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ST0272737

ENVIRONMENTAL RESEARCH 24, 167–191 (1981)

Comparative Pulmonary Responses to Inhaled Inorganic Fibers with Asbestos and Fiberglass

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Received April 25, 1980

One group of rats, hamsters, and guinea pigs was exposed to amosite asbestos by inhalation of fibers of lengths greater than 5 μm at 3.1×10^6/liter, fiberglass at 0.7×10^6/liter, potassium octatitanate (Fybex, Du Pont's registered trademark for inorganic reinforcing titanate fibers) at 2.9×10^6/liter, and pigmentary potassium titanate (PKT) at 2.0×10^6/liter, respectively, for 6 hr/day for 3 months. One group of animals exposed to air alone was used as control. In addition, three groups of rats, hamsters, and guinea pigs were exposed to Fybex at 2.9×10^6/liter, 13.5×10^6/liter, and 41.8×10^6/liter, respectively, for 3 months. One group served as control. Asbestos, Fybex, and PKT produced essentially similar pulmonary responses but with marked differences in fibrogenicity and species differences. Asbestos was the most potent fibrogenic agent and was more than 10 times more fibrogenic than Fybex in terms of exposure concentration. Dose-related fibrogenic activity was found in the animals exposed to Fybex. PKT was the least fibrogenic and produced very minute pulmonary fibrosis in rats and hamsters but not in guinea pigs. Fine fiberglass particles were not fibrogenic. Rats revealed more fibrogenic pulmonary reactions than guinea pigs or hamsters. A few pulmonary tumors developed in animals exposed to asbestos, Fybex, and fiberglass, but their numbers were too small to allow any conclusions on carcinogenicity to be drawn. Fybex produced mesotheliomas in a few hamsters and its possible carcinogenic potential cannot be ruled out.

INTRODUCTION

The literature concerning the hazardous effects of asbestos exposure has been well reviewed by the U.S. Environmental Protection Agency (1971), the U.S. Department of Health, Education and Welfare (1972, 1976), the World Health Organization (1973), and recently by Becklake (1976) and Kannerstein *et al.* (1977). The most common detrimental health effects were pulmonary fibrosis, pleural plaque, bronchogenic carcinoma, mesothelioma, and possibly gastrointestinal carcinoma.

Although animal experiments on asbestos have progressed in 40 years, little is known concerning the pathogenicity of pulmonary fibrosis and carcinogenesis. Asbestos carcinogenesis was thought to be related to trace metals, polycyclic aromatic hydrocarbons, other organic materials, and carcinogenic agents absorbed on asbestos. Recently, however, physical factors such as fiber length and diameter were considered to play an important role in carcinogenesis. Stanton *et al.* (1977) induced pleural sarcoma and mesotheliomas in rats by implanting fibrous dust particles of fiberglass, attapulgite, dawsonite, aluminum oxide, silicon carbide, and potassium titanate. Other animal experiments indicated that the car-

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cinogenicity of dust particles depended on the size and physical configuration rather than physiochemical properties (Maroudas *et al.*, 1973; Smith *et al.*, 1972; Pott and Friedrichs, 1972; Wagner *et al.*, 1976). Fibrogenicity of asbestos and fiberglass was also related to the dimensional configuration. Short fibers (<5 μm in length) produced only a macrophage reaction without fibrosis while longer fibers produced foreign body granuloma with fibrosis (Webster, 1970; Kuschner *et al.*, 1976).

Since the test compounds in the present study are similar to the asbestos fiber in size, diameter, and shape, the potential risk of pulmonary fibrosis and lung cancer cannot be ruled out. To the best of our knowledge, there were no reports of long-term inhalation experiments for these inorganic fibers, and their fibrogenicity and carcinogenicity are unknown. This investigation describes the pulmonary response to inorganic fibers in comparison to asbestos and fiberglass by inhalation exposure in experimental animals.

MATERIALS AND METHODS

Dust Characterization

The test compounds were potassium octatitanate (Fybex),[1] pigmentary potassium titanate (PKT), fiberglass, and amosite of UICC standard reference asbestos sample. Detailed fiber length distribution of the test compounds is shown in Table 1. Fiber lengths were measured by a method described by Johnson and Rosen (1978). Fiber diameters were determined from scanning electron micrographs. Fybex had an average size of 6.7 × 0.2 μm (Fig. 1), PKT averaged 4.2 × 0.2 μm (Fig. 2), and amosite asbestos averaged 5.0 × 0.4 μm (Fig. 3). Fiberglass (Fig. 4) was ball milled to prepare fine respirable dust particles. The size distribution of the original fiberglass sample is shown in Table 1 and that of airborne fiber is presented in Fig. 5. The dust particles had an average diameter of 1.2 μm and most particles were less than 2 μm in length (Fig. 5). Only 7% of the dust particles had an aspect ratio of 3/1 or greater and could be considered fibrous in shape. Marked variation in diameter distribution was observed in both the asbestos (0.18–8 μm)

TABLE 1
DISTRIBUTION OF FIBERS LONGER THAN 3 μm

Test material (μm)	Fybex	PKT	Fiberglass	Amosite asbestos
<3.0	209 (19.1%)	1632 (45.8%)	382 (24.2%)	815 (38.1%)
3.0–4.9	260 (23.8%)	894 (25.1%)	441 (27.9%)	633 (29.6%)
5.0–6.9	207 (18.9%)	456 (12.8%)	208 (13.2%)	259 (12.1%)
7.0–9.9	221 (20.2%)	365 (10.2%)	188 (11.9%)	220 (10.3%)
10.0–14.9	146 (13.4%)	171 (4.8%)	254 (16.1%)	128 (6.0%)
15.0–19.9	34 (3.1%)	31 (0.9%)	75 (4.7%)	41 (1.9%)
20.0–29.9	15 (1.4%)	12 (0.3%)	30 (1.9%)	38 (1.8%)
30.0>	1 (0.1%)	0	2	4 (0.2%)
Total fiber	1093	3561	1580	2138

[1]Fybex is DuPont's registered trademark for inorganic reinforcing titanate fibers.

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FIG. 1. Fybex fibers presenting needle shape with relatively uniform diameter, ×3000.

FIG. 2. PKT fibers showing fine rod shape with uniform diameter and no longitudinal splitting as shown in Fig. 1, ×10,000

FIG. 3. Asbestos fibers illustrating longitudinal splitting (arrows) and formation of thin fibers (wavy arrows), ×3000

FIG. 4. Fiberglass fibers showing large diameter and marked variation in both the diameter and length, ×3000.

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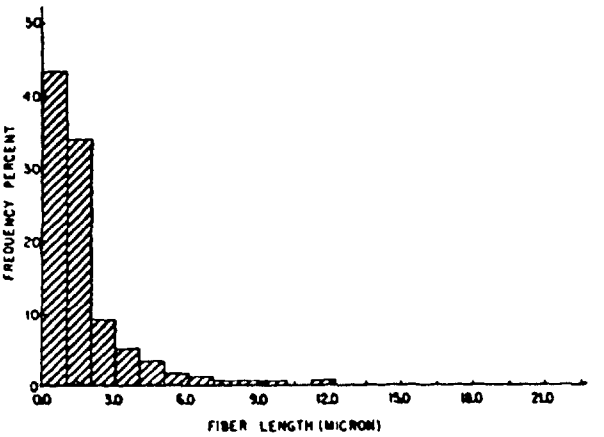

FIG. 5. Fiber length distribution of ball-milled fiberglass obtained from measuring 833 fibers by a particle analyzer (Zeiss TGZ-3). Most fibers are shorter than 2 μm in length.

and fiberglass (0.2–6.5 μm) samples. Fybex diameter distribution varied moderately (0.12–0.7 μm) while PKT was relatively uniform (0.12–0.4 μm). Asbestos fibers showed longitudinal splitting, and, consequently formed fine needle-shaped fibers (0.18 μm in diameter) while the inorganic fibers exhibited horizontal fractures.

Dust Generation

The dust reservoir was conical in shape with a flattened base (Fig. 6). Carrier air was introduced through inlet jets which were located near the top of the reservoir, positioned tangentially, and pointed slightly downward. Smaller inlet ports located at right angles to, and on the lower half of, the reservoir could be used to introduce pulses of air when needed to keep the material from packing.

The top of the reservoir had a centrally located port for filling and an outlet port located near its perimeter. A soft rubber tube was connected to this outlet through

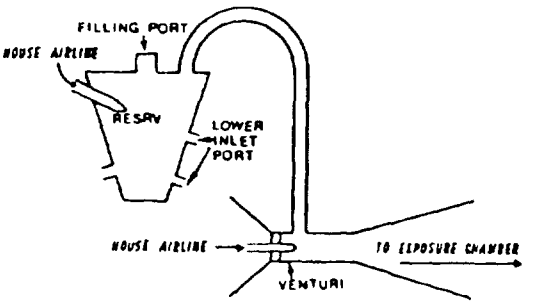

FIG. 6 Block diagram of dust generating apparatus.

which the dust, mostly as agglomerates, passed to the venturi. Here a second jet of air broke up the agglomerates and drew diluting air in from the room. This dust-laden atmosphere was blown into the flared end of a 4-in.-diameter pipe leading to the chamber. Since the venturi–delivery connection was open so that additional makeup air could be drawn from the room, a trap was designed to avoid room air contamination in the event of an exhaust system failure. It consisted of an open acrylic box which encased the connection. The top was covered with a low-pressure-drop fiberglass filtering material.

Dust Concentration and Size Analysis

Five-cubic-meter exposure chambers were used for Fybex, PKT, and asbestos, and two one-cubic-meter chambers for fiberglass. Two atmospheric sampling methods were employed. Samples for gravimetric analysis were taken two to four times during each exposure. All filters were dried in a desiccator for at least 24 hr prior to use and after sampling. For each sample, a known volume of chamber air was drawn through a tared fiberglass filter. Time, sample volume, and pressure drop over the filter were recorded for each sample. Barometric pressure (BP) was also recorded daily. After drying in a desiccator, the filters were weighed and gravimetric concentrations were calculated as follows:

$$\text{Conc. (mg/liter)} = \frac{\text{sampled filter (mg)} - \text{tared filter (mg)}}{\text{volume samples (liter)} \times (BP - \Delta P)/BP}.$$

Samples for fiber size and concentration were taken one to two times during each exposure. For each sample, a known volume of chamber air was drawn through a cellulose acetate filter. Time, sampling rate, and duration were recorded for each sample. Preparation of filter sections and enumeration of fibers by phase contrast microscopy were carried out following the procedure of Edward and Lynch (1968). Concentrations of total fibers, fibers >5 μm < 10 μm, and fibers >10 μm were calculated using the formula

$$\text{Conc. (fibers/liter)} = \frac{\text{avg. fibers/field} \times \dfrac{\text{area filter (mm}^2\text{)}}{\text{area field (mm}^2\text{)}}}{\text{volume samples (l)}}$$

All analytical data was tabulated for statistical analysis.

Experimental Design

Experiment 1. A total of 230 young adult male rats (Charles River, Caesarian-Derived Sprague–Dawley) were divided into five equal groups of 46. The groups were exposed to fibers greater than 5 μm in length in concentrations of 3.1×10^6/liter (asbestos), 0.7×10^6/liter, (fiberglass), 2.9×10^6/liter (Fybex), and 2.0×10^6/liter (PKT). Details of dust concentrations based on fiber length are shown in Table 2. Gravimetric concentrations were 0.3 mg/liter (asbestos), 0.4 mg/liter (fiberglass), 0.07 mg/liter (PKT), and 0.08 mg/liter (Fybex). One group of 46 rats exposed to air served as a control. Similarly, 167 albino male guinea pigs were divided into five groups: 32 were exposed to fiberglass; 35 to asbestos, Fybex, and PKT, respectively; and 30 were used as a control. A total of 170 hamsters were

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TABLE 2
EXPOSURE CONCENTRATIONS OF ASBESTOS, FIBERGLASS, PKT, AND FYBEX

Compound, group	Gravimetric concentration (mg/liter)	Total number of fibers/liter of air	Number of fibers >5 μm/liter of air	Number of fibers >10 μm/liter of air
Experiment 1				
Asbestos 1	0.300	13.25×10^6	3.09×10^6	1.09×10^6
Fiberglass 1	0.424	6.54×10^6	0.73×10^6	0
PKT 1	0.073	6.53×10^6	2.00×10^6	0.36×10^6
Fybex 1	0.079	8.53×10^6	2.91×10^6	0.73×10^6
Experiment 2				
Fybex 2	0.039	6.72×10^6	2.94×10^6	1.09×10^6
Fybex 3	0.082	36.10×10^6	13.49×10^6	4.00×10^6
Fybex 4	0.371	101.50×10^6	41.76×10^6	18.34×10^6

divided into five equal groups of 34 and exposed to the test compounds, with one group serving as control. The exposure time was 6 hr/day, 5 days/week for 90 days. Actual exposure numbers are shown in Table 3.

Four rats from each group were killed at 20 days of exposure and five animals per species of each group were killed at 50 and 90 days of the exposure period. At the end of the exposure, the remaining animals were placed in holding rooms. Subsequently, animals per species were killed at 6, 12, 18, and 24 months postexposure as shown in Table 3. In addition, some animals which were killed *in extremis* or died during the exposure or postexposure periods were added to the number of animals killed at the regular sacrifice schedule.

Experiment 2. A total of 160 male rats were divided into four equal groups of 40; three groups were exposed to Fybex (fiber length > 5 μm) at exposure concentrations of 2.9×10^6/liter, 13.5×10^6/liter, and 41.8×10^6/liter. One group was used as a control. Dust concentrations according to fiber length are shown in Table 2. Gravimetric concentrations were 0.04 mg/liter, 0.08 mg/liter, and 0.37 mg/liter. Similarly, 139 guinea pigs were divided into four groups. Two groups of 35 guinea pigs were each exposed at the two lower levels, respectively, one group of 34 was exposed at the highest level, and one group of 35 served as control. A total of 146 hamsters were divided into four groups; two groups of 37 were each exposed at the two lower levels, respectively, with one group of 36 at the highest level and one group of 36 serving as control. The exposure time was 6 hr/day, 5 days/week for 3 months. On test Day 35 (25 exposure number), the exposure was interrupted for 1 week because of problems with the exhaust systems in the exposure chambers but was then resumed. The exposure was extended to 95 days (63 exposure number) as opposed to 90 days used in Experiment 1. Since animals at the highest exposure level showed high incidences of weight loss and mortality on 74 days of testing, the exposures were reduced to 4 days/week for the remainder of the exposure period (Table 4).

The animal sacrifice schedule was similar to Experiment 1 except the first animal sacrifice started on exposure Day 50 and animal numbers at various sacrifice schedules are shown in Table 4.

After gross examination, the lungs were filled with Bouin's fixative by intratracheal instillation at low pressure. The paraffin sections were prepared according to routine histologic techniques. The sections were stained by hematoxylin and eosin, modified trichrome, silver impregnation, periodic acid-Schiff (PAS), and Perl's stain.

RESULTS

General Observation

In Experiment 1, the mean body weights for all exposed groups were significantly less than control values during the exposure period. Rats exposed to asbestos had the lowest weights that persisted for 15 weeks after the last exposure. After the end of the exposure, the other test groups had body weights similar to controls. Hamsters exposed to asbestos had a greater mortality than controls.

In Experiment 2, during exposure to various concentrations of Fybex, all species had a dose-related decrease in body weight gain. After the end of the

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TABLE 3

NUMBER OF ANIMALS AT VARIOUS EXPOSURE AND POSTEXPOSURE PERIODS WITH LUNG TUMOR INCIDENCE IN EXPERIMENT 1

Species, test material, group	Exposure concentration (fiber length >5 μm)	Number of animals and lung tumors examined by microscopy								Tumor type
		Exposure number	Exposure: 20 days	Exposure: 50 days	Exposure: 90 days	Postexposure: 6 mo	Postexposure: 12 mo	Postexposure: 18 mo	Postexposure: 24 mo	
Rat										
Fiberglass 1	(0.7 × 10⁹/liter)	61	4	5	5	6	7	8	11(2)	Bronchioloalveolar adenoma (2)
Asbestos 1	(3.1 × 10⁹/liter)	52	4	10	5	6	5	5	11(3)	Bronchioloalveolar adenoma (2) Epidermoid carcinoma (1)
Fybex 1	(2.9 × 10⁹/liter)	64	4	5	5	5	5	7	14(1)	Bronchioloalveolar adenoma (1)
PKT 1	(2.0 × 10⁹/liter)	64	4	5	5	5	7	1	19	
Control 2	Air	0	4	5	5	6	7	6	13	
Guinea Pig										
Fiberglass 1	(0.7 × 10⁹/liter)	61	—	5	5	7	6	1	7(2)	Bronchioloalveolar adenoma (2)
Asbestos 1	(3.1 × 10⁹/liter)	54	—	5	5	9	5	2	5	
Fybex 1	(2.9 × 10⁹/liter)	64	—	5	5	5	6	0	11	
PKT 1	(2.0 × 10⁹/liter)	64	—	5	7	8	5	1	6	
Control 2	Air	0	—	5	5	10	5	1	5	
Hamster										
Fiberglass 1	(0.7 × 10⁹/liter)	61	—	5	5	5	5	1	9	
Asbestos 1	(3.1 × 10⁹/liter)	54	—	5	5	5	8	7	5	
Fybex 1	(2.9 × 10⁹/liter)	64	0	5	5	5	5	4(1)	8	Pleural mesothelioma, epithelial type (1)
PKT 1	(2.0 × 10⁹/liter)	64	—	5	5	6	6	5	8	
Control 2	Air	0	—	5	5	5	5	3	7	

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TABLE 4
NUMBER OF ANIMALS AT VARIOUS EXPOSURE AND POSTEXPOSURE PERIODS WITH LUNG TUMOR INCIDENCE IN EXPERIMENT 2

Species, group, exposure concentration (fiber length >5 μm)	Number of animals and lung tumors examined by microscopy							Tumor type
	Exposure number	Exposure		Postexposure				
		50 day	90 day	6 mo.	12 mo.	18 mo.	24 mo.	
Rat, Fybex								
2 (2.9 × 10^6/liter)	63	5	5	5	3	7	14	
3 (13.5 × 10^6/liter)	63	5	5	5	0	4	21 (3)	Bronchioloalveolar adenoma (2) adenocarcinoma (1)
4 (41.8 × 10^6/liter)	58	10	5	5	0	6	13 (1)	Epidermoid carcinoma (1)
5 Control, air	0	6	5	5	0	14	9	
Guinea pig, Fybex								
2 (2.9 × 10^6/liter)	63	6	5	5	2	1	16	
3 (13.5 × 10^6/liter)	63	5	5	5	2	4	14	
4 (41.8 × 10^6/liter)	58	7	5	6	0	6	10	
5 Control, air	0	5	5	7	2	3	14 (2)	Bronchioloalveolar adenoma (2)
Hamster, Fybex								
2 (2.9 × 10^6/liter)	63	5	5	5	4	12	6	
3 (13.5 × 10^6/liter)	63	5	5	8	6	10 (1)	3	Pleural mesothelioma, biphasic type (1)
4 (41.8 × 10^6/liter)	58	5	5	5	5	12 (1)*	4 (1)	* Pleural mesothelioma, epithelial type (1) bronchioloalveolar adenoma (1)
5 Control, air	0	6	5	6	9	7	3	

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exposure, body weights for all groups were similar to controls. A high mortality rate was observed in the rats and guinea pigs exposed to Fybex at 41.8 × 10^6/liter during the 50-day exposure. Subsequently, rats and guinea pigs showed a dose-related increase in mortality.

Gross Pathology

In Experiment 1, the lungs of some rats exposed to fiberglass revealed numerous miliary gray foci throughout the visceral pleura at 90 days exposure. The gray foci were much less prominent in hamsters than in rats, but none were seen in guinea pigs. The gray foci had disappeared by 6 months postexposure. The hilar and satellite lymph nodes of all animals were swollen and studded with tiny gray foci during the entire recovery period. No gross changes were found in animals exposed to Fybex or PKT. Some rats exposed to asbestos revealed tiny emphysematous plaques which were more prominent when the lungs collapsed after releasing a string tied to the trachea and which persisted throughout the recovery period. The white plaques were not remarkable in the hamsters but were not seen in guinea pigs. The hilar and satellite lymph nodes of animals exposed to asbestos, Fybex, and PKT were swollen and mottled but much less than those of animals exposed to fiberglass. In Experiment 2, the gross findings of animals exposed to Fybex at 41.8 × 10^6/liter were occasional emphysematous plaques in rats, fewer in hamsters, and none in guinea pigs. No remarkable gross findings were observed in the lungs of animals at lower exposure levels.

Histopathology

It was difficult to identify tiny fiberglass dust particles in the macrophages because they had a refractive index similar to the microscopic slide or cover glass and were neither refractive nor polarized. In contrast, Fybex and PKT were readily recognized as dark brown, needle-shaped fibers and birefringent under polarized microscopic examination, while asbestos fibers were pale yellow, transparent, and birefringent. The fiber length of asbestos was markedly longer than Fybex and the shortest fiber was PKT. In Experiment 1, asbestos, Fybex, and PKT produced essentially similar pulmonary reactions but significant differences in magnitude of tissue responses. There were some species differences in the response to inhaled dust particles. In contrast, the pulmonary reactions to fiberglass were different from other test compounds. In Experiment 2, the pulmonary responses of animals exposed to Fybex were dose-related pulmonary responses. A comparison of the pulmonary response to each of the test compounds is shown in Table 5. At 20 days exposure, inhaled dust particles were mostly phagocytized within intraalveolar macrophages and foreign body giant cells in the respiratory bronchiolar region. Most dust cells were free within the alveoli which were lined by hyperplastic alveolar lining cells. The giant-cell reaction was most prominent in asbestos, much less in Fybex, and least prominent in the PKT exposure. Some alveoli adjacent to the terminal bronchioles were lined with ciliated columnar epithelium (bronchiolarization) (Fig. 7). In contrast, fiberglass provoked numerous neutrophils and foamy dust cells but no giant-cell reaction or bronchiolarization. On Day 50 of the exposure, the dust cell, giant cell, and

TABLE 5
SUMMARY OF COMPARATIVE PULMONARY RESPONSES TO INHALED DUST PARTICLES

Pathological lesions	Test material and groups						
	FG	AB	PKT	FB(1)	FB(2)	FB(3)	FB(4)
Dust-laden macrophages	+++	+−+	±	+	+	++	+++
Proliferation, granular pneumocyte	±	+−+	±	±	±	+	++
Bronchiolarization	−	+−+	±	±	±	+	++
Collagenized fibrosis	−	+−+	±*	±	±	+	++
Alveolar proteinosis	±	−	−	−	−	−	−
Ferruginous body	±	+++	±	±	±	+	++
Dust cell accumulation, tracheobronchial lymph node	+++	+	±	±	±	+	++

Note. FG = Fiberglass, AB = asbestos, FB(1) = group 1 (Fybex), FB(2) = group 2 (Fybex), FB(3) = group 3 (Fybex), FB(4) = group 4 (Fybex), PKT = pigmentary potassium titanate. − = No abnormalities detected, ± = very slight change, − = slight change, ++ = moderate change, +++ = marked change, * = negative for guinea pig.

hyperplastic granular pneumocytes increased slightly. Some alveolar air spaces were occluded with dust cells, giant cells, hyperplastic alveolar cells, fibroblasts, and inflammatory cells. The respiratory bronchioles were thickened with dust cells and proliferating fibroblasts. Areas of asbestos accumulation showed a reticulin fiber network with thick wavy collagenous fibers. Obliterative bronchiolitis was observed in rats and hamsters exposed to Fybex at 41.8 × 10^6/liter but not in guinea pigs. Slightly increased dust cell and alveolar cell hyperplasia was found in the animals exposed to fiberglass.

On exposure Day 90, rats and hamsters exposed to fiberglass revealed marked hyperplastic granular pneumocytes and dust-cell reactions which extended to the peripheral alveoli far from the respiratory bronchioles. In contrast, guinea pigs showed no remarkable dust-cell reactions. The dust cells were heavily concentrated in the respiratory bronchiolar region. Some dust cells disintegrated and released granular cellular debris and dust particles. The alveoli were filled with eosinophilic granular material which was PAS positive with diastase resistance (Fig. 8) and stained light green with trichrome stain. The microscopic findings were consistent with the alveolar proteinosis. There was no collagenized fibrosis in the dust-accumulated sites.

In asbestos-exposed rats, the respiratory bronchiolar regions showed patchy thickening due to foreign-body granulomas with marked proliferation of fibroblasts and fibrocytes (Fig. 11). The granulomas contained loosely interwoven collagenous and reticulin fibers. The fibroblast proliferation of granulomas induced by Fybex at 41.8 × 10^6/liter was much less prominent than in asbestos granuloma (Fig. 12). The granulomatous lesions induced by Fybex at various exposure levels were dose related (Figs. 9, 10). The alveolar cell hyperplasia and dust-cell reaction with minute collagen fiber deposition were observed in the respiratory bronchiolar region. The bronchiolarization of alveoli adjacent to the

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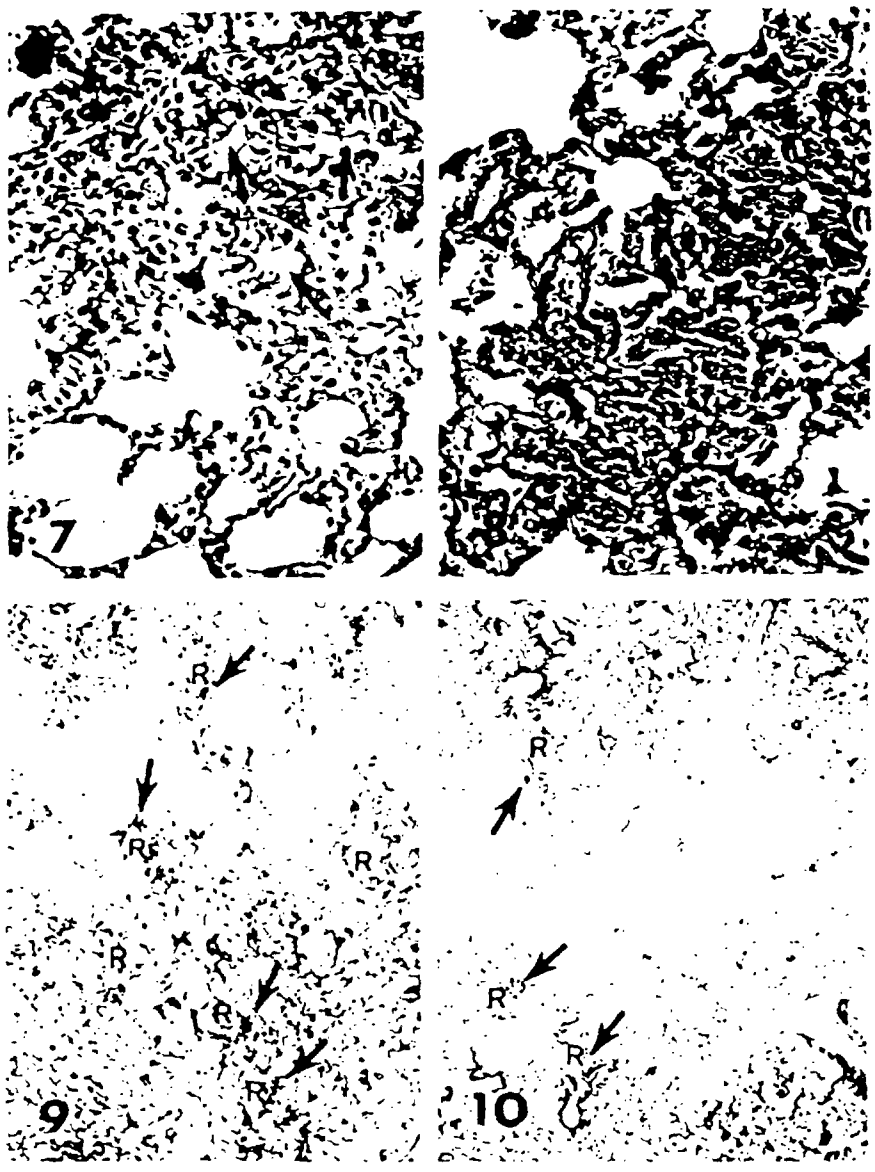

FIG. 7. The alveolar walls (arrows) adjacent to the terminal bronchiole are lined with ciliated columnar epithelium (bronchiolarization). Rat, Fybex (41.8 × 10⁶/liter), 90 days exposure, H&E stain. ×300.

FIG. 8. The alveolar air spaces are filled with PAS-positive granular material. Rat, fiberglass (0.7 × 10⁶/liter), 90-days exposure, PAS method, ×300.

FIG. 9. Low-power micrograph illustrating dense Fybex fiber accumulation (arrows) mainly in the respiratory bronchiolar region (R). Rat, Fybex (41.8 × 10⁶/liter), 90 days exposure, H&E stain, ×30.

FIG. 10. Low power micrograph showing dose-related dust deposition (arrows) mainly in the respiratory bronchiolar region (R). Rat, Fybex (2.9 × 10⁶/liter), 90 days exposure, H&E stain, ×30.

terminal bronchioles was increased (Fig. 7). By the 90-day exposure, there was no significant increase in PKT dust cells when compared with the 50-day inhalation. The pulmonary response was mainly dust cell and alveolar cell hyperplasia and was less than that seen in Fybex exposure at 2.9 × 10^6/liter. Marked species differences were observed among animals exposed to asbestos, Fybex, and PKT at the 90-day exposure. In rats, some dust-laden giant cells in the alveoli were partially or completely integrated into either bronchiolar or adjoining alveolar walls with proliferating fibroblasts and hyperplastic alveolar lining cells (Figs. 11, 12). In contrast, most dust-laden giant cells were free in alveolar spaces and surrounded by hyperplastic alveolar lining cells in the hamsters and guinea pigs (Figs. 13, 14). The hamsters showed marked neutrophil infiltration in comparison with rats or guinea pigs.

By 6 months postexposure, fiberglass-laden dust cells and eosinophilic granular material in the alveoli were markedly reduced in the rats and hamsters, but the granular material was absent in the guinea pigs. There was no collagenized fibrosis in the fiberglass dust-deposited sites. Asbestos or Fybex granulomas revealed a marked reduction in cellularity by the disappearance of fibroblasts and inflammatory cells but increased collagen deposition. Free intraalveolar dust cells were markedly reduced in number, and most hyperplastic granular pneumocytes in the alveoli were replaced by flattened epithelial cells. Some of the alveoli that enclosed the dust particles showed bronchiolarization and were adenomatous in appearance. The respiratory bronchioles and alveolar ducts revealed patchy thickening with dust cells and collagenization. The dust-free alveoli were restored to normal structure. The dust-laden granulomas in the rats reduced cellularity markedly with increased collagen deposition while hamsters and guinea pigs showed still active cellular granulomas with markedly proliferating fibroblasts, inflammatory cells, and hyperplastic alveolar lining cells. Collagen deposition of granuloma was slightly more prominent in hamsters than in guinea pigs but less prominent in rats. PKT induced markedly fewer fibrotic lesions than Fybex at 2.9 × 10^6/liter. PKT exposure induced minute collagenized fibrosis in rats and occasionally in hamsters but not in guinea pigs.

At 1 year postexposure, foamy fiberglass-laden macrophages were tightly packed within the alveoli that were lined with hyperplastic granular pneumocytes. Dust cells were sharply localized in the respiratory bronchiolar region. The eosinophilic granular material had disappeared from all exposed animals. There were no remarkable differences in the pulmonary responses to asbestos, Fybex, and PKT when compared with those seen at 6 mo postexposure. The cellularity of the granulomas and the number of dust cells decreased slightly while collagen deposition became slightly more prominent.

By 2 years postexposure, most fiberglass-laden dust cells were eliminated from the alveoli and some were sharply aggregated in the alveoli showing hyperplastic granular pneumocytes adjacent to the respiratory bronchioles and alveolar ducts. Negligible collagenous deposition was found occasionally in the interstitium where dust cells were trapped. The asbestos- or Fybex-laden granulomas in rats were replaced with collagenized fibrotic lesions in the respiratory bronchiolar region (Figs. 15, 16, 17). The collagenized fibrosis (Fig. 16, 19) of Fybex at 41.8 × 10^6/liter was slightly less than that of asbestos (Figs. 15, 18). The Fybex-laden

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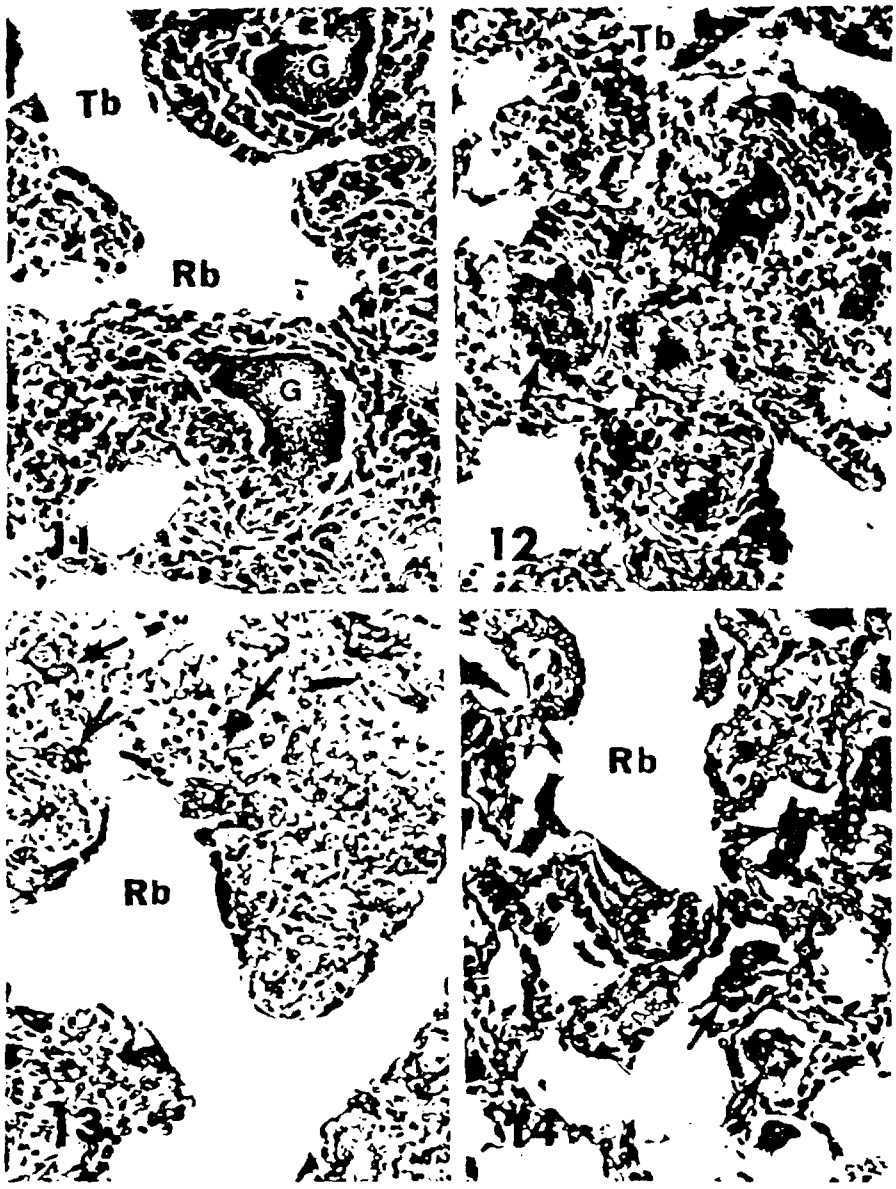

FIG. 11. Asbestos-laden giant cells (G) are surrounded by markedly proliferating fibroblasts and inflammatory cells, mainly lymphocytes in the respiratory bronchiolar region (Rb). Terminal bronchiole (Tb), rat, asbestos (3.1×10^6/liter), 90 days exposure, H&E stain, ×300.

FIG. 12. Fybex-laden giant cells (G) are enclosed by mostly proliferating alveolar lining cells and inflammatory cells. Note fibroblast proliferation is not as remarkable as that shown in Fig. 11. Dust cell aggregates (arrow) in the alveolar air space are surrounded by hyperplastic alveolar lining cells and obliterate the air spaces. Terminal bronchiole (Tb), rat, Fybex (41.8×10^6/liter), 90 days exposure, H&E stain, ×300.

collagenized fibrosis was dose related. No distinct collagenized fibrosis was found in hamsters or guinea pigs exposed to PKT, but rats showed some occasional slight collagenized fibrosis in the respiratory bronchiolar region. In asbestos- or Fybex-exposed guinea pigs, most dust-laden giant cells were free in the alveolar air spaces and were surrounded by a few inflammatory cells, proliferating fibroblasts, and hyperplastic granular pneumocytes (Fig. 20). Hamsters (Fig. 21) showed tissue responses similar to those of guinea pigs. Collagenized fibrosis in the respiratory bronchiolar region was slightly more marked in hamsters than in guinea pigs but less than in rats (Fig. 19).

In fiberglass exposures, ferruginous bodies were first detected by the 6th month postexposure in hamsters and by the 12th month postexposure in guinea pigs. The number of ferruginous bodies increased markedly after 12 months postexposure. Ferruginous bodies from asbestos had already developed at 50 days exposure in hamsters and had increased markedly after 90 days exposure in guinea pigs. Both Fybex and PKT formed ferruginous bodies at 90 days exposure. The number of ferruginous bodies in the Fybex group was dose related, though they were much less numerous than those in the asbestos group. No typical ferruginous bodies were found in rats exposed to any test compounds.

The dust particles were already apparent in the tracheobronchial lymph nodes by 50 days exposure and increased gradually thereafter in all animals. Most particles were less than 2 μm and fiberglass dust deposition was most prominent of all test compounds. Fibrosis was observed in dust-deposited areas of the lymph nodes.

The incidence of lung tumors and pleural mesothelioma in animals exposed to the test compounds is shown in Tables 3 and 4. In Experiment 1, after 18 and 24 months postexposure, 2 of 19 rats exposed to fiberglass developed bronchioloalveolar adenomas. Three of 16 rats exposed to asbestos showed lung tumors. Two of three had bronchioloalveolar adenomas and one had epidermoid carcinoma. One of 21 rats exposed to Fybex developed bronchioloalveolar adenoma. None of the 20 rats exposed to PKT nor any of the 19 control rats had any primary lung tumors. One of 12 hamsters exposed to Fybex in Experiment 1 developed an epithelial type of pleural mesothelioma. No lung tumor was found in the other groups. The mesothelioma nodules were scattered throughout the pleural surfaces of the lungs, thoracic wall, and diaphragm. The tumor nodules consisted of a central core of collagenized connective tissue surrounded by layers of hyperplastic or pleomorphic mesothelial cells. Dust-laden macrophages and inflammatory cells infiltrated the tumor nodules.

In Experiment 2, after 18 and 24 months postexposure to Fybex, 3 of 25 rats in group 3 (13.5 × 10^6/liter) had lung tumors. Two of three were bronchioloalveolar adenomas and the other was an adenocarcinoma. One of 19 rats in group 4 (41.8 ×

FIG. 13 Alveolar air spaces adjoining the respiratory bronchiole (Rb) are obliterated by Fybex-laden cells (arrows), hyperplastic alveolar lining cells, and inflammatory cells, mostly neutrophils. Note absence of proliferating fibroblasts at dust-deposited sites. Hamster, Fybex (41.8 × 10^6/liter), 90 days exposure, H&E stain, ×300.

FIG. 14. Alveolar air spaces adjacent to the respiratory bronchiole (Rb) contain free dust cells (arrows) and the alveolar walls are lined with proliferating alveolar lining cells. Fibroblast proliferation is not remarkable. Guinea pig, Fybex (41.8 × 10^6/liter), 90 days exposure, H&E stain, ×300.

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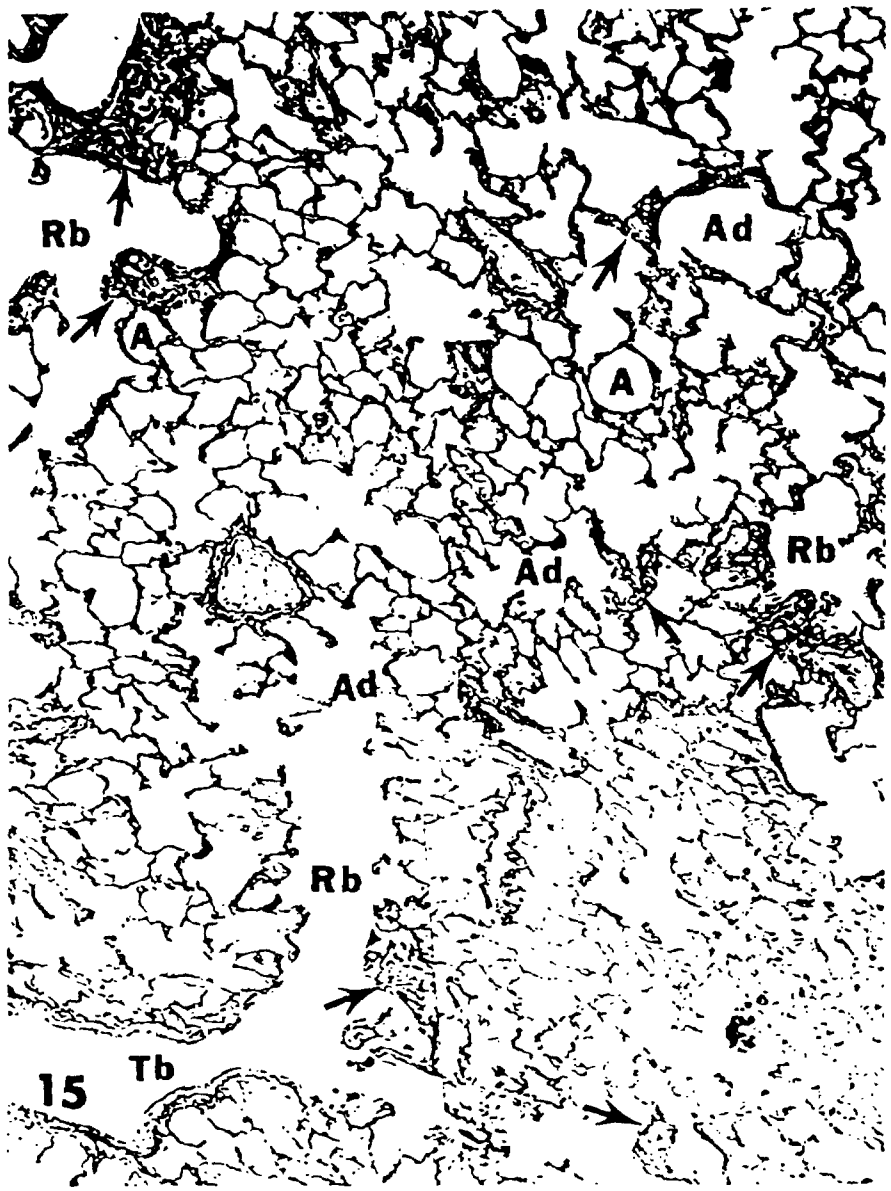


FIG. 15. Montage of low-power micrograph illustrating an overview of collagenized fibrosis (arrows) mainly in the respiratory bronchioles (Rb), alveolar ducts (Ad), and adjoining alveoli (A). Terminal bronchiole (Tb), rat, asbestos (3.1×10^6/liter), 2 years postexposure, silver impregnation technique, ×100.

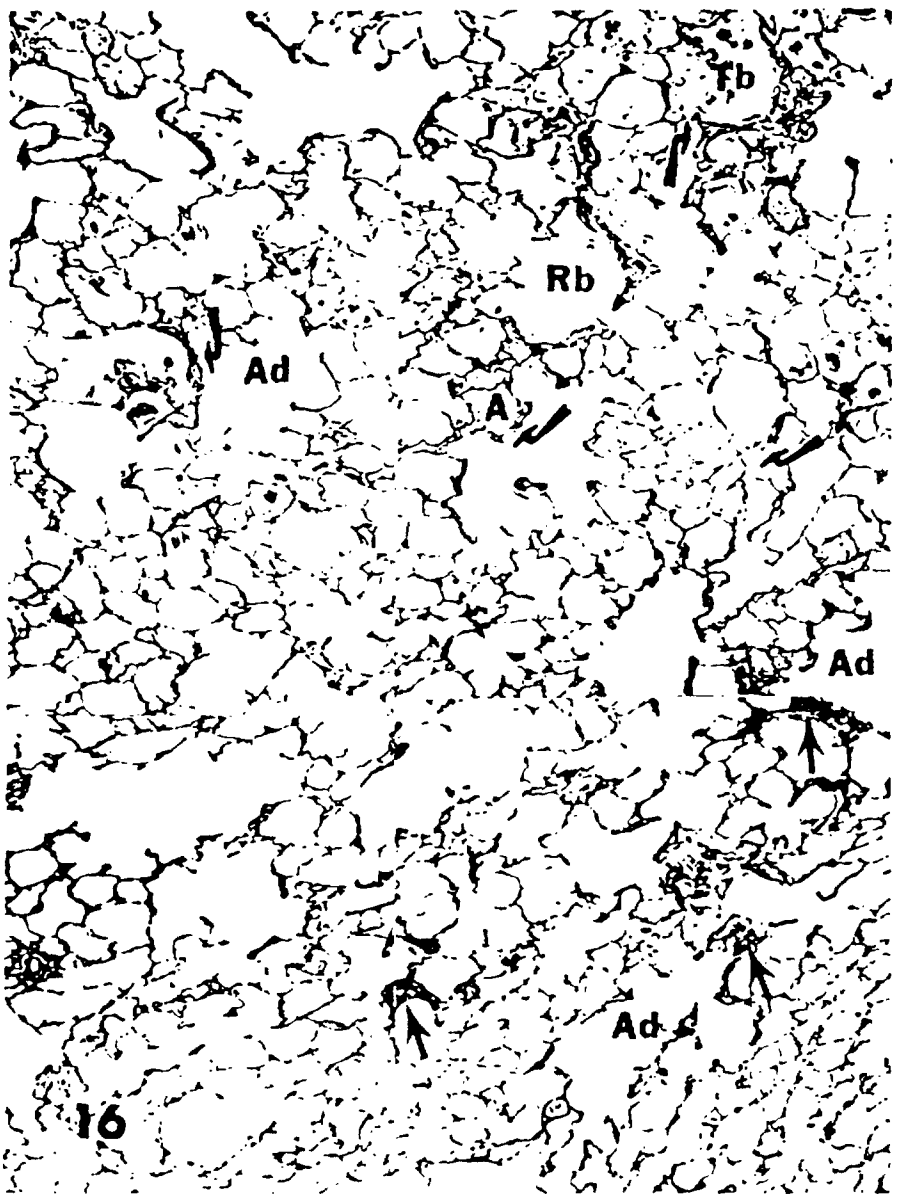


FIG. 16. Montage of low-power micrograph showing an overview of collagenized fibrosis (bent arrows) mainly in the respiratory bronchiole (Rb), alveolar ducts (Ad), and alveoli (A). Note collagenization is not as remarkable as that shown in Fig. 15. Dust-accumulated areas (arrows) show dark staining due to pigmented fibers and resemble collagenization. Terminal bronchiole (Tb), rat, Fybex (41.8×10^6/liter), 2 years postexposure, silver impregnation technique, ×100.

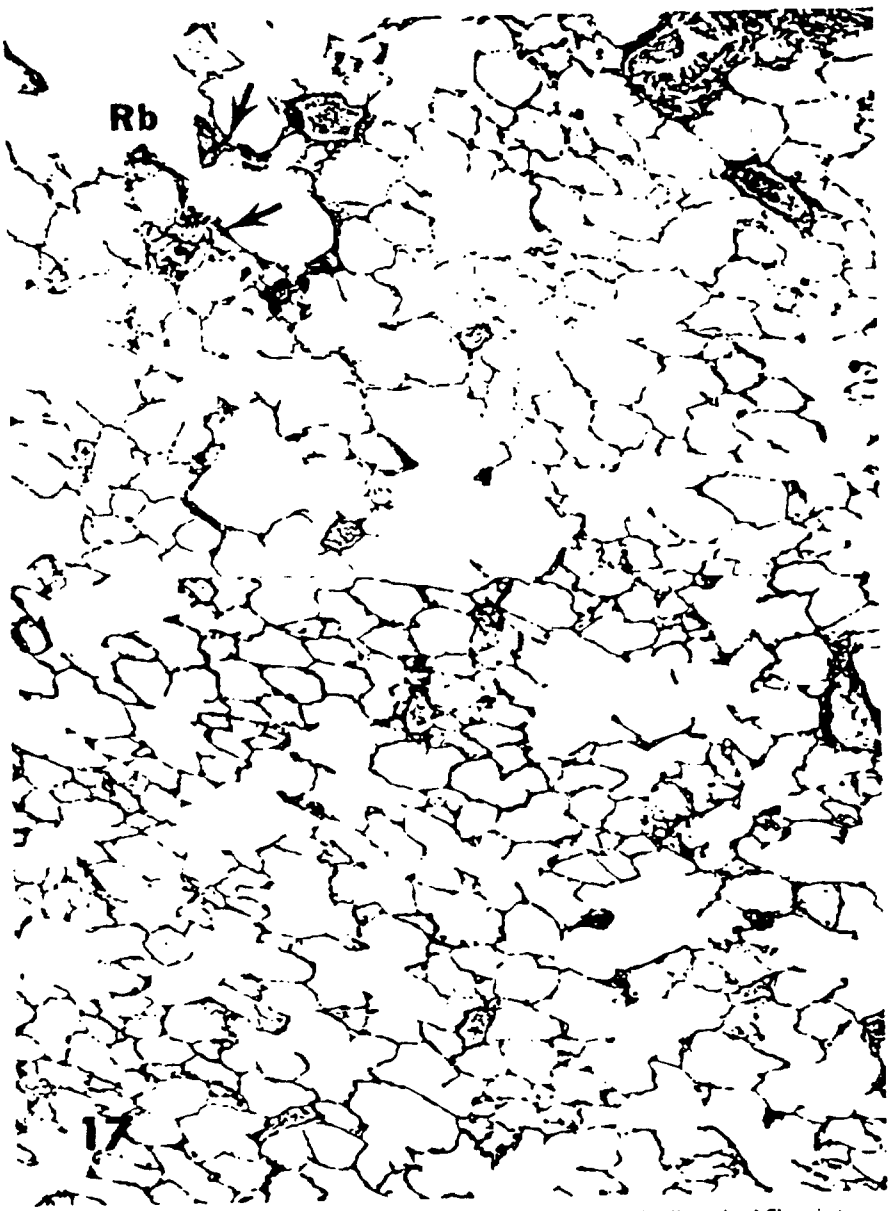


FIG. 17. Montage of low-power micrograph presenting an overview of collagenized fibrosis (arrow) in the respiratory bronchiolar (Rb), region. Note minute and dose-related collagenization in comparison to that shown in Fig. 16. Rat, Fybex (2.9×10^6/liter), 2 years postexposure, silver impregnation technique, ×100.

10^6/liter) had an epidermoid carcinoma. No lung tumors were found in either control or group 2 (2.9×10^6/liter). Two of 17 guinea pigs in the control group revealed bronchioloalveolar adenomas, but there were no lung tumors in the Fybex-exposed groups. One of 13 hamsters in group 3 developed a biphasic pleural mesothelioma consisting of mesenchymal and epithelial cells. Of 16 hamsters in group 4, 1 exhibited a pleural mesothelioma (Figs. 22, 23, 24, 25) and 1 had a bronchioloalveolar adenoma. No lung tumors were found in either the control or group-2 hamsters. The pleural mesotheliomas spread throughout the thoracic wall, pericardium, diaphragm, and mediastinal adipose tissue. One case of mesothelioma showed a metastasis in the mediastinal lymph nodes. The mesotheliomas were infiltrated with dust-laden macrophages. Fybex-exposed hamsters showed a high incidence of fibrotic pleuritis with dust cells and variable mesothelial hyperplasia when compared to rats or guinea pigs (Table 6).

DISCUSSION

Although considerable understanding of the biological effects of asbestos has been achieved during the last four decades, the basic mechanisms of pulmonary fibrosis and carcinogenesis is still an enigma. The biological effects of asbestos are thought to be related not only to physical properties (Stanton and Wrench, 1972) but also to trace metals (Cralley and Lainhart, 1973), polycyclic aromatic hydrocarbons (Shabad *et al.*, 1974), and other carcinogenic substances (Dixon *et al.*, 1970) adsorbed on the asbestos fibers during mining, handling, and milling. The carcinogenicity of asbestos is markedly enhanced by cofactors such as smoking (Selikoff *et al.*, 970).

Recently, however, the importance of the physical dimensions of fibrous dust has been widely acknowledged for induction of pulmonary fibrosis and lung tumors. Asbestos fibers with an average length of less than 5 μm are devoid of fibrogenic potential (Webster, 1970) as well as carcinogenic potential (Smith *et al.*, 1972; Maroudas *et al.*, 1973). In contrast, long asbestos fibers are not only carcinogenic but also fibrogenic in experimental asbestosis. Similarly, fiberglass and other man-made fibers revealed size-related carcinogenic potential (Stanton and Wrench, 1972; Stanton *et al.*, 1977; Davis, 1976; Pott *et al.*, 1976) and fibrogenic potential (Kuschner and Wright, 1976). Fibers less than 1.5 μm in diameter and greater than 8 μm in length produced the highest carcinogenic potential and fibers less than 5 μm in length provoked mainly a macrophage reaction (Stanton *et al.*, 1977; Pott *et al.*, 1976).

There was no marked difference between the length of asbestos and Fybex based on the fiber length analysis of individual fibers (Table 1). However, the asbestos fibers were significantly longer than Fybex fibers in the tissue sections and often reached more than 100 μm. Asbestos fibers also showed marked variations in length and diameter in comparison to those of Fybex or PKT. The long asbestos fibers produced prominent giant-cell reaction and severe pulmonary fibrosis. Fractured asbestos fibers exhibited polyfilamentous structure, longitudinal splitting, and adhesion of fine split fibers. The split fibers were not completely separated from the thick fibers and therefore extended the overall fiber length by dislocation or adhesion (Fig. 2). The persistence of numerous long fibers in the

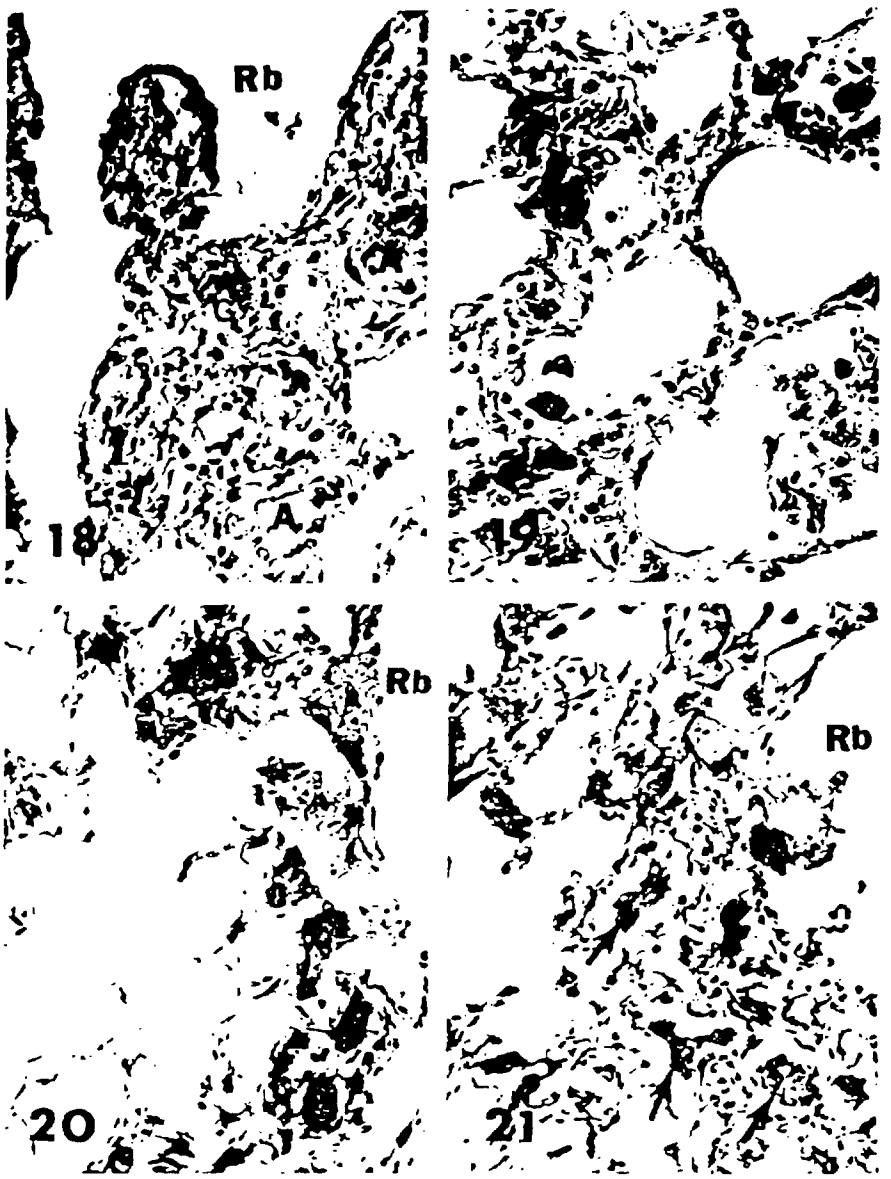


FIG. 18. The respiratory bronchiole (Rb), and adjacent alveoli (A), show prominent collagenization. Dust-laden giant cells (G) and macrophages are embedded in the collagen with scanty fibrocytes. Note markedly decreased cellularity in the collagenized area in comparison to that of the 90-day exposure (Fig. 11). Rat, asbestos (3.1×10^6/liter), 2 years postexposure. H&E stain, ×300.

FIG. 19. The collagenization of alveolar walls is not as prominent as that shown in Fig. 18. Note markedly reduced cellularity of collagenized area in comparison to that of the 90-day exposure shown in Fig. 12. Rat, Fybex (41.8×10^6/liter), 2 years postexposure. H&E stain, ×300.

lung tissue seems to be related with the unique polyfilamentous structure, longitudinal splitting, and adhesion. In contrast, Fybex and PKT fibers showed monofilamentous structure and transverse fracture with reduction of the fiber length when the fibers were broken (Figs. 3, 4).

In our experiments, asbestos was the most potent fibrogenic fiber. In terms of collagenized fibrosis at 2 years postexposure, the fibrogenicity of asbestos at 3.1 $\times$ 10^6/liter was slightly more severe than that of Fybex at 41.8 $\times$ 10^6/liter. PKT was the least fibrogenic and only minimal fibrosis was observed in rats and hamsters but not in guinea pigs. Large asbestos granuloma contained longer fibers (up to 100 μm) whereas Fybex granulomas were smaller and contained shorter fibers than asbestos granuloma. Short PKT fibers mainly produced a macrophage reaction with minimal giant-cell granuloma. Fine fiberglass dust particles provoked only a macrophage reaction and no giant-cell response.

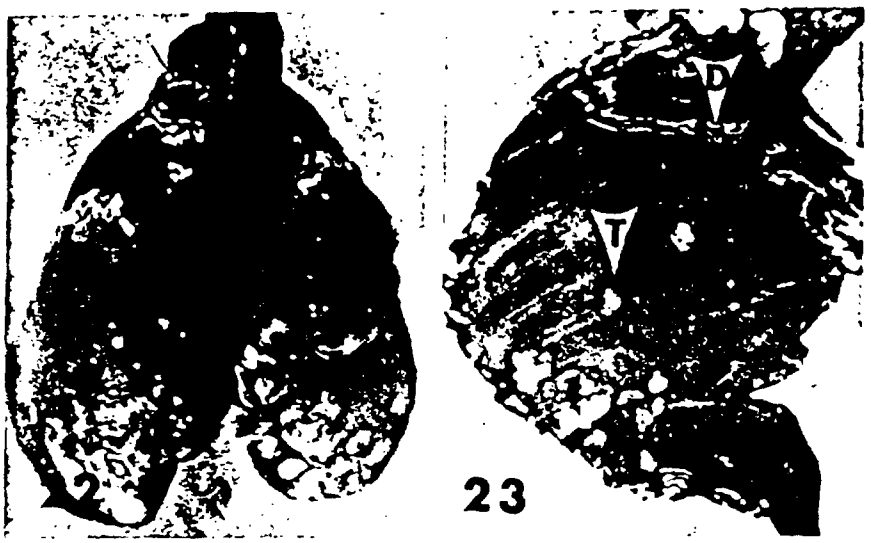
The exposure concentration of Fybex at 41.8 $\times$ 10^6/liter was so high that the lungs were literally loaded with dust, especially in the respiratory bronchiolar regions, causing obliterative bronchiolitis and high mortality in rats and hamsters but not in guinea pigs during the 50-day exposure. On the ba.. s of the microscopic findings and high mortality, this dust concentration was unrealistically high and overloaded the lung-clearing mechanisms.

Although the gravimetric concentrations of asbestos and Fybex (group 4) were similar, 0.3 and 0.37 mg/liter, respectively (Table 2), the total number of Fybex fibers per liter of air was 7.7 times that of asbestos, containing 13.5 times more fibers longer than 5 μm and 16.8 times more fibers longer than 10 μm. The weight of an average-length (6 μm) asbestos fiber was approximately 15 $\times$ 10^{-12} g while the weight of an average-length (10 μm) Fybex fiber was 0.9 $\times$ 10^{-12} g. Theoretically, at least 10–15 times more Fybex fibers would be collected from air containing equal weights of asbestos and Fybex fibers. Based on the severity of the pulmonary fibrosis observed at various exposure concentrations, asbestos fibers appeared to be at least 10 times more fibrogenic than Fybex fibers.

Kuschner and Wright (1976) reported that the giant-cell reaction and pulmonary fibrosis developed when the guinea pigs were instilled with longer glass fibers (50% of fibers >10 μm in length) with a thin diameter. Short fibers (<5 μm in length and <1 μm in diameter) produced only a dust-cell reaction but no fibrosis. In our experiments, animals exposed to fine fiberglass particles (mostly <2 μm) at 420 mg/m^3 revealed a macrophage reaction with hyperplastic granular pneumocytes and slight alveolar proteinosis which appeared at 90 days exposure but disappeared by 1 year postexposure. Massive dust-cell reactions with subsequent disintegration and releasing of dust particles, ingested surfactant, and cellular

FIG. 20 The alveolar collagenization of the guinea pig is similar to that of the hamster (Fig. 21) but much less than that of the rat (Fig. 19). Guinea pig, Fybex (41.8 $\times$ 10^6/liter), 2 years post-exposure, H&E stain, $\times$300.

FIG. 21 The alveolar collagenization of the hamster is not as remarkable as that shown in the rat in Fig 19. The alveolar air spaces are patent, but the alveolar walls are thickened due to slight collagen deposition. Note most dust cells (arrows) are free in the alveolar air spaces and reduced cellularity in comparison to the 90-day exposure (Fig 13). Respiratory bronchiole (Rb), hamster, Fybex (41.8 $\times$ 10^6/liter), 2 years postexposure, H&E stain, $\times$300.



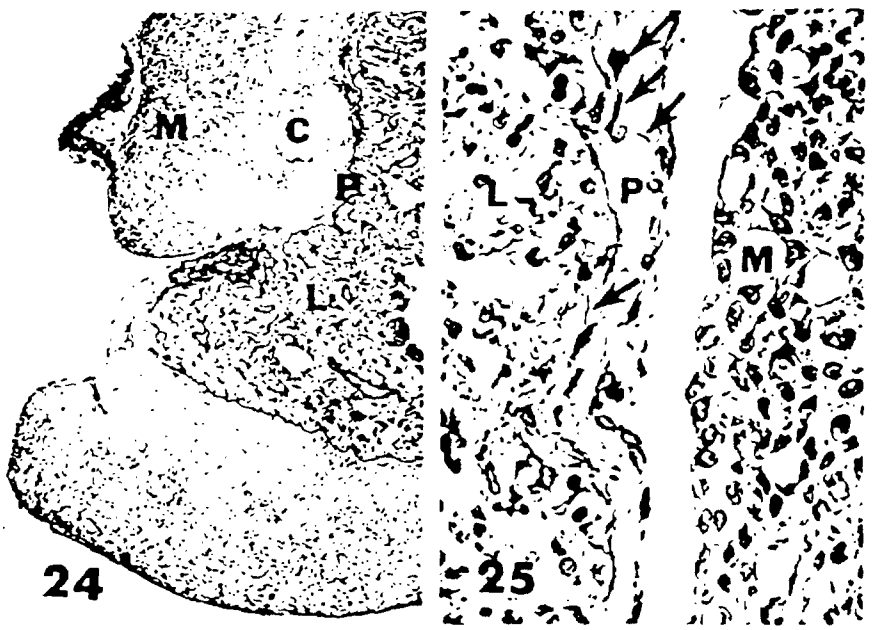


FIG. 22. White glistening tumor nodules (arrows) are scattered throughout the visceral pleura of the lung. Hamster, Fybex (41.8 × 10⁶/liter), 18 months postexposure.

FIG. 23. Same hamster shown in Fig. 22. Well-circumscribed white glistening tumor nodules (arrows) are distributed throughout the parietal pleura of the thoracic wall (T) and diaphragm (D).

FIG. 24. Same hamster shown in Fig. 22. The pleural surface (P) is covered with tumor nodules showing collagenized connective tissue (C) adjacent to the pleura and neoplastic mesothelial cells (M). Note collapsed lung parenchyma (L) is free of tumor invasion. Hamster, Fybex (41.8 × 10⁶/liter), 18 months postexposure, trichrome stain, ×30

crocidolite followed by amosite, chrysotile, and anthophyllite (McDonald and McDonald, 1977). Since amosite and crocidolite are similar in chemical composition, the physical characteristics of crocidolite appeared to be associated with a high incidence of pleural mesothelioma (NIOSH, 1976).

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TABLE 6
INCIDENCE OF FIBROTIC PLEURITIS IN ANIMALS EXPOSED TO FYBEX AT 18 MONTHS POSTEXPOSURE

Group, exposure concentration	Rat	Hamster	Guinea pig
Control	0/23	0/10	0/17
2 (2.9 × 10^6/liter)	1/21	0/18	1/17
3 (13.5 × 10^6/liter)	0/25	4/13	2/18
4 (41.8 × 10^6/liter)	3/19	8/16	2/16

debris into the air spaces appeared to play an important role in inducing alveolar proteinosis (Lee *et al.*, 1979). At 2 years postexposure, our experimental animals exposed to fine fiberglass particles satisfied the criterion of a biologically inert dust (Gross *et al.*, 1970).

Of the 10 lung tumors in rats exposed to fiberglass, asbestos, and Fybex at 2 years postexposure, 7 were bronchioloalveolar adenomas, 2 were epidermoid carcinomas, and 1 was an adenocarcinoma (Tables 3, 4). The incidence of lung tumors in Charles River C-D Sprague–Dawley-derived rats used as controls in our laboratory over the 10 years (1962–1972) is 4 bronchioloalveolar adenomas and 2 adenocarcinomas in 365 male rats, and only 1 bronchioloalveolar adenoma in 365 female rats. The number of animals exposed to the test compounds at the 2-year postexposure sacrifice was too small to draw any meaningful conclusions for carcinogenic potential (Tables 3, 4). In the guinea pigs exposed to fiberglass at 24 months postexposure, 2 out of 7 developed bronchioloalveolar adenoma while no lung tumors were found in 5 control animals in Experiment 1 (Table 3). Two of 14 control guinea pigs, however, showed bronchioloalveolar adenoma at 24 months postexposure in Experiment 2 (Table 4).

It is noteworthy that intrapleural injections of asbestos or fibrous dust particles produced mostly fibrosarcomatous mesothelioma (Stanton and Wrench, 1972; Stanton *et al.*, 1977; and Reeves *et al.*, 1971). Neither fibrosarcomatous nor adenomatous features were found in our experimental hamsters exposed to Fybex. Two cases of mesothelioma were the epithelial type and there was one case of biphasic mesothelioma consisting of mesenchymal and epithelial cells. There were no other primary tumors in other organs which could cause a serosal spread in all three cases of mesotheliomas. Considering the fact that the natural incidence of mesothelioma in hamsters is relatively rare and considering the high incidence of fibrotic pleuritis with hyperplastic mesothelium and dust-laden macrophage infiltration (Table 6), the possibility that the mesothelioma is related to the Fybex exposure cannot be ruled out. It is well known that a chronic pleurisy of both the visceral and parietal pleura was common in asbestos patients (Sluis-Cremer and Webster, 1972). The different mineral types of asbestos fiber have different mesothelioma-producing potential. The one with the most potential was

FIG. 25. Note migrated Fybex fibers (arrows) in the collagenized pleura (P) with hyperplastic mesothelial cells. Lung parenchyma (L), mesothelioma (M), hamster, Fybex (41.8 × 10^6/liter), 18 months postexposure. H&E stain, ×500.

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