A REPORT ON THE SURFACTANT SYSTEM OF THE LUNG

by

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I. INTRODUCTION

In the past 10-15 years, basic biological research has progressed greatly in defining the pulmonary surfactant system. The reason for specialized focus on this system is because it plays a particularly significant role in the survival of newborn infants. The lack of pulmonary surfactant is intimately linked to the respiratory distress syndrome (RDS) in infants which is also called the hyaline membrane disease (HMD). This disease appears to be the cause of 30% of all neonatal deaths and 50-70% of premature infant deaths (Crofton and Douglas, 1969). According to Northway and Daily (1972), 25% of "liveborn premature infants," and 1-2% of all newborn infants develop this disease. Because this disease accounts for such a large portion of infant deaths, scientists are involved in research which may allow better treatment and perhaps prevention of this disease.

The basic cause of this disease is not known. However, it appears as though the "maturity" of the pulmonary surfactant system at birth is an important factor in this disease. From the statistics cited above, it appears as if prematurity predisposes an infant to develop this disease. As will be shown in this report, in premature animals the surfactant system is not fully developed. However, treatment with corticosteroids appears to accelerate the development of the system and may prolong survival of animals (Avery, 1973).

Avery (1973) constructed a table from various sources showing things which are known for certain, or are probable, or are possible in relation to this disease. A few things which are known with certainty will be mentioned here. The disease occurs near the time of birth, and death or recovery occurs in 3-5 days. Reduced lung compliance (elasticity), low systemic blood pressure, reduced effective pulmonary blood flow, and cyanosis are additional characteristics. Pathological observations include regions of atelectasis (collapse) of alveoli, and formation of hyaline membranes. The hyaline membrane lines the alveoli and is made up of "sloughed cell debris in a protein matrix" (Crofton and Douglas, 1969). These membranes contain fibrins (Avery, 1973). The etiology of the disease is definitely linked to surfactant deficiency (Avery, 1973). Evidence for this comes from the "effectiveness of continuous distending airway pressure" (Avery, 1973). These and other clinical and pathological symptoms are characteristic of this disease. The general consensus appears to be that alveolar collapse due to a lack of pulmonary surfactant is a primary factor of the disease. Bates et al. (1971) (citing various sources) gave possible causes of this disease which include "asphyxia, hypoperfusion of the pulmonary vasculature, or a fibrinolytic-enzyme defect."

This report will focus on the pulmonary surfactant system itself. Four basic topics will be covered. The first topic will mainly define the system which includes the

composition, structure, and synthesis of surfactant. Secondly, the discussion will center on the type II cell and model systems. The third topic will deal with varicus treatments of animals and/or lung tissues in order to determine what agents or conditions might control surfactant synthesis and secretion. A discussion of proposed research will be the fourth topic. Due to the rather broad scope of this paper, an attempt will be made to cover significant points about this subject. The reader should keep in mind that this report, as with any publication, is limited by the author's biases toward certain information and is limited to this author's knowledge of the field. Even though some portions of this subject are covered superficially, the report should provide a thorough background for anyone interested in studying the pulmonary surfactant system.

References for Introduction

Avery, M.E. (1973) "Respiratory Distress Syndrome: State of the Art," In: Respiratory Distress Syndrome (Villee, C.A., Villee, D.B., and Zuckerman, J., eds.) Academic Press, Inc., New York, pp. 1-6.

Bates, D.V., Macklem, P.T., Christie, R.V. (1971) In: Respiratory Function in Disease, W.B. Saunders Co., Philadelphia, pp. 462-463.

Crofton, J. and Douglas, A. (1969) In: Respiratory Diseases, Blackwell Scientific Publications Ltd., Oxford and Edinburgh, Great Britain, pp. 591-592.

Northway, W.H., Jr., and Daily, W.J.R. (1972) "Pulmonary Disorders of the Perinatal Period," In: Bronchopulmonary Diseases and Related Disorders, Vol. I (Holman, C.W., and Muschenheim, C., eds.) Harper and Row, Hagerstown, Maryland, pp. 210-217.

II. LUNG DEVELOPMENT

Before plunging into a discussion of pulmonary surfactant, lung development will be briefly outlined. This will provide some orientation as to the time and place of the events leading up to and following surfactant production.

Lung development as described here will be very general. Development will be discussed mainly in terms of the human lung. (Most of the material here comes from books or review articles). Most animal lungs share the same general pattern of development with some variation (Williams, 1977). Engel (1962) has compared the alveolar diameter in lungs of different species (see Table 1) and he found that the diameter range is very small even though they differ greatly in body size. He (Engel, 1962) also noted that rat and mouse lungs have "true acinar structure," but the mouse acini are primitive. Engel (1962) stated that the lungs of mice and rats did not require a bronchial tree due to their small size, and termed their branched structure as a "bronchiolar tree."

In humans, the lung first appears as a shallow groove in the ventral wall of the foregut, just cephalad to the liver and stomach, by 22-26 days after fertilization (5mm long embryo) (Strang, 1977). The lung epithelial lining cells are derived from endoderm, and the "auxilliary" tissues such as pleura, muscle, cartilage, and blood vessels are mesodermal and mesenchymal derivatives (Williams, 1977). Pulmonary elastic tissue and lymphatic tissue are also derived from mesenchyme (Emery, 1969a). In humans, the caudal end of the groove froms a single small pouch which later divides (Emery, 1969a; Strang, 1977). However, in the mouse, there are initially two separate lung buds which later fuse at their bases forming a "primary branch point" (Spooner and Wessells, 1970). Later, the gut tube gives rise to the dorsal esophagus and ventral trachea at the primary branch point (Spooner and Wessells, 1970). The epithelial component of these buds form a sheet of cells which grow more rapidly than the surrounding mesoderm, and branch to form the bronchial tree (Strang, 1977). The branching pattern of lungs depends on the interaction of epithelium and its surrounding mesoderm (Williams, 1977; Strang, 1977). For a short review of how mesoderm, and collagen produced by the mesoderm, influence lung branching, see the review by Strang (1977). The formation of alveoli is the final and one of the most important steps in lung development. The type II alveolar epithelial cell, believed to produce surface-active material which prevents collapse of alveoli in the air-breathing infant, appears at various stages of development depending on the animal studied. Osmiophilic inclusion bodies (OIB's), presumably the surfactant storage sites in type II cells, appear at the following times:

18 days in the mouse fetus (19 days = term)
120 days in the lamb fetus (147 days = term)
24 days in the rabbit fetus (31 days = term)

22-26 weeks in the human fetus (40 weeks = term) (Strang, 1977).

Much alveolar growth apparently occurs postnatally in humans but some alveolar ducts, alveolar sacs, and alveoli appear before birth (or term) (Northway and Daily, 1972; Emery, 1969b).

Lung development is generally broken down into three stages: 1) The glandular period - characterized by respiratory tubules lined by tall columnar epithelia, unassociated with capillaries (Williams, 1977). This stage occurs up to about the fourth month of human fetal gestation (Emery, 1969a). 2) The canalicular period - characterized by respiratory tubules with broad lumens becoming "apposed to the capillary network," according to Williams (1977). Emery (1969a) described this as a period during which bronchi are dividing, sometimes "dichotomously," and often monopodially. There is increased vascularization but capillaries are not yet "intimately" in contact with the epithelium. 3) The alveolar period - type I cells apparently form thin cytoplasmic extensions which are in contact with capillaries to form "attenuated blood-gas barriers" (Williams, 1977). In humans, this period begins around six months (Emery, 1969a) and alveolarization continues through to postnatal life (Northway and Daily, 1972; Emery, 1969b). For a view of human lung anatomy at the cellular level (electron microscopic) throughout development see Conen and Balis (1969).

Table 1. (Engel, 1962)

Animal	Alveolar Diameter
Mouse	0.005
Rat	0.1
Guinea Pig	0.05
Rabbit	0.1
Goat	0.07
Man	0.2
Elephant	0.25

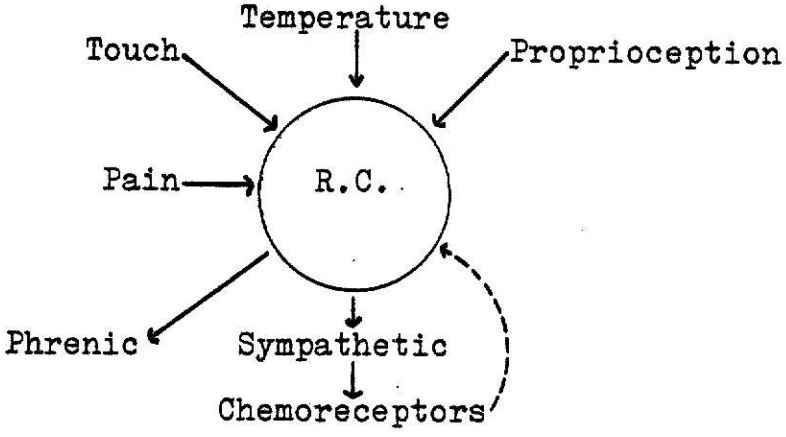
The factors which control the onset of respiration are not fully understood. During delivery, lung fluid is expelled and the remaining fluid in the lungs is thought to be removed through the lymphatic (Northway and Daily, 1972; Strang, 1977) and capillary circulations (Northway and Daily, 1972). Purves (1967) has performed studies on the initiation of respiration. Figure 1 after his drawing (Purves, 1967) diagrams the proposed events at the onset of respiration. The hypothesis involves chemoreceptors in the regulation of breathing. A number of stimuli apparently activate the respiratory center of the brain at birth, then the sympathetic activity results in activation of the chemoreceptors (Purves, 1967). The review by Strang (1977) provides more up-to-date information on the initiation and control of neonatal breathing

which includes evidence that chemoreceptors are depressed in the fetus.

The whole of lung development is much more complex than presented here. However, it gives one an idea of where the events of surfactant production fit in to the overall pattern of development. This report will deal mainly with the activities of alveolar type II cells and the surface-active material which these cells produce.

Figure 1. (after Figure I from Purves, 1967)

Sequence of Events at Onset of Respiration



"The respiratory centre (R.C.) is activated by sensory impulses from the periphery which, in turn, causes rhythmic activity in phrenic and efferent sympathetic nerves. One consequence of this may be activation of the peripheral chemoreceptors."

(Note: Much of the material used in the introduction was derived from reviews and books - original papers were not cited here).

References for Lung Development

Conen, P.E., and Balis, J.U. (1969) "Electron Microscopy in Study of Lung Development" In: The Anatomy of the Developing Lung (Emery, J., ed.) Wm. Heinemann Medical Books Ltd. in Assoc. with Spastics International Medical Publications, Printed in England, pp.18-48.

Emery, J. (1969a) "Embryogenesis" In: The Anatomy of the Developing Lung (Emery, J., ed.) Wm. Heinemann Medical Books Ltd. in Assoc. with Spastics International Medical Publications, Printed in England, pp. 1-7.

Emery, J. (1969b) "The Postnatal Development of Alveoli" In: The Anatomy of the Developing Lung (Emery, J., ed.) Wm. Heinemann Medical Books Ltd. in Assoc. with Spastics International Medical Publications, Printed in England, pp. 8-17.

Engel, S. (1962) In: Lung Structure, Charles C. Thomas, Springfield, Ill., pp. 227-234.

Northway, W.H., Jr., and Daily, W.J.R. (1972) "Pulmonary Disorders of the Perinatal Period," In: Bronchopulmonary Diseases and Related Disorders, Vol. I (Holman, C.W., and Muschenheim, C., eds.) Harper and Row, Hagerstown, Maryland, pp. 210-217.

Purves, M.J. (1967) "Initiation of Respiration" In: Development of the Lung - Ciba Foundation Symposium (deReuck, A.V.S., and Porter, R., eds.) Little, Brown, and Co., Boston, pp. 317-325.

Spooner, B.S., and Wessells, N.K. (1970) J. Exp. Zool. 175: 445-454.

Strang, L.B. (1977) Ann. Rev. Physiol. 39: 253-276.

Williams, M.C. (1977) Fed. Proc. 36: 2653-2659.

III. DEFINING THE SURFACTANT SYSTEM

In the past several years, the biochemical make-up of pulmonary surfactant has been studied carefully. Pulmonary surfactant is a combination of mainly lipid and protein molecules (Farrell and Avery, 1975) which are secreted by the type II alveolar epithelial cell during late gestation in most experimental animals, as will be seen in future sections (OIB's occur around mid-gestation in humans - see Strang (1977) from the development section). The purpose of the surfactant appears to be that of lowering surface tension at the air-liquid interface in the alveoli, thus preventing their collapse (King, 1974).

In this section of the report, the phospholipid and protein compositions of adult lung surfactant will be discussed, with brief attention to carbohydrate composition. A monograph written by Clements and King (1976) provides a good overview of the lipid and carbohydrate composition but emphasizes the protein composition. After establishing the composition, proposed structures for intracellular and extracellular surfactant will be covered. Finally, ideas on the synthesis (including enzyme and lipid profiles during development) of surfactant will be presented.

A. Phospholipid Composition of Adult Lung

The isolation and identification of surface-active material from lung washings has made it possible to determine the content of pulmonary surfactant itself. Lung wash fractions with surface tension lowering capabilities appear to be primarily lipid in nature and, as will be seen in the protein section, there may be specific proteins associated with surfactant (King, 1974).

According to a reveiw by Farrell and Avery (1975) surfactant is composed of 85% lipid, 13% protein, <1.7% hexose, <0.7% nucleic acid, and <0.5% hexosamine. Phosphatidylcholine (PC) makes up 75% of the surfactant lipid (Farrell and Avery, 1975). This class of lipids has been sutdied to a much greater extent than the other lipid classes of surfactant. Other phospholipids include phosphatidylehtanolamine (PE), sphingohyelin (SP), and lysolecithin (lyso-PC) which make up 6.3, 2.1, and 0.9% of surfactant lipid, respectively (Farrell and Avery, 1975). Neutral lipids account for 5-13% of surfactant lipid, which is mostly cholesterol (King, 1974). For an example of the surfactant components from canines, see Figure 2.

The following material was derived from a few studies of the surfactant lipid composition. They yield examples of the types of data obtained when analyzing surfactant lipids. Many studies similar to these have been performed.

Because phospholipids are the primary lipid component of surfactant, most of the material presented in this section

will deal with this class of lipids. The phospholipids will be viewed from three different levels - phospholipid classes, degree of saturation and unsaturation as indicated by fatty acid (FA) composition, and molecular classes. The biological half-life of these phospholipids will also be covered. It is important to note that the information found in the following studies is derived from adult animals. A discussion on the synthesis of these lipids especially during development, will be found in the synthesis section of this report.

Table 2 contains data on the surfactant phospholipids found in various cell fractions. Compositions of the alveolar wash (extracellular surfactant) and lamellar body (surfactant storage sites) fractions are considered to be indicative of surfactant. It is interesting to note that some of the minor surfactant components such as SP, PS, and PE are found in greater relative abundance in other cell fractions.

Phosphatidylcholine is the most abundant phospholipid in surfactant or in any other lung fraction studied (Hallman and Gluck, 1975; Jobe et al., 1978a). In general, PG appears to be the next most abundant phospholipid of surfactant based on a comparison of alveolar wash and lamellar body fractions (Hallman and Gluck, 1975; Jobe et al., 1978a). (Note: PI ≅ PG in alveolar wash from the Jobe et al., 1978a) study). PE appears to rank third based on the same comparison (Hallman and Gluck, 1975) or PI may rank third according to Jobe et al. (1978a). Both PI and PE comprise a very similar percentage of lamellar body and alveolar wash phospholipid (Hallman and Gluck, 1975; Jobe et al., 1978a). Bis-(monoacylglyceryl)-phosphate, cardiolipin, PS, lyso-PC, phosphatidic acid, and SP make up the rest of surfactant phospholipids as far as the studies cited in Table 2 show.

Figure 2. (after Figure 2 from Clements, 1973)

Composition of Canine Surfactant

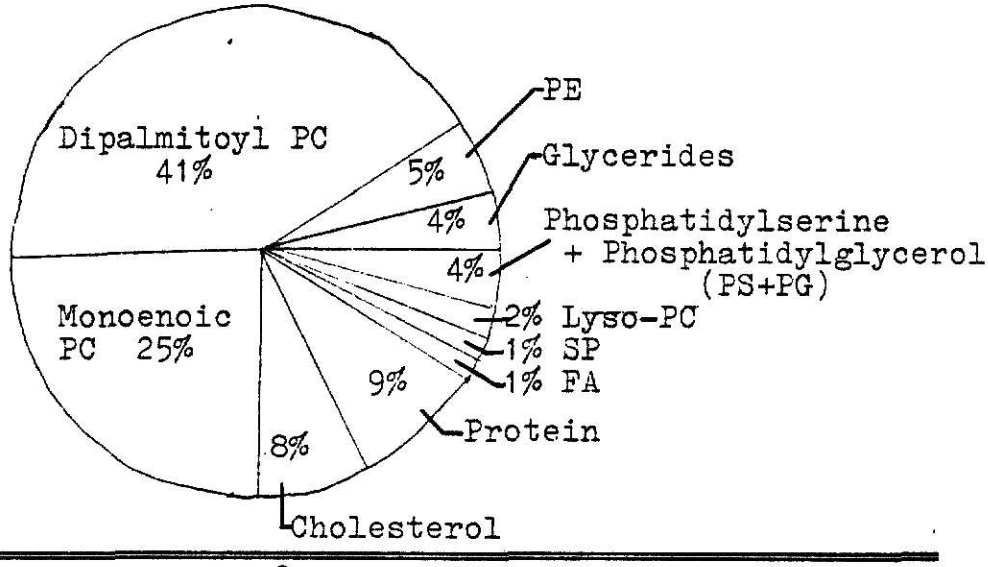


Table 2. Phospholipid Composition in Subcellular Fractions of Lung (Note: Applies to Adult Lungs)

Phospholipid	Lung Homogenate	Microsomes (endoplasmic reticulum)	Lamellar bodies	Alveolar wash	Mitochondria	Ref.
Phosphatidyl-choline (PC)	0.06 μmol/ mg protein	0.17 μmol/ mg protein	2.6 μmol/ mg protein	7 μmol	0.19 μmol/ mg protein	a
	11.01 ± 1.16 μmol/ g wet tissue	---	---	---	---	b
	57.5 ± 1.4% *	41.9 ± 1.7% **	77.0 ± 1.7% ***	75.5 ± 1.7% ****	44.8 ± 1.8% *****	c
	52.5 ± 2.0% *	51.8 ± 4.0% **	86.0 ± 5.1% ***	83.8 ± 2.2 ****	---	d
Phosphatidyl-glycerol (PG)	0.08 ± 0.07 μmol/g wet tissue	---	---	---	---	b
	3.3 ± 0.5% *	1.7 ± 0.3% **	11.2 ± 1.2% ***	11.0 ± 0.9% ****	1.7 ± 0.2% *****	c
	1.6 ± 0.5% *	1.2 ± 0.6% **	5.0 ± 1.1% ***	4.7 ± 1.9% ****	---	d
Bis-(monoacyl-glyceryl)-phosphate (Structural relative of PG)	1.0 ± 0.2% *	0.7 ± 0.2% **	1.5 ± 0.1% ***	1.7 ± 0.4% ****	0.5 ± 0.3% *****	c
Diphospha-tidylglycerol (cardiolipin)	1.4 ± 0.2% *	0.2 ± 0.1% **	0.3 ± 0.2% ***	0.1 ± 0.0% ****	7.3 ± 1.4% *****	c

Table 2. (Continued)

Phospholipid	Lung Homogenate	Microsomes (endoplasmic reticulum)	Lamellar bodies	Alveolar wash	Mitochondria	Ref.
Phosphatidyl-ethanolamine	5.29 ± 0.11 μmol/g wet tissue	---	---	---	---	b
	16.6 ± 0.7% *	24.6 ± 1.0% **	4.2 ± 0.2% ***	4.6 ± 0.2% ****	26.5 ± 1.4% *****	c
	18.4 ± 1.7% *	20.4 ± 5.3% **	3.5 ± 0.5% ***	3.6 ± 1.3% ****	---	d
Phosphatidyl-serine (PS)	9.3 ± 0.6% *	12.8 ± 1.0% **	1.2 ± 0.1% ***	1.9 ± 0.5% ****	8.9 ± 0.5% *****	c
	3.2 ± 0.9% *	4.7 ± 1.7% **	0.4 ± 0.1% ***	0.4 ± 0.2% ****	---	d
Phosphatidyl-inositol (PI)	0.93 ± 0.09 μmol/g wet tissue	---	---	---	---	b
	2.6 ± 0.4% *	3.1 ± 0.2% **	3.2 ± 0.2% ***	3.0 ± 0.4% ****	2.2 ± 0.1% *****	c
	3.1 ± 0.4% *	3.6 ± 0.8% **	4.2 ± 1.9% ***	5.2 ± 1.6% ****	---	d
Lyso-PC	0.5 ± 0.2% *	0.5 ± 0.2% **	0.4 ± 0.4% ***	0.2 ± 0.0% ****	0.0 ± 0.0% *****	c
	1.3 ± 0.6% *	not detected (ND)	0.7 ± 0.3% ***	ND	---	d
Phosphatidic acid (PA)	0.3 ± 0.1% *	0.8 ± 0.1% **	0.3 ± 0.3% ***	0.5 ± 0.2% ****	0.6 ± 0.1% *****	c

Table 2. (Continued)

Phospholipid	Lung Homogenate	Microsomes (endoplasmic reticulum)	Lamellar bodies	Alveolar wash	Mitochondria	Ref.
Sphingomyelin (SP)	7.5 ± 0.2% *	13.7 ± 0.6% **	0.7 ± 0.3% ***	1.5 ± 0.2% ****	7.5 ± 0.2% *****	c
	13.2 ± 2.3% *	10.8 ± 1.4% **	0.5 ± 0.4% ***	0.6 ± 0.4% ****	---	d
Diacylglycerol (not a phospholipid)	0.34 ± 0.03 μmol/g wet tissue					b

* % of phospholipids in homogenate
** % of phospholipids in microsomes
*** % of phospholipids in lamellar bodies
**** % of phospholipids in alveolar wash
***** % of phospholipids in mitochondria

Refs.
a - Jobe (1977)
b - Okano et al. (1978)
c - Hallman and Gluck (1975)
d - Jobe et al. (1978a)

A note about PG should be made here concerning its significance in surfactant. PG is second in relative abundance to PC as seen above. PG is also found in surfactant from other mammalian species (other references cited by Hallman and Gluck, 1975). PG is present in trace amounts in animal tissues, mostly in the mitochondria (Strickland and Benson, 1960; Gray, 1964; Ray et al., 1969 - from Hallman and Gluck, 1975). These observations led Hallman and Gluck (1975) to the conclusion that PG is a characteristic component of surfactant in adult animals. The importance, if any, of the structural relatives of PG such as bis-(monoacylglyceryl)-phosphate as found in lamellar bodies and alveolar wash is not known (Hallman and Gluck, 1975). Its presence in lavage has not been detected by some investigators (Pfleger et al., 1972 - from Hallman and Gluck, 1975). Further discussion on the significance of PG will be seen later in this discussion on lipids.

Table 3 shows the percentages of different fatty acids attached to the major phospholipids of surfactant and gives an overall percentage saturation of those phospholipids or of fatty acids in the various lung fractions.

Focusing again on the lamellar body and alveolar wash fractions, one can see that PC contains over 50% palmitic acid and around 67% of its fatty acids are saturated (Jobe et al., 1978a). In general, PC has the highest percentage of palmitic acid and highest percentage of saturated fatty acids compared to the other surfactant phospholipids (see Table 3). PG, the second most abundant phospholipid contained similar amounts of palmitic acid in the lamellar body and alveolar wash fractions, compared to PC (Hallman and Gluck, 1975; Jobe et al., 1978a). Fatty acids of PG were about 61% saturated in lamellar body and alveolar wash fractions (Jobe et al., 1978a). A relatively high percentage of palmitic acid was found in PG from mitochondria, also (Hallman and Gluck, 1975). PG found in mitochondria is thought to contribute to cardiolipin formation according to references cited by Hallman and Gluck (1975). Both PC and PG contained relatively high percentages of oleic acid (18:1) in the lamellar body and alveolar wash fractions (Jobe et al., 1978a; Hallman and Gluck, 1975). PC contained high amounts of linoleic (18:2) acid in these two fractions (Jobe et al., 1978a). PI contained about 42% saturated fatty acids in the lamellar body and alveolar wash fractions, and palmitic acid and oleic acid were the most abundant FA's of this phospholipid in these fractions (Jobe et al., 1978a). Arachidonic (20:4) acid was the most plentiful FA of PI in the lung homogenate and microsomal fractions (Jobe et al., 1978a). Stearic (18:0) acid was the major FA at the 1-position of PI and arachidonic at the 2-position (Okano et al., 1978) in lung homogenates. PI contained about 30% saturated FA's in the homogenate and microsomal fractions (Jobe et al., 1978a). PE plus PS had mostly oleic and linoleic FA's associated with them in the alveolar and lamellar body fractions (Jobe et al., 1978a). Large amounts of palmitoyl- and stearoyl-aldehydes were found at the 1-position of PE and mostly polyunsaturated FA's at the