ENVIRONMENTAL RESEARCH 8, 178–202 (1974)

Inhalation Carcinogenesis from Various Forms of Asbestos[1]

ANDREW L. REEVES, HENRY E. PURO, AND RALPH G. SMITH[2]

Department of Occupational and Environmental Health, School of Medicine, Wayne State University, Detroit, Michigan 48201

Received November 23, 1973

Rats, rabbits, guinea pigs, gerbils, and mice were exposed to the inhalation of chrysotile, crocidolite, or amosite for 2 years. Mean atmospheric concentrations were 47.9–50.2 mg/m³, but only 0.08–1.82% of the dusts retained fibrous morphology during the dissemination procedure which involved hammer milling. Trace contamination especially by chromium and nickel was also increased. Light microscopic fiber counts per ml chamber air were 54 (chrysotile), 1105 (crocidolite), and 861 (amosite). A fibrogenic response to these dusts was observed in all five animal species, the severity corresponding to the extent of exposure (with reaction to chrysotile frequently very slight). Gerbils developed frequent alveolar proteinosis. Mice developed spontaneous papillary carcinomas in the lungs. Disregarding the latter species, carcinogenic response to asbestos inhalation was restricted to rats and occurred in all three exposure groups. There were 2 lung cancers and 1 pleural mesothelioma after chrysotile inhalation; 4 lung cancers after crocidolite inhalation; and 1 lung cancer and 2 pleural mesotheliomas after amosite inhalation. These cases constituted 7–9% incidence of malignancy among rats with adequate survival record. Hypotheses of asbestos carcinogenesis are reviewed and it is suggested that different etiologic principles may be involved in the causation of lung cancer and of pleural mesothelioma.

The carcinogenic potential of asbestos in the environment has become one of the leading public health concerns of our time. The hazards of asbestos exposure were well demonstrated recently in miners and mill workers by McDonald *et al.* (1971), in pipe insulation workers by Selikoff *et al.* (1970), in shipyard workers by Harries *et al.* (1972), in coke oven operators by Selikoff and Hammond (1971), in cigarette filter manufacturers by Goff and Gaensler (1972), and in automobile brake repairmen by Hickish and Knight (1970). Asbestos hazard was also considered to originate from battery boxes (Greenberg, 1970), protective clothing (Bamber and Butterworth, 1970), and certain types of soils during agricultural work (Burilkov and Michailova, 1970). Nonoccupational and nonrespiratory exposure to potentially significant quantities of asbestos was postulated for consumers of talc-coated rice in view of the tremolite asbestos in the talc (Merliss, 1971); various bottled beverages including beer (Cunningham and Pontefract, 1971); and parenteral drugs (Nicholson *et al.*, 1972). In the two latter cases, the asbestos content of the fluids was believed to be contributed from the filters during filtration, and it was speculated that migration of the fibers from the gastrointestinal tract to mesothelial tissue might occur in a similar way

[1] Supported by Grant No. 5-RO-1-EC 00240 from the U. S. Public Health Service.

[2] Present address: School of Public Health, University of Michigan, Ann Arbor, Mich. 48104.

as from subcutaneous injection sites (Westlake *et al.*, 1965; Kanazawa *et al.*, 1970; Pontefract and Cunningham, 1973).

The presence of asbestos fibers in urban air is now well established (Selikoff *et al.*, 1972b), although whether or not the ambient concentration is high enough to constitute a hazard is uncertain (Rickards and Badami, 1971). In the lung of city dwellers, both uncoated asbestos (Langer *et al.*, 1971), and ferruginous bodies (Gross *et al.*, 1969) are common findings, although the central core of the latter is not always asbestos (Gross *et al.*, 1970). Much of the atmospheric asbestos in the cities probably originates from building demolition and weathering. The significance of vehicle brake emissions is uncertain, since at the temperature of brake application part or all of asbestos undergoes crystallographic transformation into nonfibrous pyroxenes and forsterite (Speil and Leineweber, 1969). However, the degree of completion of this transformation during actual vehicle operation is not known, nor has the chronic inhalation toxicity of the heat decomposition products of asbestos been thus far adequately investigated.

Malignant neoplasia now recognized to result from the inhalation of asbestos fibers includes cancer of the lung, and mesothelioma of the pleura and peritoneum (Wagner *et al.*, 1971). Also suspected are links to cancers of the gastrointestinal tract (Selikoff *et al.*, 1967), hematopoietic system (Gerber, 1970) and female reproductive system (Graham and Graham, 1967). Some of the former lesions were reproduced experimentally by several workers during the past decade. Pleural and peritoneal mesotheliomas were readily produced through local implantation of asbestos fibers into various laboratory animals by Wagner (1962), Smith *et al.* (1965), Stanton *et al.* (1969), Wagner and Berry (1969), Donna (1970), and Reeves *et al.* (1971); a few lung cancers were also produced in rats by inhalation exposure to chrysotile by Gross *et al.* (1967) or to crocidolite by Reeves *et al.* (1971). In this paper, we report on the continued production of various neoplasms in the rat after inhalation of chrysotile and crocidolite; and on the production of papillary carcinoma of the lung and fibrous or fibrosarcomatous mesothelioma of the pleura in the rat after inhalation of amosite.

MATERIALS AND METHODS

1. Dust Characterization

Crude samples of amosite, crocidolite, and chrysotile[3] were ballmilled in glazed ceramic jars with stainless-steel balls for 10 days, and the resultant dusts were forced through a #8 mesh screen for the distintegration of larger clumps. These specimens were fed into the hopper of hammer mills and blown into the exposure chambers as described previously (Reeves *et al.*, 1971).

The milling procedure caused not only substantial reduction of particle size which was intended, but also a loss of fibrous structure in a great proportion of particles which was not intended. By using an axis–diameter ratio of 3:1 or more to define a fiber, we found that only 0.08–1.82% of the dust as collected on Millipore filters in the chambers answered this definition during light microscopic

[3] Amosite, W-3 fibers; crocidolite, 5-blue fibers; and chrysotile, 3-T fibers. These samples were obtained by courtesy of the Johns-Manville Corporation.

examination. The upper limit of fiber length observable under the light microscope was 55–130 μm (comprising 0.001–0.015% of the dust) in various samples, and the fiber count per ml air was 864 (amosite), 1105 (crocidolite), or 54 (chrysotile). It is thus apparent that the great majority of light-microscopically visible fibers were destroyed or transformed into submicronic fibrils or apparently nonfibrous crystals. Similar results were observed using X-ray crystallographic technique after a grinding procedure involving cutting action by Occella and Maddalon (1963). It is well known that chrysotile is changed into forsterite at 810°C, and the amphiboles into pyroxenes at 900°C. Whether or not such high impact temperatures might have occurred during ball milling is not known. If they have, this might be a plausible explanation for the loss of fibrous structure in 98–99% of the particles in the light microscopic range.

However, it should be emphasized that even with this degree of destruction of fibrous structure, the animals still inhaled substantial quantities of apparently unchanged asbestos. It can be estimated that atmospheric concentration of the fibrous component alone in the chamber was 40–900 γ/m^3, and the fiber counts as reported above were 11–220 times higher than the current threshold limit value for asbestos as established by the American Conference of Governmental Industrial Hygienists (Stokinger *et al.*, 1973). Moreover, a similar destruction of fibrous structure was not observed in the electron microscopic range; under the electron microscope it appeared that the majority of particles were fibrous and average length and diameter of dust samples collected in the chambers were as follows: amosite, 3–5 and 0.2–0.5 μm; crocidolite, 3–6 and 0.4–0.5 μm; and chrysotile, 6–15 and 0.2 μm.

X-ray diffraction line positions of asbestos samples agreed well with those reported in the literature (Timbrell, 1970). The loss of line intensity (measured as difference in peak heights on samples obtained before and after the dissemination procedure) was computed as percent of the UICC reference value. The results (Table 1) show about 70% loss of diffractivity for amosite, and about 35% for crocidolite and chrysotile. These values are uncorrected for the effect of particle size diminution and they indicate the extent of loss of crystallinity only in an approximate manner.

Another artefact introduced during dust preparation was heavy metal contamination. The ballmilling procedure was accomplished with stainless steel balls of ½–2 in. diameter, having the following trace element composition:[4] Cr 1.3–1.6%; C 0.95–1.1%; Si 0.2–0.35%; Mn 0.25–0.45%; Ni $< 0.35\%$; Cu $< 0.25\%$; Mo $< 0.08\%$; P $< 0.025\%$; S $< 0.025\%$.

It is apparent that during the prolonged contact of the balls with the asbestos samples there was potential hazard of trace element adsorption.

Analytical–chemical studies were conducted using atomic absorption spectrophotometry in order to compare raw and processed asbestos samples and to relate each to UICC standard reference samples (Roy-Chowdhury *et al.*, 1973). About 1 g specimens of each dust were digested with the minimum amount of

[4] According to analysis of the manufacturer (Atlas Ball Division of SKF Industries, Philadelphia, Pa.)

TABLE 1
X-RAY DIFFRACTION DIAGRAM OF ASBESTOS DUSTS

Sample	X-ray line (degrees)[a]	UICC[b] (units)	J Mc crude (units)	J Mc crude (% of UICC[b])	J Mc disseminated (units)	J Mc disseminated (% of UICC[b])	% loss of peak ht during grinding	
Amosite	10.7–10.9	78	78	100	30	38	62	av
	27.3–27.7	13	13	100	4	31	69	70%
	29.1–29.4	39	41	105	10	26	77	
	32.3–32.8	8	10	125	3	38	70	
Crocidolite	10.5–10.8	67	65	97	33	50	50	av
	19.7–19.9	9	9	100	5	55	44	35%
	26.0–26.7	35	14	40	18	52	---	
	28.7–29.0	19	20	105	9	48	55	
	32.9–33.2	14	14	100	12	86	14	
	34.4–34.7	5	5	100	3	60	40	
	35.3–35.7	8	7	88	6	75	14	
Chrysotile	12.0–12.4	55	60	111	41	74	32	av
	18.7–19.8	10	10	100	6	60	40	35%
	24.3–24.7	32	40	125	16	50	60	
	60.2–60.7	6	6	100	5	83	17	

[a] In Cu$K\alpha$-radiation, $\lambda = 1.54$ Å.
[b] Union Internationale Contre le Cancer reference specimens.
[c] Johns-Manville Corporation commercial specimens.

HF in Pt crucibles and slowly evaporated to dryness. The ash was twice digested with concentrated HNO_3 and then dissolved in 1 N HCl. The elements Co, Ni, Cr, Mn, and Fe were determined using the 2407, 2302, 3579, 4030, and 3470 Å resonance lines, respectively. Iron was also determined spectrophotometrically as hydroxylamine–HCl and *o*-phenanthroline complex. The results are summarized in Table 2.

It appears that with UICC samples, our results agreed well with those of Timbrell *et al.* (1968; 1970), and in most cases there was no great discrepancy between these values vs those obtained on crude commercial samples. JM-3T chrysotile resembled UICC-B chrysotile, although its nickel concentration was appreciably lower.

Substantial contamination by chromium was introduced during grinding into amosite and crocidolite, and by nickel into crocidolite, the factors being 3×, 9×, and 5×, respectively. The other values appear to be within or close to experimental error limits.

2. *Chamber Maintenance and Monitoring*

Three "walk-in chambers" (12 × 12 ft rooms with perforated walls) were utilized in this experiment, one each for the dissemination of amosite, crocidolite, and chrysotile. These were described in more detail previously (Reeves *et al.*, 1971). The dusts were blown into these chambers through a network of ducts

TABLE 2
HEAVY METAL CONTENT OF ASBESTOS DUSTS

Specimen and method				Cr (ppm)	Mn (ppm)	Fe (%)	Co (ppm)	Ni (ppm)
Amosite	UICC	As reported	Atomic abs.	32	13,000	15.1	11	34
		by Timbrell	Neutron act.	35	15,000	28	7	<100
		As analyzed in our lab		38 ± 8	13,600 ± 500	25 ± 1	0 ± 1	36 ± 2
	Johns-Manville		Crude	40 ± 3	14,000 ± 400	24 ± 1	10 ± 1	52 ± 2
	W-3		Processed	120 ± 5	14,000 ± 400	25 ± 1	12 ± 1	58 ± 2
Crocidolite	UICC	As reported	Atomic abs.	22	864	15.1	9	13
		by Timbrell	Neutron act.	16	870	27	2	<100
		As analyzed in our lab		16 ± 5	820 ± 15	23 ± 4	10 ± 2	12 ± 2
	Johns-Manville		Crude	13 ± 1	1,030 ± 50	33 ± 6	6 ± 1	12 ± 1
	S-Blue		Processed	120 ± 7	1,320 ± 70	33 ± 6.5	7 ± 1	58 ± 2
Chrysotile	UICC	As reported	Atomic abs.	1395	366	0.88	54	1445
	A	by Timbrell	Neutron act.	1390	430	1.70	55	1250
		As analyzed in our lab		1400 ± 60	360 ± 40	2.2 ± 0.1	40 ± 1	880 ± 80
	UICC	As reported	Atomic abs.	316	443	1.14	45	795
	B	by Timbrell	Neutron act.	490	510	2.60	46	990
		As analyzed in our lab		460 ± 80	460 ± 40	2.8 ± 0.4	41 ± 2	700 ± 60
	Johns-Manville		Crude	320 ± 80	450 ± 10	2.5 ± 0.3	30 ± 1	330 ± 40
	3-T		Processed	500 ± 60	480 ± 10	2.8 ± 0.4	33 ± 2	490 ± 40

with the aid of hammer mills and fan systems. The chambers were in operation 4 hours/day, 4 days/week, with each Friday reserved as cleanup day. The exposure lasted 2 years with 1480 cumulative total exposure hours.

Dust concentration was monitored regularly throughout the experiment, through weekly air samples collected in each of the chambers on Millipore filters. The measurements yielded an overall mean (in mg/m^3, ±SD) of 48.6 ± 3.0 for amosite; 50.2 ± 3.6 for crocidolite; and 47.9 ± 3.0 for chrysotile. The aerosols appeared as dense dust clouds readily visible to the naked eye but with little tendency to settle on surfaces. The used asbestos was exhausted from each chamber into plastic collection bags.

3. *The Animal Colonies*

207 rats, 96 guinea pigs, 60 rabbits, 90 mice, and 204 gerbils[5] of both sexes were evenly divided among the amosite, crocidolite, and chrysotile chambers,

TABLE 3
SURVEY OF THE ANIMAL COLONIES

Exposure	Specimen count (Inventory of live animals at) (months)	Mice	Gerbils	Rats	Rabbits	Guinea pigs
Amosite	0	30	68	69	20	32
	6	23	64	62	14	20
	12	17	60	56	12	20
	18	0	51	46	10	13
	24	0	46	19	10	8
	No. slides prepared	19	58	66	20	30
Crocidolite	0	30	68	69	20	32
	6	26	63	63	14	20
	12	18	58	57	12	20
	18	0	49	46	9	14
	24	0	46	22	9	6
	No. slides prepared	22	49	66	20	31
Chrysotile	0	30	68	69	20	32
	6	26	64	62	16	22
	12	19	56	56	13	20
	18	0	50	43	11	14
	24	0	44	14	11	13
	No. slides prepared	17	59	57	20	32
Control	0	10	12	12	12	12
	6	8	11	9	7	8
	12	6	10	7	6	8
	18	0	8	5	4	5
	24	0	8	3	3	4
	No. slides prepared	10	12	12	12	11
Total no. of animals		100	216	219	72	108

[5] Charles River CD rats; Camm–Hartley guinea pigs; Shankin Farms Dutch rabbits; Stout Farms Swiss mice; and Hasenau Mongolian gerbils.

respectively, with a smaller number (10–12 of each species) being retained as unexposed controls. The animals were 3–6 weeks old when shipped. Sample necropsies were obtained after 3 and 6 months, respectively, with the majority of animals allowed to survive the complete course of exposure. Altogether, out of a total animal count of 715, there were 417 animals necropsied on schedule; 249 animals died in the course of the experiment through attrition; and 49 were lost to cannibalism or otherwise unaccounted for. Attritional losses were heaviest among rats during the fourth semester of exposure when 25–30 animals were lost for unexplained reasons. There were no indications of infectious epizootic and no antibiotic medication was given. It may be that the mortality was the consequence of fully developing pulmonary fibrosis, perhaps in combination with higher summertime temperatures. Mortality among the other animal species was normal, and there was no gross differential effect of exposure to any of the dusts on survival. The complete animal inventories are summarized in Table 3.

Necropsy procdures were as reported before (Reeves *et al.*, 1971). Lung weight was obtained after excision, in wet state, but blotted between sheets of filter paper. Histopathologic slides were obtained from the lungs and pleura of altogether 643 animals, with varying length of exposure. Hematoxylin–eosin, Van Gieson's elastic, and $K_4Fe(CN)_6$ stains were applied to the sections.

RESULTS

Table 4 summarizes the essential histopathology findings and lung weight/body weight ratios. It may be seen that exposure to various kinds of asbestos has increased the latter in each species in the following ascending order: chrysotile; amosite; and crocidolite. Severest effects were seen with gerbils (crocidolite-control difference $t = 3.14$, significant at 99.5% confidence level); and with rats (crocidolite-control difference $t = 2.36$, significant at 95% confidence level). With the other species, the differences were of low or borderline significance, and in each species amosite and chrysotile caused successively less effect than crocidolite. Tumor-bearing animals were excluded from this computation, so that the results are indicative of the degree and extent of asbestosis. The details of histopathologic impressions with each animal species were as follows.

(*a*) *Rats*

Among control animals there was one bacterial pneumonia but no other significant pathological changes. Among exposed animals, histiocytic and giant cell response was universal and gradually increasing in the course of exposure. Fibrotic response was most severe in the crocidolite group. Fiber deposits were frequently seen but there were few readily apparent ferruginous bodies and almost no necrosis. A few cases of pneumonia and bronchitis were seen. In the crocidolite group, frequent pleural reaction with collagen accumulation occurred, as well as hemosiderin accumulation in the lymph nodes. There was filling of some alveoli with histiocytes, giant cells, and cholesterol.

A proliferative response was especially frequent in the crocidolite group, where 8 animals (on the 567, 585, 691, 693, 727, 772, and 785th day, respectively) had squamous metaplasia of the alveolar lining. In 2 crocidolite-exposed animals, epithelial papillomata were seen in the bronchi, which appeared histologically

benign (Fig. 1). There were also the following definite malignant neoplasms in all groups:

(*1*) *Amosite.* Rat No. 1206-23, male, found dead after 539 days of exposure. The lung and pleura contained a large neoplasm of fibrosarcoma pattern with adhesions to the chest wall (Figs. 2 and 3). Rat No. 1206-26, male, found dead after 564 days of exposure. The lung contained an osteosarcoma, probably metastasis of a primary bone tumor. Rat No. 1206-42, female, necropsied after 584 days of exposure. The pleura contained a large fibrous mesothelioma (Fig. 4). Rat No. 1206-53, female, found dead on the 18th day after completion of exposure. The lung contained a bronchoalveolar carcinoma with papillary pattern, composed of tall columnar cells (Fig. 5). A similar neoplasm was seen on the pleural surface.

(2) *Crocidolite.* Rat No. 1208-48, female, found dead on the 12th day after completion of exposure. The lungs contained a bronchogenic adenocarcinoma. Rat No. 1208-49, male, found dead on the 18th day after completion of exposure. The lungs contained a squamous cell carcinoma. Rat No. 1208-55, female, sacrificed 45 days after completion of exposure. The lungs contained a squamous cell carcinoma (Figs. 6 and 7). Rat No. 1208-69, female, necropsied 56 days after completion of exposure. The lungs contained a squamous cell carcinoma.

(3) *Chrysotile.* Rat No. 1207-41, male, found dead after 617 days of exposure. The upper mediastinum contained an area of proliferative fibrosis histologically classified as a low-grade fibrosarcoma of probably pleural origin. Rat No. 1207-48, male, found dead after 608 days of exposure. The lungs contained a papillary carcinoma. Rat No. 1207-61, male, necropsied 43 days after completion of exposure. The lungs contained a well differentiated squamous cell carcinoma (Fig. 8).

(*b*) *Rabbits*

In control animals there were no significant pathological changes. Amosite and crocidolite caused histiocytic and foreign-body responses, with the extent of reaction well correlated to the length of exposure. Light to intermediate fibrosis was observed in animals surviving the complete course of exposure. Inflammatory reaction was infrequent, and chrysotile caused remarkably fewer lesions. There were no malignancies.

(*c*) *Guinea Pigs*

Control animals had no significant pathological changes. Among exposed animals, this species was remarkable for conspicuous abundance of ferruginous bodies, especially after crocidolite exposure. Foreign-body and giant-cell response, as well as gradually developing fibrosis, were common in all exposure groups. There were a few cases of adenomatosis but no malignant neoplasia.

(*d*) *Gerbils*

Control animals had no significant pathological changes. Exposed animals showed foreign-body response, light to moderate fibrosis, and a conspicuously high incidence of focal and generalized alveolar proteinosis, especially in the amosite and crocidolite groups. There were no cases of malignant neoplasia.

TABLE 4
SUMMARY OF PATHOLOGICAL FINDINGS

Group		Asbestosis			Neoplasia	
		No. of specimens	$\frac{LW}{BW}$ (mg/g + SD)	Microscopic appearance of slides	No. animals surviving 18 months	Incidence and type of malignancy
Mice	Control	4	11.5 ± 1.5	Normal	6[a]	1 papillary carcinoma of bronchus
	Chrysotile	1	10.6	Near normal	19[a]	None
	Crocidolite	4	14.4 ± 2.8	Fibrosis	18[a]	2 papillary carcinoma of bronchus
	Amosite	5	12.2 ± 3.1	Fibrosis	17[a]	None
Gerbils	Control	7	6.7 ± 0.3	Normal	8	
	Chrysotile	40	6.4 ± 1.6	Histiocytes; Fibrosis	50	
	Crocidolite	31	15.3 ± 7.0[b]	Alveolar proteinosis	49	None
	Amosite	45	8.5 ± 5.1	Histiocytes; Fibrosis	51	
Rats	Control	6	8.5 ± 4.2	Normal	5	None
	Chrysotile	34	8.8 ± 5.1	Histiocytes; Fibrosis	43	1 papillary carcinoma of lung 1 squamous carcinoma of lung 1 mesothelial fibrosarcoma of mediastinum

	Crocidolite	43	14 ± 6.5[c]	Many histiocytes; Fibrosis	46	1 adenocarcinoma of lung 3 squamous carcinoma of lung 1 papillary carcinoma of lung
	Amosite	43	10.5 ± 5.3	Histiocytes	46	1 metastic osteosarcoma of lung 1 mesothelial fibrosarcoma of lung and pleura 1 fibrous mesothelial of pleura
Rabbits	Control	5	7.3 ± 3.2	Normal	4	
	Chrysotile	11	5.8 ± 0.9	Near normal	11	
	Crocidolite	10	8.0 ± 1.0	Histiocytes; Fibrosis	9	None
	Amosite	9	5.3 ± 1.3	Histiocytes; Fibrosis	10	
Guinea pigs	Control	6	14.1 ± 2.5	Normal	5	
	Chrysotile	15	13.2 ± 2.9	Histiocytes; Slight fibrosis	14	
	Crocidolite	13	15.5 ± 5.4	Histiocytes; Fibrosis	14	None
	Amosite	15	14.4 ± 3.6	Histiocytes; Fibrosis	13	

[a] 12-month survival.
[b] Difference from control significant at 99.5% level ($t_{36df} = 3.14$).
[c] Difference from control significant at 95% level ($t_{47df} = 2.36$).

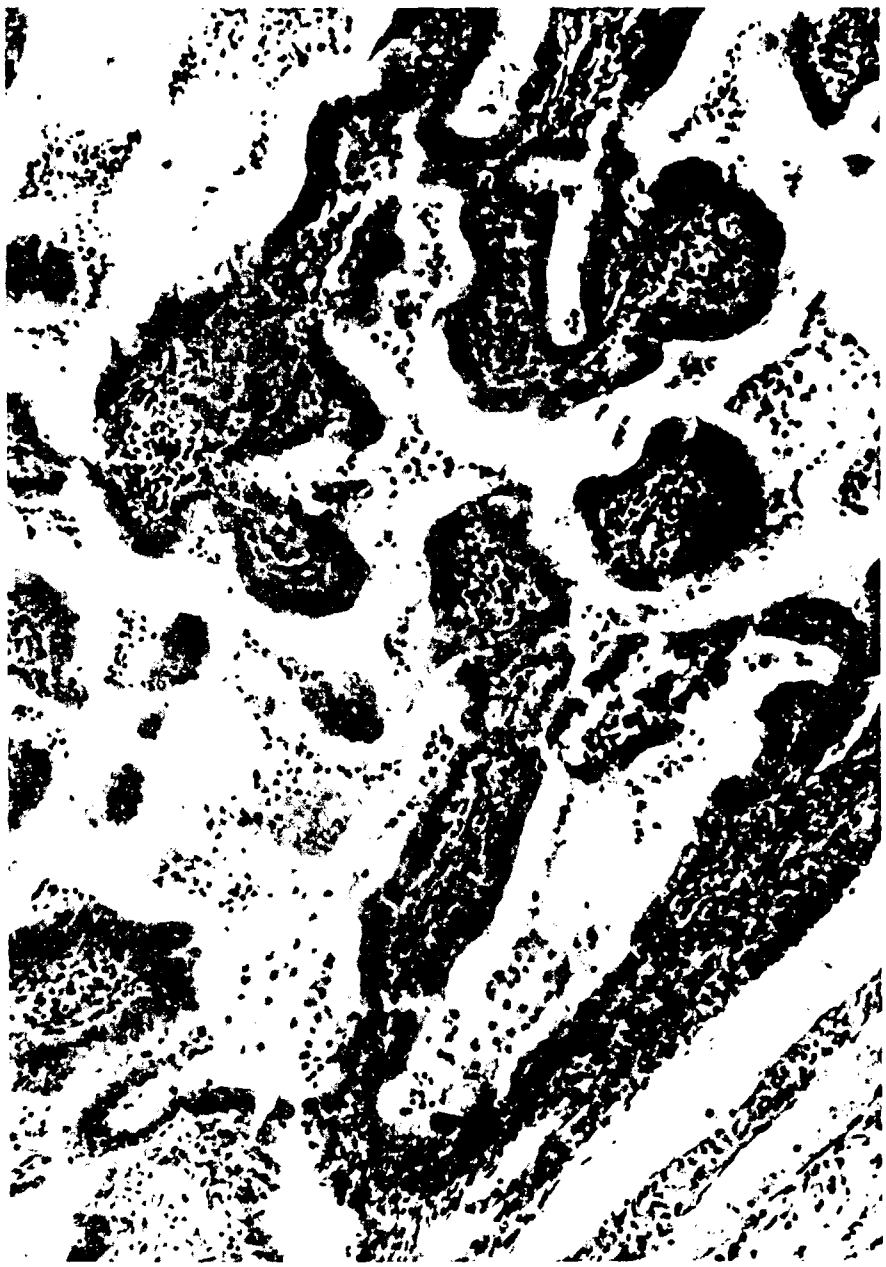

FIG. 1. (Rat No. 1208-62). Benign papilloma of the bronchus after crocidolite exposure. Hematoxylin–eosin stain, ×40.

(*e*) *Mice*

Control animals had generally no significant pathological changes, except one female (No. 1213-8, necropsied on the 388th day of exposure) which exhibited a papillary carcinoma of the bronchus. Two histologically identical neoplasms

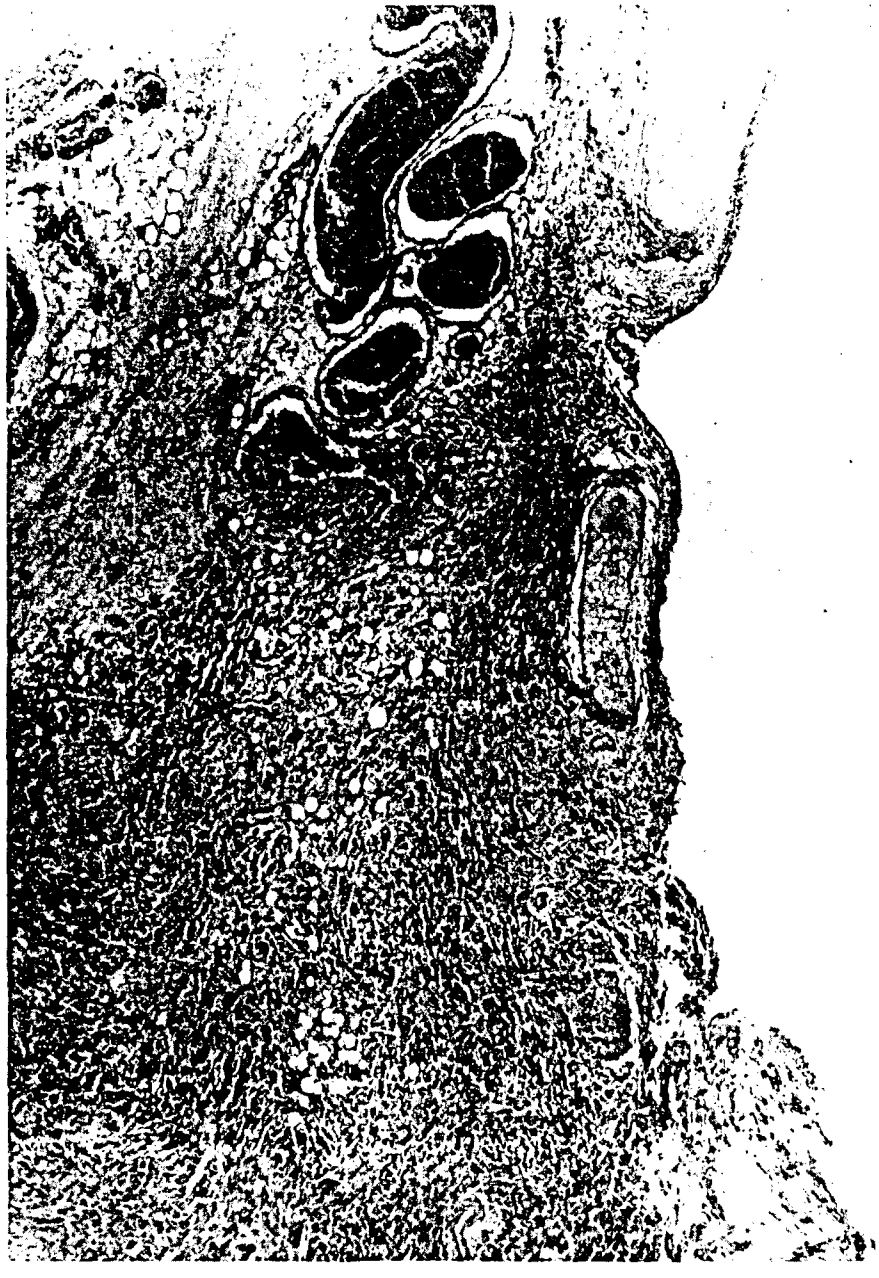

FIG. 2. (Rat No. 1206-23). Mesothelial fibrosarcoma invading the wall of a bronchus, after amosite exposure. Hematoxylin–eosin stain, ×10.

were also seen in 2 females of the crocidolite group (Nos. 1216-20 and 1216-24, necropsied on the 360th and 388th day of exposure, respectively). Animals exposed to amosite and crocidolite, and to a much lesser degree those exposed to chrysotile, had mild to moderate fibrosis associated with pulmonary deposits of asbestos fibers.

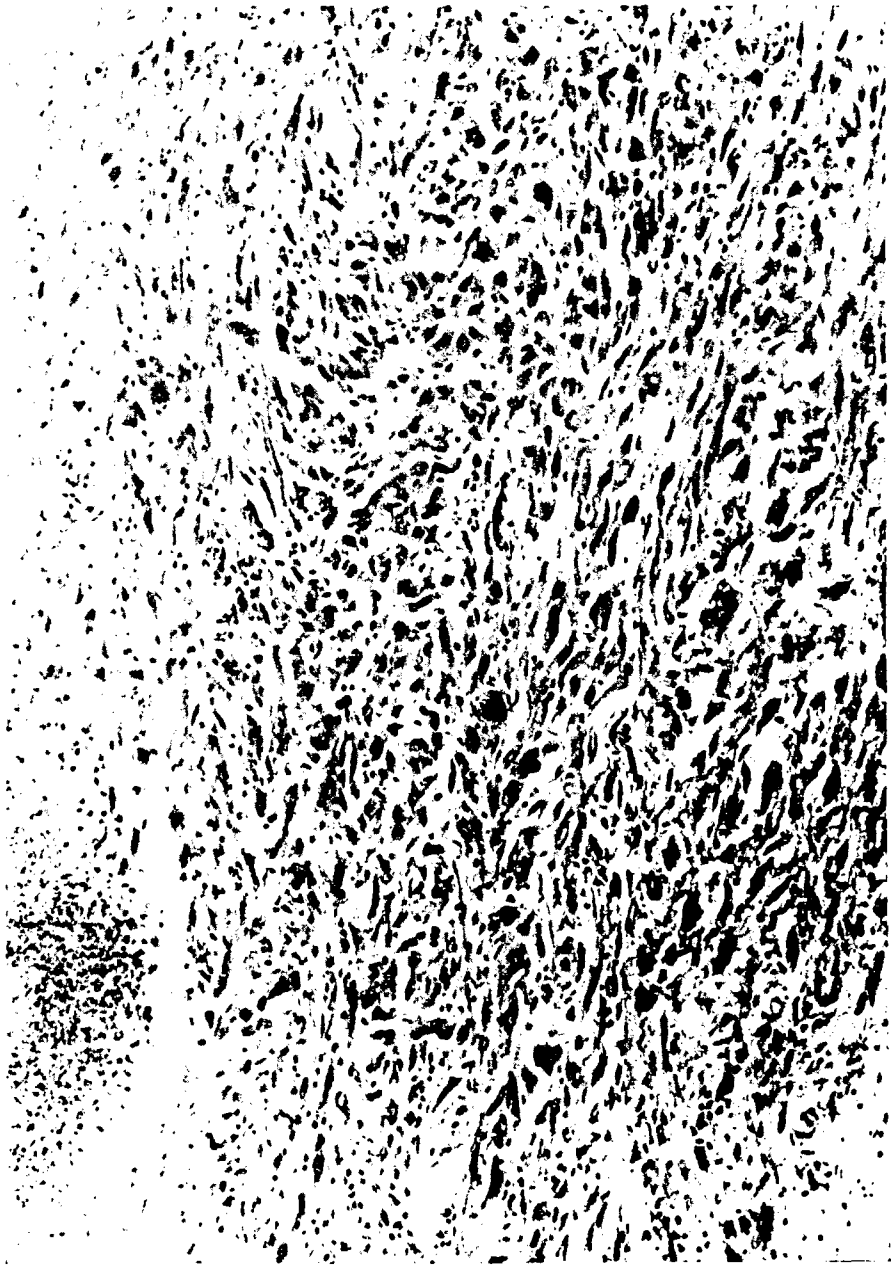

FIG. 3. (Rat No. 1206-23). Same specimen as in Fig. 2, under high magnification, showing cytologic pleomorphism and mitotic figures. Hematoxylin–eosin stain, ×100.

DISCUSSION

Of the 13 malignant neoplasms observed in this study following inhalation of asbestos, one was pulmonary metastatic osteosarcoma in a rat and two were bronchial papillary carcinomas in mice, with a similar carcinoma also occurring

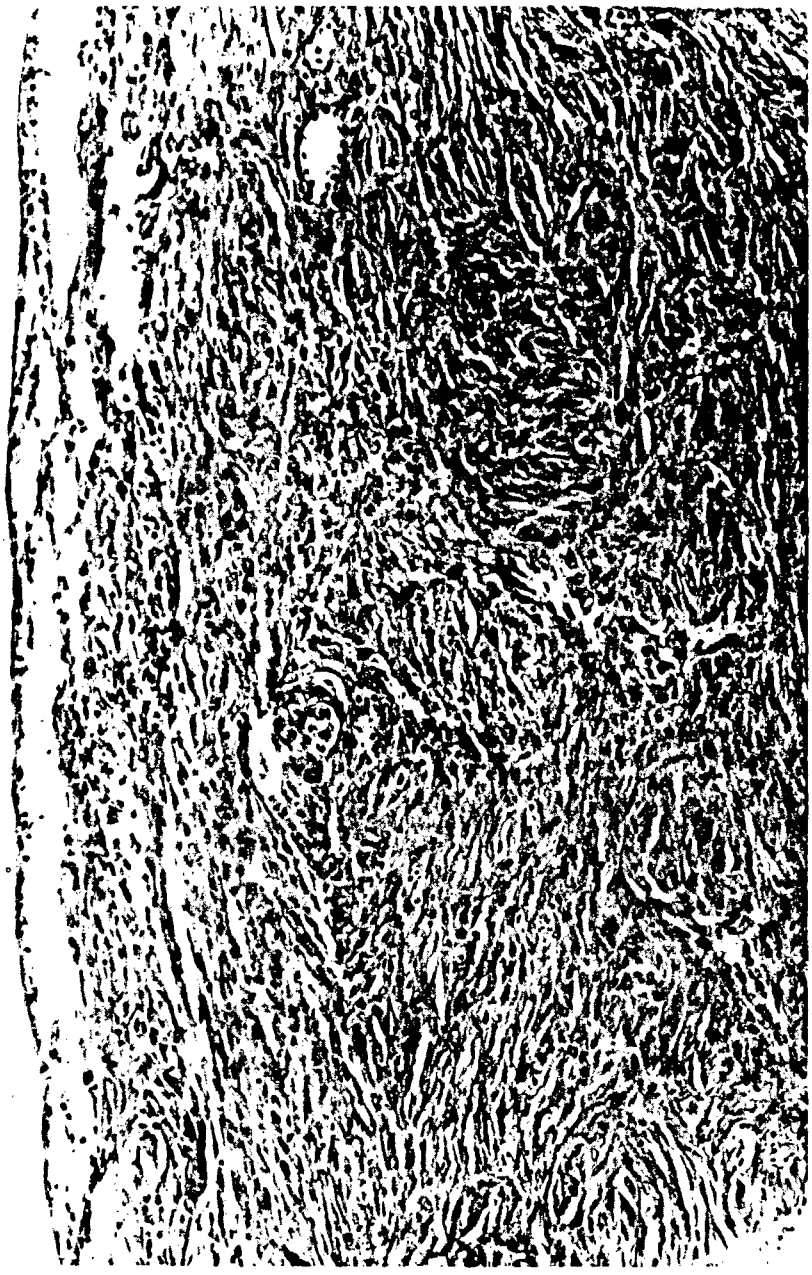

FIG. 4. (Rat No. 1206-42). Fibrous mesothelioma of the pleura after amosite exposure. Hematoxylin–eosin stain, ×40.

in a control mouse. Excluding these tumors as doubtful in relation to an asbestos etiology, there is a 7–9% incidence of malignant neoplasia in rats exposed to the inhalation of finely ground chrysotile, crocidolite, or amosite. The significance of this finding is threefold.

First, the results show that under the conditions of this experiment there was

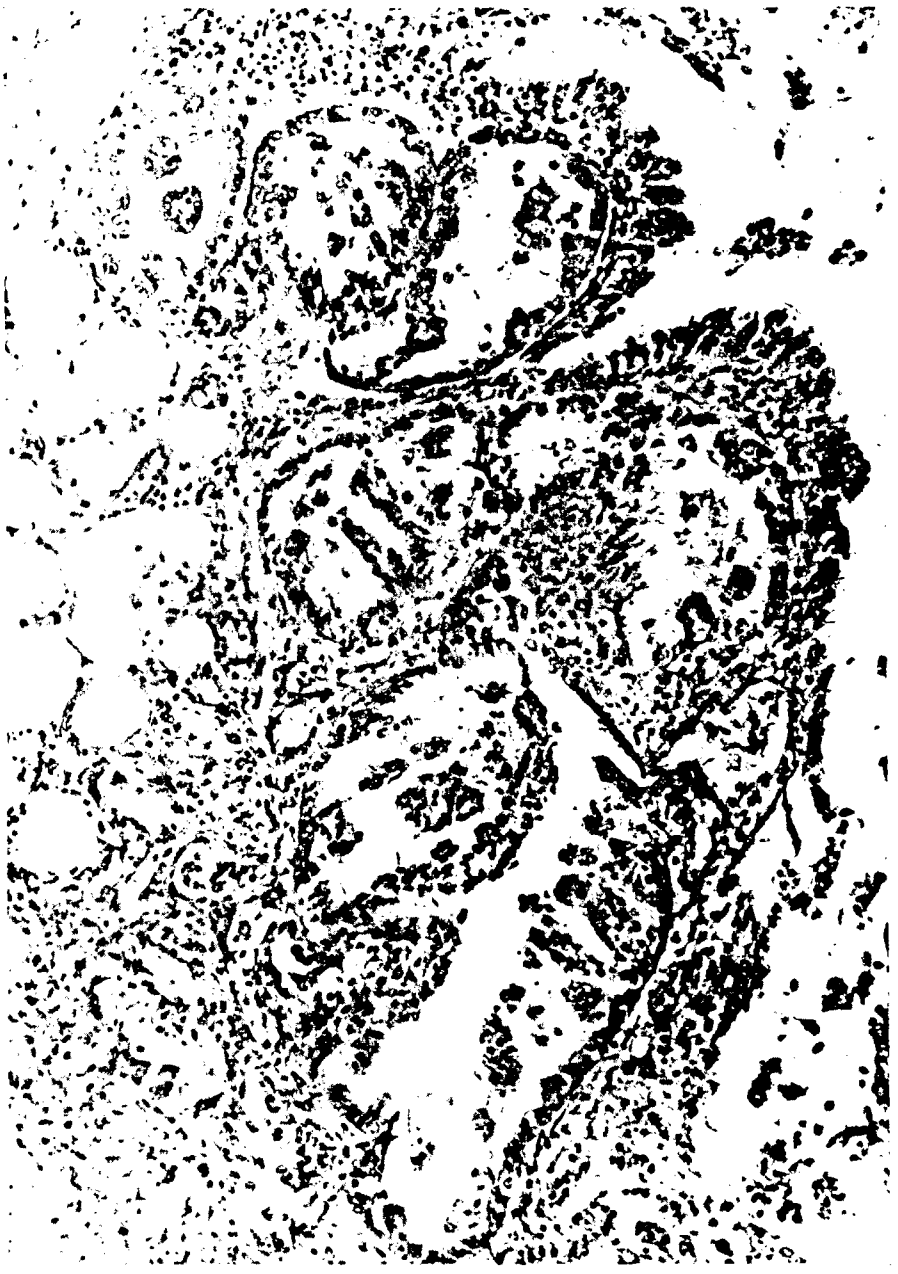

FIG. 5. (Rat No. 1206-53). Bronchoalveolar carcinoma after amosite exposure. Hematoxylin–eosin stain, ×40.

no important difference in the carcinogenic capacities of three asbestiform minerals including amosite, which in previous experience (Wagner and Berry, 1969; Reeves *et al.*, 1971, 1972) appeared to be less carcinogenic than crocidolite or chrysotile. Roe (1968) could also obtain pleural and peritoneal mesotheliomas

FIG. 6. (Rat No. 1208-55). Squamous cell carcinoma of bronchogenic origin after crocidolite exposure. Hematoxylin–eosin stain. ×40.

in mice after subcutaneous injection of crocidolite as well as amosite, although the carcinogenic potential was abolished by solvent extraction; Stanton and Wrench (1972) have produced mesotheliomas after pleural implantation of amosite, crocidolite, chrysotile, or fibrous glass. Selikoff *et al.* (1972a) have called

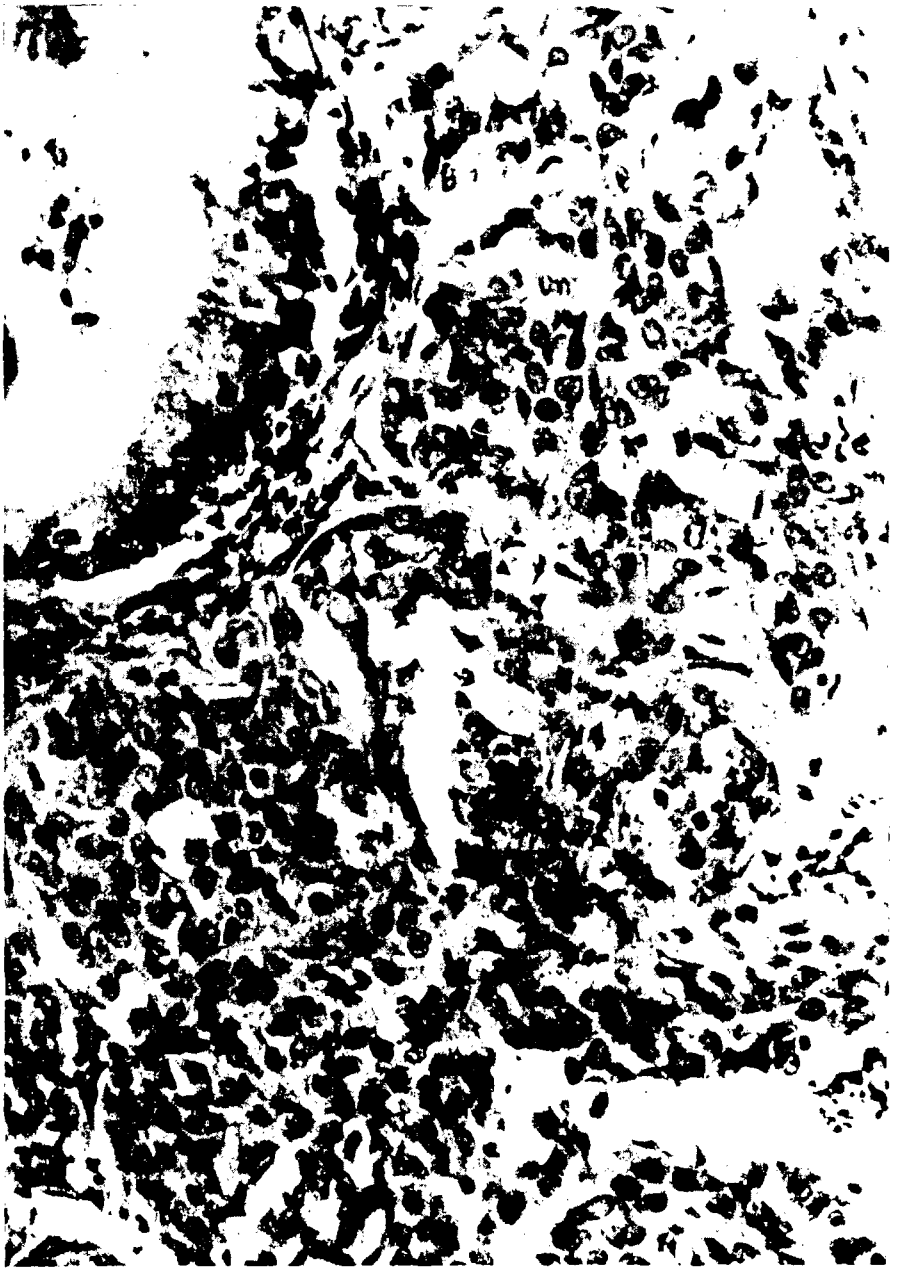

FIG. 7. (Rat No. 1208-55). Same specimen as in Fig. 6, under high magnification, showing cytologic pleomorphism. Hematoxylin–eosin stain, ×100.

attention to the carcinogenicity of amosite in man. In the study reported here, we have obtained the first carcinoma of lung and mesothelioma of pleura after experimental inhalation exposure to amosite.

Second, it is remarkable that while the fibrogenic capacity of chrysotile in

FIG. 8. (Rat No. 1207-61). Well-differentiated squamous cell carcinoma of the lung after chrysotile exposure. Hematoxylin–eosin stain, ×40.

this experiment was much less than that of crocidolite or amosite, the carcinogenic capacities appeared to be about the same. Even though the quantities of the three disseminated dusts were comparable (48.6–50.2 mg/m^3), it appeared that fiber destruction was much more extensive with chrysotile than with crocidolite or amosite (54 vs 864–1105 light microscopically visible fibers per ml cham-

ber air). The sharply reduced fiber concentration of chrysotile is well reflected in the histopathologic data showing marked reduction or near-absence of the fibrotic response in animals exposed to chrysotile. The fact that the carcinogenic response was not comparably reduced suggests either or both of the following postulates. (1) The carcinogenic threshold of chrysotile fibers is orders of magnitude lower than their fibrogenic threshold. (2) Destruction of the fibrous geometry of chrysotile diminished its fibrogenic potential but did not diminish its carcinogenic potential. Both of these alternatives have highly significant potential public health implications.

Third, this study is remarkable for the finding that among four rodent species exposed to identical aerosols of asbestos and comparable survival record, only rats developed respiratory neoplasia (mice which developed spontaneous bronchial cancers are excluded from this consideration). Mesotheliomas were produced previously with implanted asbestos fibers in hamsters (Smith *et al.*, 1965), fowls (Peacock and Peacock, 1965), rabbits (Reeves *et al.*, 1971) as well as rats, but experimental inhalation carcinogenesis from asbestos has thus far been restricted to the rat (Gross *et al.*, 1967; Reeves *et al.*, 1971). In some cases this may have been due to relative efficiencies of pulmonary clearance, and for instance the unsuitability of hamsters to develop cancers from inhaled asbestos was attributed to a swift and severe fibrotic response which curtailed the life expectancy of the exposed animals (Gross *et al.*, 1967; Reeves *et al.*, 1971). However, guinea pigs showed adequate survival and no carcinogenic response after either asbestos implantation or inhalation. It is also remarkable that after equal asbestos exposure, pulmonary ferruginous bodies were abundant in the guinea pig and rare in the rat.

There is now growing evidence to suggest that the formation of ferruginous bodies is the result of a phagocytic process involving several macrophages at a site, which fuse to form giant cells (Suzuki and Churg, 1969; Davis, 1970a). This process results in the coating of fibrous particles with a mucopolysaccharide layer containing hemosiderin granules (Governa and Rosanda, 1972), irrespective of whether the phagocytized fiber was asbestos, fibrous glass, or other ceramic silicate (Davis, 1970b; Botham and Holt, 1971). Ferruginous body formation is thus perhaps a protective reaction of the organism. In rodents, there appears to be an inverse relation between the frequency of these bodies and the carcinogenic effect attributable to inhaled asbestos. The existence of an immunologic factor in the causation of asbestosis or asbestos neoplasia has been considered (Massey *et al.*, 1971) but serum immunoelectrophoretic studies in asbestos cement workers (El-Sewefy and Hassan, 1971) and pulmonary function studies in asbestos-exposed rabbits with and without administration of the immunosuppressive drug Imuran (Ford, 1971) were thus far inconclusive.

Essentially, the carcinogenic entity present in asbestos may be either physical or chemical. The physical hypothesis, according to which the mechanical irritation attributable to the embedded fibers or to the ensuing biological response (Oppenheimer effect) is the key factor in the etiology of asbestos cancers was not viewed with favor by the early investigators (e.g., Harington, 1965), but received new support from the studies of Stanton and Wrench (1972), who ob-

tained mesotheliomas with fibrous glass if the latter was milled to approach the size range of carcinogenic asbestos fibers. These authors therefore concluded that carcinogenicity was primarily related to the structural shape of these materials rather than to physicochemical properties.

The chemical hypothesis is espoused by numerous other authors who believe that a chemical or physicochemical factor, associated either with the silicate core of asbestos fibers or with certain adventitious factors, is the ultimate carcinogenic principle. Thus far, the question of adventitious factors has received the most attention. Harington (1962) discovered that certain virgin samples of asbestos contained cyclohexane-extractable oils composed of aromatic hydrocarbons, one of which was 3,4-benzpyrene. Furthermore, it was pointed out that besides natural trace constituents which apparently became adsorbed on asbestos fibers during their geological genesis, additional opportunities for contamination exist during commercial handling. Jute bags (Roe *et al.*, 1966) as well as polythene bags (Commins and Gibbs, 1969) were shown to release aromatic compounds of possible or proven carcinogenic potential. Removal of these contaminants reduced (Harington *et al.*, 1967) or eliminated (Roe *et al.*, 1966) the carcinogenic activity of asbestos, and addition of 3,4-benzpyrene to chrysotile sometimes did and sometimes did not increase its carcinogenic potential (Pott *et al.*, 1972; Rylev, 1972).

Metals present in asbestos include iron, aluminum, and magnesium as major constituents and chromium, nickel, cobalt, manganese, and others as trace constituents. Several of the latter are known respiratory carcinogens although it was argued (Stanton and Wrench, 1972) that their levels in asbestos are too small to be biologically significant. However, it was pointed out by Cralley *et al.* (1967) that during manufacture of asbestos textile products the concentration of carcinogenic metals in asbestos increases substantially through contact with the weaving machinery. Similar increase in heavy metal content of asbestos was also experienced during chamber dissemination as practiced by Gross and DeTreville (1967) and Reeves *et al.* (1971). Dixon *et al.* (1970) have attempted to explain the carcinogenic activity of asbestos-associated trace metals on the grounds of inhibition of benzpyrene hydroxylase, a detoxifying enzyme that presumably protects the organism from the effects of benzpyrene. Cralley (1971) suggested that adsorbed metal on asbestos fibers may become the poles of miniature electrolytic cells, and the ensuing electromotive forces may produce high levels of biologically active cations at localized tissue sites.

The *in vitro* action of asbestos on various model systems including tissue culture was studied as a possible adjunct to the understanding of its fibrogenic and carcinogenic properties. Pernis and Castano (1971) have found no cytotoxicity for chrysotile or crocidolite to mouse peritoneal macrophages under ideal culture conditions, and no interruption of cellular lactate biosynthesis was observed (Beck *et al.*, 1971a). However, membrane permeability as measured by eosin uptake was increased after contact with asbestos, which could lead to lysis of the cells in the absence of protective factors (Allison, 1971). Glass fiber, but not glass powder, was shown to have similar effects (Turnock *et al.*, 1971). It was also observed that passage through asbestos filters conferred a growth-inhibitory

property on the medium used in the culture of HeLa cells (Litterst and Lichtenstein, 1970). Bey and Harington (1971), Miller and Harington (1972), as well as Robock and Klosterkötter (1971) have found higher general cytotoxicity for chrysotile than for the amphiboles; and chrysotile, but not amosite or crocidolite, was found to be a powerful hemolytic agent (MacNab and Harington, 1967; Schnitzer and Pundsack, 1970; Harington *et al.*, 1971). That amphibole dusts (amosite and crocidolite) should nonetheless appear to be more hazardous upon inhalation than chrysotile was explainable on the grounds that the shorter and harsher fibrils of the former have a better opportunity to penetrate into the alveoli or pleura while chrysotile, in view of its curved shape, is more likely to get arrested high in the respiratory tract (Robock and Klosterkötter, 1971).

The *in vivo* solubility of chrysotile was investigated with radioactive tracer techniques by Morgan *et al.* (1971), detecting migration of certain fractions to the liver. Special vulnerability of the pleural mesothelium to chrysotile was shown by measuring the turnover of tritiated thymidine (Bryks and Bertalanffy, 1971); collagen biosynthesis, measured as rate of proline hydroxylation, was highest in the early stages of tissue contact with asbestos (Davis and Reeves, 1971).

The toxicity of modified asbestos fibers was investigated only very incompletely thus far. Beck *et al.* (1971b) have treated chrysotile with 0.1 N HCl, leaving a SiO_2 surface on the fibers, and observed increased acute cytotoxicity as manifested by reduced lactate biosynthesis. Heating an impure specimen of chrysotile (fibrous component about 50%, the rest mainly antigorite) to 1000°C for 3 hours caused greatly increased acute toxicity after intraperitoneal injection into mice (Jagatic *et al.*, 1967). The heat treatment caused appearance of forsterite and enstatite detectable by X-ray diffraction, but the acute toxicity may have been due to a volatile component.

Selikoff *et al.* (1968) as well as Berry *et al.* (1972) have observed that asbestos exposure in man has caused significantly increased mortality from lung cancer only among cigarette smokers. Mortality from mesotheliomas, on the other hand, was apparently independent from cigarette smoking, much less dose-related, and frequently occurred in subjects with little or no asbestosis (Selikoff *et al.*, 1967; Wagner *et al.*, 1971). It is possible that different etiologic entities in the asbestos fiber are responsible for the causation of these two diseases. Mesothelioma may be a consequence of mechanical irritation by the fibers or by the fibrous plaques formed in response to the presence of the fibers (Bryson and Bischoff, 1967); pulmonary carcinoma may be related to chemical carcinogens present in or adsorbed on the fibers. More work is needed to throw light on this interesting question.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Anil Roy-Chowdhury for chemical analysis; to Kenneth R. Mikel for fiber counts; to Duane VanAcker for operation of the exposure facility; to Leonard Pickett for histological processing; and to Clarence J. Hickey and Walter Dybicki for animal colony maintenance.

REFERENCES

ALLISON, A. C. (1971). Effects of silica and asbestos on cells in culture. *In* "Inhaled Particles III" (W. H. Walton, Ed.), pp. 437–445. Unwin Bros. Ltd., Old Woking, Surrey.

BAMBER, H. A., AND BUTTERWORTH, R. (1970). Asbestos hazard from protective clothing. *Ann. Occup. Hyg.* **13**, 77–79.

BECK, E. G., BRUCH, J., FRIEDRICHS, K. H., HILSCHER, W., AND POTT, F. (1971a). Fibrous silicates in animal experiments and cell culture–Morphological cell and tissue reactions according to different physical chemical influences. *In* "Inhaled Particles III" (W. H. Walton, Ed.), pp. 477–487. Unwin Bros. Ltd., Old Woking, Surrey.

BECK, E. G., HOLT, P. F., AND NASRAKAH, E. T. (1971b). Effects of chrysotile and acid-treated chrysotile on macrophage cultures. *Brit. J. Ind. Med.* **28**, 179–185.

BERRY, G., NEWHOUSE, M. L., AND TUROK, M. (1972). Combined effects of asbestos exposure and smoking on mortality from lung cancer in factory workers. *Lancet* **II**, 476–478.

BEY, E., AND HARINGTON, J. S. (1971). Cytotoxic effects of some mineral dusts on Syrian hamster peritoneal macrophages. *J. Exp. Med.* **133**, 1149–1169.

BOTHAM, S. K., AND HOLT, P. F. (1971). The development of glass-fibre bodies in the lungs of guinea pigs. *J. Pathol.* **103**, 149–156.

BRYKS, S., AND BERTALANFFY, F. D. (1971). Cytodynamic reactivity of the mesothelium–pleural reaction to chrysotile asbestos. *Arch. Environ. Health* **23**, 469–472.

BRYSON, G., AND BISCHOFF, F. (1967). Silicate-induced neoplasms. *Progr. Exp. Tumor Research* **9**, 77–164.

BURILKOV, T., AND MICHAILOVA, L. (1970). Asbestos content of the soil and endemic pleural asbestosis. *Environ. Res.* **3**, 443–451.

COMMINS, B. T., AND GIBBS, G. W. (1969). Contaminating organic material in asbestos. *Brit. J. Cancer* **23**, 358–362.

CRALLEY, L. J. (1971). Electromotive phenomenon in metal and mineral particulate exposures: relevance to exposure to asbestos and occurrence of cancer. *Amer. Ind. Hyg. Assoc. J.* **32**, 653–661.

CRALLEY, L. J., KEENAN, R. G., AND LYNCH, N. R. (1967). Exposure to metals in the manufacture of asbestos textile products. *Amer. Ind. Hyg. Assoc. J.* **28**, 452–461.

CUNNINGHAM, H. M., AND PONTEFRACT, R. (1971). Asbestos fibers in beverages and drinking water. *Nature (London)* **232**, 332–333.

DAVIS, H. V., AND REEVES, A. L. (1971). Collagen biosynthesis in rat lungs during exposure to asbestos. *Amer. Ind. Hyg. Assoc. J.* **32**, 599–602.

DAVIS, J. M. G. (1970a). Asbestos dust as a nucleation center in the calcification of old fibrous tissue lesions, and the possible association of this process to the formation of asbestos bodies. *Exp. Mol. Pathol.* **12**, 133–147.

DAVIS, J. M. G. (1970b). Further observations on the ultrastructure and chemistry of the formation of asbestos bodies. *Exp. Mol. Pathol.* **13**, 346–358.

DIXON, J. R., LOWE, D. B., RICHARDS, D. E., CRALLEY, L. J., AND STOKINGER, H. E. (1970). The role of trace metals in asbestos carcinogenesis: asbestos cancers. *Cancer Research* **30**, 1068–1074.

DONNA, A. (1970). Tumori Sperimentali da Amianto di Crisotilo, Crocidolite, e Amosite in Ratto. *Med. Lavoro* **61**, 1–32.

EL-SEWEFY, A. Z., AND HASSAN, F. (1971). Immunoelectrophoretic pattern changes in asbestosis. *Ann. Occup. Hyg.* **14**, 25–28.

FORD, J. E. (1971). The effect of pulmonary macrophage suppression in developing asbestosis. Dissertation, Wayne State University, Detroit.

GERBER, M. A. (1970). Asbestosis and neoplastic disorders of the hematopoietic system. *Amer. J. Clin. Pathol.* **53**, 204–208.

GOFF, A. M., AND GAENSLER, E. A. (1972). Asbestosis following brief exposure in cigarette filter manufacture. *Respiration* **29**, 83–93.

GOVERNA, M., AND ROSANDA, C. (1972). A histochemical study of the asbestos body coating. *Brit. J. Ind. Med.* **29**, 154–159.

GREENBERG, M. (1970). Asbestos release from battery boxes. *Ann. Occup. Hyg.* **13**, 79–80.

GRAHAM, J., AND GRAHAM, R. (1967). Ovarian cancer and asbestos. *Environ. Res.* **1**, 115–128.

GROSS, P., AND DETREVILLE, R. T. P. (1967). Experimental asbestosis. *Arch. Environ. Health* **15**, 638–649.

GROSS, P., DETREVILLE, R. T. P., TOLKER, E. B., KASĆHAK, M., AND BABYAK, M. A. (1967). Experimental asbestosis–The development of lung cancer in rats with pulmonary deposits of chrysotile dust. *Arch. Environ. Health* **15**, 343–355.

GROSS, P., DETREVILLE, R. T. P., AND HALLER, M. N. (1969). Pulmonary ferruginous bodies in city dwellers: a study of their central fiber. *Arch. Environ. Health* **19**, 186–188.

GROSS, P., TUMA, J., AND DETREVILLE, R. T. P. (1970). Fibrous dust particles and ferruginous bodies. *Arch. Environ. Health* **21**, 38–46.

HARINGTON, J. S. (1962). Occurrence of oils containing 3,4-benzpyrene and related substances in asbestos. *Nature (London)* **193**, 43–45.

HARINGTON, J. S. (1965). Studies of carcinogenesis of asbestos fibers and their natural oils. *Ann. N. Y. Acad. Sci.* **132**, 439–450.

HARINGTON, J. S., MILLER, K., AND MACNAB, G. (1971). Hemolysis by asbestos. *Environ. Res.* **4**, 95–117.

HARINGTON, J. S., ROE, F. J. C., AND WALTERS, M. (1967). Studies on the mode of action of asbestos as a carcinogen. *S. African Med. J.* **41**, 800–804.

HARRIES, P. G., MACKENZIE, F. A. F., SHEERS, G., KEMP, J. H., OLIVER, T. P., AND WRIGHT, D. S. (1972). Radiological survey of men exposed to asbestos in naval dockyards. *Brit. J. Ind. Med.* **29**, 274–279.

HICKISH, E. E., AND KNIGHT, K. L. (1970). Exposure to asbestos during brake maintenance. *Ann. Occup. Hyg.* **13**, 17–21.

JAGATIC, J., RUBNITZ, M. E., GODWIN, M. C., AND WEISKOPF, R. W. (1967). Tissue response to intraperitoneal asbestos with preliminary report of acute toxicity of heat-treated asbestos in mice. *Environ. Res.* **1**, 217–230.

KANAZAWA, K., BIRBECK, M. S. C., CARTER, R. L., AND ROE, F. J. C. (1970). Migration of asbestos fibers from subcutaneous injection sites in mice. *Brit. J. Cancer* **24**, 96–106.

LANGER, A. M., SELIKOFF, I. J., AND SASTRE, A. (1971). Chrysotile asbestos in the lungs of persons in New York City. *Arch. Environ. Health* **22**, 348–361.

LITTERST, C. L., AND LICHTENSTEIN, E. P. (1970). Toxicity of HeLa cell growth medium after passage through asbestos filters. *Lab. Pract.* **19**, 1221–1223.

MACNAB, G., AND HARINGTON, J. S. (1967). Haemolytic activity of asbestos and other mineral dusts. *Nature (London)* **214**, 522–523.

MCDONALD, J. C., MCDONALD, A. D., GIBBS, G. W., SIEMIATYCKI, J., AND ROSSITER, C. E. (1971). Mortality in the chrysotile asbestos mines and mills of Quebec. *Arch. Environ. Health* **22**, 677–687.

MASSEY, D., LEMONDE, J., AND FOURNIER-MASSEY, G. (1971). Les facteurs immunologiques dans l' amiantose. *Union Med. Canada* **100**, 1588–1591.

MERLISS, R. R. (1971). Talc-treated rice and Japanese stomach cancer. *Science* **173**, 1141–1142.

MILLER, K., AND HARINGTON, J. S. (1972). Some biochemical effects of asbestos on macrophages. *Brit. J. Exp. Pathol.* **53**, 397–405.

MORGAN, A., HOLMES, A., AND GOLD, C. (1971). Studies of the solubility of constituents of chrysotile asbestos *in vivo* using radioactive tracer techniques. *Environ. Res.* **4**, 558–570.

NICHOLSON, W. J., MAGGIORE, C. J., AND SELIKOFF, I. J. (1972). Asbestos contamination of parenteral drugs. *Science* **177**, 171–173.

OCCELLA, E., AND MADDALON, G. (1963). X-ray diffraction characteristics of some types of asbestos in relation to different techniques of comminution. *Med. Lavoro* **54**, 628–636.

PEACOCK, P. R., AND PEACOCK, A. (1965). Asbestos-induced tumors in white Leghorn fowls. *Ann. N. Y. Acad. Sci.* **132**, 501–503.

PERNIS, B., AND CASTANO, P. (1971). Effect of asbestos on cells *in vitro*. *Med. Lavoro* **62**, 120–129.

PONTEFRACT, R. D., AND CUNNINGHAM, H. M. (1973). Penetration of asbestos through the digestive tract of rats. *Nature* (*London*) **243**, 352–353.

POTT, F., HUTH, F., AND FRIEDRICHS, K. H. (1972). Tumors of rats after i.p. injection of powdered chrysotile and benzo(*a*)-pyrene. *Zbl. Bakt. Hyg.* B **155**, 463–469.

REEVES, A. L., PURO, H. E., SMITH, R. G., AND VORWALD, A. J. (1971). Experimental asbestos carcinogenesis. *Environ. Res.* **4**, 496–511.

REEVES, A. L., PURO, H. E., SMITH, R. G., AND VORWALD, A. J. (1972). Fibrogenicity and carcinogenicity of amosite, crocidolite, and chrysotile in animal experiment. *Amer. Ind. Hyg. Assoc. J.* **33**, A 79.

RICKARDS, A. L., AND BADAMI, D. V. (1971). Chrysotile asbestos in urban air. *Nature* (*London*) **234**, 93–94.

ROBOCK, K., AND KLOSTERKÖTTER, W. (1971). Biological action of different asbestos dusts with special respect to fibre length and semiconductor properties. *In* "Inhaled Particles III" (W. H. Walton, Ed.), pp. 465–475. Unwin Bros. Ltd., Old Woking, Surrey.

ROE, F. J. C. (1968). Experimental asbestos carcinogenesis. *Food and Cosmet. Toxicol.* **6**, 566–568.

ROE, F. J. C., WALTERS, M. A., AND HARINGTON, J. S. (1966). Tumor initiation by natural and contaminating asbestos oils. *Intern. J. Cancer* **1**, 491–495.

ROY-CHOWDHURY, A. K., MOONEY, T. F., AND REEVES, A. L. (1973). Trace metals in asbestos carcinogenesis. *Arch. Environ. Health* **26**, 253–255.

RYLEV, L. N. (1972). Morphological lesions in rat lungs induced by intratracheal injection of pure chrysotile asbestos and a chrysotile asbestos–benzo(*a*)pyrene mixture. *Vop. Oncol.* **18**, 40–45.

SCHNITZER, R. J., AND PUNDSACK, F. L. (1970). Asbestos hemolysis. *Environ. Res.* **3**, 1–13.

SELIKOFF, I. J., AND HAMMOND, E. C. (1971). Asbestos exposure to coke oven operators. *J. Occup. Med.* **13**, 496–497.

SELIKOFF, I. J., BADER, R. A., BADER, M. E., CHURG, J., AND HAMMOND, E. C. (1967). Asbestos and neoplasia. *Amer. J. Med.* **42**, 487–496.

SELIKOFF, I. J., HAMMOND, E. J., AND CHURG, J. (1968). Asbestos exposure, smoking and neoplasia. *J. Amer. Med. Assoc.* **204**, 104–110.

SELIKOFF, I. J., HAMMOND, E. C., AND CHURG, J. (1970). Mortality experiences of asbestos insulation workers. *In* "Proceedings of the International Conference of Pneumoconiosis" (H. A. Shapiro, Ed.), pp. 180–186. Oxford Univ. Press, Cape Town.

SELIKOFF, I. J., HAMMOND, E. C., AND CHURG, J. (1972a). Carcinogenicity of amosite asbestos. *Arch. Environ. Health* **35**, 183–186.

SELIKOFF, I. J., NICHOLSON, W. J., AND LANGER, A. M. (1972b). Asbestos air pollution. *Arch. Environ. Health* **25**, 1–13.

SMITH, W. E., MILLER, L., CHURG, J., AND SELIKOFF, I. J. (1965). Mesotheliomas in hamsters following intraperitoneal injection of asbestos. *J. Mt. Sinai Hosp. N. Y.* **32**, 1–8.

SPEIL, S., AND LEINEWEBER, J. P. (1969). Asbestos minerals in modern technology. *Environ. Res.* **2**, 166–208.

STANTON, M. F., BLACKWELL, R., AND MILLER, E. (1969). Experimental pulmonary carcinogenesis with asbestos. *Amer. Ind. Hyg. Assoc. J.* **30**, 236–244.

STANTON, M. F., AND WRENCH, C. (1972). Mechanisms of mesothelioma induction with asbestos and fibrous glass. *J. Nat. Cancer. Inst.* **48**, 797–821.

STOKINGER, H. E. (Chairman) (1973). Threshold limit values for chemical substances in workroom air. Am. Conf. Govt. Indust. Hygienists, Cincinnati.

SUZUKI, Y., AND CHURG, J. (1969). Formation of the asbestos body–A comparative study with three types of asbestos. *Environ. Res.* **3**, 107–118.

TIMBRELL, V. (1970). Characteristics of the International Union Against Cancer Standard Reference Samples of Asbestos. *In* "Proceedings of the International Conference on Pneumoconiosis" (H. A. Shapiro, Ed.). pp. 28–38. Oxford Univ. Press, Cape Town.

TIMBRELL, V., GILSON, J. C., AND WEBSTER, I. (1968). UICC standard reference samples of asbestos. *Inter. J. Cancer* 3, 406–408.

TURNOCK, A. C., BRYKS, S., AND BERTALANFFY, F. D. (1971). The synthesis of tritium-labeled asbestos for use in biological research. *Environ. Res.* 4, 86–94.

WAGNER, J. C. (1962) Experimental production of mesothelial tumors of the pleura by implantation of dusts in laboratory animals. *Nature (London)* **196**, 180–181.

WAGNER, J. C., AND BERRY, G. (1969). Mesotheliomas in rats following inoculation with asbestos. *Brit. J. Cancer* 23, 567–581.

WAGNER, J. C., GILSON, J. C., BERRY, G., AND TIMBRELL, V. (1971). Epidemiology of asbestos cancers. *Brit. Med. Bull.* **27**, 71–76.

WESTLAKE, G. E., SPJUT, H. J., AND SMITH, M. N. (1965). Penetration of colonic mucosa by asbestos particles. *Lab. Invest.* **14**, 2029–2033.