Pulmonary Edemagenesis in F-344 Rats Exposed to SFE (formulation A) Atmospheres

EC Kimmel[1], EA Smith', S Prues[1], K Zepp', JH English', and RL Carpenter[3]
1. Geo-Centers, Inc., 2. Army Medical Research Unit - Toxicology Division, 3. Naval Medical Research Institute Detachment (Toxicology)
NMRI/TD, Bldg 433, Area **B, 2612** Fifth St., Wright-Patterson **AFB. OH 45433-7903**

ABSTRACT

Pulmonary edemagenesis in F-344 rats exposed to atmospheres of pyrolyzed SFE (Formulation A) fire suppressant was evaluated by analysis of histopathological examination of respiratory tract tissues and enzyme activity in bronchoalveolar lavage (BAL) fluids. Twelve experimental groups of I2 animals each were exposed either to air or to **80 g/m^3** nominal concentration of SFE for one hour, Exposures were conducted in a **700** L inhalation exposure chamber operated in a static mode. Exposure atmospheres were analyzed for actual aerosol mass concentration and size distribution and for the major gaseous components of SFE atmospheres. CO and CO_2. In addition to the aforementioned analyses clinical observations were made during the exposures. Time course of edemagenesis and resolution of the insult was determined by serial evaluation of animals at 1, 6, 12 and **24** hrs, as well as 2, 3, 4, 5, 6 and 7 days post exposure. Histopathological examination included nasal turbinate and pulmonary tissues. BAL analyses included total protein, acid and alkaline phosphatase, lactate dehydrogenase, and β-glucuronidase enzyme activity determinations.

INTRODUCTION

Pyrolysis of Spectronix Fire Extinguishant (formulation A - SFEA) produces atmospheres consisting of a dry particulate aerosol phase and gas phase combustion by-products. At nominal concentrations of **80 g** of SFEA ignited per m^3 of space the aerosol phase has a particle distribution with an initial mass median aerodynamic diameter (MMAD) of **2.4** to **3.0 µm** and a standard geometric deviation (og) of **1.7.** Particle growth by coagulation forms 3.2 to **4.0 µm** MMAD particles without significant change of the og, generally within **30** min. At this nominal concentration the initial aerosol mass concentration varies as a function of the proportion of bulk SFEA constituents. The non-stoichiometric formulation (SFEAl) produces initial mass concentrations of 8.4 to **10.1** g/m^3, whereas the stoichiometric formulation (SFEAZ) produces **8.5** to **9.0 g/m^3** initial mass concentrations. Under static conditions, gravitational settling of the particles reduces the aerosol mass concentration exponentially, with $T_{\frac{1}{2}}$ ranging from 16 to 18 min., as a function initial concentration and coagulation rate. Regardless of formulation, the aerosol particles are nearly pure KCl (98+ %). The gas phase of the atmospheres also varies as a function of formulation. SFEA produces both CO_2 and CO, whereas SFEAZ does not produce CO above exposure system limits of detection (≅ **20** ppm).

In our exposure system, **3.0** to **3.5 µm** MMAD is the average particle size range over the course of a **1** hr test period. Particles in this size range are highly respirable with a **0.90** to **0.95** total lung fractional deposition in humans. Human regional fractional deposition in the lung is approximately as follows; naso-pharyngeal region (NP) - **0.12** to **0.24** , tracheobronchial (TB) - **0.08** to 0.16, and pulmonary (P, aka alveolar) - **0.60** to **0.45;** with the remainder depositing in the oro-nasal vestibule. Consequently, a significant portion of the aerosol penetrates deep within the respiratory tree to the parenchymal tissues of the P region which are highly susceptible to damage and for which clearance mechanisms are the least rapid. A common sequelae of irritation of tissues is edema which is an integral and initial part of the inflammatory response along with hystiocytosis (extravasiation and migration of blood cellular components - neutrophils, eosinophils, monocytes, plasma cells, and lymphocytes) and migration of activated interstitial macrophages.

Historically, pulmonary edema was defined by Laennec (1816) as "an infiltration of serum into the pulmonary tissue. camed to the extent such that it significantly diminishes its permeability to air." However, there is substantial pathology associated with edema prior to extensive flooding of the alveoli and significant interference with gas exchange. Thus, a generalized definition of edema is excessive translocation of serum fluids (water and plasma proteins) from the vasculature to extravascular spaces. Lung extravascular space consists of two compartments, the interstitial space between cellular tissues and the air space in the alveoli. Figure 1 shows a schematic representation of the pulmonary vascular, interstitial and alveolar spaces along with a synopsis of the

pathogenesis of edema. pulmonary edema is considered to have two distinct stages, an accumulation of fluid in the interstitial space (interstitial edema = IE) which usually, but not necessarily, precedes flooding of the air spaces in alveolar edema (AE). IE which is detrimental to function does not necessarily progress to fluid accumulation in the air spaces, particularly if the alveolar epithelium remains intact and maintains its normally low permeability. pulmonary edema often presages other non-neoplastic pulmonary lesions and diseases, particularly interstitial pneumonitis; and may have many causes other than direct insult to the lung, such as neurogenic edema and Cor pulmonale.

Numerous alkali, halide, and other ionic salts and acids have been found to induce pulmonary edema and subsequent pulmonary pathology; these include HCl, H_2SO_4, KOH, CrCl, $(NH_4)_2SO_4$, $CdCl_2$, and NH_4Cl. The latter two often are used in laboratory animal models for the investigation of the pathogenesis of pulmonary edema.

The roles potassium and chloride ions play in the physiology of various organ, tissue and cellular level systems are sufficiently numerous to be far beyond the scope of *this* discussion. However, the delicate homeostatic balance of these ions, acting in concert with other ions and biological macromolecules, is crucial for maintenance of normal lung function; particularly with regard to transport and control of fluids. pulmonary epithelial tissue secretes small amounts of fluid necessary to maintain hydration of air space tissues which are subject to evaporative water loss during breathing. The so–called chloride pump which regulates the composition and volume of the epithelial surface fluid (*Qwl*) providing this hydration is driven by active membrane transport of Na^+ and Cl^- ions. K^+ not only participates in the active transport of Na', it is thought that K^+ is a principal participant in the maintenance of osmotic balance between the mucosal and serosal sides of the epithelium during this process. Ciliary beat frequency and velocity in the sol (water) layer of the mucociliary escalator which lines the conducting airways, and is the principal mechanism of early clearance of aerosol particles, is regulated primarily through a balance between K^+ and Ca^{++} ions. Excessive deposition of ionic species in the sol layer can produce hypertonicity with subsequent migration of fluid to *this* layer thereby expanding it and decoupling the sol and gel layers of the mucociliary escalator thus causing stasis of mucociliary transport. KCl, depending on the concentration and whether it is applied either intra- or extralumenally, influences both tone (dilation or constriction) and permeability of pulmonary endothelial tissues (both bronchial and vascular). KCl mediates vesicular transport and release from epithelial tissues *of* Major Basic Protein (MBP a large cationic molecule) which is responsible for *this* action on endothelial tissue, but at physiologic concentrations does not directly effect epithelial permeability. However, both K^+ and Cl^- are thought to participate in the balance of the anionic barrier which along with cellular tight junctions maintains the relative impermeability of alveolar epithelium. Cellular tight junctions are rather more like gates in which glycoproteins act to "police" transport. K^+ and Cl^- are thought to participate in *this* "policing" action. Finally, but not exhaustively, K is thought to have an important role, ancillary to the intravascular proteins, in maintaining the osmotic gradient within and between the various tissues thereby permitting normal fluid flow from the vasculature through the interstitium to the pulmonary lymphatic capillaries. Consequently, there is sufficient reason to *suspect* that SFEA aerosol particles which are composed almost exclusively of KCl and which are deposited in high fractions directly on the pulmonary epithelium can significantly alter fluid balance in the lung leading to pulmonary edema. The present investigation was prompted by this speculation and the observation of prevalent buccal mucus accumulation and at least one case of very severe pulmonary edema in animals exposed during a pilot investigation of SFEAl toxicity

METHODS

Test Materials. SFEAl and SFEA2 *(numeric designators ours)* was supplied by Spectronix Ltd. Tel Aviv, Israel.

Test Subjects. Male Fisher CDF(F-344)/CrlBr rats, between 200 and 250 g body weight, were supplied by Charles River Breeding Laboratories (Wilmington, MA). The animals were housed in AAALAC accredited facilities and were provided food (Formula Lab Chow 5008 - Purina Mills Inc., St. Louis, MO) and water *ad libitum.*

Experimental Design. *Animals* were randomized into 12 experimental groups of 12 animals each (Table 1). Two groups were exposed to room air only and served as controls (24 hrs and 7 day post exposure). Ten groups of 12 animals each were exposed to SFEAZ pyrolysis atmospheres for 1 hr. at a nominal concentration of SO g/m^3. Clinical observations were performed during exposure at 1, 5, 15, 30, 45 and 60 min and once every 12 hours post exposure where applicable. Animals were euthanized at either 1, 6, 12, 24 hrs or 3, 4, 5, 6, and 7 *days* post exposure with an ip injection of Ketamine™/Xylazine™ mixture (70 and 6 mg/kg respectively). Six of

the animals from each group were necropsied for gross and histopathological examination of tracheal, lung. heart, and abdominal organ tissues. Six animals from each group were sacrificed for subsequent bronchoalveolar lavage (BAL) for analysis of enzyme activity and protein content in *Qwl*. Enzyme analyses included determination of acid and alkaline phosphatase, lactate dehydrogenase, and β-glucuronidase activities all of which are indicators of tissue damage, increased epithelial permeability, or elevated macrophage and leukocyte activity

Inhalation Exposures (system and operation), Atmosphere Generation. Aerosol Characterization, and Gas Analysis. Descriptions of these procedures and systems have been previously reported. For detailed descriptions see "Physical and Chemical Characterization of SFE Fire Suppressant Atmospheres: Comparison of Small with Large Scale Laboratory Atmospheres.", Kimmel, et al., 1995 Proceedings of HOTWC, or "Physical and Chemical Characteristics of SFE Fire Suppressant Atmospheres in Small vs Large Scale Tests: Implications for Pulmonary Deposition and Toxicology.", Kimmel, et al.- these proceedings.

BAL. After euthanization a midline thoracotomy was performed and the trachea exposed and cannulated. The lungs were infused three times with 5 mL of Ca^{++}-Mg^{+} free phosphate-buffered saline (PBS). The aliquots of PBS were pooled and centrifuged at 300 g for 10 min to separate cellular and fluid components. The cell pellet was suspended in 5 mL of RPMI 1640 (Grand Island, NY) containing 25 mM Hepes buffer, and refrigerated for analysis at a later date. Total protein content of the BAL was determined colorimetrically with bovine serum as a standard. Enzyme activities were determined using a COBAS-Bio automatic analyzer using commercially available reagent kits with appropriate substrates.

Histopathology. After gross examination of the thoracic and abdominal organs, the trachea and lungs were excised and trimmed. Nasal turbinates were harvested intact via a transverse cut at the incisive papilla and second palatal ridge. The nasal turbinates, trachea, pharynx, larynx, and lungs were collected for histopathological examination. The lungs were not perfused or inflated prior to fixation to preserve alveolar fluid accumulation. Tissue sections were fixed in 10% neutral-buffered formalin and the nasal tissue sections were decalcified for 3 days in 10% ethylenediaminetetracetic acid. Tissue sections 3-4 μm thick were embedded in paraffin and stained for light microscopic examination with hematoxylin and eosin.

Statistical Analysis A one factorial analysis of variance with Bonferoni Multiple comparison and Levene's test of equivalence of variance was used to evaluate the enzyme analyses. A $p \leq 0.05$ was considered statistically significant.

RESULTS

Pilot Investigation - SFEA1

In an initial pilot investigation of general acute toxicity one of the exposure groups of animals was exposed to 80 g/m³ SFEAI for 60 minutes using procedures identical to those employed for this experiment. Actual initial aerosol concentration was 8.4 g/m³ decaying exponentially to 0.86 g/m³ at end exposure. Particle size ranged from an initial 2.09 to 2.85 μm MMAD with σgs from 1.5 to 2.1. Gas concentrations were steady-state at 9,731±300 ppm CO_2 and 6,675±220. None of the animals died Over the course of a 14 day observation period at this level. however one animal due for sacrifice at 6 hr post exposure was moribund. Lesions in the nasal turbinates were observed in 80 to 100 % of the animals necropsied at 1, 6 (80%), and 24 hr post exposure. No lesions were observed 7 or 14 days post exposure, no intervening (1 to 7 day) observations were made. Nasal lesions consisted of multifocal non-supparative rhinitis of the respiratory epithelium of the ventral-distal turbinates. Necrosis and sloughing of the ethmoid epithelial tissue (Figure 2) was observed in some of the animals. Pulmonary edema was observed in 40 %, 80 %, and 60 % of the animals at 1,6 and 24 hr post exposure (Figure 3). The edema was classified as moderate to severe and was characterized by perivascular and peribronchiolar IE (pvIE, pbIE) and definite serofibrinous, exudative AE. Alveolar spaces contained cellular debris, numerous macrophages, and histiocytes (lymphocytes, neutrophils and few eosinophils). Severe edema was noted in one animal at 6 hr post exposure in which pvIE, pbIE and diffuse AE, filling most of the alveoli, was observed Moderate to severe interstitial pneumonia was observed in 2 animals at 24 hr and 1 animal at 7 day. Alveolar flooding was patchy with foci of coalescing alveoli adjacent to sites of moderate to severe pbIE and pvIE with hemorrhage. Numerous macrophages were observed in the alveoli. Most of the animals were clear of AE at day 7 with mild IE. There were no significant histopathological findings at day 14.

SFEA2 Exposures

Exposure Atmomhere The average aerosol mass concentration was 8.61 g/m³ and decayed exponentially ($T_{1/2}$ = 18.3 min) to 0.5 g/m³ in 60 min. Despite gravitational settling, particle sue grew from 2.9 to 3.58 µm MMAD with respective σgs of 1.5 to 1.6. CO_2 concentration in the atmospheres was steady-state and averaged 21,500±860 ppm Combustion of SFEA2 was complete therefore no CO was found in the exposure atmospheres.

Clinical Observations All animals exhibited dyspnea, lethargy, lack of wordination, and a cough/choking response which subsided within minutes upon removal from the chamber.

Histopathological Observations *(see* **Figure 4-6d)** Table 2 synopsizes the development and resolution of nasal and pulmonary lesions.

Controls - no lesions of nasal turbinates or lung in either the 24 hr or 7 day control animals (Figures 4a & 6a).

1 hr uost exposure A single animal demonstrated minimal AE with serofibrinous exudate which was limited to alveoli surrounding foci of moderate pbIE **Figure** 6b).

6 hr uost exposure No pulmonary lesions were evident. Five of 6 animals exhibited minimal focal erosion of the respiratory epithelium in the ventraldistal turbinates.

12 hr post exuosure Focal suppurative rhinitis with sloughing of epithelial tissue in the distal turbinates was observed in 2 of the 6 animals. Four of 6 animals had moderate pbIE with AE of the mounding area. Numerous alveoli showed a moderately elevated macrophage population and some histiocytosis including eosinophils. Alveoli adjacent to foci of pbIE and pvIE showed an accumulation of eosinophillic fibrillar material admixed with the serous exudate **(Figures** 4b & 6c).

24 hr post exposure Half of the animals exhibited unilateral suppurative rhinitis with denuding of the respiratory epithelia of the turbinates **(Figure 5).** Fragments of plant matter were found embedded in turbinate epithelial tissue. All animals exhibited multifocal AE with serofibrinous exudate containing occasional red blood cells (RBCs), and distended pvIE and pvIE with coalescence of adjacent alveoli. Macrophages were numerous and foamy macrophages containing phagocytosed debris were observed. Occasional polymorphonuclearleukocytes (PMNs) were Observed. Multifocal type II pneumocyte hyperplasia was present.

2 davs uost exposure Two of *6 animals* had mild unilateral suppurativerhinitis. All animals had diffuse AE with mild histiocytosis similar to lungs at 24 hr with exception that fibrillar material was not present. Multifocal pv and pbIE was observed with AE most prevalent . Macrophages occasionally contained phagocytosed RBCs and other debris. Multifocal type II pneumocyte hyperplasia was present.

3 days post exposure Nasal turbinates appeared normal with regeneration of epithelial tissues. Six of 6 animals exhibited minimal pulmonary histiocytosis, with minimal pv and pbIE and interstitial pneumonitis. Type II *cell* proliferation is present. No AE observed.

4,5,6 & 7 days post exposure Nasal turbinates appeared normal. No evidence of AE or JE was present. There was apparent restructuring of parenchymal architecture by replacement of type I with type II pneumocytes.

There was a mild irritant effect on the ventral-distal respiratory epithelium which is an area of high localized aerosol deposition (in rats) in the nasal turbinates due to very turbulent flow. The effect was transient; first appearing in 1 animal at 6 hr and in one third of the animals at 12 hr. The lesion was predominantly unilateral and associated with plant material at 24 hrs and 2 days, suggesting a pruritic effect (possibly associated with a resolving lesion) inducing animals to rub their noses in their bedding. Lung lesions were moderate but distinctive suggesting an early edematogenic response, probably between 6 and 12 hrs, with some evidence for appearance as early as 1 hr. Severity of the lung lesions peak in the 24 hr to 2 day time frame. Diffuse alveolar flooding was not noted but thickening peribronchiolar and perivascular interstitial tissues, accompanied by histiocytosis and focal serofibrinous exudative AE was consistent with an edematogenic, physiologic response to irritation. Lesions were resolved with some type II pneumocyte hyperplasia evident. Both nasal and pulmonary lesions were in resolution by the 4th day post exposure. Resolution of an insult is a function of the repair process rate and dissipation of the insult. SFE aerosol particles are highly soluble and with elevated "lymph flows" ($\dot{Q}f$) the KCl could be cleared from the lung fluids quickly; the rate limiting steps being sufficiency of $\dot{Q}wl$ for panicle dissolution and low $\dot{Q}f$ (wen though significantly elevated by IE). The time course of the resolution of the insults is consistent with the normal turnover rate for most pulmonary epithelial tissues which is 3 - 10 days, with

exception of type I pneumocytes (≅ 30 days). However newly divided type II pneumocytes can differentiate, if necessary, into type I pneumocytes in 2 days.

BAL. Fluid Analyses (Figure 7)

Total protein (Tp) An increase was observed at 1 hr post exposure and peaked at 12 hr nith return to normal levels by day 4. The peak elevation was ≅ 5 fold. Concentrations were statistically significant at 1 hr through 2 days post exposure.

Add phosphatase (aPhos) There was a statistically significant increase in aPhos activity 6 hr post exposure.

Alkaline phosphatase (alkP) There was a slight but statistically significant increase in alkP activity at 1 hr post exposure, followed by slight but statistically significant depression of alkP activity at 12 and 24 hr post exposure with a similar second depression at days 6 and 7.

β-glucuronidase (β-gluc) A generalized elevation of β-gluc activity was noted with significant increases at 12 hr and 3 day post exposure. Activity 3 - 4 times control levels.

Lactate Dehydrogenase (LDH) LDH activity was slightly increased from 1 hr to 2 day post exposure with a statistically significant peak at day 2.

Elevated protein levels (particularly serum albumin) are pathognomonic of edema indicating an increase in endothelial permeability and a breach of the epithelium either through epithelial cell damage or disruption of the intercellular tight junctions. The moderate increase in protein levels observed are consistent with moderate epithelial damage and tight junction disruption and the time course of the protein elevation is consistent with increasing interstitial pressure associated with accumulation of fluid and protein in IE. The minimal changes in enzyme activities, although statistically significant, and the paucity of information provided by an noncohesive pattern of changes are not given to clear-cut definitive interpretation of the results. Changes in enzyme activity of 200 to 600 % of normal values in response to toxic insult are not uncommon. Elevations as high as 3900 % (β-gluc) times control values and which persist for 1 yr (at 1500 %) have been observed, however this was in response to sub-chronic exposure to insoluble aerosol particles. In addition, enzyme levels in "normal", control subjects are highly variable having coefficients of variation of 15 to 20 %. Never-the-less, the observed elevations (and depressions) and their time course is consistent with some initial moderate epithelial damage, gradual development of hydrostatic (osmotic) IE, then IE induced (pressure) additional disruption of the epithelial bamer and moderate focal AE. Alkaline phosphatase is a plasma membrane enzyme found in type II pnuemocytes; consequently the early elevation of this enzyme may indicate moderate initial, direct irritation and damage by SFE to these and other epithelial cells. AlkP also can be released by epithelial cell memhrane during the transudation of protein. Both are consistent with the early influx of protein and the histopathological findings. The depressions of alkP could be equivocal or due to subsequent removal by elevated Qf. The latter is consistent with the diminishing protein level pattern, but with the bimodal pattern would imply separate discrete releases (initial cell damage - then type II cell proliferation), other enzyme levels do not follow a similar pattern. LDH is a cytosolic enzyme, the release of which is indicative of cell damage. It is found in epithelial. endothelial and inflammatory cells. However moderate amounts are thought to exist free in the sera. The initial trend toward elevation of LDH may represent moderate epithelial damage or may reflect an influx accompanying the protein influx, seen histologically as early focal AE. Lysis of inflammatory cells affects LDH release, however, if this were the primary source of LDH it would he expected that the LDH profile would track with that of lysolytic enzymes such as β-gluc, and it does not. The LDH peak at day 2 may represent a culmination of the combined effects of initial direct epithelial damage and serum transudation, hydrostatic epithelial damage which is consistent with the histopathological findings and by release due to cell damage associated with phagocytosis of aerosol particles. β-gluc and aPhos are lysosomal enzymes found in activated PMNs and to a lesser extent in macrophages. They are released during the phagocytic process and by lysis of these cells. Initial 6 and 12 hr increase of these enzyme activities coincides with histopathological evidence of elevated macrophage and PMN cell population in the airspace and release due to phagocytosis of undissolved SFE particles. The second peak of β-gluc activity at day 3 is attributable to increased phagocytic clearance of cellular dehris subsequent to peak IE, AE and resultant cell damage which is consistent with the histopathological findings and with the LDH peak.

DISCUSSION

There is normally a small (≅ 5.0 mL/hr) net fluid flow from the vasculature through the interstitium to the lymphatics. According to Starling's principle this flow is maintained and regulated through a balance between hydrostatic and osmotic form of the interstitium and the microvasculature. Described as follows:

$$Qf = Kf\ [(Pmv - Ppmv) - \sigma f\ (\Pi mv - \Pi pmv)]$$

where: Qf = the transvascular fluid flow

Kf = the fluid filtration coefficient - representing endothelial permeability to ultrafiltrate

σf = the reflection coefficient - a measure of the plasma retaining efficiency of the endothelium

Pmv, Ppmv = the hydrostatic pressure of the microvasculature and the interstitium respectively

Πmv, Πpmv = the osmotic pressure of the microvasculature and the interstitium respectively,

Rearranged and combined with Fick's law ($Jsv = \alpha\ Qv\ \Delta C$, describing membrane transport). Total solute transport of plasma protein and other large molecules, thought to be the principal mediators of the epithelial/interstitial/endothelial osmotic gradient, can be described as a function of different transport mechanisms:

$$Js = \dot{Q}f\ (1-\sigma)Cp + PA\Delta CFx + \alpha Qv\Delta C$$

Convection Diffusion Vesicular

where: Js = solute transport

Cp, ΔC = Plasma protein solute concentration and concentration difference respectively

PA = permeability-surface area product

Fx = nonlinear correction function based on the ratio of convective solute and diffusion solute entry to the interstitium = $x(e^{-x} - 1)$ where $x = Qf\ (1-\sigma)/PA$

Qv = volumetric flow of vesicular (transmembranevacuolar) transport

α = the solute partition coefficient between plasma and the vesicles.

These functions also essentially describe the relationships of the osmotic and hydrostatic forces and mechanisms, which when disrupted result in pulmonary edema. Inherent in the maintenance of these fluid and solute relationships is a delicate intercompartmental balance between small ionic species (such as K^+ and Cl^-) as well as balance of the larger intravascular plasma proteins which are the predominant factor in balance of the osmotic forces.

Although there are no methods to directly measure the quantity of Qwl three separate techniques of indirect measurement estimate the amount of extravascular surface fluid in the human lung to be ≥ 0.35L. In the P lung region K^+ concentration in animals and humans is ≅ 56 meq/L (56 mOsmol/L) or 2.18 g/L, and Cl- concentration is 106 meq/L (106 mOsmol/L) 3.71 g/L. Corresponding total Qwl quantities of K^+ and Cl^- are 0.77 g and 1.30g respectively. Average aerosol deposition rate in the lung as a result of breathing an 80 g/m^3 SFE atmosphere was determined to be 5.2×10^{-2} g/min by integration of the equation describing overall aerosol mass deposition rate change as a function of exponential decay of aerosol mass concentration. The function also compensated for CO_2 stimulated hypercapnea factors (see Kimmel et al. these proceedings). The atmosphere and equation are correspondent to the (SFEAI) exposure atmosphere in the present investigation. Thus for a 60 minute exposure period total deposition of KCI aerosol in humans would be 3.10 grams, corresponding to 1.63 g and 1.46 g of K^+ and Cl^- respectively. This represents an increase of K^+ by a factor of 2.11 and an increase of Cl^- by a factor of 1.12. The result would be an increase of Qwl osmolarity due to K^+ and Cl^- to 175 and 225 mOsmol/L respectively. The combined increase of 238 mOsmol/L is nearly as large as the original total Qwl osmolarity of 304 mOsmol/L.

There is relatively free communication of water and small molecules to and from the interstitial space across the microvascular endothelium, consequently interstitial fluid and blood serum are nearly identical in composition and are nearly isotonic. The slight osmotic pressure gradient between the endothelial and interstitial compartments is maintained by the large intravascular proteins balanced by with K^+, Cl^- and Na^+ balance on the apical and serosal sides of the endothelium. With K^+ having a much higher affinity of the active channels of this

balance than Na^+. SFE (KCl) deposited in the airway lumen disrupts the ionic balance of the chloride pump effectively shutting down epithelial secretion of fluid to the alveolar surface. This establishes a hydrostatic drive for movement of fluid from the interstitium to the airspace. This, in turn, causes an interstitial/endothelilial hydrostatic gradient favoring movement of fluid from the microvasculature to the interstitium; which is facilitated by increased endothelial permeability due to K^+ stimulated release of epithelial BMP. Because of the permeability difference between the epithelia and endothelia the net effect is an increase in interstitial hydrostatic pressure, increased Qwl and IE.

The very large increase in osmotic pressure of the Qwl from SFE aerosol deposition coupled with the relatively slow rate of ionic transport across the epithelial membrane establishes a large descendmg osmotic gradient from the air space to the microvascular lumen This osmotic gradient is maintained by the interstitial ground substance which is composed of mucopolysaccharide and glycoproteins that are very sensitive to local hydrostatic pressure and behave as a gel filtration media, resulting in graded retention of fluid to facilitate fluid movement from the vasculature to the interstitium. In addition to K^+ mediated increase in endothelial permeability, this ion also fosters vesicular transport of proteins out of the epithelial and endothelial cells further disrupting the osmotic pressure in favor of IE. Whether from transudation leakage, through ionic "loosening" of tight junctions or throughvesicular transport, the net effect of the translocation of protein from the microvasculature is fnrther disruption of the osmotic balance and increased net flow of water into the interstitium. Although counter-intuitive, the lymphatic capillaries are not collapsed by the high interstitial pressure but are, in fact, dilated. Qf can increase under edematogenic conditions from 5.0 to 30.5 mL/hr. The increase may start immediately after a traumatic edematogenic event and peak in as late as 3 days subsequent to the insult. Highly elevated Qf may persist for as much as 14 days while clearing IE fluid. Therefore increased Qf provides a certain countermeasure for the elevated interstitial hydrostatic pressure. Elevated Qf, the relative impermeability of epithelium, and the fluid reservoir of the perivascular and peribronchiolar-loose connective tissue interstitium provide the so-called "edema safety net" that allows interstitial pressure to increase by factors of 3 to 4 without epithelial rupture and subsequent AE formation. Although epithelial integrity is for the most part retained (no ruptures), the hydrostatic pressure renders the membrane more permeable (further loosening of the tight junctions). As noted. there also is evidence to suggest that K^+ and Cl^- are a factor in moderation of the effectiveness of the gel material which acts to regulate tight junctions efficiency. Excessive K^+ also will interact to weaken the anionic "barrier" on the epithelial membrane which contributes to it's relative impermeability. With increased endothelial permeability, histiocytes can migrate from the vasculature and are potent cationic sources which further decrease the anionic "barrier" hence decreasing epithelial permeability. Macrophages and polymorphonuclearleukocytes (PMNs) are activated and migrate in response to chemotatic factors the release of which is stimulated by aerosol particle deposition. In the process of phagocytosing particles and debris from directly injured tissues, these cells release enzymes which further degrade and digest alveolar epithelial tissue. Subsequently, these combined factors may lead to rupture of the epithelium resulting in AE. Once the epithelial membrane is significantly ruptured all the aforementioned hydrostatic and osmotic balances no longer control fluid transport and fluid will follow the path of least resistance (ie move to the air spaces). IE and moderate non-complicated AE can be resolved as long as the epithelium remains reasonably intact. Part of the resolution process is through dissipation of the edematogenic driving forces by return to ionic balance through diffusion and an elevated lymphatic drainage. As noted in animals, this process may require up to two weeks depending on the severity and continuity of the insult and the absence of a complicating infection. As shown above. in rats the enzyme activities and protein content profiles BAL as well as the histopathological evidence demonstrate the presence, as well as the resolution, of fairly persistent IE, moderate AE, and interstitial pneumonia induced by SFE aerosols.

CONCLUSION

SFE aerosol particles deposited by turbulent flow in the nares induces direct damage to nasal turbinate epithelial tissue which is moderate and is resolved by normal repair processes in the absence of repeated insult. In the lung, SFE (SFEAZ) aerosol deposition induces progressive IE and moderate focal to multifocal AE with concomitant hystiocytosis, direct and indirect damage to epithelial tissue, mild interstitial pneumonia, and restructuring of the parenchymal architecture. These lesions also are resolved in the absence of repeated insult and complication. However, the incidence, time course and severity of the induced pulmonary edema is increased when the SFE (SFEAI) atmospheres include CO, a known pulmonary imtant. These lesions also are resolvable,

however, the severity and diffuse nalure of the AE represents a fertile substrate for additional complications such respiratory infection.

References References are available on request.

Acknowledgement/Disclaimer. The authors gratefully acknowledge the contributions of Mr. Larry Bowen to this effort. This research was sponsored by *the* Navy CFC/Halon Replacement Program; NAVSEASYSCOMCode 03V2. The experiments described herein were performed in a —dance with the principles and guidelines set forth by the "Guide for the Care and Use of Laboratory Animals." Institute *of* Laboratory Animal Resources, National Research Council. Opinions contained herein are those *of the* authors and are not to be construed as official *or* reflecting the views of the Department of the Navy or the Naval Service at large. Mention *of* commercial products or services does not constitute endorsement by the Department of the Navy or the Naval Service at large.

TABLE 1

EXPERIMENTAL DESIGN

Exposure Group	Animals per Group	Nominal Conc. (g/m^3)	Exposure Duration (min.)	Evaluation Times	Post-Exposure Evaluations	
					BAL*	Necropsy
1	12	Control	60	24 hrs	6	6
2	12	Control	60	7 days	6	6
3	12	80	60	1 hr	6	6
4	12	80	60	6 hrs	6	6
5	12	80	60	12 hrs	6	6
6	12	80	60	24 hrs	6	6
7	12	80	60	2 days	6	6
8	12	80	60	3 days	6	6
9	12	80	60	4 days	6	6
10	12	80	60	5 days	6	6
11	12	80	60	6 days	6	6
12	12	80	60	7 davs	6	6

TABLE 2.

TimePost-Exp	CON	1 hr	6hr	12 hr	24hr	2 d	3d	4d	5d	6d	7d
Nasal turbinates	*016*	016	516	2/6	316	*216*	016	016	016	016	*016*

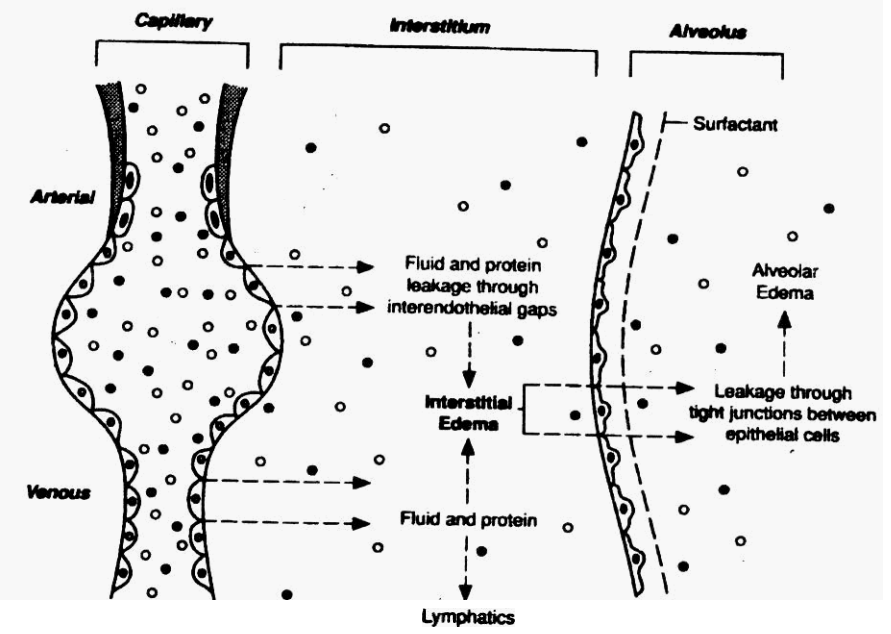
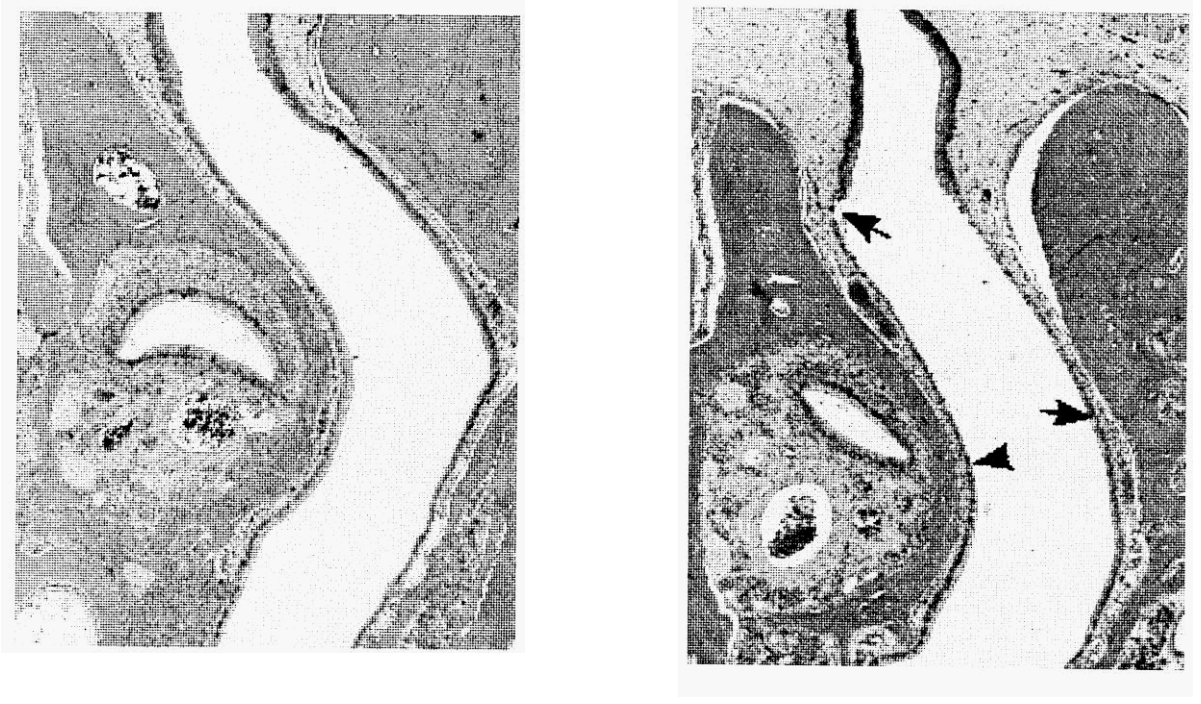


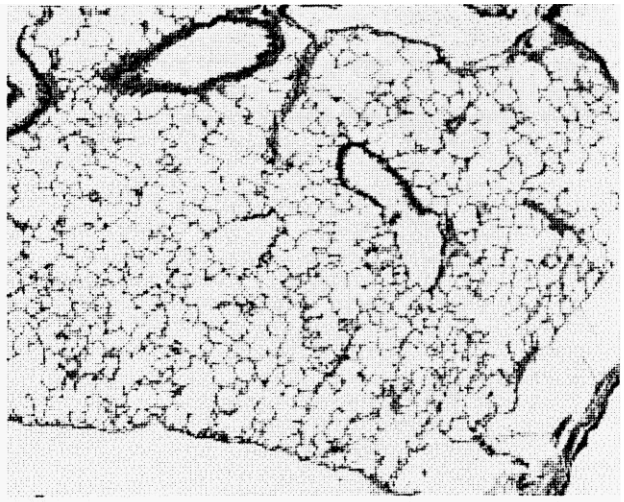
Figure 1. Schematic representation of the pulmonary microvasculature-interstitial space-alveolar lumen-lymphatics.



Control - normal

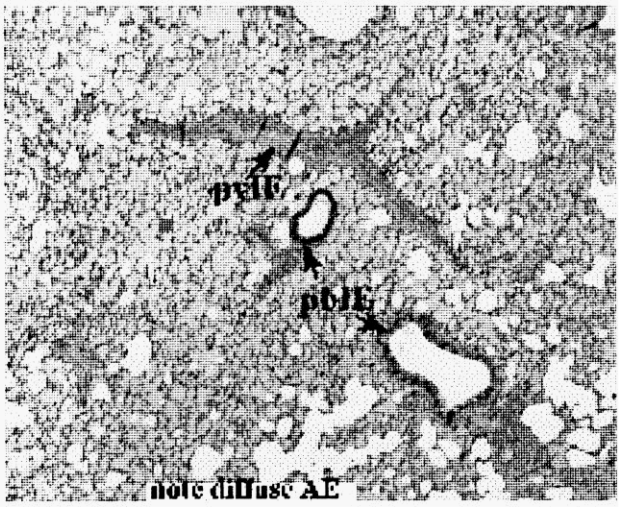
1 hr post exposure
arrows denote denuded epithelium & intralumenal cellular debris

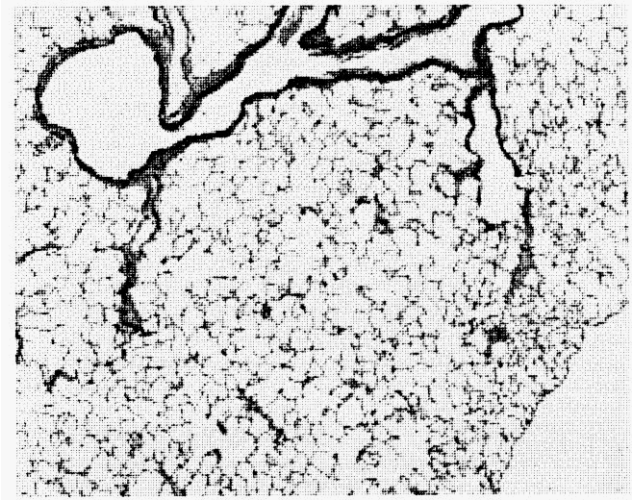
Figure 2. Nasal turbinates - SFE A1 - 80 g/m^3 - 60 min



Control - normal

6 hr post exposure
pv IE = perivascular interstitial edema
pb IE = peribronchiolar interstitial edema
severe diffuse Alveolar Edema prevalent





7 days post exposure
normal appearance
lesions resolved
minor repair evident

Figure 3. Lung tissue - SFE A1 - 80 g/m^3 - 60 min.

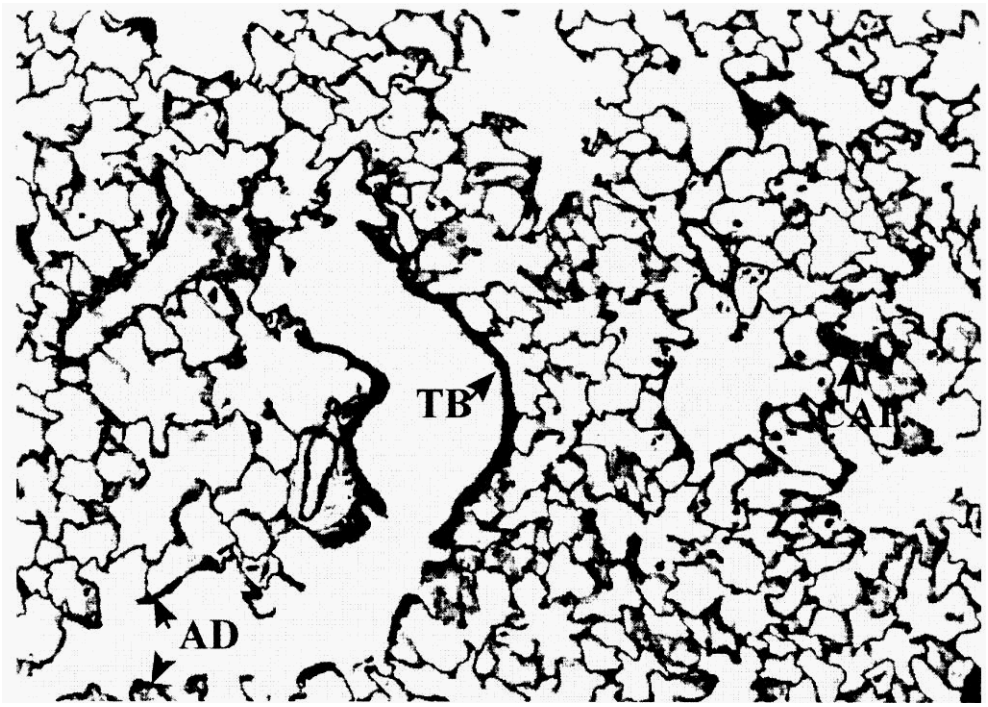


Figure 4a. Lung tissue - SFE A2 - 80 g/m^3 - 60 min - Control - normal
TB = terminal bronchiole, AD = alveolar duct

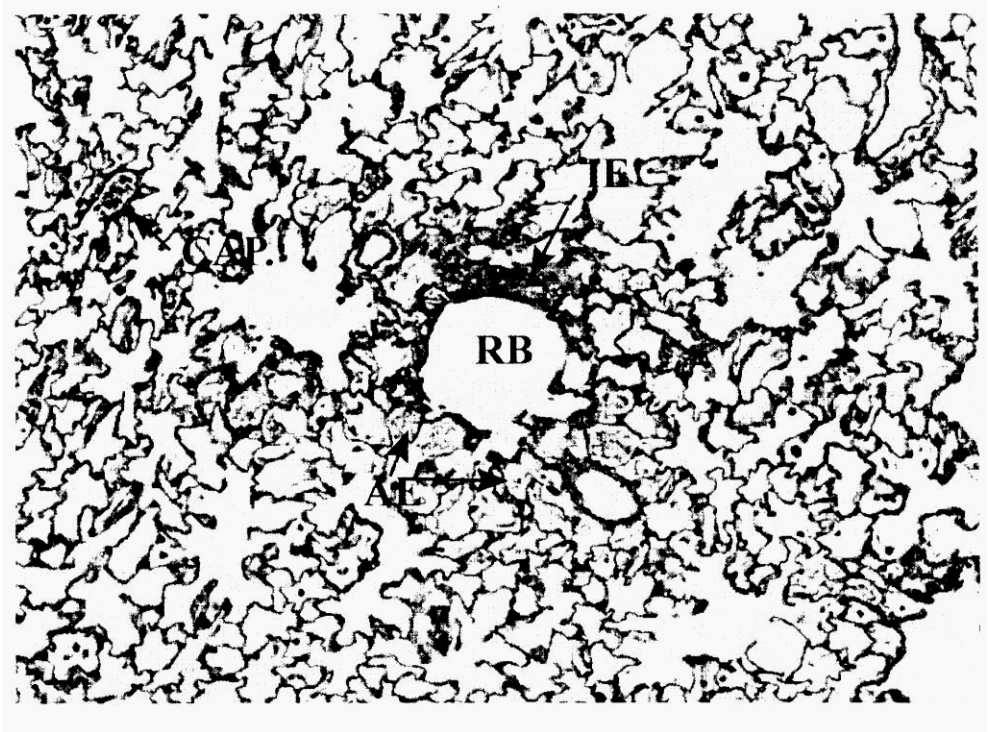


Figure 4b. Lung tissue SFE A2 - 80 g/m^3 - 60 min - 12 post exposure
CAP = capillary, RB = respiratory bronchiole, IE = interstitial edema (peribronchiolar), AE =alveolar edema

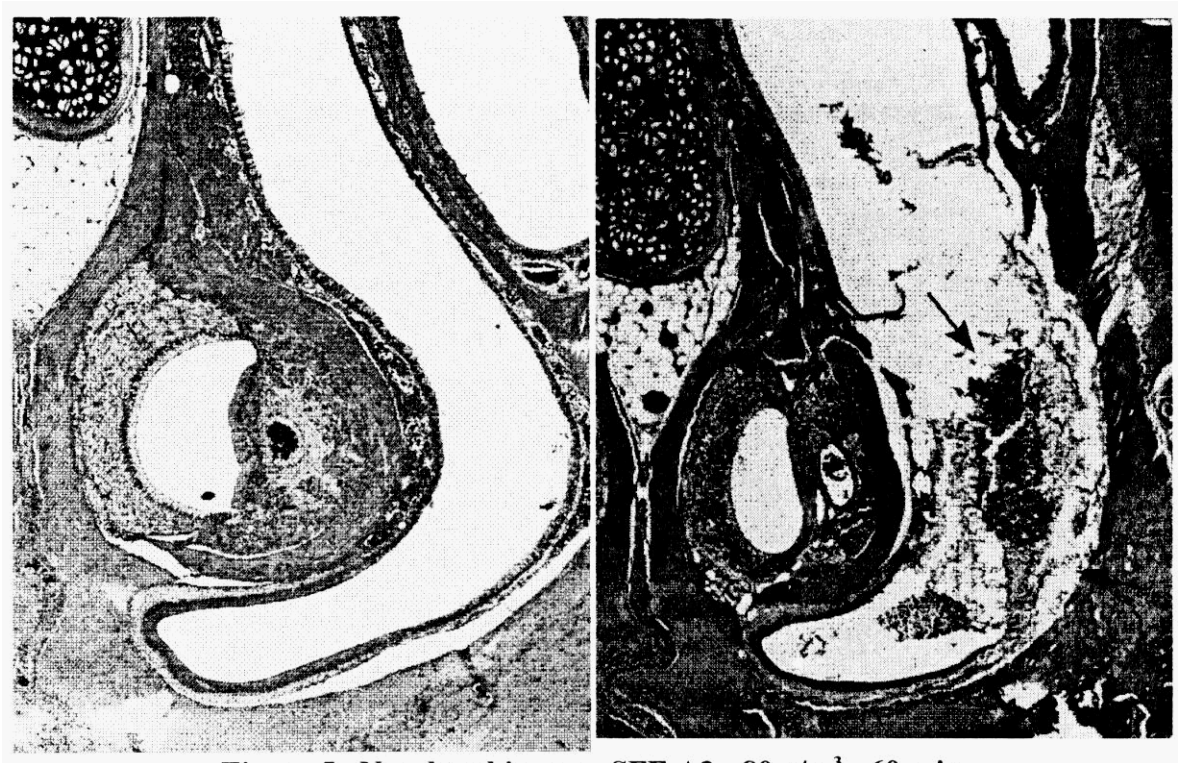


Figure 5. Nasal turbinates - SFE A2 - 80 g/m^3 - 60 min

left = normal, right = 24 hr post exposure, arrows show epithelial denuding and intralumenal fluid and cellular debris

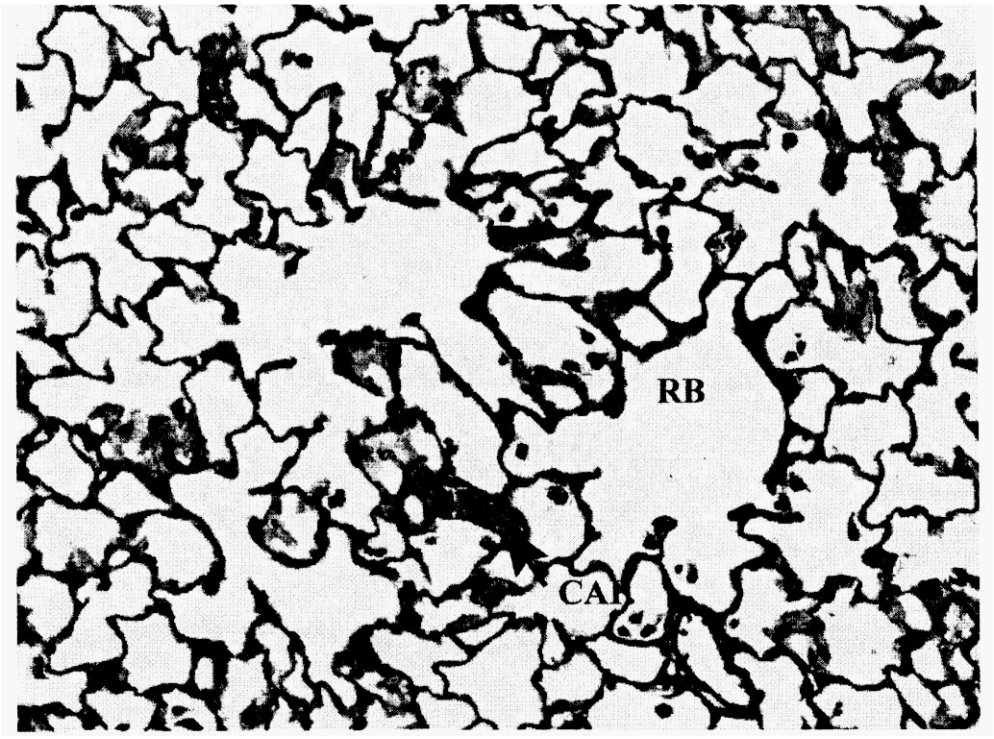
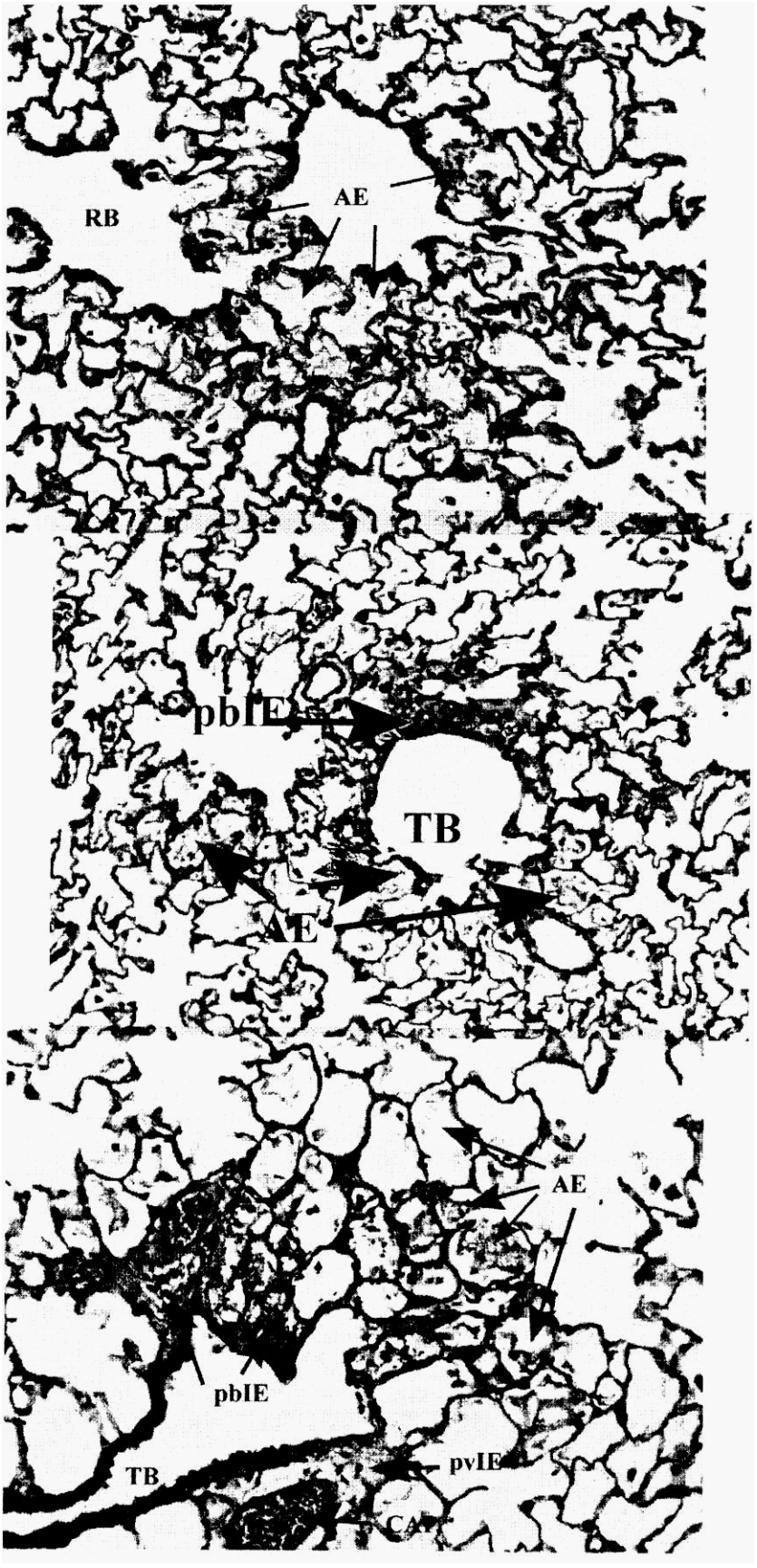


Figure 6a. Normal lung tissue - 7 day control



b. 1 hr post exposure
moderate AE with fibrin deposition

c. 12 hr post exposure
pb IE with focal AE

d. 24 hr post exposure
pbIE, pv IE, focal AE with cellular debris

Figure 6 b-d. Lung tissue SFE A2- - 80g/m^3 - 60 min - see previous figures for definitions

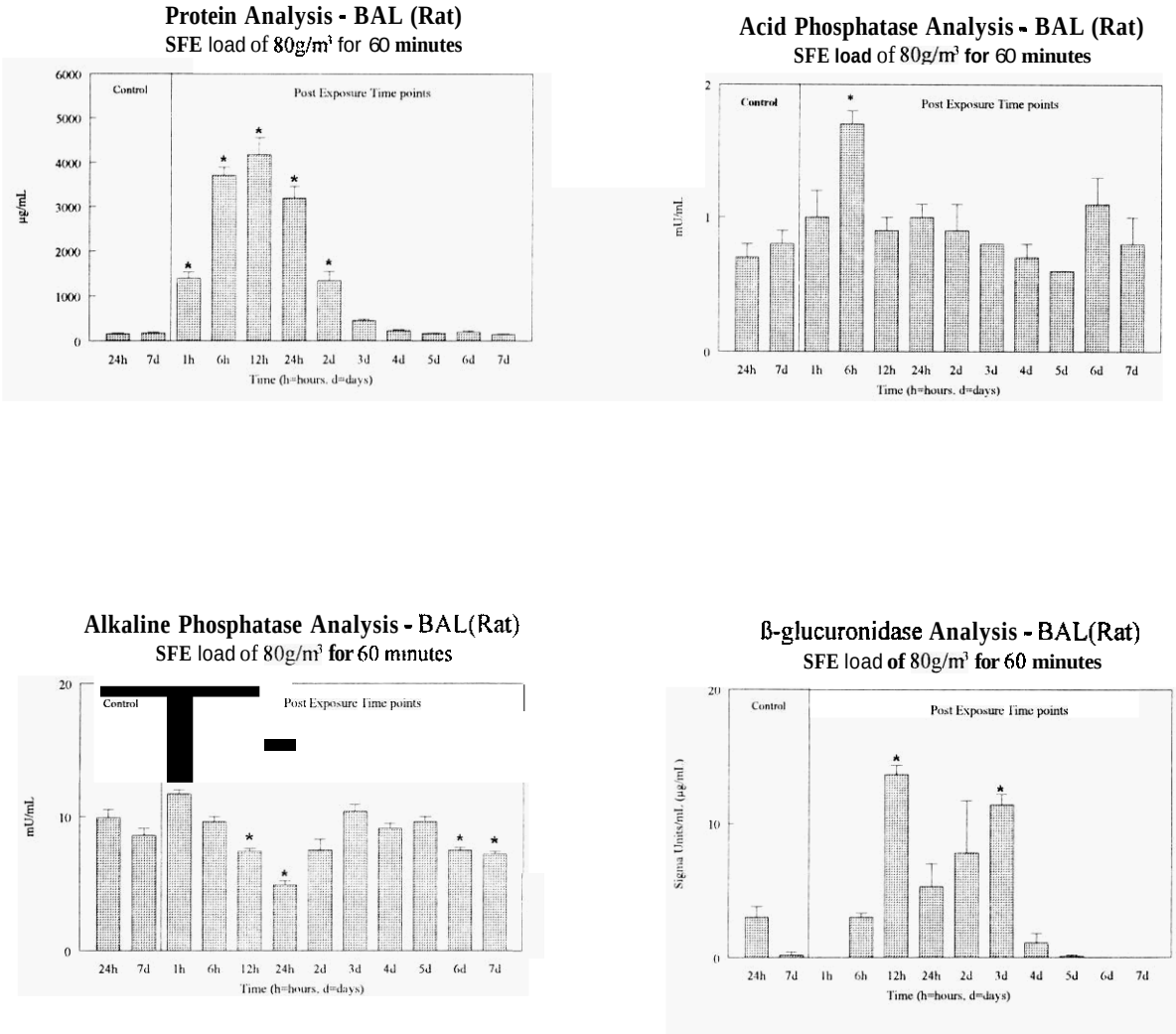


Figure 7. Bronchoalveolar lavage fluid - enzyme activities and protein content