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Smoke inhalation has been identified as a major cause of lung injury and death in fires with a mortality rate of approximately 75%. Soldiers regularly occupy enclosed spaces and travel near flammable fuels. The combination of burning material and an enclosed space are major factors that lead to smoke inhalation. We used a combination of diesel fuel and polycarbonate plastic to generate smoke-induced lung injury. Rabbits were exposed to 60 tidal volume breaths of smoke in approximately 10 minutes. Acute smoke exposure caused changes in broncho-alveolar lavage (BAL) and plasma eicosanoid concentration, especially at 0.5 hours post-smoke exposure. In addition, there were decreases in technetium-labelled diethylenetriamine pentaacetic acid (99mTcDTPA) T1/2, increases in BAL total white cell count and alveolar macrophage acid phosphatase activity, and pathological evidence of pulmonary edema and type II pneumocyte injury. We conclude that lung eicosanoids are involved in the inflammatory process caused by severe smoke inhalation. However, the specific roles these lung eicosanoids play in the smoke-induced injury process are not known at this time.

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FOREWORD

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INTRODUCTION

Smoke inhalation has been identified as a major cause of lung injury and death in fires with a mortality rate of approximately seventy-five percent. The combination of burning material and an enclosed space are major factors that lead to smoke inhalation. Soldiers regularly occupy enclosed spaces such as airplanes, helicopters, and tanks. During my recent visit to the United States Army Institute of Surgical Research Burn Center at Fort Sam Houston, I observed soldiers who had suffered smoke inhalational injury due to helicopter crashes, a car bomb explosion in Panama, and fuel storage tank fire.

Smoke inhalation has been identified as a major cause of Adult Respiratory Distress Syndrome (ARDS). ARDS is associated with an acute lung injury to lung permeability. This increased permeability leads to a rapid and large accumulation of protein-rich fluid in the lung.

We have noticed many of the anatomic and physiologic features of the early stages of ARDS in our rabbit model of acute cigarette smoke exposure (CSE) (1,2). The CSE rabbits had increased pulmonary clearance of aerosolized technetium-labeled diethylenetriamine pentaacetic acid (99mTcDTPA), focal amounts of protein-rich alveolar edema accompanied with hemorrhage, and hypoxemia in spite of mechanical ventilation. Furthermore, the majority of rabbits pretreated with ibuprofen (prostaglandin cyclooxygenase pathway inhibitor) (CSE-I) had these same features of ARDS with a significant amount of edema and hyaline membranes. In our last cigarette smoke exposure study, we followed the same smoke exposure protocol as our previous projects except a broncho-alveolar lavage (BAL) was performed at the end of the experiment. The CSE-I group was divided into rabbits that survived the 30-breath smoke exposure (CSE-IL) and those that died during the 30-breath smoke exposure (CSE-ID). We found in the CSE, CSE-IL, and CSE-ID groups that 99mTcDTPA biologic half-life (T1/2) as well as BAL fluid concentrations of leukotriene B4 (LTB4) were significantly decreased. Plasma and BAL 6-keto-PGF1 alpha (stable metabolite of prostacyclin, PGI2) increased in CSE rabbits compared to other groups. Alveolar macrophages were lower in concentration in the CSE-ID rabbits than in the CSE-IL group. CSE and CSE-IL BAL lymphocyte concentrations were decreased compared to control values. These findings suggest that eicosanoids could cause and/or mediate the manifestations of lung injury after acute smoke inhalation.

Alveolar macrophages, polymorphonuclear leucocytes, lymphocytes, and other cells are involved in inflammatory processes in the lungs. Therefore, it is reasonable to speculate that these cells may release chemical mediators, such as eicosanoids, i.e., prostaglandins and leukotrienes, that play important roles in smoke-induced lung injury. The purpose of the present study was to determine-

(1) If acute smoke inhalation was associated with time-related changes in 99mTcDTPA pulmonary clearance and BAL eicosanoid concentration.
(2) To characterize the lung injury process after acute smoke inhalation by pathologic determination of pulmonary edema as well as light and electron micrographic evidence of lung injury.
(3) To determine changes in BAL inflammatory cells and alveolar macrophage enzyme activity.

BODY

The over-all project design was for five groups of rabbits. The five groups were the following-

(1) Controls (C) who did not receive any smoke exposure or pharmacologic blockers.
(2) A smoke exposure (SE) group that did not receive any pharmacologic blockers.
(3) Rabbits that were pre-treated with ibuprofen (a prostaglandin cyclo-oxygenase pathway blocker) (SE-I) before smoke exposure.
(4) Rabbits that were pre-treated with piriprost (a leukotriene synthesis blocker) (SE-P) before smoke exposure.
(5) Rabbits that were pre-treated with both ibuprofen and piriprost (SE-IP) before smoke exposure.

All rabbits underwent aerosolization of 99mTcDTPA and smoke or sham smoke exposure. The rabbits were monitored 0.5, 1, 2, or 4 hours after smoke exposure. The rabbits were killed by exsanguination of the abdominal aorta and their lungs were removed for broncho-alveolar lavage or pathological examination.

The rabbits given ibuprofen received a daily intramuscular injection (25 mg/kg body weight) for six days prior to the experiment. The final ibuprofen injection was administered immediately prior to smoke exposure. Piriprost has a short plasma half-life. Consequently, 0.03 grams of piriprost was mixed with an equal amount of THAM (Tris hydroxymethyl aminomethane) in 2.94 mls of normal saline and aerosolized into the rabbits' lungs with a DeVilbiss Pulmo Sonic nebulizer (Model 25, Somerset, PA) for three minutes immediately before smoke exposure (3).

The nebulizer is inserted between the distal end of the inspiratory limb of the ventilator circuit and the endotracheal (ET) tube using a separate set of ventilator tubing. This nebulizer circuit produced droplets with a mass median diameter of 1.58 (+-0.7) microns as measured with a cascade impactor (4). The output of the nebulizer circuit was 0.5 ml/minute at a tidal volume of 30 mls and 40 breaths/minute.

The rabbits were anesthetized intramuscularly with Ketamine HCL (50 mg/kg; Parke-Davis, Morris Plains, NJ), Xylazine (8 mg/kg; Miles, Shawnee, KS), and Acepromazine Maleate (1 mg/kg; Aveco, Fort Dodge, IA). A 2.5 F catheter was placed in the rabbit's pulmonary artery through the right external jugular vein for measurements of pulmonary artery pressure. A polyethylene catheter (PE-240) with a 2 cm long latex balloon at its tip was placed in the distal esophagus and positioned where the changes in esophageal pressure with spontaneous inspiration were most negative. Balloon volume was determined using the technique of Lemen et al (5). We measured airway pressure with a polyethylene catheter placed at the proximal end of the ET tube. The airway and esophageal pressure catheters were connected to opposite sides of a differential pressure transducer (Validyne MP45-2, Northridge, CA) for measurement of transpulmonary pressure (Ptp). The rabbits were paralyzed with 20 mg of intravenous gallamine triethiodide (Flaxedil; Lederle, Carolina, Puerto Rico) and ventilated with a piston-type ventilator (Model 665, Harvard Apparatus Co., South Natick, MA) set to deliver a tidal volume of 14 ml/kg of body weight at a rate of 40 breaths/minute. All recordings were made using an Electronics for Medicine (Model ER-4) recorder.

The smoke exposure protocol was 60 tidal volume breaths of diesel fuel-polycarbonate plastic smoke or sham smoke (air drawn through an empty smoke chamber) delivered in the following manner. The diesel fuel-plastic smoke was generated by the combustion of 20 mls diesel fuel and 0.02 grams of polycarbonate plastic shavings in a ceramic crucible that was set in a stainless steel smoke chamber. The smoke chamber was constructed of #302 stainless steel with ports for air intake, sampling, and a thermometer. A tidal volume breath of smoke was drawn from the sampling port with a 60 ml syringe. The smoke-filled air was injected with the syringe into the rabbit's lungs via its ET tube while the rabbit was disconnected from the ventilator. Four to six ventilated breaths of room air were given to the rabbit after each breath of smoke. The temperature of the smoke chamber was recorded at 15, 30, 45, and 60 breaths of smoke exposure to ensure uniformity of the smoke. The combination of diesel fuel-polycarbonate plastic burned for approximately 15 minutes and the 60 smoke breaths were usually administered in 10 minutes. We chose these materials as representative of the types of compounds that a soldier might encounter in combat. We chose polycarbonate plastic after personal communication with Dr. Karl Luther of the General Dynamics Tank Plant in Michigan. Dr. Luther informed us that polycarbonate plastic is a component of the M-1 battle tank.

Technetium-labeled diethylenetriamine pentaacetic acid (99mTcDTPA, MW=492 daltons) was aerosolized into the rabbit's lungs at 25 minutes before the end of the study session for 10 minutes. Pulmonary 99mTcDTPA (physical half-life of 6.03 hours) in 2 ml normal saline was aerosolized with a nebulizer circuit identical to that used for piriprost delivery. Gamma radiation was measured with two Ludlum (Model 44-2, Sweetwater, TX) portable scintillation probes. The range of the scintillation probes was 0 to 500,000 counts/minute. One probe was placed over the right lung in the sagittal plane. The other probe was placed over the medial aspect of the right thigh excluding the bladder.

Our nuclear medicine technician prepared 99mTcDTPA using standard kits (Medi-Physics, Emeryville, CA). Binding of 99mTc and DTPA was consistently greater than 97%, as determined by thin-layer paper chromatography. The aerosolization, pulmonary uptake, and passage of the 99mTcDTPA into the rabbit's urine does not affect binding of 99mTc and DTPA.

Pulmonary clearance of 99mTcDTPA was established over a 15-minute counting period beginning immediately after aerosolization. Gamma counts were recorded for the first 30 seconds of every minute. The 99mTcDTPA pulmonary clearance curves were corrected for lung blood concentration of 99mTcDTPA over a 10 minute period at the end of the study session by intravenous injection of 200 microCuries 99mTcDTPA. The 99mTcDTPA clearance curves were used to determine biologic half-life (T1/2) and 99mTcDTPA T1/2 was corrected for residual background radiation.

The heart-lung block was removed immediately after death. Broncho-alveolar lavage was performed by placing a #16 angiocatheter distal to the cross-clamped trachea. Washes were repeated twice with 20 ml aliquots of sterile 0.85% saline solution (6). The collected fluid was decanted into chilled plastic tubes and centrifuged at 1000 rpm for 10 minutes at 4 degrees centigrade. The supernatant was decanted from the cell pellet and stored at -70 degrees centigrade without preservative until used for chemical mediator assay.

We assayed for LTB4, LTC4, 6-keto-PGF1 alpha, PGE2, and TxB2 (stable metabolite of thromboxane A2) in the BAL fluid. Blood samples (6 mls) for plasma measurements of LTB4, LTC4, 6-keto-PGF1 alpha, PGE2, and TxB2 were drawn from the abdominal aorta into plastic syringes containing 0.16% indomethacin (0.01 ml/ml blood) and EDTA (0.09 ml/ml blood) before and after smoke exposure. The blood was immediately transferred to chilled polypropylene tubes and centrifuged at 2000 x g, 4 degrees centigrade, for 30 minutes. The plasma was pipetted into polypropylene tubes and frozen at -70 degrees centigrade until analysis.

Plasma eicosanoids were purified using a Waters reverse-phase high pressure liquid chromatography system (7). The percent recovery was measured for each eicosanoid and used to calculate plasma concentration. Eicosanoid concentration was determined by radioimmunoassay using single antibody techniques (8). All samples were run in duplicate.

The cell pellet was resuspended with normal saline. Total cell counts were performed using a standard hemocytometer. Differential counts were done with both a wet-mount and Wright-Giemsa stained slide under light microscopy.

The tissue for electron micrographic studies was fixed in 3%

gluteraldehyde for two hours, transferred to phosphate buffer, imbedded in Epon, postfixed in osmium tetroxide, and stained with uranyl acetate-lead citrate. Sections were examined with a Joel electron microscope (X 19,000 maximal magnification). Pulmonary edema formation was measured in the pathology rabbits by wet lung weight/body weight ratio (WL/BW) and histology slides.

An aliquot of 0.2 ml BAL fluid was attached to several glass slides in a Shandon Cytospin II centrifuge. The presence of alveolar macrophage acid phosphatase enzyme was determined in fixed BAL-derived samples as described by Dannenberg and Moritaka (9). A portion of the cell-free BAL fluid was assessed for the release of plasminogen activator enzyme, a neutral protease that is highly correlated with alveolar macrophage activation and capable of degrading elastin and activating humoral enzyme cascades (10).

Mean data was calculated for each group of rabbits. The statistical design we used in the majority of analyses in this study was analysis of variance (ANOVA) (11). A Student-Newman-Keuls test was utilized for a posteriori contrasts if the ANOVA was significant ($p<0.05$). The Statview statistical program for Apple Macintosh SE microcomputers was used for all the statistical analyses in this study (12).

RESULTS

A total of 148 rabbits were completed in the study. The number of rabbits in each group is presented in the Appendix, Table 1.

Acute smoke exposure caused changes in BAL and plasma eicosanoid concentration, especially at the 0.5 hour time interval. Furthermore, these changes in eicosanoid levels were accompanied by decreases in 99mTcDTPA T1/2, increases in BAL total white cell count (BTWCC) and alveolar macrophage acid phosphatase enzyme activity, and pathological evidence of pulmonary edema and type II pneumocyte injury.

The 0.5 hour SE rabbits had less than 0.25 pg/0.1 ml of BAL fluid concentration of PGE2 (Appendix, Table 2). The 0.25 pg/0.1 ml of BAL fluid value is the lowest detectable limit of the PGE2 radioimmunoassay (8). Furthermore, the 0.5 hour SE group had a significant increase in BAL 6-keto-PGF1 alpha levels compared to controls (Appendix, Table 3). Other 0.5 hour groups also had changes in BAL eicosanoid concentration.

The 0.5 hour SE-I rabbits had decreases in 6-keto-PGF1 alpha but, surprisingly, showed no change in PGE2 levels compared to controls. As one might expect, the 0.5 hour SE-P group had no detectable levels of LTB4 (Appendix, Table 4). On the other hand, the 0.5 SE-P rabbits also showed a marked decrease in BAL TxB2 concentration (Appendix, Table 5). These findings were also present in the 0.5 hour SE-IP rabbits.

At the 1.0 hour post-smoke time interval; SE, SE-I, SE-P, SE-IP, and piriprost control (PC) groups all had decreases in BAL LTB4. The only other change in eicosanoid concentration at 1.0 hour was a decrease in BAL TxB2 in the SE-IP rabbits. The 2.0 hour SE and SE-P groups also had decreases in BAL LTB4 levels. We did not demonstrate any changes in BAL LTC4 concentration at any time interval.

There were no detectable levels of PGE2 or LTC4 in the pre-smoke exposure or post-smoke plasma samples. In addition, there were no changes in pre-vs-post smoke exposure concentrations of 6-keto-PGF1 alpha or TxB2. However, post-smoke LTB4 plasma concentrations were elevated in the 0.5 hour SE-P and 1.0 hour SE-I rabbits. In contrast, LTB4 post-sham smoke plasma concentration was decreased compared to pre values in 1.0 hour PC rabbits (Appendix, Table 6).

The 99mTcDTPA T1/2 values followed an erratic pattern compared to the changes in eicosanoid concentration. However, at the 0.5 hour time interval, the SE rabbits had a decreased mean 99mTcDTPA T1/2 value compared to controls (Appendix, Table 7). Surprisingly, the 0.5 hour SE-IP group had a higher mean 99mTcDTPA T1/2 than controls. The 1.0 hour control rabbits had a somewhat higher mean 99mTcDTPA T1/2 compared to 0.5, 2.0, and 4.0 hour control groups. Consequently, the 1.0 hour SE, SE-I, SE-P, and SE-IP groups had decreased 99mTcDTPA T1/2 compared to the 1.0 hour control rabbits. There were no changes in 99mTcDTPA T1/2 at 2.0 or 4.0 hours.

Piriprost pre-treatment caused a very large increase in BTWCC; however, there were no changes in white cell differentials between any groups (Appendix, Table 8). This increase was also noted in the 1.0 hour PC group. In addition, the 1.0 hour SE-I and 2.0 hour SE rabbits had an increase in BTWCC compared to controls. At 0.5 hours, the SE-P group had a mean of 78.1% post-vs-pre systemic white cells compared to 82.6% for controls. However, at 2.0 hours the SE-P rabbits had 46.4% post-vs-pre systemic white cells while the SE and C means were 76.8% and 111.7%, respectively. Thus, it appears at the 2.0 hour time interval, the large BTWCC may be due to an influx of white cells from the systemic circulation.

All rabbits exposed to smoke had increases in alveolar macrophage acid phosphatase enzyme activity (Appendix, Table 9). In contrast, there were no changes in BAL plasminogen activator enzyme activity.

The WL/BW ratio demonstrated that the 0.5 hour SE-P rabbits had an increase in WL/BW compared to controls (Appendix, Table 10). This pattern continued in the 1.0 hour and 2.0 hour SE-P groups. The 1.0 hour SE-I and 2.0 hour SE rabbits also had increases in WL/BW. The WL/BW ratio was not corrected for lung blood volume. However, our experimental protocol included a second 200 microCurie injection of 99mTcDTPA given through an ear vein catheter at the end of the study session so that it would be possible to correct lung 99mTcDTPA counts for lung blood volume. We did not demonstrate any increase in 99mTcDTPA lung/99mTcDTPA thigh ratio in the rabbit groups that had increases in WL/BW. Thus, we conclude these rabbits had increases in WL/BW due to fluid accumulation in the lung interstitium and alveolar spaces rather than an increase in lung blood volume. This conclusion is supported by light microscopy which demonstrated interstitial and alveolar edema.

Electron micrographs of 1.0 hour SE and SE-I rabbits showed alveolar spaces that contained small amounts of granular material consistent with edema fluid and scattered red blood cells (Appendix, Figure 1). Smoke particles were seen in alveoli and small airways and were present within phagocytic vacuoles and alveolar macrophages (Appendix, Figures 2 and 3). There were fragments of cellular debris which suggests the destruction of cells, possibly alveolar macrophages.

The 1.0 hour SE-P rabbits demonstrated minor focal alveolar septal alterations, consisting of swelling in type I epithelium (Appendix, Figure 4). A low-power view of alveolar septa showed numerous polymorphonuclear leucocytes within capillaries and the lack of prominence of type II pneumocytes (Appendix, Figure 5). There were infrequent intravascular inflammatory cells.

The 2.0 hour SE-P rabbits had type II pneumocytes which appeared much more prominent than in the 0.5 or 1.0 hour SE-P groups. The density of these pneumocytes (Appendix, Figure 6) can be compared with Figure 5. The increased prominence of the type II pneumocytes may represent increased recognition due to discharge of their secretory products and easier recognition of the cytoplasmic organelles that characterize type II pneumocytes.

The 4.0 hour SE-P rabbits had the same prominence of type II pneumocytes that was observed in the 2.0 hour SE-P group. However, the 4.0 hour SE-P rabbits' alveolar spaces contained a dense granular material associated with intact and disintegrating alveolar macrophages containing phagocytized material (Appendix, Figure 7).

DISCUSSION

Acute smoke exposure produced many changes in eicosanoid concentration as well as pathologic and physiologic parameters of lung injury. Several of our findings in the present study with diesel fuel-generated smoke were similar to our previous studies with acute cigarette smoke exposure (1,2,4).

Principally, acute exposure with either diesel fuel or cigarette smoke caused decreases in BAL LTB4 and 99mTcDTPA T1/2. Alveolar macrophages are the chief source of LTB4 in the lung (13). Furthermore, several investigators theorize that LTB4 may regulate alveolar macrophage communication by controlling interleukin-1 (IL-1) release (14,15). We have videotape evidence that alveolar macrophages play an early critical role in the inflammatory process after severe smoke inhalation. However, we cannot prove conclusively that the decrease in BAL LTB4 is due to alveolar macrophage involvement in acute smoke inhalation.

Severe smoke exposure is accompanied by a decrease in pulmonary 99mTcDTPA T1/2. Many investigators postulate that a decrease in 99mTcDTPA T1/2 demonstrates an increase in alveolar epithelial permeability (16,17). There is ample evidence that the epithelial layer of the alveolar-capillary barrier is much less permeable than the endothelial layer. Thus, an increase in epithelial permeability could make the formation of alveolar edema by the movement of fluid across the alveolar-capillary barrier easier to accomplish.

Unfortunately, our data shows a poor correlation between a decrease in 99mTcDTPA T1/2 and an increase in WL/BW ratio at the 0.5 hour time interval. However, the 1.0 hour SE-I and SE-P groups both had decreases in 99mTcDTPA T1/2 and increases in WL/BW ratio compared to controls.

Our 1.0 hour control mean 99mTcDTPA T1/2 of 88 minutes is higher than our 0.5, 2.0, or 4.0 hour control values of 48.4, 57.3, 52.3 minutes, respectively. We have performed the 99mTcDTPA T1/2 measurement in approximately 250 rabbits and have observed a wide variability in rabbits. Other investigators have demonstrated a wide variability in 99mTcDTPA T1/2 in normal humans (18,19). Three rabbits in the 1.0 hour control group had 99mTcDTPA T1/2 values of greater than 100 minutes. There was no pathological evidence of any lung abnormality and, consequently, we decided to include these rabbits in our statistical analyses. We have no explanation why these rabbits had an unusually high 99mTcDTPA T1/2.

The BAL PGE2 data at the 0.5 hour time interval is very interesting. All of the SE rabbits had no detectable levels of PGE2 in the BAL fluid. Gerrard believes that PGE2 may play an important role in the activation of alveolar macrophages (20). Once again, we have videotape evidence of alveolar macrphages that have ingested smoke particles and thus it is reasonable to conclude these alveolar macrophages underwent activation. However, we cannot prove conclusively that alveolar macrophages in the 0.5 hour SE group metabolized PGE2 in the activation process.

We were surprised that the 0.5 hour SE-I rabbits had normal levels of BAL PGE2. Ibuprofen is a cyclooxygenase prostaglandin inhibitor and one might expect it to block PGE2. There was a decrease in 6-keto-PGF1 alpha 0.5 hour SE group concentration compared to controls, indicating that ibuprofen caused a decrease in another cyclooxygenase prostaglandin, prostacyclin. We have no explanation why ibuprofen blocked 6-keto-PGF1 alpha production but not PGE2.

Piriprost pre-treatment caused decreased BAL TxB2 as well as BAL

LTB4 in the 0.5 hour SE-P and SE-IP rabbits and 1.0 hour SE-IP group. Thus, it appears that piriprost has cyclooxygenase pathway inhibitor properties in addition to the expected lipoxygenase pathway inhibition. Recently, there is evidence that injection of LTB4 into blood can induce changes in blood TxB2 concentration (21). Thus, it appears there is a "synergism" between LTB4 and TxB2 activity with piriprost pre-treatment and the physiological significance of this observation is unknown at this time.

The plasma eicosanoid data was unremarkable except for LTB4. It has been theorized that all lung prostaglandins and leukotrienes, with the exception of PGI2, would be metabolized immediately in the lung and would not change plasma eicosanoid levels (22). Our LTC4 data supports this hypothesis. We did not detect any LTC4 in BAL fluid or plasma and this is probably due to the relatively short (4 hour) time interval we have studied to date does not allow for the development of changes in LTC4 concentration. Other BAL eicosanoid concentrations show changes after smoke exposure and/or inhibitor treatment while plasma levels of PGE2 were not detectable or unchanged as with TxB2 and 6-keto-PGF1 alpha. We conclude that BAL and plasma eicosanoid levels demonstrate a poor correlation and the physiological importance of plasma eicosanoid concentrations after severe smoke exposure is minimal.

The over-all significance of BAL eicosanoid levels and their importance in the inflammatory process after severe smoke exposure is unclear at this time. LTB4 is thought to regulate IL-1 release by alveolar macrophages and there is evidence that IL-1 can increase vascular permeability by stimulating the formation of an unknown prostaglandin (23). Furthermore, PGE2 can cause the release of tumor necrosis factor (TNF alpha) and vice versa. In addition, PGE2 in low-dose concentrations can activate alveolar macrophages (24). Thus, the changes in LTB4 and PGE2 BAL levels at the various time intervals could mediate the release of lymphokines or powerful chemical substances that could play substantial roles in the lung injury process after severe smoke inhalation.

The BAL eicosanoids could control physiologically important lung processes such as alveolar epithelial permeability. The four rabbits in the 0.5 hour SE-IP group had a higher mean 99mTcDTPA T1/2 (59.8 minutes -vs- 48.4 minutes) than did controls. We realize that it is not scientifically valid to make comparisons between the 0.5 hour SE-IP group (N=4) and 0.5 C group (N=12). However, there is evidence that ibuprofen treatment after synthetic smoke inhalation can attenuate pulmonary edema (25). Perhaps, eicosanoid blockade can decrease alveolar epithelial permeability by changing the physiological or anatomical characteristics of the lung epithelial cells.

Piriprost treatment can cause a large influx of inflammatory cells into the lungs even without smoke exposure as demonstrated by the 1.0 hour PC group's increase in BTWCC. It appears the white cell influx is from the systemic circulation. However, we do not have any pathology piriprost control rabbits at this time. Consequently, we cannot determine how these cells cross the alveolar-capillary barrier. This observation could have important physiological and anatomical considerations. Many inflammatory processes in the lungs are characterized by an influx of white cells into the lungs. Obviously, it would be very important to learn how these cells are "called" into the lungs and by what method they use to cross the alveolar-capillary barrier.

The 1.0 hour SE-I and 2.0 hour SE rabbits also had increases in BTWCC compared to controls. Thus, it appears that piriprost and ibuprofen treatment before smoke exposure can cause an influx of white cells into the lungs with piriprost acting more quickly as evidenced by the increase in BTWCC in the 0.5 hour SE-P group. It seems that the normal course of an increase in BTWCC is at 2.0 hours following smoke exposure as evidenced by the 2.0 hour SE group. It is also interesting to note that the 0.5 and 1.0 hour SE-IP groups had the highest BTWCC. Consequently, we can conclude that piriprost pre-treatment alone can induce a large increase in BTWCC and that ibuprofen and piriprost together cause the highest BTWCC. At this point in time, we have no explanation how piriprost causes the large increase in BTWCC. However, we can speculate that BAL eicosanoids could possibly control this process based on the 0.5 hour and 1.0 hour SE-IP groups having the highest BTWCC in their respective time intervals.

The large influx of inflammatory cells in the BAL fluid of the 0.5 hour SE-IP, 1.0 hour SE-I and SE-P, and 2.0 hour SE and SE-P groups was also accompanied by an increase in WL/BW. Thus, it appears the large increase in BTWCC is accompanied by pulmonary edema. It is possible that the white cells change the permeability of the alveolar-capillary barrier as they travel into the alveolar spaces; thereby causing fluid to leave the capillary and move into the lung interstitium and alveolar spaces. On the other hand, the two events could be unrelated. At this point in our study, we cannot make any linkage between the increase in BTWCC and WL/BW. We can conclude that both phenomena are part of the inflammatory process after smoke exposure.

Electron micrographic studies demonstrated that piriprost pre-treatment caused increased prominence of type II pneumocytes presumably due to the discharge of their secretory products. The major question this observation raises is whether piriprost "speeds" up the inflammatory process or selectively activates type II pneumocytes. We need to study the other groups at longer time intervals to answer this question.

In summary, acute smoke exposure caused changes in BAL eicosanoid concentration, especially at the 0.5 hour time interval. In addition, there were decreases in 99mTcDTPA T1/2, pathological evidence of pulmonary edema and activation of type II pneumocytes by piriprost pre-treatment.

We conclude that lung eicosanoids are involved in the inflammatory process caused by severe smoke inhalation. However, the specific roles these lung eicosanoids play in the smoke-induced inflammatory process are not known at this time.

REFERENCES

(1) Witten ML, Lemen RJ, Quan SF, et al. Acute cigarette smoke exposure increases alveolar permeability in rabbits. Am Rev Respir Dis 132: 321-325, 1985.

(2) Witten ML, Lemen RJ, Quan SF, et al. Acute cigarette smoke exposure causes lung injury in rabbits treated with ibuprofen. Exp Lung Res 13: 113-126, 1987.

(3) Personal communication with Dr. Michael Bach, senior research scientist, of Upjohn Pharmaceutical Co.

(4) Witten ML, Lemen RJ, Quan SF, et al. Acute cigarette smoke exposure alters lung eicosanoid and inflammatory cell concentrations in rabbits. Exp Lung Res (in press).

(5) Lemen R, Benson M, Jones JG. Absolute pressure measurements with hand-dipped and manufactured esophageal balloons. J Appl Physiol 37: 600-603, 1974.

(6) Reynolds HY, Thompson RE. Analysis of protein and lipids in bronchial secretions and antibody responses after vaccination with pseudomonas aeruginosa. J Immunol 111: 358-368, 1973.

(7) Burgoyne B. Arachidonic acid cascade: technical backgrounder. Milford, MA. Waters Corporation, 1985.

(8) Standard radioimmunoassay kits for eicosanoids. Boston, MA. Dupont New England Nuclear Co., 1988.

(9) Dannenberg AM, Moritaka S. Histochemical stains for macrophages in cell smears and tissue sections. In: Methods for studying mononuclear phagocytes. D.O. Adams, ed. Academic Press, Inc., 1981, pp. 375-396.

(10) Personal communication with Dr. Andrea Hubbard, research assistant professor, of the University of Arizona Health Sciences Center.

(11) Nie NH, Hull CH, Steinbrenner K, et al. Statistical package for the social sciences, (2nd ed.), New York, McGraw-Hill, 1975.

(12) Statview statistical software. Cupertino, CA., Apple Computer Co., 1987.

(13) Hutchinson-Ford AW. Leukotrienes: their formation and role as inflammatory mediators. Federation Proc 44: 25-29, 1985.

(14) Bonney RJ, Opas EE, Humes JL. Lipoxygenase pathways of macrophages. Federation Proc 44: 2933-2936, 1985.

(15) Unanue ER, Allen PM. The immunoregulatory role of the macrophage. Hospital Practice April 15, 1987, pp. 63-80.

(16) Symposium on non-invasive measurement of lung water/Section on pulmonary circulation/Scientific assembly on respiratory structure, function, and metabolism. Presented on May 12, 1985 at the American Thoracic Society meetings, Anaheim, CA.

(17) Rizk NW, Luce JM, Hoeffel JM, et al. Site of deposition and factors affecting clearance of aerosolized solute from canine lungs. J Appl Physiol 56: 723-729, 1984.

(18) Jones JG, Lawler P, Crawley JCW, et al. Increased alveolar epithelial permeability in cigarette smokers. Lancet 1: 66-68, 1980.

(19) Jefferies AL, Coates G, Webber CE, et al. Measurement of pulmonary clearance of radioaerosol using a portable sodium iodide probe. J Appl Physiol 57: 1908-1912, 1984.

(20) Gerrard JM. Prostaglandins and leukotrienes: blood and vascular cell function. New York, Marcel Dekker, 1985.

(21) Miller RF, Lefferts PL, Christman BW, et al. Meclofenamate attenuates leukotriene B4 induced pulmonary hypertension in sheep. The FASEB Journal 2: A1559, 1988.

(22) Nowak, J. Eicosanoids and the lungs. Annals of Clinical Research 16: 269-286, 1984.

(23) Schnizlein-Bick CT, Roepke JE, Barnard JW, et al. Interleukin-1 interstitial edema in perfused rat lungs: role of cyclooxygenase metabolites. The FASEB Journal 2: A1058, 1988.

(24) Barthelson R, Vaione FH, Debs R, et al. Synergy in interleukin-1 (IL-1) release by human monocytes stimulated with platelet-activating factor (PAF) plus gamma interferon (IFN gamma) or tumor necrosis factor (TNF). The FASEB Journal 2: A1228, 1988.

(25) Shinozawa Y, Hales CA, Jung W, et al. Ibuprofen prevents synthetic smoke-induced pulmonary edema. Am Rev Respir Dis 134: 1145-1148, 1986.

APPENDIX

TABLE 1

0.5 C	N=12
0.5 SE	N=12
0.5 SE-I	N=11
0.5 SE-P	N=12
0.5 SE-IP	N=5
1.0 C	N=12
1.0 SE	N=12
1.0 SE-I	N=9
1.0 SE-P	N=12
1.0 SE-IP	N=2
1.0 PC	N=6
2.0 C	N=11
2.0 SE	N=12
2.0 SE-P	N=10
4.0 C	N=3
4.0 SE	N=1
4.0 SE-P	N=6

The number of rabbits in each of the study groups. There were a total of 148 rabbits in the study.

APPENDIX

TABLE 2

BAL PGE2 CONCENTRATION

0.5 C	22.8 (4.6)
0.5 SE	0 (0)*
0.5 SE-I	21.6 (2.1)
0.5 SE-P	22.9 (0.3)
0.5 SE-IP	21.6 (0.5)
1.0 C	13.4 (5.6)
1.0 SE	8.4 (5.3)
1.0 SE-I	24.1 (0.7)
1.0 SE-P	15.8 (5.0)
1.0 SE-IP	21.2 (1.5)
1.0 PC	23.1 (0.4)
2.0 C	11.2 (5.4)
2.0 SE	16.1 (5.1)
2.0 SE-P	20.1 (4.0)

Values are means with standard errors of the mean in parenthesis. The concentrations of PGE2 are pg/0.1 ml of BAL fluid. * $p<0.05$ compared to the control group.

APPENDIX

TABLE 3

BAL 6-KETO-PGF1 ALPHA CONCENTRATION

0.5 C	122.2 (18.2)
0.5 SE	169.4 (14.3)*
0.5 SE-I	72.6 (19.4)*
0.5 SE-P	97.5 (8.2)
0.5 SE-IP	114.5 (3.1)
1.0 C	95.3 (28.5)
1.0 SE	117.1 (18.2)
1.0 SE-I	133.6 (8.0)
1.0 SE-P	124.4 (5.1)
1.0 SE-IP	96.5 (7.5)
1.0 PC	125.0 (5.2)
2.0 C	127.7 (13.2)
2.0 SE	121.0 (5.4)
2.0 SE-P	125.2 (7.8)

Values are means with standard errors of the mean in parenthesis. The concentrations of 6-keto-PGF1 alpha are pg/0.1 ml of BAL fluid. *$p<0.05$ compared to the control group.

APPENDIX

TABLE 4

BAL LTB4 CONCENTRATION

0.5 C	39.2 (25.3)
0.5 SE	37.1 (23.9)
0.5 SE-I	11.0 (11.0)
0.5 SE-P	0 (0)*
0.5 SE-IP	0.7 (0.7)*
1.0 C	112.9 (30.0)
1.0 SE	7.8 (6.3)*
1.0 SE-I	5.7 (5.7)*
1.0 SE-P	2.9 (2.9)*
1.0 SE-IP	4.6 (2.1)*
1.0 PC	3.3 (2.8)*
2.0 C	61.5 (38.5)
2.0 SE	17.4 (12.0)*
2.0 SE-P	5.0 (3.0)*

Values are means with standard errors of the mean in parenthesis. The concentrations of LTB4 are pg/0.1 ml of BAL fluid. $^*p<0.05$ compared to the control group.

APPENDIX

TABLE 5

BAL TxB2 CONCENTRATION

0.5 C	72.8 (9.2)
0.5 SE	81.0 (5.9)
0.5 SE-I	52.8 (8.3)
0.5 SE-P	35.5 (6.6)*
0.5 SE-IP	34.2 (10.6)*
1.0 C	72.1 (3.1)
1.0 SE	67.6 (7.4)
1.0 SE-I	56.8 (5.3)
1.0 SE-P	49.7 (5.6)
1.0 SE-IP	36.8 (4.7)*
1.0 PC	58.7 (6.8)
2.0 C	67.0 (14.0)
2.0 SE	66.2 (5.9)
2.0 SE-P	55.9 (4.5)

Values are means with standard errors of the mean in parenthesis. The concentrations of TxB2 are pg/0.1 ml of BAL fluid. *$p<0.05$ compared to the control group.

APPENDIX

TABLE 6

PLASMA LTB4 CONCENTRATIONS

	PRE-SE	POST-SE
0.5 SE-P	126.7 (13.3)	143.8 (9.4)
1.0 SE-I	123.7 (15.1)	174.9 (16.1)
1.0 PC	150.7 (25.8)	105.2 (14.8)

Values are means with standard errors of the mean in parenthesis. The concentrations of LTB4 are pg/0.1 ml of plasma fluid. There was a significant difference, $p<0.05$, between the pre-se and post-se values.

APPENDIX

TABLE 7

99mTcDTPA T1/2

0.5 C	48.4 (6.2)
0.5 SE	32.6 (4.7)*
0.5 SE-I	41.4 (3.3)
0.5 SE-P	39.2 (4.7)
0.5 SE-IP	59.8 (12.1)
1.0 C	88.0 (10.4)
1.0 SE	57.4 (10.2)*
1.0 SE-I	46.8 (6.6)*
1.0 SE-P	57.3 (4.9)*
1.0 SE-IP	33.0 (9.8)*
1.0 PC	67.8 (12.9)
2.0 C	57.3 (8.4)
2.0 SE	48.3 (4.8)
2.0 SE-P	52.6 (9.6)
4.0 C	52.3 (6.5)
4.0 SE	45.7 (3.3)

Values are means with standard errors of the mean in parenthesis. 99mTcDTPA T1/2 is in minutes. *$p<0.05$ compared to the control group.

APPENDIX

TABLE 8

BAL TOTAL WHITE CELL COUNT

0.5 C	740 (331)
0.5 SE	333 (64)
0.5 SE-I	1240 (221)
0.5 SE-P	18093 (7830)*
0.5 SE-IP	47869 (22227)*
1.0 C	333 (86)
1.0 SE	537 (144)
1.0 SE-I	28009 (13802)*
1.0 SE-P	35372 (10158)*
1.0 SE-IP	41736 (2267)*
1.0 PC	33282 (6883)*
2.0 C	851 (214)
2.0 SE	16317 (1004)*
2.0 SE-P	42402 (7381)*

Values are means with standard errors of the mean in parenthesis. The BAL total white cell concentration is in cells per cubic mm of BAL fluid. *$p<0.05$ compared to the control group.

APPENDIX

TABLE 9

ALVEOLAR MACROPHAGE ACID PHOSPHATASE ACTIVITY

0.5 C	10.4 (2.9)
0.5 SE	98.2 (20.7)*
0.5 SE-I	159.3 (28.1)*
0.5 SE-P	222.3 (5.4)*
0.5 SE-IP	144.0 (56.0)*
1.0 C	12.6 (2.7)
1.0 SE	179.0 (18.5)*
1.0 SE-I	146.0 (32.9)*
1.0 SE-P	144.4 (13.4)*
1.0 SE-IP	118.7 (12.5)*
1.0 PC	67.0 (20.4)
2.0 C	23.2 (8.3)
2.0 SE	114.0 (86.0) (N=2)
2.0 SE-P	151.5 (14.7) *

Values are means with standard errors of the mean in parenthesis. The concentrations of alveolar macrophage acid phosphatase enzyme are based on a numerical grading scale of 0-3. *$p<0.05$ compared to the control group.

APPENDIX

TABLE 10

WET LUNG WEIGHT (g) / BODY WEIGHT (kg) RATIO

0.5 C	3.59 (0.2)
0.5 SE	3.86 (0.2)
0.5 SE-I	3.93 (0.6)
0.5 SE-P	6.18 (0.5)*
1.0 C	3.75 (0.2)
1.0 SE	3.32 (0.8)
1.0 SE-I	5.23 (0.5)*
1.0 SE-P	4.80 (0.4)*
2.0 C	3.82 (0.2)
2.0 SE	5.49 (0.4)*
2.0 SE-P	5.41 (0.5)*
4.0 C	4.26 (0.1) (N=3)
4.0 SE-P	5.95 (0.1) (N=2)

Values are means with standard errors of the mean in parenthesis.
*$p<0.05$ compared to the control group.

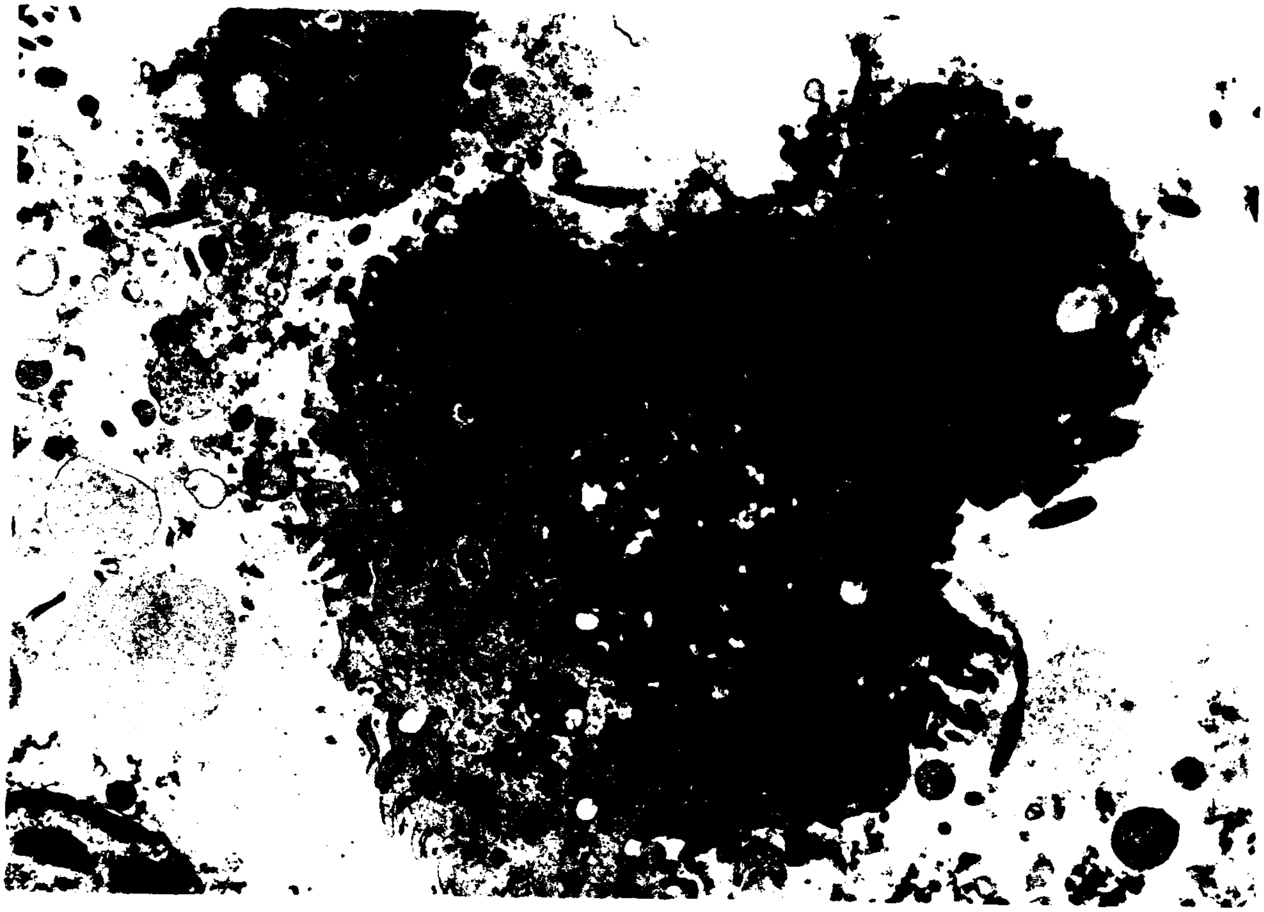
APPENDIX

FIGURE 1

Magnification is X 3800.

APPENDIX

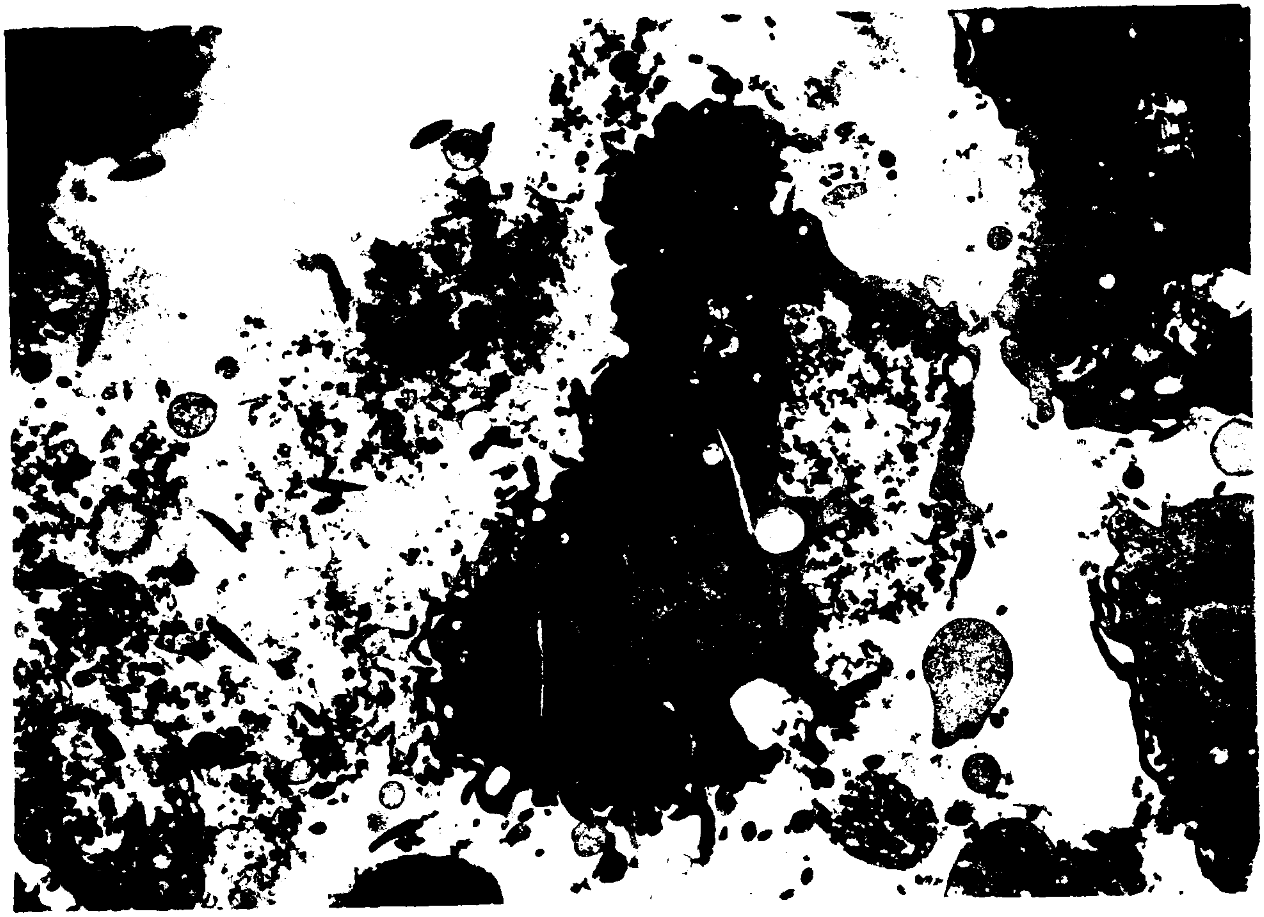
FIGURE 2



Magnification is X 9600.

APPENDIX

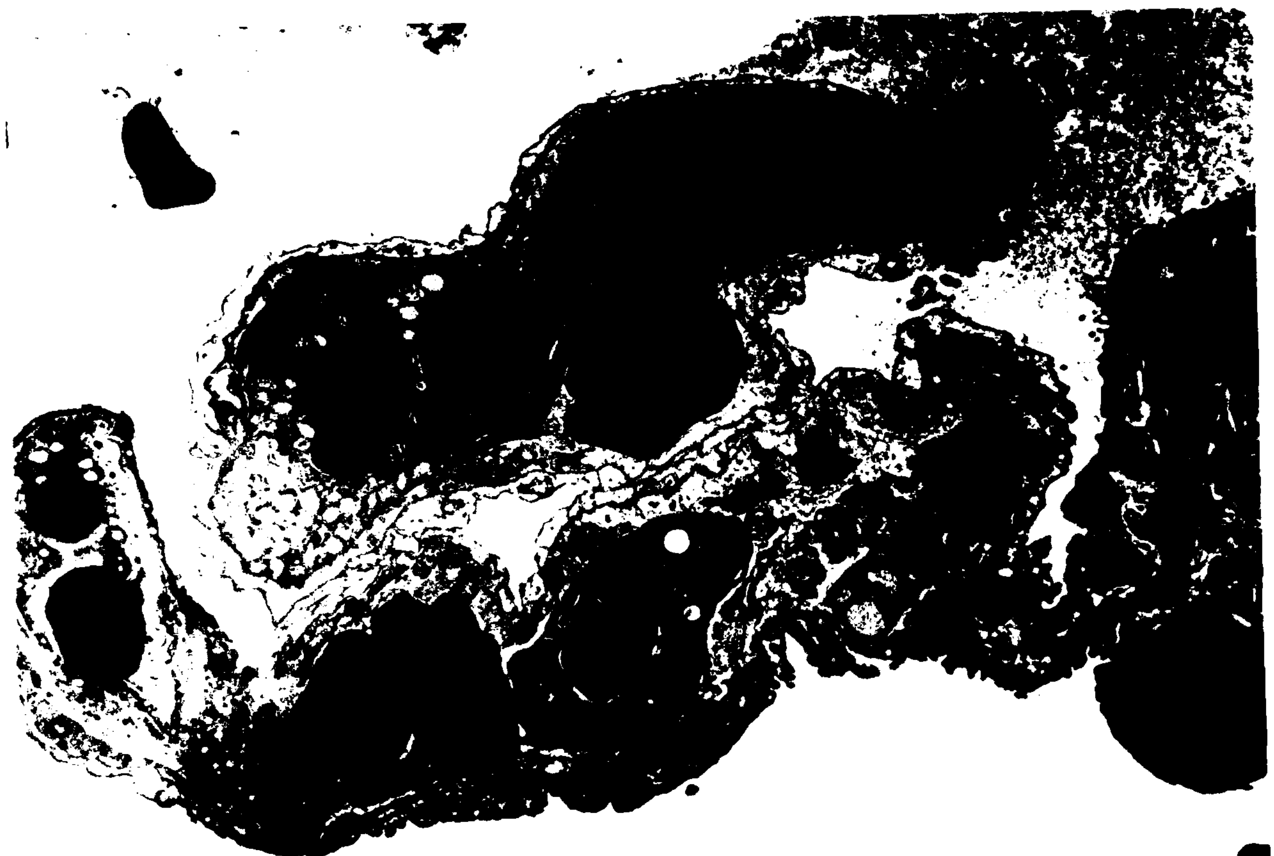
FIGURE 3



Magnification is X 7200.

APPENDIX

FIGURE 4



Magnification is X 5800.

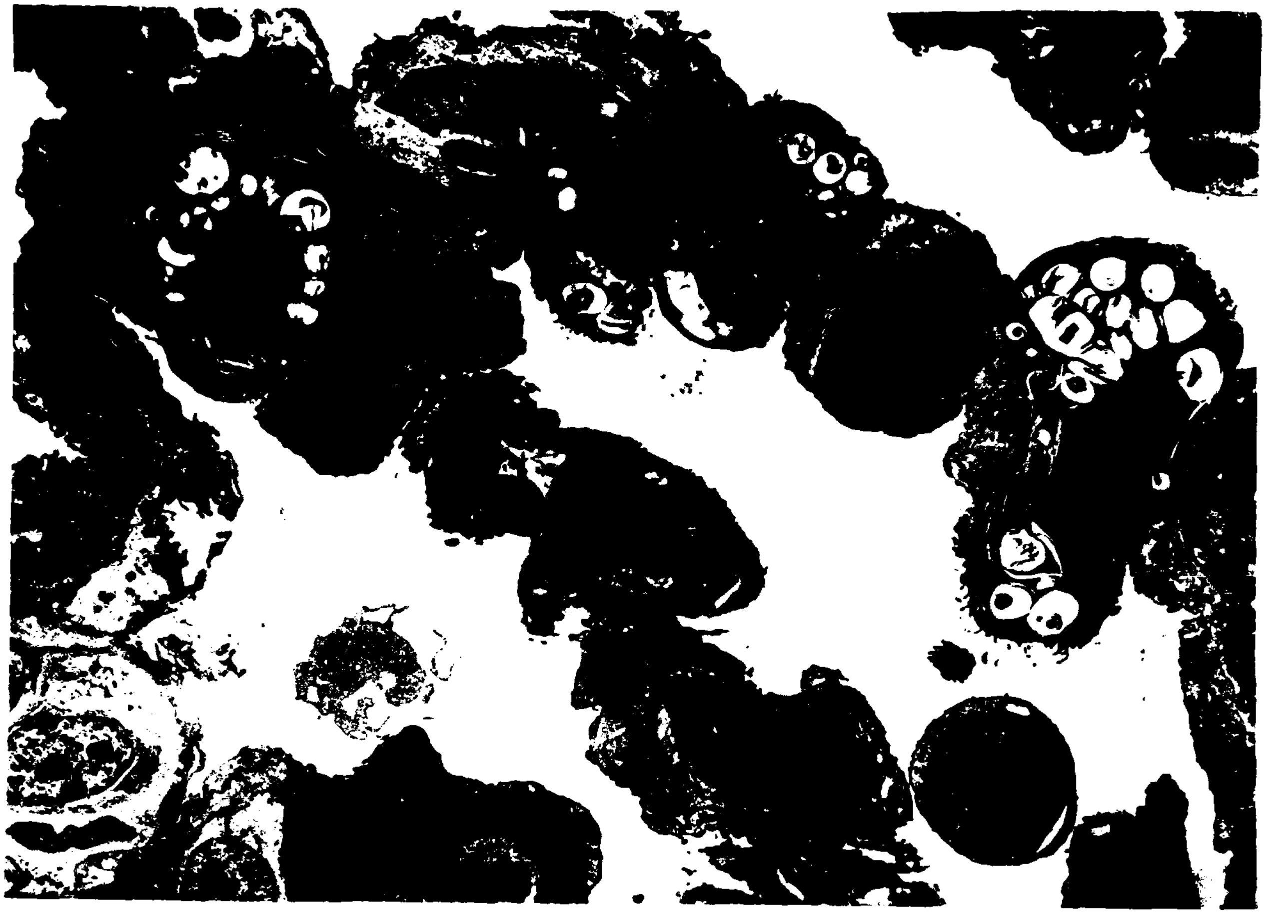
APPENDIX

FIGURE 5

Magnification is X 3800.

APPENDIX

FIGURE 6



Magnification is X 3800.

APPENDIX

FIGURE 7

Magnification is X 3800.

FINAL REPORT DOCUMENT

(1) Abstract resulting from this contract at the present time-

(1) Witten M, Sobonya R, Hubbard A, et al. Acute smoke exposure alters lung eicosanoid and alveolar macrophage (AM) activity in rabbits. THE FASEB JOURNAL, Volume 2, 1988.

(2) Personnel paid by this contract-

(1) Mark L. Witten, Ph.D. 6-4-87 to 7-4-88.
(2) Cynthia Luedke 6-11-87 to 2-1-88.

(3) No graduate degrees resulted from this contract support.