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Detection of Early Changes in Lung Cell Cytology by Flow-Systems Analysis Techniques

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DETECTION OF EARLY CHANGES IN LUNG CELL CYTOLOGY BY FLOW-SYSTEMS ANALYSIS TECHNIQUES

by

J. A. Steinkamp, J. S. Wilson, and Z. V. Svitra

ABSTRACT

This report summarizes results of ongoing experiments designed to develop automated flow-analysis assay methods for discerning damage to exfoliated respiratory tract cells in model test animals exposed by inhalation to physical and chemical agents associated with the production of synthetic fuels from oil shale and coal, the specific goal being the determination of atypical changes in exposed alveolar macrophages and epithelial cells. Animals were exposed to oil shale particles (raw and spent), silica, and polystyrene latex spheres via intratracheal instillation. Respiratory tract cells were obtained by lavaging the lungs with normal saline, stained with mithramycin for DNA content, and analyzed using flow cytometric analysis methods. In addition to measuring DNA content, differential and total cell counts were made on all samples analyzed. DNA content frequency distribution histograms and cytology showed definite atypical changes resulting from exposure to shale and silica particulates when compared to the controls. To continue development of fluorescence staining methods for measuring intracellular enzymes in alveolar macrophages, studies were initiated for determining β-glucuronidase using naphthol AS-BI-β-d-glucuronic acid as a fluorogenic substrate. As this new technology becomes adapted to analyzing pulmonary macrophages and epithelial cells, the measurement of physical and biochemical properties as a function of exposure to particulate and gaseous toxic agents related to the production of synthetic fuels will be increased. This analytical approach is designed to assist in the establishment of future guidelines for estimating the risks to exposed humans.

I. INTRODUCTION

The application of advanced flow cytometric instrumentation to measure cytological and biochemical properties of pulmonary macrophages and epithelial cells provides a new approach for assaying damage to lung epithelium exposed by inhalation to toxic environmental pollutants associated with the production of synthetic fuels from oil shale and coal.[1-3] This includes the development of automated cytological methods for determining atypical changes in exfoliated respiratory tract cells from experimental animals exposed to particulate and gaseous agents, the end objective being to assist in estimating the risks, evaluating the incipient damage, and establishing guidelines for determining exposure levels of various toxic agents to occupationally exposed workers and society-at-large. To develop analytical flow-analysis methods for quantitative assessment of cellular damage in animal models, automated cell-analysis and sorting instrumentation[4-6] is presently being applied to study respiratory tract cells from hamsters exposed to particulates of oil shale and silica. This includes the exposure of experimental animals to physical and chemical toxicants; the acquisition of exfoliated lung cells by lavaging the respiratory tract with normal saline; and the utilization of fluorescence staining methods to measure cellular biochemical parameters using flow cytometric methods. Recent efforts have been directed toward (1) measurement of DNA content in respiratory tract cells exposed to raw and spent oil shale particulates, silica, and polystyrene latex spheres over a period ranging to 90 days; (2) correlation of cell counts and total numbers of macrophages, leukocytes, and epithelial cells as a function of time after exposure; (3) cytological observations of rosettes of macrophages formed around oil shale particulates and giant cell formation; and (4) the

preliminary evaluation of a derivative of naphthol AS-BI as a fluorogenic substrate for measuring β-glucuronidase activity in lung cells.

II. MATERIALS AND METHODS

To continue studying cellular changes in animals exposed to particulates of oil shale and silica, 30 Syrian hamsters were injected intratracheally with 10 mg of ball-milled (2- to 7-μm diameter range) raw oil shale suspended in 0.2 mℓ of normal saline and 30 with the same amount and size of spent shale. Thirty hamsters were similarly instilled with 10 mg of silica (4-μm mean diameter) and 30 with 10 to 20 x 10^6 polystyrene latex spheres (5.7-μm mean diameter) suspended in 0.2 mℓ of normal saline. Ten hamsters were instilled with 0.2 mℓ of normal saline alone, and 10 were used as controls. The raw and spent oil shale was obtained from Anvil Points, Colorado, and silica from the Pennsylvania Glass and Sand Corporation. Hamsters were anesthetized with "Brevital" (5 mg) prior to intratracheal instillation of particulates and saline via the oral cavity and then were returned to the colony. Animals exposed to particulates of oil shale, silica, and latex spheres were then sacrificed by pentobarbital injection in groups of three for each type of particulate exposure 4, 7, 21, 28, 35, 42, 49, 60, and 90 days later. The lungs were lavaged four times with saline to obtain exfoliated macrophages, leukocytes, and epithelial cells, which were fixed in 35% ethanol prior to staining for DNA content with mithramycin.[7,8] Cell samples were then excited at 457 nm wavelength (argon-ion laser) and analyzed for fluorescence on a cell-by-cell basis and displayed as frequency distribution histograms using a multichannel pulse-height analyzer. Cell counts (cells/mℓ) were made on all lavage samples using a hemocytometer. Cytology also was performed to determine the percentage of the different types of cells present, including giant cells and macrophage rosettes.

To begin development of using fluorogenic substrates to measure β-glucuronidase activity in alveolar macrophages and other cell types,[9] naphthol AS-BI-β-d-glucuronic acid (1 mg) was dissolved in 1 mℓ of acetone and then diluted to 25 mℓ with 0.2 M acetate buffer (pH 4.6). A solution of 5 mℓ was then added to the cells (pellet), which were analyzed by exciting in the uv (333, 351, and 363 nm wavelength) and measuring fluorescence.[1,6]

III. RESULTS AND DISCUSSION

During this report period (January 1-June 30, 1979), major emphasis was placed on exposing respiratory tract cells of Syrian hamsters to oil shale particulates and silica and on beginning the development of methods for measuring hydrolytic enzyme activity (acid hydrolases) in alveolar macrophages using fluorogenic substrates.

A. DNA Measurements: Respiratory Tract Cells Exposed to Saline, Latex Spheres, Oil Shale Particulates, and Silica

Figure 1 shows a typical DNA content distribution of cells taken from lavaging the respiratory tract of a normal (control) hamster. Peak 1 represents cells having 2C DNA content and peak 2 binucleated cells and doublets.[10] DNA content distributions of lung cells exposed to normal saline, polystyrene latex spheres, raw and spent oil shale particulates, and silica for 90 days maximum are shown below in Figs. 4-9. Cell counts in the lavage fluid from normal and exposed animals for the period 4 to 90 days after exposure are illustrated in Fig. 2. The mean cell count for the control hamsters was 1.66 x 10^6 cells/mℓ.

The total numbers of macrophages, leukocytes, and epithelial cells also were determined from

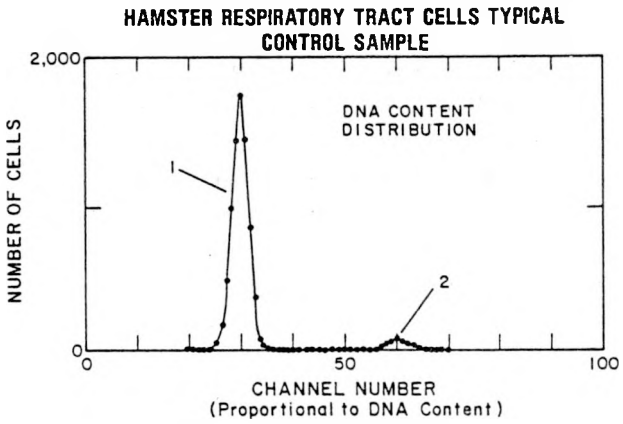


Fig. 1. Frequency distribution histogram (DNA content per cell) of normal hamster respiratory tract cells fixed in 35% ethanol and stained with mithramycin.

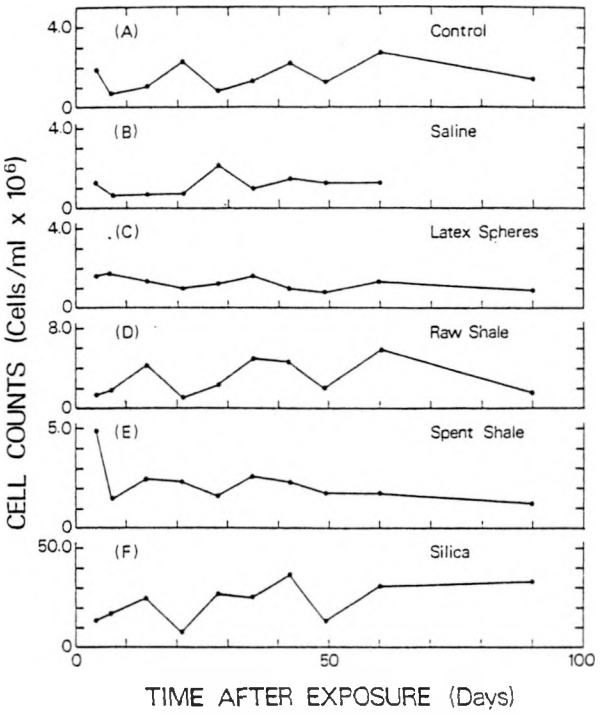


Fig. 2. Plots of cell counts vs time after exposure of hamster respiratory tract cells from controls and hamsters exposed to saline, latex spheres, raw shale, spent shale, and silica. Each data point for the control and saline-exposed hamsters represents one animal, whereas each point on the remaining plots represents the average cell count from three hamsters.

differential cell counts (see the appendix) and plotted as a function of time after exposure for control and exposed hamsters. These data are shown in Fig. 3. For control hamsters (Fig. 3A), the number of macrophages ranged from 0.9 x 10^6 to 4.4 x 10^6, with a mean of 1.97 x 10^6. The number of leukocytes and epithelial cells ranged from 0.1 x 10^6 to 0.8 x 10^6 and 0.8 x 10^6 to 2.0 x 10^6, respectively, with means of 0.38 x 10^6 and 0.97 x 10^6.

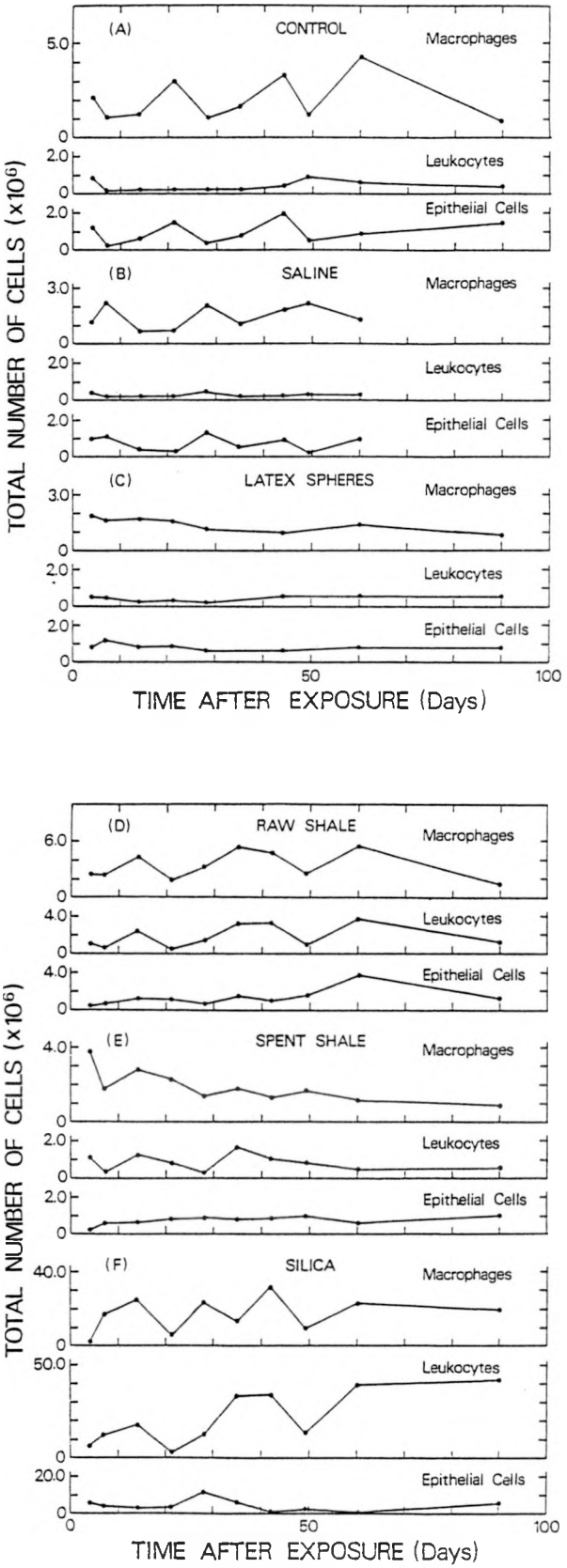


Fig. 3. Plots of total numbers of macrophages, leukocytes, and epithelial cells vs time after exposure of hamster respiratory tract cells from controls and hamsters exposed to saline, latex spheres, raw shale, spent shale, and silica. Each data point for the control and saline-exposed hamsters represents one animal, whereas each point on the remaining plots represents the average numbers of cells from three hamsters obtained by multiplying the percentages shown in the appendix times the individual animal cell counts.

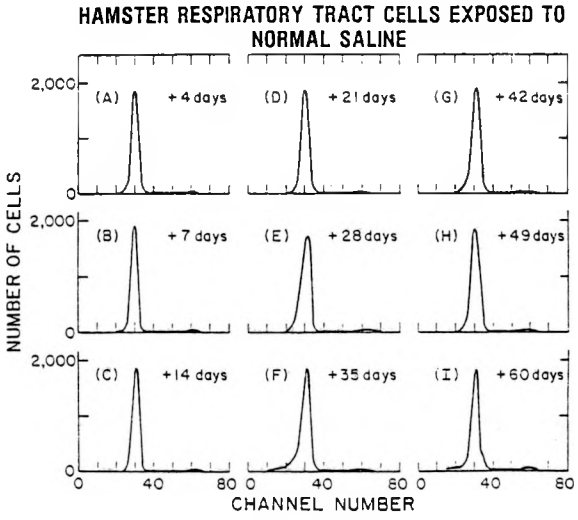
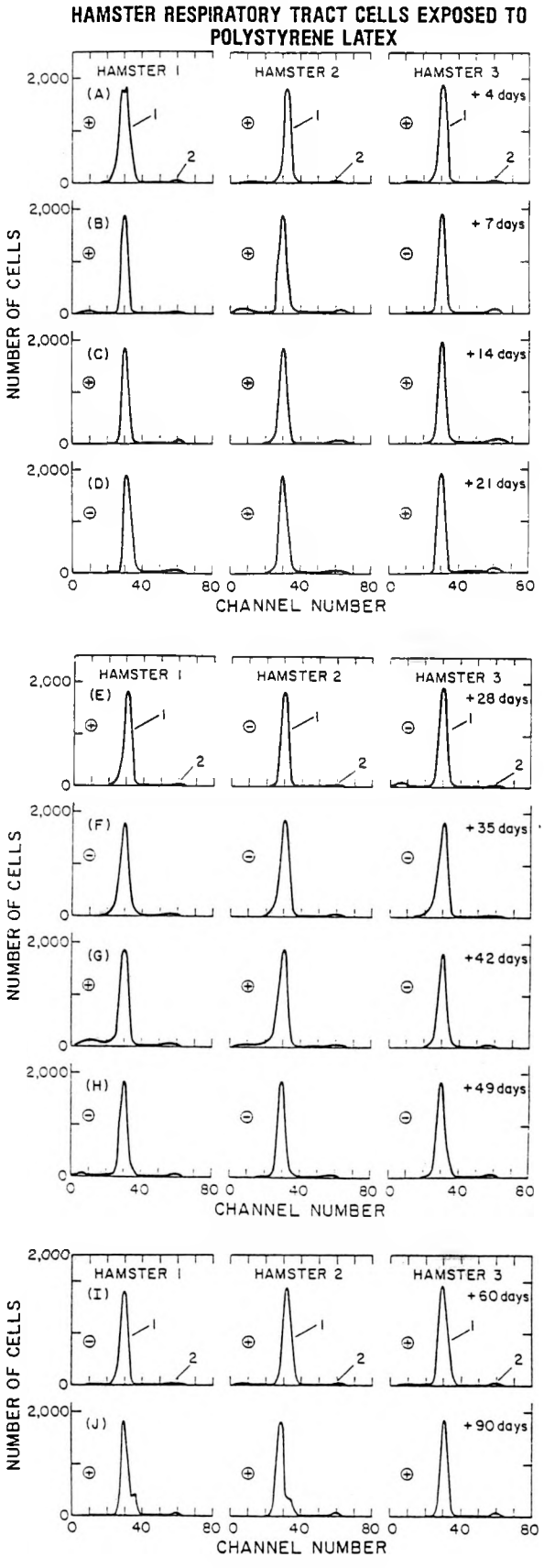


Fig. 4. DNA content frequency distribution histograms of hamster respiratory tract cells exposed to saline (0.2 mℓ intratracheal injection at day 0) and sacrificed 4, 7, 14, 21, 28, 35, 42, 49, and 60 days later. Cell samples were obtained by lung lavage, fixed in 35% ethanol, stained with mithramycin, and analyzed for fluorescence.

The DNA content distributions of hamster respiratory tract cells exposed to normal saline are shown in Fig. 4. These distributions are similar to the controls (Fig. 1). Cell counts (Fig. 2B) and total numbers of cells (Fig. 3B) recorded on hamsters exposed to saline alone were nearly identical to the controls. Hamsters were next exposed to 5.7-μm diameter polystyrene latex spheres for comparison to oil shale particulates and silica. The DNA content distributions (Fig. 5) are nearly normal, with the exception that a region of cells to the left side of peak 1 is present. These are most likely dead cells and debris. The circled "+" and "-" symbols next to each distribution indicate a "positive" or "negative" exposure of the hamster, observed by locating particles in the lavaged cell sample x days after exposure.

Fig. 5. DNA content frequency distribution histograms of hamster respiratory tract cells exposed to 5.7-μm diameter polystyrene latex spheres (10 to 20 x 10^6 spheres suspended in 0.2 mℓ saline) via intratracheal injection at day 0. The hamsters were sacrificed in groups of three 4, 7, 14, 21, 28, 35, 42, 49, 60, and 90 days later. Cell samples were obtained by lung lavage, fixed in 35% ethanol, stained with mithramycin, and analyzed for fluorescence.



Some of the DNA content distributions, which appear abnormal (e.g., see Figs. 5A and 5B), correlate well with positive or negative exposures. However, in other cases (e.g., Fig. 5C), this was not true. The shape of the DNA content distribution generally reflected a positive or negative exposure to latex particles. Cell counts from hamsters exposed to latex spheres (Fig. 2C) were slightly less (1.27×10^6 cells/mℓ mean) compared to controls. The total numbers of macrophages and epithelial cells (Fig. 3C) remained at a nearly constant value throughout the exposure period. This phenomenon was not present in the control animals.

Figure 6 shows the DNA content distributions of lung cells from hamsters exposed to raw oil shale particulates. DNA content distributions (Figs. 6A through 6H) for the period 4 to 49 days postexposure appear normal with the exception that, in the majority of samples, there is a region of cells (or debris) to the left side of peak 1 and the distribution is skewed. The DNA distributions also are broadened in some cases. Peak 2 (binucleates and cell doublets) is present in all distributions. By 60 days after exposure (Fig. 6I), the DNA content distributions began to show other changes. Peak 1 is split into two regions (bimodal distribution), thus indicating a change in DNA content, differences in stainability between cell types, or other factors. Cell counts from hamsters exposed to raw shale (Fig. 2D) showed a definite increase (3.21×10^6 cells/mℓ mean) compared to controls and animals exposed to saline and latex spheres. Plots of total cell numbers (Fig. 3D) also reflect an increase in leukocytes and epithelial cells present in the lavaged samples. Macrophages increased, but at a smaller percentage.

DNA content distributions of hamsters exposed to spent oil shale particulates are shown in Fig. 7. The distributions recorded from animals 4 through 21 days postexposure (Figs. 7A through 7D) appear

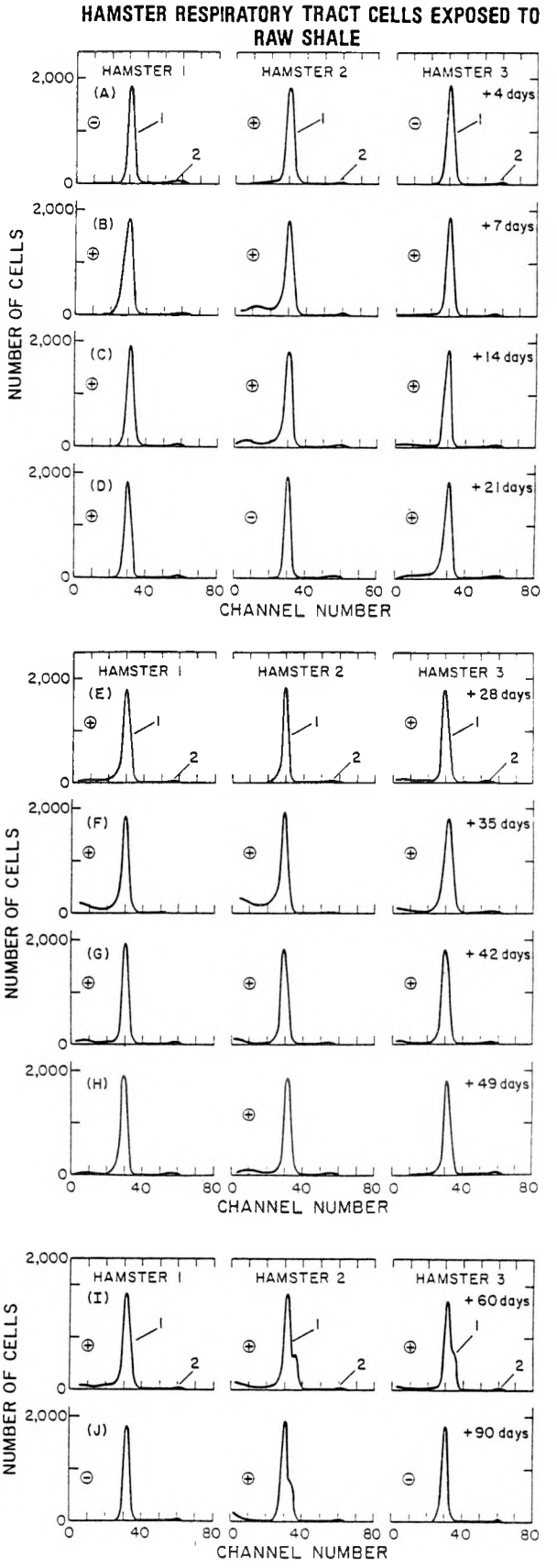


Fig. 6. DNA content frequency distribution histograms of hamster respiratory tract cells exposed to 2- to 7-μm diameter range raw shale particulates (10 mg suspended in 0.2 mℓ saline) via intratracheal injection at day 0. The hamsters were sacrificed in groups of three 4, 7, 14, 21, 28, 35, 42, 49, 60, and 90 days later. Cell samples were obtained by lung lavage, fixed in 35% ethanol, stained with mithramycin, and analyzed for fluorescence.

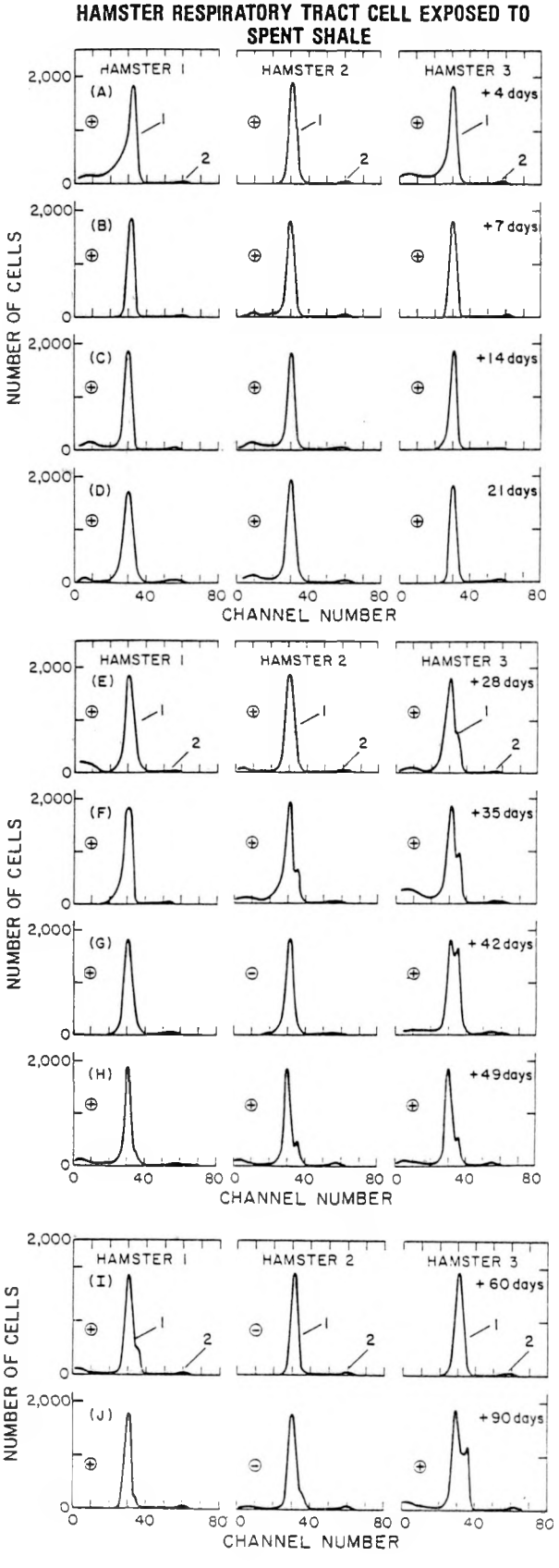


Fig. 7. DNA content frequency distribution histograms of hamster respiratory tract cells exposed to 2- to 7-μm diameter range spent shale particulates (10 mg suspended in 0.2 mℓ saline) via intratracheal injection at day 0. The hamsters were sacrificed in groups of three 4, 7, 14, 21, 28, 35, 42, 49, 60, and 90 days later. Cell samples were obtained by lung lavage, fixed in 35% ethanol, stained with mithramycin, and analyzed for fluorescence.

similar to those determined for hamsters exposed to raw shale (Fig. 6). There is a general broadening of peak 1 and a region of cells and/or debris to the left side of the peak. Peak 1 is skewed in some animals. During the period 28 to 90 days postexposure (Figs. 7E through 7J), peak 1 of many of the DNA content distributions split into two distinct peaks, similar to respiratory tract cells exposed to raw shale for 60 days (Fig. 6I). Cell counts (Fig. 2E) initially were elevated slightly when compared to controls (Fig. 2A) but then began to decrease with time. Total numbers of macrophages and leukocytes also were initially elevated (Fig. 3E), whereas epithelial cells remained nearly constant throughout the exposure period.

Figure 8 shows the DNA content frequency distribution histograms of hamster respiratory tract cells exposed to silica particles. The DNA content distributions recorded from lung cells exposed through the period up to 21 days (Figs. 8A through 8D) appear similar to hamsters exposed to raw and spent oil shale (Figs. 6 and 7). At 28 days postexposure, peak 1 of two of the DNA content distributions (Fig. 8E) split into two distinct peaks. The DNA distributions 35 and 42 days postexposure did not exhibit this phenomenon. However, at 49, 60, and 90 days after exposure, the DNA distributions again were abnormal. Cell counts performed on hamsters exposed to silica (Fig. 2F) were elevated by an order of magnitude (21.8 x 10^6 cells/mℓ) compared to controls (Fig. 2A). The results of total cell number counts (Fig. 3F) showed that macrophages and leukocytes greatly increased, remaining high throughout the 90-day exposure period. Epithelial cells also increased in number during the initial 30 days after exposure and then decreased to nearly zero at 40 to 60 days.

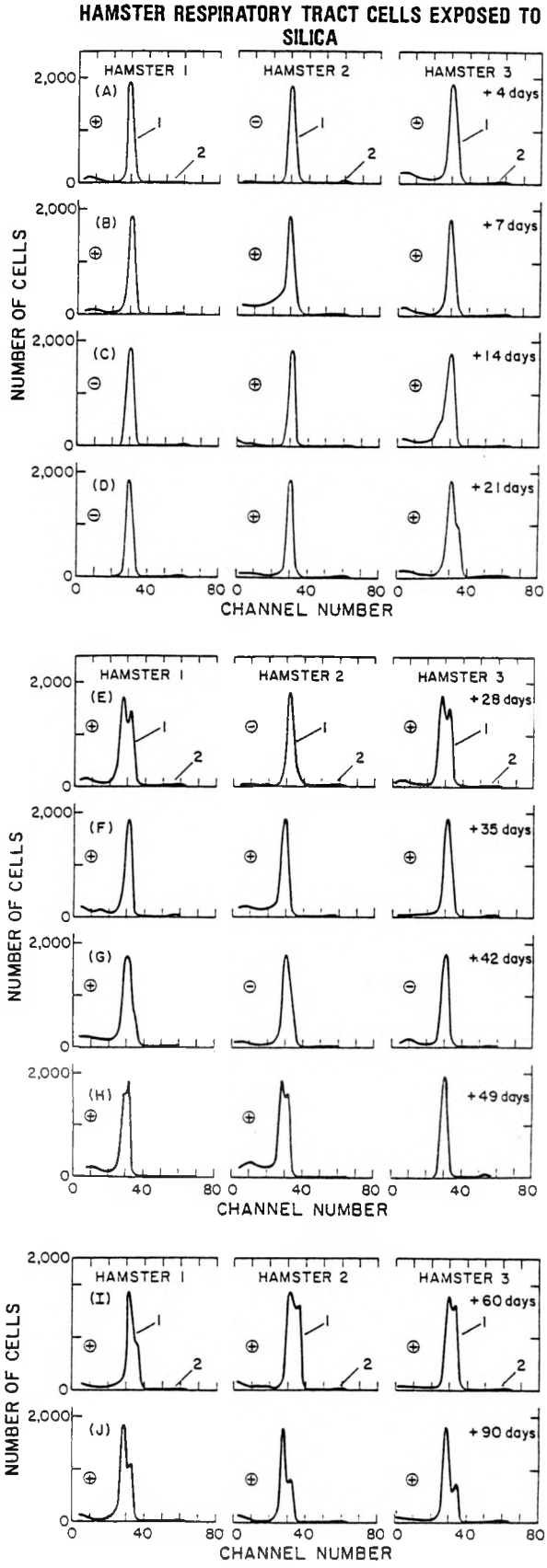


Fig. 8. DNA content frequency distribution histograms of hamster respiratory tract cells exposed to 4-μm diameter silica particles (10 mg suspended in 0.2 mℓ saline) via intratracheal injection at day 0. The hamsters were sacrificed in groups of three 4, 7, 14, 21, 28, 35, 42, 49, 60, and 90 days later. Cell samples were obtained by lung lavage, fixed in 35% ethanol, stained with mithramycin, and analyzed for fluorescence.

These results demonstrate the potential of using flow cytometric analysis to study the effects of exposure and recovery to toxic agents. Future experiments will be designed to correlate cytology with DNA content measurements and to couple these observations with other cellular parameters using multiparameter methods by sorting to identify cells within peak 1 (bimodal distribution). From cytology alone (see the appendix), we have been unable to identify positively the cells with the bimodal distribution of peak 1.

B. Cytology: Macrophage Rosettes and Giant Cells

From microscopic examination of cytocentrifuge sample preparations of exposed hamster respiratory

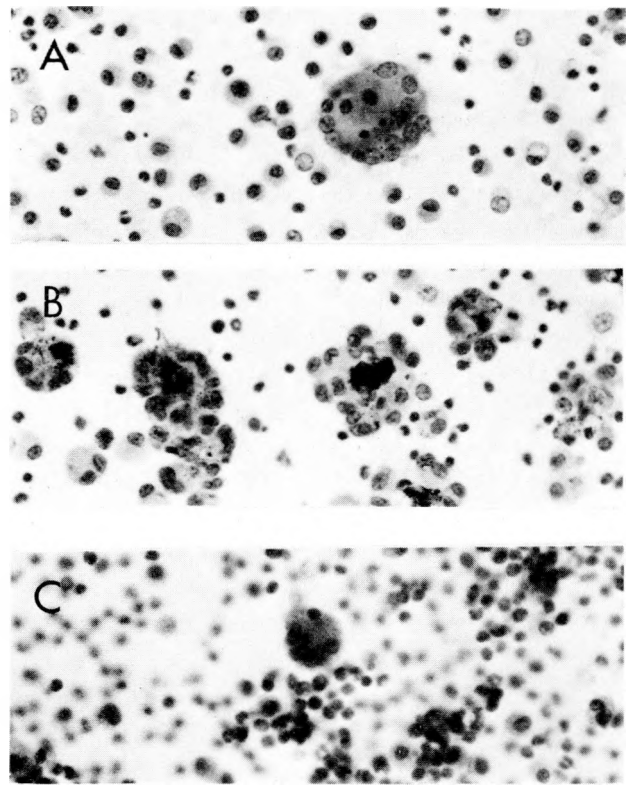


Fig. 9. Photomicrographs of macrophage rosettes located around (A) raw oil shale, (B) spent oil shale, and (C) giant cells (multinucleated) (x320).

TABLE I

NUMBER OF MACROPHAGE ROSETTES AS A FUNCTION OF TIME AFTER EXPOSURE TO RAW SHALE, SPENT SHALE, AND SILICA

Time after Exposure (days)	Number of Rosettes (raw shale)	(spent shale)	(silica)
4	5	35	-
7	20	25	3
14	9	51	-
21	8	7	-
28	29	5	-
35	17	30	8
42	26	--	1
49	3	7	-
60	9	--	-
90	1	--	-
Total	127	160	12

tract cells, there also appears to be a "chemical attraction" between macrophages and raw and spent shale.[11] This takes place in the form of rosettes of macrophages that form around pieces of oil shale particles that are too large to be injected (Fig. 9). Very little "attraction" was found with macrophages and large silica particles. These data are summarized in Table I.

Increased multinucleation (giant cells) also was observed in lung cell samples from hamsters exposed to raw oil shale and silica at 7 to 14 days postexposure. Although "giant cells" were not observed at other times or in cell samples exposed to spent shale, they were most likely present.

C. Flow Cytometric Quantitation of β-Glucuronidase Activity: Preliminary Evaluation

Fluorogenic substrates that are hydrolyzed by intracellular enzymes into fluorescent compounds offer a new method for quantitating various enzyme activities in alveolar macrophages and epithelial cells. For example, total esterase activity has been measured in macrophages and leukocytes using fluorescein diacetate (FDA) as a fluorogenic substrate (nonfluorescent), which is converted to free fluorescein by the enzymatic action of esterases.[12,13] Naphthol AS-BI similarly has been used to quantitate alkaline phosphatase in rat respiratory tract cells.[1] It has been well established that certain inhaled particles and gaseous agents alter the susceptibility of experimental animals to pulmonary infection by cytotoxic damage to alveolar macrophages.[14,15] Since lysosomal enzymes play a prominent role in the cellular bacterial function, attempts will be made to locate suitable fluorogenic substrates for quantitating primarily those enzymes related to alveolar macrophage function (i.e., acid hydrolases).

To begin development of a method for studying β-glucuronidase activity in macrophages, naphthol AS-BI-β-glucuronic acid was selected as a fluorogenic substrate.[9] Figure 10 shows the fluorescence excitation and emission curves of Chinese hamster ovary (line CHO) cells fixed in 10% neutral formalin and then treated with the substrate (20 min). Since the excitation maxima occur near 340 nm wavelength, either the high-power argon or krypton laser was used to excite the fluorochrome.[6] The kinetics of β-glucuronidase activity were done on unfixed CHO cells incubated with the substrate at room temperature (80 to 85°F). Cells were analyzed (fluorescence intensity/cell) at different time intervals (Fig. 11) by recording frequency distribution histograms and plotting the peak channel numbers. As illustrated in Fig. 11, fluorescence intensity (enzyme hydrolyzed substrate) is linear to about 30 min and, by 80 min, saturation is obtained.

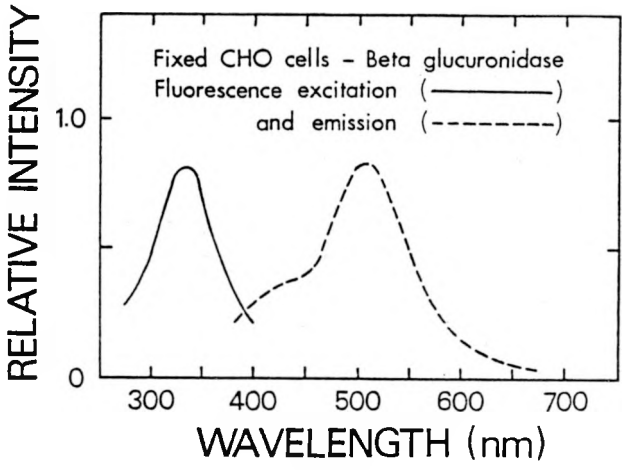


Fig. 10. Fluorescence excitation (——) and emission (---) spectra for β-glucuronidase measured in unfixed Chinese hamster ovary cells using naphthol AS-BI-β-d-glucuronic acid as a fluorogenic substrate.

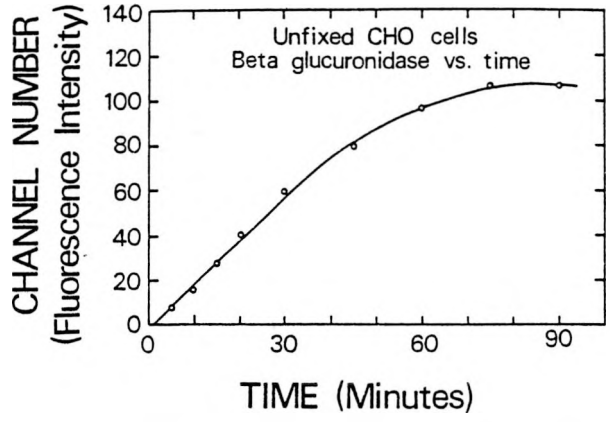


Fig. 11. Plot of β-glucuronidase in unfixed CHO cells vs time after adding fluorogenic substrate (naphthol AS-BI-β-d-glucuronic acid) to cells. Fluorescence intensity was measured by recording the peak channel number from the frequency distribution histograms at different time intervals.

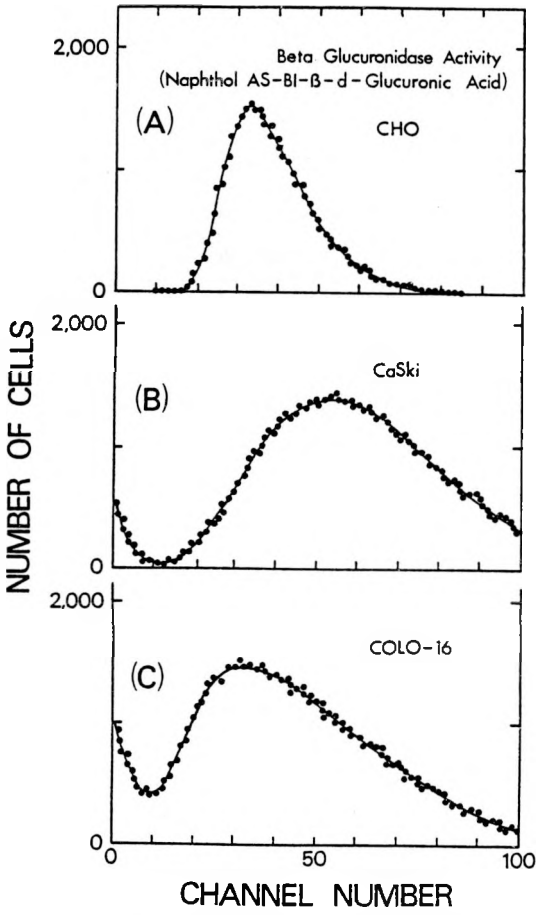


Fig. 12. Frequency distribution histograms of β-glucuronidase activity in unfixed (A) CHO, (B) CaSki, and (C) COLO-16 cells 30 min after reacting with naphthol AS-BI-β-d-glucuronic acid.

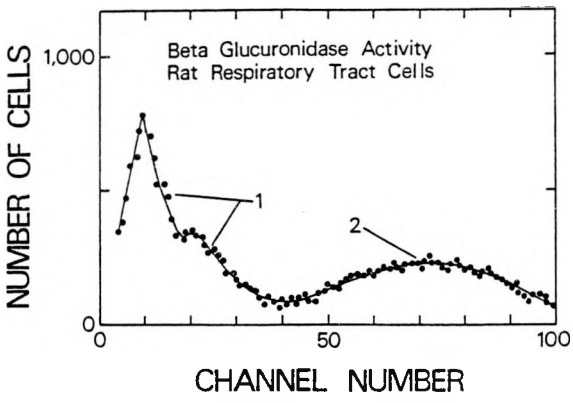


Fig. 13. Frequency distribution histogram of β-glucuronidase activity in unfixed respiratory tract cells from a normal rat 30 min after reacting with naphthol AS-BI-β-d-glucuronic acid.

To demonstrate the measurement of β-glucuronidase activity in the cultured cell lines, unfixed CHO and two epithelial lines (CaSki and COLO-16) were stained (i.e., reacted with the substrate for 30 min) and then analyzed. β-Glucuronidase activity in the CaSki cell line was higher than in CHO and COLO-16. Normal rat (Sprague-Dawley) respiratory tract cells also were analyzed for β-glucuronidase activity. Figure 13 shows a frequency distribution histogram of β-glucuronidase per cell. Although peaks 1 and 2 have not been positively identified, they most likely represent leukocytes and macrophages, respectively. These preliminary data illustrate the potential for quantitating enzymes in alveolar macrophages using fluorescence methods. Future studies will involve determining the effects of cell fixation, concentration of cells/mℓ, cell volume, temperature, and other factors on enzyme measurement prior to exposing the animals to toxic agents that have been shown to alter enzymatic action in pulmonary macrophages and epithelial cells.

IV. FUTURE PLANS

Experimental goals during the next 6 months are to (1) continue exposing hamsters to particulates of oil shale and silica, with emphasis on DNA content measurements and the possibility of combining these data with other cellular parameters to differentiate macrophages, leukocytes, and epithelial cells, correlate cytology and cell counts

with flow cytometric measurements, and sort cells within the different regions of the histograms for identification; (2) improve the present experimental setup for exposing animals to gaseous agents such as ozone[1] and possibly nitrogen dioxide; (3) continue the development of using fluorescent microspheres to measure phagocytic activity of alveolar macrophages;[1] and (4) continue the development of enzyme staining methods for measuring hydrolytic enzymes in respiratory tract cells.

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APPENDIX

TABLE A-I

DIFFERENTIAL CELL COUNTS OF RESPIRATORY TRACT CELL SAMPLES FROM HAMSTERS EXPOSED TO SALINE (ALONE), LATEX SPHERES, RAW AND SPENT OIL SHALE, AND SILICA[a]

Hamster Number[b]	Exposure (agent)	Time after Exposure (days)	Macrophages (%)	Leukocytes (%)	Epithelial Cells (%)
C-1	Control	--	53	17	30
S-1	Saline	4	47	15	38
LX-1	Latex spheres	4	55	13	32
LX-2	Latex spheres	4	64	16	20
LX-3	Latex spheres	4	53	17	30
PRS-1	Raw shale	4	56	11	33
PRS-2	Raw shale	4	57	25	18
PRS-3	Raw shale	4	42	6	52
PSS-1	Spent shale	4	75	22	3
PSS-2	Spent shale	4	66	9	25
PSS-3	Spent shale	4	75	22	3
Q-1	Silica	4	--	--	--
Q-2	Silica	4	60	7	33
Q-3	Silica	4	43	30	27
C-2	Control	--	75	6	19
S-1	Saline	7	75	6	19
LX-4	Latex spheres	7	43	11	46
LX-5	Latex spheres	7	63	19	18
LX-6	Latex spheres	7	69	9	22
PRS-4	Raw shale	7	53	27	20
PRS-5	Raw shale	7	65	16	19
PRS-6	Raw shale	7	71	11	18
PSS-4	Spent shale	7	74	5	21
PSS-5	Spent shale	7	54	34	12
PSS-6	Spent shale	7	69	0	31
Q-4	Silica	7	60	36	4
Q-5	Silica	7	45	41	14
Q-6	Silica	7	57	28	15
C-3	Control	14	58	13	29
S-3	Saline	14	57	15	28
LX-7	Latex spheres	14	63	12	25
LX-8	Latex spheres	14	58	7	35
LX-9	Latex spheres	14	73	2	25
PRS-7	Raw shale	14	63	25	12
PRS-8	Raw shale	14	55	31	14
PRS-9	Raw shale	14	45	29	26
PSS-7	Spent shale	14	44	47	9
PSS-8	Spent shale	14	66	21	13
PSS-9	Spent shale	14	67	15	18
Q-7	Silica	14	56	19	25
Q-8	Silica	14	57	43	0
Q-9	Silica	14	46	29	25
C-4	Control	--	64	4	32
S-4	Saline	21	61	8	31
LX-10	Latex spheres	21	56	9	35
LX-11	Latex spheres	21	53	14	33
LX-12	Latex spheres	21	58	8	34
PRS-10	Raw shale	21	53	19	28
PRS-11	Raw shale	21	69	3	28
PRS-12	Raw shale	21	56	15	29
PSS-10	Spent shale	21	59	14	27
PSS-11	Spent shale	21	54	28	18
PSS-12	Spent shale	21	55	14	31
Q-10	Silica	21	69	4	27
Q-11	Silica	21	46	23	31
Q-12	Silica	21	51	16	33

TABLE A-I (cont)

Hamster Number[b]	Exposure (agent)	Time after Exposure (days)	Macrophages (%)	Leukocytes (%)	Epithelial Cells (%)
C-5	Control	--	60	12	28
S-5	Saline	28	49	13	38
LX-13	Latex spheres	28	57	10	33
LX-14	Latex spheres	28	64	9	27
LX-15	Latex spheres	28	51	8	41
PRS-13	Raw shale	28	63	25	12
PRS-14	Raw shale	28	66	13	21
PRS-15	Raw shale	28	53	28	19
PSS-13	Spent shale	28	57	5	38
PSS-14	Spent shale	28	56	12	32
PSS-15	Spent shale	28	54	16	30
Q-13	Silica	28	50	26	24
Q-14	Silica	28	54	9	37
Q-15	Silica	28	--	--	--
C-6	Control	35	60	10	30
S-6	Saline	35	58	8	34
LX-16	Latex spheres	35	50	5	45
LX-17	Latex spheres	35	65	17	18
LX-18	Latex spheres	35	66	10	24
PRS-16	Raw shale	35	63	25	12
PRS-17	Raw shale	35	45	32	23
PRS-18	Raw shale	35	54	38	8
PSS-16	Spent shale	35	51	10	39
PSS-17	Spent shale	35	47	35	18
PSS-18	Spent shale	35	64	36	0
Q-16	Silica	35	10	80	10
Q-17	Silica	35	35	55	10
Q-18	Silica	35	48	26	26
C-7	Control	42	73	5	22
S-7	Saline	42	64	4	32
LX-19	Latex spheres	42	42	26	32
LX-20	Latex spheres	42	43	21	36
LX-21	Latex spheres	42	59	8	33
PRS-19	Raw shale	42	45	37	18
PRS-20	Raw shale	42	70	22	8
PRS-21	Raw shale	42	54	41	5
PSS-19	Spent shale	42	50	17	33
PSS-20	Spent shale	42	64	8	28
PSS-21	Spent shale	42	37	37	26
Q-19	Silica	42	48	52	0
Q-20	Silica	42	35	59	6
Q-21	Silica	42	26	42	32
C-8	Control	49	46	33	21
S-8	Saline	49	83	14	3
LX-22	Latex spheres	49	51	10	39
LX-23	Latex spheres	49	49	15	36
LX-24	Latex spheres	49	55	14	31
PRS-22	Raw shale	49	56	19	25
PRS-23	Raw shale	49	55	37	8
PRS-24	Raw shale	49	56	24	19
PSS-22	Spent shale	49	52	23	25
PSS-23	Spent shale	49	54	26	20
PSS-24	Spent shale	49	42	25	33
Q-22	Silica	49	42	53	5
Q-23	Silica	49	38	62	0
Q-24	Silica	49	53	8	39

TABLE A-I (cont)

Hamster Number[b]	Exposure (agent)	Time after Exposure (days)	Macrophages (%)	Leukocytes (%)	Epithelial Cells (%)
C-9	Control	60	75	10	15
S-9	Saline	60	50	10	40
LX-25	Latex spheres	60	60	9	31
LX-26	Latex spheres	60	52	18	30
LX-27	Latex spheres	60	43	20	37
PRS-25	Raw shale	60	51	27	22
PRS-26	Raw shale	60	48	31	21
PRS-27	Raw shale	60	38	45	17
PSS-25	Spent shale	60	48	28	24
PSS-26	Spent shale	60	54	9	37
PSS-27	Spent shale	60	63	7	30
Q-25	Silica	60	27	44	29
Q-26	Silica	60	37	63	0
Q-27	Silica	60	36	64	0
C-10	Control	90	34	13	53
S-10	Saline	90	--	--	--
LX-28	Latex spheres	90	33	26	41
LX-29	Latex spheres	90	5	19	46
LX-30	Latex spheres	90	54	18	28
PRS-28	Raw shale	90	58	11	31
PRS-29	Raw shale	90	40	30	30
PRS-30	Raw shale	90	52	10	38
PSS-28	Spent shale	90	51	15	34
PSS-29	Spent shale	90	42	14	44
PSS-30	Spent shale	90	18	49	33
Q-28	Silica	90	32	68	0
Q-29	Silica	90	27	63	10
Q-30	Silica	90	30	58	12

[a]Differential counts were determined microscopically from samples by lavaging the lungs x days after exposure.

[b]C = control; S = saline; LX = polystyrene latex particles; PRS = raw shale; PSS = spent shale; Q = silica.

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