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PARTICULATE OIL SHALE INHALATION AND PULMONARY INFLAMMATORY RESPONSE IN RATS

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INTRODUCTION

Oil shale deposits located in the western United States provide an unexploited source of domestic crude oil. Development of this resource has been limited by economics; however, recent reduction of recovery costs coupled with the possibility of liquid fuel shortages encourage continued development of improved recovery technologies. Along with technology development, a parallel effort to define the potential occupational and environmental hazards associated with extraction of oil from oil shale is ongoing at the Life Sciences Division of the Los Alamos National Laboratory. The present status of this industry and the prospect that it could expand to full capacity present a singular opportunity to define hazards which will facilitate industrial hygiene and environmental controls as part of the development, growth and expansion of the industry. This study presents one facet of our efforts to define the hazards and to understand the mechanisms that may result from inhalation of the several dusts inherent in the processing of oil shale.

In this study we investigate: 1) the progression of biological response following shale- and quartz-dust inhalation; 2) recovery following cessation of exposure and 3) the potential for permanent injury. Two endpoints were of primary importance: fibrosis and tumorigenesis, which can occur separately or in

parallel. Our study concentrated on the events resulting in pulmonary fibrosis. In addition to collecting data at 1, 3, 6 and 12 months of exposure, we included sufficient animals to harvest samples at several intervals after the exposure ended. Each sacrifice point has three sample elements: 1) those taken immediately after termination of exposure; 2) those taken 7 days after termination of exposure; and 3) those taken 30 days after termination of exposure.

MATERIALS AND METHODS

Female Fischer-344 rats were exposed to well characterized aerosols of raw oil shale or quartz (Minusil, Pittsburgh Sand and Glass Co.). Groups of rats were exposed to the aerosols in a nose-only configuration (1). The exposures were conducted for 6 hours on each of 4 days/week for periods of 1, 3, 6 and 12 months. The mass concentration of shale dust aerosol was 10 mg/m^3. Equal numbers of animals were exposed as positive controls for fibrogenesis to an aerosol of quartz for the same 1-, 3-, 6- and 12-month periods. The quartz concentration was established at 1 mg/m^3, because raw oil shale contains about 10% quartz, therefore, exposing all groups to approximately equivalent quartz levels. The quartz aerosol had a mass median aerodynamic diameter (MMAD) of approximately one micron (S.D.=1.8). The MMAD for shale dust was approximately two microns (S.D.=1.8), as determined by cascade impactor sampling. To complete the study a group of age-matched cage controls were maintained in the same animal rooms.

Animals were randomly selected for sacrifice with four animals from each of the two exposure groups and the control group euthanized by intraperitoneal injection of pentabarbitol sodium (25 mg). The lungs were surgically exposed and the left lung isolated by ligating the left bronchus. The lobes of the

right lung were lavaged four times with 5 mL of normal saline and subsequently fixed in 10% formalin for histological evaluation. The left lung was frozen for collagen analysis. The collagen assay has been described (2). Briefly, it involves acid hydrolysis and purification of hydroxy-proline, a building block of collagen, followed by colorimetric quantitation. The lavage fluids were separated into fluid and cellular components. The fluid portion was frozen (4°C) and later thawed and assayed for elastase by the Enzyme Labelled Substrate Assay (ELSA) method (3). Total and differential cell counts were determined for the cellular fractions. Microscopic examination of the right lobes was performed on all lungs.

RESULTS

We proposed that presence of increased elastase levels in the lavage fluids could be a measure of evolving lung tissue injury. Decreased elastase levels were observed in the quartz-exposed animals when compared to controls at the 3- and 6-month exposure points (Fig. 1). The shale-exposed animals had lavage fluid elastase levels higher than those from the quartz-exposed and comparable to control values at both these points. At 12 months, the shale-treated animals exhibited values significantly higher than controls, while the values of the quartz-exposed animals compared to controls were not significantly different.

Exfoliative cells from the lung have long been used to evaluate responses to inhaled materials in an effort to predict dysfunction or disease (4). Pulmonary alveolar macrophages (PAM) comprise the majority of the cells in lavage fluids. Other constituents are polymorphonuclear cells (PMN), lymphocytes, and epithelial cells. Two important criteria for assessing change or response are total cell yield and the relative numbers of each cell type.

The average cell yield from control animals was about two million cells at each time point over the course of the experiment (Table I). The total cell yield for the shale-treated animals was not significantly different from the age-matched controls at any of the sacrifice dates. For the quartz-exposed animals, the harvests at 6 and 12 months were significantly different, being elevated to 2.7-2.9 million cells ($p<.05$). The volumes retrieved in all cases were comparable, between 16 and 19 mL. The number of PMN steadily increased with time for both shale- and quartz-treated animals with the latter showing the greatest increases. Table I shows the increasing cell yields from quartz-exposed animals, a decreasing number of PAM and increasing numbers of lymphocytes and PMN. Numbers of PAM are not significantly different from controls for either shale- or quartz-exposed animals except for the 3-month exposures, at which time PAM are significantly depressed from shale-exposed and controls. All of these observations are for the samples taken from the animals sacrificed immediately after cessation of exposure.

Levels of hydroxy-proline, a constituent of collagen, showed a small but steady increase over the course of the experiment in all groups, but there were no significant differences between the controls and the treated animals (Fig. 2). The last collagen data taken for the 12-month exposures showed a significant increase in the quartz-exposed group compared to controls. Shale-exposed animals exhibited increased levels that were not statistically significant.

When the animals were allowed a recovery period of 7 or 30 days, the changes in PAM and PMN abundance were even more dramatic. Again, at the earliest sacrifices (1 and 3 months) there were no changes in relative numbers of PAM or PMN as reflected in the total cell yields (Fig. 3). However, at six months the total cell yields increased almost exclusively as a result of an

increase in PMN (Fig. 4); macrophage numbers for both shale and quartz-treated samples remained near control levels (Table II). By 12 months of exposure, the total cell yields after 7 days recovery were elevated for both groups compared to controls and were even more so at the 30-day recovery point. These increases are due to elevated numbers of lymphocytes and PMN after 7 days recovery and after 30 days both shale- and quartz-exposed have significant increases in PAM, lymphocytes and PMN. In conjunction with this, no significant lesions were noted microscopically in the shale-exposed animals, but at 6 months several quartz-exposed animals had pneumonia, granulomas or fibrosis, alone or in combination (Fig. 5). At 12 months of exposure, all the quartz-exposed animals had developed granulomas, while the shale-exposed animals remained devoid of significant lesions (Fig. 6).

DISCUSSION

Our goal in this endeavor was to define the temporal response of the lung to inhaled shale dust and to compare these findings with those in animals exposed to a known fibrogenic agent. During the course of the experiment, lavaged cells indicated inflammatory responses to both shale and quartz, but the responses to quartz were more pronounced. When the animals were allowed a recovery period after treatment, those from the later time points had a greater influx of PMN for both treated groups. The increase in the number of cells harvested resulted almost exclusively from an increase in PMN and lymphocytes.

Both macrophages and PMN contain lysosomal enzymes, including elastase, a neutral protease with specific lytic activity for native soluble collagen and active at neutral pH. We assayed the lavage fluids for elastase expecting to find increases at some time during the course of data collection. However, there were no significant differences between controls and treated groups. At

this time it's unclear whether the lack of any specific change was the result of technique insensitivity or actually reflected biological response. Quantification of collagen present in the left lung failed to indicate any temporal gradations or differences in effects between groups. It is conceivable that 12 months of exposure was the point at which, under the conditions of this experiment, excess collagen would become apparent. Analysis of lungs from animals exposed 18 and 24 months might reveal differences.

CONCLUSIONS

In summary, this experiment indicates that long-term inhalation of shale dusts by rats elicits a limited inflammatory response in the lung less profound than that observed in animals exposed to equivalent levels of quartz alone. This observation is significant because, while the concentration of quartz used was equivalent to the amount found in the shale, shale contains organic and inorganic constituents that may provide a protective effect. The implications for fibrogenic disease are two-fold: 1) inhalation of oil shale dusts appeared to be less detrimental than the inhalation of quartz alone, and 2) there was no apparent synergistic action of quartz and the complex of organic materials present in shale. Animals exposed to shale dusts failed to develop any significant lung lesions, while all of the animals exposed to quartz developed granulomas and some frank fibrosis. Apparently, it takes more than three months but less than six months of continuous quartz exposure at 1 mg/m^3 to elicit an inflammatory response in the lungs, but longer than six months for a similar response in animals exposed to shale, even though the quartz levels were roughly equivalent. Additionally, the numbers of recruited PAM and PMN are persistent after six months of exposure and even increase when the insult is discontinued. This increase in cell numbers after discontinuation of exposure suggests release

from a macrophage-leukocyte recruitment inhibition. The release of lytic enzymes from PAMs and PMNs into lung parenchyma is one mechanism that could explain the presence of fibrosis. However, we currently have no information about the functional capacity of these cells. We intend to investigate this very important aspect of the injury-repair mechanism in future work. After the 12 months of inhalation, the fibrogenic action of quartz alone was emphasized by the observation of silica-positive granulomas in the quartz-exposed animals when no similar lesions were observed in shale-exposed animals.

Autopsy data acquired by Seaton et al. on Scottish shale miners demonstrates increased incidence of both pneumoconiosis and neoplasia following decades of occupational exposure to native shale dusts (5). We also have data from lifetime, high-concentration inhalation and intratracheal instillation experiments in which fibrosis and tumors were observed in shale-exposed rats (6). We are continuing our efforts to understand the progression and specific nature of the toxicity of shale dusts as well as the responses of specific cell types involved in disease processes.

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Figure 1. Elastase levels (μg/mL) in lavage fluids from quartz-exposed, shale-exposed, and control animals. There were no significantly different values for either of the treated groups compared to the controls, except for the shale-exposed animals at 12 months.

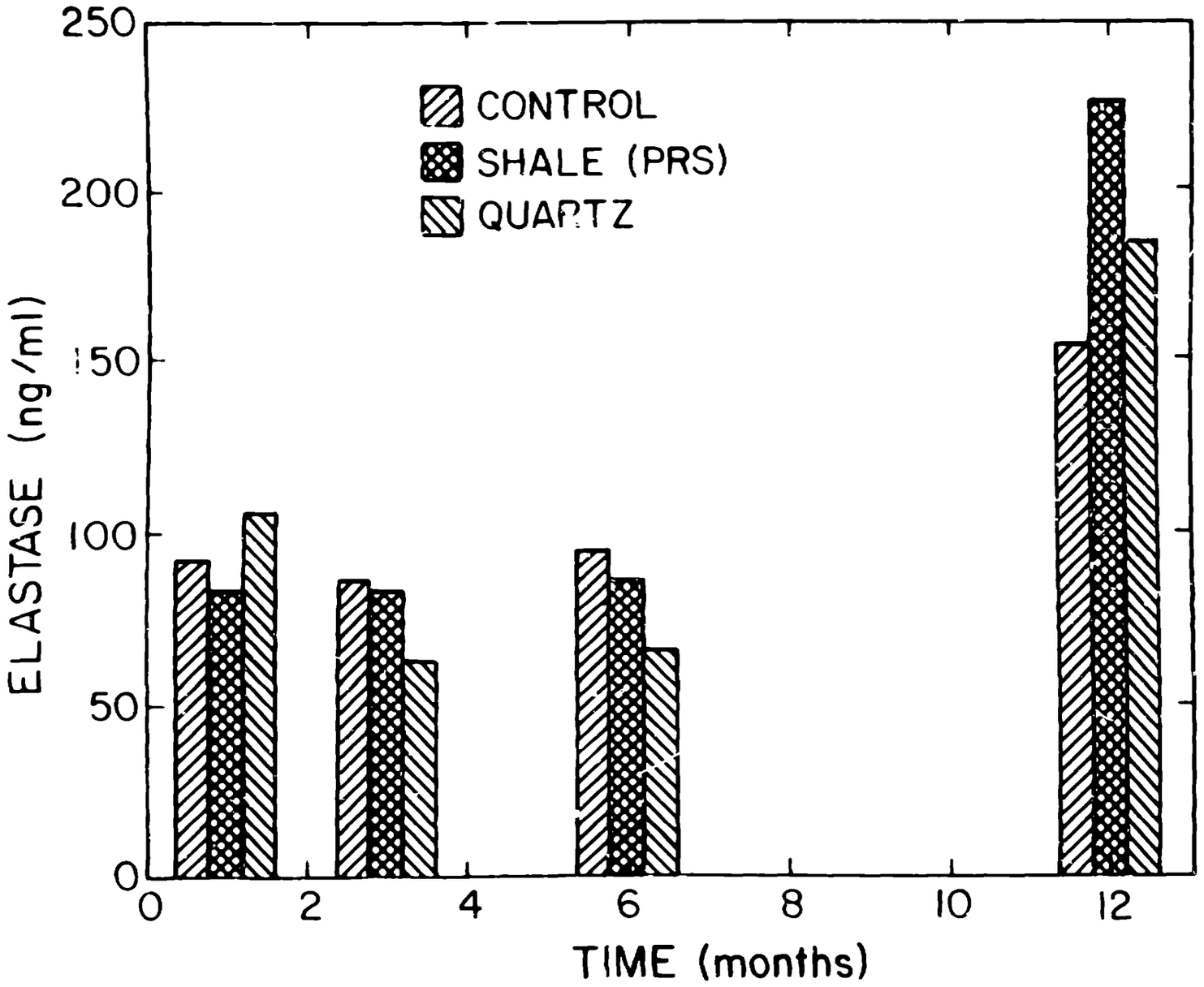

250
200
150
100
50
0
ELASTASE (ng/ml)
CONTROL
SHALE (PRS)
QUARTZ
0
2
4
6
8
10
12
TIME (months)

Figure 2. Hydroxy-proline levels in the left lungs of all exposed and control animals. Comparisons at 1, 3, 6, and 12 months of exposure show no significant differences for either of the treated groups versus the controls.

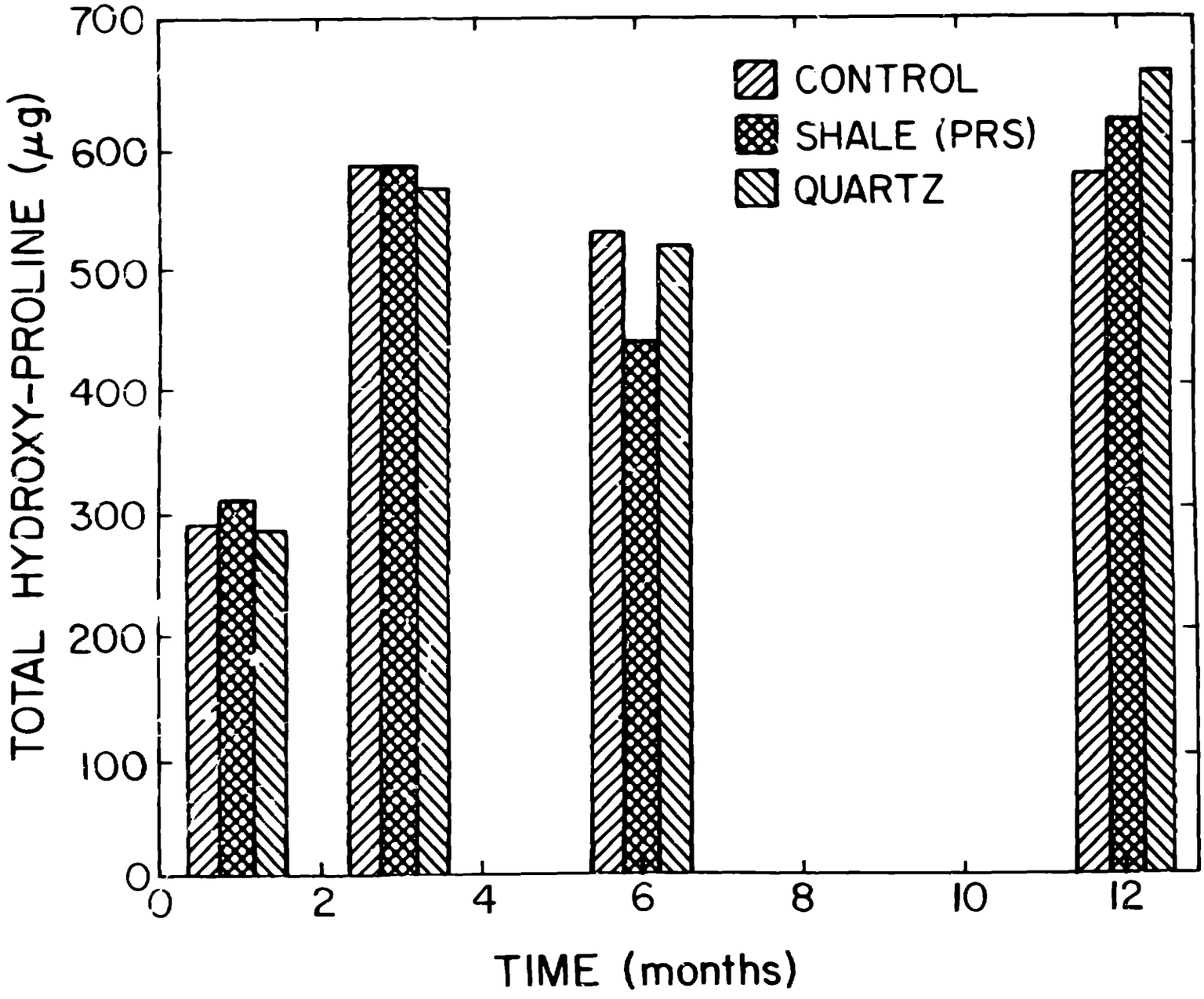

700
600
500
400
300
200
100
0
0
2
4
6
8
10
12
TOTAL HYDROXY-PROLINE (μg)
TIME (months)
CONTROL
SHALE (PRS)
QUARTZ

Figure 3. Lavaged cell yields after 1 and 3 months of exposure to quartz (□) or shale (△) along with age-matched controls (○) show no significant differences. At t = 0, treatment is discontinued and subsequent data points are plotted after recovery periods of 7 and 30 days.

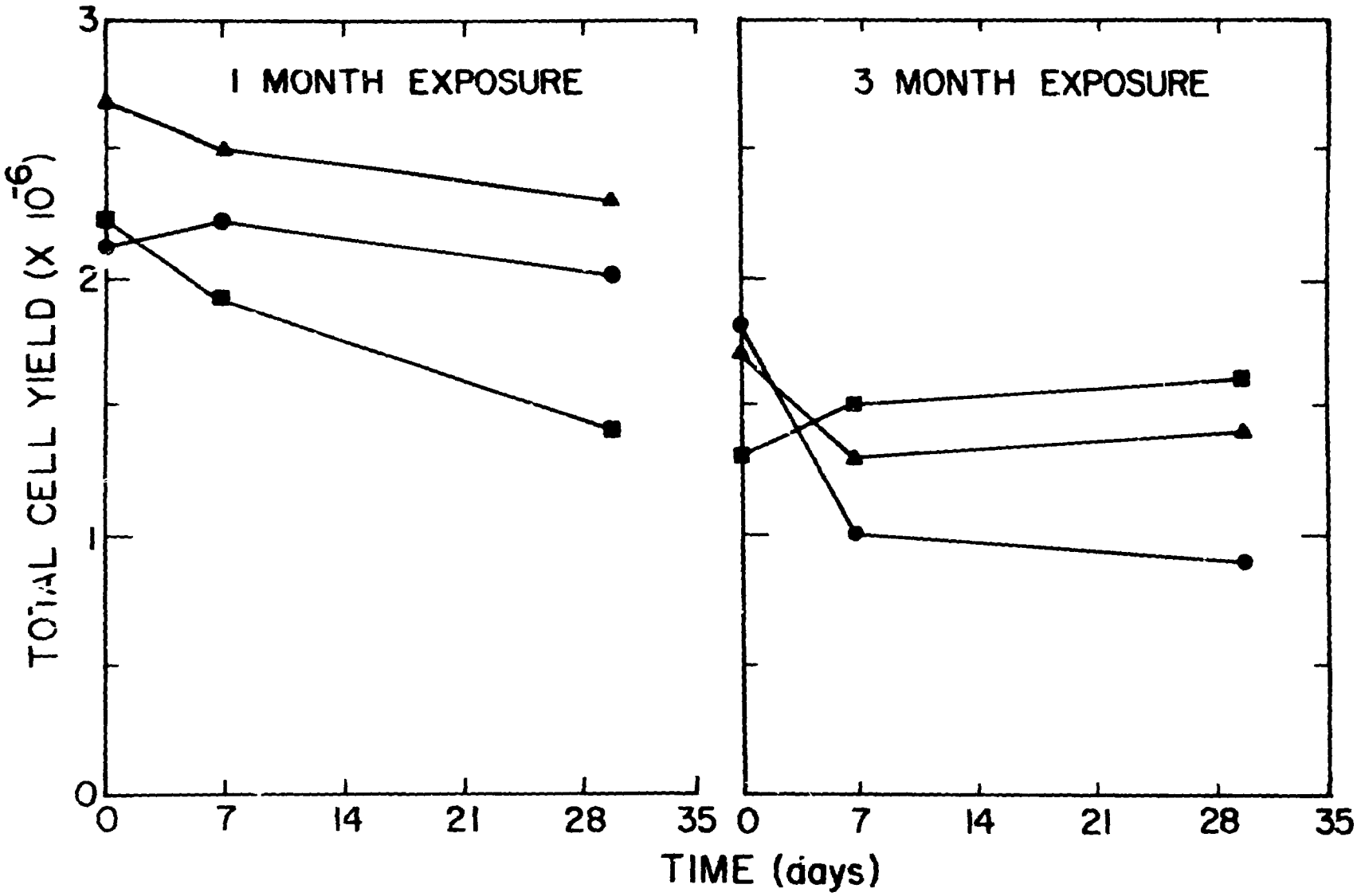

1 MONTH EXPOSURE
3 MONTH EXPOSURE
TOTAL CELL YIELD (X 10⁻⁶)
3
2
1
0
0
7
14
21
28
35
TIME (days)

Figure 4. After a 6-month exposure to quartz (□) or shale (△), the number of PMNs havested from the quartz-exposed, t = 0, is elevated. Treatment was discontinued and the number of PMN's harvested after 7 and 30 days recovery continued to rise for the quartz-treated animals. Controls (○) show no changes.

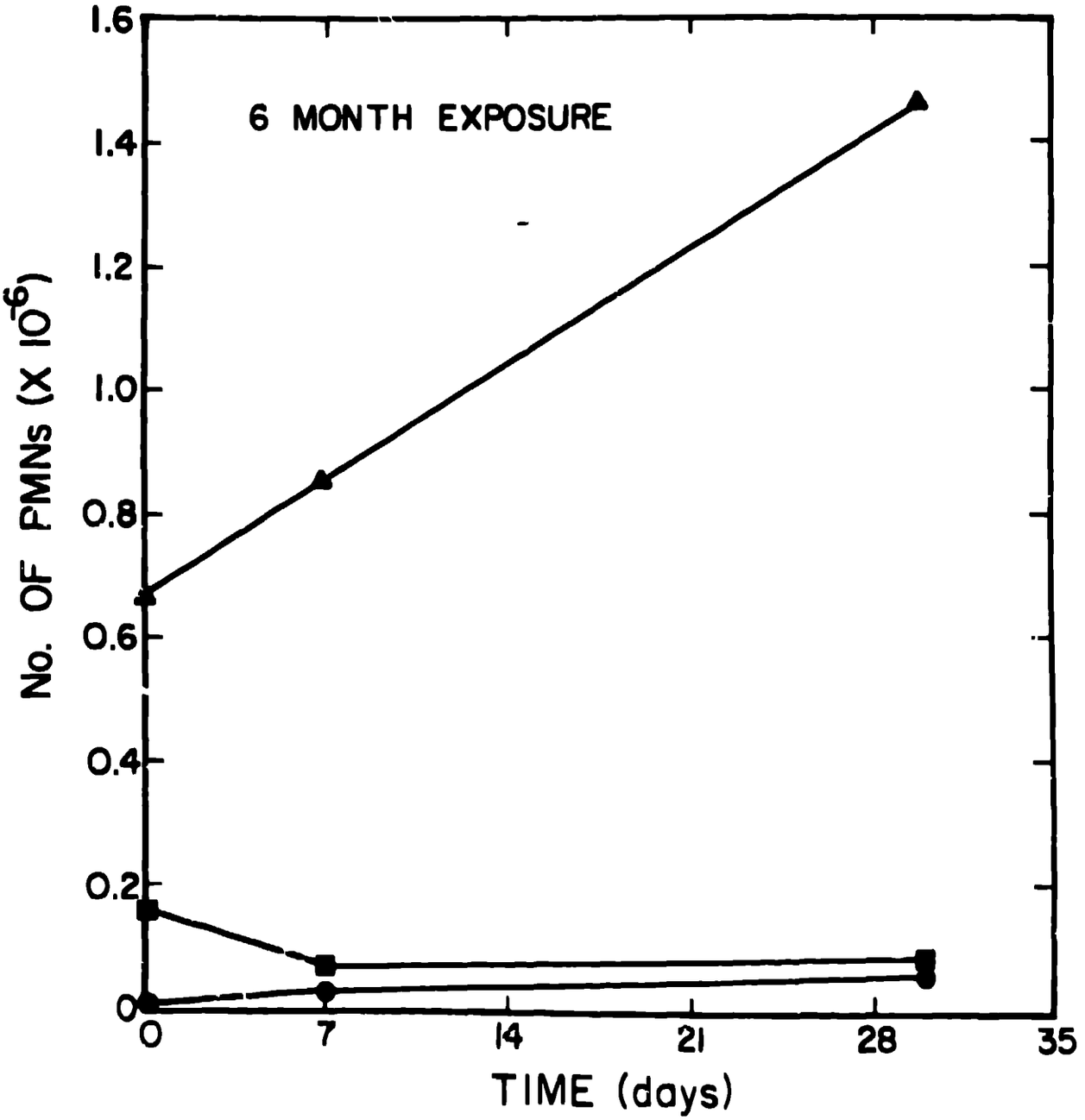
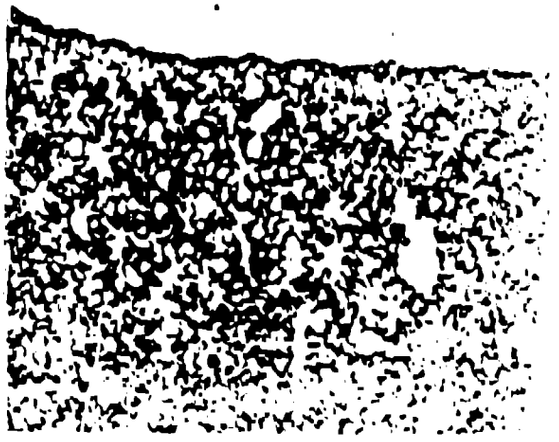

6 MONTH EXPOSURE
No. OF PMNs (X 10⁻⁶)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
7
14
21
28
35
TIME (days)

Figure 5. Photomicrographs of sections of lungs from controls (A), shale-exposed (B), and quartz-exposed (C) animals after 6 months of treatment. A pleural plaque (nidus of inflammatory cells, characteristic of 6- to 12-month quartz exposures) can be seen at the area indicated by the arrow in (C).

(A)

(B)



(C)

Figure 6. Photomicrograph of a section of lung from a rat exposed to quartz for 12 months with a mature, well-organized pleural plaque. Multiple plaques at the pleural surface were observed in both gross and microscopic specimens.

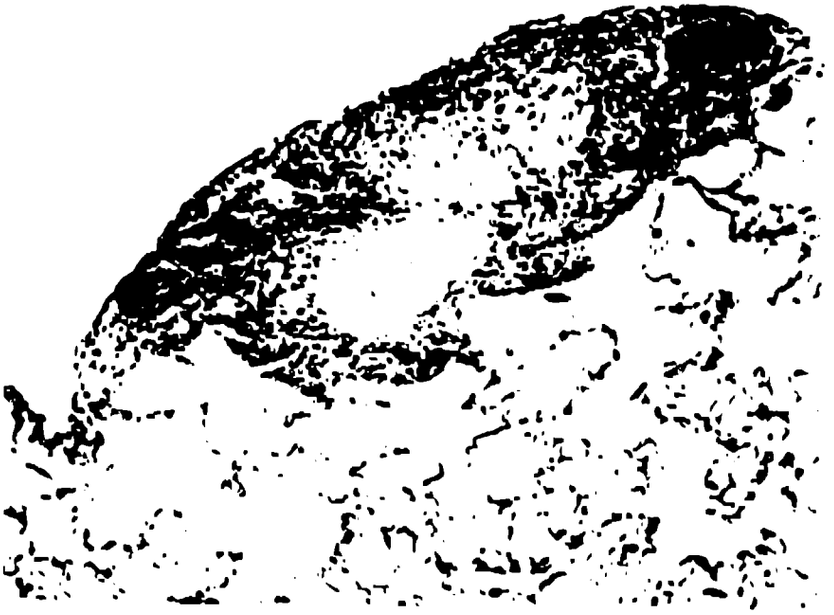


TABLE I. Total cell yields and differentials for treated and control animals after 1-, 3-, 6-, and 12-month exposures. Total cell yields increased for the quartz-exposed animals by 6 and 12 months as a result of increases in lymphocytes and PMNs.

Length of Exposure	Treatment Group	Total Cell Yield	PAM	Numbers of Lymphs	PMN
(months)		($\times 10^6$)		($\times 10^6$)	
1	Control	2.1 ± 0.2	1.7 ± 0.1	0.3 ± 0.1	0
1	Shale	2.7 ± 0.5	2.0 ± 0.1	0.5 ± 0.5	0.04 ± 0.4
1	Quartz	2.2 ± 0.7	1.5 ± 0.1	0.6 ± 0.6	0.02 ± 0.02
3	Control	1.8 ± 0.1	1.6 ± 0.1	0.2 ± 0.02	0
3	Shale	1.7 ± 0.3	1.5 ± 0.3*	0.1 ± 0.05	0
3	Quartz	1.3 ± 0.1	0.9 ± 0.1**	0.3 ± 0.1	0.03 ± 0.03
6	Control	1.5 ± 0.3	1.2 ± 0.2	0.1 ± 0.1	0.01 ± 0.02
6	Shale	1.3 ± 0.5*	1.0 ± 0.4	0.1 ± 0.4	0.16 ± 0.08**
6	Quartz	2.7 ± 0.5**	1.5 ± 0.3	0.4 ± 0.3**	0.67 ± 0.19**
12	Control	1.5 ± 0.5	1.2 ± 0.5	0.1 ± 0.03	0.03 ± 0.01
12	Shale	1.5 ± 0.1*	1.0 ± 0.1	0.2 ± 0.05*	0.20 ± 0.11**
12	Quartz	2.9 ± 1.2**	1.2 ± 0.7	0.7 ± 0.30**	0.85 ± 0.30**

*Value is significantly different from quartz value at the 95% confidence level by student's t test.

**Value is significantly different from control at the 95% confidence level by student's t test.

TABLE II. Total cell yields and differentials for 6-, and 12-month exposures following 7 or 30 days of recovery. Persistent increases in numbers of PMN and lymphocytes are evident for the quartz-exposed animals after six months, but only at 12 months of exposure for the shale-treated animals.

Time*	Treatment Group	Total Cell Yield ($\times 10^6$)	PAM	Numbers of Lymphs ($\times 10^6$)	PMN
6/7	control	1.6 ± 0.2	1.4 ± 0.2	0.12 ± 0.03	0.03 ± 0.03
6/7	Shale	1.4 ± 0.3	1.2 ± 0.3	0.10 ± 0.04**	0.07 ± 0.03*
6/7	Quartz	2.1 ± 0.6	0.9 ± 0.3***	0.24 ± 0.1***	0.86 ± 0.4***
6/30	Control	2.4 ± 0.5	3.2 ± 0.2	0.23 ± 0.3	0.06 ± 0.2
6/30	Shale	2.2 ± 0.7	1.7 ± 0.6**	0.10 ± 0.02	0.09 ± 0.02
6/30	Quartz	5.7 ± 1.4***	2.9 ± 0.7	1.17 ± 0.5***	1.47 ± 0.6***
12/7	Control	1.6 ± 0.4	1.3 ± 0.4	0.15 ± 0.03	0.04 ± 0.02
12/7	Shale	2.4 ± 2.0	0.81 ± 0.2	0.15 ± 0.07	0.1 ± 0.07**
12/7	Quartz	3.6 ± 0.6	1.6 ± 0.6	0.67 ± 0.4***	1.11 ± 0.2***
12/30	Control	1.8 ± 0.5	1.4 ± 0.3	0.13 ± 0.10	0.06 ± 0.05
12/30	Shale	3.7 ± 1.4	2.8 ± 1.2***	0.38 ± 0.18**	0.32 ± 0.13**
12/30	Quartz	5.9 ± 1.3	2.9 ± 0.8***	0.74 ± 0.3***	2.1 ± 0.6***

*Months of Exposure/Days or Recovery.

**Value is significantly different from quartz value at the 95% confidence level by student's t test.

***Value is significantly different from control at the 95% confidence level by student's t test.