PULMONARY SURFACTANT PHOSPHOLIPIDS IN INTERSTITIAL PULMONARY FIBROSIS

THESIS

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(CHEST DISEASES)

BY

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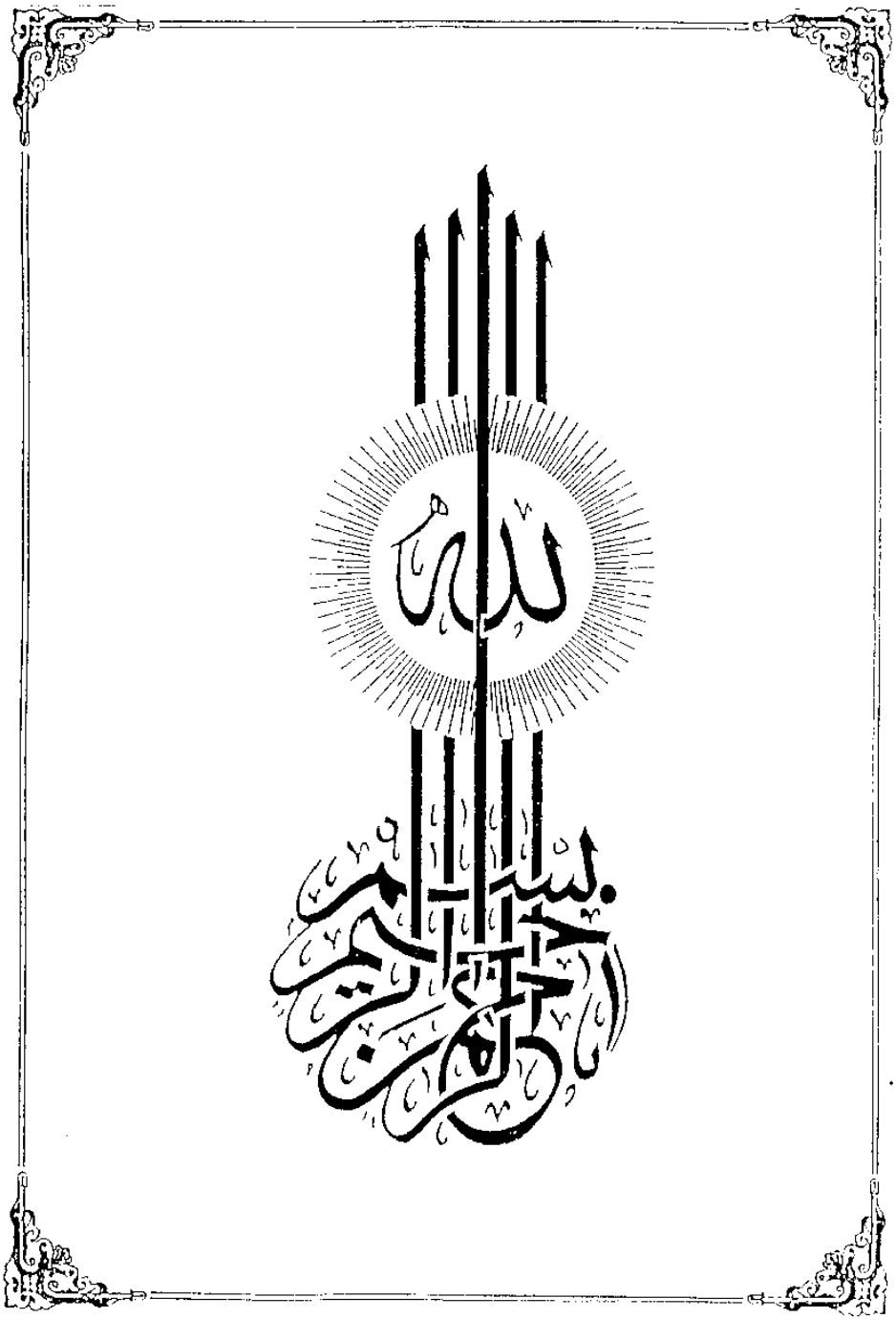

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INTRODUCTION
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INTRODUCTION:

A deficiency of pulmonary surface active material [Avery and Mead,1959] and more recently abnormalities in its composition [Hallman et al.,1977] have been demonstrated in infants with hyaline membrane disease. However,alterations in amount and composition of pulmonary surface active material in adult pulmonary diseases have been less well defined [Kikkaway and Smith,1983]. Alterations in composition and physical properties of surfactant have been reported in patients with the adult respiratory distress syndrome (ARDS) [Ashbaugh et al.,1967].

Hallman and co-workers,1982,reported that the phospholipids recovered by lavage from patients with adult respiratory distress syndrome (ARDS) had a lower percentage of phosphatidylglycerol and saturated phosphatidylcholine than did those from control subjects or from patients with other lung diseases. A decrease in total bronchoalveolar lavage fluid (BALF) phospholipids and abnormal phospholipid

composition, which include a decrease in phosphatidylglycerol and an increase in phosphatidylinositol, have been reported in a patient with bleomycin induced lung fibrosis [Baker et al.,1983].

Abnormalities in surface active material have also been demonstrated in animals with acute lung injury. In dogs injected with N-nitroso-N-methylurethane to produce acute lung injury,there was a decrease in the percentages of phosphatidylglycerol and phosphatidylinositol but not in percentage of saturated phosphatidylcholine to the total phosphatidylcholine and the change in lipids correlated with a decreased pulmonary compliance [Ryan et al.,1981].

Recently,Thrall and co-workers,1987,reported that in rats treated with bleomycin there was a decrease in the percentage of phosphatidylglycerol and an increase in the percentage of phosphatidylinositol in the lavage phospholipids. In rats treated with bleomycin,the total phospholipid recovered in lavage increased and the percentage of phosphatidylcholine that was saturated increased slightly.

Because histologic alterations in the alveolar epithelium occur in patients with I.P.F. [Kawanami et al.,1982],it is reasonable to speculate that the major secretory product of alveolar type II cells, surfactant,might be altered.

On the basis of the available data on abnormalities in the composition of surface active material in human and in animal after acute lung injury,it is reasonable to question if surfactant abnormalities occur in patients with intersitial lung fibrosis.

AIM OF THE WORK:

Estimation of total bronchoalveolar lavage phospholipids in patients with interstitial lung fibrosis as a marker of lung surfactant.

REVIEW OF LITERATURE

NORMAL ALVEOLAR STRUCTURE

In order to understand how alveolitis in interstitial disorders of unknown cause alters normal lung architecture,it is necessary to understand the inflammatory cells,parenchymal cells and matrix elements that are normal components of the alveolar walls. In the normal lung,there are approximately 80 effector cells per alveolus [Grapo et al.,1982]. These cells are located both within the alveolar interstitium and on the alveolar epithelial surface. More than 90% are alveolar macrophages [Reynolds and Newball,1974]. Although macrophages can replicate in the normal lung,most are derived from blood monocytes that migrate through the alveolar wall [Golde,1977].

In the normal lung,lymphocytes make up nearly all the remaining 10% (or less) of the effector cells [Hunninghake et al.,4,1979]. As a general rule,the distribution of lymphocyte subtypes in lung is similar to that in blood,although the lung has relatively more T cells and fewer B cells. Unlike mononuclear phagocytes and lymphocytes, polymorphonuclear leucocytes are present in very low proportions (less than 1%) [Hunninghake et al.,1981].

* Parenchymal Cells & Matrix of Normal Alveolar Wall:

The normal adult lung contains 300 * 10^6 alveoli, which are grape-like structures that branch off terminal bronchioles. Typical alveoli have an internal diameter of 200 to 300 Um and wall 5 to 10 Um in width [Grapo et al.,1982]. The walls are lined with a single layer of epithelial cell resting on a basement membrane (a thin continuous structure that contains type IV collagen),laminin (a glycoprotein that mediate the attachment of epithelial cells to type IV collagen) and other macromolecules including proteoglycans [Rennard and Crystal,1982].

One third of the epithelial cells are type I cells:pancake-like cells that cover more than 90% of the epithelial surface. The remainder are type II cells:cuboidal cells that secret the surface acting material that prevent alveolar collapse [Grapo et al.,1982].

The pulmonary capillaries form a branching net-work of tubes that weave through the interior of the alveolar walls. The capillaries comprise a single layer of endothelial cells lying on a continuous basement membrane. Although,the endothelial and

epithelial basement membranes are distinct structures, they are just at the location where the capillaries come closest to the airspace;these are the sites of gas exchange. The epithelial and the endothelial basement membranes define the boundaries of the alveolar interstitium,a region made up of fibroblasts and connective tissue matrix that together represent almost 50% of the tissue volume of the alveolar wall [Grapo et al.,1982].

In the normal lung,fibroblasts account for 37% of all parenchymal cells and occupy approximately two thirds of the volume of the interstitium. The most abundant interstitial matrix components are collagen type I and III,which are structural macromolecules normally present in a ratio of 2:1 [Sryer et al., 1976]. Fibrils composed mostly of type I collagen are thick,whereas fibrils rich in type III collagen are thinner [Rennard and Crystal,1982]. In addition to collagen, the interstitial matrix include elastin, proteoglycans,fibronectin [Rennard and Crystal,1982]. Although,the basement membranes determine the topography of their respective cell types,the fibroblasts and interstitial connective tissue matrix provide the structural frame-work that defines

alveolar shape and to a large extent the mechanical properties that modulate the volume-pressure characteristic of the lung during respiration.

* Pulmonary Surfactant:

The pulmonary surfactant consists of acellular material lining the lung alveoli and the cells that produce this material. since the type II epithelial cells are the only known source of surface active lipids (dipalmitoyl phosphatidylcholine (DPPC), phosphatidylglycerol (PG) and lung specific apoprotein), at present the surfactant system is defined to include both the alveolar lining layer and type II cells. The ability of the material to lower surface tension which would otherwise be generated by the presence of as fluid-gas interface in the alveolus has led to the designation "surfactant" [Yutaka and Fred,1983].

It is difficult to draw firm conclusions about the composition and metabolism of surfactant system and its elements from analysis of the whole lung tissue,since the type II cells and the alveolar lining layer do not comprise a major fraction of the total volume or mass of the lung. A cellular alveolar

lining material can be obtained by saline lavage of lung parenchyma through the airways,however the resulting sample is diluted many times over by the lavage medium and is contaminated by upper airway secretions and by components of the circulating blood [Reifenrath and Zimmerman,1973].

More gentle lavage procedures appear to minimize contamination from the blood [Skoza et al.,1983]. The elution pattern of instilled exogenous marker materials and endogenous enzymes indicate five successive lavage are sufficient to recover more than 90% of the phospholipids of the lining layer [Skoza et al.,1983].

A variety of techniques,most employing ultra-centrifugation,has been used to obtain fractions of lung lavage fluid which are highly enriched in surface activity. Such concentrates,frequently referred to in the literature as surfactant [Harwood et al.,1975],could likely differ from the native alveolar lining material,for a number of reasons. First,some constituents of the lining material might be destroyed or altered during the fractionation procedures. Second,constituents might not fractionate identically with other constituents. Third,extraneous

contamination might artificially bind to constituents of the lining material during fractionation [Shelley et al.,1975]. Fourth,supernatant contains some remaining surfactant lipids [Shelley et al.,1975].

Direct aspiration of lining material by alveolar micropuncture [Reifenrath and Zimmerman,1973], a sampling technique that theoretically overcomes these objections,yields such minute sample volumes that only major components can be reliably identified. Although,the later technique has been used only in experimental animal,lung lavage fluid can be obtained from humans at fiberoptic bronchoscopy [Low et al., 1978].

Inspite of its theoretical drawbacks,since there is no alternative practical method for recovering lining material in quantity,bronchoalveolar lavage remains one of the major tools in the study of pulmonary surfactant.

* Maturation of Surfactant System in Fetal Lung:

In general,in the lung of various mammalian species,cytology and biochemical maturation of the type II cell population closely parallels the