CHAPTER 9

Surfactant Replacement Therapies in Children

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1. Introduction/Historical Perspective

In 1806 Laplace described the mathematical expression of surface tension. He described it as the attraction between molecules at a surface that contracts the area of an air-liquid interface so that the net force exerted by the surface tension is determined by the curvature. Over a century later von Neergaard [1] applied Laplace's law to the lung. In his classic experiment, Neergaard filled lungs with a liquid so that the lung retractile forces were a function of tissue elasticity (because the alveolar air-liquid interface would have been removed). From this experiment he concluded that 75% of the retractile force of the air-filled lung was the result of surface tension. In 1933 it was discovered that the initial force required for air expansion of the newborn's lung was considerably greater than for the subsequent breaths [2]. In 1947 it was found that this force could be reduced by one-half with

the addition of amyl acetate vapor. This reduction in force correlated with amyl acetate's surface tension-lowering ability [3].

In 1954 Macklin [4] described a thin, aqueous, flexible, lining layer rich in myelin figures in the alveolar space. Macklin [4] and Schlipkoter [5] both described lamellar osmophilic inclusion bodies in type II pneumocytes. These lamellar bodies have been temporally correlated with the appearance of the surface active material, suggesting that these were the precursors of the alveolar lining fluid [6, 7].

In 1955 Pattle [8] discovered an insoluble protein-rich layer lining the lungs. This lining reduced the surface tension at the alveolar-air interface to nearly zero. Clements [9, 10] measured change of surface tension during compression and decompression of a film of lung lavage fluid. He correlated the shape of the tension-area curve of the lung extract with the pressure-volume curve of the air-inflated lungs. Clements concluded that the hysteresis of the lung pressure-volume curve could be attributed to variations of surface tension at different degrees of stretch due to the presence of a unique surface active material in the lung extract.

2. Endogenous Surfactant Composition

Endogenous surfactant is a mixture of lipid, protein and carbohydrate. The major component is lipid, accounting for 80–90% of surfactant by weight. The majority of this lipid is phospholipid (80%) [11]. The individual components include: phosphatidylcholine (PC) (60–70%); phosphatidylglycerol (PG) (5–10%); phosphatidylethanolamine (PE) (5–10%); phosphatidylinositol (PI); and phosphatidylserine (PS) (3–6%). These phospholipids are not unique to surfactant, but surfactant is unique in that it contains an unusually high proportion of PG and saturated PC. The saturated PC is in the form of dipalmitoylphosphatidylcholine (DPPC) [12].

Surfactant also has a protein component and to date four surfactant-associated proteins have been identified. They constitute approximately 10% of the surfactant weight. There are two groups of proteins. The first group consists of the hydrophilic surfactant-associated proteins A and D (SP-A, SP-D), and the second group consists of the hydrophobic surfactant-associated proteins B and C (SP-B, SP-C) [13].

SP-A (synonyms: Apoprotein A, Glycoprotein A, PSP-A, SAP 28–36, SP 26–36, SP 28–36) is the most abundant surfactant protein. SP-A is a glycoprotein with a molecular weight of 26 to 35 kilodaltons (kDa) that is present in alveolar surfactant as a 650 kDa multimer [14]. Two genes located on chromosome 10 encode human SP-A [15, 16]. SP-A contains a distinct amino-terminal domain that consists of an extensive collagen-like peptide, rich in hydroxylated proline residues [17]. Cysteine residues near the amino-terminus of the protein form intermolecular sulfhydryl bonds that account for the larger oligomeric forms of the protein [18].

SP-A contains an amphipathic helical domain located C-terminal to the collagenous segment that is closely followed by the most hydrophobic segment of SP-A. The C-terminal regions of the molecule may facilitate its association with surfactant phospholipids [19]. The carboxy-terminal region of SP-A has significant homology to mammalian lectins. This region has specific structural similarity to the C1q subunit of complement and interacts with glycoproteins and glycolipids on the surface of prokaryotes and with other surfactant components [20, 21].

SP-D was first recognized in 1983 but was not characterized until 1989 [22]. The organizational structure is remarkably similar to that of SP-A with an amino-terminal globular domain, a collagen-like region, a carbohydrate recognition domain and the linking sequence between the collagen domain and the carbohydrate recognition domain [23]. SP-D has a monomeric molecular weight of 43 kDa [24]. SP-D differs from SP-A in that SP-D contains hydroxylysine, hydroxylysine glycosides, a longer collagen-like domain, and different monosaccharide [25] and phospholipid [26, 27] binding specificities. Subunits of SP-D assemble into a tetramer of disulfide bonded trimers in a cruciform structure reminiscent of bovine conglutinin [23]. The gene for SP-D is located close to the gene for SP-A on chromosome 10 [28].

SP-B (synonyms: PSP-B, SAP-(PHE), SPL-(PHE), SP-18) is one of two hydrophobic proteins and is found in surfactant as a nonglycosylated, sulfhydryl-dependent dimer with a molecular weight of 18 kDa. The gene for SP-B has been isolated to chromosome 2 and encodes a 381 amino acid proprotein [29]. The mature form is a 79 amino acid mature protein [30].

SP-C (synonyms: PSP-6, SAP(ILE), SAP(LEU), SAP(PVAL), SP5-8, SP5, SP1(PVAL)) is the other hydrophobic protein. Its gene is found on chromosome 8 [31]. The primary translation product of this gene is a 22 kDa protein that undergoes a proteolytic post-translational modification to a 32–33 amino acid (4–5 kDa) hydrophobic protein rich in valine [32].

3. Function of the Endogenous Surfactant System

The primary role of surfactant is to lower alveolar surface tension at low lung volumes, preventing alveolar collapse at the end of expiration. DPPC lowers surface tension and is present in sufficient quantity to cover the entire alveolar surface. DPPC contains a three carbon 'backbone' that has nonpolar long-chain fatty acids esterified to the first two carbon atoms and a polar phosphorylcholine bound to the third carbon atom. The polar portion of the molecule partitions into the aqueous layer, while the nonpolar, long, straight-chain fatty acids project into the air above the liquid. Because the saturated fatty acids are rigid, they can be tightly compressed when aligned side to side. This tight packing of the DPPC molecules lowers the surface tension by interfering with the intermolecular

attractive forces of the aqueous layer. This provides great stability to the surface film [12].

Although DPPC accounts for most of the surface tension lowering ability of surfactant, it adsorbs very slowly to the air-liquid interface in the alveolus. Because of this slow adsorption, other components of the endogenous surfactant system must aid in the spreading and adsorption of DPPC to the air-liquid interface. These include unsaturated phosphatidylcholines [33] and the surfactant-associated proteins. The surfactant proteins are vital for the ability of the phospholipids to form a monolayer at the air-liquid interface. Purified SP-B, SP-C or mixtures of the two proteins greatly enhance the rate of formation of a phospholipid layer at an air-liquid interface *in vitro* [34, 35]. SP-B appears to be more active than SP-C in surfactant's ability to reduce the surface tension [34]. SP-A and SP-B are also involved in the formation of tubular myelin [36, 37]. The ability of surfactant to rapidly adsorb to an air-liquid interface and to decrease surface tension results in several physiological actions: increased pulmonary compliance that decreases the work of breathing [38], increased alveolar stability on expiration [39], decrease in the driving force that leads to the development of pulmonary edema [40], and more uniform alveolar recruitment on inspiration [41]. SP-A also inhibits both the basal and secretagogue-induced release of PC by type II cells *in vitro* [42].

Surfactant has immunomodulatory functions, first demonstrated by increased killing of *Staphylococcus aureus* by alveolar macrophages in the presence of surfactant both *in vitro* and *in vivo* [43, 44]. Alveolar lining fluid directly kills *Streptococcus pneumoniae* [45], and it renders both blood and alveolar lymphocytes hyporesponsive to mitogens and allogeneic cells [46, 47]. Some of this immunomodulatory function has been assigned to SP-A and SP-D since they belong to a calcium-dependent lectin family. This family also includes the mannose-binding protein-A and C1q. This family of proteins appears to have a role in the innate, nonspecific immune system. They act as opsonins, have bactericidal activity and inhibit human immunodeficiency virus *in vitro* [48–51]. Children deficient in mannose-binding protein have opsonic defects and inadequate phagocytosis resulting in an increased susceptibility to infections [52, 53].

SP-A binds and neutralizes influenza A viruses [54]. It enhances opsonization and aggregation of hemophilus type A bacteria [55] and enhances phagocytosis of a variety of microorganisms including *Staphylococcus aureus* [56], *Herpes simplex virus* [57], *Escherichia coli* and *Pseudomonas aeruginosa* [58]. SP-A promotes *Mycobacterium tuberculosis* attachment to alveolar macrophages [59]. There is also evidence that SP-A stimulates the production of reactive oxygen species by alveolar macrophages, which is lethal to invading microorganisms [56]. SP-A stimulates alveolar macrophage chemotaxis [60] and production of cytokines and immunoglobulins [61]. SP-D also appears to be involved in host defenses. SP-D binds to *E. coli*, stimulates agglutination of bacteria [62], acapsular yeast cells [63]

and *Pneumocystis carinii* [64], and it stimulates production of reactive oxygen species by alveolar macrophages [65].

The role of surfactant phospholipids in host defense is unclear. There are conflicting studies regarding the effect of surfactant lipids on chemotaxis, phagocytosis and production of reactive oxygen species. These conflicts may be due to the different techniques used for surfactant isolation that result in different combinations of surfactant components, or to contamination by serum proteins and immunoglobulins [53].

4. Synthesis, Secretion, and Metabolism of the Endogenous Surfactant System

Pulmonary surfactant is synthesized and assembled by alveolar epithelial type II cells. This process involves multiple steps: (1) synthesis of the individual surfactant components, (2) packaging of these components, (3) intracellular trafficking of the components to the apical surface of the cell, (4) secretion of the components into the alveolar space, (5) formation of tubular myelin, (6) formation of a lipid monolayer, (7) clearance of the pulmonary surfactant from the alveolar space and (8) recycling or metabolism of surfactant components.

4.1. Synthesis of the Phospholipids

Glucose from the circulation is the major source of glycerol that serves as the backbone for surfactant lipid synthesis [66]. During the late fetal period, lung glucose moieties derived from the breakdown of lung glycogen may be a substrate for pulmonary PC synthesis. Increases in lamellar body content in alveolar type II cells coincide with the disappearance of glycogen from fetal lung epithelial cells [67]. Glycerol stored in the type II cell plays only a minor role as a substrate for surfactant lipid synthesis in adults [68], but may have a more significant role in the early neonatal period when circulating concentrations of glycerol increase markedly [69].

Fatty acids required for surfactant phospholipid synthesis are derived from the circulation as free fatty acids or triacylglycerols in lipoproteins. The uptake of fatty acids from the blood into the alveolar type II cell is mediated by a membrane-bound fatty acid binding protein on the surface of the type II cells [70]. Fatty acids also become available upon hydrolysis of phospholipids that are absorbed from the alveolar space [71]. Adult type II cells synthesize fatty acids *de novo* at high rates, and lactate is preferred over glucose as the source of acetyl units [72]. Glycogen is also an important precursor for fatty acid synthesis during the late fetal period [73]. Newborns largely utilize acetoacetate for fatty acid synthesis [74]. Choline required for phosphatidylcholine synthesis in type II cells is probably

derived from the diet [75], and the myo-inositol required for phosphatidyl-inositol formation can be synthesized by a variety of tissues in the body. Dietary inositol is sometimes required [76].

The *de novo* synthesis of surfactant phospholipids begins with the formation of phosphatidic acid (PA) from glucose or glycogen to form glycerol 3-phosphate or dihydroxyacetonephosphate. After production of the (PA) backbone, individual phospholipids are synthesized. Phosphatidylcholine is formed by the addition of choline to a diacylglycerol backbone (derived from PA) by the enzymatic action of cholinephosphotransferase [77]. Direct synthesis *de novo* is not responsible for the lung's entire production of DPPC. Estimates are that only 25–45% of surfactant DPPC is produced by *de novo* synthesis while 55–75% is formed by recycling of pre-existing DPPC taken up from the alveolar space [78, 79].

There are two mechanisms proposed for surfactant phospholipid remodeling. Both mechanisms involve deacylation of the 2-position of PC by phospholipase A2 followed by reacylation. The reacylation step is where the two mechanisms diverge. The first mechanism has the 1-palmitoyllysophosphatidylcholine acylated with palmitoyl-CoA by the enzyme lysophosphatidylcholine acyltransferase. The second mechanism involves a transacylation of the 1-palmitoyllysophosphatidylcholine by the enzyme lysophosphatidylcholine: lysophosphatidylcholine acyltransferase [77].

Both PG and PI are synthesized from CDP diacylglycerol. CDP diacylglycerol is produced from PA by phosphatidate cytidyltransferase [80]. The biosynthesis of the minor phospholipids of surfactant PE and PS has received little attention. PE is formed by ethanolaminephosphotransferase from diacylglycerol [81]. PS is probably produced from PC or PE by an enzyme-catalyzed exchange of head groups [82]. The synthesis of the individual surfactant-associated proteins has been described earlier.

After the phospholipids are synthesized in the endoplasmic reticulum they are transferred via the Golgi system to lamellar bodies [83]. Lamellar bodies appear to be packages of folded phospholipid whose composition is very similar to that of surfactant [84]. The transfer of the phospholipids to the lamellar bodies involves multiple participants. Small lamellar bodies are probably intermediates that carry phospholipid between the Golgi system and mature lamellar bodies [85]. Phospholipid transfer proteins may be involved in the transport of surfactant lipids from the endoplasmic reticulum to the lamellar bodies [86]. These proteins facilitate the transfer of phospholipids between membranes [77].

The PC in lamellar bodies contains a higher percentage of the saturated DPPC species than the PC found in the endoplasmic reticulum. This higher concentration does not appear to be due a selective recycling of alveolar DPPC to the lamellar bodies [87]. Likewise, PC remodeling in the lamellar bodies does not account for the higher concentration of DPPC [88]. The enrichment of lamellar bodies in DPPC may be caused by selective transfer of phospholipids from the endoplasmic reticulum to the lamellar body [77].

4.2. Regulation of Synthesis and Secretion

There are a variety of hormonal and physiological factors that regulate surfactant synthesis and secretion (Tables 1–4). These factors influence DPPC synthesis, surfactant-associated protein RNA expression and synthesis and surfactant secretion into the alveolar space. Many of these factors increase intracellular cAMP.

Table 1. Agents that stimulate surfactant phospholipid or surfactant-associated protein synthesis

Agent	Reference(s)	Component regulated
Glucocorticoids	[89]	SP–A
Glucocorticoids	[90]	SP–B, SP–C
cAMP	[91]	Proteins
Dibutyl cAMP	[91]	SP–A
8-Bromo cAMP	[91]	SP–A
Dibromo cAMP	[91]	SP–A
Forskolin	[90]	SP–A
Terbutaline	[90]	SP–A
Isobutylmethylxanthine	[90]	SP–A
Oxygen	[92]	SP–A, SP–C
Epideral growth factor	[93–95]	SP–A, DPPC
Keratinocyte growth factor	[96]	SP–A, SP–B
Interferon gamma	[97]	SP–A
Thyroid hormone	[98]	Phospholipid
Estrogens	[99]	Phospholipid
Insulin	[99]	Phospholipid

Table 2. Agents that inhibit surfactant phospholipid or surfactant-associated protein synthesis

Agent	Reference(s)	Component regulated
Glucocorticoids	[90]	SP–A
Transforming growth factor- β	[93]	SP–A
Insulin	[100]	SP–A
12-O-tetradecanoylphorbol-13-acetate	[101]	SP–A, SP–B
Tumor necrosis factor- α	[102–103]	SP–A, SP–B, SP–C
Androgens	[90, 104]	Phospholipid

Table 3. Agents that stimulate surfactant phospholipid or surfactant-associated protein secretion

Agent	Reference(s)	Agent	Reference(s)
Epidermal growth factor	[95]	Pilocarpine	[105]
α and β -Adrenergic agents	[106]	Isobutylmethylxanthine	[106]
cAMP	[106]	Dibromo cAMP	[106]
Theophylline	[106]	Cholera toxin	[107]
Forskolin	[107]	Terbutaline	[107]
Purinoreceptor agonists	[108–110]	Calcium ionophores	[111–112]
Calcium channel blockers	[113]	Vasopressin	[114]
Hyperventilation	[115]	Leukotriene E4	[116]
Prostaglandin E2	[117]	Colchicine	[119]
Increased tidal volume breathing	[118]	Vinblastine	[119]
Histamine	[120]	Antihistamines	[121]

Table 4. Agents that inhibit surfactant phospholipid or surfactant-associated protein secretion

Agent	Reference	Agent	Reference
SP-A	[42]	Cold temperatures	[117]
Vinblastine	[119]	Colchicine	[119]
Compound 48/80	[122]	Substance P	[123]
Lectins	[124]	Purinoreceptor agonists	[125]

4.3. Clearance and Metabolism

Approximately 50% of the alveolar surfactant is degraded [126], the remainder is recycled back into type II cells for resecretion [127]. This recycling process is approximately 90% efficient [128] and appears to be regulated by SP-A. Phospholipid that is internalized in the presence of SP-A is not significantly degraded, whereas the phospholipid that is internalized in the absence of SP-A is degraded [129].

Surfactant clearance from the alveolar space is also regulated. *In vitro* SP-A and PG enhance clearance of the surfactant by both the type II pneumocyte and the alveolar macrophage [129–132]; however, *in vivo* studies have failed to demonstrate the importance of SP-A. Many of the factors that stimulate the synthesis or secretion of surfactant also enhance the clearance of the surfactant from the alveolar space. Isoproterenol, terbutaline, metaproterenol and hyperventilation enhance secretion and clearance of surfactant [130–135]. Recently, granulocyte-macrophage colony-stimulating factor (GM-CSF) was shown to be important in the regulation of the surfactant pool size [136], but the mechanism remains unclear. Until the endogenous surfactant system can be manipulated in clinically significant ways, exogenous surfactant will be required to treat the disease states resulting in or from surfactant deficiency.

5. Exogenous Surfactant Preparations

Exogenous surfactant preparations can be categorized as either natural or synthetic (Table 5). Pure DPPC was the first synthetic surfactant used in clinical trials. DPPC reduces surface tension at an air-liquid interface but fails to disperse rapidly in the absence of surfactant proteins or spreading agents. Pure DPPC alone has failed in clinical trials [137, 138]. Artificial Lung Expanding Compound (ALEC[®], Britannia Pharmaceutical, Surrey, United Kingdom) is another synthetic surfactant. ALEC is a mixture of 70% DPPC and 30% PG [139]. It is a simple and inexpensive preparation to produce but it does not rapidly reduce surface tension as compared to natural surfactants [140, 141]. Exosurf[®] (Burroughs-Wellcome, Triangle-Park, NC) is a mixture of DPPC and PG to which hexadecanol and tyloxapol (spreading agents/emulsifiers) have been added [142]. Its clinical efficacy for the treatment of IRDS has been established by large multicenter trials

Table 5. Exogenous surfactant preparations

Source	Proprietary name
Synthetic surfactants	Artificial lung Expanding Compound (ALEC®) Exosurf® KL4 combined with DPPC
Natural surfactants	Survanta® Infasurf® Bovine Lung Expanding Substance (BLES®), Curosurf® Alveofact®

[143]. Another synthetic surfactant consisting of a mixture of 30 mg DPPC and 2 mg high-density lipoprotein failed to decrease the severity of RDS or improve mortality over control groups in a clinical trial [144].

There are several natural exogenous surfactant preparations. Most have been obtained by extraction of the surfactants into an organic solvent from either total lung lavage material or minced cow lungs. This technique provides a replacement surfactant that contains SP-B and SP-C but a relatively low concentration of protein [145–149]. These natural surfactants include: Survanta® (Abbott/Ross Laboratories; Columbus, Ohio), Infasurf® (ONY; Buffalo, NY), Bovine Lung Expanding Substance (BLES®, BLES, Inc.; London, Ontario, Canada), Curosurf® (Chiesi Pharmaceutica; Parma, Italy) and Alveofact® (Thomae, Biberach, Germany) [150]. Natural surfactants have a greater ability to reduce the surface tension and resist inactivation by serum proteins than do the synthetic surfactants [151].

SP-B has recently been shown to be of great importance in sustaining lung inflation. Several infants with congenital deficiency of SP-B have suffered lethal respiratory distress syndrome [152, 153]. Two patients with selective SP-C deficiency have also died from lethal respiratory distress syndrome. A transgenic mouse model of selective SP-B deficiency has corroborated these reports [154]. KL4, an amphipathic peptide with properties similar to SP-B, has been combined with DPPC [155] and is being tested as a surfactant preparation at the time of writing.

6. Issues Related to Surfactant Replacement

6.1. Delivery Techniques

Surfactant replacement therapy for Infant Respiratory Distress Syndrome (IRDS) in neonates has been by instillation into the endotracheal tube. This treatment has required large volumes of surfactant (50–100 mg/kg body weight/dose), that has been adequately tolerated by these infants. There have been attempts to deliver the surfactant by mechanisms other than endotracheal instillation. Aerosolized surfactant delivery would have several

advantages over tracheal instillation. First, it would be more cost effective since a smaller dose would be required for therapy than currently required for tracheal instillation. Second, treatment could be performed in older children and adults with illness associated with surfactant deficiency without concerns about the large volumes currently used in the treatment of IRDS. Third, surfactant replacement could be initiated prior to the development of respiratory failure. With these considerations, aerosolized delivery has been compared to intratracheal instillation in a variety of lung injury models. Initially, aerosolized surfactant was thought to be more efficacious than intratracheal instillation [156]. However, in subsequent studies that evaluated the efficacy of aerosolized surfactant in nonuniform lung injuries it was determined that a majority of the aerosolized surfactant was deposited in the noninjured regions of the lung and that the surfactant-deficient areas of the lung received little exogenous surfactant [157]. There was better distribution to all areas of the lung by direct intratracheal instillation. Methods of intratracheal instillation have been evaluated. Methods utilizing rapid instillation, instillation over 5 minutes and instillation over 45 minutes have all been studied. Rapid instillation demonstrated the best improvement in oxygenation and an even pulmonary distribution, while the 5 minute instillation time provided a transient improvement in oxygenation and an uneven pulmonary distribution; the 45 minute instillation time had no effect on oxygenation and had an uneven pulmonary distribution [158, 159]. Gravity is also a major determinant of surfactant distribution [160]. Until a better aerosol delivery system is available to deliver exogenous surfactants, intratracheal instillation will continue to be the preferred method of delivery. Surfactant has also been successfully administered to fetuses *in utero* during preterm labor, but the efficacy has to be assessed [161].

In animal models of Adult Respiratory Distress Syndrome (ARDS), response to exogenous surfactant therapy was improved by lung lavage prior to surfactant administration [162]. This response was thought to be due to the removal of proteins in edema fluid that could inactivate the exogenous surfactant [163]. Lung lavage prior to surfactant administration has not been studied in clinical trials.

6.2. *Turnover of Exogenous Surfactant*

Initially, synthetic surfactants were thought to be cleared from the alveolar space more quickly than the natural surfactants. This was based on the finding that PG from synthetic surfactant-treated infants persisted in tracheal aspirates for 30 hours compared to the 12 hours of the natural surfactants [164, 165]. Ashton [166] challenged this finding and measured exogenous surfactant turnover by a different method: measurement of DPPC/palmitoyllecithin (POPC) ratios. POPC is produced by premature infants as an immature surfactant phospholipid. DPPC represented

both endogenous and exogenous surfactant while POPC represented only the endogenous surfactant. The findings demonstrated a similar duration of the DPPC/POPC ratio in both natural and synthetic surfactant-treated infants, indicating that turnover of both exogenous surfactants is similar.

6.3. Immunological Consequences of Surfactant Therapy

One of the original concerns of surfactant replacement therapy was the potential immunological response to the foreign protein content of the natural surfactants. There are studies that documented the transepithelial flux of exogenous surfactant into the pulmonary interstitium and the pulmonary circulation, exposing the immune system of the host to the exogenous surfactant [167–169]. Infants as young as 24 weeks' gestation have the ability to produce an immune response to almost all foreign proteins [170, 171].

There were early reports that surfactant was immunogenic [172, 173] and that circulating immune complexes consisting of surfactant and anti-surfactant antibodies had been detected in the serum of premature infants treated with amniotic fluid surfactant [174, 175]. Unfortunately the analysis for surfactant antibodies is fraught with complications. First, the presence of blood group A determinants on the carbohydrate moieties of human SP-A [176] and the ELISA commonly used for antibody detection would detect both protein and blood group A antigen epitopes. They would falsely identify 'antisurfactant' antibodies. Second, the ELISA used by others reporting the presence of anti-surfactant antibodies could be complicated by the nonspecific associations between immunoglobulins and the hydrophobic surfactant antibodies used in the plating assays [177, 178]. Because of the difficulties with the ELISA assay, Western blot was performed for the detection of anti-SP-B and anti-SP-C antibodies in serum samples from 1,428 infants treated with a natural surfactant. Serum samples were obtained at one week, four weeks and six months following surfactant therapy. A total of 3,730 samples was analyzed without detecting any antibodies [179]. This finding has been confirmed by a subsequent study [180]. Studies evaluating surfactant antibody formation in adults who received natural surfactant have not been reported.

7. Clinical Applications of Exogenous Surfactant Therapy

7.1. Infant Respiratory Distress Syndrome (IRDS)

IRDS is largely attributable to inadequate surfactant production and function, although disordered maturation of lung cellular architecture, interstitial matrix formation and antioxidant systems probably play a role in the progression of IRDS to bronchopulmonary dysplasia (BPD) [181].

Safety and efficacy of both prophylactic and therapeutic administration of exogenous surfactants have been established [140, 182]. Surfactant replacement therapy has been beneficial in decreasing the mortality due to IRDS, the severity of IRDS, the development of air leaks and the length of hospital stay [183–186]. Exogenous surfactant improves pulmonary compliance and reduces pulmonary edema with subsequent improvements in chest radiographs [187–190].

Pulmonary hemorrhage has been associated with surfactant replacement therapy. In a large multicenter trial involving 2,831 infants with IRDS receiving either surfactant or an air placebo, pulmonary hemorrhage occurred in 1.9% of the surfactant-treated group versus 1.0% in the placebo group. Autopsy evaluation of 159 of these infants demonstrated no histological difference between the two groups. The only factor that was shown to be related was an inverse relationship of birth weight to the likelihood of developing pulmonary hemorrhage [191].

The effect of surfactant therapy on the incidence of BPD cannot be clearly defined. The difficulty in establishing this correlation is that the current definitions of BPD are inadequate. Until a more sophisticated definition of BPD is available and widely accepted, the impact of surfactant replacement on the development of BPD will remain unclear [143, 181]. Surfactant replacement therapy has reduced the overall hospital costs for premature infants by reducing the intensity of required services [192].

7.2. Adult Respiratory Distress Syndrome (ARDS)

Most information concerning exogenous surfactant replacement therapy for ARDS is based on the investigation of surfactant replacement for the treatment of IRDS, its associated surfactant deficiency, and on studies involving animal models of acute lung injury. A variety of animal models of ARDS have been used to evaluate the efficacy of exogenous surfactant therapy. These animal models have established the ability of exogenous surfactant replacement to improve oxygenation, lung compliance, pulmonary mechanics, gas exchange and alveolar expansion, and to protect against histological damage [193–200].

Several clinical trials have assessed surfactant replacement therapy for the treatment of ARDS. The largest series utilized an aerosol delivery system. The phase I and II trials of this series gave encouraging results, indicating that the use of surfactant for the treatment of ARDS may be beneficial [201, 202]. However, when the trial was expanded to a phase III investigation there was no difference in mortality between the surfactant-treated group and the placebo group [203]. Other studies of surfactant replacement therapy by intratracheal instillation in adult patients with ARDS have suggested that this therapy may be beneficial; however, the number of patients studied to date has been insufficient to declare statistical significance [204–211].

There have been even fewer pediatric patients studied. Clinical trials in children have demonstrated an improvement in oxygenation [212–214], trends toward improved survival [215, 216], and increases in phosphatidylcholine and phosphatidylglycerol by 4.4- and 34.7-fold respectively [217]. All these trials used intratracheal instillation of surfactant.

These clinical trials have established the safety of exogenous surfactant replacement therapy for patients of all ages with ARDS, and suggest that surfactant replacement may be beneficial for the treatment of ARDS. The one large multicenter trial using an aerosolized synthetic surfactant may have failed due to technical problems with the delivery system, as opposed to failure of the surfactant [218]. Further investigation will determine the efficacy of exogenous surfactant replacement therapy for ARDS as well as the patient populations with ARDS most likely to benefit from surfactant therapy.

7.3. *Meconium Aspiration Syndrome (MAS)*

MAS affects about 5% of all infants born with meconium-stained amniotic fluid and continues to carry a considerable morbidity and mortality [219]. The pathophysiology of MAS is multifactorial involving mechanical obstruction of the airways by meconium, chemical injury to the respiratory epithelium, and surfactant inhibition [220–222]. Animal models of meconium aspiration have shown a physiological response consistent with surfactant inhibition which improved following surfactant replacement therapy [223, 224]. Recently, clinical trials have demonstrated that early administration of exogenous surfactant may be beneficial for the therapy for MAS [220, 225]. Infants with MAS who received exogenous surfactant demonstrated improvements in oxygenation, resolution of persistent pulmonary hypertension, decreased air leaks, and shorter duration of mechanical ventilation, oxygen therapy and hospital stay.

7.4. *Pneumonia: Viral, Pneumocystis carinii, Bacterial and Aspiration*

Many of the animal models of acute lung injury used to demonstrate the effectiveness of exogenous surfactant replacement therapy involve viral pneumonias [226–228]. Recently, surfactant alterations were reported in children with respiratory failure secondary to respiratory syncytial virus infection [229]. Currently there is a multicenter trial investigating the efficacy of exogenous surfactant replacement therapy for the treatment of RSV bronchiolitis.

Surfactant abnormalities are well documented in patients with human immunodeficiency virus infection and *Pneumocystis carinii* pneumonia [230–233]. Animal models of *Pneumocystis carinii* pneumonia have

demonstrated improvement in oxygenation following exogenous surfactant therapy [234]. A clinical trial of surfactant replacement in a child with *Pneumocystis carinii* pneumonia has been reported [235]. Bacterial pneumonias also result in abnormalities of both surfactant phospholipid and SP-A content [236–239]. Animal models of neonatal group B streptococcus pneumonia have suggested that surfactant therapy may be beneficial [240]. Animal models of aspiration pneumonia have repeatedly demonstrated the effectiveness of exogenous surfactant therapy for this acute lung injury [200, 241, 242].

7.5. Lung Transplantation

Surfactant abnormalities after lung transplantation have been reported. Some studies have documented decreases in pulmonary compliance and surfactant function within the first 48 hours following transplantation [243]. These early defects were thought to be related to the ischemia time. Other studies have reported alterations in the surfactant function for 20–90 days, associated with delayed graft rejection [244]. These observations have been confirmed in several follow-up studies [245–249]. In an animal model of lung transplantation, the alteration in oxygenation following an ischemia time of 36 hours was prevented by exogenous surfactant treatment [250]. Recently, a trial of nebulized synthetic surfactant replacement was performed in a lung transplant patient. This demonstrated improved lung compliance in a patient with reperfusion injury following a single lung transplant [251].

7.6. Cardiopulmonary Bypass

Cardiopulmonary bypass results in physiological and morphological alterations of the lungs that resemble those seen after shock, trauma and ARDS [252, 253]. Recently, bronchoalveolar lavage fluid taken pre- and post-cardiopulmonary bypass demonstrated alterations in the surfactant that correlated with alterations in pulmonary function [254]. These alterations were similar to those previously described in an animal model of acute lung injury [255]. Since the description of surfactant abnormalities associated with cardiopulmonary bypass, there have been two clinical trials of exogenous surfactant replacement following cardiopulmonary bypass [256, 257]. These two trials reported conflicting results of the effect of surfactant replacement on oxygenation and lung compliance.

7.7. Cystic Fibrosis (CF)

CF is characterized by the production of hyperviscous and hyperelastic secretions that contribute to a chronic progressive airway obstruction.

There are many contributors to the hyperviscosity of the secretions but leukocyte DNA and phospholipids appear to be the main factors. Phospholipid quantities and composition are abnormal in CF airway secretions [258, 259]. Alterations in phospholipid content has been correlated with abnormalities in the surface active properties and the viscosity of the mucus [260]. These changes in viscoelasticity and sputum surface tension have a direct effect on cough clearability through its effect on adhesion of mucus to the respiratory tract [261]. The addition of saturated PG to the mucus from patients with CF has been shown to decrease adhesion of the mucus to the airway, increase mucus transport velocity and increase cough clearance [262]. Recently there was a clinical trial of a nebulized synthetic surfactant in patients with CF that reported an improvement in lung function [263].

7.7. *Asthma*

Asthma is characterized by airway obstruction, hyperresponsiveness and inflammation. These characteristics are mediated through a variety of inflammatory responses involving inflammatory cells [264], chemical mediators [265], and excessive secretion of mucus with abnormal rheological properties [266]. Along with the inflammation an exudate develops in the airways. The exudate contains protein that can inactivate endogenous surfactant [267]. Asthmatic patients were also found to have an abnormal SP-A/phosphatidylcholine ratio when compared to controls. One clinical trial of aerosolized surfactant in the treatment of an asthma attack demonstrated significant improvement in forced vital capacity, forced expiratory volume in one second and maximal midexpiratory flows in surfactant-treated patients compared to controls [268].

There are many other clinical conditions that have been associated with surfactant deficiency or that have been shown to be responsive to exogenous surfactant therapy in animals and humans (Table 6). Multicenter,

Table 6. Clinical conditions associated with surfactant deficiency or that may be responsive to exogenous surfactant treatment

Pulmonary alveolar proteinosis	[269]	Lung cancer	[270]
Congenital diaphragmatic hernia	[271]	Cigarette smoking	[272]
Anticancer chemotherapy	[273]	Early chronic lung disease of neonates	[274]
Neonatal pulmonary hemorrhage	[275]	Wood smoke inhalation	[276]
Idiopathic pulmonary fibrosis	[277]	Silicosis	[278]
Respiratory failure due to COPD	[279]	Hypersensitivity pneumonitis	[280]
ALTE and SIDS	[281]	IRDS	[183–186]
ARDS	[204–214]	Lung transplantation	[243–251]
Cardiopulmonary bypass	[252–257]	Asthma	[268]
CF	[258, 259, 263]	Pneumonia	[226–242]
MAS	[220, 225]	Surfactant protein B deficiency	[152, 153]

placebo-controlled clinical trials are needed to evaluate the efficacy of exogenous surfactant replacement for these diseases.

8. Conclusion

Advances in surfactant biology have underscored the importance of a healthy endogenous surfactant system in maintaining normal pulmonary function. The ability to study the surfactant cycle in type II cells and the alveolus has enhanced our understanding of the variety of functions that surfactant possesses. The variety of animal models of acute lung injury has provided the opportunity to study the pathophysiology of surfactant depletion or inhibition. Surfactant biology and animal models have been directly responsible for the success of surfactant replacement therapy for the treatment of IRDS and the possibility of using exogenous surfactants for other pulmonary injuries associated with surfactant abnormalities. The animal models have also been instrumental in the evaluation of delivery techniques for surfactant administration to the airways.

Recent advances in molecular genetics have provided the ability to develop transgenic animal models in which the endogenous surfactant system can be manipulated in a controlled manner. This will provide the opportunity to evaluate the mechanism of surfactant regulation in an intact system and the development of therapies that control the endogenous surfactant system. The ability to manipulate the endogenous surfactant system would potentially make surfactant replacement therapy obsolete. However, there are other areas in which exogenous surfactant therapy may have a role for. Limited studies have been performed on the ability of surfactant to deliver medications such as antibiotics for the treatment of lower respiratory tract infections. Delivery of DNA to the distal lung with the aid of an exogenous surfactant preparation has a potential role in attempts to correct an underlying genetic abnormality responsible for diseases such as CF or alpha-1 antitrypsin deficiency. Progress has been made since Macklin's discovery of a thin, aqueous, flexible layer rich in myelin figures in the alveolar space in 1954 but there are still many new opportunities for surfactant therapy for lung disorders that remain to be investigated.

References

1. Von Neergaard K (1929) Neue Retraktionskraft über einen Grundbegriff der Attemechanik. *Z Ges Exp Med* 66: 373–394.
2. Wilson JL, Farber S (1933) Pathogenesis of atelectasis of the newborn. *Am J Dis Child* 46: 590–603.
3. Gruenwald P (1947) Surface tension as a factor in the resistance of neonatal lungs to aeration. *Am J Obstet Gynecol* 53: 966.
4. Macklin CC (1954) The pulmonary alveolar mucoid film and the pneumocytes. *Lancet* 1: 1099–1104.

5. Schlipkoter H (1954) Elektronenoptische Untersuchungen ultradünner Lungenschnitte. *Dtsch Med Wochenschr* 79: 1658.
6. Woodside GL, Dalton AJ (1958) The ultrastructure of lung tissue from newborn and embryo mice. *J Ultrastruct Res* 2: 28–54.
7. Buckingham S, Avery ME (1965) Time of appearance of lung surfactant in the fetal mouse. *Nature* 193: 688–689.
8. Pattle RE (1955) Properties, function and origin of the alveolar lining layer. *Nature* 175: 1125–1126.
9. Clements JA (1957) Surface tension of lung extracts. *Proc Soc Exp Biol Med* 95: 170–172.
10. Clements JA, Brown ES, Johnson RP (1958) Pulmonary surface tension and the mucus lining of the lungs: some theoretical considerations. *J Appl Physiol* 12: 262–268.
11. King JR, Clements JA (1985) Lipid synthesis and surfactant turnover in the lungs. In: Geiger SR (ed). *Handbook of Physiology*, Vol 1. Washington, DC: Am Physiol Soc, 309–336.
12. Dobbs LG (1989) Pulmonary surfactant. *Am Rev Med* 40: 431–446.
13. Possmayer F (1988) A proposed nomenclature for pulmonary surfactant-associated proteins. *Am Rev Respir Dis* 138: 990–998.
14. King RJ, Simon D, Horowitz PM (1989) Aspects of secondary and quaternary structure of surfactant protein A from canine lung. *Biochim Biophys Acta* 1001: 294–301.
15. Fisher JH, Kao FT, Jones C, White RT, Benson BJ, Mason RJ (1987) The coding sequence for the 32,000 Dalton pulmonary surfactant-associated protein A is located on chromosome 10 and identifies two separate restriction-fragment-length polymorphisms. *Am J Hum Genet* 40: 503–511.
16. Bruns G, Stroh H, Veldman GM, Latt SA, Floros J (1987) The 35 kd pulmonary surfactant-associated protein is encoded on chromosome 10. *Human Genet* 76: 58–62.
17. Benson B, Hawgood S, Schilling J, Clements J, Damm D, Cordell B, White RT (1985) Structure of canine pulmonary surfactant apoprotein: cDNA and complete amino acid sequence. *Proc Natl Acad Sci USA* 82: 6379–6383.
18. Ross GF, Meuth J, Ohning B, Kim Y, Whitsett JA (1986) Purification of canine surfactant-associated glycoproteins A. Identification of a collagenase resistant domain. *Biochim Biophys Acta* 870: 267–278.
19. Ross GF, Notter RH, Meuth J, Whitsett JA (1986) Phospholipid binding and biophysical activity of pulmonary surfactant-associated protein (SAP) 35 and its non-collagenous COOH-terminal domains. *J Biol Chem* 261: 14283–14291.
20. Drickamer K, Dordal MS, Reynolds L (1986) Mannose-binding proteins isolated from rat liver contain carbohydrate-recognition domains linked to collagenous tails. *J Biol Chem* 261: 6878–6887.
21. Haagsman HP, Hawgood S, Sargeant T, Buckley D, White RT, Drickamer K, Benson BJ (1987) The major lung surfactant protein, SP 28–36, is a calcium dependent, carbohydrate-binding protein. *J Biol Chem* 262: 13877–13880.
22. Persson A, Chang D, Rust K, Moxley M, Longmore W, Crouch E (1989) Purification and biochemical characterization of CP4 (SP–D), a collagenous surfactant association protein. *Biochemistry* 28: 6361–6367.
23. McCormack FX (1995) Molecular biology of the surfactant apoproteins: *Seminars Respir Crit Care Med* 16: 29–38.
24. Hawgood S, Shiffer K (1991) Structures and properties of the surfactant-associated proteins. *Ann Rev Physiol* 53: 375–394.
25. Persson A, Chang D, Crouch E (1990) Surfactant protein D is a divalent cation-dependent carbohydrate-binding protein. *J Biol Chem* 265: 5755–5760.
26. Ogasawara Y, Kuroki Y, Akino T (1992) Pulmonary surfactant protein D specifically binds to phosphatidylinositol. *J Biol Chem* 267: 21244–21249.
27. Persson AV, Gibbons BJ, Shoemaker JD, Moxley MA, Longmore WJ (1992) The major glycolipid recognized by SP-D in surfactant is phosphatidylinositol. *Biochemistry* 31: 12183–12189.
28. Crouch E, Rust K, Veile R, Donis-Keller H, Grosso L (1993) Genomic organization of human surfactant protein D (SP–D). *J Biol Chem* 268: 2976–2983.
29. Pilot-Matias TJ, Kister SE, Fox JL, Kropp K, Glasser SW, Whitsett JA (1989) Structure and organization of the gene encoding human pulmonary surfactant proteolipid SP–B. *DNA* 8: 75–86.

30. Weaver TE, Whitsett JA (1989) Processing of hydrophobic pulmonary surfactant protein B in rat type II cells. *Am J Physiol* 257: L100–108.
31. Glasser SW, Korfhagen TR, Weaver TE, Clark CJ, Pilot-Matias T, Meuth J, Fox JL, Whitsett JA (1988) cDNA, deduced polypeptide structure and chromosomal assignment of human pulmonary surfactant proteolipid, SPL(pVAL). *J Biol Chem* 263: 9–12.
32. Jacobs KA, Phelps DS, Steinbrink R, Fisch J, Kriz R, Mitsock L, Dougherty JP, Taeusch HW, Floros J (1987) Isolation of a cDNA clone encoding a high molecular weight precursor to a 6-kDa pulmonary surfactant associated protein. *J Biol Chem* 262: 9808–9811.
33. King RJ, Clements JA (1985) Lipid Synthesis and Surfactant Turnover in the Lungs. In: Geiger SR, *Handbook of Physiology*, Vol I, American Physiological Society, Washington DC, 309–336.
34. Revak SD, Merritt TA, Degryse E, Stefani L, Courtney M, Hallman M, Cochrane CG (1988) Use of human surfactant low molecular weight apoproteins in the reconstitution of surfactant biologic activity. *J Clin Invest* 81: 826–833.
35. Whitsett JA, Ohning BL, Ross G, Meuth J, Weaver T, Holm BA, Shapiro DL, Notler RH (1986) Hydrophobic surfactant-associated protein in whole lung surfactant and its importance for biophysical activity in lung surfactant extracts used for replacement therapy. *Pediatr Res* 20: 460–467.
36. Hawgood S, Benson BJ, Hamilton RL (1985) Effects of surfactant-associated protein and calcium ions on the structure and surface activity of lung surfactant. *Lipids* 24: 184–190.
37. Suzuki Y, Fujita Y, Kogishi K (1989) Reconstitution of tubular myelin from synthetic lipids and proteins associated with pig pulmonary surfactant. *Am Rev Respir Dis* 140: 75–81.
38. Notter RH (1988) Biophysical behavior of lung surfactant: implications for respiratory physiology and pathophysiology. *Seminars in Perinatology* 12: 180–212.
39. Clements JA, Husted RF, Johnson RP, Gribetz I (1961) Pulmonary surface tension and alveolar stability. *J Appl Physiol* 16: 444–450.
40. Neiman GF, Bredenberg CE (1985) High surface tension pulmonary edema induced by detergent aerosol. *J Appl Physiol* 58: 129–136.
41. Notter RH, Taubold RD, Mavis RD (1982) Hysteresis in saturated phospholipid films and its potential relevance for the lung *in vivo*. *Exp Lung Res* 3: 109–127.
42. Dobbs LG, Wright JR, Hawgood S, Gonzales R, Venstrom K, Nellenbogen J (1987) Pulmonary surfactant and its components inhibit secretion of phosphatidylcholine from cultured rat alveolar type II cells. *Proc Natl Acad Sci USA* 84: 1010–1014.
43. LaForce FM, Kelly WJ, Huber GL (1973) Inactivation of Staphylococci by alveolar macrophages with preliminary observations on the importance of alveolar lining material. *Am Rev Respir Dis* 108: 784–790.
44. Juers JA, Rogers RM, McCurdy JB, Cook WW (1976) Enhancement of bactericidal capacity of alveolar macrophages in human alveolar lining material. *J Clin Invest* 58: 271–275.
45. Claypool WD, Fisher AB (1983) The immune function of the lung alveolar lining material. *Surv Synth Pathol Res* 2: 34–40.
46. Ansfield MJ, Kaltreider HB, Benson BJ, Caldwell JL (1980) Immunosuppressive activity of canine pulmonary surface active material. *J Immunol* 122: 1062–1066.
47. Ansfield MJ, Kaltreider HB, Benson BJ, Shalaby MR (1980) Canine surface active material and pulmonary lymphocyte function studies with mixed-lymphocyte culture. *Exp Lung Res* 1: 3–11.
48. Voss T, Eistetter H, Schafer KP, Engle J (1988) Macromolecular organization of natural and recombinant lung surfactant protein SP 28-36. Structural homology with complement factor C1q. *J Mol Biol* 201: 219–227.
49. Schweinle JE, Ezekowitz RAB, Tenner AJ, Kuhlman M, Joiner KA (1989) Human mannose-binding protein activates the alternative complement pathway and enhances serum bactericidal activity on a mannose-rich isolate of salmonella. *J Clin Invest* 84: 1821–1829.
50. Kuhlman M, Joiner K, Ezekowitz RAB (1989) The human mannose-binding protein functions as an opsonin. *J Exp Med* 169: 1733–1745.
51. Ezekowitz RAB, Kuhlman M, Groopman JE, Byrn RA (1989) A human serum mannose-binding protein inhibits *in vitro* infection by the human immunodeficiency virus. *J Exp Med* 169: 185–196.

52. Super M, Thiel S, Lu J, Levinsky RJ, Turner MW (1989) Association of low levels of mannose-binding protein with a common defect of opsonisation. *Lancet* 2: 1236–1239.
53. Wright JR (1995) Immunomodulatory functions of surfactant. *Seminars Respir Crit Care Med* 16: 61–68.
54. Benne CA, Kraaijeveld CA, Strijp JAG, Brouwer E, Harmsen M, Verhoef J, van Golde LMG, van Iwaarden JF (1995) Interactions of surfactant protein A with influenza A viruses: binding and neutralization. *J Inf Dis* 171: 335–341.
55. McNeely TB, Coorod JD (1994) Aggregation and opsonization of type A but not type B Hemophilus influenzae by surfactant protein A. *Am J Respir Cell Mol Biol* 11: 114–122.
56. Van Iwaarden F, Welmers B, Verhoef J, Haagsman HP, van Golde LMG (1990) Pulmonary surfactant protein A enhances the host-defence mechanism of rat alveolar macrophages. *Am J Respir Cell Mol Biol* 2: 91–98.
57. Van Iwaarden F, Strijp JAG, Ebskamp MJM, Welmers AC, Verhoef J, van Golde LMG (1991) Surfactant protein A is opsonin in phagocytosis of herpes simplex virus type I by rat alveolar macrophages. *Am J Physiol* 262: L204–L209.
58. Manz-Keinke H, Egenhofer C, Plattner H, Schlepper-Schafer J (1991) Specific interaction of lung surfactant protein A (SP-A) with rat alveolar macrophages. *Exp Cell Res* 192: 597–603.
59. Downing JF, Pasula R, Wright JR, Twigg HL III, Martin WJ II (1995) Surfactant protein A promotes attachment of Mycobacterium tuberculosis to a alveolar macrophages during infection with human immunodeficiency virus. *Proc Natl Acad Sci USA* 92: 4848–4852.
60. Wright JR, Youmans DC (1993) Pulmonary surfactant protein SP-A stimulates chemotaxis of alveolar macrophage. *Am J Physiol* 264: L338–L344.
61. Kremlev SG, Phelps DS (1994) Surfactant protein A stimulation of inflammatory cytokine and immunoglobulin production. *Am J Physiol* 267: L712–L719.
62. Kuan SF, Rust K, Crouch E (1992) Interactions of surfactant protein D with bacterial lipopolysaccharides. Surfactant protein D is an *Escherichia coli*-binding protein in bronchoalveolar lavage. *J Clin Invest* 90: 97–106.
63. Schelenz S, Malhotra R, Sim RB, Holmskov U, Bancroft GJ (1995) Binding of collectins to the pathogen yeast cryptococcus neoformans: human surfactant protein D acts as an agglutinin for acapsular yeast cells. *Infection Immunity* 63: 3360–3366.
64. O'Riordan DM, Standing JE, Kwon KY, Chang D, Crouch EC, Limper AH (1995) Surfactant protein D interacts with Pneumocystis carinii and mediates organism adherence to alveolar macrophages. *J Clin Invest* 95: 2699–2710.
65. Van Iwaarden F, Shimizu H, van Golde PHM, Voelker DR, van Golde LMG (1992) Rat surfactant protein D enhances the production of oxygen radicals by rat alveolar macrophages. *Biochem J* 286: 5–8.
66. Batenburg JJ, Longmore WJ, van Golde LMG (1978) The synthesis of phosphatidylcholine by adult rat lung alveolar type II epithelial cells in primary culture. *Biochim Biophys Acta* 529: 160–170.
67. Williams MC, Mason RJ (1977) Development of the type II cell in the fetal rat lung. *Am Rev Respir Dis* 115: 37s–47s.
68. Fisher AB, Chander A (1982) Glycerol kinase activity and glycerol metabolism of rat granular pneumocytes in primary culture. *Biochim Biophys Acta* 711: 128–133.
69. Mims LC, Zee P (1971) Utilization of glycerol by the newborn mammalian lung. *Biol Neonate* 18: 356–362.
70. Maniscalco WM, Stremmel W, Heeney-Campbell M (1990) Uptake of palmitic acid by rabbit alveolar type II cells. *Am J Physiol* 259: L206–L212.
71. Chander A, Reichert J, Fisher AB (1987) Degradation of dipalmitoyl phosphatidylcholine by isolated rat granular pneumocytes and reutilization for surfactant synthesis. *J Clin Invest* 79: 1133–1138.
72. Maniscalco WM, Finkelstein JN, Parkhurst AB (1983) De novo fatty acid synthesis by freshly isolated alveolar type II epithelial cells. *Biochim Biophys Acta* 751: 462–469.
73. Bourbon JR, Rieutort M, Engle MJ, Farrell PM (1982) Utilization of glycogen for phospholipid synthesis in fetal rat lung. *Biochim Biophys Acta* 712: 382–389.
74. Sheehan PM, Yeh YY (1985) Pulmonary surfactant lipid synthesis from ketone bodies, lactate and glucose in newborn rats. *Lipids* 20: 835–841.

75. Zeisel SH (1981) Dietary choline: biochemistry, physiology and pharmacology. *Ann Rev Nutr* 1: 95–121.
76. Sherman WR (1989) Inositol homeostasis, lithium and diabetes. In: Mitchell RH, Drummond AH, Downes PC (eds) *Inositol lipids in cell signaling*. London: Academic Press, 39–79.
77. Batenburg JJ (1992) Surfactant phospholipids: synthesis and storage. *Am J Physiol* 262: L367–L385.
78. Den Breejen JN, Battenburg JJ, van Golde LMG (1989) The species of acyl-CoA in subcellular fractions of type II cells isolated from adult rat lung and their incorporation into phosphatidic acid. *Biochim Biophys Acta* 1002: 277–282.
79. Mason RJ, Nellenbogen J (1984) Synthesis of saturated phosphatidylcholine and phosphatidylglycerol by freshly isolated rat alveolar type II cells. *Biochim Biophys Acta* 794: 392–402.
80. Hallman M, Gluck L (1980) Formation of acidic phospholipids in rabbit lung during perinatal development. *Pediatr Res* 14: 1250–1259.
81. Crecelius CA, Longmore WJ (1984) A study of the molecular species of diacylglycerol, phosphatidylcholine and phosphatidylethanolamine and of cholinephosphotransferase and ethanolaminephosphotransferase activities in the type II pneumocyte. *Biochim Biophys Acta* 795: 247–256.
82. Kennedy EP (1986) The biosynthesis of phospholipids. In: Op den Kamp JAF, Roelofsens B, Wirtz KWA (eds) *Lipids and biomembranes. Past, present and future*. Amsterdam: Elsevier, 171–206.
83. Chevalier G, Collet AJ (1972) In vivo incorporation of choline-3H, leucine-3H, and galactose-3H in alveolar type II pneumocytes in relation to surfactant synthesis. A quantitative radioautographic study in mouse by electromicroscopy. *Anat Rec* 174: 289–310.
84. Chander A, Fisher AB (1990) Regulation of lung surfactant secretion. *Am J Physiol* 258: L241–L253.
85. Jobe A, Ikegami M, Sarton-Miller I, Jones S, Yu G (1981) Characterization of phospholipids and localization of some phospholipid synthetic and subcellular marker enzymes in subcellular fractions from rabbit lung. *Biochim Biophys Acta* 666: 47–57.
86. Lumb RH (1989) Phospholipid transfer proteins in mammalian lung. *Am J Physiol* 257: L190–L194.
87. Lewis J, Jobe A (1989) Metabolism of intratracheally administered unsaturated phosphatidylcholines in adult rabbits. *Biochim Biophys Acta* 1005: 277–281.
88. Magoon MW, Wright JR, Baritussio A, Williams MC, Goerke J, Benson BJ, Hamilton RL, Clemens JA (1983) Subfractionation of lung surfactant-implications for metabolism and surface activity. *Biochim Biophys Acta* 750: 18–31.
89. Ballard PL, Hawgood S, Liley H, Wellenstein G, Ganzales LW, Benson B, Cordell B, White RT (1986) Regulation of pulmonary surfactant apoprotein SP 28-36 gene in fetal human lung. *Proc Natl Acad Sci USA* 83: 9527–9531.
90. Liley HG, White RT, Warr RG, Benson BJ, Hawgood S, Ballard PL (1989) Regulation of messenger RNAs for the hydrophobic surfactant proteins in human lung. *J Clin Invest* 83: 1191–1197.
91. Whitsett JA, Pilot T, Clark JC, Weaver TE (1987) Induction of surfactant protein in fetal lung. *J Biol Chem* 262: 5256–5261.
92. Acarregui MJ, Brown JJ, Mallampalli RK (1995) Oxygen modulates surfactant protein mRNA expression and phospholipid production in human fetal lung in vitro. *Am J Physiol* 268: L818–L825.
93. Whitsett JA, Weaver TE, Lieberman MA, Clark JC, Daugherty C (1987) Differential effects of epidermal growth factor and transforming growth factor β on synthesis of Mr = 35,000 surfactant associated protein in fetal lung. *J Biol Chem* 262: 7908–7913.
94. Gross I, Dynia DW, Rooney SA, Smart DA, Warshaw JB, Sissom JF, Hoath SB (1986) Influence of epidermal growth factor on fetal rat lung development in vitro. *Pediatr Res* 20: 473–477.
95. Haigh RM, Hollingsworth M, Micklewright LA, Boyd RDH, D'Souza SW (1988) The effect of human urogastrone on lung phospholipids in fetal rabbits. *J Dev Physiol* 10: 433–443.

96. Sugahara K, Rubin JS, Mason RJ, Aronsen EL, Shannon JM (1995) Keratinocyte growth factor increases mRNAs for SP-A and SP-B in adult rat alveolar type II cells in culture. *Am J Physiol* 269: L344–L350.
97. Whitsett JA, Ross G, Weaver TE, Rice W, Dion C, Hull W (1985) Glycosylation and secretion of surfactant-associated glycoprotein A. *J Biol Chem* 269: 15273–15279.
98. Ballard PL (1982) Hormonal aspects of fetal lung development. In: Farrell PM (ed.). *Lung development: biological and clinical perspectives*. New York: Academic Press, 205–253.
99. Smith BT (1984) Pulmonary surfactant during fetal development and neonatal adaptation: hormonal control. In: Robertson B, van Golde LMG, Batenburg JJ (eds). *Pulmonary Surfactant*. Amsterdam: Elsevier, 357–381.
100. Synder JM, Mendelson CR (1987) Insulin inhibits the accumulation of the major lung surfactant apoprotein in human fetal lung explants maintained in vitro. *Endocrinology* 120: 1250–1257.
101. Pryhuber BS, O'Reilly MA, Clark JC, Hull WM, Fink I, Whitsett JA (1990) Phorbol ester inhibits surfactant protein SP-A and SP-B expression. *J Biol Chem* 265: 20822–20828.
102. Whitsett JA, Clark JC, Wispe JR, Pryhuber GS (1992) Effects of TNF-alpha and phorbol ester on human surfactant protein and MnSOD gene transcription in vitro. *Am J Physiol* 262: L688–L693.
103. Bachurski CJ, Pryhuber GS, Glasser SW, Kelly SE, Whitsett JA (1995) Tumor necrosis factor- α inhibits surfactant protein C gene transcription. *J Biol Chem* 270: 19402–19407.
104. Liley HG, White RT, Benson BJ, Ballard PL (1988) Glucocorticoids both stimulate and inhibit production of pulmonary surfactant protein A in fetal human lung. *Proc Natl Acad Sci USA* 85: 9096–9100.
105. Goldenberg VE, Buckingham S, Sommers SC (1969) Pilocarpine stimulation of granular pneumocyte secretion. *J Histochem Cytochem* 16: 102–109.
106. Brown LA, Longmore WJ (1981) Adrenergic and cholinergic regulation of lung surfactant secretion in the isolated perfused lung and in the alveolar type II cell in culture. *J Biol Chem* 256: 66–72.
107. Rice WR, Hull WM, Dion CA, Hollinger BA, Whitsett JA (1985) Activation of cAMP dependent protein kinase during surfactant release from type II pneumocytes. *Exp Lung Res* 9: 135–149.
108. Gilfillian AM, Rooney SA (1987) Functional evidence for adenosine A2 receptor regulation of phosphatidylcholine secretion in cultured type II pneumocytes. *J Pharmacol Exp Ther* 241: 907–914.
109. Gilfillian AM, Rooney SA (1988) Functional evidence for involvement of P2 purinoceptors in the ATP stimulation of phosphatidylcholine secretion in type II alveolar cells. *Biochim Biophys Acta* 959: 31–37.
110. Rice WR, Singleton FM (1986) Purinoreceptors regulate surfactant secretion from rat isolated alveolar type II cells. *Br J Pharmacol* 89: 485–491.
111. Dobbs LG, Gonzales RF, Marinari LA, Mescher EJ, Hawgood S (1986) The role of calcium in the secretion of surfactant by rat alveolar type II cells. *Biochim Biophys Acta* 877: 305–313.
112. Hill DJ, Wright TC, Andrews ML, Karnovsky MJ (1984) Localization of calmodulin in differentiating pulmonary type II epithelial cells. *Lab Invest* 51: 297–306.
113. Corbet A, Voelker R, Murphy F, Owens M (1991) Effect of calcium and calcium antagonists on phospholipid secretion induced by lung inflation in newborn rabbits. *Am J Med Sci* 301: 102–114.
114. Brown LA, Wood LH (1989) Stimulation of surfactant secretion by vasopressin in primary cultures of adult rat type II pneumocytes. *Biochim Biophys Acta* 1001: 76–81.
115. Oyarzun MJ, Clements JA (1978) Control of lung surfactant by ventilation, adrenergic mediators, and prostaglandins in the rabbit. *Am Rev Respir Dis* 117: 879–891.
116. Rooney SA, Gobran LI (1988) Adenosine and leukotrienes have a regulatory role in lung surfactant in the newborn rabbit. *Biochim Biophys Acta* 960: 98–106.
117. Marino PA, Rooney SA (1980) Surfactant secretion in a newborn rabbit lung slice model. *Biochim Biophys Acta* 620: 509–519.
118. Nicholas TE, Barr HA (1981) Control of release of surfactant phospholipids in the isolated perfused rat lung. *J Appl Physiol* 51: 90–98.

119. Brown LAS, Pasquale SM, Longmore WJ (1985) Role of microtubules in surfactant secretion. *J Appl Physiol* 58: 1866–1873.
120. Chen M, Brown LAS (1990) Histamine stimulation of surfactant secretion from rat type II pneumocytes. *Am J Physiol* 258: L195–L200.
121. Gilfillian AM, Lewis AJ, Rooney SA (1987) Effects of thiazinamium chloride and other antihistamines on phosphatidylcholine secretion in rat type II pneumocyte cultures. *Biochem Pharmacol* 36: 277–281.
122. Rice WR, Whitsett JA (1984) Inhibition of surfactant release from isolated type II cells by compound 48/80. *Biochim Biophys Acta* 805: 261–267.
123. Rice WR, Singleton FM (1986) Regulation of surfactant secretion from isolated type II cells by substance P. *Biochim Biophys Acta* 889: 123–127.
124. Rice WR, Singleton FM (1986) Regulation of surfactant phospholipid secretion from isolated rat alveolar type II cells by lectins. *Biochim Biophys Acta* 958: 205–210.
125. Gobran LI, Rooney SA (1990) Adenosine A1 receptor-mediated inhibition of surfactant secretion in rat type II pneumocytes. *Am J Physiol* 258: L45–L51.
126. Rider ED, Ikegami M, Jobe AH (1990) Intrapulmonary catabolism of surfactant-associated phosphatidylcholine in rabbits. *J Appl Physiol* 69: 1856–1862.
127. Rider ED, Ikegami M, Jobe AH (1992) Localization of alveolar surfactant clearance in rabbit lung cells. *Am J Physiol* 263: L201–L209.
128. Jacobs H, Jobe AH, Ikegami M, Conway D (1983) The significance of reutilization of surfactant phosphatidylcholine. *J Biol Chem* 258: 4159–4165.
129. Wright JR, Wager RE, Hawgood S, Dobbs L, Clements JA (1987) Surfactant apoprotein Mr = 26,000–36,000 enhances uptake of liposomes by type II cells. *J Biol Chem* 262: 2888–2894.
130. Rice WR, Sarin VK, Fox JL, Baatz J, Wert S, Whitsett JA (1989) Surfactant peptides stimulate uptake of phosphatidylcholine by isolated cells. *Biochim Biophys Acta* 1006: 237–245.
131. Oyarzun MJ, Clements JA, Baritussio A (1980) Ventilation enhances pulmonary alveolar clearance of radioactive dipalmitoylphosphatidylcholine in liposomes. *Am Rev Respir Dis* 121: 709–721.
132. Bates SR, Ibach PB, Fisher AB (1989) Phospholipids co-isolated with rat surfactant protein C account for the apparent protein-enhanced uptake of liposomes into lung granular pneumocytes. *Exp Lung Res* 15: 695–708.
133. Fisher AB, Dodia C, Chander A (1985) β -Adrenergic mediators increase pulmonary retention of instilled phospholipids. *J Appl Physiol* 59: 743–748.
134. Fisher AB, Dodia C, Chander A (1989) Secretagogues for lung surfactant increase lung uptake of alveolar phospholipids. *Am J Physiol* 257: L248–L252.
135. Pettenazzo A, Jobe A, Ikegami M, Abra R, Hogue E, Mihalko P (1989) Clearance of phosphatidylcholine and cholesterol from liposomes, liposomes loaded with metaproterenol, and rabbit surfactant from adult rabbit lungs. *Am Rev Respir Dis* 139: 752–758.
136. Dranoff G, Crawford AD, Sadelain M, Ream B, Rashid A, Bronson RT, Dickersin GR, Bachurski CJ, Mark EL, Whitsett JA, et al. (1994) Involvement of granulocyte-macrophage colony stimulating factor in pulmonary homeostasis. *Science* 264: 713–716.
137. Robillard E, Alarie Y, Dagenais-Perusse P, Baril E, Guilbeault A (1964) Microaerosol administration of synthetic B-g-dipalmitoyl-L-a-Lecithin in the respiratory distress syndrome. *Can Med Assoc J* 90: 55–57.
138. Chu J, Clements JA, Colton EK, Klaus MH, Sweet AY, Tooley WH (1967) Neonatal pulmonary ischemia: clinical and physiologic studies. *Pediatrics* 40: 709–782.
139. Morely CJ, Miller N, Bangham A, Davis J (1981) Dry artificial lung surfactant and its effect on very premature babies. *Lancet* 1: 64–68.
140. Ten Centre Study Group (1987) Ten centre trial of artificial surfactant (artificial lung expanding compound) in very premature babies. *Br Med J* 294: 991–996.
141. Jobe A, Ikegami M (1987) Surfactant for the treatment of respiratory distress syndrome. *Am Rev Respir Dis* 135: 1256–1275.
142. Durand DJ, Clyman RI, Heymann MA, Clements JA, Murray F, Kitterman J, Ballard P (1985) Effects of a protein-free, synthetic surfactant on survival and pulmonary function in premature lambs. *J Pediatr* 107: 775–780.
143. Long W, Thompson T, Sundell H, Schumacher R, Volberg R, Guthrie R (1991) The American Exosurf Neonatal Study Group I and the Canadian Exosurf Neonatal Study Group.

- Effects of two rescue doses of a synthetic surfactant on mortality rate and survival without bronchopulmonary dysplasia in 700 to 1360 gm infants with respiratory distress syndrome (see comments). *J Pediatr* 118: 595–605.
144. Halliday HL, Reid M, Mehan C, McClure G, Lappin T, Thomas P (1984) Controlled trial of artificial surfactant to prevent respiratory distress syndrome. *Lancet* 1: 476–478.
 145. Fujiwara T, Chida S, Watabe Y, Maeta H, Morita T, Abe T (1980) Artificial surfactant therapy in hyaline membrane disease. *Lancet* 1: 55–59.
 146. Metcalfe IL, Pototschnik R, Burgoyne R, Enhorning G (1982) Lung expansion and survival in rabbit neonates treated with surfactant extract. *J Appl Physiol* 53: 838–843.
 147. Notter RH, Egan EA, Kwong MS, Holm BA, Shapiro DL (1985) Lung surfactant replacement in premature lambs with extracted lipids from bovine lung lavage: effects of dose, dispersion technique and gestational age. *Pediatr Res* 19: 569–577.
 148. Whitsett JA, Ohning BL, Ross G, Meuth J, Weaver TE, Holm BA, Shapiro DL, Notter RH (1986) Hydrophobic surfactant-associated protein in whole lung surfactant and its importance for biophysical activity in lung surfactant extracts used for replacement therapy. *Pediatr Res* 20: 460–467.
 149. Shapiro DL (1988) The development of surfactant replacement therapy and the various types of replacement surfactants. *Seminar Perinatol* 12: 174–179.
 150. Holm BA, Waring AJ (1993) Desigern surfactants: the next generation in surfactant replacement. *Clin Perinatol* 20: 813–829.
 151. Hall SB, Venkitaraman AR, Whitsett JA, Holm BA, Notter RH (1992) Importance of hydrophobic apoproteins as constituents of clinical exogenous surfactants. *Am Rev Respir Dis* 145: 24–30.
 152. Hamvas A, Cole FS, deMello DE, Moxley M, Whitsett JA, Colten HR, Noguee LM (1994) Surfactant protein B deficiency: antenatal diagnosis and prospective treatment with surfactant replacement. *J Pediatr* 125: 356–361.
 153. Noguee LM, deMello DE, Dehner LP, Cotton HR (1993) Brief report: deficiency of pulmonary surfactant protein B in congenital alveolar proteinosis. *N Engl J Med* 328: 406–410.
 154. Clark JC, Wert SE, Bachurski CJ, Stahlman MT, Stripp BA, Weaver TE, Whitsett JA (1995) Targeted disruption of the surfactant protein B gene disrupts surfactant homeostasis, causing respiratory failure in newborn mice. *Proc Natl Acad Sci USA* 92: 7794–7798.
 155. Merritt TA, Amirkhanian JD, Helbock H, Halliwell B, Cross CE (1993) Reduction of the surface-tension-lowering ability of surfactant after exposure to hypochlorous acid. *Biochem J* 295: 19–22.
 156. Lewis J, Ikegami M, Higuchi R, Jobe A, Absolom D (1991) Nebulized versus instilled exogenous surfactant in an adult injury model. *J Appl Physiol* 71: 1270–1276.
 157. Lewis J, Ikegami M, Tabor B, Jobe A, Absolom D (1992) Aerosolized surfactant is preferentially deposited in normal versus injured regions of the lung in a heterogenous lung injury model. *Am Rev Respir Dis* 145: A184.
 158. Segerer H, van Gelder W, Angenent FWM, van Woerkens LJPM, Curstedt T, Obladen M, Lachman B (1993) Pulmonary distribution and efficacy of exogenous surfactant in lung-lavaged rabbits are influenced by instillation technique. *Pediatr Res* 34: 490–494.
 159. Segerer H, Scheid A, Wagner M, Obladen M (1994) Tracheal surfactant infusion during 5 minutes is less effective than bolus instillation in rabbits. *Pediatr Res* 36: 37A.
 160. Broadbent R, Fok TF, Dolovich M, Watts J, Coates G, Bowen B, Kirpalani H (1995) Chest position and pulmonary deposition of surfactant in surfactant depleted rabbits. *Arch Dis Child Fetal and Neonatal* 72: F84–F89.
 161. Petrikovsky BM, Lysikiewicz A, Markin LB, Slomko Z (1995) In utero surfactant administration to preterm human fetuses using endoscopy. *Fetal Diagnosis and Therapy* 10: 127–130.
 162. Kobayashi T, Ganzuka M, Taniguchi J, Nitta K, Murakami S (1990) Lung lavage and surfactant replacement for hydrochloric acid aspiration in rabbits. *Acta Anaesthesiol Scand* 34: 216–221.
 163. Kobayashi T, Nitta K, Ganzuka M, Inui S, Grossman G, Robertson B (1991) Inactivation of exogenous surfactant by pulmonary edema fluid. *Pediatr Res* 29: 353–356.
 164. Hallman M, Merritt TA, Pohjavuori M, Gluck L (1986) Effect of surfactant substitution on lung effluent phospholipids in respiratory distress syndrome: Evaluation of surfactant phospholipid turnover, pool size, and the relationship to severity of respiratory failure. *Pediatr Res* 20: 1228–1235.

165. Wilkinson A, Jenkins PA, Jeffrey JA (1985) Two controlled trials of dry artificial surfactant: early effects and later outcomes in babies with surfactant deficiency. *Lancet* 2: 287–291.
166. Ashton MR, Postle AD, Hall MA, Austin NC, Smith DE, Normand ICS (1992) Turnover of exogenous artificial surfactant. *Arch Dis Child* 67: 383–387.
167. Matalon S, Holm BA, Notter RH (1987) Mitigation of pulmonary hyperoxic injury by administration of exogenous surfactant. *J Appl Physiol* 62: 756–761.
168. Ikegami M, Berry D, Elkady T, Pettenazzo A, Seider S, Jobe A (1987) Corticosteroids and surfactant change lung function and protein leaks in the lungs of ventilated premature rabbits. *J Clin Invest* 79: 1371–1378.
169. Peltanazzo A, Seider SR, Ikegami M, Jobe A (1987) Surfactant clearance in 3-day-old rabbits: effect of lipid extraction and resuspension on clearance kinetics of phosphatidylcholine. *Pediatr Res* 21: 463.
170. Lawton AR (1984) Ontogeny of the immune system. In: Ogra PL (ed.) *Neonatal infections: Nutritional and immunologic interactions*. Philadelphia: Grune and Stratton, 3–20.
171. Toivanen P, Uksila J, Leino A, Lassila O, Hirvonen T, Ruuskanen O (1981) Development of nitrogen responding T cells and natural killer cells in the human fetus. *Immunol Rev* 57: 191–105.
172. Strayer DS, Merritt TA, Makunike C, Hallman M (1989) Antigenicity of low molecular weight surfactant species. *Am J Path* 134: 723–732.
173. Strayer DS, Vitetta ES, Kohler H (1975) Anti-receptor antibody I. Isolation and characterization of immunoglobulin receptor for phosphorylation. *J Immunol* 114: 722–727.
174. Strayer DS, Merritt TA, Lwebuga-Mukasa J, Hallman M (1986) Surfactant-anti-surfactant immune complexes in neonatal respiratory distress syndrome. *Am J Path* 122: 353–362.
175. Merritt TA, Strayer D, Hallman M, Spragg R, Wozniak P (1988) Immunologic consequences of exogenous surfactant administration. *Semin Perinatol* 12: 221–230.
176. Stahlman MT, Gray ME, Ross GF, Hull WM, Wikenheiser K, Dingle S, Zelenski-Low KR, Whitsett JA (1992) Human surfactant protein-A contains blood group A antigenic determinants. *Pediatr Res* 31: 364–371.
177. Chida S, Phelps DS, Soll RF, Taeusch HW (1991) Surfactant proteins and anti-surfactant antibodies in sera from infants with respiratory distress syndrome with and without surfactant treatment. *Pediatrics* 88: 84–89.
178. Weaver TE, Whitsett JA (1995) Antigenicity of surfactant proteins A, B, and C. In: Robertson B, Taeusch HW (eds) (1995) *Surfactant therapy for lung disease*. New York: Marcel Dekker, 293–306.
179. Whitsett JA, Hull WM, Luse S (1991) Failure to detect surfactant protein-specific antibodies in sera of premature infants treated with Survanta, a modified bovine surfactant. *Pediatrics* 87: 505–510.
180. Bartmann P, Bamberger U, Pohlandt F, Gortner L (1992) Immunogenicity and immunomodulatory activity of bovine surfactant (SF–RI 1). *Acta Pediatr* 81: 383–388.
181. Allred TF, Auten Jr. RL (1995) Surfactant therapy in newborns. *Semin Respir Crit Care Med* 16: 39–46.
182. Collaborative European Multicenter Study Group (1988) Surfactant replacement therapy for severe neonatal respiratory distress syndrome: an international, randomized clinical trial. *Pediatrics* 82: 683–691.
183. Merritt TA, Hallman M, Berry C, Pohjavuori M, Edwards DK, Jaaskelainen J, Grafe MR, Vaucher Y, Wogniak P, Heldt G, et al. (1991) Randomized, placebo-controlled trial of human surfactant given at birth versus rescue administration in very low birth weight infants with lung immaturity. *J Pediatr* 118: 581–594.
184. Shapiro DL, Notter RH, Morin F, Deluga KS, Golub LM, Sinkin RA, Weiss KI, Cox C (1985) Double-blind, randomized, trial of calf lung surfactant extract administered at birth to very premature infants for prevention of respiratory distress syndrome. *Pediatrics* 76: 593–599.
185. Long W, Corbet A, Cotton R, Courtney S, McGuinness G, Walter D, Watts J, Smyth J, Bard H, Chernick V, et al. (1991) The American Exosurf Neonatal Study Group I and the Canadian Exosurf Neonatal Study Group. A controlled trial of synthetic surfactant in infants weighing 1250 g or more with respiratory distress syndrome. *N Engl J Med* 325: 1696–1703.

186. Hoekstra RE, Jackson JC, Myers TF, Frantz III ID, Stern ME, Powers WF, Maurer M, Raye JR, Carrier ST, Gunkel JH, et al. (1991) Improved neonatal survival following multiple doses of bovine surfactant in very premature neonates at risk for respiratory distress syndrome. *Pediatrics* 88: 10–18.
187. Cummings JJ, Holm BA, Hudak ML, Hudak BB, Ferguson WH, Egan EA (1992) A controlled comparison of four different surfactant preparations in surfactant-deficient preterm lambs. *Am Rev Respir Dis* 145: 999–1004.
188. Edberg KE, Ekstrom-Jodal B, Hallman M, Hjalmarson D, Sandberg K, Silberberg A (1990) Immediate effects on lung function of instilled human surfactant in mechanically ventilated newborn infants with IRDS. *Acta Paediatr Scand* 79: 750–755.
189. Halliday HL, McCord FB, McClure BG, Reid MM (1989) Acute effects of instillation of surfactant in severe respiratory distress syndrome. *Arch Dis Child* 64: 13–16.
190. Kendig JW (1988) A multicenter randomized trial of surfactant replacement with calf lung surfactant extract: effects of pre- and post-ventilatory instillation and of multiple doses. In: Lachman B (ed) *Surfactant replacement therapy*. Rotterdam: Springer-Verlag, 108–122.
191. Phibbs RH, Ballard RA, Clements JA, Heilborn DC, Phibbs CS, Scluer MA, Sniderman SH, Tooley WH, Wakeley MA (1991) Initial clinical trial of EXOSURF, a protein free synthetic surfactant, for the prophylaxis and early treatment of hyaline membrane disease. *Pediatrics* 88: 1–9.
192. Raju TN, Vidyasagar D, Bhat R, Sobel D, McCulloch KM, Anderson M, Maeta H, Levy PS, Furner S (1987) Double-blind controlled trial of single-dose treatment with bovine surfactant in severe hyaline membrane disease. *Lancet* 1: 651–656.
193. Van Houten J, Long W, Mullett M, Finer N, Derleth D, McMurray B, Peliowski A, Walker D, Wold D, Sankaran K, et al. (1992) The American Exosurf Neonatal Study Group I and the Canadian Exosurf Neonatal Study Group. Pulmonary hemorrhage in premature infants after treatment with synthetic surfactant: an autopsy evaluation. *J Pediatr* 120: S40–S44.
194. Maniscalco WM, Kendig JW, Shapiro DL (1989) Surfactant replacement therapy: impact of hospital charges for premature infants with respiratory distress syndrome. *Pediatrics* 83: 1–6.
195. Lachmann B, Fujiwara T, Chida S, Morita T, Konishi M, Nakamura K, Maeta H (1981) Improved gas exchange after tracheal instillation of surfactant in the experimental adult respiratory distress syndrome. *Crit Care Med* 9: 158.
196. Kobayashi T, Kataoka H, Ueda T, Murakami S, Takada Y, Kokubo M (1984) Effects of surfactant supplement and end-expiratory pressure in lung lavaged rabbits. *J Appl Physiol* 57: 995–1001.
197. Oetomo S, Reijngoud DJ, Ennema JJ, Okhen A, Wildevuur CH (1988) Surfactant replacement therapy in surfactant deficient rabbits: early effects on lung function and biochemical aspects. *Lung* 166: 65–73.
198. Berggren P, Lachmann B, Curstedt T, Grossman G, Robertson B (1986) Gas exchange and lung morphology after surfactant replacement in experimental adult respiratory distress syndrome induced by repeated lung lavage. *Acta Anaesthesiol Scand* 30: 321–328.
199. Fracica PJ, Piantadosi CA, Young SL, Crapo JD (1992) Artificial surfactant diminishes pathologic injury in hyperoxia in primates. *Am Rev Respir Dis* 145: A609.
200. Huang YC, Fawcett TA, Moon RE, Fracica PJ, Siminon SG, Sane AC, Piantadosi CA, Young SL (1992) Exogenous surfactant treatment improves Va/Q abnormalities in hyperoxic lung injury. *Am Rev Respir Dis* 145: A609.
201. Simonson SG, Huang YC, Fracica PJ, Sane AC, Piantadosi CA, Young SL (1992) Exogenous surfactant improves oxygenation in hyperoxic lung injury. *Am Rev Respir Dis* 145: A610.
202. Lamm WJE, Albert RK (1990) Surfactant replacement improves lung recoil in rabbit lungs after acid aspiration. *Am Rev Respir Dis* 142: 1279–1283.
203. Weg J, Reines H, Balk R, Tharratt R, Kearney P, Killian T, Scholten D, Zaccardelli D, Horton J, Pattishall E (1991) The Exosurf-ARDS Sepsis Study Group. Safety and efficacy of an aerosolized surfactant (Exosurf) in human sepsis-induced ARDS. *Chest* 100: 137S.

204. Wiedemann H, Baughman R, deBoisblanc B, Schuster D, Caldwell E, Weg J, Balk R, Jenkinson S, Wiegelt J, Tharratt R, et al. (1992) The Exosurf ARDS-Sepsis Study Group. A multicenter trial in human sepsis-induced ARDS of an aerosolized synthetic surfactant (Exosurf). *Am Rev Respir Dis* 145: A184.
205. Anzueto A, Baughman R, Guntupalli K, DeMaria E, Davis K, Weg J, Long W, Horton J, Pattishall E (1994) The Exosurf ARDS sepsis study group. An international randomized, placebo-controlled trial evaluating the safety and efficacy of aerosolized surfactant in patients with sepsis-induced ARDS. *Am J Respir Crit Care Med* 149: A567.
206. Richman PS, Spragg RG, Merritt TA, Robertson B, Curstedt T (1987) Administration of porcine-lung surfactant to humans with ARDS: initial experience. *Am Rev Respir Dis* 135: A5.
207. Nosaka S, Sakai T, Yonekura M, Yoshikawa K (1990) Surfactant for adults with respiratory failure. *Lancet* 336: 947–948.
208. Gregory TJ, Longmore WJ, Moxley MA, Cai GZ, Gadek JE, Weiland JE, Hyers TM, Crime, Hudson LD, Steinberg KP, et al. (1994) Surfactant repletion following Survanta supplementation in patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med* 149: A124.
209. Spragg RG, Gilliard N, Richman P, Smith RM, Hite D, Pappert D, Robertson B, Curstedt T, Strayer D (1994) Acute effects of a single dose of porcine surfactant on patients with the adult respiratory distress syndrome. *Chest* 105: 195–202.
210. Weg JG, Balk RA, Tharratt RS, Jenkinson SG, Shah JB, et al. (1994) Safety and potential efficacy of an aerosolized surfactant in human sepsis-induced adult respiratory distress syndrome. *J Am Med Assoc* 272: 1433–1438.
211. Heikkinheimo M, Hynynen M, Rautiainen P, Andersson S, Hallman M, Kukkonen S (1994) Successful treatment of ARDS with two doses of synthetic surfactant. *Chest* 105: 1263–1264.
212. Lachmann B (1989) Surfactant therapy. *Resuscitation* 18: S37–S49.
213. Wilson D, Jiao JH, Zaritsky A, Bauman L, Craft H (1994) The use of exogenous surfactant in pediatric respiratory failure (Abstract). *Pediatric Critical Care Colloquium*.
214. Lewis J, Frewen T, Hutchinson J, Simons J, Splinter W, Kovesi T (1994) Exogenous surfactant therapy for pediatric ARDS patients (Abstract). *Pediatric Critical Care Colloquium*.
215. Lewis J, Dhillon J, Frewen T (1994) Exogenous surfactant therapy in pediatric patients with ARDS. *Am J Respir Crit Care Med* 149: A125.
216. Smyth JA, Metcalfe IL, Duffy P, Possmayer F, Bryan MH, Enhorning G (1983) Hyaline membrane disease treated with bovine surfactant. *Pediatrics* 71: 913–917.
217. Haslam PL, Hughes DA, MacNaughton PD, Baker CS, Evans TW (1994) Surfactant replacement therapy in late stage adult respiratory distress syndrome. *Lancet* 343: 1009–1111.
218. Evans DA, Wilmott RW, Whitsett JA (1996) Surfactant replacement therapy for adult respiratory distress syndrome in children. *Pediatr Pulmonol* (in press).
219. Wiswell TE, Tuggle JM, Turner BS (1990) Meconium aspiration syndrome: have we made a difference? *Pediatrics* 85: 715–721.
220. Findlay RD, Taeusch HW, Walther FJ (1996) Surfactant replacement therapy for meconium aspiration syndrome. *Pediatrics* 97: 48–52.
221. Moses D, Holm BA, Spitale P, Liu M, Enhorning G (1991) Inhibition of pulmonary surfactant function by meconium. *Am J Obstet Gynecol* 164: 477–481.
222. Sun B, Curstedt T, Robertson B (1993) Surfactant inhibition in experimental meconium aspiration. *Acta Pediatr* 82: 182–189.
223. Al-Mateen KB, Dailey K, Grimes M, Gutcher GR (1994) Improved oxygenation with exogenous surfactant administration in experimental meconium aspiration. *Pediatr Pulmonol* 17: 75–80.
224. Giuseppetti MM, Wiswell TE (1994) The use of saline lavage and surfactant therapy in the management of a piglet model of the meconium aspiration syndrome (MAS). *Ped Res* 35: 333A.
225. Halac E, Arquello B, Bono GC, Perez R, Carriedo OAL (1994) Early exogenous surfactant therapy (EST) in severe meconium aspiration syndrome (MAS). *Ped Res* 35: 336A.

226. Lachmann B, Bergmann KC (1987) Surfactant replacement improves thorax-lung compliance and survival rate in mice with influenza infection. *Am Rev Respir Dis* 135: A6.
227. Van Daal GJ, So KL, Gommers D, Eijking EP, Fievez RB, Sprenger MJ, et al. (1991) Intratracheal surfactant administration restores gas exchange in experimental adult respiratory distress syndrome associated with viral pneumonia. *Anesth Analg* 72: 589–595.
228. Van Daal GJ, Bos JAH, Eijking EP, Gommers D, Hannappel E, Lachmann B (1992) Surfactant replacement therapy improves pulmonary mechanics in end-stage influenza A pneumonia in mice. *Am Rev Respir Dis* 145: 859–863.
229. LeVine AM, Lotze A, Stanley S, Stroud C, Pollack M, O'Donnel R, Whitsett JA (1995) Surfactant content in children with acute viral bronchiolitis. *Crit Care Med* 23: A185.
230. Pelps DS, Rose RM (1991) Increased recovery of surfactant protein A in AIDS-related pneumonia. *Am Rev Respir Dis* 143: 1072–1075.
231. Escamilla R, Prevost MC, Hermant C, Caratero A, Cariven C, Krempf M (1992) Surfactant analysis during pneumocystic carinii pneumonia in HIV-infected patients. *Chest* 101: 1558–1562.
232. Hoffman AGD, Lawrence MG, Ognibene FP, Suffredini AF, Lipschik GY, Kovacs JA, Masur H, Shelhamer JH (1992) Reduction of pulmonary surfactant in patients with human immunodeficiency virus infection and pneumocystis carinii pneumonia. *Chest* 102: 1730–1736.
233. Steinberg RI, Whitsett JA, Hull WM, Baughman RP (1995) Pneumocystis carinii alters surfactant protein A concentrations in bronchoalveolar lavage fluid. *J Lab Clin Med* 125: 462–469.
234. Eijking EP, van Daal GJ, Tenbrink R, Sluiters JF, Hannappel E, Erdman W, Lachmann B (1992) Improvement of pulmonary gas exchange after surfactant replacement in rats with Pneumocystis carinii pneumonia. In: Goldstick TK (ed) *Oxygen transport to tissue XIII*. New York: Plenum Press, 293–298.
235. Slater AJ, Nichani SH, Macrae D, Wilkinson KA, Novelli V, Tasker RC (1995) Surfactant adjunctive therapy for Pneumocystis carinii pneumonia in an infant with acute lymphoblastic leukemia. *Intens Care Med* 21: 261–263.
236. Sachse K (1989) Changes in relative concentrations of surfactant phospholipids in young pigs with experimental pneumonia. *J Vet Med* 36: 385–390.
237. Brogden KA (1991) Changes in pulmonary surfactant during bacterial pneumonia. *Antonie von Leeuwenhoek* 59: 215–223.
238. Baughman RP, Stein E, MacGee J, Rashkin M, Sahebajani H (1984) Changes in fatty acids in phospholipids of the bronchoalveolar lavage fluid in bacterial pneumonia and in adult respiratory distress syndrome. *Clin Chem* 30: 521–523.
239. Baughman RP, Steinberg RI, Hull W, Buchsbaum JA, Whitsett JA (1993) Decreased surfactant protein A in patients with bacterial pneumonia. *Am Rev Respir Dis* 147: 653–657.
240. Herting E, Jarstrand C, Rasool O, Curstedt T, Sun B, Robertson B (1994) Experimental neonatal group B Streptococcal pneumonia: effect of a modified porcine surfactant on bacterial proliferation in ventilated near term rabbits. *Pediatr Res* 36: 784–791.
241. Strohmaier W, Redl H, Schlag G (1990) Studies of the potential role of a semisynthetic surfactant preparation in an experimental aspiration trauma in rabbits. *Exp Lung Res* 16: 101–110.
242. Strohmaier W, Schlag G (1993) Experimental aspiration trauma: comparison of steroid treatment versus exogenous natural surfactant. *Exp Lung Res* 19: 397–405.
243. Waldhausen JA, Giammona ST, Kilman JW, Daly WJ (1965) Effect of transplantation of canine lung on pulmonary compliance and surfactant. *J Am Med Assoc* 191: 1002–1005.
244. Yeh TJ, Toyohara H, Ellison LT, Parker JL, Ellison RG (1966) Pulmonary function in dogs after lung homotransplantation. *Ann Thorac Surg* 2: 195–203.
245. Yakeishi Y, Nozaki M, Joseph WL, Rangel DM, Stevens GH, Fonkalsrud EW (1968) Effect of homotransplantation of the dog lung on pulmonary surfactant. *Surg Forum* 19: 236–237.
246. Yakeishi Y, Nozaki M, Rangel DM, Stevens GH, Adams FH, Fonkalsrud EW (1968) Effect of allotransplantation of the canine lung on pulmonary surfactant. *Surg Gynecol Obstet* 128: 1264–1268.
247. Jurmann MJ, Obladen M, Schaefer HJ, Dammenhayn L, Haverich A (1989) Analysis of bronchoalveolar lavage and pulmonary alveolar surfactant for diagnosis of rejection and infection in heart-lung transplantation. *Transplant Proc* 21: 2581–2582.

248. Klepetko W, Lohninger A, Wissner W, Mueller MR, Khunl-Brady G, Windisch A, Eckersberger F, Wolner E (1990) Pulmonary surfactant in bronchoalveolar lavage after canine lung transplantation: effect of L-carnitine application. *J Thorac Cardiovasc Surg* 99: 1048–1058.
249. Veldhuizen RAW, Lee J, Sandler D, Hull W, Whitsett JA, Lewis J, Possmayer F, Novick RJ (1993) Alterations in pulmonary surfactant composition and activity after experimental lung transplantation. *Am Rev Respir Dis* 148: 208–215.
250. Veldhuizen R, McDougall K, Lewis J, Novick R, Possmayer F (1993) Surfactant supplementation in experimental lung transplantation. *Am Rev Respir Dis* 147: A261.
251. Struber M, Cremer J, Harringer W, Hirt SW, Costard-Jackle A, Haverich A (1995) Nebulized synthetic surfactant in reperfusion injury after single lung transplantation. *J Thorac Cardiovasc Surg* 110: 563–564.
252. Pennock JL, Pierce WS, Waldhausen JA (1977) The management of the lungs during cardiopulmonary bypass. *Surg Obstet Gynecol* 145: 917–927.
253. Ghia J, Andersen NB (1970) Pulmonary function and cardiopulmonary bypass. *J Am Med Assoc* 212: 593–597.
254. McGowan FX, Ikegami M, del Nido PJ, Motoyama EK, Kurland G, Davis PJ, Siewers RD, Griffith BP (1993) Cardiopulmonary bypass significantly reduces surfactant activity in children. *J Thorac Cardiovasc Surg* 106: 968–977.
255. Lewis JF, Ikegami M, Jobe AH (1990) Altered surfactant function and metabolism in rabbits with acute lung injury. *J Appl Physiol* 69: 2303–2310.
256. Do Campo JL, Bertranou EG, De Lorenzi A, Hager AA (1994) Nebulised exogenous natural surfactant after cardiac surgery. *Lancet* 343: 482.
257. Macnaughton PD, Evans TW (1994) The effect of exogenous surfactant therapy on lung function following cardiopulmonary bypass. *Chest* 105: 421–425.
258. Galabert C, Jacquot J, Zahm JM, Puchelle E (1987) Relationship between the lipid content and the rheologic properties of airway secretions in cystic fibrosis. *Clin Chim Acta* 164: 139–149.
259. Gilljam H, Andersson O, Ellin A, Robertson B, Strandvik B (1988) Composition and surface properties of the bronchial lipids in adult patients with cystic fibrosis. *Clin Chim Acta* 176: 29–38.
260. Girod S, Galabert C, Lecuire A, Zahm JM, Puchelle E (1992) Phospholipid composition and surface active properties of tracheobronchial secretions from patients with cystic fibrosis and chronic obstructive pulmonary diseases. *Pediatr Pulmonol* 13: 22–27.
261. Rubin BK (1992) A superficial view of mucus and the cystic fibrosis defect. *Pediatr Pulmonol* 13: 4–5.
262. Girod de Bentzmann S, Pierrot D, Zahm JM, Puchelle E (1992) Enhancement of cystic fibrosis mucus clearance by liposomes of distearoyl phosphatidylglycerol. *Am Rev Respir Dis* 145: A689.
263. Rubin BK, Ramirez OE, Smith E, Barrett J (1995) Early results of clinical trials of Exosurf for the therapy of cystic fibrosis lung disease. *Pediatr Pulmonol* 20: 234.
264. Djukanovic R, Roche WR, Wilson JW, Beasley CRW, Twentyman OP, Howarth PH, Holgate ST (1990) Mucosal inflammation in asthma. *Am Rev Respir Dis* 142: 434–457.
265. Lee T (1992) Mediators of bronchial responsiveness. In: Clark TJH, Godfrey S, Lee T (eds): *Asthma*, Third edition. London: Chapman and Hall Medical, p 162.
266. O'Riordan T, Zwang J, Smaldone GC (1992) Mucociliary clearance in adult asthma. *Am Rev Respir Dis* 146: 598–603.
267. Brogan TD, Ryley HC, Neale L, Yassa J (1975) Soluble protein of bronchopulmonary secretions from patients with cystic fibrosis, asthma and bronchitis. *Thorax* 30: 72–79.
268. Kurashima K, Ogawa H, Ohka T, Fujimura M, Matsuda T, Kobayashi T (1991) A pilot study of surfactant inhalation in the treatment of asthmatic attack. *Jap J Allergol* 40: 160–163.
269. Jennings VM, Dillehay DL, Webb SK, Brown LA (1995) Pulmonary alveolar proteinosis in SCID mice. *Am J Respir Cell Mol Biol* 13: 297–306.
270. Betz C, Papadopoulos T, Buchwald J, Dammrich J, Muller-Hermelink HK (1995) Surfactant protein gene expression in metastatic and micrometastatic pulmonary adenocarcinomas and other non-small cell lung carcinomas: detection by reverse transcriptase-polymerase chain reaction. *Cancer Res* 55: 4283–4286.

271. Wilcox DT, Glick PL, Karamanoukian HL, Azizkhan RG, Holm BA (1995) Pathophysiology of congenital diaphragmatic hernia. XIII: Amniotic fluid lecithin/sphingomyelin ratio and phosphatidylglycerol concentrations do not predict surfactant status in congenital diaphragmatic hernia. *J Pediatr Surg* 30: 410–412.
272. Zetterberg G, Curstedt T, Eklund A (1995) A possible alteration of surfactant in bronchoalveolar lavage fluid from healthy smokers compared to non-smokers and patients with sarcoidosis. *Sarcoidosis* 12: 46–50.
273. Karam H, Hurbai-Kosmath I, Housset B (1995) Direct toxic effect of bleomycin on alveolar type II cells. *Toxicol Lett* 76: 155–163.
274. Pandit PB, Dunn MS, Kelly EN, Pearlman M (1995) Surfactant replacement in neonates with early chronic lung disease. *Pediatrics* 95: 851–854.
275. Pandit PB, Dunn MS, Colucci EA (1995) Surfactant therapy in neonates with respiratory deterioration due to pulmonary hemorrhage. *Pediatrics* 95: 32–36.
276. Nieman GF, Paskanik AM, Fluck RR, Clark WR (1995) Comparison of exogenous surfactants in the treatment of wood smoke inhalation. *Am J Respir Crit Care Med* 152: 597–602.
277. McCormack FX, King TE Jr., Bucher BL, Nielsen L, Mason RJ (1995) Surfactant protein A predicts survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 152: 751–759.
278. Lesur O, Bouhadiba T, Melloni B, Cantin A, Whitsett JA, Begin R (1995) Alterations of surfactant lipid turnover in silicosis: evidence of a role for surfactant-associated protein A. *Int J Exper Path* 76: 287–298.
279. Wirtz H, Habscheid W, Ertl G, Schmidt M, Kochsiek K (1995) Exogenous surfactant application in respiratory failure due to chronic obstructive pulmonary disease. *Respiration* 62: 157–159.
280. Lesur O, Mancini NM, Janot C, Chabot F, Boitout A, Polu JM, Gerard H (1994) Loss of lymphocyte modulatory control by surfactant lipid extracts from acute hypersensitivity pneumonitis: comparison with sarcoidosis and idiopathic pulmonary fibrosis. *Eur Respir J* 7: 1944–1949.
281. Masters IB, Vance J, Hills BA (1994) Surfactant abnormalities in ALTE and SIDS. *Arch Dis Child* 71: 501–505.