This indicated that the lyso oleoyl phosphatidylcholine may have promoted the insertion of lipid into the monolayer. When the amount of lyso PC was increased to 9 mole percent a surface tension of only 9-10 $mN \cdot m^{-1}$ could be achieved on maximum compression. The addition of more lyso PC evidently increased the "fluidity" of the monolayer so that insufficient material could be excluded on compression to maintain a rigid surface monolayer. It is of note that with the mixture containing DPPC/SOPC/lyso oleoyl PC (61.5/34/5.5), the time needed to achieve the equilibrium value of 27-28 $mN \cdot m^{-1}$ increased with successive compressions. This demonstrated that the mechanism by which reinsertion occurs after each compression was not sufficient to maintain a low surface tension on cycling and that some material was lost into the subphase. Apparently, there may be a fine balance in the molar ratios of these components in bulk dispersion to attain optimum results for the parameters examined. Such a balance must exist in the mammalian surfactant system so

that alveolar lipid in the bulk phase will be fluid enough to promote insertion of DPPC, but the monolayer which is formed will be rigid enough to stabilize the lung.

Phosphatidylglycerol has been thought to play a possible part in surfactant function (Hallman & Gluck, 1976) and may aid in the promotion of DPPC to the surface. A 3:1 molar mixture of DPPC and egg phosphatidyl glycerol gave very encouraging results for spreadability in experiments done by Bangham et al., (1979) when the material was spread from a dry state on the surface. A mixture of DPPC/egg PG/ lyso oleoyl PC (65/20/15) showed very poor results with respect to the ability to reach low γ_{min} (Fig. 21A). Low surface tensions could not be achieved even at the highest compression rate of 3.6 cm^2/sec. Even when the surface was left undisturbed for an hour and the surface tension at full expansion fell to 44 $mN \cdot m^{-1}$ and subsequent compression could not achieve low surface tension. The mixture is apparently too fluid to reach values of surface tension anywhere near those necessary for lung function. Although the mixture is very very fluid, it does not promote insertion of material into the interface since a rapid fall in surface tension after re-expansion to 100 percent pool size was not seen.

Since encouraging surface characteristics were found in mixtures of DPPC with SOPC and lyso oleoyl PC, similar experiments were performed with OSPC (Figs. 21 B & C). For a mixture containing DPPC/OSPC/lyso oleoyl PC (67/24/9), low surface tension, relatively rapid reinsertion and a low

equilibrium surface tension of 21 $mN \cdot m^{-1}$ were observed. The first compression did not reach near zero surface tension perhaps because it was not compressed from γ eq. When the surface was left undisturbed for 1 hour, a γ eq of 21 $mN \cdot m^{-1}$ was achieved. Subsequent compressions reached 0 $mN \cdot m^{-1}$ although the area of the hysteresis loop decreased indicating a loss of surface active material. When the amount of OSPC was increased to 30 percent and the lyso reduced to 5 percent (frame C) low surface tensions could be reached even on the first compression. This mixture with 30 percent OSPC also displayed reproducible hysteresis loops for ten successive attempts indicating no net loss of surface active material. The results show that in these systems small variations in the proportions of OSPC and lyso oleoyl PC produce significant changes in the surface behaviour of the mixtures. The mixture containing a higher proportion of the lyso material (panel B) was capable of achieving a lower γmax indicating the promotion of more lipid to the surface. The mixture containing a higher proportion of OSPC to lyso material (C) appeared to be extremely efficient at eliminating fluid material upon compression as well as reinsertion of more material upon re-expansion as evidenced by its reproducible hysteresis indicating no net loss of surface material.

In bulk phase mixtures it is likely that the fluid lipid determines the eventual γmin. Fluid lipid is inserted with DPPC into the monolayer. The nature of this fluid moiety will determine whether it will be excluded upon

compression. A very fluid bulk mixture will promote insertion of lipid but compression will not result in low γ . This may be a result of insufficient DPPC at the interface or inefficient exclusion of the fluid component. If the bulk phase mixture is too rigid then not enough DPPC will be inserted into a monolayer to reach low γ on compression.

General considerations

The understanding of the surface behaviour of certain phospholipids have given researchers much information concerning the mechanism of action of lung surfactant. Using a Wilhelmy type balance, it has been demonstrated that the surface tension of lung surfactant decreases with surface area and hence area per molecule. The surface tension-area curves obtained for surfactant and its substituent lipids are similar in shape to that found for pressure-volume curves of exised lungs. Avery and Mead (1959) used the surface balance to detect the difference in the ability of normal surfactant to reach lower surface tension than that from infants with Hyaline Membrane Disease. The surface balance is also very useful in studies such as this one to examine the activity of certain phospholipids and their mixtures to fully understand the mechanism of action of lung surfactant.

There are limitations to the extrapolation of information from the Wilhelmy Balance to the functioning lung. First of all it is difficult to determine the rate of change of the alveolar surface area and hence the compression

rate at which to set the balance. All the alveoli do not open and close synchronously; some alveoli are opening while others are closing. For this reason several rates were used in this study and these were similar to those used by other experimenters (Hallman and Gluck, 1976, 1.6 $cm^2 \cdot sec^{-1}$; King and Clements, 1972 III, 3.06 $cm^2 \cdot sec^{-1}$; Hildebran et al., 1979, 1.7 $cm^2 \cdot sec^{-1}$). To achieve rates faster than 3.6 $cm^2 \cdot sec^{-1}$ the design of the trough would likely have to be changed to avoid formation of wave motions within the trough. The important thing is that the compression rate affects the shape of the isotherm (Villalonga, 1968) and we find that in mixed monolayers the maximum surface tension is affected by compression rate.

Concern has been expressed by some researchers that there is a certain amount of leakage around the dam in the Wilhelmy-type balance (Hildebran et al., 1979). This is a common fear when using teflon, glass or wax as a trough and dam. There exists also the possibility that surface active material can "creep" up the sides of the trough especially when subjected to high surface pressures. To partially alleviate this problem Hildebran et al., (1979) coated the inner surfaces of the trough and dam with 0.1M lanthanum chloride ($LaCl_3$). This led to reproducible isotherms especially when monolayers were kept at a high surface pressure for extended periods of time. This procedure was attempted in this laboratory but gave no noticeable differences in isotherms. $LaCl_3$ was found to influence the

transition temperature of DPPC. When a 1:2 weight:volume dispersion of DPPC and 0.1 M $LaCl_3$ was examined using differential scanning calorimetry, the phase transition temperature was elevated to 46°C as compared to 41.4°C for a similar mixture of DPPC and distilled water. Because the balance of DPPC and more fluid lipids in surfactant is a delicate one and depends on the relative phases of the components, it was decided not to attempt further use of the lanthanium chloride treatment in case any residual $LaCl_3$ might change the phase characteristics of the monolayers.

Another consideration which arises concerning this type of surface balance is the contact angle made between the dipping plate and the surface. It has been shown that the angle of contact influences the surface tension measurement (Sato & Kishimoto, 1979). Care was taken to roughen the platinum flag before usage to attain reproducible results.

Although the Wilhelmy surface balance has some limitations it has proven to be a useful tool in discerning the mechanism of surfactant function. Especially when used in conjunction with other physical-chemical techniques for examining properties of surfactant it can provide useful insight into the relationship between surfactant composition and surfactant function.

CONCLUSIONS

This study systematically examined the surface activity of monolayers of some synthetic phospholipids under a dynamic compression as a function of temperature. It was observed that the ability to reach low surface tension under high lateral surface pressure is dependent on the phase of the lipid. Because phospholipids comprise a large proportion of lung surfactant, a delicate balance of fluid and rigid lipid must be maintained to provide the correct surface activity to keep the lung stabilized.

From the results the following conculsions can be made:

1. The ability to reach low surface tensions in monolayers of phosphatidylcholines is dependent on the presence of rigid lipid. Monolayers of unsaturated and saturated lecithins are both capable of achieving very low surface tension when compressed as long as they are below their respective phase transition temperatures. Conversely monolayers of unsaturated and saturated lecithins are incapable of reaching very low surface tensions above their phase transition temperatures.

2. In binary monolayers containing rigid and fluid lipids the minimum surface tension attainable depended on the relative amounts of the lecithins. When DPPC was mixed with a lower melting lecithin and compressed at 37°C, the minimum surface tension depended on the amount of fluid lipid present. As the mole percentage of DPPC in the monolayer was

decreased the minimum surface tension attainable on compression progressively increased.

3. The minimum surface tension attainable in binary monolayers is dependent on the type of fluid lecithin used. Reaching low surface tensions upon high compression may be dependent on the ability of the lipid to pack tightly in the monolayer. Those which do not exclude readily upon compression will not form a DPPC enriched surface and hence low surface tension. Lipids which do not pack tightly in the monolayer may be "squeezed out" to reach low surface tension. The ability to form a tight packing arrangement may be dependent on the specific structure as well as the phase of the fluid moiety.

4. In binary monolayers of DPPC and fluid lecithins, low surface tension may be achieved by the exclusion of the fluid moiety during high compression. The resultant DPPC enriched surface is capable of reaching low surface tensions.

5. The minimum surface tension attainable in binary monolayers is dependent on the rate at which the surface compressed.

6. Monolayers of dipalmitoyl phosphatidylethanolamine, are incapable of reaching low surface tension even when the lipid is below its t_c.

7. Dipamitoyl N-methyl phosphatidylethanolamine and Dipalmitoyl N,N-dimethyl phosphatidylethanolamine show intermediate abilities to reach low surface tensions on

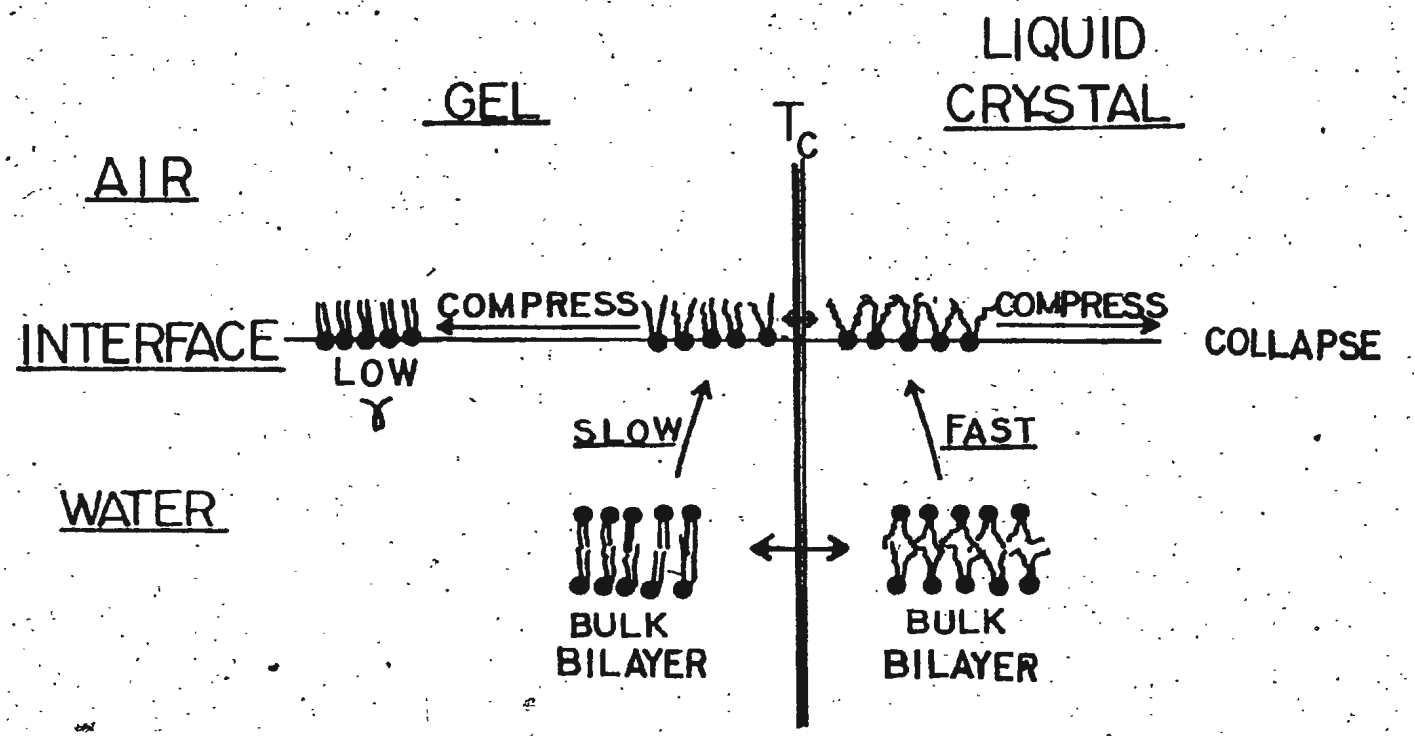
compression in comparison to those of DPPE and DPPC.

8. The minimum surface tensions of monolayers mixture of DPPC and DPPE depends on the relative molar amounts of the two components.

Overall summary

Dipalmitoyl phosphatidylcholine is the major surface active material of mammalian lung surfactant and accounts for its ability to reach the low surface tensions necessary for pulmonary stability. Although other lecithins can reach surface tensions near zero $mN \cdot m^{-1}$ under appropriate conditions, only DPPC is suited as the major surfactant material. The key to the uniqueness of DPPC is in its phase behaviour with respect to temperature. A summary of the surface behaviour of DPPC is shown in Fig. 25. DPPC undergoes a phase transition (t_c) at 41°C at which point the fatty acyl chains go from a rigid, low motion state to one which is more fluid with a high degree of mobility. Below the t_c DPPC has great difficulty in leaving the bulk bilayer to form a surface monolayer. Once in a monolayer, the rigid lecithin films can reach extremely low surface tensions when compressed. Above the t_c, DPPC spreads spontaneously to a surface monolayer but once compressed collapses at about $\gamma = 20\ mN \cdot m^{-1}$. At body temperature DPPC is in a rigid state capable of lowering surface tension if inserted into the interface. However to promote insertion the phase of DPPC must be changed either by a change of temperature or some other means.

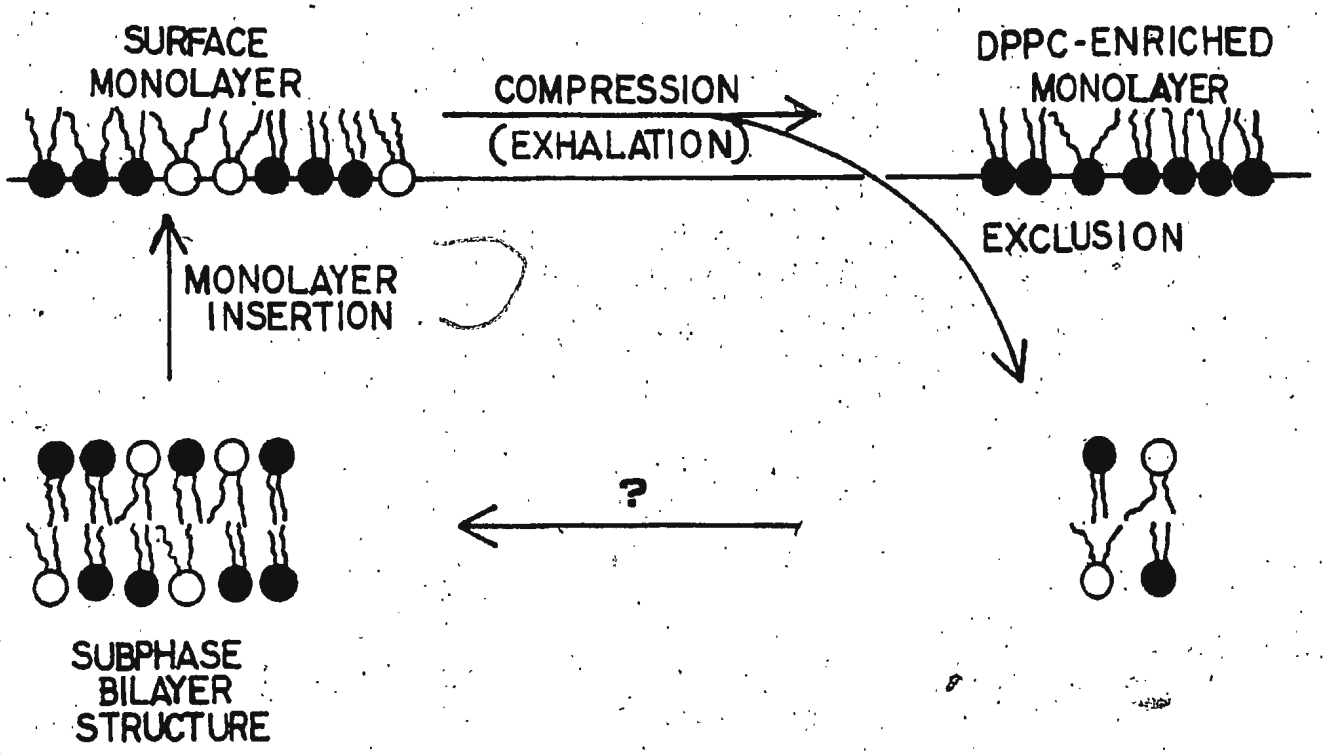
Figure 25. Summary of the monolayer and bulk phase behaviour of lecithin above and below its phase transition temperature. Above t_c promotion to the monolayer is very fast but subsequent compression results in collapse. Below t_c monolayer insertion is slow but compression results in low surface tension.


GEL
LIQUID CRYSTAL
T_c
AIR
INTERFACE
WATER
COMPRESS
COMPRESS
COLLAPSE
LOW
γ
SLOW
FAST
BULK BILAYER
BULK BILAYER

Since the body does not normally change its core temperature to any significant degree, the phase of DPPC may be altered by the other surfactant components. A hypothetical scheme is shown in Fig. 26. The other surfactant components may "fluidize" DPPC to promote insertion into the interface. Because this new fluid monolayer is liquid crystalline in nature it is incapable of achieving the low surface tension needed to provide lung stability. It has been postulated that under high compression (or a decrease in molecular surface area) the fluid lipid will preferentially exit the surface leaving a DPPC-enriched surface capable of reaching near-zero surface tension. A fine balance of lipids must exist so that the bulk phase is fluid enough to promote insertion into the monolayer but the monolayer can become sufficiently rigid to lower surface tension.

This work provides specific information about the surface and fluidity relationships of individual molecular species and their simple mixtures. These studies contribute to the detailed understanding of the complex surfactant mixture at the molecular level. These findings also provide some information about how individual molecular species and their simple mixtures may be useful in the design of replacement surfactants.

Figure 26. Possible mechanism of lowering surface tension in lipid the lung. General insertion of fluid lipid and DPPC into the monolayer; compression of the monolayer excluding some fluid lipid leaving behind a DPPC enriched monolayer capable of effectively lowering surface tension; excluded material incorporated into subphase structure.


SURFACE
MONOLAYER
COMPRESSION
(EXHALATION)
DPPC-ENRICHED
MONOLAYER
EXCLUSION
MONOLAYER
INSERTION
?
SUBPHASE
BILAYER
STRUCTURE

REFERENCES

Askin, F.B. and C. Kuhn. The cellular origin of pulmonary surfactant. Lab. Invest. 25:260-268,1971.

Avery, M.E. and J. Mead. Surface properties in relation to atelectasis and Hyaline Membrane Disease. A.M.A. Journal of Diseases of Children. 97:517-523, 1959.

Avery, M.E., N.S. Wang and H.W. Taeusch. The lung of the newborn infant. Sci. Amer. 228:75, 1973.

Bangham, A.D., C.J. Morley and M.C. Phillips. The physical properties of an effective lung surfactant. Biochim.Biophys.Acta. 573:552-556, 1979.

Blume, A. A comparative study of the phase transitions of phospholipid bilayers and monolayers. Biochim. Biophys.Acta. 557:32-44, 1979.

Brown, E.S. Lung area from surface tension effects. Proc. Soc. Expl. Biol. Med. 95:168-170, 1957.

Brown, E.S. Isolation and assay of dipalmityl lecithin in lung extracts. Am. J. Physiol. 207:402-406, 1964.

Brown, E.S., R.P. Johnson and J.A. Clements. Pulmonary surface tension. J. Appl. Physiol. 14:717-720, 1959.

Chapman, D. Phase transitions and fluidity characteristics of lipids and cell membranes. Quarterly Reviews of Biophysics. 8:185-235, 1975.

Chapman, D., R.M. Williams and R.D. Ladbrooke. Physical studies of phospholipids. VI. Thermotropic and lyotropic mesomorphism of some 1,2-diacyl-phosphatidylcholines (lecithins). Chem. Phys. Lipids. 1:445-475, 1967.

Clements, J.A. Surface tension of lung extracts. Proc. Soc. Expl. Biol. Med. 95:170-172, 1957.

Clements, J.A. Surface phenomena in relation to pulmonary function. The Physiologist. 5:11-28, 1962.

Clements, J.A. and H.J. Trahan. Effect of temperature on pressure-volume characteristics of rat lungs. Federation Proc. Abs. no. 719. 22:281, 1963.

Clements, J.A. The alveolar lining layer. In: Development of the Lung. A.V.S. de Reuck and R. Porter Eds. Little, Brown and Co., Boston 202-221, 1967.

Clements, J.A. Functions of the alveolar lining. Am. Rev. Resp. Dis. 115:67-71, 1977.

Cubero-Robles and D. Van Den Berg. Synthesis of lecithins by acylation of O-(sn-glycero-3-phosphoryl) choline with fatty acid anhydrides. Biochim. Biophys. Acta. 187:520-526, 1969.

Culls, P.R. and B.DeKruiff. Lipid polymorphism and functional roles of lipids in biological membranes. Biochim. Biophys. Acta. 559:399-420, 1979.

Davis, P.J., K.P. Coolbear and K.M.W. Keough. Differential scanning calorimetric studies of the thermotropic behaviour of membranes composed of dipalmitoyl-lecithin and mixed-acid unsaturated lecithins. Can. J. Biochem. 58:851-858, 1980.

Evans, R.W., M.A. Williams and J. Tinoco. Surface viscosities of phospholipids alone and with cholesterol in monolayers at the air-water interface. Lipids. 15: 524-533, 1980.

Fiske, C.H. and Y. Subbarow. The colorimetric determination of phosphorous. J. Biol. Chem. 66:475, 1925.

Fujiwara, T., S. Chida, Y. Watabe, H. Maeta, T. Morita and T. Abe. Artificial surfactant therapy in Hyaline Membrane Disease. The Lancet. 8159:55-59, 1980.

Fugiwara, T., Y. Tanaka, and T. Takei. Surface properties of artificial surfactant in composition with natural and synthetic surfactant lipids. Resp. Sys. 7:311, 1979.

Gershfeld, N.L. and K. Tajima. Energetics of the transition between lecithin monolayers and bilayers. J. Coll. Inter. Sci. 59:597-604, 1977.

Gershfeld, N.L. and K. Tajima. Spontaneous formation of bilayers at the air-water interface. Nature. 279:708-709, 1979.

Gikas, E.G., R.J. King, E.J. Mescher, A.C.G. Platzker, J.A. Kitterman, P.L. Ballard, B.J. Benson, W.H. Tooley and J.A. Clements. Radioimmunoassay of pulmonary

surface-active material in the tracheal fluid of the fetal lamb. Amer. Rev. Resp. Dis. 115:587-593, 1977.

Gladston, M. and D.O. Shah. Surface properties and hysteresis of dipalmitoyllecithin in relation to the alveolar lining layer. Biochim. Biophys. Acta. 137: 255-263, 1967.

Goerke, J. Lung surfactant. Biochim. Biophys. Acta. 344:241-261, 1974.

Grathwhohl, C., A. Newman, P. Phizackerley, and M. Town. Structural studies on lamellated osmophilic bodies isolated from pig lung. Biochim. Biophys. Acta. 552:509-18, 1979.

Gruenwald, P. Pulmonary surface active forces as affected by temperature. Arch. Path. 77:568-574, 1964.

Hallman, M. and L. Gluck. Phosphatidylglycerol in lung surfactant. III. Possible modifier of surfactant function. J. Lipid Res. 17:257-262, 1976.

Hildebran, J.N., Goerke, J. and J.A. Clements. Pulmonary surface film stability and composition. J. Appl. Physiol. 47:604-611, 1979.

Hildebrandt, J. Lung surfactant mechanics: Some unresolved problems. In: Research topics in physiology. Regulation of ventilation and gas exchange. D.G. Davis and D. Barnes. Eds. Academic Press. New York, 261-297, 1978.

Huang, C.H. A structural model for the cholesterol-phosphatidylcholine complexes in bilayer membranes. Lipids. 12:348-355, 1977.

Keough, K.M.W. and P.J. Davis. Gel to liquid-crystalline phase transitions in water dispersions of saturated mixed acid phosphatidylcholines. Biochemistry. 18:1453-1459, 1979.

King, R.J. The surfactant system of the lung. Federation Proc. 33: 2238-2247, 1974.

King, R.J. and J.A. Clements. Surface active materials from dog lung. I. Method of isolation. Am. J. Physiol. 223:707-714, 1972

King, R.J. and J.A. Clements. Surface active materials from dog lung. II. Composition and physiological correlations. Am. J. Physiol. 223:715-726, 1972.

King, R.J. and J.A. Clements. Surface active materials from dog lung. III. Am. J. Physiol. 223:727-1972.

King, R.J., E.G. Gikas, J. Ruch and J.A. Clements. The radioimmunoassay of pulmonary surface active material in sheep lung. Amer. Rev. Respir. Dis. 110:273-281, 1974.

King, R.J., D.J. Klass, E.G. Gikas and J.A. Clements. Isolation of apolipoproteins from canine surface active material. Am. J. Physiol. 224:788-795, 1973.

King, R.J. and C. MacBeth. Physiochemical properties of dipalmitoyl phospatidylcholine after interaction with an apolipoprotein of pulmonary surfactnat. Biochim. Biophys. Acta. 557:86-101, 1979.

King, R.J., J. Ruch, E.G. Gikas, A. Platzker and R.K. Creasy. Appearance of apolipoproteins of pulmonary surfactant in human amniotic fluid. J. Appl. Physiol. 39: 735-741, 1975.

Klaus, M.H., J.A. Clements and R.J. Havel. Composition of surface active material isolated from beef lung. Proc. Nat. Acad. Sci. 47: 1858-1859, 1961.

Lau, M.J. and K.M.W. Keough. Lipid composition of lung and lung lavage fluid from map turtles (Malaclemys geographica) maintained at different environmental temperatures. Can. J. Biochem. 59:208-219, 1981.

Luzzati, V. and A. Tardieu. Lipid phases: Structure and structural transitions. Ann. Rev. of Phys. Chem. 25:79-94, 1974.

Macklin, C.C. The pulmonary alveolar mucoid film and the pneumocytes. The Lancet. :1099-1104, 1954.

Morley, C.J., A.D. Bangham, P. Johnson, G.D. Thorburn and G. Jenkin. Physical and physiological properties of dry lung surfactant. Nature. 271:162-163, 1978.

Morley, C.J., N. Miller, A.D. Bangham and J.A. Davis. Dry artificial lung surfactant and its effect on very premature babies. Lancet. 1:64-68, 1981.

Notter, R.H. Surfactant composition in hyaline membrane disease. N. Engl. J. Med. 301:612-613, 1979.

Notter, R.H. and P.E. Morrow. Pulmonary surfactant: A surface chemistry standpoint. Ann. Biomed. Engineering. 3:119-159, 1975.

Notter, R.H., S.A. Tabak, S. Holcomb and R.D. Mavis. Postcollapse dynamic surface pressure relaxation in binary surface films containing dipalmitoyl phosphatidylcholine. J. Coll. Inter. Sci. 74:370-377, 1980.

Pattle, R.E. Properties, function and origin of the alveolar lining layer. Nature. 175:1125-1126, 1955.

Phillips, M.C. and Chapman, D. Monolayer characteristics of saturated 1,2-diacyl phosphatidylcholines (lecithins) and phosphatidylethanolamines at the air-water interface. Biochim. Biophys. Acta. 163:301-313, 1968.

Phillips, M.C., E.G. Finer and H. Hauser. Differences between conformations of lecithin and phosphatidylethanolamine polar groups and their effects on interactions of phospholipid bilayer membranes. Biochim. Biophys. Acta. 290:397-402, 1972.

Phillips, M.C. and H. Hauser. Spreading of solid glycerides and phospholipids at the air-water interface. J. Coll. Inter. Sci. 49:31-39, 1974.

Phillips, M.C., B.D. Ladbrooke and D. Chapman. Molecular interactions in mixed lecithin systems. Biochim. Biophys. Acta. 196:35-44, 1970.

Ries, H.E. Stable ridges in a collapsing monolayer. Nature. 281:287-289, 1979.

Roseman, M.A., B.R. Lentz, B. Sears, D. Gibbes and T.E. Thompson. Properties of sonicated vesicles of three synthetic phospholipids. Chem. Phys. Lipids. 21:205-222, 1978.

Sato, S. and H. Kishimoto. The contact angle of phospholipid monolayer on a Wilhelmy Plate. J. Coll. Inter. Sci. 69:188-191, 1979.

Schürch, S., J. Goerke and J.A. Clements. Direct determination of surface tension in the lung. Proc. Natl. Acad. Sci. 73:4698-4702, 1976.

Seelig, J. and H.U. Gally. Investigation of phosphatidylethanolamine bilayers by deuterium and phosphorus-31 nuclear magnetic resonance. Biochemistry. 15:5199-5204, 1976.

Shelley, S.A., M. Kovacevic, J.E. Pachia and U. Balis. Sequential changes of surfactant phosphatidylcholine in hyaline membrane disease of the

newborn. N. Engl. J. Med. 300; 112-116, 1979.

Standish, M.M. and B.A. Pethica. Surface pressure and surface potential study of a synthetic phospholipid at the air-water interface. Trans. Faraday. Soc. 64: 1113-1122, 1968.

Steim, J.M. The physics of lung surfactant. Proc. of the 70th Ross Conference on Pediatric Research. Lung maturation and the prevention of hyaline membrane disease. 10-17, 1976.

Steim, J.M., R.A. Redding, C.T. Hauck and M. Stein. Isolation and characterization of lung surfactant. Biochem. Biophys. Res. Comm. 34:434-440, 1969.

Tabak, S.A. and R.H. Notter. Factors affecting pure and mixed films of pulmonary surfactant components. Federation Proc. 34:426, 1975.

Tierney, D.F. and R.P. Johnson. Altered surface tension of lung extracts and lung mechanics. J. Appl. Physiol. 20:1253-1260, 1965.

Toshima, N. and T. Akino. Alveolar and tissue phospholipids of rat lung. Tohoku. J. Exp. Med. 108: 253-263, 1972.

Trauble, H., H. Eibl and H. Sawada. Respiration - A critical phenomenon? Lipid phase transitions in the lung alveolar surfactant. Naturwissenschaften. 61:344-354, 1974.

van Deenen, L.L.M., U.M.T. Houtsmuller, G.H. deHaas and E. Mulder. Monomolecular layers of synthetic phosphatides. J. Pharm. and Pharmacol. 14:429-444, 1962.

Vaughan, D.J. and K.M.W. Keough. Changes in phase transitions of phosphatidylethanolamine- and phosphatidylcholine-water dispersions induced by small modifications in the headgroup and backbone regions. FEBS Lett. 47:158-161, 1974.

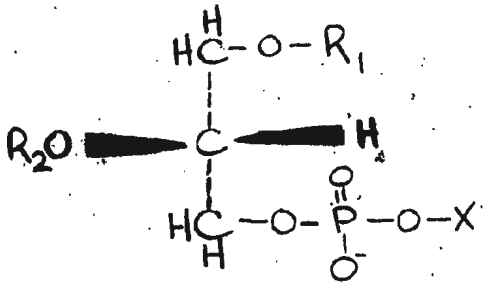
Villalonga, F. Surface chemistry of L-α-diplmitoyl lecithin at the air-water interface. Biochim. Biophys. Acta. 163:290-300, 1968.

von Neergaard, K. Neue Auffassun über einen Gundegriff. der Atemmechanik. Die Retraktionskaft der Lunge, abhängig von der Oberflächenspannung un den Alveolen. Z. Gestamte. Exp. Med. 66:373-394, 1929.

Watkins, J.C. The surface properties of pure phospholipids in relation to those of lung extracts. Biochim. Biophys. Acta. 152:293-306, 1968.

Wildeboer-Venema, Fried. A model for the study of the physical behaviour of the lung surfactant film in vitro. Respir. Physiol. 32:225-237, 1978.

APPENDIX A NAMES & STRUCTURES OF LIPIDS



R_1	R_2	X	NAME	
$CH_3(CH_2)_{14}C(=O)$	$CH_3(CH_2)_{14}C(=O)$	$CH_2CH_2\overset{+}{N}(CH_3)_3$	1,2-dipalmitoyl-sn-glycerol-3-phosphocholine	DPPC
$CH_3(CH_2)_{14}C(=O)$	$CH_3(CH_2)_2CH=CH(CH_2)_7C(=O)$	$CH_2CH_2\overset{+}{N}(CH_3)_3$	1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine	POPC
$CH_3(CH_2)_{16}C(=O)$	$CH_3(CH_2)_7CH=CH(CH_2)_7C(=O)$	$CH_2CH_2\overset{+}{N}(CH_3)_3$	1-stearoyl-2-oleoyl-sn-glycerol-3-phosphocholine	SOPC
$CH_3(CH_2)_7CH=CH(CH_2)_7C(=O)$	$CH_3(CH_2)_{16}C(=O)$	$CH_2CH_2\overset{+}{N}(CH_3)_3$	1-oleoyl-2-stearoyl-sn-glycerol-3-phosphocholine	OSPC
$CH_3(CH_2)_{16}C(=O)$	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_2C(=O)$	$CH_2CH_2\overset{+}{N}(CH_3)_3$	1-stearoyl-2-linoleoyl-sn-glycerol-3-phosphocholine	SLPC
$CH_3(CH_2)_7CH=CH(CH_2)_7C(=O)$	H	$CH_2CH_2\overset{+}{N}(CH_3)_3$	1-oleoyl-sn-glycerol-3-phosphocholine	lyso PC
$CH_3(CH_2)_{12}C(=O)$	$CH_3(CH_2)_{12}C(=O)$	$CH_2CH_2\overset{+}{N}(CH_3)_3$	1,2-dimyristoyl-sn-glycerol-3-phosphocholine	DMPC

$CH_3(CH_2)_{10}C(=O)$	$CH_3(CH_2)_{10}C(=O)$	$CH_2CH_2\overset{+}{N}(CH_3)_3$	1,2-dilauryl-<u>sn</u>-glycerol-3-phosphocholine	DLPC
$CH_3(CH_2)_{14}C(=O)$	$CH_3(CH_2)_{14}C(=O)$	$CH_2CH_2\overset{+}{N}H_3$	1,2-dipalmitoyl-<u>sn</u>-glycerol-3-phosphoethanolamine	DPPE
$CH_3(CH_2)_{14}C(=O)$	$CH_3(CH_2)_{14}C(=O)$	$CH_2CH_2\overset{+}{N}H_2CH_3$	1,2-dipalmitoyl-<u>sn</u>-glycerol-3-phospho-N-methyl ethanolamine	N-Me DPPE
$CH_3(CH_2)_{14}C(=O)$	$CH_3(CH_2)_{14}C(=O)$	$CH_2CH_2\overset{+}{N}H(CH_3)_2$	1,2-dipalmitoyl-<u>sn</u>-glycerol-3-phospho-N,N-dimethyl-ethanolamine	N,N-di-Me DPPE
70-80% $CH_3(CH_2)_{14}C(=O)$ 20-30% other chains	70-80% $CH_3(CH_2)_7CH{=}CH(CH_2)_7C(=O)$ 20-30% other chains	$-C(H)(H)-C(OH)(H)-C(H)(H)-OH$	<u>rac</u>-1,2-diacyl-<u>sn</u>-glycero-3-phospho-sn'-glycerol	Egg PG
$CH_3(CH_2)_{16}C(=O)$	$CH_3(CH_2)_{16}C(=O)$	$CH_2CH_2\overset{+}{N}H_3$	1,2-distearoyl-<u>sn</u>-glycerol-3-phosphoethanolamine	DSPE

APPENDIX B

COMPOSITION OF SOLUTIONS USED IN THE ISOLATION OF PIG LUNG SURFACTANT (King and Clements, 1972I)

Solution	Density (g/ml)	MOLARITY				
		NaCl	NaBr	$MgCl_2$	$CaCl_2$	Tris HCl pH 7.33
1	1.008	0.15	-	0.003	0.003	0.005
2	1.057	0.15	0.60	0.004	0.004	0.005
3	1.124	0.15	1.40	0.031	0.031	0.005
4	1.143	0.15	1.64	0.035	0.035	0.005
5	1.174	0.15	2.02	0.043	0.043	0.005
6	1.096	0.15	1.05	0.024	0.024	0.005

APPENDIX C

PUBLICATIONS FROM THIS WORK

1. Hawco, M.W., P.J. Davis, K.P. Coolbear and K.M.W. Keough. Lipid fluidity and pulmonary surfactant function - Models for normal and abnormal surfactant. CFBS Proc. 23: Abstract no. 385, 111, 1980.

2. Hawco, M.W., P.J. Davis and K.M.W. Keough. The role of lipid fluidity in lung surfactant mechanics. The role of some saturated and unsaturated lecithins and their mixtures. J. Appl. Phys. 51(2): 509-515, 1980.

3. Hawco, M.W., K.P. Coolbear, P.J. Davis and K.M.W. Keough. Exclusion of fluid lipid during compression of monolayers of mixtures of dipalmitoyl lecithin with some other lecithins. Biochim. Biophys. Acta. 646:185:197, 1981.