Review

The role of lipids in pulmonary surfactant

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Abstract

Pulmonary surfactant is composed of approx. 90% lipids and 10% protein. This review article focusses on the lipid components of surfactant. The first sections will describe the lipid composition of mammalian surfactant and the techniques that have been utilized to study the involvement of these lipids in reducing the surface tension at an air-liquid interface, the main function of pulmonary surfactant. Subsequently, the roles of specific lipids in surfactant will be discussed. For the two main surfactant phospholipids, phosphatidylcholine and phosphatidylglycerol, specific contributions to the overall surface tension reducing properties of surfactant have been indicated. In contrast, the role of the minor phospholipid components and the neutral lipid fraction of surfactant is less clear and requires further study. Recent technical advances, such as fluorescent microscopic techniques, hold great potential for expanding our knowledge of how surfactant lipids, including some of the minor components, function. Interesting information regarding surfactant lipids has also been obtained in studies evaluating the surfactant system in non-mammalian species. In certain non-mammalian species (and at least one marsupial), surfactant lipid composition, most notably disaturated phosphatidylcholine and cholesterol, changes drastically under different conditions such as an alteration in body temperature. The impact of these changes on surfactant function provide insight into the function of these lipids, not only in non-mammalian lungs but also in the surfactant from mammalian species. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Pulmonary surfactant; Surfactant lipid; Surfactant composition; Dipalmitoylphosphatidylcholine; Phosphatidylcholine; Phosphatidylglycerol; Neutral lipid; Non-mammalian surfactant; Surface tension; Alveolar stability

Contents

1. Introduction .......................................................... 91
2. Composition .......................................................... 92

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; FRC, functional residual capacity; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; SM, sphingomyelin; TLC, total lung capacity

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1. Introduction

A major function of pulmonary surfactant is to reduce surface tension at the air-water interface of the terminal airways, thereby decreasing the tendency for alveolar collapse. It was surfactant’s ability to reduce surface tension and thereby increase compliance (i.e., elasticity) of the excised lung that led to its initial discovery by von Neergaard in the 1920s [1]. This was truly a ‘premature’ discovery [2,3] which exceeded the scientific and medical communities’ capacity to appreciate its significance. Von Neergaard clearly understood that the lining layer of the lung possessed special surface properties superior to those of adsorbed serum. Whether he understood that phospholipids, or even lipids, were involved is not clear. Von Neergaard also recognized the importance of low surface tension for the newborn lung.

Surfactant’s capacity for reducing surface tension also led to its rediscovery in the mid-1950s by Pattle [4] and Clements [5]. These early surfactologists not only recognized the ability of pulmonary surfactant to adsorb and spread rapidly to form an insoluble surface film, but also demonstrated that reductions in surface area resulted in a corresponding fall in surface tension to surprising if not unprecedented low values. By this time, the ability of lipids to act as surface tension reducing agents was well recognized. Lecithin, or phosphatidylcholine (PC), had been discovered as a component of egg yolk in 1847. The presence of the disaturated lecithin, dipalmitoylphosphatidylcholine (DPPC) in lung tissue had been reported in 1946 [6]. Although Pattle initially intuited that pulmonary surfactant was an insoluble protein film [7], further studies soon established that phospholipids comprised the major surface tension reducing component and DPPC was a major constituent [8–10].

The rediscovery of pulmonary surfactant did not go unnoticed by physicians concerned with RDS. In 1959, Avery and Mead were able to report saline extracts of lungs from premature infants succumbing to RDS were surfactant deficient compared to infants dying of other causes [11]. This discovery spurred enormous interest in the topic of this dedicated issue.

This review will discuss the roles of the lipid components of pulmonary surfactant. Although accomplished in cooperation with the surfactant-associated proteins (SP-A, SP-B, and SP-C), it is the lipids that are ultimately responsible for surface tension reduction to low value during inhalation and expiration (see chapters by Goerke, Schürch and by Perez-Gil and Keough for further details on the roles of surfactant proteins). Furthermore, although it has never been demonstrated chemically, considerable circumstantial evidence implies that a surface monolayer highly enriched in DPPC is responsible for the critical reduction in surface tension to values near 0 mN/m that facilitates lung expansion at birth and allows
regular breathing throughout life. The roles of the other lipid components of pulmonary surfactant are less well understood. This is particularly true with surfactants recovered from non-mammalian species.

This review will try to present our best ‘educated guesses’ as to these roles and suggest potential ways of limiting the guesswork in the future. Due to limited space, other chapters in this dedicated issue and previous reviews will be used liberally to document evidence for some concepts and as sources of original references. In addition, in many cases only the most recent, key reference will be cited. Readers can use these publications to find references to earlier work.

2. Composition

The overall lipid and phospholipid compositions of surfactants isolated from a number of experimentally used species (mouse, rat, rabbit, and sheep), of bovine surfactant (which is used clinically), and of human surfactant are listed in Table 1. The authors are more impressed with the similarities than the differences. It is noteworthy that the lipid composition of lamellar bodies, the storage form of surfactant, is very similar to those given in Table 1. In all species, PC comprises approx. 80%, about half of which is DPPC. Palmitic acid is a 16 carbon acyl chain with no double bonds. Virtually no other disaturated PC species have been observed. Thus, about 40% of the total phospholipid in mammalian surfactant is composed of DPPC. Recent studies on surfactant from neonatal calves reported DPPC accounted for only 32% of the total phospholipid [12]. Although high DPPC levels have long been considered a hallmark of pulmonary surfactant, DPPC accounts for 10–20% of the phosphatidylcholine content of brain myelin and erythrocyte membranes [13,14].

The discrepancy between disaturated PC levels given here and some previous reports suggesting higher levels may have arisen in part from the use of different techniques to measure disaturated lipids. For example, it has been reported that the osmium tetroxide method of Mason [15] also detects some monoenoic (one double bond) species. This leads to artificially high estimates of disaturated PC [16]. It should also be recognized that not all of the DPPC in lungs is in surfactant. In rat and ovine lungs, only 25 and 40%, respectively, of the DPPC is surfactant associated [13,14].

The remaining PC in surfactant is composed primarily of molecular species containing monoenoic and dienoic fatty acids at the 2-position, with only minor amounts of short chains or polyunsaturated acyl groups. The acidic phospholipids, phosphatidyl-

Table 1

<table>
<thead>
<tr>
<th>Lipid composition of extracellular surfactant in selected mammalian species</th>
<th>Phospholipid composition (% total) [% disaturated]</th>
<th>Cholesterol (% chol/PL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC</td>
<td>LPC</td>
</tr>
<tr>
<td>Mus musculus</td>
<td>72.3</td>
<td>0</td>
</tr>
<tr>
<td>Mouse&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rattus norvegicus</td>
<td>82.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Rat&lt;sup&gt;b&lt;/sup&gt;</td>
<td>[49.3]</td>
<td>[32.3]</td>
</tr>
<tr>
<td>O. cuniculus</td>
<td>80.6</td>
<td>Tr</td>
</tr>
<tr>
<td>Rabbit&lt;sup&gt;b&lt;/sup&gt;</td>
<td>[52.8]</td>
<td>[38.7]</td>
</tr>
<tr>
<td>Ovis spp.</td>
<td>81</td>
<td>Tr</td>
</tr>
<tr>
<td>Ovine&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bos spp.</td>
<td>79.2</td>
<td>Tr</td>
</tr>
<tr>
<td>Bovine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>[49.9]</td>
<td>[33.3]</td>
</tr>
<tr>
<td>Homo sapiens</td>
<td>80.5</td>
<td>Tr</td>
</tr>
<tr>
<td>Human&lt;sup&gt;e&lt;/sup&gt;</td>
<td>[47.7]</td>
<td></td>
</tr>
</tbody>
</table>

PC, phosphatidylcholine; LPC, lysophosphatidylcholine; SM, sphingomyelin; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; LBPA, lyso-bis-phosphatidic acid; PL, phospholipid; chol, cholesterol; Tr, trace.

(In some cases, the authors have added ‘trace’ on the basis of their experience.)

Data from: <sup>a</sup>Langman et al., 1996 [71]; <sup>b</sup>Akino, 1992 [14]; <sup>c</sup>Possmayer, 1984 [13]; <sup>d</sup>Yu et al., 1983 [78]; <sup>e</sup>Daniels et al., 1995 [65].
glycerol (PG) and phosphatidylinositol (PI), account for 8–15% of the total surfactant phospholipid pool with most species. Adult surfactants are depicted in Table 1, so PG is high while PI is low. With surfactant from fetal and neonatal lung, PI levels are elevated and PG correspondingly lower. With some animals, such as the rhesus monkey, PI remains high into adult life. PG possesses a significant amount of disaturated lipids, although not as much as PC. However, this accounts for only a small part of the total disaturated phospholipid pool. In contrast, PI contains very low amounts of disaturated components, although this remains somewhat controversial [14].

The remaining phospholipids are present in rather small amounts. Except for the human, phosphatidylethanolamine (PE) is low with only 3–5% being present. The other phosphorus-containing phospholipids are present at very low levels. While they could represent contaminants arising from membrane lipids, the levels are quite consistent from one preparation to another and from species to species. The low phosphatidylserine (PS) level is not surprising in view of the known role of PS in triggering blood clotting reactions and in apoptosis [17].

Less information is available concerning the neutral lipid components of pulmonary surfactant. In those mammalian species reported, cholesterol appears to be the major component (80–90%). Small amounts of monoacylglycerol, diacylglycerol, and triacylglycerol are present with most species. Cholesterol esters appear to be low in most species [13,18]. Most surfactants appear to have some free fatty acids, mainly palmitate.

3. Methodology

3.1. Techniques for studying surfactant activity

Pulmonary surfactant is a unique biological system in that a major function involves the rapid formation of a highly surface-active film with an equilibrium surface tension of approx. 25 mN/m (see below). Furthermore, during breathing this film is subject to dynamic compression, resulting in lateral packing of the film to produce further lowering to near 0 mN/m. As a result, most of the information on the functional aspects of pulmonary surfactant have emerged from biophysical techniques foreign to most biochemists. In order to appreciate current investigations on the roles of surfactant lipids, it is imperative to understand the kind of information derived from these techniques and, just as importantly, their limitations. The reader can find further information in previous reviews [19–21].

The Langmuir-Wilhelmy surface balance, designed by Clements for his initial studies [5,22], consists of a trough whose surface area can be varied, and a Wilhelmy dipping plate which monitors surface tension [19,20]. This apparatus is well suited for examining area-surface tension characteristics with dilute films, that is, surfactant films with surface tensions between the 70 mN/m observed with a clean saline surface at 37°C and the equilibrium surface tension of approx. 25 mN/m for phospholipids. The equilibrium surface tension is the lowest surface tension pure or mixed phospholipid films can achieve, given sufficient lipids and a long enough time. Identical equilibrium surface tensions are observed whether the phospholipid is spread from above or adsorbed from below. Surfactant apoproteins increase adsorption rate, but to the same equilibrium surface tension.

Lateral compression of insoluble films decreases the surface area such that surface tension can fall below equilibrium. Such films are inherently unstable by definition and therefore in a metastable state. The high surface pressure required to attain low surface tensions can result in loss of film material, not only by film collapse but also onto or under the teflon barriers. This loss, termed ‘leakage’, is dependent on the sample and is more serious with increased levels of fluid lipids, i.e., lipids above their corresponding gel-to-liquid transition temperatures for bilayers, and for surfactants with neutral lipids. Such films cannot pack in an ordered fashion and are less stable than films of phospholipids below their corresponding bilayer gel-to-liquid crystalline transition temperatures. The Langmuir-Wilhelmy balance is particularly suited for studying spread films. At present, it is the only technique where precise amounts of film material can be deposited, where surface radioactivity can be monitored conveniently, and where fluorescent probes can be used to study film structure.

The pulsating bubble surfactometer introduced by
Enhorning in 1977 [23] consists of an air bubble connected by a capillary to the atmosphere. With this artificial alveolus model, a bubble created within a suspension of the surfactant sample is pulsed, normally at 20 cycles/min at 37°C, and the pressure across the bubble monitored [19]. The law of Young and Laplace, which states that the pressure across the bubble equals twice the surface tension divided by the radius, is used to calculate surface tension. The pulsating bubble monitors adsorption of surfactant during bubble expansion and its ability to obtain low surface tension during film compression generated during the 50% surface area change which occurs during reduction of bubble volume. The surface tension at maximum bubble radius is indicative of surfactant adsorption during surface expansion, while the surface at minimum radius indicates the film’s ability to achieve low surface tensions during compression. These parameters are important determinants of the quality of the surfactant and, in general, reflect the biological activity of the surfactant in vivo. The small amounts of sample required, the relatively short time needed, and the relatively straightforward technical requirements recommend this assay. It functions best at relatively high concentrations (1–10 mg/ml) and short pulsation periods because with the time bubble radius can change during pulsation. However, at low surface tensions (i.e., high surface pressures), surface material can be pushed onto the capillary so the true surface area reduction is less than 50%. Initiating pulsation while the capillary walls are wetted can also lead to increased surface area [24]. The pulsating bubble is extremely useful for monitoring the quality of surfactant relative to its biological activity, but can be problematic for mechanistic studies.

The last technique to be discussed here, the captive bubble tensiometer introduced by Schürch [25], consists of a gas-tight chamber containing a bubble floating against a hydrophilic agar gel. Increasing the pressure within the chamber compresses the bubble, thereby increasing surface area. The hydrophilic agar roof is incapable of accepting hydrophobic lipids, resulting in an apparently leak-proof system. The captive bubble tensiometer is more accurate but is very time-consuming compared to the pulsating bubble surfactometer, both experimentally and for data analysis. However, this apparatus is extremely flexible in terms of controlling surface area reduction, speed of compression, and manipulation of other bubble characteristics. One example of new information obtained with this apparatus is evidence for a surface-associated surfactant reservoir which selectively replenishes the surface film with DPPC-rich material during surface area expansion (see below).

3.2. Reconstitution studies and in vivo alterations in surfactant composition

In general, knowledge regarding the role of lipids in surfactant function arises from two approaches: reconstitution studies where purified or synthetic lipid and protein components are combined, and studies where physiological conditions occur or are imposed which result in alteration of the surfactant system in vivo. Surfactant reconstitution studies can employ simple mixtures, providing insight into the contributions made by individual components under specific conditions. Physiological conditions leading to surfactant alteration include not only acute lung injury but also transplantation after prolonged storage, mechanical ventilation, and physiological states such as exercise. As will be discussed later, with some non-mammalian species, temperature adaptation results in significant alterations in overall surfactant composition.

With acute lung injury and a number of other conditions small decreases in DPPC as a proportion of PC and increases in the PI/PG ratio are often observed. Increases in the level of so-called ‘membrane phospholipids’, PE, PS and sphingomyelin (SM) have also been noted. In some cases, lyso-PC, a product of phospholipase A2 degradation, can also increase. Alterations arising during severe lung injury are remarkably consistent among various species and different initiating causes [26,27]. These alterations in phospholipid composition are not only indicative of lung injury, but could contribute to surfactant dysfunction within the lung. It must be pointed out that studies in which organic solvent lipid extracts were altered by addition of specific lipids produced no detectable effects on pulsating bubble surfactometer tracings (I.L. Metcalfe, F. Possmayer, unpublished results; A.M. Cockshutt, F. Possmayer, unpublished results). However, surfactant isolation unavoidably results in an averaging of total lung surfactant com-
position. Even with uniform forms of acute lung injury, considerable variation is likely. Marked alterations in surfactant lipid composition in individual alveoli could lead to localized surfactant dysfunction without greatly affecting overall composition. In addition, to the best of our knowledge, the effect of combined alterations in surfactant lipids has not been examined.

Lung injuries are often, if not universally, accompanied by increases in alveolar serum protein content. Increases in lyso-PC level which did not influence the surface activity of bovine lipid extract surfactants on the pulsating bubble surfactometer sensitized this preparation to inhibition by serum proteins. Furthermore, SP-A, which prevents protein inhibition, did not reverse inhibition arising from lyso-PC [28].

It should also be recognized that interpretation of results relating alterations in surfactant lipids to surfactant function in vivo is complicated by the fact that lung injury-associated surfactant alterations do not occur in isolation. The acute lung injury changes in lipid profile are invariably accompanied by alterations in surfactant apoprotein content, and as indicated above, increases in serum proteins, which can inhibit surfactant.

4. The roles of specific lipids in surfactant function

Surfactant is a complex mixture of proteins and lipids. In the lung this complex functions to reduce the surface tension at the air-liquid interface. For some of the major lipid classes a role in this process has been indicated, but for most of the minor lipid components their exact contribution must still be established. It should also be noted that whereas most of the experimental focus has been on surface tension-reducing properties of surfactant, the lipid components of surfactant could also be involved in other metabolic aspects of the surfactant life cycle, for example lamellar body assembly and secretion. These areas require further study.

4.1. Phosphatidylcholines

The need for low surface tensions approaching 0 mN/m to stabilize the lungs during expiration was first suggested by Clements in 1957, based on theoretical calculations [5]. DPPC is not only the major component of pulmonary surfactant, but also the only major component capable of generating low surface tensions during compression on the Langmuir-Wilhelmy balance and the captive bubble tensiometer. With the captive bubble tensiometer, area reductions of 10–12% are sufficient to lower surface tension of pure DPPC films from equilibrium (approx. 25 mN/m) to less than 2 mN/m. Furthermore, if surface area is held constant, the film maintains a low surface tension and returns towards equilibrium only after many hours. In situ studies by Schürch, which used the lung as a surface balance by employing fluorocarbon microdroplets to assess alveolar surface tension, have revealed surface tensions of 30 mN/m, slightly above equilibrium at total lung capacity (TLC) [29]. Reducing lung volume to functional residual capacity (FRC) is accompanied by a 20% reduction in surface area and a fall in surface tension to near 1 mN/m. When held at FRC, surface tension remains low for several minutes, showing the alveolar surface film is stable.

The properties of DPPC mentioned above contrast with those of unsaturated PC films above the gel-to-liquid crystalline transition temperature of bilayers composed of the same lipids. Since such fluid films cannot pack in an ordered fashion, compression can lower surface tension only to 15–20 mN/m, and when dynamic compression ceases, surface tension returns towards equilibrium. Such surface tensions are incapable of stabilizing the lung at low lung volumes [29–31]. Thus it is implied that the lung contains a film highly enriched in DPPC. As indicated earlier, the alveolar surface monolayer cannot be sampled, so chemical evidence for a DPPC film is lacking.

Vesicles of pure DPPC, below the phase transition temperature of 41°C, form a surface monolayer only extremely slowly and therefore are incapable of acting directly as an adequate surfactant replacement. Addition of unsaturated PCs (or other fluid phospholipid or neutral lipid components) enhances adsorption [19] (see chapters by Goerke and Pérez-Gil and Kcough). However, in the absence of the low molecular weight hydrophobic proteins or some other adsorbance-inducing factor, adsorption of such mixtures is still inadequate. Nevertheless, it is
thought the fluidity resulting from unsaturated phospholipids and other fluid components is important for enhancing adsorption to a clean surface. Furthermore, SP-B and SP-C are very effective in promoting adsorption of surfactant lipids to partially filled monolayers so that equilibrium surface tension is rapidly attained [32].

Studies with the captive bubble tensiometer suggest two mechanisms, selective DPPC adsorption and preferential squeeze-out of unsaturated lipids, could be involved in DPPC enrichment of the interfacial film [19] (see chapters by Schürch and by Pérez-Gil and Keough). For example, bovine lipid extract surfactant (BLES) lipids are approx. 40% disaturated (Table 1). However, in the captive bubble, BLES films adsorbed to the equilibrium value of 25 mN/m, require only 30–35% surface area reduction to change surface tensions to near 0 mN/m. When SP-A is added, surface area reductions of 20–25% are sufficient. Perfect squeeze-out of the less stable unsaturated lipid components of BLES (40% disaturated) would require 60% surface area reduction. A further 4–5% surface area reduction would be required to pack the remaining DPPC to reduce surface tension from equilibrium to near 0. This indicates the initial adsorbed film is highly enriched in DPPC. With additional compression-decompression cycles, surface area reduction required to achieve low surface tension declines further to 15–20%, a value consistent with a remaining film even more highly enriched in DPPC.

The fluid components of pulmonary surfactant, such as PC and the other lipids, could also play an important role in film respreading. DPPC films can be repeatedly cycled between equilibrium and 1 mN/m. Such cycling occurs reversibly without hysteresis [29] (hysteresis implies non-reversible behaviour). Nor is hysteresis observed with the lung during quiet tidal breathing. However, overcompression of DPPC films at low surface tension results not only in hysteresis, but leads to ever-increasing surface tensions during subsequent cycles. This occurs because DPPC forced out of the monolayer into a collapsed state cannot reform the monolayer rapidly (i.e., cannot respread). As suggested above, the fluid components of pulmonary surfactant and the surfactant apoproteins could be important for respreading. As discussed further by Schürch et al. (pp. 180–202, this issue), washout experiments with the captive bubble tensiometer have provided evidence for a surface-associated surfactant reservoir, which appears to be important for feeding DPPC-enriched material into the surface monolayer during film expansion. Replenishing the surface monolayer with DPPC-enriched components is particularly important after film overcompression. This reservoir is dependent on the palmitoylated SP-C [33–35].

Although considered less often, the fluid components of pulmonary surfactant could be important in other aspects of the surfactant cycle. The processing and packaging of pure DPPC into lamellar bodies could present difficulties arising from the stiffness of this phospholipid. The effect of lipid fluidity could be important for secretion of lamellar bodies, formation of tubular myelin and/or other precursors of the surface film, and in the re-uptake of surfactant components by the type II cells [36]. It should be evident that the ancillary functions suggested for unsaturated PC, discussed here, will apply to unsaturated PG and the other fluidizing phospholipids and neutral lipid components of pulmonary surfactant.

In summary, it appears likely that the surface film covering the alveoli is highly enriched in DPPC. Unsaturated PC and other fluid components could facilitate adsorption of surfactant lipids but such a role has not been proven.

4.2. Acidic phospholipids

The observation that all pulmonary surfactants possess significant amounts of PG and/or PI suggests an important specific role. Mixing fluid acidic phospholipids with DPPC leads to enhanced adsorption, suggesting a potential role in surface tension reduction [37]. A specific interaction between PG and SP-B has been suggested by fluorescence studies [38] and by the highly surface-active properties observed with DPPC-PG-SP-B mixtures [39]. As suggested above, DPPC-enriched films could arise through the preferential adsorption of DPPC during film formation, through selective PG squeeze-out during compression, or both. With DPPC-PG mixtures, SP-B appears superior to SP-C both in promoting preferential DPPC adsorption and facilitating PG squeeze-out [34,39]. Such selective squeeze-out is not observed during compression of either spread or ad-
sorbed phospholipid films in the absence of SP-B or SP-C [40].

Decreases in the amount of PG have been observed in patients with ARDS and in other acute disease states. As with the decrease in DPPC, it has been suggested the lower PG levels could be involved in the decreased biophysical activity of recovered material. It has also been inferred that the high PI/PG ratios noted with surfactant from premature animals could result in lower activity. However, recent studies have suggested differences in SP-A could account for the lower in vitro and in vivo activity of pulmonary surfactant recovered from lambs of different gestational ages [41] (see chapter by Jobe for further discussion). Increasing the proportion of PI in surfactant by administering inositol to adult rabbits had no significant effect on surfactant function in vitro or in vivo [42,43].

In summary, acidic phospholipids represent a significant component of pulmonary surfactant. However, their precise role and why PG rather than PI is present in most adult surfactants are not understood and require further study.

4.3. Minor phospholipid components

Analysis of surfactant has revealed the consistent presence of a number of phospholipids present in relatively low amounts (Table 1). In general the role of these lipids is not clear and investigations into their contribution to surfactant function have been limited. From a surface tension reduction perspective it would be anticipated that these low amounts of phospholipids would have minimal effects on surfactant activity. The higher amounts of these lipids observed in lung injury could contribute to decreased surfactant activity, but there is no specific evidence for this. The exception is lyso-PC, which can make surfactants more susceptible to protein inhibition [28].

Since a specific surface active role for these minor lipid components of surfactant appears unlikely, other explanations for their presence in surfactant have to be sought. The presence of some of these lipids in surfactant could be related to some metabolic aspects. For example, small amounts of an acidic phospholipid thought to be lyso-bis-phosphatidic acid is present in most surfactants (Table 1), although the precise nature of this lipid has not been determined. Lysosomes contain lyso-bis-phosphatidic acid and since lamellar bodies are thought to be modified lysosomes, the presence of lyso-bis-phosphatidic acid in surfactant could be related to this origin. It could be that the lamellar body limiting membrane contains higher levels of the membrane lipids PE and SM. The limiting membrane does contain high levels of cholesterol (S. Orgeig, T.E. Nichols, unpublished results). The limiting membrane fuses with the plasma membrane during exocytosis, but some limiting membrane may be ejected with surfactant. It should also be recognized that the minor phospholipid components could be involved in signalling events related to surfactant metabolism. However, this has not been demonstrated.

4.4. Neutral lipids

Although present in significant amounts, the neutral lipids of surfactant have received far less attention experimentally than the phospholipids. Neutral lipid effects are complex and depend on the system being studied and the technique used for investigation. Addition of mono-, di-, and triacylglycerol or free palmitic acid to DPPC or DPPC-PG enhance adsorption rate, presumably by introducing minor packing defects in the membrane structure. Cholesterol can also enhance adsorption of vesicles primarily composed of DPPC, presumably by increasing fluidity, and improve film respreading [44,45]. However, it should be noted that, by increasing fluidity, cholesterol limits the minimum surface tensions obtainable during compression. This occurs because this sterol cannot be squeezed out easily from DPPC-cholesterol spread films [44,46,47]. Possibly for this reason, cholesterol is removed from most modified natural surfactants used clinically for treating neonates. Whether removing cholesterol affects physiological function significantly has not been established.

The effect of neutral lipids on the surface activity of DPPC and DPPC-PG has been examined using reconstitution studies by Tanaka et al. [48]. These investigators found the most effective reconstituted preparation contained DPPC, PG, tripalmitate or palmitic acid, and the hydrophobic surfactant proteins. DPPC-hydrophobic apoproteins and DPPC-
PG-hydrophobic apoprotein mixtures adsorbed well but were not effective in attaining low surface tensions during surface area reduction on the Langmuir-Wilhelmy balance. Surface activity was markedly improved by addition of high amounts of neutral lipids (tripalmitin and/or palmitic acid). Mixtures containing DPPC-PG-palmitic acid and hydrophobic apoproteins were also effective in promoting lung expansion in prematurely delivered rabbit pups of 27 days gestation.

Removing cholesterol from bovine surfactant lipid extracts hampers adsorption activity at 25°C but not 37°C. Interestingly, SP-A greatly retarded the ability of radioactive cholesterol in [14C]cholesterol-labelled BLES to attain the air-liquid interface during adsorption to equilibrium [46]. Furthermore, the addition of SP-A markedly enhanced the surface activity of such preparations during compression on the Langmuir-Wilhelmy surface balance and during pulsation with the pulsating bubble surfactometer. These results are consistent with SP-A enhancing DPPC adsorption while limiting cholesterol access to the air-liquid interface. SP-A also appears to bind pure cholesterol films in such a manner as to increase their surface activity during compression [47]. The exact manner in which SP-A influences surface activity of the phospholipid-cholesterol mixtures in BLES is not known, but could be related to the formation of surface-associated phospholipid aggregates rich in DPPC [46,47].

In contrast to studies conducted with the Langmuir-Wilhelmy surface balance and the pulsating bubble surfactometer, cholesterol appears to have minimal effects on surface tension reduction of BLES with the captive bubble tensiometer [49]. Thus, some of the detrimental effects attributed to cholesterol in lipid extract surfactants may be related to film leakage associated with the particular apparatus being used.

An intriguing, and as yet unexplained, aspect of surfactant lipid composition is the fact that different species possess quite different proportions of neutral lipids relative to phospholipids. Interestingly, cholesterol levels in lung surfactant can vary rapidly. For example, the hyperventilation induced in swimming rats lowers the cholesterol/disaturated PC ratio. Studies with the isolated perfused lung model indicate there may be an alternate (i.e., non-lamellar body) source of cholesterol which is rapidly mobilized by hyperventilation [50]. In addition, trained athletes demonstrated a decrease in cholesterol/disaturated PC ratio during intense activity compared with less fit individuals who demonstrated an increase in this ratio [51]. The significance of these rapid alterations in surfactant sterol levels is not known.

As indicated above, the other neutral lipids have not been extensively examined. Surfactant possesses small amounts of vitamin E which could function as an internal anti-oxidant. There is also a very small amount of plasmalogen in lung which could also function in this capacity [52].

5. Recent contributions from novel approaches

Recent technical advances hold great potential for improving our understanding of what surfactant is and how it functions. For example, the electron spray mass spectrometer tracing of BLES lipids depicted in Fig. 1, which shows individual lipid molecular species, allows for exceptional sensitivity and accuracy. Mass spectrometer analysis using matrix-assisted methodology has been applied to SP-B and SP-C [53]. The advent of novel microscopic and spectroscopic techniques capable of detecting individual lipid-lipid and lipid-protein molecular interactions and associations could provide direct means for answering fundamental questions not accessible by conventional surface chemistry (i.e., do minor lipid components play specific roles in forming and maintaining the interfacial layer? Do surfactant proteins attain and remain at the surface?).

During the past decade, fluorescent microscopic techniques have provided direct visual confirmation of earlier suggestions by Clements and others (see chapter by Goerke) that DPPC films undergo phase transitions during dynamic compression at the air-water interface. As films containing DPPC are compressed, the lipids assemble into gel-like condensed phases, and then with further packing, into a solid-like phase. Each phase transition is reflected by the appearance of a transitory aggregated lipid structure in one phase coexisting with another. A solid-like phase generated below 20 mN/m may represent structures capable of withstanding high surface pres-
sures, so as to generate low surface tensions near 0 mN/m. Condensed structures generated within DPPC films possess peculiar, yet characteristic, kidney-shaped structures (Fig. 2A,B) [54]. Such structures have recently been observed with surfactant extract preparations [55,56]. These structures are not fluorescent probe-induced artifacts, since they can also be observed at the air-water interface by non-probe-dependent techniques such as Brewster angle microscopy [55]. Similar structures are observed with solvent or vesicle-spread and also with adsorbed films [57].

It should be apparent that surface-fluorescence studies can provide unique structural detail not accessible by conventional surface techniques. As suggested by Pierre Giles DeGennes, Nobel Laureate in 1991 for studies on ‘Soft Matter’ [58], the DPPC condensed regions can be considered as a form of a liquid-crystalline structure. Such structures are formed due to differential tilt and orientation of individual molecules in the films during packing. These monomolecular model films can also be used to study the manner in which other lipids present in surfactant interact with DPPC-condensed structures at the interface. Small amounts of cholesterol change the DPPC-condensed structures from kidney shapes to elaborate fine structures (Fig. 2C–F). When added together with PG, the kidney-shaped structures are modified to appear stellate (Fig. 2G). When calcium is also present, there is increased DPPC condensa-

Fig. 1. A typical mass spectrum of phospholipid species present in BLES. The mass spectrum was obtained using electrospray ionization mass spectrometry of organic solutions of BLES. The lipid species identified as their exact molecular mass and chain distributions are shown in parentheses. DPPC, dipalmitoylphosphatidylcholine; POPC, 1-palmitoyl, 2 oleyl-PC.
Fig. 2. Various phase structures formed in DPPC and mixed lipid films as observed using fluorescence microscopy at an air-saline interface at 60 mN/m and 25°C. The structures arise due to lipid-lipid interactions, and multiple tilt and orientation of the molecules perpendicular to the plane of the air-water interface. The structures were observed in films of DPPC (A,B); DPPC with cholesterol (0.2-4 mol%) (C–F); at surface pressures near the gel-fluid phase coexistence regions of such films (3–14 mN/m). Images G and H indicate DPPC films containing PG (DPPC-DPPG), 7:3) and unsaturated POPC and cholesterol (DPPC-POPC-cholesterol, 7:2:1) respectively. The black regions indicate the gel-like liquid-condensed (LC) phase and the white the fluid liquid expanded (LE) phase. The LC phases are observed by fluorescence microscopy from the exclusion of fluorescent probes from such regions. Note the high susceptibility of the molecular packing or shape of the gel domains of DPPC in films to minor amounts of perturbants like cholesterol. The fluorescent probe was 1-palmitoyl, 2-nitrobenzoxadiole amino-dodecanoyl (NBD)-phosphatidylcholine. Scale bar is 25 μm. (From [54].)
tion, resulting in formation of multi-sized, circular domains (Fig. 2F). Recent studies with fluorescent-labelled films have confirmed the ability of unsaturated PC to promote DPPC respreading from condensed structures, providing further evidence for the reversible squeeze-out of PC and, in the presence of calcium, of PG during film compression [56]. Studies with fluorescent-labelled cholesterol have confirmed this steroid cannot be readily squeezed out of DPPC films [44,47,59].

The fluorescence approach also provides direct evidence for surfactant apoprotein interaction with lipids in surfactant films. SP-C alters DPPC and DPPC-PG packing in solvent-spread and adsorbed films [35,53,57]. The presence of multilayered structures resulting from molecular squeeze-out in the presence of SP-C has been directly demonstrated by atomic force microscopic analysis of films compressed to low surface tensions [35]. Such structures are consistent with the role of SP-C acylation in the generation of a surface-associated surfactant reservoir [34]. SP-B and SP-B-like peptides interact with DPPC-DPPG films differently from SP-C in that they form protein networks at the surface. Such protein networks could provide initiation sites for molecular squeeze-out [60], thus explaining the remarkable ability of SP-B to promote PG squeeze-out [61].

The propensity of SP-A to bind to gel-like condensed regions of DPPC has been directly demonstrated by fluorescence techniques [62]. SP-A:DPPC interactions are highly sensitive to ionic conditions. That calcium and other ions affect SP-A-DPPC interactions has also been shown with transmission electron microscopy. The latter electron microscopic investigations reveal that SP-A interacts with DPPC via its C-terminal carbohydrate recognition domains [63], as has been inferred from site-directed mutagenesis of SP-A [64]. The fluorescence microscopic investigations indicate that small amounts of surfactant apoproteins remain associated with surfactant films compressed to near zero, suggesting the proteins could be involved in respreading [53,62] as previously indicated by captive bubble tensiometric studies [33,34]. Readers are referred to chapters by Pérez-Gil and Keough and by Schürch in this dedicated issue for further details on these aspects.

As indicated above, condensed domains reminiscent of those observed with DPPC films can be ob-

Fig. 3. Fluorescence micrographs of films of porcine lipid surfactant extract, at high (50 mN/m) (A), intermediate (30 mN/m) (B) and low (10 mN/m) (C) surface tensions. The dark condensed domains seen in B are probably composed primarily of DPPC, as their total area estimates agree well with the proportion of DPPC present in porcine surfactant [54,55]. The bright structures with black spots in C appear to be multilayered squeezed-out materials from such films above the air-water interface. The fluorescent probe was 1-palmitoyl, 2-nitrobenzoxadiazole aminododecanoyl (NBD)-phosphatidylcholine.
served during lateral packing of porcine lipid extract surfactant (Fig. 3) [54]. The presence of subphase calcium produces a further increase in condensed phase area, suggesting the possibility that PG, complexed with calcium, may coexist within the DPPC-rich regions [56]. Further addition of SP-A to such systems results in coalescence of separate DPPC-like domains, resulting in a smaller number of larger condensed regions, but with a similar overall surface area. Although the actual molecular composition of these aggregated domains is unknown, it is clear they are DPPC-rich. When cholesterol is also present, calcium induces formation of novel, still not-understood multilayer structures at surface tensions near 0 mN/m (Fig. 3C). Whether such structures are related to the surface-associated reservoir [33] or to DPPC-rich cholesterol-poor surface film-associated aggregates arising during film formation [46,47] is not known. However, it appears these structures could be capable of film replenishment during surface area expansion [55,56]. Recent investigations using X-ray diffraction to examine DPPC films at low surface tensions indicate the presence of head group dehydration, which could further stabilize monolayers at low surface tensions and enhance interactions with hydrophobic areas of SP-B and with calcium [53,56].

6. Non-mammalian surfactants

The pulmonary surfactant system, including both the lipids and SP-A, is present in the lungs of air-breathing representatives of all vertebrate groups [65,66]. In fact, it appears surfactant function related to air breathing evolved at least 400 million years ago in the lungs of primitive air-breathing fish and has been highly conserved [66]. This suggests surfactant could have ‘older’ and perhaps some as yet undefined functions. Furthermore, relatively large amounts of surfactant have also been found in the swim bladders of modern teleost fish such as the goldfish [67]. The role of surfactant in the swim blad-

<table>
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<th>Table 2</th>
<th>Phospholipid profiles for lavage material for different species</th>
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<tr>
<td>Phospholipid composition (% total) [% disaturated]</td>
<td>Cholesterol (% chol/PL)</td>
</tr>
<tr>
<td>PC</td>
<td>LPC</td>
</tr>
<tr>
<td>C. auratus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75</td>
</tr>
<tr>
<td>Goldfish</td>
<td>[27.7]</td>
</tr>
<tr>
<td>L. osseus&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.4</td>
</tr>
<tr>
<td>Gar fish</td>
<td>[18.5]</td>
</tr>
<tr>
<td>N. forsteri&lt;sup&gt;c&lt;/sup&gt;</td>
<td>59.6</td>
</tr>
<tr>
<td>Australian lungfish</td>
<td>[9.0]</td>
</tr>
<tr>
<td>P. annectens&lt;sup&gt;e&lt;/sup&gt;</td>
<td>80.2</td>
</tr>
<tr>
<td>African lungfish</td>
<td>[25.4]</td>
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<tr>
<td>B. marinus&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.6</td>
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<tr>
<td>Cane toad</td>
<td>[5.6]</td>
</tr>
<tr>
<td>N. depressus&lt;sup&gt;d&lt;/sup&gt;</td>
<td>70.7</td>
</tr>
<tr>
<td>Sea turtle</td>
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<tr>
<td>C. porosus&lt;sup&gt;d&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Crocodile</td>
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<tr>
<td>C. atrox&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.6</td>
</tr>
<tr>
<td>Rattlesnake</td>
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</tr>
<tr>
<td>C. nuchalis&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Lizard</td>
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<tr>
<td>S. crassicaudata&lt;sup&gt;e&lt;/sup&gt;</td>
<td>70.7</td>
</tr>
<tr>
<td>Dunnart</td>
<td>[56.9]</td>
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Amounts of phospholipids are given as the percentage of total phospholipids. PC, phosphatidylcholine; LPC, lysophosphatidylcholine; SM, sphingomyelin; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; chol, cholesterol; PL, phospholipid.

Data from: <sup>a</sup>Daniels and Skinner, 1994 [67]; <sup>b</sup>Daniels et al., 1995 [65]; <sup>c</sup>Orgeig and Daniels, 1995 [79]; <sup>d</sup>Daniels et al., 1996 [80]; <sup>e</sup>Langman et al., 1996 [71]. All samples collected at room temperature except sea turtles (32°C) and lizards (37°C).
der, which is derived from the lungs of air-breathing fish, is not completely known. However, as the swim bladder is not required for air breathing, this finding suggests that surfactant is requisite for an inflating/de inflating organ with an air-liquid interface.

Early work on mammalian pulmonary surfactant focussed on lipid components, in particular DPPC. The relative lack of this so-called ‘active ingredient’ in the lungs of non-mammalian vertebrates led to the concept that these surfactants were less important for airway stabilization and may function as an anti-adherent (also known as an ‘anti-glue’) factor and as an anti-oedemic factor, rather than as an anti-atelectatic agent. Studies on non-mammalian surfactants not only provide interesting insights into the evolutionary aspects of pulmonary surfactant, but similarities and differences between these surfactants could prove valuable in identifying new roles for specific components. Furthermore, non-mammalian species have provided valuable model systems for studying surfactant and its functions under different physiological conditions, such as alterations in body temperature.

Table 2 presents the phospholipid composition of a number of non-mammalian surfactants. While not identical, there are obvious similarities to each other and to mammalian surfactants. Interesting differences are the higher levels of SM in many species and the extremely high levels of lyso-PC in snakes. Most of the PS/PI appears to be PI but PG tends to be low or absent. The exception appears to be the anurans (frogs and toads), which demonstrate a similar PG/PI pattern to mammals. This may be an example of convergent evolution. Depending on the species, PE can be higher or lower than with mammals.

The major differences between surfactants from mammalian and non-mammalian species are the proportion of disaturated PC and the amount of neutral lipids. Compositional differences also occur between different non-mammalian species. Among the latter species, the disaturated PC varies from approx. 10 to 30% in fish and amphibians, and up to 60% in reptiles. (Note that the disaturated PC levels in Table 2 were determined with the osmium tetroxide technique of Mason [15], which gives higher values than the molecular species methods used for the values in Table 1. For example, the Mason method indicates PC from human surfactant is 67.3% disaturated compared to the 44.7% reported by the more accurate molecular species method. Thus, the values for disaturated PC in Table 2 could be elevated by approx. 20% relative to Table 1.) Nevertheless, it is clear there is a significant effect of body temperature which affects disaturated phospholipid as a percentage of total in different species. The compositional variation among groups may reflect a balance between temperature-dependent fluidity of surfactant phospholipids due to the need to maintain homeoviscosity (similar viscosity at different temperatures) and the requisite low surface tensions during expiration. Only homeotherms and some heliothermal reptiles experience body temperatures which approach the 41°C gel-to-liquid crystalline transition temperatures of DPPC bilayers. It is generally thought that only these animals require (or can tolerate) high disaturated PC levels. However, lipid extracts of mammalian surfactant with high disaturated PC levels display fluid characteristics at 20–25°C and show similar surface activities at 25°C and 37°C on the pulsating bubble surfactometer [61,68]. This suggests the possibility that simply being in the fluid state at body temperatures may not be sufficient to meet the demands on the surfactant system in poikilotherms. Unfortunately, the nature of these demands, which impose relatively low disaturation levels, must still be defined.

A major difference between species in Tables 1 and 2 is the cholesterol content. In mammals, cholesterol relative to phospholipid varies from approx. 3% in the cow to approx. 10% in the rat and sheep. A similar 3–10% range is observed for most non-mammalian species, but this can be greater in specific species. Surfactant from goldfish swim bladders and the lungs of ancient air-breathing fish (actinopterygians) have high cholesterol (15–35%), as does the primitive Australian lungfish (*N. forsteri*). These fish also have relatively low (10–30%) disaturated PC levels. These values contrast with the high disaturated phospholipid and relatively low cholesterol values in rat and human surfactant (Table 1) and snake and lizard surfactant (Table 2). However, the African and South American lungfish have intermediate (25–38%) disaturated lipids and low (5%) cholesterol levels. Intermediate PC disaturation and low cholesterol levels are also observed with salamanders, turtles and toads. A possible explanation for the radically different lipid profiles of surfactant
from the teleost fish, primitive air-breathing fish, and Australian lungfish (high cholesterol and low disaturated PC) is that it may represent a proto-surfactant which is used infrequently in air breathing, but is important for other functions in the swim bladder. Apart from the evolutionary trends in surfactant composition between vertebrate groups, there are also differences within a particular group such as the amphibians. For example, temperature can affect surfactant composition via habitat preference, because terrestrial amphibians as a group have a surfactant lipid profile which is different from aquatic amphibians. Terrestrial amphibians with variable body temperatures possess surfactants low in both disaturated PC (13–20%) and cholesterol (0–6%), while aquatic amphibians with more stable body temperatures have high disaturated PC (26–43%) and cholesterol (8–11%) [69]. With both groups, relatively similar cholesterol to disaturated PC ratios are observed, implying the proportion of cholesterol to disaturated PC must be kept within certain limits.

Temperature also has profound effects on the relation between cholesterol and disaturated lipids within individual species. For example, decreasing the body temperature of the lizard, Ctenophorus nuchalis, from 37°C to 18°C results in a doubling of surfactant cholesterol [70]. Similarly a torpid marsupial, the fat-tailed dunnart (Sminthopsis crassicaudata), exhibits a higher ratio of cholesterol to total and disaturated phospholipid (11% and 22% respectively) at 15°C than at its normal active body temperature of 35°C (7% and 18%) [71] (marsupials are a subclass of mammals but it was thought appropriate to mention this study with non-mammalian species). These changes occur within a few hours, showing an amazing ability of the surfactant system to adapt in these animals. A possible explanation is that cholesterol functions to modulate the fluidity of the surfactant lipids, thereby enhancing surfactant adsorption, particularly at low temperatures. Certainly the larger size of the faveoli (100–1000 times larger than alveoli of similar sized mammals) would avoid the high collapsing forces thought to induce atelectasis (alveolar collapse) in the 50–100 μm mammalian alveoli. Faveoli possess an enhanced backbone consisting of elastin and collagen and an inner trabecular system which supports and stabilizes the inner connecting units [74].

These considerations have led to the suggestion that surfactant has other functions in non-mammalian vertebrates such as acting as an anti-adherent (also known as an anti-glue) factor. A water droplet between two glass microscope slides spreads readily by capillary action because hydrophilic glass binds water better than water binds itself. The surprisingly high force required to separate wetted glass slides is due to surface tension forces resisting expansion of the air-water interface. Air-breathing fish, some lizards, snakes and amphibians can partially or completely degas their respiratory units [65,75]. Reopening the wet tissue surfaces of interfolded faveoli (or collapsed alveoli) is greatly aided by reducing surface tension to the equilibrium value of approx. 25 mN/m. A similar phenomenon occurs with the first breath in newborn mammals, where by reducing surface tension surfactant facilitates lung expansion.
with air. It should be stressed there is no ‘glue’ for the anti-glue factor to counter but only surface tension forces. The anti-glue function can be determined by completely emptying the lungs of air and measuring the pressure required to open the lungs before and after lavage. In almost all species examined, there is a greater opening pressure following surfactant removal [65,75]. However, in control experiments, where gut tissue is collapsed and inflated before and after lavage, no such increase in opening pressure is observed. Surfactants contributing to respiratory unit opening must adsorb rapidly at body temperature, but this action is independent of the surfactant’s ability to attain low surface tension during compression. High cholesterol and low disaturation should enhance adsorption and respreading at low temperatures.

Another function suggested for both non-mammalian and mammalian surfactants is that of an anti-oedema factor [76]. It has been suggested this function could be important in birds which have minute rigid air capillaries [73]. The theoretical basis of this anti-oedema function is that water molecules at the air-liquid interface experience lower average attractive forces into the bulk phase, resulting in a decreased pressure difference across the interface, as indicated by the Laplace equation. However, water in the bulk phase would experience association with ions and proteins as indicated by the Starling equations, and to date this anti-oedema function of surfactant has not been experimentally proven for normal mammalian lungs. Nevertheless, considerable evidence is available for the ability of surfactant to prevent flooding of the lungs injured through surfactant deficiency arising from prematurity, lavage, or other insults which tend to increase serum protein levels [19,76]. Thus it appears likely that pulmonary surfactant could act synergistically with epithelial sodium channels to maintain wetted but not flooded alveoli [77].

7. Future directions

Since the rediscovery of pulmonary surfactant by Pattle and Clements in the 1950s, information arising from a number of scientific disciplines has greatly expanded our understanding. However, it is evident a number of crucial details are still lacking. For example, how is the DPPC-enriched monolayer generated during adsorption? What are the precise roles of PC, PG, and possibly the other minor phospholipid constituents in selective DPPC adsorption and in fluid lipid squeeze-out? What is the exact composition of the interfacial film and what structures does it adopt? Does cholesterol contribute to selective DPPC adsorption in the lung? Is cholesterol excluded from the surface, as appears likely, and if so, how does this occur? What is the mechanism by which surfactant desorbs from the interface and what controls its return to the type II cells and the subsequent cellular re-uptake?

Are the minor lipid components, the major lipid components, or the surfactant apoproteins involved in signal transduction processes responsible for controlling alveolar surfactant levels? Are surfactant lipids involved in other signalling events?

What is the reason for the differences in neutral lipid content between mammalian species, and what is the significance of the observed alterations in alveolar cholesterol content during activity? Why do some non-mammalian species have surfactants with very similar phospholipid profiles, but different cholesterol/disaturation patterns? Do non-mammalian surfactants lower surface tension in the alveoli to low values like mammalian surfactants?

We anticipate these and other issues will keep scientists in many disciplines occupied for a considerable time. We speculate that, during these investigations, they may discover other important biological functions for surfactant still not suspected. We predict a better understanding of surfactant function will prove valuable not only in contributing to our understanding of the biology of surfactant, but also as a basis for important improvements in therapy for a number of respiratory diseases.

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