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The Pharmacology of Lung Surfactant Secretion

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I. Introduction

THE TERM lung surfactant describes a highly surface-active complex found in pulmonary alveoli whose major function is prevention of lung collapse during expiration (38, 86, 166, 197). Although the presence of lung surfactant was postulated as early as 1929 (223), its existence

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was not convincingly demonstrated until the mid-1950s when Pattle (178) and Clements (37) found a highly surface-active component in lung lavages and extracts of whole lung tissue.

The surfactant complex is thought to stabilize alveoli by reducing surface tension at the air-liquid interface (86, 185, 197) but may have the alternative property of forming a rigid surfactant monolayer which "splints" the alveoli open at low internal alveolar diameters (166). Surfactant may have the additional functions of preventing lung edema and fluid transudation into alveoli (104, 179), aiding the removal of foreign particles from airways, and assisting digestion of bacteria (145, 151).

The major stimulus to the study of lung surfactant has been the demonstration that a deficiency of this complex has a fundamental role in the pathology of human respiratory distress syndrome, a major cause of death in neonates (12, 13, 79, 106, 178). The main features of the disease are development of atelectasis in the lungs within a few hours of birth followed either by formation of hyaline membranes and death or by recovery with the possibility of residual lung pathology (12). There is considerable evidence that glucocorticoids can enhance "maturation" and surfactant synthesis in the fetal lung (204). This action is the basis of the clinical use of glucocorticoids, first described by Liggins and Howie (142), for the prophylactic prevention of human respiratory distress syndrome (106).

The chemical nature of lung surfactant (86, 186, 187) and its biosynthetic pathways (61, 186, 222) have been well described and will be only briefly discussed. This review shows that the lung surfactant system shares many features with other secretory systems. Also, our current knowledge of the physiological controls and pharmacological agents that modulate lung surfactant secretion will be reviewed. The limited information on the relationship between secretion and synthesis also will be discussed with the hope of stimulating further experimentation.

A. Chemical Nature of Lung Surfactant

Lung surfactant is generally considered to consist primarily of a mixture of phospholipids and protein. The phospholipid composition of the complex is distinct from that of whole lung or other tissues, especially with regard to the relatively high concentrations of disaturated phosphatidylcholine (DSPC) and phosphatidylglycerol and relatively low concentrations of sphingomyelin and phosphatidylethanolamine (73; table 1; fig. 1). Other reported components include phosphatidylinositol, phosphatidylserine, cardiolipin, lyso(bis)phosphatidic acid, and lysophosphatidylcholine (73).

The surface-active properties of the individual species of phosphatidylcholines (PC) are determined by the nature of the fatty acids at the α and β positions (fig. 1). The disaturated lipid dipalmitoylphosphatidylcholine (DPPC) is by far the major component and is responsible for the majority of the surface-active properties (86). Whereas DPPC is a potent surface-active agent, di- or mono-unsaturated PCs exhibit little surface activity (83, 86) although they may be part of the surfactant complex. It should be noted that PCs are not unique to lung surfactant: unsaturated PCs are found in high concentrations in all cell membranes; disaturated PCs have been found in various quantities in other tissues (149) and in the lung are thought to exist in more than one pool (237).

There is evidence that carbohydrates, protein, and phospholipids other than DPPC are necessary to account for all properties of the total complex (14, 97, 187), perhaps by aiding in the adsorption and spread of DPPC at the alveolar air-liquid interface (14).

B. Biosynthetic Pathways for Lung Surfactant

Although some studies have been conducted on pathways involved in the production of minor surfactant components, most research has been aimed at synthesis of PC (222). By far the most important biosynthetic route for PC in the lung is the cytidine diphosphate choline pathway as outlined in figure 2. This pathway is considered to synthesize primarily unsaturated PC (8, 222). It is thought that DSPC is formed in the lung by remodelling of unsaturated PC, either by a deacylation-reacylation or a transacylation mechanism. There is, however, recent evidence that DSPC may be formed by de novo synthesis via the cytidine diphosphate choline pathway (183).

II. Lung Surfactant System as an Example of an Exocytotic Secretory Process

Secretion will be defined here as the energy-consuming transfer of surfactant molecules from an intracellular to an extracellular location. A criterion which must be satisfied as evidence that one is studying a secretory process is the measurement of an increase in concentration of the presumed secreted substance in an appropriate extracellular space within a short time interval (e.g., a few minutes) after application of a secretagogue (5). Enhanced secretion of surfactant has been investigated by using a variety of pharmacological and physiological stimuli and is discussed in detail in section IV. There are many examples of substances synthesised in specialised cells and stored in discrete intracellular organelles which are then secreted into the extracellular space by the process known as exocytosis. Well-investigated examples include adrenaline secretion from the adrenal chromaffin cells (135), insulin secretion from B-cells of the pancreas (10, 135), enzyme secretion from pancreatic acinar cells (33), and histamine secretion from mast cells (63). As well as the diversity of cells involved and of substances they secrete, there is a wide range of secretagogues including hormones, neurotransmitters, bacterial toxins,

TABLE 1
Lipid profiles of lung fractions

Lipid	Lavaged lungs*		Lamellar bodies†§	Lung slices‡ Secreted material§	Type 2 pneumocyte culture*	
	Lung tissue§	Lung lavage§			Cells§	Secreted material¶
Phosphatidylcholine	50.5	80.9	80.9	71.8	76.7	80.1
Sphingomyelin	10.9	1.4	3.6	4.0	3.9	3.5
Phosphatidylinositol	4.6	3.2	0.9	9.9	2.9	1.7
Phosphatidylserine	7.3	0.5	2.8		1.3	0.7
Phosphatidylethanolamine	21.2	3.2	3.5	7.2	4.4	2.8
Lysophosphatidylcholine	0.6	1.0	2.8	0.8	1.7	1.0
Phosphatidylglycerol	1.7	7.1	5.9	6.5	4.6	6.5
Lyso(bis)phosphatidic acid	1.0	1.1	ND		1.0	1.5
Cardiolipin	0.8	0.2	ND		1.0	0.9
Percent of phosphatidylcholine that is disaturated	36.6	61.6	ND	51.7	56.3	65.6

* From Gilfillan et al. (73); used with permission.
† From Spalding et al. (207); used with permission.
‡ From Marino and Rooney (147); used with permission. The data in items *, †, ‡ are based on percentage of total phospholipid.
§ Data based on phospholipid phosphorus.
¶ Data based on phospholipids labelled with sodium [^{14}C]acetate.
‖ ND, not determined.

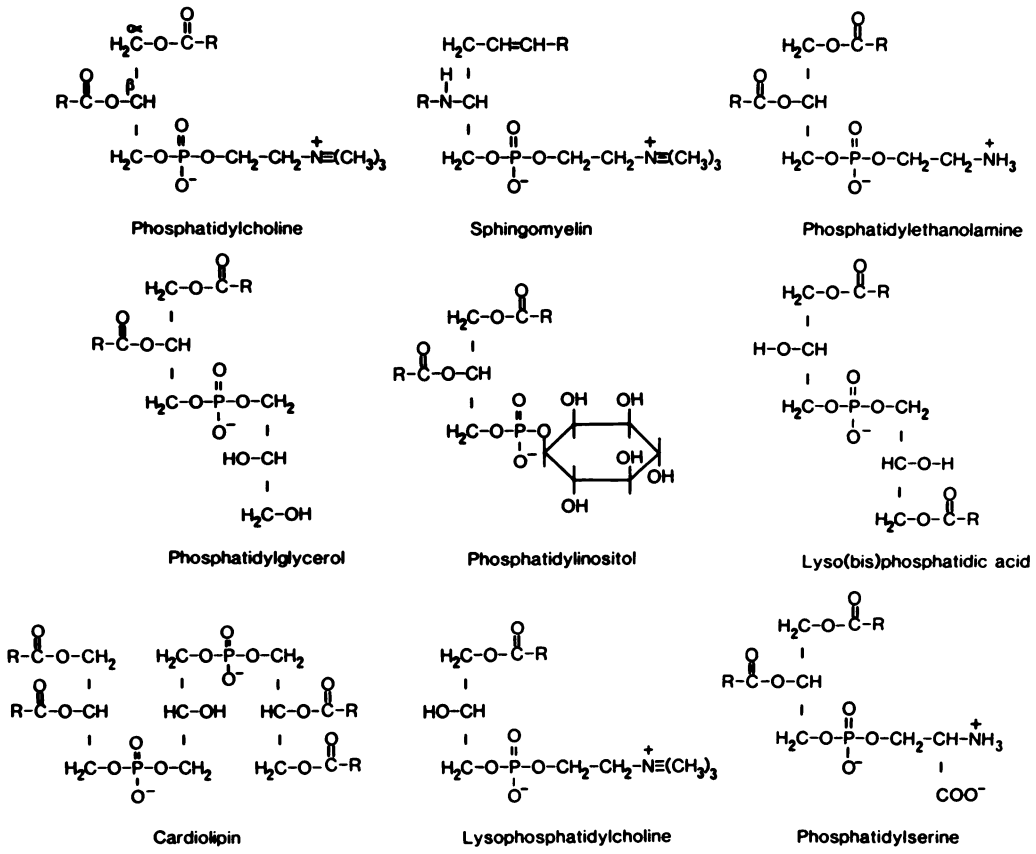

FIG. 1. Structures of phospholipids. R = fatty acid.

and antigens. There is much in common within this diversity with regard to the processes of synthesis, storage, and secretion (33, 107, 135, 221). It is our thesis that the lung surfactant system shares many characteristics with the above, better-understood secretory systems. This contention is supported by ultrastructural, biochemical, autoradiographic, radiolabelling, pharmacological, and physiological studies.

A. Ultrastructural and Biochemical Studies

Macklin (145) observed putative secretory organelles within type 2 pneumocytes and suggested these cells had an exocrine function.

It is now well accepted that the type 2 pneumocyte is the site of surfactant synthesis, storage, and secretion (163). These cells account for about 14% of the total cell mass in the adult rat lung but only occupy 2.5% of the

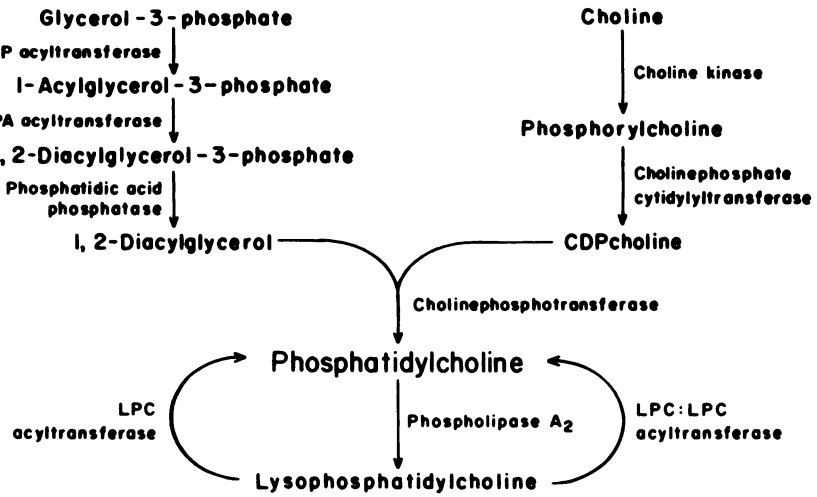

FIG. 2. Cytidine diphosphate choline pathway responsible for the synthesis of phosphatidylcholine and for the remodelling of the fatty acid constituents via lysophosphatidylcholine. Abbreviations: GP, glycerol-3-phosphate; LPA, lysophosphatidic acid; CDP, cytidine diphosphate; LPC, lysophosphatidylcholine. (After Rooney and Wai-Lee (192) and Lung; used with permission of Springer-Verlag, Heidelberg.)

alveolar epithelial surface. The remainder of the alveolar epithelium comprizes type I pneumocytes (94). Type 2 pneumocytes, large cells with a diameter between 7 and 14 μm, project into alveolar spaces or lie in niches in alveolar walls surrounded by type I pneumocytes. The former type of cells are rich in Golgi elements, endoplasmic reticulum, and mitochondria; their nuclei are large and they possess microvilli on their free surfaces.

A feature of all exocytotic secretory systems is the presence of discrete intracellular organelles which are the storage form of the secreted substance. The most characteristic feature of a type 2 pneumocyte is the presence of organelles known as osmiophilic lamellar bodies (OLB, fig. 3). These organelles have been described in lungs from a wide range of species (7, 20, 52, 88, 130). Many observational and experimental studies support the concept that OLB are the storage form of lung surfactant. Buckingham and Avery (29) speculated that there was a parallelism in the morphological appearance of OLB in the fetal mouse lung during late pregnancy and the appearance of lung surfactant. This observation has been confirmed in the fetal lamb (28, 174) and fetal rabbit (84, 124). Supportive evidence comes from the observation that there was a parallel increase during gestation in the DSPC concentration in lungs of fetal rats and the number of OLB (230). Treatment of fetal rabbits with glucocorticoids also advanced lung maturity and increased surface activity and the numbers of type 2 pneumocytes and OLB in lungs (167, 225). Conversely, early studies showed that treatment of guinea pigs with carbon dioxide or rats with pilocarpine led to a loss of both surfactant and OLB from lung tissue (20, 88). From a series of ultrastructural and freeze-fracture studies, Stratton (210–213) suggested that the lamellae of OLB are probably 6.6 nm (66 Å) apart, which is compatible with the lamellae being composed of a series of lipid bilayers. There have been several observations of intact OLB or lamellar material passing from type 2 pneumocytes into alveoli (7, 20, 72, 98, 124, 133, 194) thereby providing the clearest evidence that OLB secretion is by exocytosis (fig. 4).

Several biochemical studies have shown that OLB and type 2 pneumocytes have a high content of lipid, specifically PC or DSPC and phosphatidylglycerol, with a low content of sphingomyelin compared to whole lung tissue (11, 70, 73, 123, 125, 154, 190, 191; table 1). This phospholipid profile closely parallels that of lung surfactant secretions thereby strongly supporting the OLB as the intracellular store of surfactant. It is likely that OLB are solely a storage form of surfactant as they lack the key enzymes necessary for DPPC biosynthesis (68, 191), although they may possess enzymes involved in phospholipid remodelling (54).

B. Autoradiographic and Radiolabelling Studies

Radiolabelled precursors and electron microscopy have been used to demonstrate that the peak appearance of grains over different organelles in the type 2 pneumocyte is in the order: rough endoplasmic reticulum, Golgi body, lamellar body, and then within a few hours, appearance in the alveolar space (11, 36, 157). This order is similar to the sequence of events first reported in detail in the pancreatic acinar cell (109).

Numerous in vivo studies (111, 112, 114, 116, 165, 217, 236, 237) have shown that the time of peak appearance of radiolabel in phospholipids, PC, or DSPC in homogenates of lung tissue precedes the appearance of label in lung lavages. Jobe et al. (100, 115, 116), Young et al. (236), and Baritussio et al. (15) have shown the sequential appearance of radiolabel in microsomal and OLB subcellular fractions and lung lavage after injection of [^{14}C]palmitate, [^{3}H]choline, or [^{32}P]phosphate. These patterns are very consistent with the autoradiographic

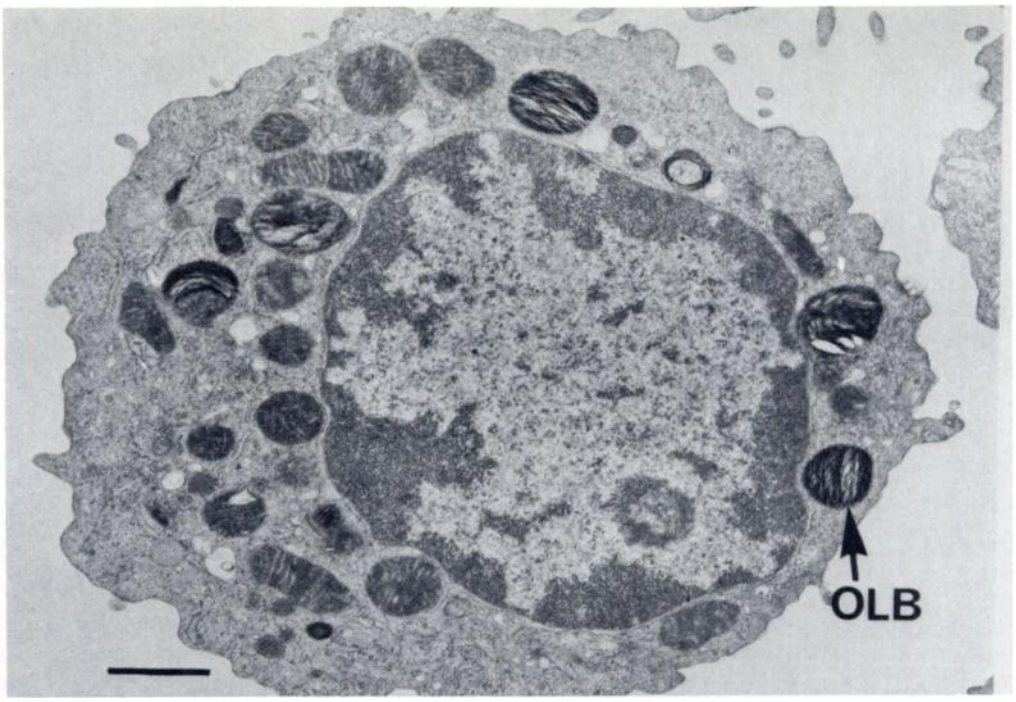

FIG. 3. An electron micrograph of a type 2 pneumocyte isolated from adult rat lung. Note especially the osmiophilic lamellar bodies (OLB). Bar = 1 μm, magnification × 11,500. (After Mason and Williams (151) and American Review of Respiratory Diseases; used with permission.)

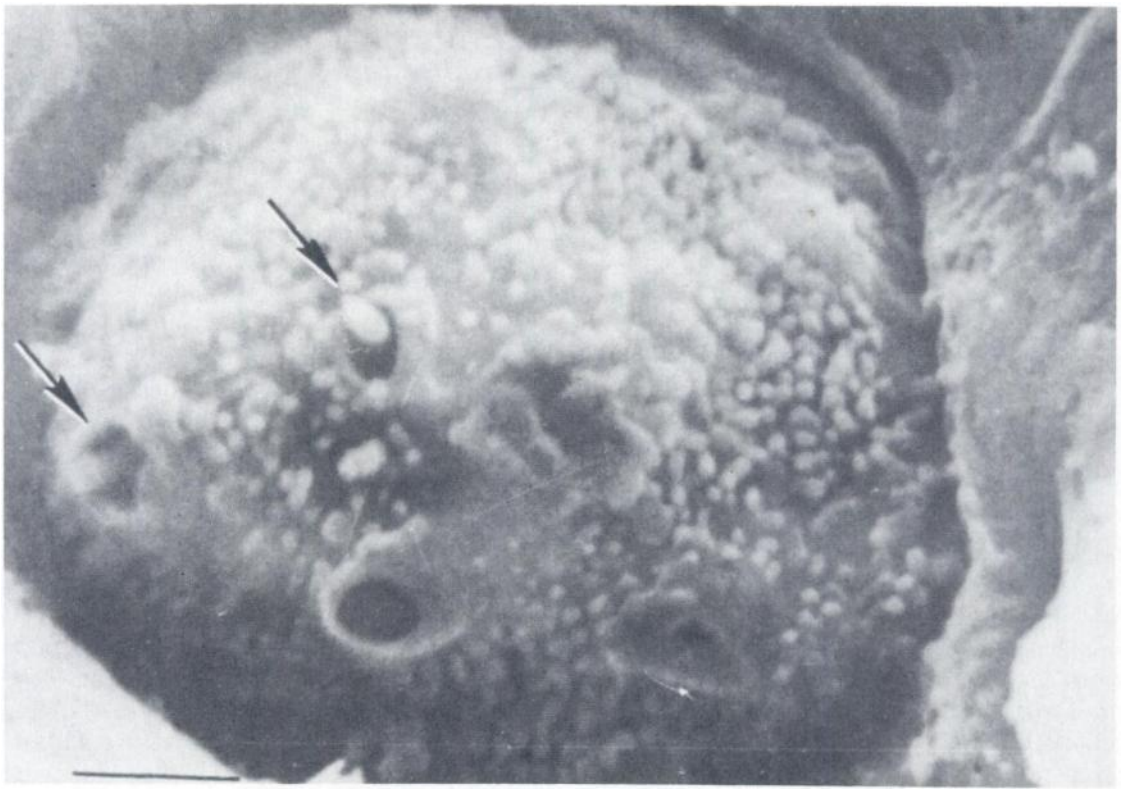

FIG. 4. Scanning electron micrograph of a type 2 pneumocyte in hamster lung. Two lamellar bodies can be apparently seen in the process of exocytosis (arrows). An empty secretary vacuole surrounded by a pouting rim of cytoplasm is visible near the bottom of the micrograph. Bar = 1 μm, magnification × 24,000. (After Kuhn (133) and Academic Press, Inc. (London) Ltd.; used with permission.)

studies and suggest a pathway of sequential biosynthesis, storage, and secretion. The radiolabelling studies do not, of course, allow specification of the responsible cell type.

C. Postsecretory Events

Lamellar bodies, after secretion into alveoli, reorganize to form a layer at the alveolar air-liquid interface, thought to be the active form of surfactant. Ultrastructural observations of alveolar spaces have shown lamellar bodies unravelling to produce a lattice-work of tubular myelin. This myelin is probably the intermediate in the formation of the functional layer at the air-water interface (194, 196, 230).

The processes of elimination of lung surfactant have been relatively little investigated. It has been suggested that surfactant is only active in a "dry" form and when "wetted" becomes functionally ineffective (166). Three main routes of surfactant elimination have been suggested: surfactant can pass up the trachea into the pharynx and then swallowed (13) but this pathway is no longer thought to be a major route. Secondly, metabolism of surfactant may occur either in alveolar fluid or by alveolar macrophages (222). Phospholipases have been found in alveolar macrophages (64, 65) and alveolar lavage (198). According to Goerke (86) these phospholipases could play an important role in the degradation of surfactant phospholipids. Thirdly, surfactant may be reabsorbed through the alveolar epithelium (78, 96, 108). Current studies would suggest reabsorption is by far the most important route for surfactant elimination. It has been estimated (108) that as much as 90% to 95% of DSPC is reutilized in an intact, rather than a metabolized, form.

It is, therefore, important to recognize that surfactant within alveoli may be newly secreted but nonfunctional, functionally active at the interphase, and in a postfunctional form.

D. Conclusion

In summary, there is convincing evidence for the type 2 pneumocyte as the major site of lung surfactant biosynthesis and storage. Type 2 pneumocytes appear to possess many features seen in other exocrine cells.

III. Methods for the Study of Lung Surfactant Secretion

By analogy with other secretory tissues, it can be reasoned that there may be physiological and pharmacological modulators of the rate of secretion which are distinct from those factors modulating synthesis. Methods, therefore, have had to be developed to study secretion separate from synthesis with the aim of identifying the major regulators and determining when the physiological controls are most important.

Since secretion is the transfer of surfactant molecules from an intracellular to an extracellular location, it is necessary to be able to sample these sites (i.e., the type 2 pneumocyte and the alveolus, respectively) discretely. The concept of separate pools of alveolar and lung tissue surfactant arose from the observation that the phospholipid composition of lung lavages differed from that of lung tissue (66, 84).

The study of lung surfactant secretion has been complicated by two main problems. Firstly, the type 2 pneumocyte is only one of some 40 cell types within the lungs and comprises only about 14% of lung volume (94). Secondly, and perhaps most important, there is no single and unique chemical marker for lung surfactant. As a consequence of these complications, a wide variety of in vivo and in vitro methods have been developed. Each method has its own advantages and disadvantages and each relies on several assumptions for interpretation. Method development is, therefore, currently an area of active investigation.

A. In Vivo and ex Vivo Methods

1. Lung Lavage Technique. Until recently, the lung lavage technique has been the most widely used technique for studying surfactant secretion. This method involves injecting physiological salt solution via the trachea into the intact lung. The solution is removed and the lavage repeated several times. Pooled material is then analysed for surfactant content by surface tension measurement or biochemical analysis (see section III C).

The assumption underlying the interpretation of data obtained this way is that the lavage fluid contains the total functional surfactant derived from the alveolar lining; therefore, factors stimulating surfactant secretion will increase the amount of surfactant recoverable by lavage. It is clear that lavage fluid reaches the alveoli (200) and is, therefore, capable of removing material lining alveolar surfaces. The lavage lipid appears not to be derived from the major airways (237). The lavaging technique does not seem, on the basis of ultrastructural studies (200), to damage cells and lactate dehydrogenase activity, a cytoplasmic enzyme, in lavages is low (170, 176). The lavage lipid composition is distinct from lung tissue but closely parallels that of lamellar bodies (table 1) and so is likely to represent a pool derived from secreted OLB rather than cell membranes.

As indicated above, alveoli may contain other than functional surfactant. It is a common practice to centrifuge lung lavages to remove cells and cell debris, especially macrophages which may have engulfed intact OLB (238). This procedure, however, has the disadvantage of removing some of the newly secreted intact OLB which may sediment at low centrifugal forces (175).

Henderson and Hackett (100) suggested that lavaging of lungs induces surfactant release, a finding compatible with evidence that lung inflation results in surfactant secretion (section IV E). The demonstration that there is an approximately exponential decrease of phospholipid content in successive lavages (2, 71, 103) may indicate that this stimulated release is minimal. Release induced

by air inflation can be reduced by cooling to 4°C (103), so it would seem wise to use cold solutions for lavaging.

The lavage content of surfactant at any time must reflect the balance between secretion into alveoli and elimination from that site. Probably the major drawback of the lung lavage technique is the uncertainty that changes in surfactant contents of lavages are primarily due to changes in secretion rather than secondary to alterations in synthesis or elimination. The case has been well argued that the former reason is generally true (176) and most likely to be so when there is a short time interval (minutes rather than hours) between stimulus application and measurement of effect (5).

Despite the many assumptions discussed above, the lavage technique has been widely used in the past primarily because of its simplicity. Even if the lavage contains other than the "true" alveolar pool of surfactant, the technique is able to detect changes in the lavage pool. Most secretagogues have been identified by using this method and certain experiments can only be performed in vivo.

2. Static Lung Compliance and Lung Stability. These parameters are examined by slow inflation of excised lung at known pressures with air and simultaneous measurement of the displaced volume. These pressure-volume studies assess distensibility, the maximum volume of air the lung can contain without rupture, and the deflation stability, the air retained on decreasing pressure (131). These parameters are modified by the amount of surfactant able to be recruited to the air-liquid interface (131, 223) and are therefore a measure of the functional alveolar pool of surfactant.

The pressure-volume technique was used by Avery and Mead (13) to identify surfactant deficiency as a pivotal factor in respiratory distress syndrome of human neonates. The method has its drawbacks, however, as it is insensitive to small changes in alveolar surfactant and the parameters are altered by many factors other than surfactant. The method has proved most valuable in studies of enhancement of alveolar surfactant in fetal animals by drugs where large changes in alveolar surfactant were produced from low unstimulated values (e.g., 234).

3. Ultrastructural Studies. Secretion of lung surfactant can be monitored by ultrastructural observation of OLB disappearance from type 2 pneumocytes and their increased appearance in alveoli. Early qualitative studies were able to detect marked secretions occurring after pilocarpine treatment (88) and at birth (124). More recently, ultrastructural observations have been made quantitative by counting either the number of OLB per cell (55, 60, 172) or the area occupied by these bodies per area of type 2 pneumocytes, the "volume density" (156, 172). As the number of OLB per cell can vary considerably even in controls (236), the former method is fairly insensitive to induced changes. Although the latter method would appear to be more sensitive (156, 201), it is an extremely lengthy procedure and can only realistically be used as an adjunct to other methods.

B. In Vitro Methods

To circumvent some of the problems of technique and interpretation associated with in vivo methods, a variety of in vitro techniques have been developed. In common with other areas of pharmacology, in vitro techniques are often more suitable for defining certain aspects of the sites and mechanisms of action of drugs.

1. Isolated Perfused Lung. Several isolated lung preparations have been devised for studies of lung surfactant secretion (1–5, 26, 144, 156, 170). All involve perfusion of the isolated lung via the pulmonary artery with a physiological salt solution. Lungs are lavaged before and after addition of drugs to the perfusion medium. Biochemical criteria for viability have been described (144) but, perhaps, for pharmacological experiments, consistency in drug responses may be as appropriate. Surfactant has been assayed biochemically; the sensitivity has been increased by labelling the lipids with radioactive precursors (section III C 2). It must be remembered that drugs could still be acting on cells other than type 2 pneumocytes to induce surfactant secretion indirectly in the isolated lung. Also, extensive pharmacological studies may be time consuming with the perfusion technique.

2. Lung Slice Technique. Lung slices have been used extensively to examine biosynthetic pathways for the major lipids of surfactant (e.g., 56). Recently, this technique has been modified to study lung surfactant secretion (44, 45, 146, 147). Animals are pretreated with [^{3}H] choline to label lipid stores and lung slices prepared. The amount of a suitable labelled phospholipid released into the medium is used as an index of surfactant secretion and this can be increased by drug addition to the medium. Marino and Rooney (146) demonstrated that the phospholipid profile in the medium more closely resembled that of lung washes than lung tissue an indication that the material assayed was surfactant-related.

A great advantage of the slice technique is simplicity of preparation. Also, several slices can be prepared from the same lung, thereby enabling the design of experiments with concurrent controls. With pharmacological criteria for viability, preparations will usually respond to drugs for several hours. The technical considerations for using lung slices for studies on metabolism have been discussed by O'Neil and Young (173).

A variation of the lung slice technique described by Gilfillan and coworkers (72, 76, 77) uses a perifusion technique similar to that used for studying other secretory systems such as insulin secretion (136). Lung slices are incubated in [*methyl*-^{3}H]choline chloride for 3 hr. Slices are then transferred to new chambers, perifused, and the perifusate collected in 5- or 10-min fractions. Each fraction and the tissue at the end of perifusion are assayed for [^{3}H]DSPC and the overflow of [^{3}H]DSPC

expressed as a rate coefficient. Drugs can be added to the perifusing medium for short (or long) periods. One advantage of this method is that the time-course of drug effect can be readily monitored.

3. Isolated Type 2 Pneumocytes. Probably the most important innovation in the study of lung surfactant secretion has been the development of culture techniques for isolated type 2 pneumocytes. These cells can be readily isolated by treating with either elastase (48, 150) or trypsin (27, 125) and purified by density gradient and preferential attachment. It is possible to produce preparations that are greater than 95% type 2 pneumocytes. The lipids are labeled by incubation in radiolabelled acetate (49), choline (48, 61), glycerol (49), or palmitate (27). Surfactant secretion is measured by the release of radiolabelled phospholipid, PC or DSPC.

The distinct advantage of this system is the greater certainty that any site of drug action is the type 2 pneumocyte. The major limitation is that injury to the cells during preparation may render their characteristics very different from those in vivo, for example the loss of receptors (48). The cells cannot be used after more than a few days of culture as they lose their lamellar bodies and start to dedifferentiate.

4. Other Methods. Other isolated tissue techniques, such as lung explants (184), have only limited use to date in surfactant secretion studies. Readers are referred to a review by Douglas and Smith (50) for discussion of other methods under development.

C. Monitoring Lung Surfactant Secretion

1. Surface Tension Measurements. Lung surfactant is defined by its ability to lower surface tension. Therefore, ideally the assay of lung surfactant should use this property. Goerke (86) discussed the many practical problems encountered with the most widely used surface tension assay method: the Langmuir trough with a Wilhelmy plate. This method has often been used qualitatively to determine the presence or absence of significant amounts of surfactant in lavages but can be used quantitatively (5, 86, 140). Surface tension assays are rather insensitive and consequently are more applicable to amniotic fluid, lung lavages, or lung homogenates and have been less used with medium from in vitro techniques. Prior to assay, it is usually necessary to extract surfactant from these lavages or homogenates. This extraction will inevitably alter the surfactant complex and so complicate interpretation. The practical problems, the lack of sensitivity, and the lengthy nature of the assay procedure compared to biochemical methods have limited the application of surface tension assays.

2. Biochemical Measurements. In most studies, secretion of lung surfactant has been monitored with biochemical criteria. The lack of a unique marker for the surfactant complex has meant that a variety of substances has been used as criteria for assessing changes in surfactant secretion. These substances have included total phospholipids, total PC or DSPC. As DPPC is the most important component of lung surfactant (see section I A) and over 80% of the saturated fatty acids present in lung PC are palmitate (195), then measurement of DSPC is a reasonable measure of surfactant. In animal models, data are usually normalized per milligram of protein or DNA or per gram of dry lung weight. For human lavage and amniotic fluid, data are usually presented as relative percentages of individual phospholipids. There is a good correlation between total PC (5) or DSPC (139) concentration and the surface tension lowering properties of lavages.

Several groups have determined the lipid profile in medium bathing isolated tissues or in lavages to determine whether their release is a consequence of stimulation of exocytosis rather than cell damage. If surfactant secretion is being specifically monitored, the lipid profile should resemble that of the lamellar body rather than total lung tissue, as reflected in relatively high proportions of DSPC and phosphatidylglycerol and low proportions of phosphatidylethanolamine and sphingomyelin (table 1).

In several secretory systems it has been shown that turnover of phospholipid, especially polyphosphoinosides, is increased by calcium-mobilizing secretagogues. This increased turnover may be related to the stimulatory response (63). It is important to differentiate between this enhanced turnover and secretion of phospholipids specifically related to surfactant.

Conventional biochemical assays are not sufficiently sensitive to detect the small amounts of DSPC secreted into medium from lung slices or type 2 pneumocyte cultures. Sensitivity is increased by labelling the DSPC with a precursor such as [^{14}C]- or [^{3}H]palmitate or choline (47, 72, 146, 147). Radiolabelled precursors have also been used in combination with in vivo studies and lung lavages (129, 156).

D. Conclusions on Methodology

As with other secretory processes, no single method is likely to prove suitable for all pharmacological and physiological studies of the surfactant system. In vivo methods will probably continue to be valuable for investigating certain aspects of factors regulating secretion. The combination of in vivo, isolated lung, lung slice, and type 2 pneumocyte culture techniques will prove necessary to identify whether drug effects in vivo result from a direct or indirect effect on the type 2 pneumocyte. An important feature of most of the in vitro methods is that pharmacological effects on secretion can be studied separately from their effects on synthesis. It would seem appropriate to minimize the time interval between application of a stimulus and determination of effect to be more certain one is measuring secretion. In other tissues secretory events can be readily observed within seconds or a few minutes. The actual event of exocytosis of a single OLB may be extremely rapid. Despite the diversity of methods

and assay end-points used for the study of lung surfactant secretion, there has been a considerable consistency in the conclusions drawn as to the important secretatogues.

IV. Modulation of Lung Surfactant Secretion Rate

In this section the evidence for the action of secretatogues at specific receptors will be discussed. Physiological influences and mechanisms occurring after drug-receptor combination will also be described.

A. Autonomic Nervous System

1. Agonists and Antagonists at β-Adrenoceptors. A. IN VIVO STUDIES. There is now substantial evidence that agonists at β-adrenoceptors are able to induce the secretion of lung surfactant. This was first suggested by the work of Wang et al. (224) who provided ultrastructural evidence of the loss of OLB from type 2 pneumocytes after injection of adrenaline into mice. The first definitive experiments were those by Olsen (172) who found that the phospholipid content of lung lavages in rats was increased 30 min after the injection of isoproterenol. He also reported that the number of OLB per type 2 pneumocyte and the volume density of OLB were decreased. These effects were antagonised by propranolol.

It can be seen (table 2) that results compatible with enhanced surfactant secretion after β-adrenoceptor agonists have been observed in several species and in fetal, neonatal, and adult animals. Where investigated, the effects of the β-adrenoceptor agonist were antagonised by propranolol. Wyszogrodski et al. (234) and Lawson et al. (140) found that the secretory response to a constant dose of agonist increased with gestational age in fetuses. Whether this change is a consequence of enhanced sensitivity to the agonists or an increase in the maximal possible response has still to be elucidated. A few in vivo studies (4, 5, 156, 172) have recorded changes by 30 min after treatment. This is in line with in vitro observations (see below) and would be expected from the rapid effects produced by β-adrenoceptor agonists in other systems. The doses of β-adrenoceptor agonists used in these studies would be expected to produce cardiovascular effects such as tachycardia and hypotension (e.g., 177). The peripheral vasodilator hydralazine given to pregnant does in a dose that produced the same fall in maternal blood pressure as salbutamol had no effects on the indices of fetal lung surfactant secretion (Nicholas, personal communication). Apart from this study, which suggests that the secretory effects were not secondary to cardiovascular changes, there have been no in vivo experiments to investigate the site of action of the β-adrenoceptor agonists; it has been assumed to be the type 2 pneumocyte.

If there is tonic activation of the system via β-adrenoceptors, then administration of an antagonist at β-adrenoceptors should reduce the rate of surfactant secretion. Propranolol slightly decreased the phospholipid content of lavages of anaesthetized rabbits (177). However, anaesthesia is known to increase catecholamine secretion from the adrenal medulla so surfactant secretion might not have been in an unstimulated state. In other studies, with nonanaesthetized fetal rabbits (43) and 1- to 3-day-old rabbits (5), no changes were seen after propranolol. Tonic regulation of surfactant secretion may be minimal but requires confirmation with studies using radiolabelled precursors. As the turnover of the alveolar pool of surfactant is of the order of 17 h in the adult rabbit (110), a long interval between drug injection and assay would be necessary to detect inhibition of tonic secretion.

B. IN VITRO STUDIES. Dobbs and Mason (46) were the first to substantiate these in vivo findings of β-adrenoceptor agonists increasing surfactant secretion with in vitro observations. They found that terbutaline increased the release of [^{14}C]DSPC from cultures of type 2 pneumocytes of adult rats, an effect which was antagonised by propranolol. They later extended their study (48) to show that isoproterenol and terbutaline were potent secretagogues (EC50 = 4 nM and 800 nM, respectively), phenylephrine was less potent (EC50 = 13 μM), and isoproterenol exhibited stereoselectivity ([−]isoproterenol 200 times as potent as [+]-isoproterenol). The effects of terbutaline and phenylephrine were antagonised by propranolol but not by phentolamine. These results have been supported by several other groups with a variety of agonists at β-adrenoceptors (table 2). Where examined, responses to agonists were reduced by antagonists at β-adrenoceptors (26, 77, 161).

Lands et al. (138) have subdivided β-adrenoceptors into β_1- and β_2-subtypes based on the order of potencies of agonists. The moderately high potencies of terbutaline (48) and salbutamol (72, 77) in vitro and the lack of effect of prenalterol in vivo (21) suggest that the β-adrenoceptor involved here is of the β_2-subtype, but this classification requires more rigorous pharmacological examination.

In vitro methods are usually more suitable for detailed pharmacological studies than in vivo techniques, but have been applied only recently. For example, the secretory response can be observed within 10 min of application of a β-adrenoceptor agonist (26, 72, 77). Dobbs and Mason (48) suggested that the increased release of [^{14}C] DSPC from isolated type 2 pneumocytes by terbutaline occurred over several hours but, by examination of figure 1 from their paper, it can be seen that the increase was largely complete by 1 h. Unstimulated release of radiolabelled DSPC or PC from lung slice preparations and type 2 pneumocyte cultures has varied between 0.2% and 3.5% of tissue content per hour (26, 45, 48, 72, 76, 77, 120, 147). The maximum rate of secretion during stimulation by β-adrenoceptor agonists would appear to be only some 2- to 4-fold greater than during unstimulated

TABLE 2

Enhancement of surfactant secretion by agonists at β-adrenoceptors

Agonist	Animals/Preparation	Effect	Reference
A. In vivo			
Epinephrine	Adult mice	↓ OLB* in type 2 pneumocytes	224
	Fetal sheep	↓ Surface tension of pulmonary fluid	140
	Neonatal rabbits	↑ Lavage PC content; ↓ surface tension of lavages	4, 5
Isoproterenol	Adult rats	↓ OLB in type 2 pneumocytes; ↑ lavage PL content	172
	Adult rats	↑ Lavage [^{14}C]DSPC content	156
Isoxsuprine	Fetal rabbits	↑ Lung distensibility	43
	Fetal rabbits	↓ Surface tension of pulmonary fluid; ↑ PC:sphingomyelin ratio of pulmonary fluid; ↓ OLB in type 2 pneumocytes	55
	Fetal rabbits	↑ Lung stability	234
	Fetal rabbits	↑ PC:sphingomyelin ratio of lavages	99
	Fetal rabbits	↑ Lung stability; ↑ PC:sphingomyelin ratio of lavages	119
Terbutaline	Fetal rabbits	↑ Lung distensibility; ↑ lavage PC content	21
	Fetal rabbits	↑ Lung distensibility; ↑ lavage PL content	53
	Adult rabbits	↑ Lavage PL content	177
Salbutamol	Fetal rabbits	↑ Lung distensibility; ↑ lavage PC content	171
Hexoprenaline	Fetal rabbits	↑ Lung stability; ↑ PC:sphingomyelin ratio of lavage	143
B. In vitro			
Isoproterenol	Adult rat type 2 pneumocyte culture	↑ [^{14}C]DSPC content of medium	26, 48
	Adult rat perfused lung	↑ [^{14}]DSPC content of lavage	26
	Cell line A-549	↑ [^{3}H]DSPC content of medium	152, 169
Terbutaline	Adult rat type 2 pneumocyte culture	↑ [^{14}C]DSPC, [^{3}H]PC content of medium	46, 48, 161
	Newborn rabbit lung slice	↑ [^{3}H]PC content of medium	147
Epinephrine	Neonatal rabbit perfused lung	↑ PC content of lavage	4, 5
Salbutamol	Adult rat perfused lung	↑ PL content of lavage	170
	Adult rat perifused lung slice	↑ [^{3}H]DSPC content of perifusate	72, 77

* The abbreviations used are: OLB, osmiophilic lamellar bodies; PC, phosphatidylcholine; PL, phospholipid; DSPC, disaturated phosphatidylcholine.

release, much less than the increment seen with other secretory systems such as the pancreatic acinar cell (33). This small difference may in part be a consequence of unstimulated secretion being continuously enhanced by endogenously released substances. For example, indomethacin reduced unstimulated release of [^{3}H]PC from lung slices of neonatal rabbits (146) and it has been shown that cultures of type 2 pneumocytes can produce a variety of prostanoids (216). However, indomethacin, at the concentration used (100 μM), has actions in addition to inhibition of prostaglandin synthetase (25). Young and Tierney (237) suggested that there is more than one tissue pool of DSPC and more recently Young et al. (236) have calculated that only 20% of tissue DSPC in rat lungs is found in OLB. Therefore, it is quite likely that there are "releasable" and "nonreleasable" pools of DSPC as there are with other secretory tissues. The relative sizes of these pools varies widely between other secretory tissues, for example pancreatic islet β-cells (10) and anterior pituitary cells (107), and the "releasable" pool may be small in the type 2 pneumocyte.

As discussed in section III C, measurement of radiolabelled DSPC release is probably the best current index of lung surfactant secretion. If this contention is accepted, there is then good evidence for β-adrenoceptor modulation of secretion rate of lung surfactant. Keller and Ladda (120) have argued that, since release of [^{3}H]PC can occur from tissues other than lungs (e.g., liver), there is no specific marker for lung surfactant. However, they only studied unstimulated release and not drug-induced release which would increase specificity. There is limited information on whether other components of the surfactant system are co-secreted with DSPC after addition of β-adrenoceptor agonists. Terbutaline enhanced release from type 2 pneumocytes of several labelled phospholipids, the relative proportions of which did not differ from those seen with unstimulated release (49). Massaro, Weiss, and Simon (158) found that release of labelled protein in a surface-active fraction from lung slices was increased by adrenaline. It is uncertain whether these proteins are related to the lung surfactant system.

There is insufficient quantitative information to deduce whether all of the surfactant secretion measured in vivo after β-adrenoceptor agonists is due to an action on type 2 pneumocytes or whether part of the action involves another cell type.

C. RADIOACTIVE LIGAND BINDING STUDIES. Another recent approach to the investigation of β-adrenoceptors and the lung surfactant system has been the study of the

binding of radioactive ligands. Cheng et al. (34) showed rapid, saturable, high affinity and stereoselective binding of (−)-[^{3}H]dihydroalprenolol to membranes from lungs of fetal rabbits, which was competitively antagonised by propranolol. Other studies (69, 227) with this ligand and (−)-[^{3}H]iodohydroxybenzylpindolol have confirmed these findings and found the number of binding sites increased with gestational age and in adults compared to fetal rabbits. These latter findings parallel the increase in responses to β-adrenoceptor agonists with gestational age (section IV A 1 a). Treatment with betamethasone in vivo (34) or with dexamethasone in vitro (69, 145a) increased the number of β-adrenoceptor ligand binding sites.

Radioactive ligand binding studies have the advantage of being able to measure readily the consequences of experimental changes. However, the binding sites may not reflect the biologically active receptors. This comment is particularly true here as type 2 pneumocytes are only a small proportion of total lung cell number. For example, it is well recognized that there are β-adrenoceptors mediating dilatation of the airways. Recently it has been shown that there are binding sites with characteristics of β-adrenoceptors on isolated type 2 pneumocytes (206).

2. Sympathetic Efferents and Agonists at α-Adrenoceptors. Beckman et al. (19) described decreases in pulmonary static compliance and surfactant content of lavages in monkeys after head injury, effects which could be antagonised by phenoxybenzamine. They (19, 22, 51) reported that, following stellate ganglion stimulation of anaesthetized monkeys and cats, there was decreased static compliance and this effect could be prevented by phentolamine, phenoxybenzamine, or reserpine. Wyszogrodski and Taeusch (233) were unable to repeat these latter observations.

Phenylephrine, a relatively selective agonist for α-adrenoceptors, has been shown in vitro at high concentrations to stimulate [^{14}C]DSPC release from cultures of type 2 pneumocytes via β-adrenoceptors (26, 48); no action via α-adrenoceptors has been reported. It is therefore uncertain whether neuronally released noradrenaline or α-adrenoceptors have any effects on lung surfactant secretion. As far as we are aware, no one has attempted to investigate in vitro inhibition of stimulated surfactant secretion by agonists at α-adrenoceptors. The recently developed in vitro methods would be suitable for investigating this possible action further.

3. Parasympathetic Efferents and Agonists at Muscarinic Receptors. A. IN VIVO STUDIES. Early studies indicated that vagotomy affected the surfactant system of the lung. Miller et al. (164) had shown that vagotomy in rabbits was followed by the appearance of hyaline membranes in alveoli. Later workers found that vagotomy in rats and guinea pigs resulted in increases in surface tension of lung extracts and a loss of OLB from lung tissue (23, 87, 130, 134, 218). The simplest explanation of these observations would be that the vagus has a tonic inhibitory influence on surfactant secretion which is removed by vagotomy. This is in conflict with the lack of effect of muscarinic antagonists alone on the lavage pool of surfactant and the secretory actions of stimulation of the vagus (176) and of muscarinic agonists (see below). It is possible that changes in lung surfactant induced by vagotomy were due to the lung edema which also occurred.

There are many studies demonstrating that muscarinic agonists can increase the secretion of lung surfactant in vivo but there is some debate as to whether their action is direct on type 2 pneumocytes or indirect via another cell type. The initial study by Goldenberg et al. (88) demonstrated an increase in the number of OLB in alveoli in adult rats after injection of the muscarinic agonist, pilocarpine. Pilocarpine injection into adult rats has also been shown to decrease the number of OLB per type 2 pneumocyte and the volume density of OLB (172), to increase the phospholipid and [^{14}C]DSPC content of lung lavages (156, 172), and to produce an earlier appearance of [^{14}C]phospholipid in lung lavages (165). Lung stability and phospholipid content of lavages was increased in fetal rabbits after fetal injection of pilocarpine (39, 42, 43). Similarly, treatment of neonatal rabbits with oxotremorine, another muscarinic agonist, increased the surfactant and PC content of lavages (3–5). Where investigated, effects of the muscarinic agonist were reduced or prevented by atropine.

Corbet et al. (42, 43) suggested that pilocarpine acted indirectly to increase lung stability of fetal rabbits because this action could be antagonised by phenoxybenzamine or propranolol. They proposed that pilocarpine released catecholamines, either from the adrenal medulla or from noradrenergic neurones within the lung, which in turn acted on adrenoceptors within the lung parenchyma. Later studies by Abdellatif and Hollingsworth (4, 5) provide support for part of this hypothesis. The increase in PC content of lavages of neonatal rabbits produced by oxotremorine was prevented by prior adrenalectomy or by pretreatment with *dl*-propranolol but not *d*-propranolol. The latter isomer has the local anaesthetic properties of the racemate but is less potent as a β-adrenoceptor antagonist. Oxotremorine has been shown to increase noradrenaline and adrenaline plasma concentrations in the adult rat, an action said to be via central muscarinic receptors (226). More recently Massaro et al. (156) have confirmed these observations by using pilocarpine in the adult rat. Pilocarpine has for many years been known to release catecholamines from the adrenal medulla (62). The suggestion by Corbet et al. (42, 43) of the indirect action of pilocarpine via local noradrenergic neurones seems unlikely in view of the very sparse innervation of the lung parenchyma, especially in the fetus (24). The antagonism of pilocarpine

action in the fetal rabbit by phenoxybenzamine is probably a consequence of an action of this latter drug at sites other than α-adrenoceptors (25) as there is no evidence for surfactant secretion being stimulated by agonists at α-adrenoceptor (section VI A 2).

In conflict with the above hypothesis is the observation by Oyarzún and Clements (176) that prolonged acetylcholine infusion into one pulmonary artery of adult rabbits slightly raised the phospholipid content of lavages of the infused lung. This finding is in conflict with an extrapulmonary site of action of muscarinic agonists described above and will be discussed further in the next section.

It should be realized that pilocarpine will enhance secretion from other cell types within the respiratory epithelium, for example the Clara cell (235). These secretions, which could contain phospholipids, may influence observations derived from techniques such as lung lavages.

B. IN VITRO STUDIES. If muscarinic agonists increase surfactant secretion solely by an indirect, extrapulmonary action, they should be ineffective in isolated lung preparations. Alternatively, muscarinic agonists could have an intrapulmonary site of action either by a direct action on type 2 pneumocytes or an indirect effect mediated by the release of a substance(s) from other lung cells; use of different lung preparations should elucidate the mechanism. Observations so far are contradictory.

Dobbs and Mason (48) did not observe any changes in [^{14}C]DSPC release from type 2 pneumocytes in culture with 100 μM pilocarpine, carbamylcholine, or methacholine despite the reproducible effects of β-adrenoceptor agonists. High concentrations of acetylcholine have been reported to increase [^{14}C]DSPC release from isolated type 2 pneumocytes (26). These latter effects were much less than those produced by β-adrenoceptor agonists and were not antagonised by atropine. Therefore, the effects were unlikely to be mediated via muscarinic receptors. In this study, acetylcholine increased cellular cyclic guanosine monophosphate concentrations, an effect antagonised by atropine, which indicates the presence of a muscarinic receptor but whose function is unclear.

The situation with isolated whole lung preparations is less clear-cut. The inconsistencies, however, can be largely resolved if the concentration of agonist, usually pilocarpine, is considered. High concentrations of pilocarpine had no effect on the phospholipid content of lavages of isolated, perfused whole lungs of the rat (10 μM; 170), on the release of [^{14}C]DSPC from isolated, perfused whole lungs of the rat (500 μM; 156), or on the release of [^{3}H]DSPC from perifused slices of rat lungs (100 μM; 72). However, lower concentrations of pilocarpine (0.2 to 1 μM) induced the release of [^{14}C]DSPC from isolated, perfused whole lungs of the rat (26, 156); the secretion of [^{3}H]DSPC from perifused slices of rat lungs (100 nM; 72) and [^{14}C]PC from explants of rat lungs (1 μM; 184); and increased the phospholipid content of lavages after intratracheal instillation to isolated lungs of the rat (10 μM; 103). It is to be noted that the EC50 for pilocarpine as a muscarinic agonist in producing contractions of a smooth muscle preparation, such as strip of rabbit stomach, is of the order of 1 μM (67). It is likely that the positive effects recorded on surfactant secretion are a consequence of the combination of pilocarpine with muscarinic receptors, particularly since in some studies the actions were antagonised by atropine (103, 156). The lack of effect of pilocarpine on in vitro preparations at higher concentrations indicates a "bell-shaped" concentration-effect curve due either to some mechanism such as receptor desensitisation or to pilocarpine possessing a second, antagonistic action at these high concentrations.

The conflicting evidence between in vivo and in vitro observations as to the mechanism of action of muscarinic agonists can similarly be largely resolved if it is considered that they act by a combination of extrapulmonary and intrapulmonary sites of action. In vivo the extrapulmonary site would appear to be paramount, involving release of catecholamines from the adrenal medulla, which then act on β-adrenoceptors. Current studies would suggest that the intrapulmonary site of action relevant to in vitro studies is not the type 2 pneumocyte, but the nature of the other cell type involved still requires resolution. Massaro et al. (156) found that the increase in [^{14}C]DSPC in lavages produced by pilocarpine in the isolated, perfused lung of the rat could be abolished by indomethacin. This suggests muscarinic agonists can act indirectly by stimulating prostaglandin synthesis, but this study does not define the cell types involved. Experience behooves investigators to use full concentration ranges of drugs and to assess the pharmacological viability of their preparations with β-adrenoceptor agonists.

Radioactive ligand studies have identified binding sites for muscarinic agonists in lungs of adult rats and their numbers were increased by dexamethasome pretreatment but their cellular location was not identified (148). By using autoradiography, binding sites for muscarinic agonists have been localised in ferret lung mainly in smooth muscle with no labelling of alveolar walls (16). Studies of binding sites for muscarinic agonists in cultures of type 2 pneumocytes would be of interest.

There is a hint of ontogeny in the secretory response to muscarinic agonists. The enhanced release of [^{3}H]PC from lung slices of adult hamsters after carbamoylcholine treatment both in vivo and in vitro was absent when the same protocol was followed in 14-day-old fetuses (45). Further quantitative studies are needed with a simpler preparation.

Since increases in surfactant have been observed in extracellular sites within 30 min of application of a muscarinic agonist (3–5, 72, 156, 202), it is likely that their primary action is on secretion as opposed to synthesis.

B. Prostanoids

The abilities of indomethacin or sodium meclofenamate to prevent ventilation-induced increases in phos-

pholipid content of lungs lavages of adult rabbits (177) suggest that prostanoids are involved in surfactant secretion. However, prostaglandins E_1, E_2, and $F_{2\alpha}$, U-46619 (a thromboxane A_2-like agonist), and arachidonic acid administered to adult rabbits were without effect on lavage phospholipid content (177). There have been few studies with isolated preparations. Mason et al. (153), in one of their early studies with isolated type 2 pneumocytes, briefly reported that prostaglandins had no effect on [^{14}C]DSPC release. However, with that early culture technique, they did not demonstrate any effects of β-adrenoceptor agonists. More recently, prostaglandin E_2 has been reported to induce [^{3}H]PC release from lung slices of prematurely delivered neonatal rabbits (146) and prostaglandin $F_{2\alpha}$ to increase [^{3}H]PC release from type 2 pneumocytes of the adult rat in tissue culture (9). The latter study used incubations of several hours and it is uncertain whether any effects were primarily on secretion. Further studies are obviously warranted, particularly as prostaglandins are implicated in the control of parturition.

C. Cyclic Nucleotides

A possible role for cyclic 3′,5′-adenosine monophosphate (cAMP) in lung surfactant secretion was indicated in a brief report by Stahlman et al. (208) who observed enhanced numbers of OLB in alveoli 15 min after treatment of adult rats with dibutyrl cAMP. Later studies showed increased phospholipid and phosphatidylglycerol contents of lung lavages of neonatal rabbits 1 h after treatment with dibutyrl cAMP (95) and increased lung stability and phospholipid content of lavages in fetal rabbits 2.5 h after injection of the phosphodiesterase inhibitor, aminophylline (40). Another group (53) found that a dose of aminophylline that produced some deaths in fetal rabbits had no effect on any surfactant parameters. Several other studies (18, 91) have been conducted with aminophylline but all have used longer time intervals and the drug could be affecting surfactant synthesis.

By using isolated perfused lung preparations of the rat, it has been observed that cAMP and theophylline increased the phospholipid content (170), 8-bromo-cAMP increased the [^{14}C]DSPC content (156), and dibutyrl cyclic 3′,5′-guanosine monophosphate increased the phospholipid content (129) of lavages. Adenosine triphosphate enhanced the secretion of [^{3}H]DSPC from perifused slices of rat lungs (72, 77) and 8-bromo-cAMP (48, 161) increased release of [^{14}C]DSPC from cultures of isolated type 2 pneumocytes of the rat; but 8-bromo-cyclic guanosine monophosphate and isobutylmethylxanthine, a phosphodiesterase inhibitor (48), did not.

The cAMP concentration of lobes of isolated perfused rat lungs or type 2 pneumocytes in tissue culture were increased after isoproterenol and the cyclic 3′,5′-guanosine monophosphate concentration similarly increased after pilocarpine (26). Terbutaline has been shown to increase the cAMP concentration of isolated type 2 pneumocytes of the rat (161). These studies suggest that cyclic nucleotides act as secondary messengers in β-adrenoceptor mediated stimulation of surfactant release.

D. Other Chemical Modulators

There have been limited studies on other possible secretagogues. The largest surfactant secretory response has been observed to 12-O-tetradecanoyl-13-phorbol acetate (47, 153). This substance acts as secretagogue in many tissues. Potassium chloride will produce release of [^{3}H]DSPC from perfused slices of adult rat lungs (72, 76, 77). Whether this action is by direct depolarisation of type 2 pneumocytes or by initial release of chemical mediators from other cells is not known, but the effects were not antagonised by atropine or propranolol, suggesting that it does not involve mediators acting via muscarinic receptors or β-adrenoceptors.

An increase in intracellular calcium ion concentration plays a central role in exocytosis in many secretory tissues (30, 33, 107). As far we are aware, there are no reports on the effects of changes in medium calcium concentration or of calcium antagonists on surfactant secretion from isolated lung preparations. Gilfillan and Hollingsworth (unpublished) found that deletion of calcium from the perfusing medium of rat lung slices increased the basal release of [^{3}H]DSPC and did not markedly attenuate the secretory response to potassium chloride, effects opposite to those anticipated. Several authors have investigated effects of the calcium ionophore, A23187 (Calimycin). A23187 increased the release of [^{3}H]PC from rabbit lung slices (146), of [^{3}H]PC and [^{3}H]DSPC from cell line A-549 (199), and of [^{14}C]DSPC from cultures of isolated type 2 pneumocytes (153). However, A23187 may have actions other than those due to enhancing calcium influx into cells (180).

Microfilaments and microtubules also appear to be fundamental to the process of exocytosis in other secretory tissues (107). It has been found that cytochalasin B, a microfilament disorganizer, and colchicine and vinblastine, microtubule disrupting agents, will reduce surfactant secretion. One or both of these drugs have been shown to reduce the phospholipid content of lung lavages of adult rats (35), unstimulated [^{3}H]PC release from lung slices of hamsters after treatment in vivo (44), unstimulated [^{3}H]PC release from slices of neonatal rabbit lungs (146), pilocarpine-stimulated release of ^{14}C-surface active fraction protein from slices of rabbit lungs (155), and 12-O-tetradecanoyl-13-phorbol acetate (47) and isoproterenol stimulated release of [^{14}C]DSPC (27) from isolated type 2 pneumocytes in tissue culture. Unstimulated release of [^{14}C]DSPC from isolated type 2 pneumocytes was either not affected or increased by colchicine and vinblastine (27, 161).

Finally, secretory processes are usually inhibited by metabolic inhibitors and by reduced temperature. Massaro et al. (158) observed that the unstimulated release of surface active fractions of ^{14}C-labelled protein from

rabbit lung slices after labelling in vivo with [^{14}C]leucine was decreased by potassium cyanide and incubation at 4°C. Later studies have found that the unstimulated release of [^{3}H]PC from neonatal rabbit lung slices (146) and from slices of adult hamster lungs (44) and unstimulated (47, 126) and 12-O-tetradecanoyl-13-phorbol-acetate-stimulated (47) release of [^{14}C]DSPC from isolated type 2 pneumocytes of the rat were inhibited by incubation at 4°C. These studies suggest that surfactant secretion is truly an energy-dependent process.

E. Lung Inflation

It would seem physiologically appropriate that secretion of a substance intended to act within alveoli should be in part regulated by the extent of ventilation. Faridy et al. (59) and McClenahan and Urtnowski (159) demonstrated that increased ventilation of isolated lung of the rat and dog, respectively, led to loss of compliance assumed to be due to a reduction in surfactant. Increasing the tidal volume of anaesthetized cats led to decreased lung compliance and surfactant in lung extracts and increased radiolabelled PC in lung lavages (231–233). Therefore, hyperventilation may promote release of surfactant from lung tissue into alveoli and enhance elimination rate. The decrease in compliance and lung surfactant content were prevented by an increase in end-expiratory pressure thought to be due to a reduction in elimination. These mechanisms have been well supported by later observations. The surfactant content of the trachea and bronchi of isolated, ventilated lungs of the rat was increased by raising the tidal volume, compatible with increased movement up the airways (58). Constant air inflation for 3 h increased the surfactant content of lavages of isolated lung of the dog (57) and air inflation raised the phospholipid content of lavages of isolated lung of the rat and rabbit (39a, 101–103). The viability of these isolated, nonperfused lung preparations might be questioned but the inflation-induced increases in surfactant and phospholipid were reduced by cooling (57, 103) and by a high concentration (10 μM) of atropine (102, 103) or propranolol (39a). Increase in tidal volume raised the phospholipid content of lung lavages of anaesthetized rabbits (176, 177), the [^{14}C]phospholipid (129) and [^{14}C]DSPC (156) content of anaesthetized rats, and the [^{14}C]DSPC (156) and [^{3}H]phospholipid (170) content of isolated, perfused lungs.

There is also evidence that inflation of the lung with physiological salt solutions results in surfactant secretion. Faridy, Thliveris and Morris (60) suggested that multiple lavaging of lung of the fetal rat results in the loss of about 12% of lung tissue DSPC, as assayed by ultrastructural observation of numbers of OLB, although this figure relies on many assumptions. Hildebran et al. (103) concluded that air inflation results in an approximately 3-fold greater phospholipid release than an equivalent expansion with a physiological salt solution. It is unclear why this difference should exist. It is difficult to distinguish experimentally between air and lavage-induced secretion of surfactant.

There has been speculation as to the mechanism of inflation-induced surfactant secretion; possibilities include direct deformation of type 2 pneumocytes or deformation of other cells within the lung resulting in the release of chemical mediators which are secretagogues for type 2 pneumocytes (103, 170, 176, 177). A neural reflex has been suggested (39a, 170). Oyarzún and Clements (176, 177) found that the rise in phospholipid content of lavages after increased ventilation of anaesthetized adult rabbits could be reduced by atropine, propranolol, sotalol, indomethacin, or sodium meclofenamate pretreatment. They concluded that agonists at muscarinic and β-adrenoceptors and prostaglandins were involved. However, the site of release or action of these substances cannot be deduced from their experiments. Inflation-induced increases in phospholipid content of lavages of isolated lung of the rat were reported to be antagonized by atropine but not by propranolol (102, 103). By contrast, in lungs of newborn rabbits the inflation-induced increase in phospholipid content of lavages was antagonized by propranolol but not atropine (39a), although it should be noted that control phospholipid contents were rather variable. Also, Nicholas and Barr (170) found in their isolated, perfused lung preparation that air inflation-induced increases in lavage content of phospholipid were not antagonized by atropine, propranolol, tetrodotoxin, indomethacin, or cyproheptadine. They concluded that increased secretion of lung surfactant from lung inflation is most likely a consequence of a direct deformation of type 2 pneumocytes. These observations (170) can only be reconciled with those of Oyarzún and Clements (176, 177) if the chemical mediators released in vivo have an extrapulmonary origin, which is unlikely. There is currently a confused picture as to the nature, or existence, of any chemical mediators involved in distension-induced surfactant secretion. Surfactant secretion in the perinatal period (section IV G) may in part be due to lung inflation.

F. Fetus

There has been a considerable number of studies of the maturation of the lung surfactant system in the fetus. After differentiation of type 2 pneumocytes, there is an accumulation of intracellular surfactant as OLB (124, 209, 229). Surfactant is then secreted into alveoli during and after delivery.

There is some secretion of lung surfactant into alveoli before labor. Kikkawa et al. (124) recognised the increasing presence of lamellar structures in alveoli of fetal rabbits in late gestation and speculated that these structures represented secreted surfactant. Jost and Policard (118) observed that ligation of the trachea of fetal rabbits resulted in swelling of the lungs due to accumulation of lung fluid suggesting that this fluid could contribute to amniotic fluid. Lung surfactant secreted into alveoli is

therefore continuously transported up the bronchii and trachea. There is an efflux of pulmonary surfactant from the chronically catheterised trachea of fetal sheep measured by surface tension balance or biochemically as DSPC (128, 140, 160). Some of this surfactant will then pass into amniotic fluid. Consequently, measurement of the PC:sphingomyelin ratio of amniotic fluid has proved valuable as an aid to estimation of fetal lung maturity and the likelihood of that fetus, if delivered, to develop respiratory distress syndrome (80, 81, 219). It can be predicted that there will be a delay between any increase in alveolar surfactant content and an increase in human amniotic fluid content (106). This delay has been found with the fetal rabbit (220).

Only a small proportion of total fetal lung surfactant is within alveoli and airways. Gluck et al. (84, 85, 124), in their pioneering biochemical and anatomical studies of the rabbit lung, found that surfactant appeared in the parenchyma around day 24 of gestation whether assayed on a surface tension balance or as acetone-precipitable (mainly saturated) PC or by low-power and ultrastructural observation of the accumulation of OLB in type 2 pneumocytes. However, there was a delay of 2 to 3 days before the appearance of surfactant in lung lavages. From then until term, an approximately constant 1% of total lung acetone-precipitable PC was found in lavages in contrast to the 12% found in adult rabbits (83). Others, by contrast, have found increases in the proportion of total lung DSPC in lavages and in the DSPC to sphingomyelin ratio of lavages in fetal rabbits with increase in gestational age (188, 193, 220). Also, there was a 2.5-fold increase in unstimulated release of [^{3}H]PC from slices of rabbit lungs from days 29 to 31 of gestation (147). These studies indicate some surfactant release from fetal lungs but probably at a low rate relative to that of the postnatal lung.

Endogenous glucocorticoids have been suggested as regulators of prenatal surfactant secretion as there was a temporal correlation between increasing surfactant efflux and plasma cortisol concentrations in the fetal sheep (127). Indomethacin and sodium meclofenamate decreased surfactant efflux in fetal sheep (126) so prostaglandins may also play a role in prenatal surfactant release. However, it is not possible to deduce from these studies whether the changes in efflux are a direct effect of any mediator or secondary to changes in synthesis rate due to the experimental design requiring long time intervals. Neither atropine, phenoxybenzamine, nor propranolol treatment of 27-day-old fetal rabbits altered lung stability 3 h later (43) thus suggesting negligible tonic control of secretion via muscarinic receptors, α-adrenoceptors or β-adrenoceptors at this time of gestation.

G. Perinatal Period

During labor and the first few hours after delivery, there occurs a large secretion of surfactant. This secretion is necessary for the formation of a functional layer at the air-liquid interface. Among the questions that can be asked are: What is the time-course of surfactant secretion and what are the regulators of the process?

Gluck et al. (83–85) were the first to demonstrate surfactant release following delivery. They observed an increase in the proportion of PC content of lavages of rabbits which was acetone-precipitable (largely saturated) and a 30-fold increase in lavage concentration of acetone-precipitable PC within 1 h of delivery. Subsequent biochemical investigations have reported increases in lavage contents of phospholipids, PC, phosphatidylglycerol, DSPC, or PC to sphingomyelin ratio in rabbits and sheep following delivery (6, 39, 71, 74, 75, 92, 97, 112, 113, 188, 189, 193). Similar biochemical observations have been made on pharyngeal and tracheal aspirates of human neonates after delivery (17, 82). Other studies in animals have described increases in surface-active material in lavages (139, 141) and greater lung stability (215) after delivery. Morphological studies have reported a loss of OLB from type 2 pneumocytes and the appearance of OLB in alveoli (60, 93, 124, 132, 215).

There are suggestions that surfactant secretion rate starts to increase in the fetus just before or during labor. An approximately 30% reduction in the "index of intracellular DSPC," calculated from quantitative ultrastructural observations, has been described in rat lungs between early on day 22 of gestation and the time of nest-building behaviour, some 1 to 2 h before delivery (60). However, this decrease of intracellular DSPC was reported to be followed by a marked reduction in total lung DSPC content and an increase in intracellular DSPC by delivery. A mechanism for this observation is difficult to envisage. Rooney et al. (189) showed that the phospholipid content of lung lavages of rabbits at delivery after oxytocin-induced labor was almost doubled compared to animals delivered by Caesarian section.

There are also suggestions of increased PC to sphingomyelin ratios in amniotic fluid or pharyngeal aspirates related to labour in humans (31, 32, 228). Further studies are clearly necessary to define surfactant secretion during the parturient process and to elucidate the control mechanisms.

With regard to immediate postnatal surfactant secretion, in many studies observations have been made at a single time point after delivery and so information on time course cannot be deduced. Biochemical data (71, 74, 75, 189) suggests a biphasic increase in phospholipids, PC, and DSPC in lavages of rabbit lung in the first 24 h after delivery. During the first phase, lasting for about 3 h, the rises in lavage contents of PC and DSPC were associated with a fall in PC and DSPC contents of residual lung tissue. Gluck et al. (83) had reported successive reductions in acetone-precipitable PC in residual lung tissue by 1 h and 6 h after delivery. These results indicate a gradual release of stored surfactant over a few

hours following delivery and not over a few minutes as might be expected to be necessary to prevent immediate alveolar collapse. Morphological studies (60, 93, 124, 132) concur in suggesting a gradual reduction in OLB in type 2 pneumocytes over a few hours. It is possible that, in each alveolus or group of alveoli, release of surfactant is almost immediate as expansion occurs after delivery, but in the entire lung, surfactant is gradually released as areas are expanded (71, 74).

Biochemical and lung compliance investigations indicate a slower rate and total amount of surfactant secreted after delivery of premature compared to term rabbits (84, 85, 189, 215). The PC contents of lung tissue only doubles between day 28 of gestation and term (day 32) in the rabbit. These studies indicate in the prematurely delivered postnatal rabbit that the low lavage contents of phospholipids may be in part a failure to secrete phospholipids, as well as indicating low surfactant stores. Similarly, the rise in PC contents of lavages after delivery was much less in lambs suffering respiratory distress compared to nondistressed lambs, despite a lung tissue PC content of 50% of unaffected animals (6).

Between 6 h and 24 h after delivery in rabbits, there are further rises in lavage contents of phospholipids, PC, and DSPC plus a return of residual lung tissue contents to delivery values (72, 74, 75, 189). During this period, morphological studies have demonstrated the reappearance of OLB in type 2 pneumocytes (60, 132). These changes suggest a second phase of surfactant release.

Among the possible stimuli that have been considered as controls of surfactant secretion immediately postpartum have been lung inflation, changes in gas partial pressures, and chemical mediators. Lawson et al. (139) found that the surfactant content of lavages was increased in newborn rabbits after 30 min of air or nitrogen breathing but not if tracheal occlusion was performed at delivery. They concluded that lung expansion per se had a role in early postnatal surfactant release. Atropine treatment of the doe or fetus shortly before Cesarean delivery of rabbits or treatment of the neonate immediately after delivery prevented the rise in lavage contents of phospholipids, PC, DSPC, or surfactant by 0.5 to 1.5 h after delivery (39, 41, 72, 74, 139). Conversely propranolol failed to prevent these biochemical changes in newborn rabbits (39, 41, 72, 74, 141). It can therefore be concluded that endogenous substances acting via muscarinic receptors, but not via β-adrenoceptors, are involved in early postnatal surfactant secretion. There are difficulties in reconciling these observations with those of muscarinic agonists in vivo (section IV A 3 a) where it has been suggested that muscarinic agonists have an indirect mechanism of action involving catecholamine release from the adrenal medulla and subsequent effects at β-adrenoceptors. The lack of involvement of β-adrenoceptors is clear despite evidence that agonists at β-adrenoceptors can enhance surfactant secretion (section IV A 1 a) and that there is a rise in fetal catecholamine plasma concentrations as labor is approached in the sheep and human (117, 137). However, Lawson et al. (139) speculated that an intrapulmonary reflex mechanism may be involved, utilizing the vagus nerve, and this conclusion is consistent with the suggestion of an additional intrapulmonary site of action of agonists at muscarinic receptors (section IV A 3 b). Also, morphological studies (93, 124, 132) suggest secretion of OLB only as each region of lung is expanded which is more compatible with a local rather than a circulating chemical mediator. Respiratory distress syndrome was not induced by atropine treatment. Presumably this lack of effect was because the dose given was insufficient to prevent all surfactant release or because sufficient secretion had occurred predelivery or postdelivery by other mechanisms. As neither phentolamine (2 mg/kg), mepyramine (5 mg/kg), nor cimetidine (5 mg/kg) treatment at delivery prevented the postnatal rise in lavage contents of DSPC in rabbits (72), it is unlikely that α-adrenoceptors or histamine H_1 or H_2 receptors are involved in any chemical pathways to stimulate surfactant secretion after delivery. Possibly surfactant secretion in utero during, but not after, labour is mediated via β-adrenoceptors while muscarinic receptors have a greater role postnatally.

V. Modulation of Lung Surfactant Synthesis Rate

There has been extensive study of the influence of drugs and hormones on differentiation of type 2 pneumocytes and the rate of surfactant synthesis. It is not the primary purpose of this review to discuss these areas and readers are referred to articles by Hitchcock (105), Smith and Bogues (204), and Rooney (186) for more detailed accounts. The majority of these studies have been aimed at examining factors influencing surfactant production in the fetus and newborn and only limited information exists as to the situation in the adult animal under normal physiological conditions.

Various indices have been used to determine the influence of hormones or drugs on lung surfactant synthesis and on fetal lung maturation, namely rates of incorporation of labelled precursors into phospholipids, enzyme analysis, lung tissue phospholipid contents, amniotic fluid phospholipid content, and morphological changes. As indicated earlier, lung lavage phospholipid content may also reflect increased synthesis as well as increased secretion. Agents that have been demonstrated to increase lung surfactant as determined by one or other of the above indices are listed in table 3. It should be noted that the effects of these agents, with the exception of β-adrenoceptor agonists, have been most clearly demonstrated in fetal animals. Glucocorticoids and thyroxine have only a limited, if any, effect in adult animal models (121, 181, 182) and, therefore, may only be able to influence significantly surfactant synthesis in undifferentiated cell systems.

The mode of action of these agents in stimulating

TABLE 3
Pharmacological and hormonal agents that stimulate synthesis and/or lung maturation

Agonist	Species	Effect	Reference*
Glucocorticoids	Fetal rat	↑ Choline incorporation into PC†	89
Thyroid hormones	Fetal rat	↑ Choline incorproation into PC ↑ Choline phosphate cytidyltransferase activity	90
Estrogens	Fetal rabbit	↑ Choline incorporation into PC ↑ Choline phosphate cytidyltransferase activity	122
Prolactin	Fetal rat	↑ Total phospholipid ↑ Choline and glycerol incorporation into PC	168
Fibroblast pneumocyte factor	Fetal rat	↑ Choline incorporation into PC	203
Epidermal growth factor	Fetal lamb	↑ Lung maturation by morphological criteria	214
β-Adrenoceptor agonists	Adult rat	↑ Choline incorporation into PC	162
Muscarinic agonists	Fetal rat	↑ Number of osmiophilic lamellar bodies	205

* Only one reference per agent is listed. That more recent references have been selected does not imply that other work is of lesser importance.
† The abbreviation used is: PC, phosphatylcholine.

surfactant synthesis is as yet unclear. It has been suggested that glucocorticoids and estrogens at least, can stimulate one or more of the enzymes of the cytidine diphosphate choline pathway (105, 186). Others have argued that the primary site of action of glucocorticoids is at the nucleus (11a). Glucocorticoids are the pharmacological group that has been shown most clearly to produce differentiation of the fetal lung and enhance surfactant synthesis (105, 204). It has been shown that, following glucocorticoid administration to fetal animals, there is a large rise in surfactant content of lung lavages or tracheal fluids (e.g., 214a). The increased surfactant in the extracellular alveolar pool is most likely secondary to increased tissue stores. Alternatively, but less likely, these results could be a primary consequence of a direct effect on secretion. As glucocorticoids can increase the number of β-adrenoceptor ligand binding sites in fetal lungs (section IV A I c) part of these effects may also be explained in terms of an indirect action via β-adrenoceptors, increasing the response of the fetal lung to secretagogues.

If agonists at β-adrenoceptors only increased surfactant secretion then their use would lead to depletion of the tissue pool of surfactant (106). An observation compatible with this hypothesis is that 24 h after a large dose of terbutaline administered intrafetally into rabbits, fetal lung compliance was reduced compared to controls (53a). Other studies have provided limited evidence to suggest that compounds that stimulate surfactant secretion can also stimulate surfactant synthesis. Abdellatif and Hollingsworth (5) reported that adrenaline produced an increase in total lung PC contents. Isoxsuprine has been reported to increase [^{14}C]choline incorporation into DSPC and PC in fetal rabbit lung slices 1 day after maternal administration (119). Terbutaline and isoproterenol have been shown to stimulate PC synthesis in isolated type 2 pneumocytes (161). It would appear that these effects are independent of their effects on secretion. Mettler et al. (161) were able to inhibit the terbutaline-mediated release of PC from type 2 pneumocytes with colchine. Colchicine had no effect on the terbutaline-mediated increase in PC synthesis. Meyer et al. (162) demonstrated that the β-adrenoceptor agonist fenoterol increased the rate of choline incorporation into PC, an effect that appeared to be related to an increase in choline kinase activity. Thus, it seems possible that β-adrenoceptor agonists may have a direct action on the de novo cytidine diphosphate choline pathway as well as an effect on secretion.

VI. Conclusions

There is now substantial evidence that the type 2 pneumocyte is the site of lung surfactant formation. This cell type exhibits many features of other more fully investigated secretory tissues. These features include the synthesis of a specific chemical substance, DPPC; its storage in discrete organelles, the OLB, and the passage of these into the extracellular space by exocytosis.

Arising from this growing concept that lung surfactant arises from a secretory tissue has been the need to develop methods to enable the study of agents on surfactant secretion separate from their effects on synthesis. There are now available a variety of in vivo and in vitro methods that can be used with reasonable confidence.

There is overwhelming evidence that the type 2 pneumocyte possesses β-adrenoceptors; agonists acting at these receptors enhance surfactant secretion. Current studies do not indicate muscarinic receptors on type 2 pneumocytes which can mediate surfactant secretion. Increased surfactant secretion by muscarinic agonists would appear to be indirect, involving a combination of extrapulmonary and intrapulmonary sites of action. The evidence for other receptors on type 2 pneumocytes, in addition to β-adrenoceptors, is sparse. Most secretory tissues other than type 2 pneumocytes possess receptors for several secretagogues; perhaps further receptors are still to be found on type 2 pneumocytes.

A simplistic view might be that β-adrenoceptor agonists enhance lung surfactant secretion and glucocorticoids increase differentiation and synthesis. However, there is limited support for the idea that each agent can

affect both synthesis and secretion. Whether synthesis and secretion are both affected independently by the drugs or there is some feedback between increased secretion and enhanced synthesis is currently unclear.

If it is accepted that a deficiency of surfactant stores and/or synthesis has a fundamental role in the etiology of respiratory distress syndrome and drugs that can induce surfactant secretion may also enhance surfactant synthesis and storage, then the possibilities for therapeutic prophylaxis are widened.

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REFERENCES

1. ABDELLATIF, M. M.: Secretion of phosphatidylcholines into lung washes from neonatal rabbit lungs. M.Sc. Thesis, Manchester University, Manchester, U.K., 1976.
2. ABDELLATIF, M. M.: Studies on the effect of oxotremorine on lung surfactant secretion in neonatal rabbits. Ph.D. Thesis, Manchester University, Manchester, U.K., 1979.
3. ABDELLATIF, M. M., AND HOLLINGSWORTH, M.: The in vitro and in vivo effects of oxotremorine on the phosphatidylcholine content of washes of neonatal rabbit lungs (Abstract). Br. J. Pharmacol. **61:** 502P, 1977.
4. ABDELLATIF, M. M., AND HOLLINGSWORTH, M.: Mediation by adrenaline of lung surfactant secretion induced by oxotremorine in neonatal rabbits (Abstract). Br. J. Pharmacol. **66:** 142P–143P, 1979.
5. ABDELLATIF, M. M., AND HOLLINGSWORTH, M.: Effect of oxotremorine and epinephrine on lung surfactant secretion in neonatal rabbits. Pediatr. Res. **14:** 916–920, 1980.
6. ADAMS, F. H., FUJIWARA, T., AND LATTA, H.: 'Alveolar' and whole lung phospholipids of premature newborn lungs. Biol. Neonate **17:** 198–218, 1971.
7. AHMED, A., AND CHISWICK, M. L.: Origin of osmiophilic inclusion bodies in type II pneumocytes. J. Pathol. **113:** 161–163, 1974.
8. AKINO, T., ABE, M., AND ARAI, T.: Studies on the biosynthetic pathways of molecular species of lecithin by rat lung slices. Biochim. Biophys. Acta **248:** 274–281, 1971.
9. ANDERSON, G. G., CIDLOWSKI, J. A., ABSHER, P. M., HEWITT, J. R., AND DOUGLAS, W. H. J.: The effect of dexamethasone and prostaglandin F2α on production and release of surfactant in type II alveolar cells. Prostaglandins **16:** 923–929, 1978.
10. ASHCROFT, S. J. H., AND RANDLE, P. J.: The pancreas and insulin release: *In* Diabetes, Its Physiological and Biochemical Basis, ed. by J. Vallance-Owen, ch. 2, pp. 31–62, MTP Press, Lancaster, U.K., 1975.
11. ASKIN, F. B., AND KUHN, C.: The cellular origin of pulmonary surfactant. Lab. Invest. **25:** 260–268, 1971.
11a. AVERY, M. E.: Pharmacological approaches to the acceleration of fetal lung maturation. Br. Med. Bull. **31:** 13–17, 1975.
12. AVERY, M. E., FLETCHER, B. D., AND WILLIAMS, R. G.: The lung and its disorders in the newborn infant, ed. 4. W.B. Saunders, Philadelphia, 1981.
13. AVERY M. E., AND MEAD, J.: Surface properties in relation to atelectasis and hyaline membrane disease. Am. J. Dis. Child. **97:** 517–523, 1959.
14. BANGHAM, A. D., MORLEY, C. J., AND PHILLIPS, M. C.: The physical properties of an effective lung surfactant. Biochim. Biophys. Acta **573:** 552–556, 1979.
15. BARITUSSIO, A., MAGOON, M. W., GOERKE, J., AND CLEMENTS, J. A.: Precursor-product relationship between rabbit type II cell lamellar bodies and alveolar surface-active material. Surfactant turnover time. Biochim. Biophys. Acta **666:** 382–393, 1981.
16. BARNES, P. J., NADEL, J. A., ROBERTS, J. M., AND BASBAUM, C. B.: Muscarinic receptors in lung and trachea: Autoradiographic localization using [^{3}H]-quinuclidinyl benzilate. Eur. J. Pharmacol. **86:** 103–106, 1983.
17. BARR, P. A., JENKINS, P. A., AND BAUM, J. D.: Lecithin/sphingomyelin ratio in hypopharyngeal aspirate of newborn infants. Arch. Dis. Child. **50:** 856–861, 1975.
18. BARRETT, C. T., SEVANIAN, A., PHELPS, D. C., GILDEN, C., AND KAPLAN, S. A.: Effects of cortisol and aminophyllin upon survival, pulmonary mechanics, and secreted phosphatidylcholine of prematurely delivered rabbits. Pediatr. Res. **12:** 38–42, 1978.
19. BECKMAN, D. L., BEAN, J. W., AND BASLOCK, D. R.: Sympathetic influence on lung compliance and surface forces in head injury. J. Appl. Physiol. **30:** 394–399, 1971.
20. BENSCH, K., SCHAEFER, K., AND AVERY, M. E.: Granular pneumocytes: Electron microscopic evidence of their exocrinic function. Science **145:** 1318–1319, 1964.
21. BERGMAN, B.: Beta-mimetics and the preterm neonatal lung: An experimental and clinical study. Acta Physiol. Scand. **113:** suppl. 497, 1981.
22. BERGREN, D. R., AND BECKMAN, D. L.: Alpha receptor influence on lung surfactant. Proc. West. Pharmacol. Soc. **19:** 87–90, 1976.
23. BOLANDE, R. P., AND KLAUS, M. H.: The morphologic demonstration of an alveolar lining layer and its relationship to pulmonary surfactant. Am. J. Pathol. **45:** 449–463, 1964.
24. BOREUS, L. (ED.): Fetal Pharmacology, Raven Press, New York, 1973.
25. BOWMAN, W. C., AND RAND, M. J.: Textbook of Pharmacology, 2nd Edition, Blackwell Scientific Publications, Oxford, U.K., 1980.
26. BROWN, L. A. S., AND LONGMORE, W. J.: Adrenergic and cholinergic regulation of lung surfactant secretion in the isolated perfused rat lung and in the alveolar type II cell in culture. J. Biol. Chem. **256:** 66–72, 1981.
27. BROWN, L. S., AND LONGMORE, W. J.: Effects of antimicrotubular and antimicrofilament agents in alveolar type II cells (Abstract). Fed. Proc. **40:** 407, 1981.
28. BRUMELY, G. W., CHERNICK, V., HODSON, W. A., NORMAND, C., FENNER, A., AND AVERY, M. E.: Correlations of mechanical stability, morphology, pulmonary surfactant and phospholipid content in the developing lamb lung. J. Clin. Invest. **46:** 863–873, 1967.
29. BUCKINGHAM, S., AND AVERY, M. E.: Time of appearance of lung surfactant in the foetal mouse. Nature (Lond.) **193:** 688–689, 1962.
30. BUTCHER, F. R.: Regulation of exocytosis. *In* Biochemical Actions of Hormones, vol. V, ed. by G. Litwack, Academic Press, New York, 1978.
31. CABERO, L., ROSES, A., VISCASILLAS, P., QUILEZ, M., GIRALTE, E., AND DURAN-SANCHEZ, P.: Influence of labor on the lecithin, lecithin/spingomyelin (L/S) ratio and palmitic acid values in the amniotic fluid. Br. J. Obstet. Gynecol. **83:** 452–453, 1976.
32. CALLEN, P., GOLDSWORTHY, S., GRAVES, L., HARVEY, D., MELLOWS, H., AND PARKINSON, C.: Mode of delivery and the lecithin/sphingomyelin ratio. Br. J. Obstet. Gynecol. **86:** 965–968, 1979.
33. CASE, R. M.: Synthesis, intracellular transport and discharge of exportable proteins in the pancreatic acinar cell and other cells. Biol. Rev. **53:** 211–354, 1978.
34. CHENG, J. B., GOLDFIEN, A., BALLARD, P. L., AND ROBERTS, J. M.: Glucocorticoids increase pulmonary β-adrenergic receptors in fetal rabbit. Endocrinology **107:** 1646–1648, 1980.
35. CHESTERS, K., AND HOLLINGSWORTH, M.: Unpublished observations.
36. CHEVALIER, G., AND COLLET, A. J.: In vivo incorporation of choline-^{3}H, leucine-^{3}H, and galactose-^{3}H in alveolar type II pneumocytes in relation to surfactant synthesis. A quantitative radio-autographic study in mouse by electron microscopy. Anat. Rec. **174:** 289–310, 1972.
37. CLEMENTS, J. A.: Surface tension of lung extracts. Proc. Soc. Exp. Biol. Med. **95:** 170–172, 1957.
38. CLEMENTS, J. A.: Functions of the alveolar lining. Am. Rev. Respir. Dis. **115:** 67–71, 1977.
39. CORBET, A.: Control of surfactant secretion in fetal and newborn rabbits. Prog. Respir. Res. **15:** 78–83, 1981.
39a. CORBET, A., CREGAN, J., FRINK, J., AND RUDOLPH, A. J.: Distension-produced phospholipid secretion in postmortem in-situ lungs of newborn rabbits—inhibition by specific beta-adrenergic blockade. Am. Rev. Respir. Dis. **128:** 695–701, 1983.
40. CORBET, A. J. S., FLAX, P., ALSTON, C., AND RUDOLPH, A. J.: Effect of aminophyllin and dexamethasone on secretion of pulmonary surfactant in fetal rabbits. Pediatr. Res. **12:** 797–799, 1978.
41. CORBET, A. J. S., FLAX, P., ALSTON, C., AND RUDOLPH, A. J.: Role of autonomic nervous system controlling secretion of pulmonary phospholipids in the rabbit at birth. Aust. Pediatr. J. **15:** 238–242, 1979.
42. CORBET, A. J. S., FLAX, P., AND RUDOLPH, A. J.: Reduced surface tension in lungs of fetal rabbits injected with pilocarpine. J. Appl. Physiol. **41:** 7–14, 1976.
43. CORBET, A. J. S., FLAX, P., AND RUDOLPH, A. J.: Role of autonomic nervous system controlling surface tension in fetal rabbit lungs. J. Appl. Physiol. **43:** 1039–1045, 1977.
44. DELAHUNTY, T. J., AND JOHNSTON, J. M.: The effect of colchicine and vinblastine on the release of pulmonary surface active material. J. Lipid. Res. **17:** 112–116, 1976.
45. DELAHUNTY, T. J., AND JOHNSTON, J. M.: Neurohumoral control of pulmonary surfactant secretion. Lung **157:** 45–51, 1979.
46. DOBBS, L. G., AND MASON, R. J.: Terbutaline-induced secretion of disaturated phosphatidylcholine from pulmonary alveolar type II cells (Abstract). J. Cell Biol. **75:** 375, 1977.
47. DOBBS, L. G., AND MASON, R. J.: Stimulation of secretion of disaturated phosphatidylcholine from isolated alveolar type II cells by 12-O-tetradecanoyl-13-phorbol acetate. Am. Rev. Respir. Dis. **118:** 705–713, 1978.
48. DOBBS, L. G., AND MASON, R. J.: Pulmonary alveolar type II cells isolated

from rats: Release of phosphatidylcholine in response to β-adrenergic stimulation. J. Clin. Invest. **63:** 378–387, 1979.
49. DOBBS, L. G., MASON, R. J., WILLIAMS, M. C., BENSON, B. J., AND SUESHI, K.: Secretion of surfactant by primary cultures of alveolar type II cells isolated from rats. Biochim. Biophys. Acta **713:** 118–127, 1982.
50. DOUGLAS, W. H. J., AND SMITH, S. S.: Lung cell culture systems. *In* Lung Development: Biological and Clinical Perspectives, ed. by P. M. Farrell, vol. I, Biochemistry and Physiology, ch. 9, pp. 151–164, Academic Press, New York, 1982.
51. DROSTE, P. L., AND BECKMAN, D. L.: Pulmonary effects of prolonged sympathetic stimulation. Proc. Soc. Exp. Biol. Med. **146:** 352–353, 1974.
52. EKELUND, L., ARVIDSON, G., EMANUELSSON, H., MYHRBERG, H., AND ASTEDT, B.: Effect of cortisol on human fetal lung in organ culture: A biochemical, electron-microscopic and autoradiographic study. Cell Tissue Res. **163:** 263–272, 1975.
53. EKELUND, L., BURGOYNE, R., BRYMER, D., AND ENHORNING, G.: Pulmonary surfactant release in fetal rabbits as affected by terbutalline and aminophyllin. Scand. J. Clin. Invest. **41:** 237–245, 1981.
53a. EKELUND, L., BURGOYNE, R., AND ENHORNING, G.: Pulmonary surfactant release in fetal rabbits: Immediate and delayed response to terbutaline. Am. J. Obstet. Gynecol. **147:** 437–443, 1983.
54. ENGLE, M. J.: Cellular and subcellular sites of lung lipid metabolism. *In* Lung Development: Biological and Clinical Perspectives, ed. by P. M. Farrell, vol. I, Biochemistry and Physiology, ch. 15, pp. 239–258, Academic Press, New York, 1982.
55. ENHORNING, G., CHAMBERLAIN, D., CONTRERAS, C., BURGOYNE, R., AND ROBERTSON, B.: Isoxuprine-induced release of pulmonary surfactant in the rabbit fetus. Am. J. Obstet. Gynecol. **129:** 197–202, 1977.
56. EPSTEIN, M. F., AND FARRELL, P. M.: The choline incorporation pathway: Primary mechanism for de novo lecithin synthesis in fetal primate lung. Pediatr. Res. **9:** 658–665, 1975.
57. FARIDY, E. E.: Effect of distention on release of surfactant in excised dogs' lungs. Respir. Physiol. **27:** 99–114, 1976.
58. FARIDY, E. E.: Effect of ventilation on movement of surfactant in airways. Respir. Physiol. **27:** 323–334, 1976.
59. FARIDY, E. E., PERMUTT, S., AND RILEY, R. L.: Effect on ventilation on surface forces in excised dogs' lungs. J. Appl. Physiol. **21:** 1453–1462, 1966.
60. FARIDY, E. E., THLIVERIS, J. A., AND MORRIS, G. S.: Relationship between lung intra- and extracellular DSPC in fetal and neonatal rats. Respir. Physiol. **45:** 55–65, 1981.
61. FARRELL, P. M. (ED.): Lung Development: Biological and Clinical Perspectives; vol. I, Biochemistry and Physiology, Academic Press, New York, 1982.
62. FELDBERG, W., MINZ, B., AND TSUDZIMURA, H.: The mechanism of the nervous discharge of adrenaline. J. Physiol. (Lond.) **81:** 286–304, 1934.
63. FOREMAN, J. C.: The pharmacological control of immediate hypersensitivity. Annu. Rev. Pharmacol. Toxicol. **21:** 63–81, 1981.
64. FRANSOM, R., BECKERDITE, S., WANG, P., WAITE, M., AND ELSBACH, P.: Some properties of phospholipases of alveolar macrophages. Biochim. Biophys. Acta **296:** 365–373, 1973.
65. FRANSOM, R., AND WAITE, M.: Lysosomal phospholipases A_1 and A_2 in normal and Bacillus Calmette-Geurin induced alveolar macrophages. J. Cell Biol. **56:** 621–627, 1973.
66. FUJIWARA, T., ADAMS, F. H., SIPOS, S., AND EL-SALAWY, A.: 'Alveolar' and whole lung phosphatidylcholine of the developing fetal lamb lung. Am. J. Physiol. **215:** 375–382, 1968.
67. FURCHGOTT, R. F., AND BURSZTYN, P.: Comparison of dissociation constants and of relative efficacies of selected agonists acting on parasympathetic receptors. Ann. N.Y. Acad. Sci. **144:** 882–899, 1967.
68. GARCIA, A., SENER, S. F., AND MAVIS, R. D.: Lung lamellar bodies lack certain key enzymes of phospholipid metabolism. Lipids **11:** 109–112, 1976.
69. GIANNOPOULOS, G., AND SOMMERS-SMITH, S. K.: Hormonal regulation of β-adrenergic receptors in fetal rabbit lung in organ culture. Life Sci. **31:** 795–802, 1982.
70. GIL, J., AND REISS, O. K.: Isolation and characterisation of lamellar bodies and tubular myelin from rat lung homogenates. J. Cell Biol. **58:** 152–171, 1973.
71. GILFILLAN, A. M.: The release of lung surfactant at birth. M.Sc. Thesis, Manchester University, Manchester, U.K., 1979.
72. GILFILLAN, A. M.: Studies on the release of [^{3}]-disaturated phosphatidylcholine from superfused lung slices: A model for lung surfactant secretion. Ph.D. Thesis, Manchester University, Manchester, U.K., 1981.
73. GILFILLAN, A. M., CHU, A. J., SMART, D. A., AND ROONEY, S. A.: Single plate separation of lung phospholipids including disaturated phosphatidylcholine. J. Lipid. Res. **24:** 1651–1656, 1983.
74. GILFILLAN, A. M., HARKES, A., AND HOLLINGSWORTH, M.: Secretion of lung surfactant following delivery after uterine section. J. Dev. Physiol. **2:** 101–110, 1980.
75. GILFILLAN, A. M., AND HOLLINGSWORTH, M.: The biphasic nature of postnatal surfactant release (Abstract). J. Physiol. (Lond.) **301:** 67P–68P, 1980.
76. GILFILLAN, A. M., AND HOLLINGSWORTH, M.: Overflow of [^{3}]-disaturated phosphatidylcholines from adult rat superfused lung slices: A novel method for the study of lung surfactant secretion (Abstract). Br. J. Pharmacol. **74:** 981P, 1982.
77. GILFILLAN, A. M., HOLLINGSWORTH, M., AND JONES, A. W.: The pharmacological modulation of [^{3}]-disaturated phosphatidylcholine overflow from perifused lung slices of adult rats: A novel method for the study of lung surfactant secretion. Br. J. Pharmacol. **79:** 363–371, 1983.
78. GLATZ, T., IKEGAMI, M., AND JOBE, A. H.: Metabolism of exogenously administered natural surfactant in the newborn lamb. Pediatr. Res. **16:** 711–715, 1982.
79. GLUCK, L., AND KULOVICH, M. V.: Fetal lung development: Current concepts. Pediatr. Clin. N. Am. **20:** 367–379, 1973.
80. GLUCK, L., KULOVICH, M. V., BORER, R. C., BRENNER, P. H., ANDERSON, G. G., AND SPELLACY, W. N.: Diagnosis of the respiratory distress syndrome by amniocentesis. Am. J. Obstet. Gynecol. **109:** 440–445, 1971.
81. GLUCK, L., KULOVICH, M. V., BORER, R. C., AND KEIDEL, W. N.: The interpretation and significance of the lecithin/sphinogomyelin ratio in amniotic fluid. Am. J. Obstet. Gynecol. **120:** 142–155, 1974.
82. GLUCK, L., KULOVICH, M. V., EIDELMAN, A. I., CORDERO, L., AND KHAZIN, A. F.: Biochemical development of surface activity in mammalian lung. IV. Pulmonary lecithin synthesis in the human fetus and newborn and etiology of the respiratory distress syndrome. Pediatr. Res. **6:** 81–99, 1972.
83. GLUCK, L., LANDOWNE, R. A., AND KULOVICH, M. V.: Biochemical development of surface activity in mammalian lung. III. Structural changes in lung lecithin during development of the rabbit fetus and newborn. Pediatr. Res. **1:** 352–364, 1970.
84. GLUCK, L., MOTOYAMA, E. K., SMITS, H. L., AND KULOVICH, M. V.: The biochemical development of surface activity in mammalian lung. I. The surface-active phospholipids: The separation and distribution of surfactactive lecithin in the lung of the developing rabbit fetus. Pediatr. Res. **1:** 237–246, 1967.
85. GLUCK, L., SRIBNEY, M., AND KULOVICH, M. V.: The biochemical development of surface activity in mammalian lung. II. The biosynthesis of phospholipids in the lungs of the developing rabbit fetus and newborn. Pediatr. Res. **1:** 247–265, 1967.
86. GOERKE, J.: Lung surfactant. Biochim. Biophys. Acta **344:** 241–261, 1974.
87. GOLDENBERG, V. E., BUCKINGHAM, S., AND SOMMERS, S. C.: Pulmonary alveolar lesions in vagotomized rats. Lab. Invest. **16:** 693–705, 1967.
88. GOLDENBERG, V. E., BUCKINGHAM, S., AND SOMMERS, S. C.: Pilocarpine stimulation of granular pneumocyte secretion. Lab. Invest. **20:** 147–158, 1969.
89. GROSS, I., BALLARD, P. L., BALLARD, R. A., JONES, C. T., AND WILSON, C. M.: Corticosteroid stimulation of phosphatidylcholine synthesis in cultured fetal rabbit lung: Evidence for de novo protein synthesis mediated by glucocorticoid receptors. Endocrinology **112:** 829–837, 1983.
90. GROSS, I., DYNIA, D. W., WILSON, C. M., INGLESON, L. D., GEWOLLO, I. H., AND ROONEY, S. A.: Glucocorticoid-thyroid hormone interactions in fetal rat lung. Pediatr. Res. **18:** 191–196, 1984.
91. GROSS, I., WILSON, C. M., INGLESON, L. D., BREHIER, A., AND ROONEY, S. A.: Fetal lung in organ culture. III. Comparison of dexamethasone, thyroxine and methylxanthines. J. Appl. Physiol. **48:** 872–877, 1980.
92. GROSS, I., WILSON, C. M., AND ROONEY, S. A.: Phosphatidylcholine synthesis in newborn rabbit lung. Developmental pattern and the influence of nutrition. Biochim. Biophys. Acta **528:** 190–198, 1978.
93. GROSSMAN, G., AND ROBERTSON, B.: Lung expansion and the formation of the alveolar lining layer in the full term newborn rabbit. Acta Pediatr. (Stockh.) **64:** 7–16, 1975.
94. HAIES, D. M., GIL, J., AND WEIBEL, E. R.: Morphometric study of rat lung cells. I. Numerical and dimensional characteristics of parenchymal cell population. Am. Rev. Respir. Dis. **123:** 533–541, 1981.
95. HALLMAN, M.: Induction of surfactant phosphatidylglycerol in the lung of fetal and newborn rabbits by dibutyryl adenosine 3′:5′-monophosphate. Biochim. Biophys. Res. Commun. **77:** 1094–1102, 1977.
96. HALLMAN, M., EPSTEIN, B. L., AND GLUCK, L.: Analysis of labeling and clearance of lung surfactant phospholipids in rabbit. Evidence of bidirectional surfactant flux between lamellar bodies and alveolar lavage. J. Clin. Invest. **68:** 715–742, 1981.
97. HALLMAN, M., AND GLUCK, L.: Phosphatidylglycerol in lung surfactant. III. Possible modifier of surfactant function. J. Lipid. Res. **17:** 257–262, 1976.
98. HATAZA, K., AND NAKAMURA, T.: Electron microscopic observations of lung alveolar epithelial cells of normal young mice, with special reference to formation and secretion of osmiophilic lamellar bodies. Z. Zellforsch. **68:** 266–277, 1965.
99. HAYDEN, W., OLSEN, E. B., AND ZACHMAN, R. D.: Effect of maternal isoxsuprine on fetal rabbit lung biochemical maturation. Am. J. Obstet. Gynecol. **129:** 691–694, 1977.
100. HENDERSON, R. F., AND HACKETT, N. A.: Effect of pulmonary lavage on lung lecithin synthesis in the Syrian hamster. Biochem. Med. **20:** 98–106, 1978.
101. HILDEBRAN, J. N., GOERKE, J., AND CLEMENTS, J. A.: Air inflation releases phospholipids into the air spaces of excised rat lungs (Abstract). Fed. Proc. **34:** 387, 1975.
102. HILDEBRAN, J. N., GOERKE, J., AND CLEMENTS, J. A.: Phospholipid (PL) recovered from the airspaces (AS) of excised rat lung is increased by pilocarpine and decreased by atropine (A) (Abstract). Fed. Proc. **36:** 541, 1977.

103. HILDEBRAN, J. N., GOERKE, J., AND CLEMENTS, J. A.: Surfactant release in excised rat lung is stimulated by air inflation. J. Appl. Physiol. **51:** 905–910, 1981.
104. HILLS, B. A.: Editorial—What is the true role of surfactant in the lung? Thorax **36:** 1–4, 1981.
105. HITCHCOCK, K. R.: Lung development and the pulmonary surfactant system: Hormonal influences. Anat. Rec. **198:** 13–34, 1980.
106. HOLLINGSWORTH, M., AND DONNAI, P.: Prophylactic treatment of idiopathic respiratory distress syndrome. *In* Clinical Pharmacology in Obstetrics, ed. by P. J. Lewis, ch. 16, pp. 219–231, J. Wright and Sons Ltd., Bristol, U.K., 1983.
107. HOPKINS, C. R., AND DUNCAN, C. J.: Secretory mechanisms. Symp. Soc. Exper. Biol. **33:** 1979.
108. JACOBS, H., JOBE, A., IKEGAMI, M., AND CONAWAY, D.: The significance of surfactant phosphatidycholine. J. Biol. Chem. **258:** 4159–4165, 1983.
109. JAMIESON, J. D., AND PALADE, G. E.: Synthesis, intracellular transport and discharge of secretory proteins in stimulated pancreatic exocrine cells. J. Cell Biol. **50:** 135–158, 1971.
110. JOBE, A.: The labelling and biological half-life of phosphatidylcholine in subcellular fractions of rabbit lung. Biochim. Biophys. Acta **489:** 440–453, 1977.
111. JOBE, A.: Surfactant phospholipid metabolism in 3-day and 3-day postmature rabbits in vivo. Pediatr. Res. **14:** 319–325, 1980.
112. JOBE, A., AND GLUCK, L.: The labeling of lung phosphatidylcholine in premature rabbits. Pediatr. Res. **13:** 635–640, 1979.
113. JOBE, A., IKEGAMI, M., AND JACOBS, H.: Changes in the amount of lung and airway phosphatidylcholine in 0.5–12.5 day old rabbits. Biochim. Biophys. Acta **664:** 182–187, 1981.
114. JOBE, A., IKEGAMI, M., AND NATHANIELSZ, P. W.: The labelling of pulmonary surfactant phosphatidylcholine in 19–31-days old lambs. J. Dev. Physiol. **1:** 245–259, 1979.
115. JOBE, A., KIRKPATRICK, E., AND GLUCK, L.: Lecithin appearance and apparent biologic half-life in term newborn rabbit lung. Pediatr. Res. **12:** 669–675, 1978.
116. JOBE, A., MANNINO, F., AND GLUCK, L.: Labeling of phosphatidylcholine in the alveolar wash of rabbits in utero. Am. J. Obstet. Gynecol. **132:** 53–58, 1978.
117. JONES, C. T., AND ROBINSON, R. O.: Plasma catecholamines in fetal and adult sheep. J. Physiol. (Lond.) **248:** 15–33, 1975.
118. JOST, A., AND POLICARD, A.: Contribution expérimental a l'étude du dévéloppement prénatal du poumon chez le lapin. Arch. Anat. Microsc. Morphol. Exp. **37:** 323–332, 1948.
119. KANJANAPONE, V., HARTIG-BEECKEN, I., AND EPSTEIN, M. F.: Effect of isoxsuprine on fetal lung surfactant in rabbits. Pediatr. Res. **14:** 278–281, 1980.
120. KELLER, G. H., AND LADDA, R. L.: Quantitation of phosphatidylcholine secretion in lung slices and primary cultures of rat lung cells. Proc. Natl. Acad. Sci. U.S.A. **76:** 4102–4106, 1979.
121. KELLER, G. H., AND LADDA, R. L.: Correlation between phosphatidylcholine labeling and hormone receptor levels in alveolar type II epithelial cells: Effects of dexamethasone and epidermal growth factor. Arch. Biochem. Biophys. **211:** 321–326, 1981.
122. KHOSLA, S. S., BREHIER, A., EISENFELD, A. J., INGLESON, L. D., PARKS, P. A., AND ROONEY, S. A.: Influence of sex hormones on lung maturation in the fetal rabbit. Biochim. Biophys. Acta **750:** 112–126, 1983.
123. KIKKAWA, Y., HAHN, H. S., YANG, S. S., AND BERNSTEIN, J.: Mucopolysaccharide in the pulmonary alveolus. Lab. Invest. **22:** 272–280, 1970.
124. KIKKAWA, Y., MOTOYAMA, E. K., AND GLUCK, L.: Study of the lungs of fetal and newborn rabbits. Morphological, biochemical and surface physical development. Am. J. Pathol. **52:** 177–209, 1968.
125. KIKKAWA, Y., YONEDA, K., SMITH, F., PACKARD, B., AND SUZUKI, K.: The type II epithelial cells of the lung. II. Chemical composition and phospholipid synthesis. Lab. Invest. **32:** 295–302, 1975.
126. KING, R. J., AND MARTIN, H. M.: Effects of inhibiting protein synthesis on the secretion of surfactant by type II cells in primary culture. Biochim. Biophys. Acta **663:** 289–301, 1981.
127. KITTERMAN, J. A., LIGGINS, G. C., CAMPOS, G., CLEMENTS, J. A., FORSTER, C. S., LEE, C. H., AND CREASY, R. K.: Prepartum maturation of the lung in fetal sheep: Relation to cortisol. J. Appl. Physiol. **51:** 384–390, 1981.
128. KITTERMAN, J. A., LIGGINS, G. C., CLEMENTS, J. A., CAMPOS, G., LEE, C. H., AND BALLARD, P. L.: Inhibitors of prostaglandin synthesis, tracheal fluid and surfactant in fetal lamb. J. Appl. Physiol. **51:** 1562–1567, 1981.
129. KLASS, D. J.: Dibutyryl cGMP and hyperventilation promote rat lung phospholipid release. J. Appl. Physiol. **47:** 285–289, 1979.
130. KLAUS, M. H., REISS, O. K., TOOLEY, W. H., PEIL, C., AND CLEMENTS, J. A.: Alveolar epithelial cell mitochondria as a source of the surface-active lining layer. Science (Wash. D.C.) **137:** 750–751, 1962.
131. KOTAS, R. V.: Physiologic assessment of lung surfactant. *In* Lung Development: Biological and Clinical Perspectives, ed. by P. M. Farrell, vol. I, Biochemistry and Physiology, ch. 4, pp. 57–86, Academic Press, New York, 1982.
132. KRASNO, R. J., KNELSON, J. H., AND DALLDORF, F. C.: Changes in the alveolar lining with onset of breathing. Am. J. Pathol. **66:** 471–482, 1972.
133. KUHN, C.: The cytology of the lung: Ultrastructure of the respiratory epithelium and extracellular lining lavages. *In* Lung Development: Biological and Clinical Perspectives, ed. by P. M. Farrell, vol. I, Biochemistry and Physiology, ch. 3, pp. 27–55, Academic Press, New York, 1982.
134. KUNC, L., KUNCOVA, M., HOLUSA, R., AND SOLDAN, F.: Physical properties and biochemistry of lung surfactant following vagotomy. Respiration **35:** 192–197, 1978.
135. LACY, P. E.: Endocrine secretory mechanisms. Am. J. Pathol. **79:** 170–187, 1975.
136. LACY, P. E., WALKER, M. M., AND FINK, C. J.: Perifusion of isolated rat islets in vitro. Diabetes **21:** 987–998, 1972.
137. LAGERCRANTZ, H., AND BISTOLETTI, P.: Catecholamine release in the newborn infant at birth. Pediatr. Res. **11:** 889–893, 1977.
138. LANDS, A. M., ARNOLD, A., MCAULIFF, J. P., LUDENA, F. P., AND BROWN, T. G.: Differentiation of receptor systems activated by sympathomimetic amines. Nature (Lond.) **214:** 597–598, 1967.
139. LAWSON, E. E., BIRDWELL, R. L., HUANG, P. S., AND TAEUSCH, H. W.: Augmentation of pulmonary surfactant by lung expansion at birth. Pediatr. Res. **13:** 611–614, 1979.
140. LAWSON, E. E., BROWN, E. R., TORDAY, J. S., MADANSKY, D. L., AND TAEUSCH, H. W.: The effect of epinephrine on tracheal fluid flow and surfactant efflux in fetal sheep. Am. Rev. Respir. Dis. **118:** 1023–1026, 1978.
141. LAWSON, E. E., AND HUANG, P. S.: Neurogenic mediation of augmented surfactant secretion at birth (Abstract). Pediatr. Res. **12:** 564, 1978.
142. LIGGINS, G. C., AND HOWIE, R. N.: A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. Pediatrics **50:** 515–525, 1972.
143. LIPSHITZ, J., BROYLES, K., HESSLER, J. R., WHYBREW, W. D., AHOKAS, R. A., AND ANDERSON, G. D.: Effects of hexoprenaline on the lecithin/sphingomyelin ratio and pressure/volume relationships in fetal rabbits. Am. J. Obstet. Gynecol. **139:** 726–734, 1981.
144. LONGMORE, W. J.: The isolated perfused lung as a model for studies of lung metabolism. *In* Lung Development: Biological and Clinical Perspectives, ed. by P. M. Farrell, vol. I, Biochemistry and Physiology. ch. 6, pp. 101–110, Academic Press, New York, 1982.
145. MACKLIN, C. C.: The pulmonary alveolar mucoid film and the pneumocytes. Lancet **1:** 1099–1104, 1954.
145a. MANISCALO, W. M., AND SHAPIRO, D. L.: Effects of dexamethasone on beta-adrenergic receptors in fetal lung explants. Pediatr. Res. **17:** 274–277, 1983.
146. MARINO, P. A., AND ROONEY, S. A.: Surfactant secretion in a newborn rabbit lung slice model. Biochim. Biophys. Acta **620:** 509–519, 1980.
147. MARINO, P. A., AND ROONEY, S. A.: The effect of labor on surfactant secretion in newborn rabbit lung slices. Biochim. Biophys. Acta **664:** 389–396, 1981.
148. MARQUARDT, D. L., MOTULSKY, H. J., AND WASSERMAN, S. I.: Rat lung cholinergic receptor: Characterization and regulation by corticosteroids. J. Appl. Physiol. **53:** 731–736, 1982.
149. MASON, R. J.: Disaturated lecithin concentration in rabbit tissues. Am. Rev. Respir. Dis. **107:** 678–679, 1973.
150. MASON, R. J.: Isolation of alveolar type II cells. *In* Lung Development: Biological and Clinical Perspectives, ed. by P. M. Farrell, vol. I, Biochemistry and Physiology, ch. 8, pp. 135–150, Academic Press, New York, 1982.
151. MASON, R. J., AND WILLIAMS, M. C.: Type II alveolar cells: Defender of the alveolus. Am. Rev. Respir. Dis. **115**(suppl.): 81–92, 1977.
152. MASON, R. J., AND WILLIAMS, M. C.: Phospholipid composition and ultrastructure of A549 cells and other cultured primary epithelial cells of presumed type II cell origin. Biochim. Biophys. Acta **617:** 36–50, 1980.
153. MASON, R. J., WILLIAMS, M. C., AND DOBBS, L. G.: Secretion of disaturated phosphatidylcholine by primary cultures of type II alveolar cells. *In* 16th Annual Hanford Biology Symposium, U.S. Atomic Authority, pp. 280–297, 1977.
154. MASON, R. J., WILLIAMS, M. C., GREENLEAF, R. D., AND CLEMENTS, J. A.: Isolation and properties of type II alveolar cells from rat lung. Am. Rev. Respir. Dis. **115:** 1015–1026, 1977.
155. MASSARO, D.: In vivo protein secretion by lung: Evidence for active secretion and interspecies difference. J. Clin. Invest. **56:** 263–271, 1975.
156. MASSARO, D., CLERCH, L., AND MASSARO, G. D.: Surfactant secretion: Evidence that cholinergic stimulation of surfactant is indirect. Am. J. Physiol. **243:** C39–C45, 1982.
157. MASSARO, G. D., AND MASSARO, D.: Granular Pneumocytes. Electron microscopic radioautographic evidence of intracellular protein transport. Am. Rev. Respir. Dis. **105:** 927–931, 1972.
158. MASSARO, D., WEISS, H., AND SIMON, M. R.: Protein synthesis and secretion by lung. Am. Rev. Respir. Dis. **101:** 198–206, 1970.
159. MCCLENAHAN, J. B., AND URTNOWSKI, A.: Effect of ventilation on surfactant and its turnover rate. J. Appl. Physiol. **23:** 215–220, 1967.
160. MESCHER, E. J., PLATZKER, A. C. G., BALLARD, P. L., KITTERMAN, J. A., CLEMENTS, J. A., AND TOOLEY, W. H.: Ontogeny of tracheal fluid, pulmonary surfactant and plasma corticoids in the fetal lamb. J. Appl. Physiol. **39:** 1017–1021, 1975.
161. METTLER, N. R., GRAY, M. E., SCHUFFMAN, S., AND LEQUIRE, V. S.: β-Adrenergic induced synthesis and secretion of phosphatidylcholine by isolated pulmonary alveolar type II cells. Lab. Invest. **45:** 575–586, 1981.
162. MEYER, W., HINTZ, H., WILKE, A., AND VON WICHERT, P.: Influence of

beta-mimetic stimulation upon phosphatidylcholine biosynthesis in rat lung. Chest **81:** 66S–68S, 1982.
163. MEYRICK, B., AND REID, L.: Electron microscopic aspects of surfactant secretion. Proc. R. Soc. Med. **66:** 386–387, 1973.
164. MILLER, H. C., BERLE, F. C., AND GIBSON, D. M.: Comparison of pulmonary hyaline membranes in vagotomized rabbits with those in newborn infants. Pediatrics **7:** 611–615, 1951.
165. MORGAN, T. E., AND MORGAN, B. C.: Surfactant synthesis, storage and release by alveolar cells. *In* Respiratory Distress Syndrome, ed. by C. A. Villee, D. B. Villee, and J. Zuckerman, pp. 117–127, Academic Press, New York, 1973.
166. MORLEY, C. J., BANGHAM, A. D., JOHNSON, P., THORBURN, G. D., AND JENKIN, G.: Physical and physiological properties of dry lung surfactant. Nature (Lond.) **271:** 162–163, 1978.
167. MOTOYAMA, E. K., ORZALESI, M. M., KIKKAWA, Y., KAIBARA, M., WU, B., ZIGAS, C. J., AND COOK, C. D.: Effect of cortisol on the maturation of fetal rabbit lungs. Pediatrics **48:** 547–555, 1971.
168. MULLON, D. K., SMITH, Y. F., RICHARDSON, L. L., HAMOSH, M., AND HAMOSH, P.: Effect of prolactin on phospholipid synthesis in organ cultures of fetal rat lung. Biochim. Biophys. Acta **751:** 166–174, 1983.
169. NARDONE, L. L., AND ANDREWS, S. B.: Cell line A549 as a model for the type II pneumocyte. Phospholipid biosynthesis from native and organometallic precursors. Biochim Biophys. Acta **573:** 276–295, 1979.
170. NICHOLAS, T. E., AND BARR, H. A.: Control of release of surfactant phospholipids in the isolated perfused rat lung. J. Appl. Physiol. **51:** 90–98, 1981.
171. NICHOLAS, T. E., LUGG, M. A., AND JOHNSON, R. G.: Maternal administration of salbutamol (S) and labetalol (L) increases the amount of alveolar surfactant in lung of the day 27 fetal rabbit (Abstract). Proc. Aust. Physiol. Pharmacol. Soc. **9:** 146P, 1978.
172. OLSEN, D. B.: Neurohumoral-hormonal secretory stimulation of pulmonary surfactant in the rat (Abstract). Physiologist **15:** 230, 1972.
173. O'NEIL, J. J., AND YOUNG, S. L.: Tissue slices in the study of lung metabolism. *In* Lung Development: Biological and Clinical Perspectives, ed. by P. M. Farrell, vol. I, Biochemistry and Physiology, ch. 5, pp. 87–99, Academic Press, New York, 1982.
174. ORZALESI, M. M., MOTOYAMA, E. K., JACOBSON, H. N., KIKKAWA, Y., REYNOLDS, E. O. R., AND COOK, C. D.: The development of the lungs of lambs. Pediatrics **35:** 373–381, 1965.
175. OULTON, M.: The role of centrifugation in the measurement of surfactant in amniotic fluid. Am. J. Obstet. Gynecol. **135:** 337–343, 1979.
176. OYARZÚN, M. J., AND CLEMENTS, J. A.: Ventilatory and cholinergic control of pulmonary surfactant in the rabbit. J. Appl. Physiol. **43:** 39–45, 1977.
177. OYARZÚN, M. J., AND CLEMENTS, J. A.: Control of lung surfactant by ventilation, adrenergic mediators and prostaglandins in the rabbit. Am. Rev. Respir. Dis. **117:** 879–891, 1978.
178. PATTLE, R. E.: Properties, function and origin of the alveolar lining layer. Nature (Lond.) **175:** 1125–1126, 1955.
179. PATTLE, R. E.: The lung surfactant: An introduction. J. Reprod. Fert. Suppl. **23:** 645–650, 1975.
180. PFEIFFER, D. R., TAYLOR, R. W., AND LARDY, H. A.: Ionophore A23187: Cation binding and transport properties. Ann. N.Y. Acad. Sci. U.S.A. **307:** 402–423, 1978.
181. PICKEN, J., LURIE, M., AND KLEINERMAN, J.: Mechanical and morphologic effects of long-term corticosteroid administration on the rat lung. Am. Rev. Respir. Dis. **110:** 746–753, 1974.
182. POST, M., BATENBURG, J. J., AND VAN GOLDE, L. M. G.: Effects of cortisol and thyroxine on phosphatidylcholine and phosphatidylglycerol synthesis in adult rat lung alveolar type II cells in primary culture. Biochim. Biophys. Acta **618:** 308–317, 1982.
183. POST, M., SCHUURMANS, E. A. J. M., BATTENBURG, J. J. AND VAN GOLDE, L. M. G.: Mechanisms involved in the synthesis of disaturated phosphatidylcholine by alveolar type II cells isolated from adult rat lung. Biochim. Biophys. Acta **750:** 68–77, 1983.
184. PYSHER, T. J., KONRAD, K. D., AND REED, G. B.: Effect of hydrocortisone and pilocarpine on fetal rat lung explants. Lab. Invest. **37:** 588–594, 1977.
185. ROBERTSON, B.: Current and counter-current theories on lung surfactant. Scand. J. Respir. Dis. **57:** 199–207, 1976.
186. ROONEY, S. A.: Biochemical development of the lung. *In* The Biological Basis of Reproductive and Developmental Medicine, ed. by J. B. Warshaw, Elsevier, pp. 239–287, New York, 1983.
187. ROONEY, S. A., CANAVAN, P. M., AND MOTOYAMA, E. K.: The identification of phosphatidylglycerol in the rat, rabbit, monkey and human lung. Biochim. Biophys. Acta **360:** 56–67, 1974.
188. ROONEY, S. A., AND GOBRAN, L. I.: Alveolar lavage and lavaged lung tissue phosphatidylcholine composition during fetal rabbit development. Lipids **12:** 1050–1054, 1977.
189. ROONEY, S. A., GOBRAN, L. I., AND WAI-LEE, T. S.: Stimulation of surfactant production by oxytocin-induced labor in the rabbit. J. Clin. Invest. **60:** 754–759, 1977.
190. ROONEY, S. A., NARDONE, L. L., SHAPIRO, D. L., MOTOYAMA, E. K., GOBRAN, L. I., AND ZAEHRINGER, N.: The phospholipids of rabbit type II alveolar epithelial cells: Comparison with lung lavage, lung tissue, alveolar macrophages and a human alveolar tumor cell line. Lipids **12:** 438–442, 1977.
191. ROONEY, S. A., PAGE-ROBERTS, B. A., AND MOTOYAMA, E. K.: Role of lamellar inclusions in surfactant production: Studies on phospholipid composition and biosynthesis in rat and rabbit lung subcellular fractions. J. Lipid Res. **16:** 418–425, 1975.
192. ROONEY, S. A., AND WAI-LEE, T. S.: Cholinephosphatetransferase from rabbit lung microsomes. An improved assay and specificity towards exogenous diacylglycerols. Lung **154:** 201–211, 1977.
193. ROONEY, S. A., WAI-LEE, T. S., GOBRAN, L. I., AND MOTOYAMA, E. K.: Phospholipid content, composition and biosynthesis during fetal lung development in the rabbit. Biochim. Biophys. Acta **431:** 447–458, 1976.
194. RYAN, U. S., RYAN, J. W., AND SMITH, D. S.: Alveolar type II cells: Studies on the mode of release of lamellar bodies. Tissue & Cell **7:** 587–599, 1975.
195. SANDERS, R. L.: The chemical composition of the lung. *In* Lung Development: Biological and Clinical Perspectives, ed. by P. M. Farrell, vol. I, Biochemistry and Physiology, ch. 11, pp. 179–192, Academic Press, New York, 1982.
196. SANDERS, R. L., HASSETT, R. J., AND VATTER, A. E.: Isolation of lung lamellar bodies and their conversion to tubular myelin figures in vitro. Anat. Rec. **198:** 485–501, 1980.
197. SCARPELLI, E. M.: The Surfactant System of the Lung, Lea & Febiger, Philadelphia, 1968.
198. SCARPELLI, E. M., CONDORELLI, S., COLACICCO, G., AND COSMI, E. V.: Lamb fetal pulmonary fluid. II. Fate of phosphatidylcholine. Pediatr. Res. **9:** 195–201, 1975.
199. SHAPIRO, D. L., NARDONE, L. L., ROONEY, S. A., MOTOYAMA, E. K., MUNOZ, J. L.: Phospholipid biosynthesis and secretion by a cell line (A549) which resembles type II alveolar epithelial cells. Biochim. Biophys. Acta **530:** 197–207, 1978.
200. SHELDON, G., BRAZY, J., TUGGLE, B., CRENSHAW, C., AND BRUMLEY, G.: Fetal lamb lung lavage and its effect on lung phosphatidycholine. Pediatr. Res. **13:** 599–602, 1979.
201. SHEPARD, J. W., DOLAN, G. F., AND YU, S. Y.: Factors regulating lamellar body volume density of type II pneumocytes in excised dog lungs. J. Appl. Physiol. **53:** 555–562, 1982.
202. SMITH, B. T.: Cell line A549: A new model system for the study of alveolar type II cell function. Am. Rev. Respir. Dis. **115:** 285–293, 1977.
203. SMITH, B. T.: Lung maturation in fetal rat: Acceleration by injection of fibroblast pneumocyte factor. Science **204:** 1094–1095, 1979.
204. SMITH, B. T., AND BOGUES, W. G.: Effects of drugs and hormones on lung maturation in experimental animal and man. Pharmacol. Ther. **9:** 51–74, 1980.
205. SMITH, D. M., SHELLEY, S. A., AND BALIS, J. U.: Maturation of rabbit fetal lung following maternal administration of pilocarpine. Am. J. Anat. **154:** 163–177, 1979.
206. SOMMERS-SMITH, S. K., AND GIANNOPOULUS, G.: Beta-adrenergic receptor binding in isolated fetal, neonatal and adult type II cells (Abstract). Fed. Proc. **40:** 407, 1981.
207. SPALDING, J. W., ORTNER, M. J., TOMBROPOULOS, E. G., GILMORE, L. B., AND HOOK, G. E. R.: Isolation and characterization of rabbit lamellar bodies. Exp. Lung Res. **4:** 171–190, 1983.
208. STAHLMAN, M. T., GRAY, M. E., LIEW, S., AND CHYTIL, F.: The role of cyclic AMP in lamellar body synthesis and secretion (Abstract). Pediatr. Res. **8:** 470, 1974.
209. STRANG, L. B.: Neonatal Respiration: Physiological and Clinical Studies, 316 pp., Blackwell Scientific, Oxford, 1977.
210. STRATTON, C. J.: Multilamellar body formation in mammalian lung: An ultrastructural study utilizing three lipid-retention procedures. J. Ultrastruct. Res. **52:** 309–320, 1975.
211. STRATTON, C. J.: The three-dimensional aspect of mammalian lung multilamellar bodies. Tissue & Cell **8:** 693–712, 1976.
212. STRATTON, C. J.: The high resolution ultrastructure of the periodicity and architecture of lipid-retained and extracted lung multilamellar body lamination. Tissue & Cell **8:** 713–725, 1976.
213. STRATTON, C. J.: The three-dimensional aspect of the mammalian lung surfactant myelin figure. Tissue & Cell **9:** 285–300, 1977.
214. SUNDELL, H. W., GRAY, M. E., SEVENIUS, F. S., ESCOBEDO, M. B., AND STAHLMAN, M. T.: Effects of epidermal growth factor on lung maturation in fetal lambs. Am. J. Pathol. **97:** 393–410, 1980.
214a. TAEUSCH, H. W., BROWN, N. E., TORDAY, J. S., AND NIELSON, H. C.: Magnitude and duration of lung response to dexamethasone in fetal sheep. Am. J. Obstet. Gynecol. **140:** 452–455, 1981.
215. TAEUSCH, H. W., WYSZOGRODSKI, I., WANG, N. S., AND AVERY, M. E.: Pulmonary pressure-volume relationships in premature, fetal and newborn rabbits. J. Appl. Physiol. **37:** 809–813, 1974.
216. TAYLOR, L., POLGAR, P., MCATEER, J. A., AND DOUGLAS, W. H. J.: Prostaglandin production by type II alveolar epithelial cells. Biochim. Biophys. Acta **572:** 502–509, 1979.
217. THOMAS, T., AND RHOADES, R. A.: Incorporation of palmitate-1-^{14}C into lung tissue and alveolar lecithin. Am. J. Physiol. **219:** 1535–1538, 1970.
218. TOOLEY, W., GARDNER, R., THUNG, N., AND FINLEY, T.: Factors affecting the surface tension of lung extracts (Abstract). Fed. Proc. **20:** 428, 1961.
219. TORDAY, J. S., CARSON, L., AND LAWSON, E. E.: Saturated phosphatidylcholine in amniotic fluid and prediction of respiratory-distress syndrome. N. Engl. J. Med. **301:** 1013–1018, 1979.
220. TORDAY, J. S., AND NIELSON, H. C.: Surfactant phospholipid ontogeny in

fetal rabbit lung lavage and amniotic fluid. Biol. Neonate **39:** 266–271, 1981.
221. TRIFARO, J. M.: Common mechanisms of hormone secretion. Annu. Rev. Pharmacol. **17:** 27–47, 1977.
222. VAN GOLDE, L. M. G.: Metabolism of phospholipids in the lung. Am. Rev. Respir. Dis. **114:** 977–1000, 1976.
223. VON NEERGAARD, K.: Neue Auffassungen uber einen Grundbegriff der Atemmechanik die Retraktion-Skraft der lunge abhangig von der Oberflackenspannung in den Alveolen. Z. Gesamte Exp. Med. **66:** 373–394, 1929.
224. WANG, N. S., HUONG, S. N., SHELDON, H., AND THURLBECK, W. M.: Ultrastructural changes of Clara cells and type II alveolar cells in adrenalin-induced pulmonary edema in mice. Am. J. Pathol. **62:** 237–252, 1971.
225. WANG, N. S., KOTAS, R. V., AVERY, M. E., AND THURLBECK, W. M.: Accelerated appearance of osmiophilic bodies in fetal lungs following steroid injection. J. Appl. Physiol. **30:** 362–365, 1971.
226. WEINSTOCK, M., ZAVADIL, P., ROSIN, A. J., CHIUEH, C. C., AND KOPIN, I. J.: The role of peripheral catecholamines in oxotremorine tremor in the rat and its antagonism by beta adrenoceptor blocking agents. J. Pharmacol. Exp. Ther. **206:** 91–96, 1978.
227. WHITSETT, J. A., MANTON, M. A., DAROVEC-BECKERMAN, C., ADAMS, K. G., AND MOORE, J. J.: β-adrenergic receptors in the developing rabbit lung. Am. J. Physiol. **240:** E351–E357, 1981.
228. WHITTLE, M. J., HILL, C. M., AND HARKES, A.: Effect of labour on the lecithin/sphingomyelin ratio in serial samples of amniotic fluid. Br. J. Obstet. Gynecol. **84:** 500–503, 1977.
229. WILLIAMS, M. C.: Development of the alveolar structure of the fetal rat in late gestation. Fed. Proc. **36:** 2653–2659, 1977.
230. WILLIAMS, M. C., AND MASON, R. J.: Development of the type II cell in the fetal rat lung. Am. Rev. Respir. Dis. **115:** 37–47, 1977.
231. WYSZOGRODSKI, I., TAEUSCH, H. W., KYEI-ABOAGYE, K., AND AVERY, M. E.: Mechanical regulation of alveolar surfactant in adult cats: The effects of hyperventilation and end-expiratory pressure in vivo. Chest **67:** suppl. 2, 15–16S, 1975.
232. WYSZOGRODSKI, I., KYEI-ABOAGYE, K., TAEUSCH, H. W., AND AVERY, M. E.: Surfactant inactivation by hyperventilation: conservation by end-respiratory pressure. J. Appl. Physiol. **38:** 461–466, 1975.
233. WYSZOGRODSKI, I., AND TAEUSCH, H. W.: Prolonged sympathetic activation and pulmonary surfactant: Absence of effect in anaesthetised cats. Am. Rev. Respir. Dis. **111:** 619–622, 1975.
234. WYSZOGRODSKI, I., TAEUSCH, H. W., AND AVERY, M. E.: Isoxsuprine-induced alterations of pulmonary pressure-volume relationships in premature rabbits. Am. J. Obstet. Gynecol. **119:** 1107–1111, 1974.
235. YONEDA, K.: Stimulation of the bronchiolar Clara cell secretion by pilocarpine. Lab. Invest. **37:** 447–452, 1977.
236. YOUNG, S. L., KREMER, S. A., APPLE, J. S., CRAPO, J. D., AND BRUMLEY, G. W.: Rat lung surfactant kinetics: Biochemical and morphometric correlation. J. Appl. Physiol. **51:** 248–253, 1981.
237. YOUNG, S. L., AND TIERNEY, D. F.: Dipalmitoyl lecithin secretion and metabolism by the rat lung. Am. J. Physiol. **222:** 1539–1544, 1972.
238. ZELIGS, B. J., NERURKAR, L. S., BELLANTI, J. A., AND ZELIGS, J. D.: Maturation of the rabbit alveolar macrophage during animal development. I. Perinatal influx into alveoli and ultrastructural differentiation. Pediatr. Res. **11:** 197–208, 1977.