NITROBLUE TETRAZOLIUM TEST RESULTS IN THE DIAGNOSIS OF PLEURAL EFFUSIONS. R.V. Light and R.B. George. Department of Medicine, Louisiana State University, Shreveport, Louisiana.

The nitroblue tetrazolium (NBT) test was initially proposed as a test with which to identify bacterial infections. Although the NBT test on serum was subsequently found to be of little value because it was positive in many other conditions, the NBT test on synovial fluid is useful in identifying pyogenic arthritis. The purpose of this project was to evaluate the diagnostic utility of the NBT test on pleural fluid. Pleural fluids from 62 patients, including 9 with congestive heart failure (CHF), 27 with malignancy (CA), 9 with tuberculosis (TB), 14 with pneumonia (Pneu), and 3 with collagen vascular disease (CVD), were studied. The NBT score for each cell was semiquantitated on a scale of 0-3, the more positive, the higher the score. Cells in the pleural fluid were divided into 3 categories: polymorphonuclear leukocytes (PMN), including eosinophils and basophils, small lymphocytes, and mononuclear cells other than small lymphocytes. When possible, 100 cells in each category were counted. The total NBT score for a cell category was the sum of the individual scores for each of the cells. The means and standard deviations for the pleural fluid NBT scores were as follows:

CELL TYPE	Pneu	TB	CA	CVD	CHF
PMN	140 ± 73	198 ± 52	172 ± 107	293 ± 2	103 ± 16
Other MONO	129 ± 73	186 ± 84	162 ± 87	297 ± 2	199 ± 39
Small Lymph	45 ± 61	73 ± 91	49 ± 83	292 ± 4	6.8 ± 1.8

We conclude that the diagnostic utility of the NBT test on pleural fluid is limited. Many conditions other than infection are associated with high pleural fluid NBT scores. A very high score for all 3 cell categories is suggestive of collagen vascular disease.

THE DIAGNOSIS OF PULMONARY ALVEOLAR PROTEINOSIS WITHOUT LUNG BIOPSY. R.J. Martin,* R.M. Rogers, J.J. Coalson,* and D.C. Levin,* Dept. of Medicine and Dept. of Pathology, Univ. of Oklahoma Health Sciences Center and VA Hospital, Oklahoma City, Okla.

The clinical and radiographic findings in pulmonary alveolar proteinosis (PAP) are nondiagnostic and in the past the diagnosis has been established only by lung biopsy. The importance of establishing this diagnosis is that patients with PAP have a different prognosis and require different therapy than patients with other chronic diffuse lung diseases (DLD). We have previously reported that a shunt fraction (QS/QT) greater than 14% and an elevated serum LDH suggested the diagnosis of PAP (12 patients) as compared to other forms of DLD (35 patients). Both the QS/QT and LDH were significantly elevated in the PAP group as compared to the DLD group ($p < .001$). The purpose of this study was to determine if the diagnoses of PAP could be strengthened by combining these common laboratory studies with histologic findings from limited segmental lavage via the fiberoptic bronchoscope. In 8 PAP patients, the hematoxylin and eosin (H&E) sections showed: 1) relative absence of alveolar macrophages; 2) macrophages present had cytoplasmic eosinophilic particles; and 3) large proteinaceous aggregates interspersed with many small granules. None of these findings were seen in the control group. Periodic Acid Schiff (PAS) staining was not useful in differentiating the two groups as eosinophil material from normals stained positive. Our data suggest that the diagnosis of PAP can be made if the QS/QT is greater than 14%, the LDH is elevated and the specific H&E stained histologic findings are present in cell blocks from segmental lavage.

THE ACUTE EFFECTS OF TOBACCO SMOKE AND SYNTHETIC SMOKING MATERIALS ON PULMONARY ANTIBACTERIAL DEFENSES. V. Mohajan, G. Huber, G. Sorenberger, J. Shea*, N. Hinds* and M. First*, Dept. of Nel., Harvard Med. School and Dept. of Environ. Health Sci., HSHP, Boston, Massachusetts.

Epidemiologic association of tobacco cigarette consumption with cardiopulmonary disease has stimulated the development of low tar, nicotine-free synthetic smoking substitutes. Two synthetic smoking products, Cytrel and NSM, have recently been introduced as blends in the European cigarette trade. Because scant biologic evidence exists on the "safeness" of these products, we evaluated their effects on intrapulmonary and in vitro alveolar macrophage antibacterial ability relative to the effects of natural tobacco. Dosimetry subsequent to acute inhaled smoke exposure by male rats was monitored using smoke tracers, correlated with bacterial inactivation, and extrapolated to human smoking levels. Following an intrapulmonary challenge of *S. aureus* rats were exposed to smoke from each unfiltered material at six one-hour intervals, while macrophages lavaged from untreated animals were incubated with *S. albus* in graded amounts of ambient smoke of each type. Inhalation of tobacco smoke significantly impaired intrapulmonary antistaphylococcal activity (87% bacterial inactivation relative to controls) with exposure to Cytrel comparible to tobacco (86% inactivation) and NSM showing improvement but still significant impairment (97% inactivation). Cultured macrophages exhibited a dose-dependent depression in bactericidal ability comparable for all three products. Lack of correlation between depressed bactericidal function and inhaled smoke particulate deposition in all products suggested a gas phase cytotoxin. Potential increased human consumption of low tar smoking materials requires further evaluation of the chronic effects of correspondingly increased gas phase exposure and its potential modification for human smokers.

IMMUNOFLUORESCENT LOCALIZATION OF PURIFIED SURFACE ACTIVE MATERIAL IN RAT LUNG AND ISOLATED TYPE II CELLS. Robert Mason, Sarah Jones,* Bradley Benson,* Elias Gikas,* Mary Williams,* and John Clements*. Cardiovascular Research Institute, University of California, San Francisco.

The lipids of surface active material (SAM) are thought to be stored in the lamellar bodies of alveolar type II cells, but the cellular and subcellular localization of the proteins present in purified surface active material is much less certain. We extended the work of Klass (Am. Rev. Resp. Dis. 107:781, 1973) and localized SAM proteins (antigens) in whole lung and in isolated type II cells and macrophages by immunofluorescence. Rat SAM was purified by isopycnic centrifugation (Am. J. Physiol. 223:707, 1972) and was used to immunize rabbits. The antiserum was absorbed with rat serum and was purified by an immunoadsorbant column prepared with rat SAM. The indirect double antibody technique was used for antigen localization. Rabbit IgG was used as a control. In cryostat sections of whole lung, immunofluorescence was seen in the alveolar lumen, in cuboidal cells on the alveolar wall (type II cells), and in free cells in the alveolar lumen (alveolar macrophages). There was some fluorescence along small airways, but this fluorescence appeared to be on top of cells rather than within them. Isolated type II cells (Am. Rev. Resp. Dis. 115:1015, 1977) had diffuse cytoplasmic fluorescence. A few macrophages isolated by saline lavage showed intense fluorescence but most of them showed little.

The data are consistent with the hypothesis that the protein and the lipids of SAM are synthesized by type II cells and that part of the secreted material is ingested by macrophages. More precise cellular and subcellular localization will require purer antigens (individual proteins) and improved resolution (electron microscopic immunocytochemistry).

RESPIRATORY FUNCTION IN COPD PATIENTS DURING DIFFERENT STAGES OF SLEEP. R.J. Martin,* W.C. Orr,* C.B. Patterson, B.E. Pennock,* and R.M. Rogers. The Dept. of Medicine and Dept. of Psychiatry, Univ. of Oklahoma Health Sciences Center, and VA Hospital, Okla. City, Oklahoma.

The purpose of this study was to relate sleep patterns to arterial saturations (sat), arterial blood gases (ABG), apneas and arrhythmias in patients with COPD. Five patients with COPD (FEV_1 range .75-2.12, x=1.45) were studied at night. Continuous recordings were made of eye movements, genioglossus activity, ECG (V-5), electroencephalogram, expired CO_2, thoracic movement and sat (ear oximeter). A radial artery catheter was inserted for hourly ABG. Mean values for sleep stages and related sat (Table) reveal an extended sleep onset latency (time from start to initial sleep) with markedly decreased sleep efficiency (sleep minutes/total study time). Of particular note are the high percentage of wakefulness which is secondary to multiple spontaneous arousals and the markedly reduced REM stage. The mean $PaCO_2$ from awake to sleep (40-44 mmHg) shows the same degree of hypoventilation as in normals. Recorded apneas were comparable to normals. Arrhythmias recorded were atrial and ventricular premature beats, however, there was not a predilection to any sleep stage or sat.

	SOL*	SE	W	I1	I2	I3	I4	REM
COPD	47	55	38	22	32	4	0	7
Norms†	20	90	6	8	58	2	1	22
Mean SAT -	-	90	87	86	88	-	-	85

Age corrected. SOL = sleep onset latency in minutes. SE = sleep efficiency, W = wake, I-4 = stages, REM = rapid eye movement.

ACUTE AND CHRONIC ULTRASTRUCTURAL (US) PULMONARY CHANGES PRODUCED BY THE ADULT RESPIRATORY DISTRESS SYNDROME (ARDS). M.A. Matthay,* E.S. Overland,* P.C. Hopewell, B.C. McKay,* (Intr. by J.F. Murray**). Dept. of Medicine and Pathology, University of California, San Francisco, Ca.

In order to define the US sequence of lung injury and repair associated with ARDS we performed transmission electron microscopy studies on lungs of 5 patients dying at various times following the onset of ARDS. One patient dying without lung disease was included as a control. All patients required assisted ventilation. Multiple, percutaneous lung injections of 3% glutaraldehyde in Sorensen's buffer were made immediately after death according to Weibel's technique. Specimens were retrieved at autopsy within 5 hours. Etiology of the ARDS included shock and aspiration in 2, phenytoin overdose in 1, and pneumonia in 2. The two patients who died within 48 hours had interstitial and alveolar edema with hemorrhage, proliferating type II pneumocytes (PII), hyaline membranes, denuded epithelial surfaces, and endothelial swelling. The lungs of the patient dying after 2 weeks of ARDS showed US changes similar to the two patients dying in the acute phase as well as viral inclusion bodies. Specimens obtained from 2 patients who died at 6 and 15 weeks after onset of ARDS showed restoration of alveolar epithelium with PI and PII, endothelial cell regeneration, interstitial fibroblasts (FB) with increased collagen (FD), and general thickening of the alveolar-capillary membrane. Proliferations of PII is the earliest phase of lung repair and occurs immediately after lung injury. Late healing is characterized by re-epithelialization of the alveolar surface, interstitial accumulation of FB and CD, endothelial cell regeneration, and clearing of alveolar debris. Overall, the nature of the US changes correlated best with the duration of the patient's survival after ARDS rather than a specific etiology.

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