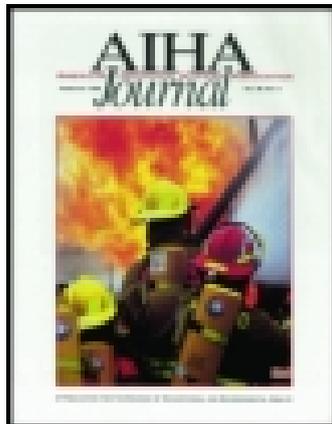


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### Fibrogenic potential of slags used as substitutes for sand in abrasive blasting operations

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The fibrogenic potentials of coal and copper slags used as substitutes for silica sand in abrasive blasting operations were assessed in rats. The test animals were given a single pulmonary intralobar instillation of 20 mg of test material and were sacrificed 10 months after dosing. Pulmonary fibrosis was seen in the coal slag-treated rats while no fibrosis was seen in the copper slag-treated animals. Granulomas were seen in the lungs from all treatment groups. The results of the study emphasize the need for bioassay of silica sand replacements for fibrogenic potential in spite of the low free silica contents of these materials.

## Fibrogenic potential of slags used as substitutes for sand in abrasive blasting operations

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### introduction

Abrasive blasting is the high velocity bombardment of a surface by a substance propelled either by hydraulic or pneumatic pressure or by centrifugal force. The abrasive blasting processes are used extensively in cleaning and preparing surfaces prior to other operations such as painting. The principal substance used for abrasive blasting has been silica sand. It has been estimated that approximately 100 000 abrasive blasting workers are exposed to free silica in their working environment in the United States.<sup>(1)</sup> The health hazard of human exposure to free silica has been well documented.<sup>(2)</sup>

During approximately the last 20 years, other materials with much lower free silica contents have been used in abrasive blasting operations. Coal and copper slags are being used as replacements for silica sand because of their low free silica content, desirable physical properties, economic feasibility, availability, and assumed health safety qualities.<sup>(3,4)</sup> These products are derived from the fritted ash of coal used in electric power generation and from copper smelting processes. The increasing use of these materials mandates an investigation of their heretofore unknown biological activity. The purpose of this study was to assess the fibrogenic potentials of coal and copper slag dusts in the lungs of rats exposed to a single high-dose intralobar instillation of the material.

### materials and methods

#### dust collection and characterization

Four dust samples were tested in this study. Two were commercial grit slag samples; one derived from coal ash and one from the ash of a primary copper ore smelter. The third sample, an airborne dust sample, was collected on-site at a coastal shipyard during an abrasive blasting operation using the above mentioned commercial copper slag as the abrasive

medium. 5.3 g of this airborne copper slag dust was collected during a four and one-half hour work cycle using a cyclone-equipped high volume electrostatic precipitator (Model ESP-100, Del Electronics Corp.)\* which was positioned five feet downwind from the ship hull being blasted. The fourth sample, a laboratory quartz standard (Min-U-Sil, Pennsylvania Glass Sand Corp.), served as a positive control for induction of silicosis.

The two commercial grit coal and copper slag samples were reduced to respirable particle size by continuous ball-milling for 16 hours in an agate mill using agate balls. All of the dust samples used in the animal exposure tests were sized with a scanning electron microscope (Model JXA-50A, JEOL USA, Inc.) equipped with an image analysis system (Model B-10, LeMont Scientific). The median diameters for the four dust samples were as follows: coal slag, milled - 1.48  $\mu\text{m}$ ; copper slag, milled - 2.67  $\mu\text{m}$ ; copper slag, airborne - 2.17  $\mu\text{m}$ ; and quartz standard - 0.98  $\mu\text{m}$ . Quantitative chemical analyses were performed on the three slag samples for 24 elements, as well as for free silica, using a variety of instrumental techniques including neutron activation, flameless atomic absorption, microwave-induced emission plasma, and x-ray diffraction. The results of these analyses are summarized in Table I.

#### animal study

Three hundred Caesarian derived male Sprague-Dawley rats (Harlan Industries, Inc.) of  $200 \pm 10$  g body weight, after a three week quarantine period, were divided into five equal treatment groups as shown in Table II. The rats were housed in conventional rooms maintained at  $72 \pm 5^\circ\text{F}$  and  $50 \pm 5\%$  relative humidity with a 12 hour dark and light cycle. Rats within a group were housed three per cage. All animals were fed a commercial pelleted diet (Purina Rat Chow) and given tap water *ad libitum* for the 10 month duration of the study.

Samples of each test material were prepared for instillation by adding 2.5 g of the appropriate dust to enough autoclaved distilled water to make 50 mL of suspension. These samples were sonicated in an ultrasonic bath (Model

\*Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

**TABLE I**  
**Inorganic Analysis of Bulk Slags,  $\mu\text{g/g}$  (or weight percent)**

Element	Copper-Milled	Copper-Airborne	Coal-Milled
Na	1030.	1650.	3520.
Mg	25 400. (2.54%)	22 000. (2.54%)	10 500. (1.05%)
Al	45 600. (4.56%)	38 600. (3.86%)	10 800. (1.08%)
Si	138 000. (13.8%)	120 000. (12.0%)	169 000. (16.9%)
Ca	158 000. (15.8%)	128 000. (12.8%)	63 000. (6.30%)
Mn	360.	3600.	730.
Fe	150 000. (15.0%)	204 000. (20.4%)	139 000. (13.9%)
Cu	2020.	2850.	52.
V	160.	160.	160.
Zn	260.	2700.	126.
Mo	11.	35.	28.
Co	52.	60.	29.
Pb	12.	890.	15.
As	34.	52.	33.
Be	1.3	1.5	9.7
Ni	26.	240.	65.
Cd	0.07	6.	0.08
Cr	61.	564.	121.
Sn	58.	57.	16.
Ag	0.92	2.4	<0.08
Ba	490.	480.	1590.
Hg	<0.008	0.42	0.018
Se	<2.	<2.	<2.
B	170.	390.	320.
SiO <sub>2</sub>	<2000.	<3000.	<2000.

3200-2, L and R Manufacturing Co.) for the duration of the respective animal dosing procedures to keep the dusts in suspension. The four dust-treated groups of rats were given a single pulmonary intralobar instillation of 20 mg of the appropriate dust by the administration of 0.4 mL of the appropriate dust suspension, followed immediately by 0.2 mL of distilled water rinse. The conventional control rats were given 0.6 mL of distilled water. The administration of the dusts and distilled water was accomplished without the benefit of anesthesia.

To administer the test materials, animals were mounted on an inclined restraining board and an illuminated laryngeal speculum was inserted into the mouth, exposing

the larynx and the trachea. A blunted 4 inch, 18 gauge spinal needle affixed with a 1 cm flexible Teflon<sup>TM</sup> extension was inserted into the left lung via the trachea. After insertion, a 1 cc tuberculin syringe containing the dust-distilled water suspension or distilled water only was inserted in the hub of the needle and the solution was delivered. The 20 mg dosage level was predicted on the animals' ability to tolerate a single heavy concentration of the dust, the positive pulmonary fibrotic reaction to the quartz standard at the 20 mg dosage level, and the long-term survival of the quartz-treated rats as determined in an earlier pilot study in this laboratory.

All surviving rats were sacrificed 10 months after dosing by an overdose of Nembutal Sodium (Abbott Laboratories)

**TABLE II**  
**Animal Treatment Groups and Disposition**

Dust Treatment	Total Number of Animals Treated	Mortality	Total Number of Animals Sacrificed	Disposition of Sacrificed Animals		
				Histology	Hydroxyproline Analyses	Free Silica Analyses
I. Copper Slag-Airborne	60	1	59	44	10	5
II. Copper Slag-Milled	60	3	57	42	10	5
III. Coal Slag-Milled	60	2	58	43	10	5
IV. Quartz (Positive control)	60	4	56	41	10	5
V. Control (Distilled water)	60	0	60	45	10	5
Totals:	300	10	290	215	50	25

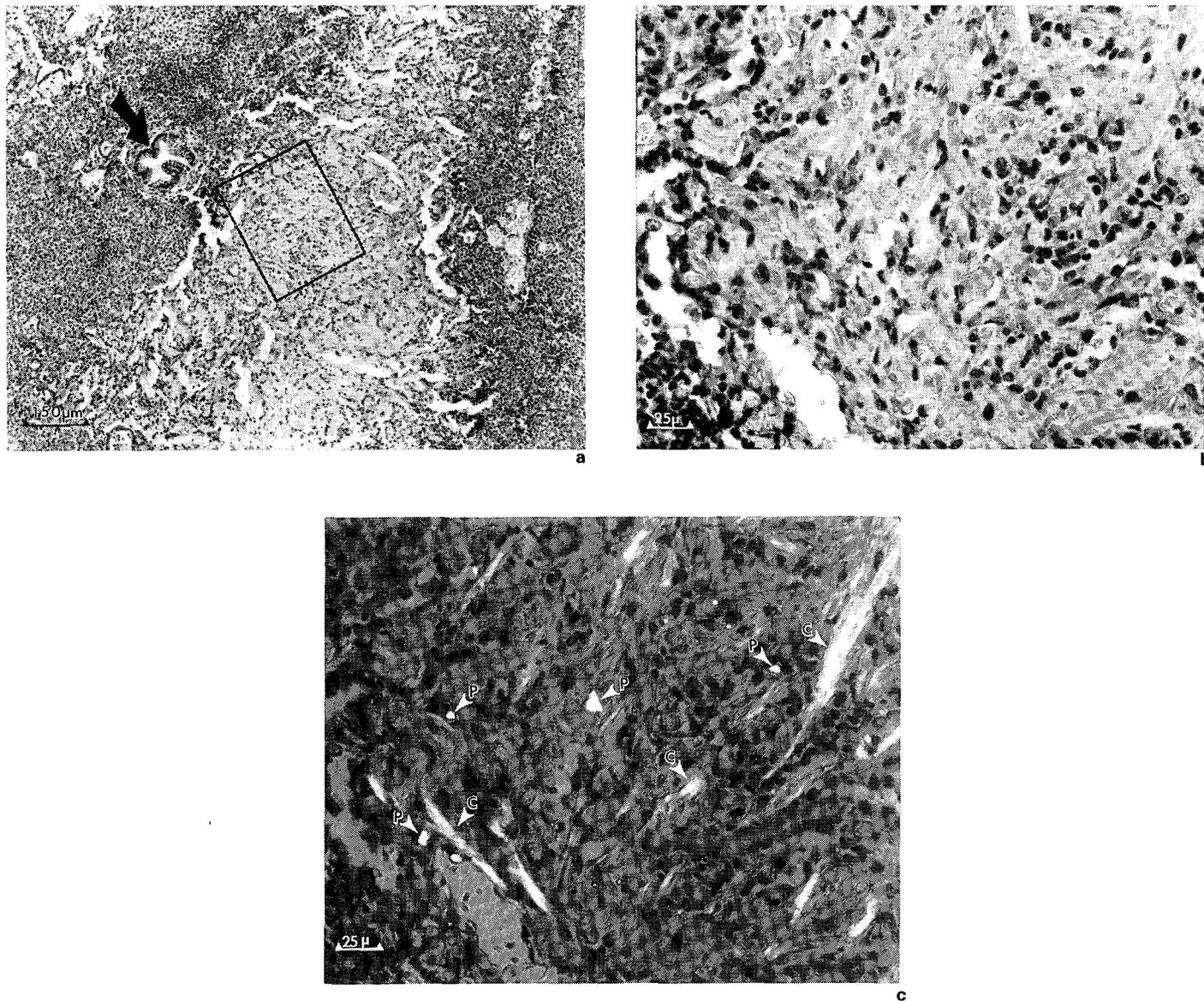


Figure 1(a) – Lung from a quartz-treated rat. A large hyalinized area is shown. A distorted bronchiole (arrow) in the middle of a densely cellular area composed primarily of macrophages and lymphocytes is also shown. ( $\times 100$ )

(b) – Higher magnification of the indicated area in Figure 1(a). Thick hyalinized bands of collagen with interspersed pyknotic nuclei are evident. ( $\times 400$ )

(c) – Same area as shown in Figure 1(b) under polarized light. The birefringence of the quartz particles (P) and the collagen (C) is shown. ( $\times 400$ )

and necropsied. Lung lobes and draining lymph nodes (tracheobronchial and mediastinal) from 15 randomly selected animals from each of the five groups were removed, weighed, and frozen for subsequent biochemical and free silica analyses. All remaining animals were necropsied for gross and histopathologic evaluation.

Lungs were inflated by gravity with phosphate-buffered, 10% formalin at 25 cm of water pressure and then immersed in formalin. Lymph nodes, heart, liver, spleen, and kidneys were fixed by immersion in formalin. Histology slides, stained with hematoxylin and eosin, were prepared from all of the above tissues and evaluated by light microscopy. The 10 spontaneous deaths which occurred during the experiment were omitted from the final pathological

evaluation since earlier evaluations determined that these deaths were not treatment related.

The lungs and lymph nodes from 10 of the 15 randomly selected animals from each group were analyzed for hydroxyproline content by standard methods.<sup>(5,6)</sup> The five remaining lungs and lymph nodes from each dust group were analyzed for free silica by x-ray diffraction.

## results

### pathology

The rate of success for the instillation of a test material into the left lung of the rats was 70%, as determined by gross inspection at autopsy. In the remaining 30% of the animals, the suspension was deposited in other lobes or in the

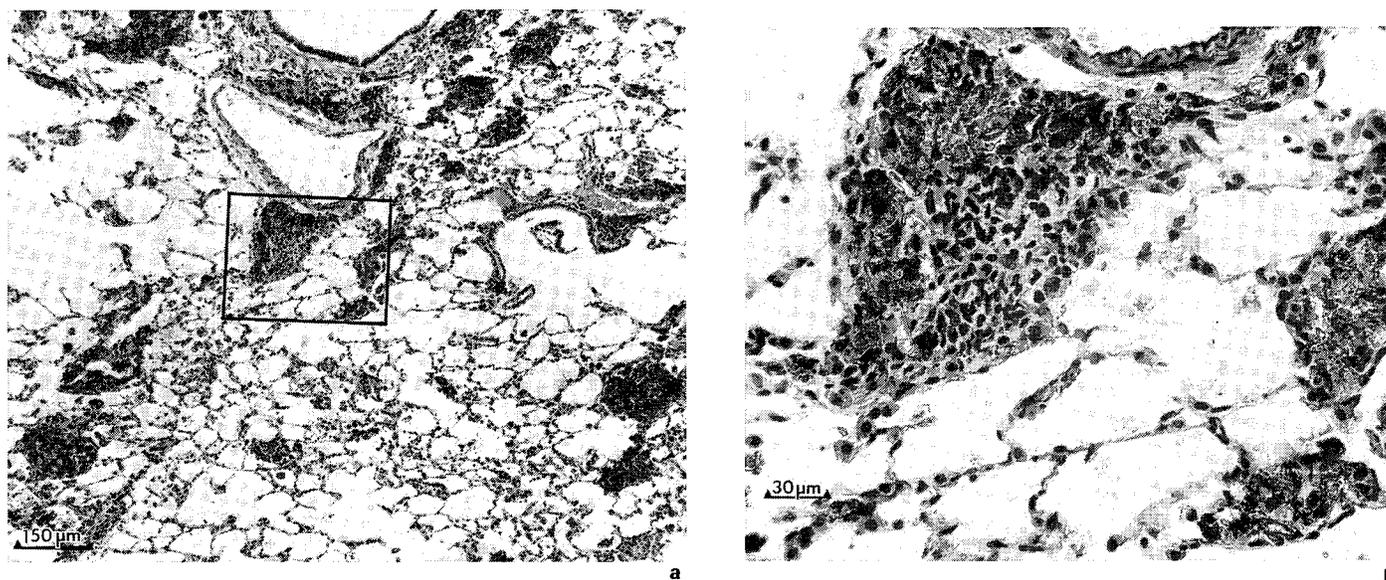


Figure 2(a) – Lung from an airborne copper slag-treated rat. Multiple granulomas are present. ( $\times 100$ )

(b) – Higher magnification of the indicated area in Figure 2(a). The granulomas are composed primarily of macrophages, some with particulates and lymphocytes. Most of the alveolar lumens are free of particulates and particulate bearing macrophages. ( $\times 400$ )

extrapulmonary spaces, *i.e.*, in the mediastinum or in the thoracic cavity.

At autopsy, the left lungs of 70% of the quartz-treated rats showed hard areas (scars) and collapse. The scars occupied from 1/4 to 1/3 of the left lung. The tracheobronchial and mediastinal lymph nodes of the quartz-treated rats were white and enlarged (0.8 to 1.5 cm in the largest dimension). Thirty percent of the quartz-treated rats showed firm, diffuse fibrinous lesions among all lobes of the lungs and in the visceral surface of the thoracic wall.

Eighty percent of the rats treated with either the milled or airborne copper slag showed discrete green plaques or slightly elevated soft areas distributed throughout the left lung. In 40% of these rats, the draining lymph nodes were soft, green, and enlarged, measuring 0.6 to 1.0 cm. Sixty-five percent of the rats treated with milled coal slag had green tinted scar tissue in the left lung. The draining lymph nodes measured 0.3 to 0.5 cm in diameter.

The gross pathologies of rats in which the dust suspensions were inadvertently deposited in the lobes of the right lung were similar (for each test substance) to those of rats in which the suspension was deposited in the left lung. In those animals in which the dust suspension was deposited in the extrapulmonary spaces, firm nodules measuring 0.4 to 2.0 cm in their largest dimension were seen on the parietal surface of the lung and on the pleura.

Granulomas were induced by all four test substances as determined by light microscopy evaluations. These granulomas were seen only in the lobes of the lungs and in extrapulmonary sites which also contained particulate material. This particulate was present both intra- and extracellularly within these granulomas as well as proximal to the granulomas.

In the rats treated with quartz, large granulomas were seen. In most instances, a single granuloma occupied an

entire field of view at 100 $\times$  magnification (Figure 1[a]). All groups of the slag-treated rats showed multiple granulomas varying from 100 to 400  $\mu\text{m}$  in diameter (Figures 2[a] and 3[a]). There were from 6 to 10 of these small granulomas per field of view at 100 $\times$ .

The cell types and populations of the granulomas found in the four treatment groups varied. The quartz-induced granulomas consisted primarily of macrophages and a few lymphocytes (Figure 1[a,b]). Equal numbers of macrophages and lymphocytes were present in the granulomas produced by the copper slags, both milled and airborne (Figure 2[b]). Macrophages, lymphocytes and foreign body giant cells were seen in the granulomas induced by milled coal slag (Figures 3[b] and 4). All granulomas lacked a definite capsule. Internal scaffolding of fibrous tissue was present in all of the granulomas found in the lining parenchyma (alveolar region), lymph node, and pleura.

Large accumulations of macrophages were seen in both the medulla and cortex of the tracheobronchial lymph nodes of the rats from all four dust treatment groups. These macrophages contained particulates similar in appearance to those found in the respective lungs. Particulate material was also present extracellularly in the tracheobronchial lymph nodes. The degree of macrophage accumulation and particulate deposition in these lymph nodes was similar for all four dust treatment groups.

Frank fibrous tissue was apparent in the rats treated with quartz and milled coal slag. However, distinct differences in the locality and degree of deposition of this connective tissue was observed between these two groups. Intense collagen formation (Figure 1[a, b, and c]) with attendant replacement and distortion of the pulmonary parenchyma was evident in the rats treated with quartz. This marked fibrosis seen in the quartz-treated rats would be rated at 10 on a scale of 1 to 10.

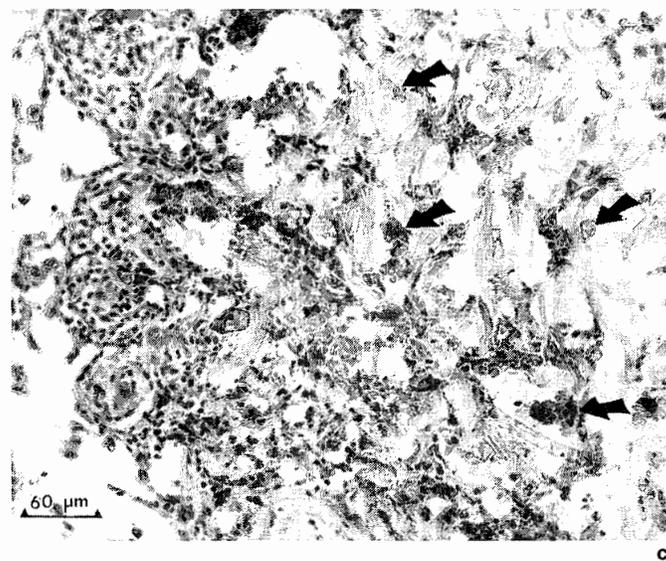
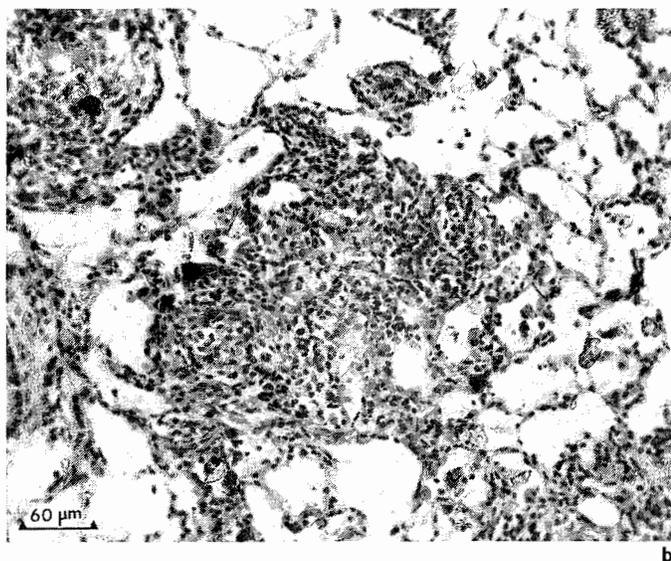


Figure 3(a) – Lung from a coal slag-treated rat. Multiple granulomas (left half) and marked fibrosis (right half) are shown. (×100)  
 (b) – Higher magnification of area (B) in Figure 3(a). Both intra- and extracellular particles are found in the granulomas. (×200)  
 (c) – High magnification of area (C) in Figure 3(a). Fibrosis of alveolar walls has resulted in reduction of alveolar lumens, lack of alveolar lining epithelium, and vascularity of the alveoli. Some of the particulate material present is denoted by arrows. (×200)

Quartz particles were present in the hyalinized area of the lungs (Figure 1[c]). Eighty percent of the quartz-treated rats showed dystrophic calcification in the hyalinized areas. Calcification was also present in the pleural lesions.

The milled coal slag induced marked thickening of the alveolar walls due to formation of fibrous tissue that showed varying degrees of hyalinization (Figures 3[a] and 3[c]). This resulted in retention of the apparent alveolar architecture

**TABLE III**  
**Free Silica Content of Lung and Lymph Node,  $\mu\text{g/g}$  Tissue (5 Animals per Dust Group)**

	Copper-Airborne		Copper-Milled		Coal-Milled		Quartz		Distilled Water	
	Lung	Lymph Node	Lung	Lymph Node	Lung	Lymph Node	Lung	Lymph Node	Lung	Lymph Node
	<100	<400	<70	<500	<90	<1000	3200	13 000	<350	<600
	<70	<1000	<100	<800	<200	<1000	*	12 000	<100	<600
	<60	<400	<70	<1000	<50	<2000	13 000	15 000	<100	<400
	<50	<500	<100	<1000	<300	<1000	*	8000	<100	<100
	<100	<600	<100	<800	<200	<1000	4500	13 000	*	<800
Avg.	<74	<580	<88	<820	<168	<1200	6900	12 200	<162.5	<500

\*Samples were lost

with reduced alveolar lumens. The fibrosis seen in the coal slag-treated rats would be rated at 4 on the scale of 1 to 10. The alveolar lining epithelium and alveolar wall capillaries were not visible by light microscopy. The fibrosing alveolar walls contained scattered macrophages filled with particulate (Figure 3[c]).

All tissues examined from the distilled water control rats were unremarkable.

#### free silica analyses

Results of the free silica analyses of the lungs and mediastinal lymph nodes of the treatment groups and controls are given in Table III.

The average values of recovered free silica (expressed in micrograms per gram of tissue) in the quartz-treated group and the distilled water-treated controls demonstrate the wide range of free silica detected in the test animals. The average values for all of the slag and distilled water-treated rats are of the same order of magnitude for both lung and lymph node and at least one order of magnitude less than that found for the quartz-treated rats.

#### hydroxyproline analyses

The results of the hydroxyproline analyses for lung and lymph nodes are summarized in Table IV. A separate statistical analysis was done for each site, *i.e.*, lungs and lymph nodes. Each analysis consisted of a one-way analysis of variance to determine whether differences existed among group means. An assumption required by this analysis is homogeneity of group variances which is verified using Bartlett's test for homogeneity.<sup>(7)</sup> For the lymph node data, Bartlett's test indicated homogeneity ( $P = 0.638$ ). A Dunnett's test was then run to determine possible differences between the exposed lymph node groups compared to the control group.<sup>(8)</sup> No significant differences were found.

The lung data showed heterogeneity of variance (Bartlett's  $\chi^2 = 16.28$ ,  $P = 0.003$ ) caused by an unusually large variance in the quartz exposed groups. With the quartz group removed, a Dunnett's test applied to the four remaining groups showed no significant difference between the exposed and control. The quartz group and the control group were then compared separately using a t-test with the Behrens-Fisher correction for unequal variance between

groups.<sup>(9)</sup> No significant difference was indicated between the two groups.

#### discussion

The results of the chemical analyses performed for each of the slag samples would suggest a non-fibrogenic response in animals treated with these materials. This was proven true for the two copper slag samples. However, even though the free silica levels as well as the major constituent elements found in all of the slags were relatively similar, pulmonary fibrosis was found in the coal slag-treated group. The pathological responses seen in the quartz-treated rats were the classical silicotic lesions seen by other workers in similarly treated rats.<sup>(10,11)</sup> The pulmonary fibrosis found in the coal slag-treated animals, while not as extensive as that seen in the quartz-treated animals, is significant.

The granulomas produced by the three slag materials are qualitatively similar which suggests that their production is a nonspecific response of the animals to the test materials. The granulomas present in the lungs of the slag-treated rats, while not as destructive as those produced by quartz, do displace the normal lung tissue and would be expected to reduce pulmonary function. Ten months after deposition, the particulate materials were abundant in the lungs of all treatment groups which suggests a low degree of pulmonary

**TABLE IV**  
**Lung Hydroxyproline in  $\mu\text{g/g}$  Nitrogen**

Group	$\bar{X}$	S.D.
Control	42.97	6.31
Quartz	54.25	19.01
Coal Slag-milled	46.10	9.28
Copper Slag-airborne	42.87	6.02
Copper Slag-milled	42.84	10.47

**Lymph Node Hydroxyproline in  $\mu\text{g/g}$  Nitrogen**

Group	$\bar{X}$	S.D.
Control	27.16	7.25
Quartz	23.93	7.73
Coal Slag-milled	19.84	5.14
Copper Slag-airborne	32.29	7.75
Copper Slag-milled	28.32	5.48

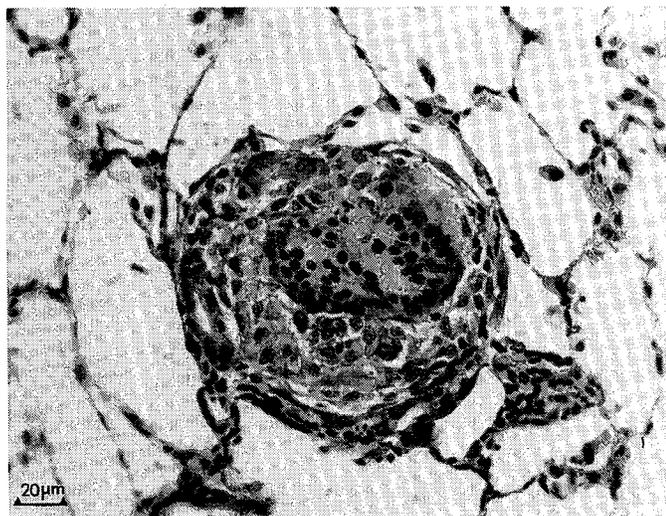


Figure 4 - Lung from a coal slag-treated rat. A microgranuloma with a foreign body giant cell is shown.

clearance for these materials. With this constant physical presence of these particulates, the induced granulomas would most likely have progressed and further adversely affected the pulmonary function.

It would be reasonable to expect differences in the trace elements chemistry of the three slag samples to account for the differences in fibrogenic response found, since the major components and free silica contents of all three slags are similar. In particular, those trace elements present in higher concentrations in the coal slag would be suspect. Of these trace elements, only the barium and beryllium concentrations are higher in coal slag than in both of the copper slags. The total amounts of barium and beryllium in the dose of coal slag given to the rats are calculated to be 32  $\mu\text{g}$  and 0.2  $\mu\text{g}$ , respectively, while the average amounts of these two elements in the copper slags given to the rats are 9.7  $\mu\text{g}$  and 0.03  $\mu\text{g}$ , respectively. Of these two elements, beryllium is known to cause pulmonary fibrosis.<sup>(12)</sup> However, it is doubtful whether 0.2  $\mu\text{g}$  of beryllium could produce the fibrosis seen in the coal slag-treated rats. In an experiment performed in this laboratory, no fibrosis was produced in rats given 0.4  $\mu\text{g}$  of  $\text{Be}(\text{OH})_2$  by intratracheal instillation.<sup>(13)</sup> Since the differences in the fibrotic responses seen in the slag-treated rats do not appear to be related in an obvious fashion to differences in chemistry between the slags, no conclusion can be made regarding why fibrosis was found in the coal slag-treated rats and not in the two groups of copper slag-treated rats.

Since epidemiological studies show the presence of a continuing health hazard for workers exposed to free silica

in the abrasive cleaning industry, the search for nontoxic sand substitute cleaning materials is needed. Coal slags and copper slags such as those used in this study offer the desired physical properties as well as a low free silica content. However, the free silica content of the prospective substitute as the basis for acceptance or rejection of any material may be misleading. Judgment made without benefit of bioassay negates the opportunity to determine potential biological hazards from the non-free silica components of these materials. The various slags used as substitutes may have carcinogenic as well as other systemic effects in addition to fibrogenic potential. The slags do contain considerable amounts of trace metals which have been identified by experimental animal or epidemiological studies as suspected human carcinogens. Until such time as data become available on worker health effects from exposure to the slags, chronic inhalation exposure experiments with appropriate animal models should be conducted to further assess the biological response from such materials.

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