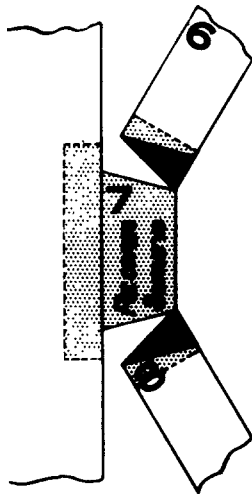


**HOW TO USE
THESE SEPARATORS**

Use one page for
each separation.

Select appropriate
tab, add further
identification if
desired, and cover
it with scotch
tape.

Cut off and discard
all tabs except the
one covered by tape.



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(2-56)

Experimental Asbestos Carcinogenesis¹

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Laboratory rodents were exposed to one of three mineralogical types of asbestos dust, amosite, crocidolite, or chrysotile. Inhalation exposures were accomplished in chambers where ballmilled specimens of these dusts were disseminated for 4 hours daily, 4 days weekly, at a mean atmospheric concentration of about 15 mg/m³. Additional animals were injected with these dusts intratracheally, intrapleurally, or intraperitoneally. Histopathologic studies showed a fibrotic reaction in all species to all three types of dusts, with amosite provoking the strongest such response especially in guinea pigs. Two pulmonary cancers were produced in rats exposed to the inhalation of crocidolite. Local injection into the pleural or peritoneal cavities caused 5 mesotheliomas in rats after chrysotile treatment and 6 mesotheliomas in rats and rabbits after crocidolite treatment. Guinea pigs and hamsters developed no tumors in this experiment, and with the dose used, there were no tumors in any species in the amosite group.

Ever since Lynch and Smith in 1935 suggested the association between asbestos exposure and lung cancer in man, this possible factor in environmental carcinogenesis has continued to attract attention. During the next 25 years, over 100 cases of distinct coexistence of asbestosis and pulmonary carcinoma were reported and evidence accumulated to show that at least certain types of asbestos, in addition to their fibrogenic property, apparently also possess carcinogenic potential (e.g., Doll, 1955).

In 1960 an additional aspect developed when Wagner and coworkers reported 32 cases of pleural mesothelioma, otherwise a rare tumor, among persons exposed to crocidolite in South Africa. Subsequently, numerous other cases were discovered in all parts of the world and connected in some cases to chrysotile exposure as well (e.g., Selikoff *et al.*, 1965). A third type of neoplasia which showed association with asbestos exposure was peritoneal tumors, of which 11 were described by Keal (1960) and 9 by Enticknap and Smither (1964). It was speculated that transfer of the carcinogen to the peritoneum was by hematogenic spread, and the specific vulnerability of this organ resulted from a stimulus due to movements between the peritoneal surfaces. Recently, possible association between asbestos exposure and cancer of the gastrointestinal tract (Hammond *et al.*, 1965) or of the hematopoietic system (Gerber, 1970) were also reported.

Experimental work on the asbestos lesion was commenced by Gloyne as early as 1930 and continued on a larger scale by Vorwald *et al.* (1951). The early work showed the principal action of asbestos fibers to be the elicitation of a foreign-

body reaction with giant bodies," leading to extensive vervet monkeys, and guinea pigs in the stated order with amosite. Holt and associates reported various kinds of asbestos fibrosis, and it was suggested that the situation in 1967, 1970), who also reported a pulmonary response to asbestos by macrophages to giant cells.

Gross and DeTreville (1967) and found the lesion nonproductive. This difference was attributed to the differential reactivity of the asbestos fiber was not a glass under similar exposure (Treville, 1969). On the other hand, it occurred after different names (when the name referring to their iron content).

Attention was also directed to the length. There was much evidence that much of its harmful potential was its transportability (Hirschman, 1953) indicate that considerable damage may result from grinding below the threshold of effective inactivation (1951). 10 μ by Wagner *et al.* (1966) considered fibrous for pathogenicity; he found that fibrogenic, the somewhat larger surface area.

In 1967, MacNab and Finkelstein reported that chrysotile dust, which was suspended less well to the inhaled air, both of which produced lesions (crocidolite, amosite, and chrysotile) (Secchi and Rezzonico, 1966). The hemolytic action of chrysotile and other sodium salt. It was observed that the surface of chrysotile fibers had catalytic activity, suggesting that the membrane lipoproteins, or if they were the key properties in the lesion (Schnitzer and Bunesco, 1970).

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Asbestosis¹

AND ARTHUR J. VORWALD¹

School of Medicine,
18207

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body reaction with giant cells, granulation tissue, and appearance of "asbestos bodies," leading to extensive collagenous fibrosis. Wagner (1963) exposed rabbits, vervet monkeys, and guinea pigs and found these species increasingly vulnerable in the stated order with chrysotile; all three species responded equally severely to amosite. Holt and associates (1964-1966) exposed rats and guinea pigs to various kinds of asbestos. Phagocytosis of the dust led to "asbestos body" formation, and it was suggested that phagocytosis is a prerequisite to the fibrogenicity of asbestos. The situation was examined electron microscopically by Davis (1963, 1967, 1970), who also concluded that phagocytosis was a key event in the pulmonary response to asbestos, and postulated the coalescence of dust-laden macrophages to giant cells or their conversion to fibroblasts.

Gross and DeTreville (1967) produced experimental asbestosis with chrysotile, and found the lesion nonprogressive in rats but progressive and lethal in hamsters. This difference was attributed to the relative efficiencies of pulmonary clearance as well as to differential reactivity of pulmonary tissue. It appears that geometry of the asbestos fiber was not a paramount factor in pathogenicity, since filamentous glass under similar exposure conditions was essentially inert (Gross and DeTreville, 1969). On the other hand, "asbestos bodies" appeared to be nonspecific and occurred after different kinds of fibrous dust inhalation so that the change of their name (when the nature of the fiber was not known) to "ferruginous bodies," referring to their iron content, was suggested (Gross *et al.*, 1967, 1969).

Attention was also directed at the correlation between fibrogenicity and fiber length. There was much evidence to suggest that very finely ground asbestos loses much of its harmful potential, presumably because of its improved lymphogenic transportability (Hilscher *et al.*, 1970), but the studies of Occella and Maddalon (1953) indicate that considerable loss of crystallinity (up to 85% for chrysotile) may result from grinding, especially if accomplished by cutting action. The threshold of effective median fiber length was regarded as 20 μ by Vorwald (1951), 10 μ by Wagner (1963), and 2 μ by Holt *et al.* (1964). However, Kogan *et al.* (1966) considered fiber length or indeed fibrous structure as nonessential for pathogenicity; he found chrysotile and associated serpentine rock both fibrogenic, the somewhat greater effect of chrysotile attributable to its larger surface area.

In 1967, MacNab and Harington called attention to the hemolytic activity of chrysotile dust, which was comparable to that of quartz dust although it responded less well to the inhibitory action of polyvinylpyridine-N-oxide or aluminum, both of which protected cells against lysis by silica. The amphiboles (crocidolite, amosite, and antophyllite) were much less hemolytic than chrysotile (Secchi and Rezzonico, 1968) and Schnitzer and Pundsack (1970) observed that the hemolytic action of chrysotile could be inhibited by carboxymethyl-cellulose ether sodium salt. It was observed that substances which were well adsorbed on the surface of chrysotile fibers were the most potent antagonists of the hemolytic activity, suggesting that the adsorptive capacity of the fiber possibly involving membrane lipoproteins, or its chelation potential involving magnesium hydroxide, were the key properties in this phenomenon (MacNab and Harington, 1967; Schnitzer and Bunesco, 1970).

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The first experimental production of lung tumors with asbestos apparently goes back to Nordmann and Sorge in 1941 and similar experiments were conducted later by Lynch *et al.* (1957). These experiments were done on mice which inhaled chrysotile dust, and the positive results consisted of accentuation of the natural tendency of these animals to develop pulmonary adenomas. More definitive results were obtained with implantation of dusts into the pleural cavity of rats (Wagner, 1962; Wagner and Berry, 1969; Berry and Wagner, 1969; Donna, 1970), hamsters (Smith *et al.*, 1965), or into the air sacs of fowls (Peacock and Peacock, 1935), and in 1967 Gross and DeTreville obtained pulmonary cancers in rats exposed to the inhalation of chrysotile. In this paper, we report two cases of pulmonary carcinoma in rats after inhalation of ercidolite and several pleural and peritoneal mesotheliomas in rats or rabbits after implantation of either ercidolite or chrysotile.

MATERIALS AND METHODS

DUST SPECIMENS

Commercial samples¹ of the three most important mineralogical varieties of asbestos, i.e., amosite, ercidolite, and chrysotile, were ballmilled in glazed ceramic jars with stainless steel balls for about 10 days, and the resultant samples were forced through a $\frac{1}{4}$ inch mesh screen for the disintegration of larger clumps. The dusts so obtained were examined by light and electron microscopy, x-ray diffraction, and atomic absorption spectrophotometry.²

Electron microscopic particle sizing gave the following range of values based on numerous counts for the mean length and mean diameter of the several fibers, respectively: Amosite, 3–5 and 0.2–0.5 μ ; ercidolite, 3–6 and 0.4–0.5 μ ; and chrysotile, 6–15 and 0.2 μ . The upper limit of fiber length, as determined with the light microscope, was 55–130 μ , comprising 0.001–0.015% of the dust. X-ray diffraction study gave the values shown in Table 1 as compared to those reported for the standard reference samples of the International Union Against Cancer [UICC] (Timbrell *et al.*, 1963).

Table 2 summarizes the results of chemical analysis, showing that substantial amounts of nickel, and in some cases also of manganese and/or chromium but not cobalt, were added to the dusts during experimental preparation. Analysis was also performed for beryllium, showing values under 1 ppm in all cases.

INHALATION EXPOSURES

In three 12 $\times$ 12 ft rooms with 2 perforated walls, 206 rats, 106 rabbits, 139 guinea pigs, and 214 hamsters, of both sexes and 2–3-months old, were about evenly distributed. One wall served as the supply, and the opposite wall as the exhaust. The asbestos dusts were disseminated into these chambers with the aid of hammer mill and fan systems essentially according to Holt *et al.* (1965), and

¹ Amosite, W-3 fibers; Erclidolite, S-Blue fibers; and Chrysotile, 3-T fibers. These samples were obtained by courtesy of the Johns-Manville Corporation.

² R. C. A. electron microscope, model EMU 3-E; G. E. X-ray diffraction apparatus, Model XRD-5; and Perkin-Elmer atomic absorption spectrophotometer, Model 303.

FEATURES OF

Asbestos type	Pe No
Amosite	1
	2
	3
	4
	5
Erclidolite	1
	2
	3
	4
	5
	6
	7
Chrysotile	1
	2
	3
	4
	5

^a In CuK α -radiation, $\lambda = 1.54$.

^b Principal diffraction angles

^c Interplanar lattice spacings

CHEM

Asbestos type	Elem
Amosite	Co
	Cr
	Mn
	Ni
Erclidolite	Co
	Cr
	Mn
	Ni
Chrysotile	Co
	Cr
	Mn
	Ni

^a As shipped.

^b As collected on millipore filter

^c Timbrell *et al.* (1968).

TABLE 1
FEATURES OF X-RAY DIFFRACTION DIAGRAM OF ASBESTOS DUSTS

Asbestos type	Peak No. ^a	2 θ ^b		d ^c	
		UICC Std	Exp.	UICC Std	Exp.
Amosite	1	10 7°	10 7°	8.26	8.26
	2	27 3°	—	3 27	—
	3	29 1°	29 2°	3 07	3.06
	4	32 3°	32 5°	2.77	2.75
	5	—	34 2°	—	2.62
Crocidolite	1	10.5°	10 5°	8.43	8.42
	2	19.7°	—	4 51	—
	3	26 0°	—	3.43	—
	4	28.7°	28 6°	3.11	3 12
	5	32 9°	32 9°	2.72	2.72
	6	34.4°	—	2.61	—
	7	35 3°	35 3°	2.54	2 54
Chrysotile	1	12.0°	12.4°	7.38	7 19
	2	19.5°	—	4.55	—
	3	24 3°	24 7°	3 66	3.62
	4	36 5°	36 8°	2.46	2 44
	5	60 2°	60 5°	1.54	1 53

^a In CuK α -radiation, $\lambda = 1.51 \text{ \AA}$.

^b Principal diffraction angles.

^c Interplanar lattice spacings in $\text{\AA}$.

TABLE 2
CHEMICAL ANALYSIS OF ASBESTOS SAMPLES

Asbestos type	Element	Concentration ppm		
		Raw ^a	Processed ^b	UICC std ^c
Amosite	Co	13	14	7-11
	Cr	—	—	32-35
	Mn	688-851	824-990	—
	Ni	40	97-108	34
Crocidolite	Co	11	12	2-9
	Cr	—	—	16-22
	Mn	—	—	864-870
	Ni	70-73	88-111	13-100
Chrysotile	Co	36-39	38-41	45-46
	Cr	287-361	382-402	316-490
	Mn	173-219	139-153	443-510
	Ni	222-294	374-461	795-990

^a As shipped.

^b As collected on millipore filters in chamber.

^c Timbrell *et al.* (1968).

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experiments were conducted
are done on mice which in-
sted of accentuation of the
ary adenomas. More defini-
s into the pleural cavity of
and Wagner, 1969; Donna,
sacs of fowls (Peacock and
obtained pulmonary cancers
paper, we report two cases
acidolite and several pleural
ter implantation of either

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sallmilled in glazed ceramic
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ving range of values based
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t, 3-6 and 0.4-0.5 μ ; and
gth, as determined with the
15% of the dust. X-ray dif-
ompared to those reported
nal Union Against Cancer

s, showing that substantial
e and/or chromium but not
preparation. Analysis was
ppm in all cases.

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3-months old, were about
d the opposite wall as the
ese chambers with the aid
to Holt *et al.* (1965), and

tile, 3-T fibers. These samples

y diffraction apparatus, Model
, Model 303.

achieved fairly even distribution. To insure that all animals received equivalent dosages, all cage racks were reoriented routinely and cages on the racks were repositioned with respect to their height from the floor. The used asbestos was exhausted from each chamber into plastic collection bags.

The chamber atmospheres were checked daily by membrane filter sampling technique throughout the experiment. The obtained values were remarkably uniform, giving the following means ($\pm$ SD) for the three rooms: amosite, 48.2 ± 1.4 , crocidolite, 48.7 ± 2.4 , and chrysotile, 47.4 ± 1.7 mg/m³. Fiber count by light microscopy with a resolution of 0.8μ gave the following results: amosite 864, crocidolite 1105, and chrysotile 54 million/m³. This included only particles with an axis/diameter ratio of about 3:1 or more. In addition, there was also substantial nonfibrous component in the dusts. The exposures to these atmospheres were conducted for 4 hour/day, 4 days/week, with each Friday reserved as clean-up day.

INJECTIONS

Intratracheal, intrapleural, and intraperitoneal injections of all three types of asbestos dusts were given to certain additional animals in order to study the tissue reaction. For these purposes, the dusts were prepared according to Badollet and Gantt (1965) by treating the specimens in a Waring Blendor, sieving, and regrinding in an agate mortar for about 15 minutes. The asbestos was then suspended in isotonic saline at a concentration of 20 mg/ml, autoclaved, and administered in simple doses as shown in Table 3.

NECROPSIES AND ATTRITIONAL MORTALITY

Periodic killings were scheduled at 1, 3, 6, 12, 18 and 24 months after injection or commencement of inhalation, consuming about one-half of the total animal complement in each group. The other half of the animals was allowed to live to an age of approximately 3 years. Total cumulative exposure hours in the chambers were 1496 (amosite), 1555 (crocidolite), and 1644 (chrysotile).

Attritional mortality was varied with the 4 animal species under study,^a the rats

TABLE 3
INJECTION DOSAGES OF ASBESTOS DUSTS^a

Species	Intratracheal				Intrapleural				Intraperitoneal			
	ml		No. animals		ml		No. animals		ml		No. animals	
	dose	a	b	c	dose	a	b	c	dose	a	b	c
Rats	0.3	16	13	15	0.5	15	13	12	1.0	11	13	13
Rabbits	0.5	12	15	14	0.8	13	13	14	—	—	—	—
Guinea pigs	0.3	16	15	15	0.5	11	17	11	—	—	—	—
Hamsters	0.2	13	10	—	0.5	14	12	9	0.5	—	—	5

^a a—Amosite, b—Crocidolite, c—Chrysotile.

^a Charles River CD rats; Shankin Farms Dutch rabbits; Lightner-Hartley guinea pigs; and Lakeview Syrian Golden hamsters.

being apparently the hard some endemic pneumonia numbers. Guinea pig loss early and the typical preventive medication was together with the total m

The attritional mortality examined. It appears that injurious (one-year survival crocidolite (0.840). Sim Strongest effects were seen halving amosite vs crocidolite at the 99.9% confidence

In view of extensive p under study, the number posed animals was small. Ten such rats, 10 rabbit received no exposure or rat, at about 1 year of monary tissue; and one the terminal bronchiole in the control colony. Pi (1971) showed that endo bronchiolitis in rats and Pulmonary fibrosis was not observed among sex here in the past.

THE

All three types of asb tiocytes as the earliest p This was followed by animals. Squamous met the crocidolite group, w hyperplasia (Fig. 1). B metaplasia, and out of longer, 5 displayed pr killed at the end of cr lung, (Fig. 2). There w

^a No survivors at 1 year

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MORTALITY

and 24 months after injection half of the total animal com- s was allowed to live to as- sure hours in the chambers (chrysotile). species under study; the rats

RESULTS

Intraperitoneal				
	ml	No. animals		
	dose	a	b	c
2	1.0	11	13	1
4	—	—	—	—
1	—	—	—	—
9	0.5	—	—	—

thinner-Hartley guinea pigs

being apparently the hardest and showing the best survival record. Rabbits had some endemic pneumonia but they also survived to their senescence in sufficient numbers. Guinea pig losses were heavier, and hamsters, especially, tended to die early and the typical survival for the latter species was 13–15 months. No preventive medication was given. The inventory of live animals at different times, together with the total malignancy incidence (cf. Results) is collated in Table 4.

The attritional mortality of each species in the three chambers was statistically examined. It appears that in terms of overall lethality, amosite inhalation was most injurious (one-year survivorship ratio 0.765),¹ followed by chrysotile (0.775) and crocidolite (0.840). Similar trends were seen in the injected animals also. Strongest effects were seen in guinea pigs, where the comparison of animals inhaling amosite vs crocidolite ($X^2 = 10.02$) showed a difference that was significant at the 99.9% confidence level.

RESULTS

CONTROL ANIMALS

In view of extensive previous departmental experience with the animal strains under study, the number of controls set aside from the same shipment as the exposed animals was small, in order to allow maximum numbers to receive exposure. Ten such rats, 10 rabbits, 12 guinea pigs, and 8 hamsters served as controls and received no exposure or treatment. These animals survived up to 1–3 years. One rat, at about 1 year of age, showed epithelial hyperplasia and necrosis of pulmonary tissue; and one rabbit, at 10 months, showed histiocytic proliferation at the terminal bronchiole with inflammation. There was no other pathology noted in the control colony. Previous experience with these animal strains (e.g. Reeves, 1971) showed that endemic conditions unrelated to any exposure include purulent bronchiolitis in rats and alveolar epithelial hyperplasia in rats as well as rabbits. Pulmonary fibrosis was infrequent, and metaplasia or neoplasia in the lungs was not observed among several thousand unexposed animals of each species studied here in the past.

THE RESPONSE TO INHALATION EXPOSURES

All three types of asbestos caused accumulation of hemosiderin-containing histiocytes as the earliest pulmonary response, associated with inflammatory reaction. This was followed by fibrosis in the region of the terminal bronchiole in a few animals. Squamous metaplasia in this area was also often prominent especially in the crocidolite group, where in some rats it progressed to a pseudo-epitheliomatous hyperplasia (Fig. 1). Both crocidolite and chrysotile also induced columnar-type metaplasia, and out of 40 rats which survived chrysotile inhalation for 2 years or longer, 5 displayed prominent benign pulmonary adenomatosis. Out of 31 rats killed at the end of crocidolite-exposure, 2 had squamous-cell carcinoma of the lung (Fig. 2). There was no neoplasia in the amosite group.

¹ No survivors at 1 year/No. all animals at start.

TABLE 4
MORTALITY AND MALIGNANCY INCIDENCE OF ANIMALS EXPOSED TO ASBESTOS

Species	Route	Amosite				Incidence of malignancy	Crocidolite				Incidence of malignancy	Chrysotile				Incidence of malignancy
		Inventory					Inventory					Inventory				
		0	6	12	24		0	6	12	24		0	6	12	24	
		months					months					months				
Rats	Inhalation	77	67	63	42	—	69	61	61	31	2 pul. ca. ^a both at 29 mo.	60	49	49	40	—
	Intratracheal	16	10	6	1	—	13	6	3	1	—	15	8	6	0	—
	Intrapleural	15	6	4	0	—	13	5	2	0	1 pleu. meso. ^b at 16 mo.	12	6	4	1	2 pleu. meso. ^b at 9 & 14 mo.
	Intraperitoneal	11	3	0	0	—	13	7	4	0	3 peri. meso. ^c at 12, 13, & 17 mo.	13	7	0	0	3 peri. meso. ^c at 7, 9, & 11 mo.
Rabbits	Inhalation	38	23	20	13	—	32	21	18	15	—	36	30	26	23	—
	Intratracheal	12	10	8	6	—	15	8	8	3	—	14	6	6	4	—
	Intrapleural	13	4	2	2	—	13	6	3	0	2 pleu. meso. ^b at 22 & 24 mo.	14	5	4	2	—
Guinea pigs	Inhalation	45	20	17	5	—	50	35	33	4	—	44	23	21	0	—
	Intratracheal	16	8	4	3	—	15	7	3	2	—	15	6	3	0	—
	Intrapleural	11	2	2	0	—	17	3	1	0	—	11	2	1	0	—
Hamsters	Inhalation	73	57	50	0	—	67	51	45	0	—	74	56	49	0	—
	Intratracheal	13	6	6	0	—	10	2	2	0	—	0	0	0	0	—
	Intrapleural	14	8	6	0	—	12	2	1	0	—	9	2	2	0	—
	Intraperitoneal	0	0	0	0	—	0	0	0	0	—	8	3	0	0	—

^a Pulmonary carcinoma (squamous cell pattern)

^b Pleural mesothelioma (fibrosarcoma pattern).

^c Peritoneal mesothelioma (fibrous, papillary, osteogenic, or epithelial patterns).

FIG. 2. Specimen No. 1012 of crocidolite for 1588 cumulative cell carcinoma, well-differentiate

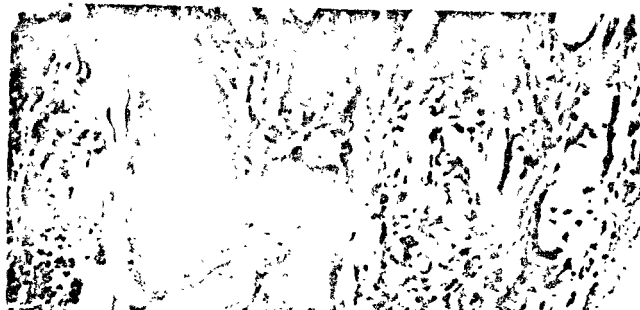
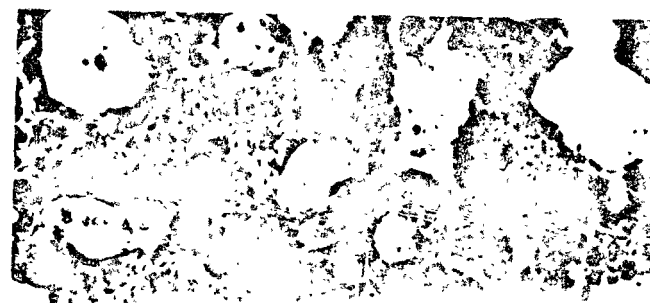


FIG. 1. Specimen No. 101 of crocidolite for 1588 cumulative cell carcinoma, well-differentiate



Species	Site of tumor	Number of animals with tumor															
		45	20	17	5	—	50	35	33	4	—	44	23	21	0	—	—
Rats	Intratracheal	16	8	4	3	—	15	7	3	2	—	15	6	3	0	—	—
	Intrapleural	11	2	2	0	—	17	6	1	0	—	11	2	1	0	—	—
	Intraperitoneal	0	0	0	0	—	0	0	0	0	—	0	0	0	0	—	—
Hamsters	Intratracheal	13	6	6	0	—	10	2	2	0	—	0	0	0	0	—	—
	Intrapleural	14	8	6	0	—	12	2	1	0	—	9	2	2	0	—	—
	Intraperitoneal	0	0	0	0	—	0	0	0	0	—	8	3	0	0	—	—

^a Pulmonary carcinoma (squamous cell pattern).

^b Pleural mesothelioma (fibrosarcoma pattern).

^c Peritoneal mesothelioma (fibrous, papillary, osteogenic, or epithelial patterns).

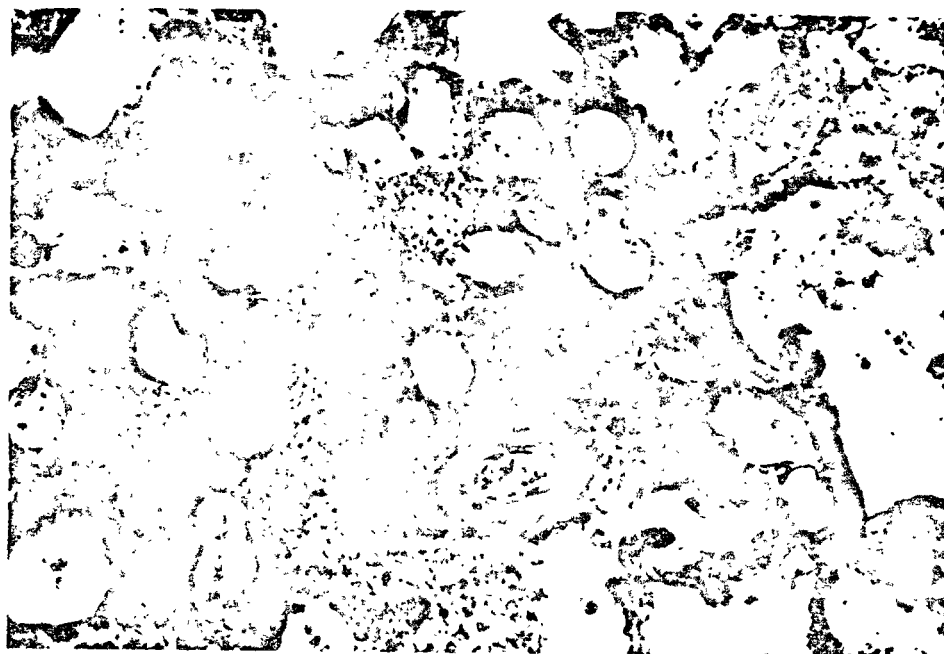


FIG. 1. Specimen No. 1012-50. Lung of female rat, age 140 weeks, exposed to inhalation of crocidolite for 1585 cumulative hours. The bronchiolar epithelium shows squamous metaplasia. $\times 230$.



FIG. 2. Specimen No. 1012-67. Lung of female rat, age 140 weeks, exposed to inhalation of crocidolite for 1585 cumulative hours. Portion of 5 mm lesion, cavitating at center. Squamous cell carcinoma, well-differentiated. $\times 200$.

THE RESPONSE TO INTRATRACHEAL INJECTIONS

Generally, the early response was similar to that seen after inhalation. In addition, necrosis of the bronchiolar epithelium in the region of the fibers was present in most animals, especially in the chrysotile group. Giant-cell accumulation followed in some instances. Several guinea pigs also showed terminal bronchiolar epithelial proliferation. One rat of the chrysotile group showed focal fibrous adhesion to the diaphragm. There was no neoplasia in any of the groups.

THE RESPONSE TO INTRAPLEURAL INJECTIONS

Histiocytic and foreign-body response was typical of all groups especially among rabbits. Fibrosis followed in most cases, occasionally with benign proliferative features. One rat (486 days after crocidolite injection) and two rabbits (666 and 725 days after crocidolite injection, respectively) had spindle-cell mesotheliomas of the pleura. Two rats (278 and 419 days after chrysotile injection, respectively) had fibromatous mesotheliomas of the pleura (Fig. 3) with invasion of the diaphragm (Fig. 4), presenting as dense proliferative lesions attached to the diaphragm. There was no neoplasia in the amosite group.

THE RESPONSE TO INTRAPERITONEAL INJECTIONS

The response was generally similar to that seen in the pleura after the intrapleural injections. All animals showed initial inflammatory response associated with histiocyte and giant-cell accumulation, followed by fibrosis. The fibrosis was



FIG. 3. Specimen No 977-39. Pleura and lung of male rat, age 76 weeks, sacrificed 419 days after an intrapleural injection of chrysotile. The pleural surface contains a fibrous mesothelioma presenting a dense, fibrous-appearing adhesion to the diaphragm. $\times 59$.

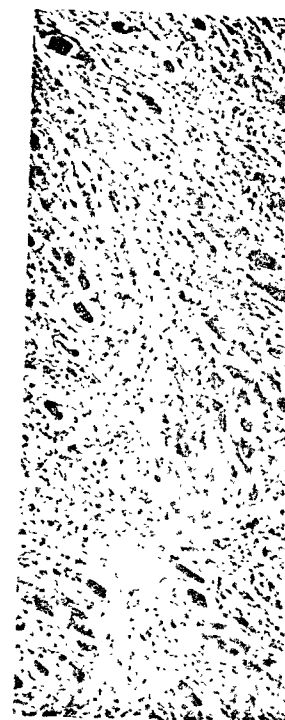


FIG. 4. Same specimen as Fig. 3, showing invasion of the diaphragm.

particularly advanced in the case of the rat which survived crocidolite injection. The following are the results of the study: One with fibrous mesothelioma and osteogenic sarcoma of the humerus and spindle-cell sarcoma of the muscle; and one, on the 263rd day, respectively, of muscle); and one, on the 261st day, of muscle). There were no neoplasia. There were no

Among the 13 cases of lung cancer among more than 500 animals (cf. Table 4), 2 were put to the inhalation of crocidolite and elsewhere, the cause of lung cancer is clear, but it must be emphasized (cf. Table 4) vs 0 in 40-42 (in different according to the

INJECTIONS

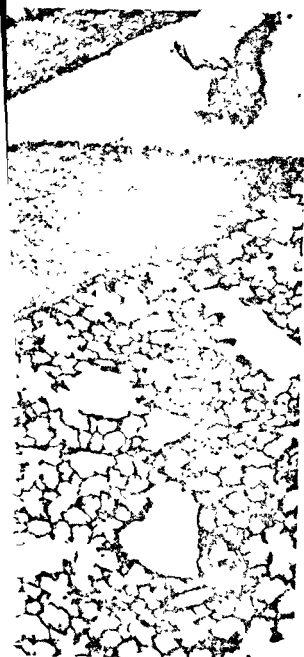
en after inhalation. In addition of the fibers was present. Giant-cell accumulation followed terminal bronchiolar up showed focal fibrous ad- of the groups.

INJECTIONS

all groups especially among with benign proliferative fea- and two rabbits (666 and 725 indle-cell mesotheliomas of tile injection, respectively)) with invasion of the dia- esions attached to the dia-

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the pleura after the intra- natory response associated y fibrosis. The fibrosis was



age 76 weeks, sacrificed 419 face contains a fibrous meso- iaphragm. $\times 50$.

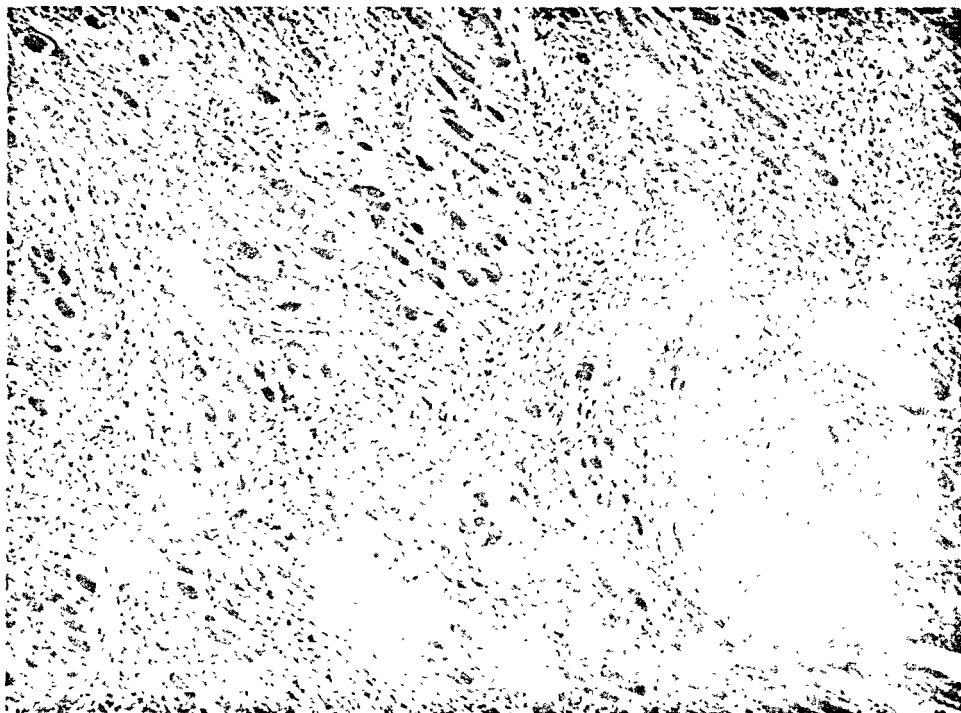


FIG. 4. Same specimen, diaphragm. Invasion of tumor from the pleura $\times 50$.

particularly advanced in animals of the amosite group. One rat, 263 days after crocidolite injection, showed mesothelial proliferation and 3 out of 4 rats which survived crocidolite injection one year or longer had peritoneal mesotheliomas as follows: One with fibrous and papillary pattern on the 366th day (Fig. 5); one with osteogenic sarcoma pattern on the 372nd day (Fig. 6); and one with reticulum- and spindle-cell sarcoma pattern on the 521st day (Fig. 7). In the chrysotile group, there were also 3 cases of mesothelioma. Two of these, on the 211th and 263rd day, respectively, had spindle-cell sarcoma pattern (the latter with invasion of muscle); and one, on the 333rd day, was of solid epithelial type. One additional animal, on the 261st day, had atypical fibrosis suggestive of but not confirmed as neoplasia. There were no tumors in the amosite group.

DISCUSSION

Among the 13 cases of malignant neoplasia which were observed in this study among more than 500 animals of various species with adequate survival record (cf. Table 4), 2 were pulmonary carcinomas. Both of these were in rats exposed to the inhalation of crocidolite. In view of the known lack of spontaneous incidence of lung cancer in the Charles River CD rat as observed in this laboratory and elsewhere, the cause-and-effect relation between exposure and cancer seems clear, but it must be emphasized that the incidence of 2 in 31 (in the crocidolite group) vs 0 in 40-42 (in the amosite and chrysotile groups) is not significantly different according to the chi-square test ($X^2 = 2.12$). Therefore, it cannot be said

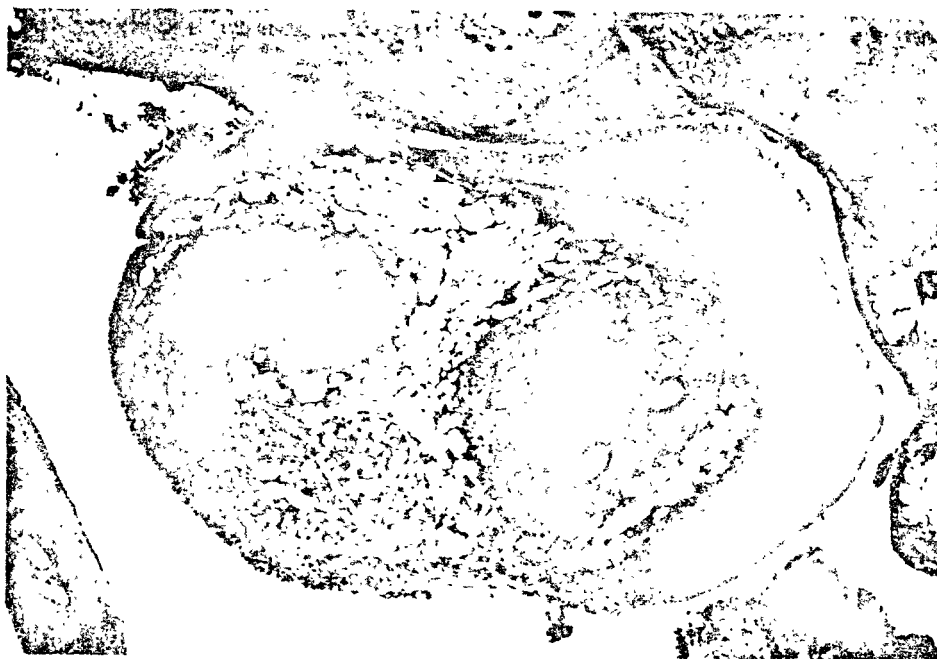


FIG. 5. Specimen No. 981-10. Peritoneum of male rat, age 63 weeks, sacrificed 366 days after an intraperitoneal injection of crocidolite. Mesothelioma, fibrous type, presenting grossly as extensive intraperitoneal spread of a white neoplasm. $\times 50$.



FIG. 6. Specimen No. 981-11. Peritoneum of male rat, age 64 weeks, sacrificed 372 days after an intraperitoneal injection of crocidolite, showing firm, pale, nodular lesions on the peritoneal surfaces. Mesothelioma, osteogenic sarcoma type. $\times 50$.

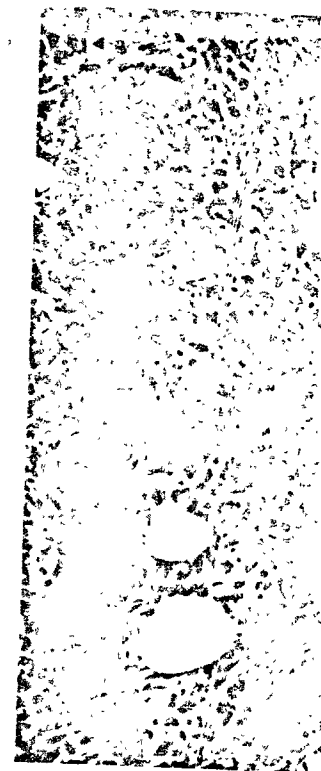


FIG. 7. Specimen No. 981-12. Peritoneum of male rat, age 64 weeks, sacrificed 372 days after an intraperitoneal injection of crocidolite, showing dense fibrous adhesions.

on the basis of these results than either amosite or chrysotile.

No mesotheliomas were produced in the pleura (Tables 3 and 4). This result they included rabbits as well as animals treated with crocidolite. The study (33) shows a difference in the fibrogenic response to crocidolite or chrysotile, with crocidolite being more fibrogenic than chrysotile, especially in guinea pigs than in rats.

No neoplasms were obtained in the number of animals so treated.

The operative principle of the present study is based on the speculations in the recent past.



63 weeks, sacrificed 366 days
 fibrous type, presenting grossly



64 weeks, sacrificed 372 days
 pale, nodular lesions on the

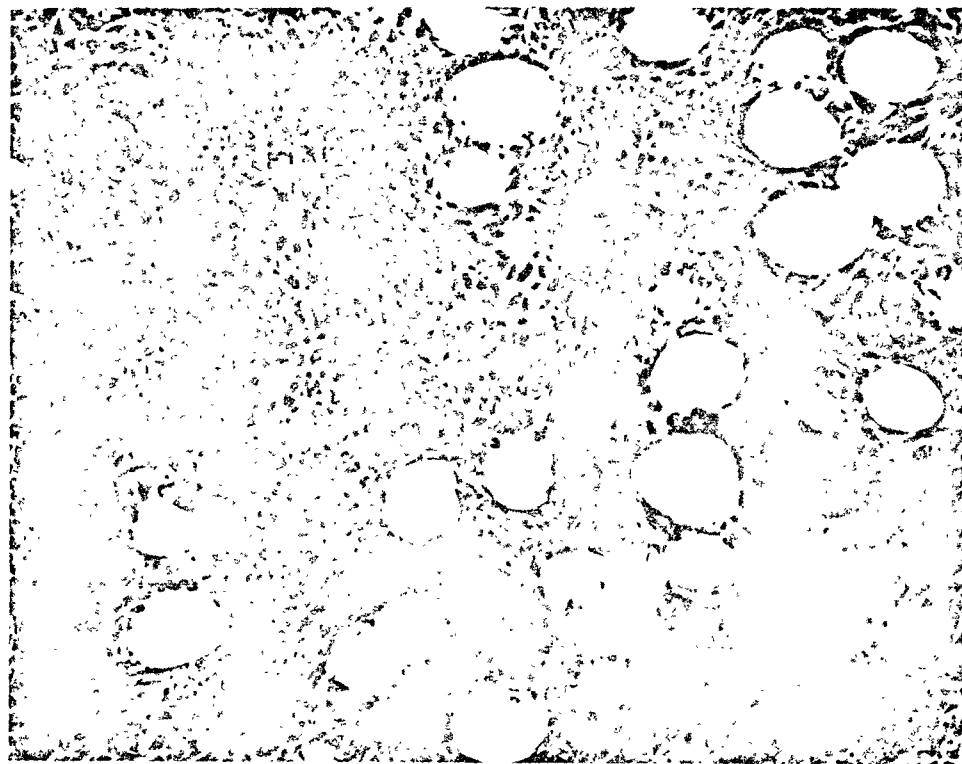


FIG. 7. Specimen No. 981-13. Peritoneum of male rat, age 85 weeks, sacrificed 521 days after an intraperitoneal injection of crocidolite. Mesothelioma, spindle cell sarcoma type, which showed dense fibrous adhesions between intestinal loops. $\times 200$.

on the basis of these results that crocidolite is more carcinogenic upon inhalation than either amosite or chrysotile.

No mesotheliomas were obtained after inhalation, but 5 such neoplasms were produced in the pleura and 6 in the peritoneum after local implantation (cf. Tables 3 and 4). This response occurred as early as 7 months after treatments, they included rabbits as well as rats, and were about evenly distributed between animals treated with crocidolite and chrysotile. No neoplastic lesions were produced with amosite. The statistical comparison of these figures (5-6 in 32 vs 0 in 33) shows a difference that is significant at the 98% confidence level ($X^2 = 5.95$). These studies therefore support the conclusion that amosite is less carcinogenic following local implantation into the pleura or peritoneum than either crocidolite or chrysotile, which was also observed by Wagner and Berry (1969). These observations are in interesting contradistinction to the finding that amosite was more fibrogenic in this study and caused much higher early mortality especially in guinea pigs than either crocidolite or chrysotile.

No neoplasms were obtained after intratracheal injection. It is possible that the number of animals so treated (10-16 of each species) was too small.

The operative principle of asbestos carcinogenesis was subject to numerous speculations in the recent past. The theory of purely mechanical irritation ("Op-

penheimer effect") was considered but ruled out by Harington and Roe (1965) and there is no evidence thus far that a crystallographic or chemical property inherent in the silicon-oxygen lattice of asbestos fibers is the essential carcinogenic entity. On the contrary, it may be noted that the two amphiboles, amosite and crocidolite, are not equally effective in this respect. Even though they both form harsh, relatively acid-resistant, and truly chain-structured fibers, amosite appears to be a weaker carcinogen than crocidolite. On the other hand, chrysotile which is mineralogically quite different from the amphiboles and is soft, acid-soluble, and not a chain-structured fiber but rather a microtubule, is apparently comparable in its carcinogenic potential to crocidolite. Current thinking favors the hypothesis that labile cations attached to the silicon-oxygen lattice, metallic contaminants, or organic compounds which may become adsorbed on the fibers either during their geological genesis or during commercial handling, may be the key factor in the carcinogenic potential of asbestos.

In 1962, Harington reported that certain virgin samples of asbestos contained cyclohexane-extractable oils composed of aromatic hydrocarbons, one of which was 3,4-benzpyrene. Furthermore, it was later pointed out that in addition to these natural trace constituents, asbestos may contain contaminants acquired during production, storage, or transport, such as the jute oils derived from storage bags. Tests for the tumor-promoting activity of these oils on mouse skin yielded considerable positive results with at least one variety of jute oil (Harington and Roe, 1965; Roe *et al.*, 1966). The work of Miller *et al.* (1965), Smith *et al.* (1970) as well as Pylev *et al.* (1969) confirmed the substantial and to some degree specific adsorption of 3,4-benzpyrene on asbestos fibers. However, Harington *et al.* in 1967 found that removal of these oils apparently decreased but did not abolish the carcinogenic activity of asbestos.

An alternate carcinogenic principle that may be present in or associated with asbestos are metallic elements. These may be covalently bound to the fiber, they may be attached to it by hydrogen bonding, ionic bonding, or adsorption. In many of these cases the metal may be detachable and/or exchangeable during *in vivo* interaction. The major metallic components present in asbestos are aluminum, magnesium, and iron; trace levels of chromium, nickel, and cobalt are also regularly found.

Among the major metallic components, iron has attracted interest. It was recalled that while iron itself is not regarded as carcinogenic, experimental tumors were produced by iron-dextran complexes where the bonding of iron to the polymer may have been similar to that in asbestos (Richmond, 1959).

Trace levels of chromium and nickel may play a role as these metals are suspect as environmental carcinogens. It is apparent from the results of chemical analysis (Table 2) that the dissemination methodology employed in this experiment caused substantial enrichment of the dusts in these metals, and similar factors were also noted in the experiments of Gross *et al.* (1937). Indeed, Cralley *et al.* (1967) pointed out that such exposures may be sustained by workers employed in the manufacture of asbestos textile products. Dixon *et al.* (1970) reported recently that Fe^{2+} and Cr^{6+} , both of which were extractable from a sample of chrysotile, inhibited the action of benzpyrene hydroxylase, a detoxifying enzyme that

presumably protects the induction of this enzyme (1967), suggesting intergenesis by certain metal

The authors are indebted consulting, to Kenneth R. construction and operation tenance and necropsies; and

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presumably protects the organism from the effects of benzpyrene. Inhibition of induction of this enzyme by $\text{Ni}(\text{CO})_4$ was observed previously by Sunderman (1967), suggesting interesting new possibilities in the understanding of carcinogenesis by certain metals, and conceivably by asbestos.

ACKNOWLEDGMENTS

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