

INTERACTIONS OF MINERAL DUSTS AND LUNG IN A NONHUMAN PRIMATE MODEL: BITUMINOUS COAL DUST

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ABSTRACT

Bituminous coal dust was repeatedly placed into one lung lobe of anesthetized Macaca nemestrina monkeys using a flexible fiberoptic bronchoscope. Radiographs taken at 15 day intervals showed a focal radiographic density after 90 days time when a total of 1 gram of dust had been instilled. The radiographic density progressed slightly in size until 12 months by which time 3 grams of dust had been instilled and the animals were euthanized. At necropsy, the only significant gross lesion detected was the focal black pulmonary lesion located at the dust instillation site. Histologically, the lesion was composed of coal dust laden macrophages located primarily within interstitial tissue and interspersed with a slight amount of reticulum and connective tissue fibers. The distribution of coal dust was limited to the instillation site in the right caudal lung lobe based on radiographic, gross and histologic examination. Bronchoalveolar lavage of both right and left caudal lung lobes was performed 8 times during the 12 month long experiments without significant complications. More macrophages were found in lavage fluid from the right lung than the left, and most macrophages from right lung contained coal dust particles. Based upon a limited physical examination and complete blood count at 15 day intervals the animals remained normal during the experiment. We conclude that the primate lung's response to focal experimental bituminous coal dust resulted in a marked focal increase in pulmonary alveolar macrophages, which can be recovered regularly in high numbers by bronchoalveolar lavage. The model described has significant potential for investigating the pathogenesis of various respirable dust-induced pneumoconioses, for evaluation of new therapeutic approaches, and for refining experimental and clinical diagnostic applications of bronchoalveolar lavage.

Key words: Coal Workers Pneumoconiosis, Disease model; Primate, Lung Lavage Fluids

INTRODUCTION

Coal workers' pneumoconiosis (CWP) and silicosis are 2 important medical conditions caused by the inhalation of coal mine dust (Kuhn, et al., 1985). Simple CWP is not thought to affect life span or directly cause disability, however it is a prerequisite to complicated CWP (progressive massive fibrosis), which is clearly associated with disability and premature death (Green, 1988). Radiographs, gross lesions at autopsy, and histopathology are typically used to characterize CWP and Progressive massive fibrosis (PMF). Chronic silicosis usually becomes radiographically detectable 20 to 40 years after first exposure, however accelerated silicosis may manifest itself within 5 - 10 years (Kuhn, et al., 1985). Complicated silicosis (PMF) may develop when the individual pulmonary lesions coalesce and form nodules greater than 1 cm size (Green, 1988).

The primary reaction at the lung's alveolar surface in response to respirable dusts include changes in the alveolar free cell population, the alveolar surface proteins, and the quantity of surfactant secreted by the alveolar type II cells (Richards and Curtis, 1984). The most important health effect associated with the inhalation of these dusts is pulmonary fibrosis (WHO, 1986). The alveolar macrophage, with interacts with lymphocytes and fibroblasts, appears to be pivotal in the development of fibrosis (Davis, 1986).

Bronchoalveolar lavage (BAL) is an important diagnostic and research tool which has been applied to animal (Haley, et al., 1989) as well as human investigations (European Society, 1989). We have extended the experimental capabilities of the procedure to include the placing of known quantities of characterized bituminous coal dust directly into a selected focal area of lung of nonhuman

primates. We believe that the lung's reaction to dust can be characterized over known periods of time by using BAL to recover alveolar lining cells and fluids from affected and unaffected (control) areas of lung. Biochemical, immunological, and cytochemical laboratory assays can be performed on the lavage specimens. These results can be compared directly to similar assays performed on cells and fluids recovered by BAL from the lungs of coal miners.

The first step in the development of this model was to determine if instillation of bituminous coal dust into a focal area of nonhuman primate lung was feasible and would produce a focal lesion otherwise comparable to that found in man associated with the inhalation of respirable coal mine dust. This report describes the initial results in this new approach to studying CWP.

METHODS

Two young mature female Macaca nemestrina weighing between 13 and 18 pounds were used in the study. This type of animal was chosen because it is large enough to perform fiberoptic bronchoscopy, is not an endangered species, and is similar anatomically, physiologically, and phylogenetically to man. During initial quarantine the animals were subjected to complete physical examinations, intradermal TB testing, chest radiographs, and prophylactic treatment with ivermectin for possible lung mite infection. They underwent intradermal TB testing at 6 month intervals and were cared for and housed in an AAALAC accredited laboratory animal facility in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act, as amended. Food was withheld for 12 hours prior to each anesthesia and BAL. The animals were anesthetized with an intramuscular injection of ketamine hydrochloride (10-25 mg/kg) during all procedures.

Chest radiographs, physical examinations, and complete blood counts were performed on each monkey before placement of dust, and at 15 day intervals during the study. The body weight, respiratory rate, heart rate, mucus membrane color, and the presence of any abnormal clinical signs were recorded.

Experimentally prepared and characterized bituminous coal dust, designated PSOC 1361, was obtained from the Generic Mineral Technology Center for Respirable Dust (Dunn and Hogg, 1989). We mixed 250 mg of autoclaved dust with 5 ml of sterile normal saline before pulmonary instillation with an Olympus pediatric

fiberoptic bronchoscope. The tip of the bronchoscope (3.8 mm diameter) was passed caudally and gently wedged into the right caudal bronchus before the 5 ml solution was infused. A total of 12 instillations (3 gm total dust) were made during 16 anesthetic procedures at 15 day intervals. Bronchoscopy was performed with the animals sitting slightly reclined in a custom-made chair and they were maintained in this position for at least 15 minutes after instilling the dust in an attempt to help localize the dust accumulation.

BAL was performed on both right and left caudal lobe bronchi 15 and 30 days after the fourth instillation, and 6 more times at 15 day intervals following the last instillation of dust. Two instillations of 50 ml each of normal saline at room temperature were used for each lavage and the fluid was recovered using gentle suction after each 50 ml instillation. In each case the lavage was performed on the left (control) side first. The bronchoscope was then cleaned before lavage was performed on the right (affected) side.

The pooled lavage specimens were immediately placed in crushed ice, transported to the laboratory, and processed within one hour. One ml of recently mixed lavage fluid was placed over a round coverslip within a 12 well plate for 10 minutes, fixed, and stained with Dif-Quick Stain. The remainder was spun in a refrigerated centrifuge for 10 minutes at 1300-1500 rpm. Supernatant was removed, and 2 ml of phosphate buffered saline was added to the cell pellet and agitated to resuspend the cells.

The total number of cells was determined by adding 125 microliters of suspended cells mixed with 25 microliters of trypan blue (composed of 2ml of 2% Trypan blue + 1ml of 1% sodium borate + 97 ml of distilled water) and 3 microliters of RBC lysing solution (catalogue # B3145-6a, American Scientific Products, McGraw Park, IL) to a hemocytometer chamber and counting the cells. The number of cells counted was adjusted by the following formula (# of cells X 153/125 X 104) to give the total number of cells per lavage, and divided by the amount of lavage to give total cells/ml.

Individual cell types were identified by examination of resuspended lavage cells allowed to settle onto cover slips placed in the bottom of micro-titer wells. The cells were subsequently fixed and stained with Dif-Quick Stain, placed on a glass slide, sealed with permount, and examined microscopically under oil at 1000X magnification. The differential cell counts were determined by counting 100 total cells and marking the cells as macrophages, neutrophils, lymphocytes, or

eosinophils. The percentage of macrophages containing dust was also recorded. Mean BAL values for right and left lung were compared for statistically significant difference by using the paired t-test (Bland, 1989) and statistical computer software (BMDP Statistical Software, Inc., Los Angeles, California). A probability level of 0.05 or less was considered statistically significant.

Twelve months after the first instillation of dust each animal was euthanized with an intravenous overdose (100mg/kg) of pentobarbital and all organs were dissected and examined. The lungs were perfused *in situ* through the trachea with McDowell's fixative (McDowell and Trump, 1976) under hydrostatic pressure of 25-30 cm which was maintained for 24 hours by ligating the trachea at the end of the procedure (Kleinerman, 1979). The right and left caudal lung lobes were each subsequently dissected free after fixation and sliced transversely at 0.5 cm intervals for their entire length. Each section (approximately 12/lobe) was trimmed and every other section was processed by standard histologic technique (Luna, 1968). Approximately 10 sections per lobe were stained with H&E, Trichrome, and Gomori methemine silver stains and examined using a Zeiss universal microscope with standard and polarized light.

RESULTS

Each of the two monkeys underwent 24 anesthetic procedures during the 12-month period without significant complications. Inhalation anesthesia could not be used because their tracheas were not large enough to accommodate both a tracheal tube and a bronchoscope.

Ketamine anesthesia provided an easily administered and safe anesthetic for the short and painless lavage procedures. Macaques develop a slight tolerance to the anesthetic effects of Ketamine with repeated administration, but it has been used as often as every 2 days without reported ill effects (Bree, et al., 1967). Repeated suction of the copious oral secretions was necessary because atropine was not used in these experiments. Local anesthesia was not necessary except to prevent laryngospasm in one animal.

The bronchoscope could be passed through the nares or directly through the oral cavity into the trachea. The bronchoscope could be passed to the level of the mainstem bronchus of both the right and left caudal lobes, but was too large and stiff to place it directly into the cranial and middle mainstem bronchi. The right caudal lobe mainstem bronchus became

black immediately after the instillation of coal dust. After the bronchoscope was removed, cleaned and reinserted, the right caudal lobe bronchus, but not the adjacent trachea or other bronchi, remained black. However, on bronchoscopic examination 15 days later the bronchus was no longer black and there was no evidence of coal dust within the adjacent trachea. After multiple dust instillations the bronchus lumen was observed to become slightly stenotic. Black pigment and increased mucus were evident within lavage returned from right lung compared to the left, by visual examination of the material under adequate light.

Body weight, vital signs, and results of complete blood counts remained within normal range (Henrickson, 1984) for macaques (Table 1).

Radiographic Findings

A focal area of increased radiodensity was evident at the site of dust instillation in each monkey at 90 days after 1 gm of dust had been instilled, and was more fully developed by 12 months after 3 gms of dust had been instilled (Figures 1 & 2). Suspicious radiographic lesions at the instillation site were identified 30 days after the first instillation of 250 mg of dust. There were slight variations in the pattern and density of the lesions between monkeys and between radiographic examinations that were thought to be due to atelectasis.

Postmortem findings

At necropsy each animal had similar lesions within the distal area of the right caudal lobe (Figure 3). The focal lesion in each animal was black and more firm than surrounding lung. It existed as a well demarcated wedge of tissue visible from both the anterior and posterior aspects of the lobe and extending approximately 3 cm proximal from the caudal edge of the lobe. There were mild fibrous adhesions between the visceral and parietal pleura at the lesion site in one animal which could be broken down digitally without perforating the pleura. The left caudal lung lobe contained no pigment and appeared normal in comparison.

When the lobes were dissected free of the remainder of lung after fixation, the normal portion of the right caudal lobe collapsed slightly while the lesion did not collapse significantly. On section, black pigment and a firmer texture were noted within the right lobe compared to the left (Figure 4). The pigment within the right lobe centered around the

primary bronchus proximally and was more diffusely distributed distally.

Histologically, the lesions were similar in both animals. The interstitium was markedly thickened with brown opaque coal dust particles within the right lung compared to the left lung control (Figures 5 & 6). In the areas with high concentration of dust particles much of the interstitial cellular detail was obscured by the dust, and the interstitial tissue occupied more area than alveoli. The dust particles were visible within the subpleural lymphatic vessels, within macrophages that distended the interstitium, and around blood vessels. Macrophages, free dust particles, occasional multinucleated giant cells, neutrophils, and eosinophils were also visible within a few bronchioles and alveoli (Figure 7). Some macrophage nuclei appeared reactive with prominent nucleoli and abundant euchromatin, but dividing cells and mitotic figures were not identified.

A few lymphocytes and occasional plasma cells were present within some areas of the interstitium. There were also occasional small clusters of lymphocytes with germinal center cells within the interstitium.

On close examination of Gomori reticulum stained tissues, reticulum fibers were evident in association with a few of the interstitial macrophages containing dust particles. The Trichrome stain showed a subtle increase in fibrous connective tissue in a few areas. The delicate pattern of the new reticulum and fibrous connective tissue depositions suggested a role of support tissue for the increased number of interstitial macrophages and coal dust, rather than tissue disrupting fibrosis associated with a post-necrotic repair response.

When the lesions were examined using polarized light, the dark brown coal particles were opaque. Only occasional small foci within the dust particles passed polarized light. There was no evidence of necrosis or infectious adventitious agents within the tissues examined.

Bronchoalveolar lavage findings

Most macrophages examined from the lavage fluid of the right lung were distended with dust particles compared to macrophages from the left control lung (Figures 8 & 9). Approximately 50% of the lavage fluid placed during each wash was recovered (Tables 2 & 3). Based upon the mean values of 8 separate lavages, an increased concentration of cells from right lung was evident. Since the last 6 lavages were more similar than the total of 8

they were re-examined statistically as a group of six. P values which were less than 0.05 (Tables 2 & 3) remained so except for Cells/Ml and Macrophages/Ml of macaque # 997 which increased to 0.084 and 0.110 respectively. The total number and percent of macrophages containing coal dust was greater in the right lung than left lung. The numbers of neutrophils, lymphocytes, and eosinophils were much lower than macrophages and wide variations in these cell numbers were noted between lavages.

DISCUSSION

This study indicates that selective bronchoscopic instillation of respirable bituminous coal dust causes a marked focal infiltration of alveolar macrophages in pigtail macaque monkeys. The alveolar macrophages were packed with coal dust and could be recovered in high numbers and compared to normal cells from the opposite control lung using BAL. The basic process of phagocytosis by alveolar macrophages and their location in the interstitial tissue and lymphatics appears to be similar in man and this macaque model, although the lesions created in this experiment were much more severe than those found in coal miners' lungs (Kuhn, et al., 1985; Green, 1988; Kleinerman, 1979). The creation of a focal unilateral experimental lesion and the ability to successfully perform bronchoalveolar lavage at regular intervals also appears to be feasible. The ability to extract adequate numbers of alveolar lining cells, proteins, and surfactant from both the experimental lesion as well as normal lung of the same subject should facilitate investigation of the cellular, biochemical, and immunologic basis of dust-related lung disease.

Experimental bituminous coal dust may lack some of the factors present within coal mine dust found in mines associated with CWP, for example the levels of silica associated with hazardous mining occupations, such as roof bolters and motormen (Green, 1988). We chose to use experimentally derived, but physically and chemically characterized, bituminous coal dust to establish a baseline generic bituminous coal dust to which other factors could later be added if necessary to produce pulmonary fibrosis. The availability of data analyzing this dust (Dunn and Hogg, 1989) and familiarity with the coal seam of origin of the experimental dust and its relationship to incidences of CWP in miners were also factors in choosing the experimental dust. Future experiments may be modified in terms of duration of exposure to dust, the amount of dust instilled, and the silica content within the coal dust as required to produce histologic lesions similar to those found in coal miners.

During this experiment we administered relatively high amounts of dust to insure the development of lesions on first attempt. We also attempted to compress the time of lesion formation by providing a strong stimulus for lesion development. Based upon relative body weights, the amount of dust placed into the small area of lung in the monkeys proportionally was slightly greater than the amount of dust recovered from the entire lung of deceased coal miners with radiographic category 3 pneumoconiosis (WHO, 1986). The present dust instillations were much higher than the concentrations used in recent studies of Titanium dioxide in rats which also produced minimal pulmonary fibrosis related to "lung overloading" (Muhle, et al., 1989).

Although the experimental lesions were severe compared to descriptions of CWP, they were focal and caused no clinical problems to complicate the anesthetic and pulmonary lavage requirements of the study. Deep placement of dust within the lungs bypassed the upper respiratory tract defense mechanisms and provides a technique to establish the amount of dust actually in contact with the alveolar lining cells. The elimination of dust by the upper respiratory tract defenses is important because less than 1% of inhaled dust is estimated to be actually retained within the lungs (WHO, 1986). In addition, dust located within lung areas having no ciliated epithelium is reported not to undergo the rapid phase of elimination. The administration of the dust within a relatively small amount of normal saline probably helps to keep the dust localized, and diluting dust in saline to a lower concentration would be expected to disperse the dust more evenly within a selected area of lung.

The radiographic density correlated closely with the size and location of the gross lesion. Microscopically, the density was composed of a large concentration of macrophages and coal dust particles located primarily within the interstitial tissue. It is possible that many of the macrophages and coal dust particles within alveoli were removed by multiple BAL procedures. The macrophages within the interstitium were associated with a slight amount of reticulum and fibrous connective tissue, which was more than that seen within control lung interstitium and may represent an early stage of fibrosis. This slight amount of fibrosis has also been described in other experiments where lungs were overloaded with benign nuisance dusts (Muhle, 1989). Significant fibrosis as well as necrosis did occur however when quartz dust was used in similar parallel experiments (unpublished data).

The major factors responsible for the increased radiodensity within these lesions were the space occupying interstitially located macrophages and coal dust particles, which reduced the ratio of alveolar space to interstitium.

The technique of BAL represents a brief sampling of what is present within the alveoli and indicates that most of the cells within the alveoli of the lesioned area as well as normal lung were macrophages. The major difference between lavage fluid from lesioned and normal lung was the amount of dust that the macrophages contained, and the number of macrophage cells.

A small percentage of macrophages within fluid recovered from the left normal lung of each lavage also contained coal dust particles, which we attribute to contamination of the bronchoscope working channel or spillage of lavage fluid from the right caudal lobe into the left lung. We did not observe macrophages containing coal dust within serial histologic sections of the normal area of lung where lavage was performed.

Technical recommendations and guidelines have recently been published for BAL (European Society, 1989). The procedure itself causes temporary pulmonary and systemic responses which may limit the lavage interval (Haley, et al., 1989). By using this model it should be possible to examine the significance of the inherent variables of the BAL procedure.

Some of the experimental variables associated with the investigation of CWP which can be better controlled using this model include: the type of dust actually placed into the lung, the amount of dust in contact with the lung, the time dust is in contact with lung, the location of dust within the lung, and the location of cells collected from within lung. By producing a lesion in only one side of the lung the opposite lung serves as a source of control cells and fluids. Stages of lesion development may be identified, and other health factors can be controlled. The experimental pulmonary lesion affects only a small part of the lung and causes no clinical signs in the animals. The results of analyzing the BAL specimens recovered from primates can be compared directly to similar studies in humans with respirable dust disease. New treatments and diagnostic tests for CWP can be developed and tested before being used in humans.

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REFERENCES

- Bland, M., 1989, An Introduction to Medical Statistics, Oxford University Press, Oxford.
- Bree, M. M., Feller, I., Corssen, G., 1967, "Safety and Tolerance of Repeated Anesthesia with CI 581 (Ketamine) in Monkeys," Anes Anal Curr Research, Vol 46, pp. 596-600.
- Davis, G. S., 1986, "Pathogenesis of Silicosis: Current Concepts and Hypothesis," Lung, Vol 164, pp. 139-154.
- Dunn, T. F. and Hogg, R., 1989, "Standard Respirable Dusts," Publications 1986, Generic Mineral Technology Center for Respirable Dust, Vol 5, A Report Submitted to the Office of Mineral Institutes, Frantz, R. L. and Ramani, R. V., eds., U.S. Bureau of Mines, Washington, DC, pp. 155-185.
- European Society of Pneumology Task Group Report, 1989, "Technical Recommendations and Guidelines for Bronchoalveolar Lavage (BAL)," Report of the Eur Respir J Vol 2, pp. 561-585.
- Green, F. H. Y., 1988, "Coal Worker's Pneumoconiosis and Pneumoconiosis Due to Other Carbonaceous Dusts," Pathology of Occupational Lung Disease, Churg, A. and Green, F. H. Y., eds., Igaku-Shoin, New York, pp. 89-154.
- Haley, P. J., Muggenburg, B. A., Rebar, A. H., Shopp, G. M., Bice, D. E., 1989, "Bronchoalveolar Lavage Cytology in Cynomolgus Monkeys and Identification of Cytologic Alterations Following Sequential Saline Lavage," Vet Pathol Vol 26, pp. 265-273.
- Henrickson, R. V., 1984, "Part A. Biology and Diseases of Old World Primates," Laboratory Animal Medicine, Fox, J. G., Cohen, B. J., Loew, F. M., eds. Academic Press, Orlando, pp. 306-307.
- Kleinerman, J., 1979, "Pathology Standards for Coal Worker's Pneumoconiosis," Arch Pathol Lab Med, Vol 103, pp. 375-432.
- Kuhn, C. and Askin, F. B., 1985, "Lung and Mediastinum," Anderson's Pathology, Volume One, 8th Ed., Kissane, J. M. and Anderson, W. A. D., eds., Mosby, St. Louis, pp. 907-917.
- Luna, L. G., 1968, Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, McGraw Hill, New York.
- McDowell, E. M. and Trump, B. F., 1976, "Histologic Fixatives Suitable for Diagnostic Light and Electron Microscopy," Arch Pathol Lab Med, pp. 405-414.
- Muhle, H., Mermelstein, R., Dasenbrock, C., Takenaka, S., Mohr, U., Kelpper, R., MacKenzi, J., Morrow, P., 1989, "Lung Response to Test Toner Upon 2-Year Inhalation Exposure in Rats," Exp Pathol Vol 37, pp. 239-242.
- Richards, R. J. and Curtis, C. G., 1984, "Biochemical and Cellular Mechanisms of Dust-Induced Lung Fibrosis," Environ Health Perspect, Vol 55, pp. 383-416.
- WHO Study Group, 1986, "Recommended Health-Based Limits in Occupational Exposure to Selected Mineral Dusts (Silica, Coal)," World Health Organization Technical Report Series 734, Geneva, pp. 1-81.

Table 1

VITAL SIGNS AND LABORATORY VALUES

TEST	MACAQUE # 997			MACAQUE # 318		
	N	MEAN	SD	N	MEAN	SD
Body Weight (lbs)	20	16.16	0.75	20	14.07	0.99
Temperature (deg. F)	22	99.86	0.72	22	99.68	0.74
Respiration rate/min	21	33.14	3.14	21	30.10	4.67
Heart rate/min	21	178.10	11.14	21	164.57	10.06
Red blood cell count	21	5.52	0.22	21	5.79	0.34
Hematocrit	21	39.34	1.31	21	37.46	2.28
White blood cell count	21	5.73	1.17	21	9.01	1.17
Percent Neutrophils	21	46.19	10.81	21	62.37	6.98
Percent Lymphocytes	21	49.62	10.00	21	34.94	6.67
Percent Eosinophils	21	2.14	1.53	21	1.52	0.98
Percent Basophils	21	0.62	0.59	21	0.14	0.36
Percent Monocytes	21	1.43	1.29	21	1.00	0.77

RESPIRABLE DUST

Table 2

BRONCHOALVEOLAR LAVAGE CELL CHARACTERISTICS - MACAQUE # 997

Category	N	RIGHT LUNG		LEFT LUNG		t-Test
		MEAN	SD	MEAN	SD	P
Ml of Lavage Retrieved	8	47.4	11.7	48.3	9.6	0.798
Cells/Ml	8	390481	96553	237818	65895	0.021 %
Macrophage	8	61	17	69	15	0.027
Macrophages/Ml	8	231579	82333	158060	42256	0.049 %
Macrophage with Dust	8	63.6	17.2	6.6	5.6	0.001
Macrophages/ML with Dust	8	147762	70597	10904	8942	0.002 %
Neutrophils	8	27	17	22	16	0.106
Neutrophils/ML	8	96567	60505	56528	50976	0.085 %
Lymphocytes	8	5	9	5	9	0.927
Lymphocytes/ML	8	23470	50795	11950	2063	0.412 %
Eosinophil	8	8	16	5	10	0.205
Eosinophils/ML	8	39725	83581	10975	21456	0.262

Table 3

BRONCHOALVEOLAR LAVAGE CELL CHARACTERISTICS - MACAQUE # 318

Category	N	RIGHT LUNG		LEFT LUNG		t-Test
		MEAN	SD	MEAN	SD	P
Ml of Lavage Retrieved	8	48.8	12.1	53	11.9	0.548
Cells/Ml	8	711886	231015	197583	81096	0.001 %
Macrophage	8	66	13	70	15	0.214
Macrophage No.	8	469698	177812	145292	45944	0.002 %
Macrophage with Dust	8	70.6	17.6	33.4	8.7	0.001
Macrophage/ML with Dust	8	328864	81031	49054	21867	0.001 %
Neutrophils	8	24	13	20	13	0.218
Neutrophils/ML	8	180176	126507	38472	29074	0.014 %
Lymphocytes	8	4	9	5	8	0.702
Lymphocytes/ML	8	29313	71346	7640	16318	0.333 %
Eosinophil	8	6	10	6	10	0.802
Eosinophils/ML	8	32700	61750	76388	26048	0.375

Legends for Illustrations:

Figure 1. Lateral radiograph of Pigtail Macaque chest 12 months after instillation of dust. Arrows indicate focal area of radiodensity.

Figure 2. Ventral-dorsal radiograph of Pigtail Macaque chest 12 months after instillation of dust. Arrows indicate focal area of radiodensity.

Figure 3. Right and left caudal lung lobes after necropsy showing focal unilateral pigmentation and increased density.

Figure 4. Serial sections of right and left caudal lung lobes after fixation showing distribution of pigmentation.

Figure 5. Low power photomicrograph of right caudal lung showing distribution of dust. H&E stain.

Figure 6. Low power photomicrograph of normal left caudal lung. H&E stain.

Figure 7. High power photomicrograph of affected lung. H&E stain.

Figure 8. Photomicrograph of macrophages from right caudal lung lobe showing coal dust particles within the cells. Diff-Quick stain.

Figure 9. Photomicrograph of normal macrophages from left caudal lung lobe. Diff-Quick stain.

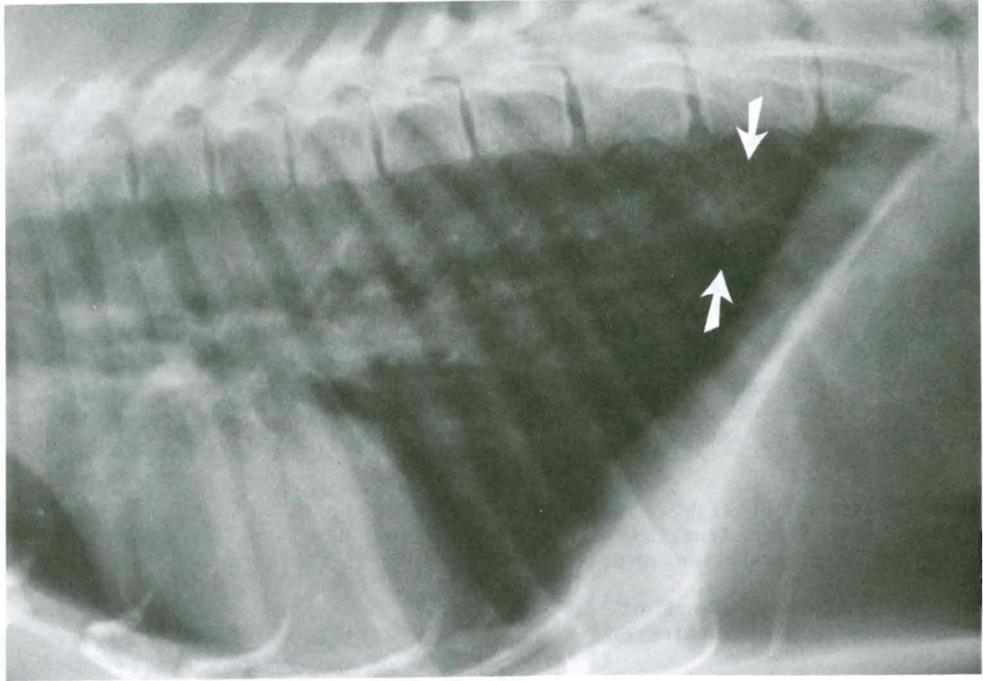


Figure 1.

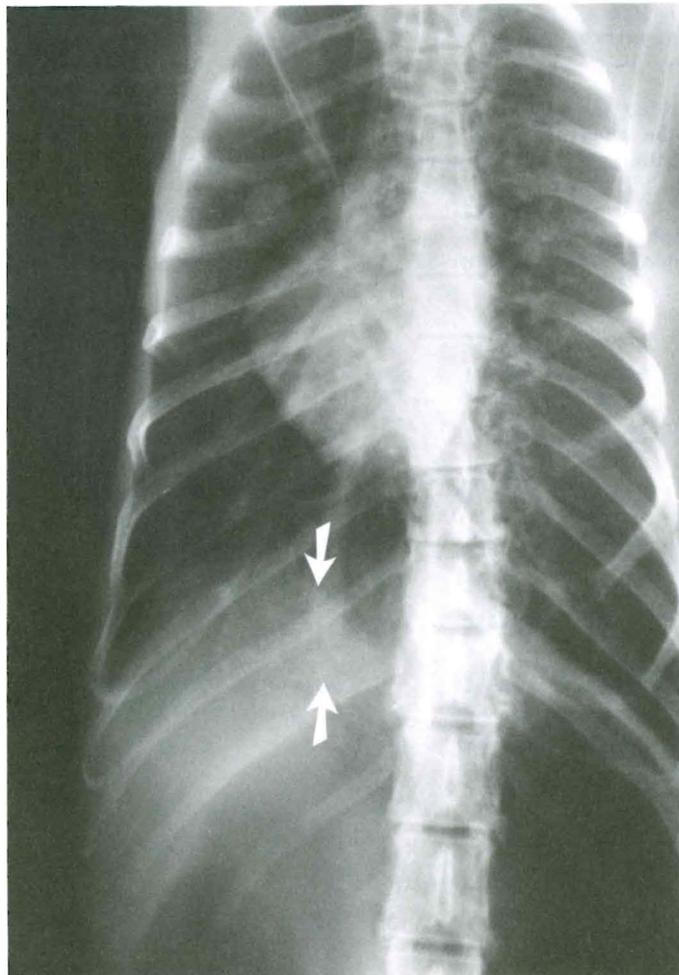


Figure 2.

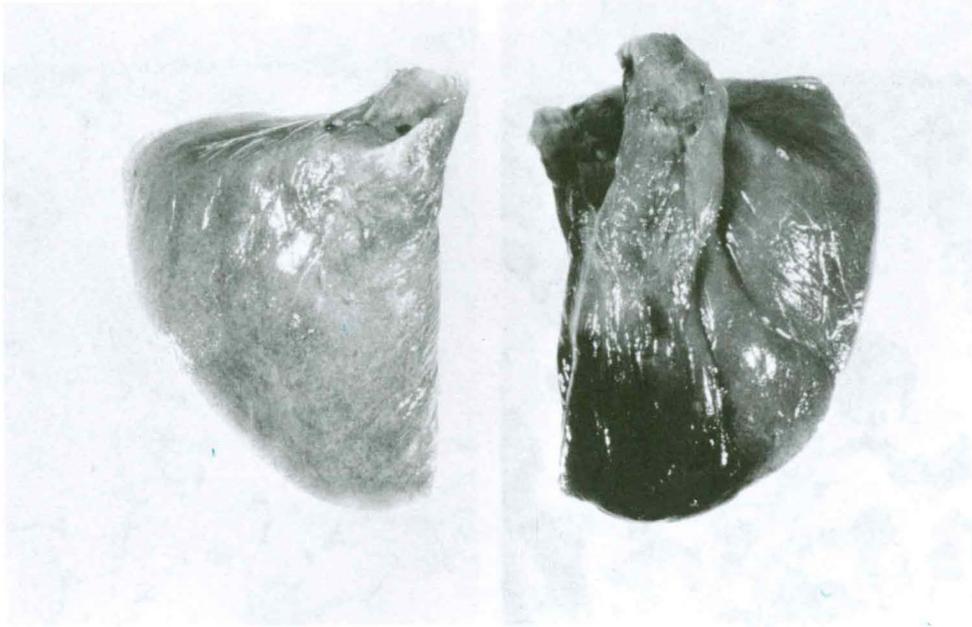


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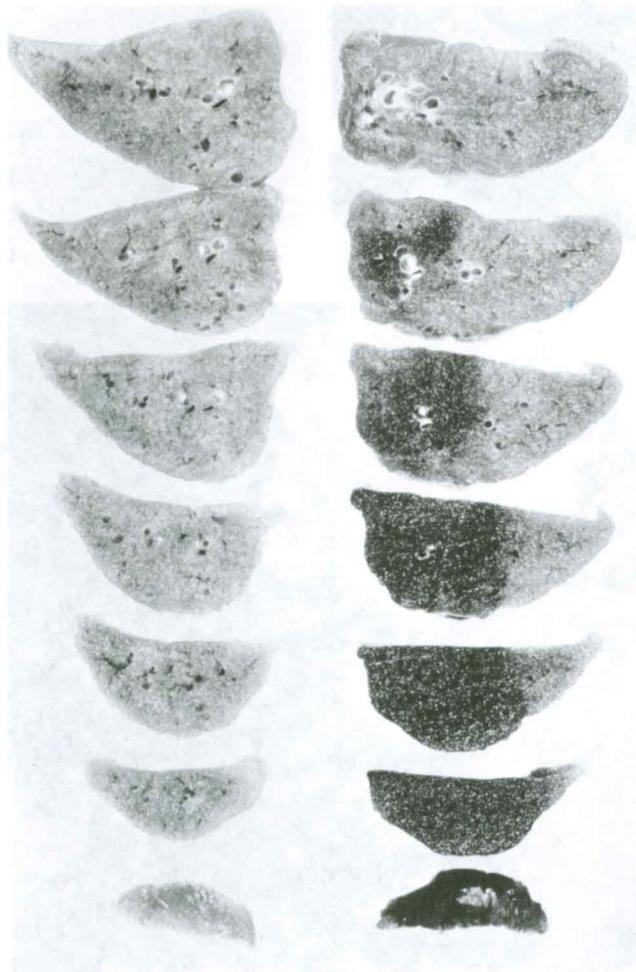


Figure 4.

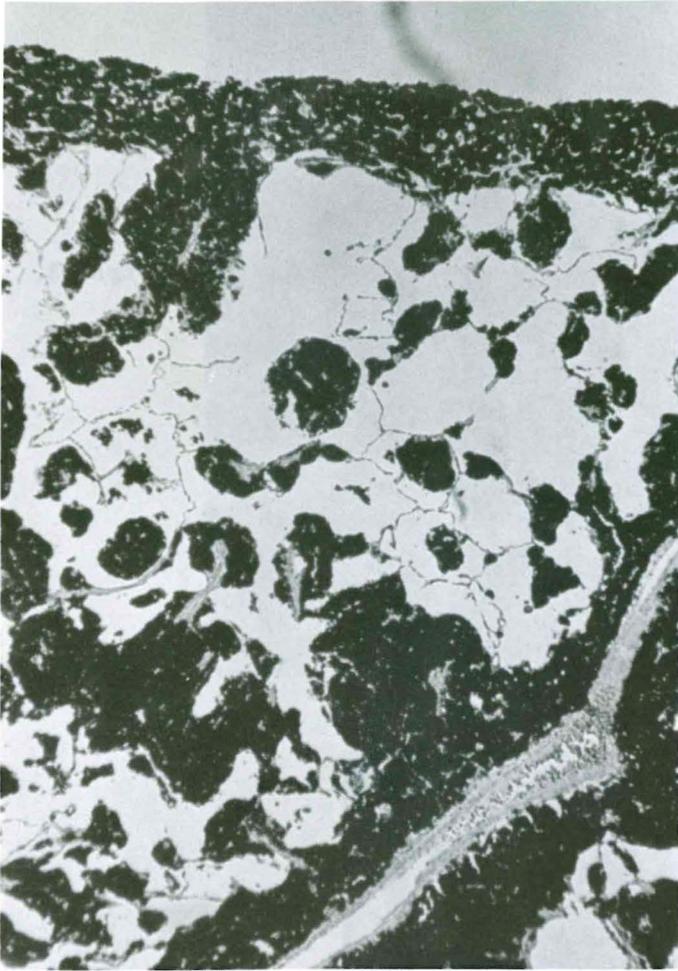


Figure 5.

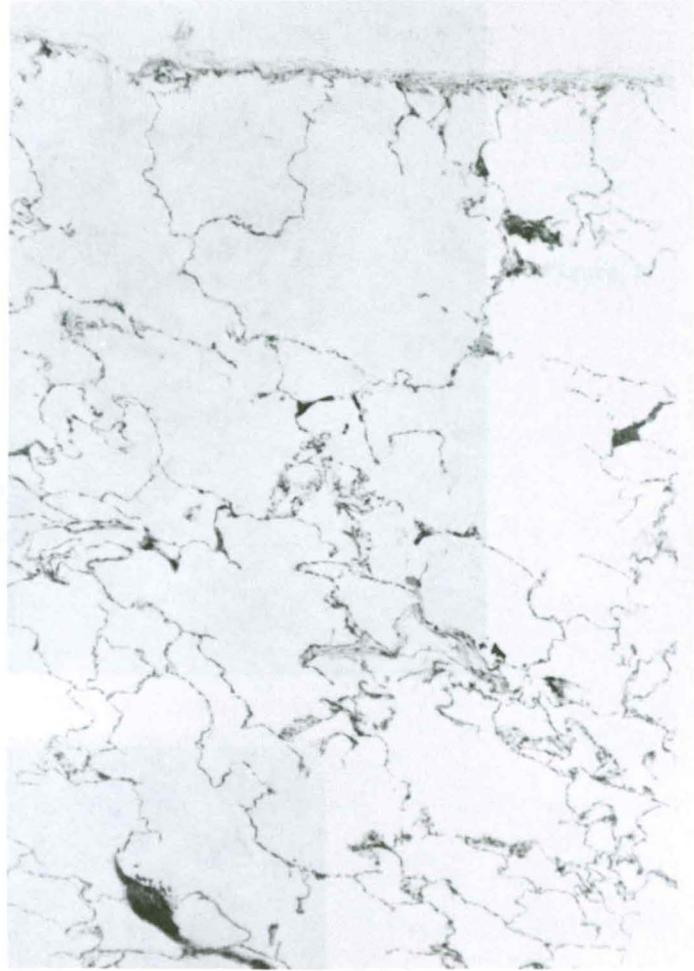


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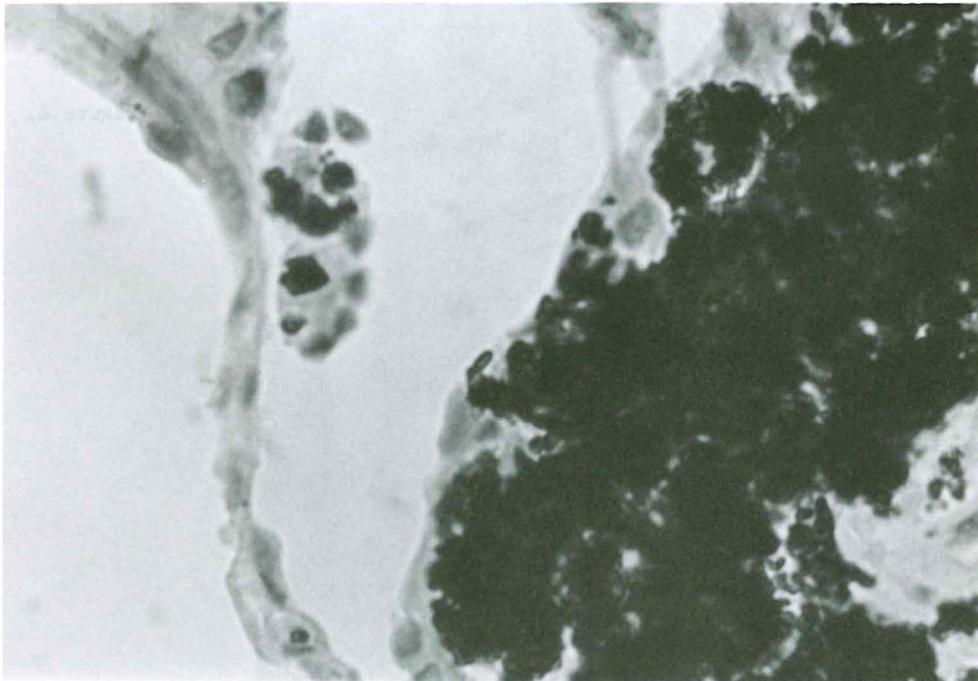


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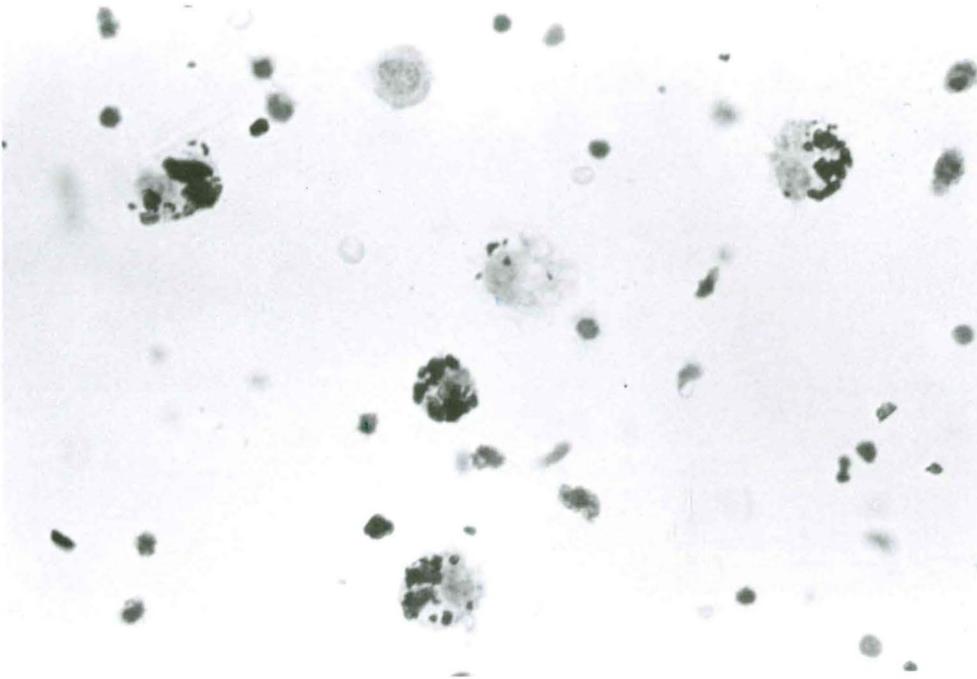


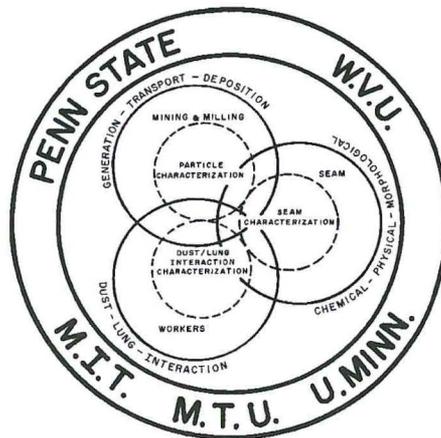
Figure 8.



Figure 9.

3rd SYMPOSIUM ON RESPIRABLE DUST IN THE MINERAL INDUSTRIES

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