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PLAINTIFF'S EXHIBIT

SA-486

Malignant Mesothelioma Induced by Asbestos and Zeolite in the Mouse Peritoneal Cavity

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The carcinogenicity of asbestos (amosite and chrysotile) and zeolite (fibrous erionite, mordenite, and synthetic zeolite 4A) were studied in the peritoneum of 586 BALB/C male mice after a single intraperitoneal or intraabdominal wall injection. As controls, 182 mice treated with and without saline solution were used. Both asbestos types and fibrous erionite frequently produced malignant peritoneal tumors after long latency; tumors developed in 93 of 394 animals (23.6%) treated with asbestos or fibrous erionite 7 months or more after administration. All of the induced peritoneal tumors were intimately associated with marked peritoneal fibrosis, in which asbestos or erionite fibers were regularly detected. Histopathologically, 83 (73 fibrous, 9 biphasic, and 1 epithelial) of 93 were consistent with malignant mesotheliomas. Other tumors consisted of 6 plasmacytomas, 1 histiocytoma, 1 liposarcoma, 1 osteosarcoma, and 1 adenocarcinoma of the pancreas. Two of the cases of mesotheliomas were associated with plasmacytoma. In many instances, the primary site of the mesotheliomas seemed to be multiple, the favorite sites being the omentum, mesentery, serosae of the gastrointestinal and genital organs, the diaphragm, the capsule of the liver and spleen, and the abdominal wall peritoneum. In these cases, asbestos or erionite-tissue burden followed by fibrosis was frequently observed. In addition to the 93 peritoneal tumors, 3 extraperitoneal tumors (1 fibrosarcoma and 2 rhabdomyosarcomas) were induced by amosite which was probably accidentally injected into the extraperitoneal connective tissue and the striated muscle tissue of the abdominal wall, respectively. These three tumors were also intimately associated with focal fibrosis in which amosite fibers were detected. Among the three different types of zeolite, only fibrous erionite showed striking carcinogenicity and marked fibrogenicity. The erionite-induced mesotheliomas were similar to those induced by asbestos in exhibiting long latency, in gross appearance, in histology, and in close association with fibrosis. Long-term persistence of asbestos or fibrous erionite around progenitor cells of the induced tumors and the consequent fibrosis seemed to be an important precondition of the malignant transformation of the progenitor cells.

INTRODUCTION

A single intraperitoneal administration of asbestos and other inorganic fibers is known as an effective experimental method for the production of malignant peritoneal mesothelioma in laboratory animals, including the mouse and rat. This animal model was used to investigate pathogenetic problems of malignant peritoneal mesothelioma, such as the development and course of the tumor after exposure to asbestos and zeolite, the incidence and latency of the tumor, favorite sites of the primary focus in the peritoneum, cell types of the tumor, and similarities and differences between the induced tumor and human malignant meso-

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thelioma. Answers to these pathogenetic problems were possible by completion of detailed anatomical and histopathological observation of the mouse peritoneums which were investigated between 0 and 23 months after exposure to asbestos or zeolite.

MATERIALS AND METHODS

A. Asbestos and zeolite. As minerals, the following were used: Amosite (U.I.C.C., milled 120 sec) and two types of chrysotile [(i) U.I.C.C., chrysotile B (milled 20 sec) and (ii) Calidria of Union Carbide Co.] were selected as asbestos samples. Two types of fibrous erionite, (natural fibrous erionite from the Resource International Company, Denver, Colo. and natural fibrous, less-contaminated erionite taken from Needle Peak, Pershing County, Nev.), mordenite (a mixture of fibrous and granular types from the Resource International Co.), and synthetic zeolite 4A (from Union Carbide Co.) were selected. In this paper, we call the former erionite Erionite I and the latter erionite Erionite II.

Size distribution and chemical analysis of all but one sample (Calidria chrysotile) were carried out by one of the authors (N.K.) by transmission electron microscopy and analytical electron microscopy. Size distribution of these mineral samples can be summarized as follows: (1) Amosite. Length; 93% were shorter than 7 μm and 6% were longer than 7.5 μm. Diameter: 99.9% were smaller than 1 μm in diameter. (2) U.I.C.C. chrysotile B. Length: 94% were shorter than 3 μm. Diameter: 98% were less than 0.1 μm. (3) Erionite I. Length: 90% were shorter than 8 μm and 6% were longer than 9.5 μm. Diameter: 82% were less than 1 μm and 8.7% were greater than 1.4 μm in diameter. (4) Erionite II. Length: 95% were shorter than 8 μm and 4% were longer than 9.5 μm. Diameter: 82% were less than 0.5 μm and 100% were less than 1 μm. (5) Mordenite. Length: 94% were less than 3 μm and 4% were longer than 38 μm. Diameter: 89% were less than 1 μm and 6.25% were longer than 1.4 μm. (6) Synthetic zeolite 4A. 2.4 μm in average length and 2.24 μm average diameter. Details of size distribution in each of the samples are shown in Charts 1 to 6.[1]

We did not characterize Calidria chrysotile. However, size distribution of this chrysotile has been reported by Langer *et al.* (1) as 92% of the fibers shorter than 3 μm and 4.6% longer than 5 μm in length, with almost all of the fibers freely separated from each other (250 to 400 Å in diameter). Chemical analysis of single fibers of the six samples was done by analytical electron microscopy. Results of the chemical analysis are shown in Table 1.

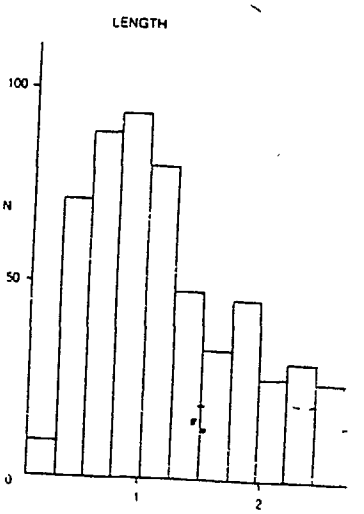
B. Doses of the mineral samples. Various doses of these mineral samples were used: 0.5 mg (Erionite II), 2 mg (amosite, U.I.C.C. chrysotile, Calidria chrysotile, and Erionite II), 10 mg (amosite, Erionite I, Erionite II, mordenite, and synthetic zeolite 4A), and 20 mg (amosite and U.I.C.C. chrysotile). These samples were suspended in 1 ml saline solution to be given to animals. Type and dose of samples which were used in each subgroup of animals are described in Table 2.

C. Animals. Seven hundred and sixty-eight male BALB/C mice were used in this study, and consisted of 291 asbestos treated, 295 zeolite treated, and 182

[1] The shaded bars in Charts 1, 3, 4, and 5 are composites representing fibers of a diameter or length greater than the number which appears above the bar in each case.

	L	D
MIN	0.16 μ	0.03 μ
MAX	53.04	1.36
AVE	2.69	0.33
SD*	4.37	0.20

*STANDARD DEVIATION



CHART

	L	D
MIN	0.09 μ	0.01 μ
MAX	9.48	0.11
AVE	1.44	0.05
SD*	1.38	0.02

*STANDARD DEVIATION

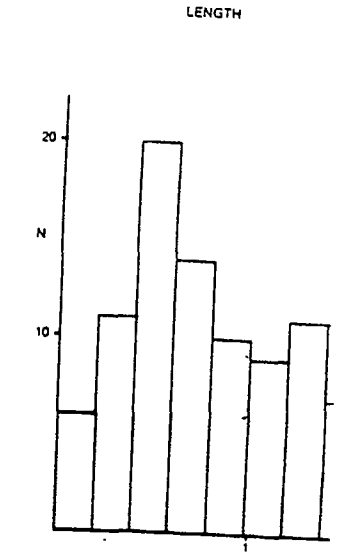


CHART 2. Cl

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. (6) Synthetic zeolite 4A. 2.4
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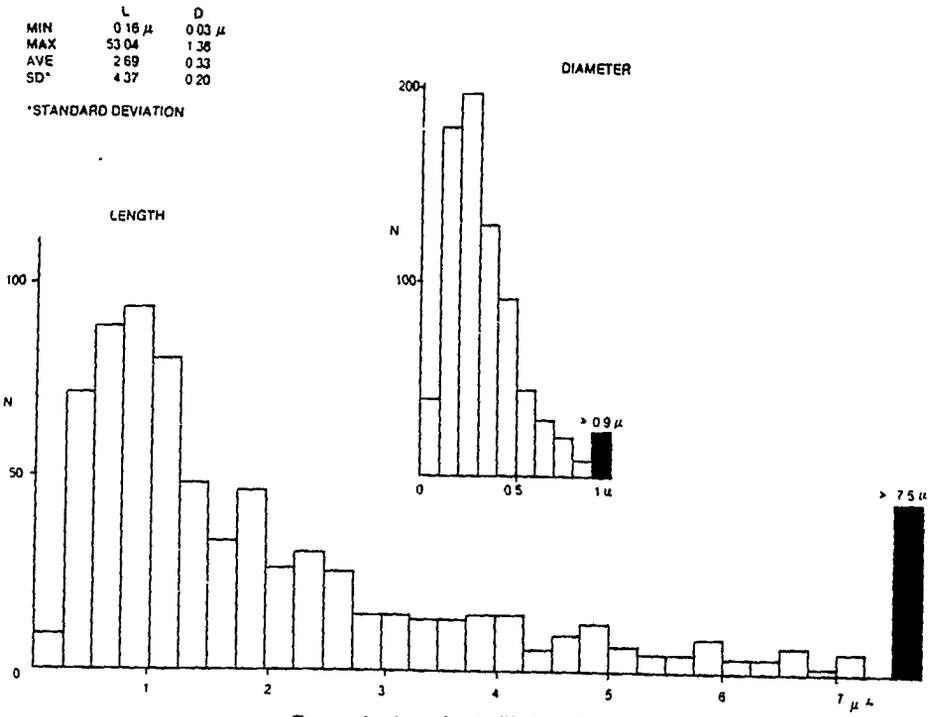


CHART 1. Amosite (milled). $N = 735$.

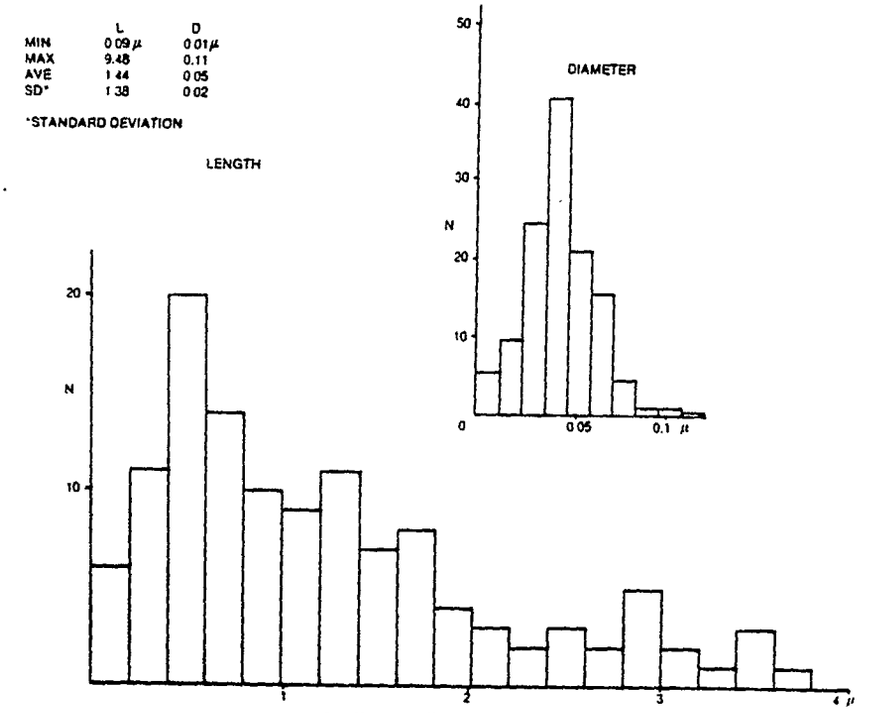


CHART 2. Chrysotile B (milled). $N = 127$.

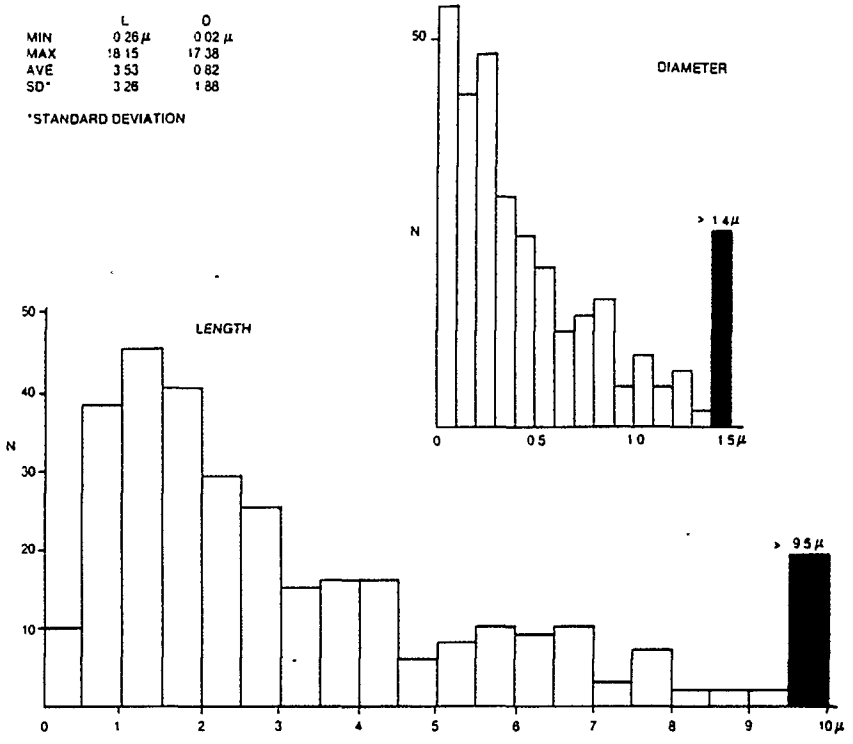


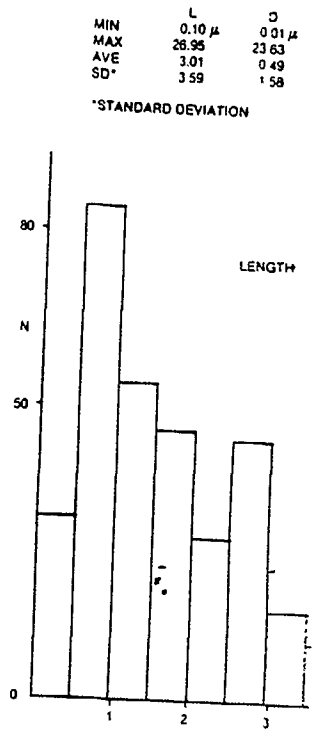
CHART 3. Erionite I. N = 312.

controls. Age of these animals was 5 to 6 weeks old when exposed to asbestos or zeolite. As shown in Tables 2 and 3, these mice were divided into 16 groups including two controlled ones. Except animals of both Groups 7 and 8, treated mice received a single intraperitoneal injection of asbestos or zeolite. Mice of Group 7 received a double intraperitoneal injection (a single injection of 2 mg U.I.C.C. chrysotile and a single injection of 2 mg amosite). Mice of Group 8 received a single intraabdominal wall injection of 10 mg amosite; instead of the mesothelium, the submesothelial connective tissue of the abdominal wall was selected as the site of exposure to amosite. Inspection of these animals was done twice a day. To avoid postmortem changes, very sick animals were sacrificed.

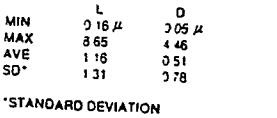
D. Pathological analyses of the animals. All animals sacrificed or found dead between 0 and 23 months after the injection were systematically necropsied. Gross anatomical and histological observation were done in all of the animals including the controls. In addition to hematoxylin eosin, Masson's trichrome, Prussian blue, and Van Gieson were used for staining. Histochemistry (colloidal iron with and without hyaluronidase and periodic acid Schiff with and without diastase) and electron microscopy were also used to investigate the induced peritoneal tumors.

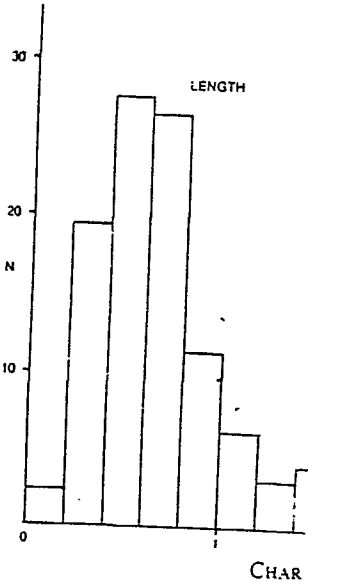
RESULTS

As shown in Table 2, peritoneal tumors were not induced by asbestos or zeolite before 7 months after exposure to these minerals. Before 7 months, a large



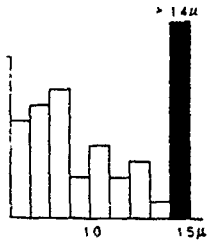
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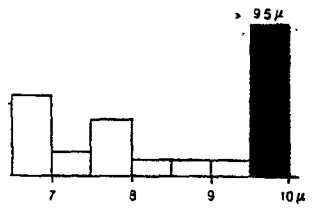




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d when exposed to asbestos were divided into 16 groups oth Groups 7 and 8, treated asbestos or zeolite. Mice of single injection of 2 mg osite). Mice of Group 8 0 mg amosite; instead of the of the abdominal wall was on of these animals was done k animals were sacrificed. als sacrificed or found dead systematically necropsied. e done in all of the animals eosin, Masson's trichrome, g. Histochemistry (colloidal cid Schiff with and without investigate the induced peri-

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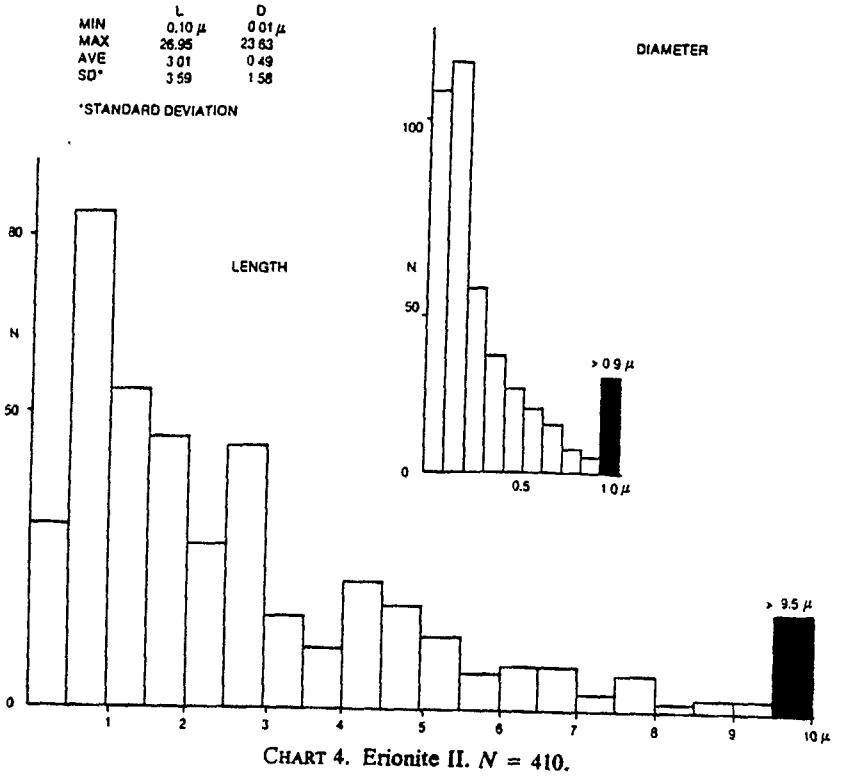


CHART 4. Erionite II. $N = 410$.

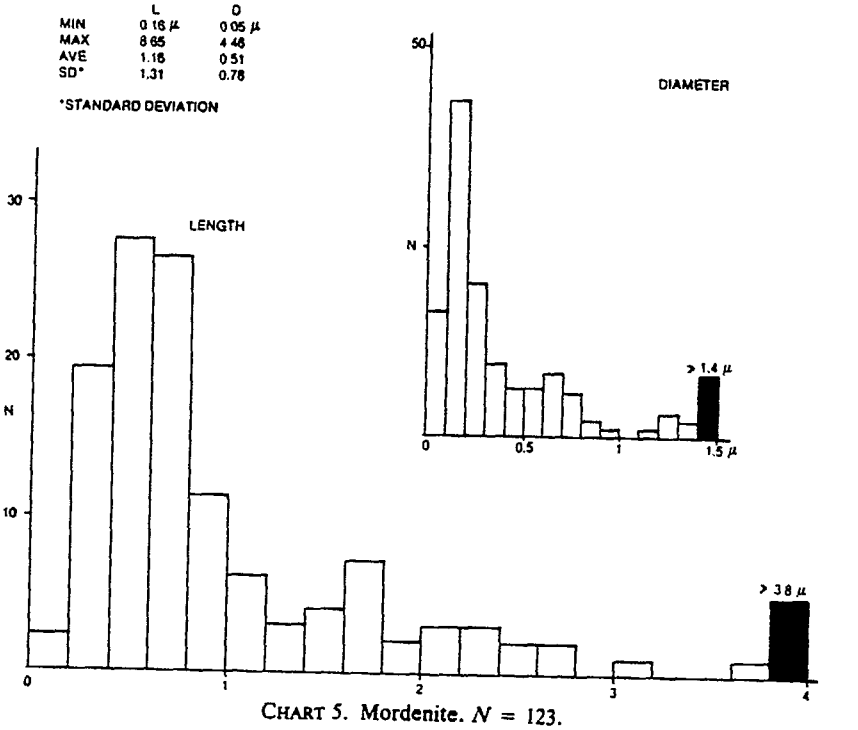


CHART 5. Mordenite. $N = 123$.

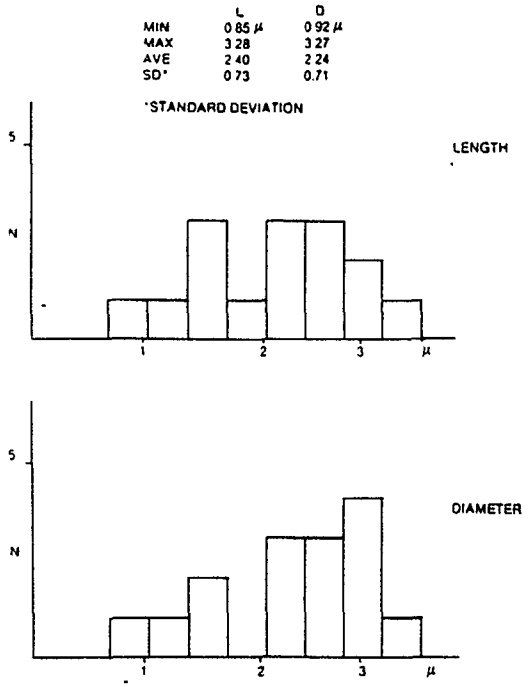


CHART 6. Zeolite 4A. $N = 15$.

number of treated animals (192 of 586) were found dead or very sick. Peritoneal fibrosis, followed by intestinal obstruction, were the most common disease responsible for death of these treated animals. As shown in Table 2, significant incidence of death or serious sickness was seen in animals exposed to high doses of amosite (43 of 58 in Group 1 and 11 of 28 in Group 2), U.I.C.C. chrysotile (23 of 55 in Group 4) and Erionite II (67 of 75 in Group 10). Since mineral fibers were histologically identified in the fibrotic peritoneum, peritoneal fibrosis seemed to

TABLE 1
CHEMICAL ANALYSIS BY ANALYTICAL ELECTRON MICROSCOPY OF SOME ASBESTOS AND ZEOLITE[a]

	UICC chrysotile ($N = 2$)	UICC amosite ($N = 5$)	Erionite I ($N = 5$)	Erionite II ($N = 5$)	Mordenite ($N = 5$)	Zeolite 4A ($N = 3$)
SiO_2	42.5 (±0.4)	48.5 (±0.6)	61.9 (±2.1)	61.0 (±0.7)	70.8 (±1.1)	44.2 (±0.6)
Al_2O_3	1.3 (±0.2)	1.1 (±0.2)	14.6 (±0.5)	14.6 (±0.3)	12.1 (±0.6)	38.7 (±0.8)
Fe_2O_3	0	0	0	0	0	0
FeO	1.4 (±0.1)	37.3 (±0.4)	0.7 (±0.2)	0.4 (±0.1)	0.6 (±0.4)	—
MnO	—	2.1 (±0.2)	—	—	—	—
MgO	39.9 (±0.3)	7.9 (±0.7)	0.7 (±0.2)	1.4 (±0.4)	0.3 (±0.3)	—
CaO	—	—	3.1 (±0.9)	2.2 (±0.2)	2.0 (±0.6)	—
Na_2O	—	—	—	—	—	17.1 (±1.1)
K_2O	—	—	1.1 (±0.6)	2.4 (±0.4)	0.2 (±0.1)	—
H_2O	[15]	[3]	[18]	[18]	[14]	[0]
Total	85.1	96.9	82.1	82.0	86.0	100.0

[a] Values represent percentages.

TABLE 2
INDUCTION OF MALIGNANT PERITONEAL TUMORS

Group	N[a]	Between 0 and 7 months after exposure[b]	Between 7 and 23 months after exposure[b]	
1. Amosite—20 mg	58	0/43 (6 sac., 37 f.d.)[c]	4/15 (8 sac., 7 f.d.)[c]	26.7
2. Amosite—10 mg	28	0/11 (3 sac., 8 f.d.)	4/17 (5 sac., 12 f.d.)	23.5
3. Amosite—2 mg	41	0/4 (f.d.)	15/37 (17 sac., 20 f.d.)	40.5
4. Chrysotile—20 mg	55	0/23 (5 sac., 18 f.d.)	6/32 (9 sac., 23 f.d.)	18.2
5. Chrysotile—2 mg	23	0/1 (f.d.)	0/22 (15 sac., 7 f.d.)	

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shown in Table 2, significant
nimals exposed to high doses
in 2), U.I.C.C. chrysotile (23
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OF SOME ASBESTOS AND ZEOLITE[a]

nite II = 5)	Mordenite (N = 5)	Zeolite 4A (N = 3)
(±0.7)	70.8 (±1.1)	44.2 (±0.6)
(±0.3)	12.1 (±0.6)	38.7 (±0.8)
	0	0
(±0.1)	0.6 (±0.4)	—
	—	—
(±0.4)	0.3 (±0.3)	—
(±0.2)	2.0 (±0.6)	—
	—	17.1 (±1.1)
(±0.4)	0.2 (±0.1)	—
	[14]	[0]
	86.0	100.0

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4. Chrysotile—20 mg	55	0/23 (5 sac., 18 f.d.)	6/32 (9 sac., 23 f.d.)	18.2
5. Chrysotile—2 mg	23	0/1 (f.d.)	0/22 (15 sac., 7 f.d.)	0
6. Chrysotile (?)[d]—2 mg	21	0/5 (f.d.)	4/16 (6 sac., 10 f.d.)	25
7. Amosite (2 mg) + chrysotile (2 mg)	10	0/0	6/10 (8 sac., 2 f.d.)	60
8. Int. abd. wall[e] amosite 10 mg	55	0/12 (10 sac., 2 f.d.)	0/43 (24 sac., 19 f.d.)	0
9. Erionite I—10 mg	50	0/8 (f.d.)	21/42 (5 sac., 37 f.d.)	50
10. Erionite II—10 mg	75	0/67 (7 sac., 60 f.d.)	3/8 (3 sac., 5 f.d.)	37.5
11. Erionite II—2 mg	50	0/6 (f.d.)	24/44 (16 sac., 28 f.d.)	54.5
12. Erionite II—0.5 mg	20	0/2 (f.d.)	6/18 (1 sac., 17 f.d.)	33.3
13. Mordenite—10 mg	50	0/6 (1 sac., 5 f.d.)	0/44 (36 sac., 8 f.d.)	0
14. Synth. zeolite 4A—10 mg	50	0/4 (f.d.)	0/46 (39 sac., 7 f.d.)	0
15. Saline control	129	0/11 (5 sac., 6 f.d.)	0/118 (97 sac., 21 f.d.)	0
16. Untreated control	53	0/16 (10 sac., 6 f.d.)	0/37 (21 sac., 16 f.d.)	0
Asbestos treated	291	0/219 (47 sac., 172 f.d.)	93/549 (310 sac., 239 f.d.)	
Zeolite treated	295			

[a] Treated, N = 586; controls, N = 182; total, n = 768.
[b] x/y = Number of peritoneal-tumor-bearing mice/Number of sacrificed or found dead mice.
[c] Sac: sacrificed, f.d.: found dead.
[d] (?): Calidria chrysotile.
[e] Intraabdominal wall injection.

TABLE 3
HISTOLOGICAL CRITERIA

Group	Induced peritoneal tumors (N = 93)							Induced extraperitoneal Tumors (N = 3)	
	Total peritoneal tumors	Mesotheliomas[a]	Plasma cytoma	Osteo-sarcoma	Histio-cytoma	Lipo-sarcoma	Pancreatic cancer	Rhabdo-myosarcoma	Fibro-sarcoma
1. Amosite—20 mg	4	4 (F)	0	0	0	0	0		1
2. Amosite—10 mg	4	4 (3F + 1B)	0	1	0	1	0		
3. Amosite—2 mg	15	13 (12F + 1B)	1	0	1	0	0		
4. Chrysotile—20 mg	6	4 (F)	0	0	0	0	0		
5. Chrysotile—2 mg	0	0	0	0	0	0	0		
6. Chrysotile(c)—2 mg	4	4 (F)	0	0	0	0	0	2	
7. Amosite + Chrysotile—2 mg (each)	6	6 (F)	0	0	0	0	0		
8. Int. Abd. Wall—10 mg	0	0	0	0	0	0	0		
9. Erionite I—10 mg	21	19 (15F[b] + 3B + 1E)	2	0	0	0	0		
10. Erionite II—10 mg	3	3 (2F + 1B)	0	0	0	0	1		
11. Erionite II—2 mg	24	21 (18F + 3B)	2	0	0	0	0		
12. Erionite II—0.5 mg	6	5 (F)	1	0	0	0	0		
13. Mordenite—10 mg	0	0	0	0	0	0	0		
14. Synth. zeolite 4A—10 mg	0	0	0	0	0	0	0		
15. Saline control	0	0	0	0	0	0	0		
16. Untreated control	0	0	0	0	0	0	1	2	1
Total	93	83 (73F + 9B + 1E)	6	1	1	1	1	2	1

[a] F: Fibrous form, B: Biphasic form, and E: Epithelial form.
[b] Two of 15 fibrous mesothelioma were associated with plasmacytoma.

be caused by fibrogenic effe
synthetic zeolite 4A (Group
was minimal in animals of
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severity of fibrosis seemed to

Between 7 and 23 months
oped in animals of all but 6
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treated control (Group 16). .
treated mice (24.2%) which
months. Incidence of perito
groups. As indicated in Tabl
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54.5% in the erionite II 2 mg
9), (4) 40.5% in the amosite
(Group 10), (6) 33.3% in the e
20 mg (Group 1), (8) 25% in C
10 mg (Group 2), and (10) 18

It was noteworthy that uni
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Gross anatomically, hemor
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capsule of the kidney, the tuni
vesicle.

As shown in Table 3, histop
sified as 83 malignant mesothe
coma, 1 extraskeletal osteosar
cell type of these 83 maligna
biphasic, and 1 epithelial for
oma with plasmacytoma was
(Group 9).

Figures 2, 3, and 4 represen
induced mesotheliomas, respe
ithelial cells seen in this study

13. Mordenite—10 mg	0	0	0	0	0	0	0	0	0	0	0	0
14. Synth. zeolite 4A—10 mg	0		0	0	0	0	0	0	0	0	0	0
15. Saline control	0		0	0	0	0	0	0	0	0	0	0
16. Untreated control	0	0	0	0	0	0	0	0	0	0	0	0
Total	93	83 (73F + 9B + 1E)	6	1	1	1	1	1	1	2	1	1

[a] F: Fibrous form, B: Biphasic form, and E: Epithelial form.
[b] Two of 15 fibrous mesothelioma were associated with plasmacytoma.

be caused by fibrogenic effects of the asbestos and zeolite. Fibrogenic effect of synthetic zeolite 4A (Group 14) seemed to be mildest since peritoneal fibrosis was minimal in animals of this group. Mordenite-treated mice (Group 14) also suffered from peritoneal fibrosis followed by intestinal obstruction. However, severity of fibrosis seemed to be milder than that of asbestos and fibrous erionite.

Between 7 and 23 months after exposure, malignant peritoneal tumors developed in animals of all but 6 groups, the U.I.C.C. chrysotile 2 mg (Group 5), the intraabdominal wall amosite 10 mg (Group 8), the mordenite 10 mg (Group 13), the synthetic zeolite 4A (Group 14), the saline control (Group 15), and the untreated control (Group 16). Malignant peritoneal tumors developed in 93 of 385 treated mice (24.2%) which were sacrificed or found dead between 7 and 23 months. Incidence of peritoneal tumor production varied among the treated groups. As indicated in Table 2, the order of incidence was as follows: (1) 60% in the double treated (2 mg U.I.C.C. chrysotile and 2 mg amosite; Group 7), (2) 54.5% in the erionite II 2 mg (Group 11), (3) 50% in the erionite I 10 mg (Group 9), (4) 40.5% in the amosite 2 mg (Group 3), (5) 37.5% in the erionite II 10 mg (Group 10), (6) 33.3% in the erionite II 0.5 mg (Group 12), (7) 26.7% in the amosite 20 mg (Group 1), (8) 25% in Calidria chrysotile (Group 6), (9) 23.5% in the amosite 10 mg (Group 2), and (10) 18.2% in the U.I.C.C. chrysotile 20 mg (Group 4).

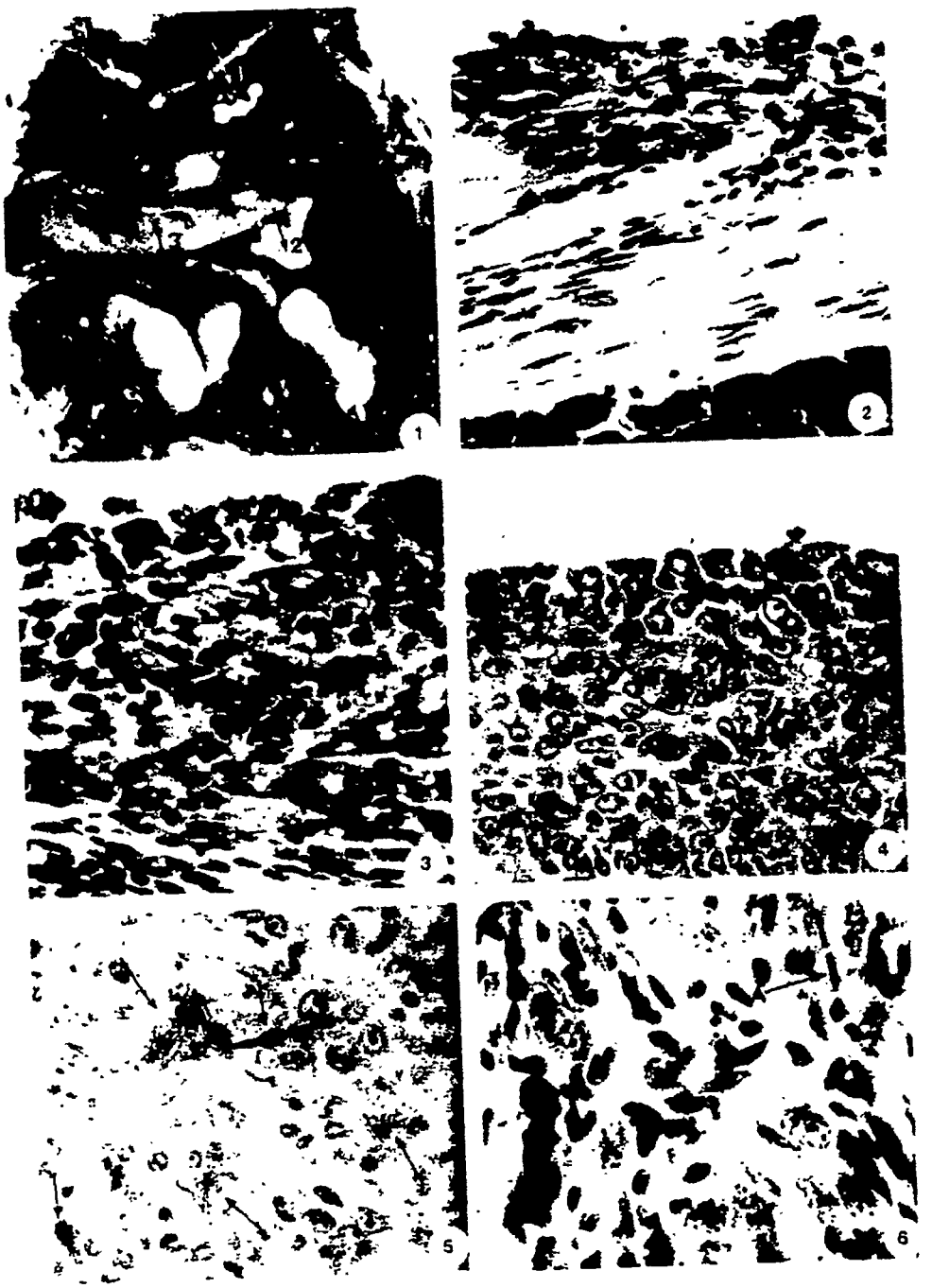
It was noteworthy that unlike asbestos or fibrous erionite (Erionite I and II), both mordenite and synthetic zeolite 4A did not induce peritoneal tumor suggesting that those subtypes of zeolite lacked carcinogenic effect for the peritoneal tissue.

Gross anatomically, hemorrhagic ascites were almost constantly observed in the induced mesothelioma cases. As shown in Fig. 1, neoplastic nodules of various sizes seen in the peritoneum were usually multiple at the site of fibrous or neoplastic adhesion. Since these macroscopic findings were also observed in the peritoneal tumors other than mesothelioma, diagnosis of the induced tumor should be confirmed by histopathological investigation.

There were certain "favorite" sites of tumor production in the peritoneum; the abdominal wall peritoneum corresponding to the site of the injection, the diaphragm, the capsule of liver and spleen, the connective tissue of pancreatic capsule, the omentum, the mesentery, serosa of the stomach and intestine, the fatty capsule of the kidney, the tunica vaginalis of the testis, and serosa of the seminal vesicle.

As shown in Table 3, histopathologically these 93 peritoneal tumors were classified as 83 malignant mesothelioma, 6 plasmacytoma, 1 histiocytoma, 1 liposarcoma, 1 extraskeletal osteosarcoma, and 1 adenocarcinoma of the pancreas. The cell type of these 83 malignant mesotheliomas was divided into 73 fibrous, 9 biphasic, and 1 epithelial forms. Coexistence of fibrous malignant mesothelioma with plasmacytoma was recognized in two cases in the Erionite I 10 mg (Group 9).

Figures 2, 3, and 4 represent fibrous, biphasic and epithelial forms seen in the induced mesotheliomas, respectively. Unlike human epithelial mesothelioma, epithelial cells seen in this study did not show tubulopapillary or cord-like fashions.



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FIG. 1. A macroscopic photograph
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FIG. 4. Epithelial mesothelioma cell
forming a nest of sheet-like fashion. T
Hematoxylin eosin. ×305.

FIG. 5. A part of an extraperitoneal
group (Group 1). An asbestos body (ar
tumor tissue. Colloidal iron stain. ×49

FIG. 6. Showing a part of a rhabdom
intra-abdominal wall amosite 10-mg grc
neoplastic cells with vesicular nuclei a







In addition to the 93 peritoneal tumors, 3 extraperitoneal tumors, which were also assumed to be induced by asbestos (by amosite), were observed. These three tumors consisted of one fibrosarcoma in the subcutaneous connnective tissue of a mouse (Group 1: amosite 20 mg) and two rhabdomyosarcomas in two mice of the intraabdominal wall 10 mg (Group 8). Since these three tumors contained coated and uncoated amosite fibers and the peritoneum of those three animals was completely free of fibrosis, it was strongly suggested that the injected amosite fibers were accidentally administrated into either the subcutaneous connective tissue or the striated muscle of the abdominal wall resulting in malignant transformation of local cells such as a fibroblast and a striated muscle cell. In Fig. 5, an asbestos body (arrow A) and partially coated asbestos fibers (arrows) are shown in a part of the subcutaneous fibrosarcoma tissue, and in Fig. 6, an asbestos body (arrow A) is seen in rhabdomyosarcoma tissue in which bizarre neoplastic cells with large vesicular nuclei are present.

Histochemically, a large majority of the induced mesotheliomas did not show high productivity of hyaluronic acid. It is known that the hyaluronic acid productivity is usually poor in human fibrous mesothelioma; accordingly, mouse fibrous mesothelioma seen as a major cell type in this study may also be not generally active in producing the substance.

Electron microscopically, fibrous cells of the induced mesotheliomas were similar to those of human mesothelioma; as shown in Fig. 7, the cell to cell attachment of two adjacent cells and well-developed rough-surfaced endoplasmic reticulums were frequently seen in this cell type. Rarely, numerous microvilli were shown in epithelial cells as seen in Fig. 8, although well-differentiated epithelial cells were not seen in the induced mesothelioma. To differentiate plasmacytomas from mesothelioma, electron microscopy seemed to be a useful method; ultrastructure of the former was similar to that of plasma cells with frequent presence of C particles.

FIG. 1. A macroscopic photograph showing an abdominal cavity in which peritoneal tumors were induced, taken from an animal of the amosite 20 mg group (Group 1). Neoplastic nodules were formed at various sites of the peritoneum. Large tumors were seen on the surface of both the liver (arrow 1) and the omentum (arrow 2). Arrow 3 indicates marked expansion of the intestine, caused by severe fibrotic and neoplastic adhesions of the peritoneum.

FIG. 2. Showing a relatively early stage of peritoneal mesothelioma (fibrous form). Spindle-shaped neoplastic cells are seen in the omentum which adhered to the fibrotic pancreatic capsule. Taken from a mouse of the Calidria chrysotile 2 mg group (Group 6). Hematoxylin eosin. ×305.

FIG. 3. Showing the biphasic form of malignant mesothelioma. Both poorly differentiated epithelial cells (shown in the upper part of this picture) and fibrous cells (in the lower part) coexisted in a tumor tissue. Taken from a mouse of the amosite 2-mg group (Group 3). Hematoxylin eosin. ×490.

FIG. 4. Epithelial mesothelioma cells are shown. The cells are poor in polarization. These cells are forming a nest of sheet-like fashion. Taken from a mouse of the Erionite I 10-mg group (Group 9). Hematoxylin eosin. ×305.

FIG. 5. A part of an extraperitoneal tumor (fibrosarcoma) induced in a mouse of the amosite 20-mg group (Group 1). An asbestos body (arrow A) and aggregated asbestos fibers (arrows) are seen in the tumor tissue. Colloidal iron stain. ×490.

FIG. 6. Showing a part of a rhabdomyosarcoma tissue seen in the abdominal wall of a mouse of the intra-abdominal wall amosite 10-mg group (Group 9). A short asbestos body (arrow A) is seen. Giant neoplastic cells with vesicular nuclei are shown. Hematoxylin eosin. ×490.





FIG. 7. Ultrastructure of fibrous mesothelioma cells seen in a mouse of the amosite 20 mg group (Group 1). Well-developed endoplastic reticulum, direct contact of a cell with another cell, elongated nucleoli, and lipid granules are seen. ×4260.

FIG. 8. Epithelial mesothelial cells, equipped with numerous microvilli are seen. Taken from an animal of the Erionite I 10 mg group (Group 9). ×9300.

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COMMENTS

Carcinogenic and fibrogenic effects of asbestos and zeolite and the development and course of malignant mesothelioma induced by these mineral fibers were studied in the mouse peritoneum. A single peritoneal administration of these fibers at various doses was used, followed by long-term observation of the peritoneum. This animal model was relatively simple to use and it produced highly reproducible results in the induction of tumors and fibrosis.

A high incidence of peritoneal tumors (mainly malignant mesothelioma) was observed in animals injected with either asbestos or fibrous erionite between 7 and 23 months after exposure; 39 of 192 (20.3%) in the asbestos group and 54 of 112 (48.2%) in the fibrous erionite group. It is noteworthy that the latency period of the induced peritoneal tumors did not seem to be related to either the type of fibers or the dose; regardless of the type or doses no tumor was seen until 7 months after exposure. This is different from that of the mouse alveologenic tumor, which can be induced at a high rate by chemical carcinogens, and appear earlier either when a stronger chemical carcinogen was used and/or when the dose of a chemical carcinogen was larger (2, 3). The asbestos-induced mesotheliomas were similar to those induced by fibrous erionite in exhibiting long latency, in gross appearance, in histology, and in close association with fibrosis. The induced mesotheliomas were also similar to human malignant mesothelioma both in exhibiting long latency and in gross appearance. A distinct difference between the animal and human mesothelioma, however, is the cell type of the malignant mesothelioma. The ratio of epithelial, biphasic, and fibrous forms was 1.2:10.8:88 in the induced mouse mesothelioma, while it was 67:26:7 in human malignant mesothelioma (4). It is not known why, unlike human mesothelioma, the epithelial form is so rare and the fibrous form so common in the induced mouse mesothelioma, although the severe focal fibrosis which constantly occurred around the mesothelium might be related to the high incidence of fibrous malignant mesothelioma.

Both asbestos and fibrous erionite were able to induce tumors other than malignant mesothelioma. In the present study, plasmacytoma, liposarcoma, osteosarcoma, histiocytoma, pancreatic cancer, subcutaneous fibrosarcoma, and rhabdomyosarcoma all were induced peritoneally or extraperitoneally by these fibers. These tumors have not been reported to occur as spontaneous neoplasms in BALB/C male mice. C-particles were frequently seen in the neoplastic cells of the induced plasmacytomas. It is possible that both C-particles and the mineral fibers participated in the induction of the plasmacytomas as cocarcinogens. It is known that human osteogenic sarcomas rarely developed extraskeletally in the osteoid matrix of the metaplastic connective tissue. A single case of peritoneal osteosarcoma induced by amosite (Group 3) may be classified as this type of tumor, since osteoid formation was not rare in fibrotic lesions induced by asbestos or fibrous erionite. Another single case of pancreatic cancer (Group 11) was also suggested to be erionite-related since focal scarring associated with erionite deposition was seen in the pancreatic parenchyma. The subcapsular pancreatic pa-

renchyma was frequently involved in a fibroplastic process induced by asbestos and fibrous erionite which were intraperitoneally administered.

Our study strongly suggested that asbestos and fibrous erionite had the potentiality for inducing neoplasms in various tissues other than the mesothelium and lung, if these fibers could reach the various tissues and remain there for long periods.

Epidemiological studies have shown that, in addition to lung cancer and malignant mesothelioma, various cancers such as cancer of the larynx, renal cell carcinoma, and gastrointestinal cancer are present with increased frequency among asbestos insulation workers (5, 6). To confirm whether individual human cases of these various cancers are caused by asbestos or not, the detection of both asbestos fibers and tissue response (such as focal fibrosis) should be attempted in the primary neoplastic focus of the individual cases. There is good reason for supporting this approach; in our study using the mouse as an experimental model, both of these conditions are met in all of asbestos- or erionite-induced tumors.

The mineral fibers which were intraperitoneally injected were located in the animal peritoneum in certain "favorite" sites. Subsequently, peritoneal fibrosis followed by the induction of malignant mesothelioma occurred in these "favorite" sites. The diaphragmatic peritoneum, one of the favorite sites, is known to have microvilli-rich mesothelium and well-developed stomata through which foreign substances can be drained out from the peritoneal cavity into the lymphatic system of the diaphragm (7). Wang (8) has described a similar anatomical characteristic in the parietal pleura, known to be the original site of pleural mesothelioma. What is not known, however, is whether all of these "favorite" sites are equipped with such a unique physico-anatomical characteristic. This may be a key question as to why tumors develop in certain "favorite" sites of the peritoneum.

Baris *et al.* (9, 10) have suggested that fibrous erionite (a type of zeolite) is the etiological factor in the development of malignant pleural mesotheliomas which occur endemically among inhabitants of several villages in south-central Turkey. Later, Rohl *et al.* (11) reported that in addition to the erionite, asbestos fibers (chrysotile and tremolite) were found in both the environment and in lung tissues of these mesothelioma patients. A question is raised therefore, whether these mesotheliomas were induced by fibrous erionite or by asbestos or by both. Our present and preliminary (12) animal studies clearly indicate that, like asbestos, fibrous erionite is also clearly carcinogenic, inducing malignant mesothelioma at a high rate in animals. Accordingly, even if asbestos fibers coexisted with the erionite in the lungs of the Turkish patients with mesothelioma, erionite can still not be excluded as a sole or cocarcinogen responsible for the induction of human mesothelioma.

Fibrosis was strongly suspected to be an important precondition for the induction of the neoplasms by asbestos and fibrous erionite; all neoplasms induced by these minerals (93 peritoneal tumors, 2 rhabdomyosarcomas of the abdominal wall, and 1 fibrosarcoma of the subcutaneous connective tissue) were intimately associated with fibrosis. This intimate relationship of the fibrogenicity with the carcinogenicity was best shown in the zeolite samples: both Erionite I and II showed striking carcinogenic and fibrogenic effects while mordenite and synthetic

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zeolite 4A did not induce any tumors, and this was accompanied by mild and minimal fibrogenicity. Another interesting finding was that animals receiving large doses of asbestos or fibrous erionite died of peritoneal fibrosis followed by intestinal obstruction before the tumor was induced. To perform long-term observations of the development and course of experimental peritoneal mesothelioma, doses of the carcinogenic mineral fibers must be carefully chosen.

Stanton and Wrench (13) have hypothesized that the carcinogenicity of asbestos, fibrous glass, and brucite was due to the durability of those fibers within a certain range of size distribution (8 μm and longer in length and 1.5 μm and smaller in width) and that the chemical nature of the fibers is not relevant for the induction of mesothelioma (13, 14). Similar results have been reported by Pott *et al.* (15, 16). Stanton's hypothesis, however, has been disputed by other investigators (17). In the present study, for example, a large majority of asbestos and fibrous erionite fibers were short and thin, as indicated in charts 1 and 6. However it is still not known whether the short, thin fibers were responsible for the high rate of mesothelioma production or whether the long thin fibers present as a minority in the mineral sample played a role. To elucidate this, the range of size distribution should be studied in the fibers detected in the primary neoplastic focus since this range may be different from the original sample.

Pathogenetic mechanisms suggested by studies on experimental mesothelioma may not be directly applicable to human mesothelioma since there are some differences in pathogenetic conditions of the human tumor as compared with the animal tumor. For instance, the life span of laboratory animals (mouse, rat, and hamster) is approximately 2 years, much shorter than that of humans. The long time latent period (20–40 years) seen in the development of human malignant mesothelioma (5, 6) cannot be reproduced in these animals. Therefore, to induce animal mesothelioma, larger doses of the mineral fibers must be used and on many occasions, investigators must apply the artificial method of fiber exposure such as inoculation or injection (12–16, 18–21). In addition, anatomical differences, such as the widely differing range of the length and diameter of the respiratory airway in human and animals, may also result in different data concerning the critical size range of the fibers responsible for the induction of the tumor.

In spite of these differences, however, animal experiments have provided valuable information on the carcinogenicity of asbestos and other inorganic mineral fibers as well as on histogenetic problems of malignant mesothelioma.

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