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Pulmonary Asbestos Body Counts and Electron Probe Analysis of Asbestos Body Cores in Patients with Mesothelioma

A Study of 25 Cases

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Malignant mesotheliomas of the pleura and peritoneum are well-recognized risks of asbestos exposure. We determined the asbestos body content of the lungs from 24 cases of malignant mesothelioma (19 pleural, five peritoneal) and compared such to the content of lungs from 50 consecutive adult autopsies and four cases of overt asbestosis using a Clorox-digestion concentration technique. The cores of 90 asbestos bodies were examined by energy dispersive x-ray analysis and compared with similar data from 120 standard asbestos fibers and 20 fiberglass fibers. The malignant mesothelioma patients had asbestos body counts intermediate between those of the general population and those of patients with asbestosis, although some of the mesothelioma cases overlapped with the general population. These latter cases often lacked an identifiable occupational exposure to asbestos. EDXA studies demonstrated an amphibole core in 88 of the 90 asbestos bodies (amosite or crocidolite in 80 of 88, anthophyllite or tremolite in eight of 88), and chrysotile in two instances.

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THE association between occupational asbestos exposure and the subsequent development of malignant mesothelioma of the pleura or peritoneum was first established by Wagner *et al.* in 1960.¹ Since this initial study, several series of mesotheliomas have been reported confirming the association with asbestos exposure.²⁻⁴ Cases have been reported in the wives and children of asbestos workers, who were apparently exposed to the dust brought home on the workers' clothes.⁴⁻⁶ An

occasional case of mesothelioma with low level occupational exposure to asbestos has been described.⁷

The increase in the number of reported cases of a malignancy previously thought to be rare led to intensified interest in asbestos and asbestos-related diseases. Quantitative methods for the concentration and isolation of ferruginous (asbestos) bodies, the hallmark of asbestos exposure, demonstrated that these structures may be found in 90-100% of the general population,⁸⁻¹⁰ and that the numbers of ferruginous bodies found in the lungs of such individuals at postmortem examination appear to have increased over the past three decades.¹¹ The specificity of the ferruginous body as a marker for asbestos exposure was challenged by the demonstration in animal models that a number of fibrous dusts other than asbestos may result in the formation of ferruginous bodies.¹² Problems of obvious importance which follow from these observations are the magnitude of asbestos exposure and tissue asbestos load in patients with mesothelioma, and the nature of the cores of ferruginous bodies found in these individuals; i.e., might fibrous dusts other than asbestos be implicated in the pathogenesis of mesotheliomas in humans.

Few studies have been reported in which quantitation of ferruginous bodies or uncoated fibers within lung tis-

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sue from mesothelioma patients was attempted.^{5,13,14,15,16} In a few instances, electron probe analysis or electron diffraction techniques were used to investigate the chemical and structural nature of the ferruginous body cores.^{5,15,17} This report is an analysis of the cases of malignant pleural and peritoneal mesotheliomas seen at our institution over the past 17 years. An attempt was made to correlate the ferruginous (asbestos) body counts in lung tissue with occupational information, and with similar data obtained from patients with asbestosis and from a control autopsy population. In selected cases, energy dispersive (electron probe) X-ray analysis (EDXA) and selected area electron diffraction (SAED) were performed on isolated ferruginous bodies to determine the nature of the fibrous core.

Materials and Methods

Patient and Control Populations

The study group consisted of all cases of malignant mesotheliomas (pleural and peritoneal) diagnosed at The Methodist Hospital from January 1963 to March 1980. Criteria for the diagnosis of malignant (diffuse) pleural mesothelioma have been previously described,¹⁸⁻²⁰ and include the following: (1) predilection to spread along the pleural surface and major fissures, with total encasement of the lung in advanced cases; (2) compatible microscopic appearance of the tumor, which includes four basic patterns: *a*: tubular or tubulopapillary epithelial type; *b*: predominantly solid, pleomorphic epithelial type; *c*: sarcomatoid or spindle cell variant; and *d*: a mixture of one or more of the previous patterns. The identification of acid mucopolysaccharide which is sensitive to hyaluronidase as demonstrated by the Alcian blue stain, and the absence of neutral mucin using the periodic acid-Schiff (PAS) with diastase, are helpful histochemical features. Malignant (diffuse) peritoneal mesotheliomas show a predilection to spread along the parietal peritoneum and serosal surface of abdominal viscera, with a similar range of microscopic and histochemical features as seen in pleural mesotheliomas.²¹⁻²² A single case of benign fibrous pleural mesothelioma included in the study showed features identical to those described by others.²³ Postmortem examination did not demonstrate a visceral primary malignancy in any of the cases included in this study. Twenty-five cases of mesothelioma were identified in our files using these criteria: 18 cases of malignant pleural mesothelioma, six cases of malignant peritoneal mesothelioma, and one localized fibrous tumor of the pleura.

The control population consisted of 50 consecutive adult autopsies with no known occupational exposure to asbestos. The mean age for this group was 59.3 years

(range, 21-89 years), and there were 29 men and 21 women. None of those individuals had overt pulmonary interstitial fibrosis, pleural plaques, or mesothelioma. Also, for the purpose of comparison with the mesothelioma group, four individuals with asbestosis were included in the study. These four men had an age range of 54-76 years, and three had carcinoma of the lung.

Occupational Data

Information concerning the occupation of individuals in the study and control groups was obtained by review of the hospital chart, and by direct phone conversation with a living relative or, in a few cases, with the private physician. Direct inquiries were made into all occupations (both recent and remote), place of residence, wartime experience, *e.g.*, shipyards, spouse's employment, and hobbies. Duration of employment was also recorded. Information was available on all 25 mesothelioma cases, all four asbestosis cases and 49 of 50 controls. From autopsy results of 57 initial controls, seven were eliminated because of occupational data obtained by phone interview with relatives: two pipefitters, two welders, one boilermaker, one engineer who had worked in shipyards intermittently, and one cabinet maker with relatively high asbestos body counts whose relatives could not be contacted for further occupational information.

Quantitation of Asbestos Bodies

Weighed samples of formalin-fixed lung (4.5-5.5 g), obtained at autopsy from 23 of 25 mesothelioma patients and at time of lobectomy for two of the mesothelioma patients, was processed by digestion with 5.25% sodium hypochlorite solution and subsequent isolation, concentration, and enumeration of asbestos bodies per gram of lung tissue (wet weight) as previously described.¹⁰ Samples were taken chiefly from the lung periphery, avoiding areas of tumor, congestion, and consolidation as much as possible. In four instances (Cases 13, 18, 20, and 21), formalin-fixed lung tissue was no longer available, and paraffin blocks of lung were deparaffinized and rehydrated for digestion-quantitation studies. One to five blocks of lung tissue were available in each of these four cases. Lung tissue from the control cases and asbestosis cases was processed in the same manner as that for the mesothelioma cases. Quantitation of asbestos bodies was performed without prior knowledge of occupational data.

Electron Microscopic Studies 10003050

Selected cases from the mesothelioma and asbestosis groups were studied by transmission (TEM) and scan-

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TABLE 1. Clinical, Occupational and Pathologic Data on 25 Cases of Mesothelioma

Case no./age/sex	Occupation	Diagnosis	Latency*	AB/g†
Pleural Mesothelioma				
1/62/M	Pipe Insulator	Malig. sar type. Lt; asbestosis	40 yr	29,700
2/80/M	Asbestos plant inspector	Malig. tubulopap & solid epithelial, Lt; asbestosis	29 yr	26,400
3/65/M	Pipefitter	Malig. tubulopap, Rt	37 yr	480
4/66/M	Shipyards worker	Malig. tubulopap, Lt	35 yr	380
5/86/M	Shipyards worker	Malig. mixed sar & pap, Rt	30 yr	280
6/59/M	Commercial fisherman	Malig. pleo solid epithelial type, Lt	—	160
7/53/M	Welder, oil refinery	Malig. sar type, RT	18 yr	130
8/62/M	Supervisor, chem company	Malig. mixed sar & solid epithelial, Lt	16 yr	91
9/61/M	Superintendent, engr corp	Malig. mixed pap-sar-pleo undiff, Lt	38 yr	80
10/84/F	FBI agent	Malig. pleo epithelial type, Lt	—	19
11/69/M	Offc worker, oil refinery	Malig. tubulopap & pleo epithelial type, Lt	—	8.1
12/70/M	Chem engr	Malig. sar type, Lt	20-30 yr	7.6
13/46/M	Tugboat capt	Malig. tubulopap, Lt	—	6.7
14/34/M	Proj engr	Malig. pleo epithelial type, Rt	—	2.0
15/62/M	Supervisor, paper fact	Malig. sar type, Lt	—	1.5
16/47/M	Engr, chem co	Malig. tubuloacinar, Lt	—	1.4
17/44/M	Accountant	Malig. mixed sar & pap, Lt	—	<1
18/80/F	Housewife	Malig. mixed sar & pap, Rt	—	<1
19/39/M	Butcher	Benign, solitary, fibrous, Rt	—	3.8
Peritoneal Mesothelioma				
20/61/M	Insulator	Mixed, sar & pap	22+ yr	380
21/60/M	Estimator	Tubular type	—	215
22/54/M	Janitor	Sar type	—	3.5
23/56/F	Housewife	Tubulopap	—	1.2
24/59/M	Mgr. produce co	Tubulopap	—	<1
25/61/F	Housewife	Pap type	—	<1
Other				
26/76/M	Guard, asbestos plant	Asbestosis, sq ca lung	20 yr	30,900
27/73/M	Insulation sprayer	Asbestosis	Many yr	10,100
28/69/M	Insulator	Asbestosis, sq ca lung	40+ yr	1500
29/54/M	Pipe insulator	Asbestosis, adenoca lung	34 yr	1200

* Time from initial occupational asbestos exposure till diagnosis of disease.

† Asbestos bodies per gram lung tissue (wet weight).

Chem co: chemical company; engr: engineer; offc: office; ref: refin-

ery; capt: captain; proj: project; fact: factory; mgr: manager; Rt: right; Lt: left; sq ca: squamous cell carcinoma; adenoca: adenocarcinoma; Pap: papillary; Tubulopap: tubulopapillary; Malig: malignant; Sar: sarcomatous; Pleo: pleomorphic; AB: asbestos bodies.

ning transmission (STEM) electron microscopy, energy dispersive x-ray analysis (EDXA), and selected area electron diffraction (SAED). Samples of lung tissue were processed by digestion in 5.25% sodium hypochlorite, the residue extracted in an equal volume mixture of chloroform and 50% ethyl alcohol, the centrifuged sediment suspended in 95% ethyl alcohol, and 5 μ l of the sediment transferred onto a nitrocellulose coated 300-mesh nylon grid. UICC asbestos standards and a sample of commercial fibrous glass were suspended in 95% ethyl alcohol, and 5 μ l samples transferred to nylon grids as described above. All specimens were examined in a JEOL-100C electron microscope equipped with a Kevex x-ray energy dispersive spectrometer (Kevex Corporation, Burlingame, California) and a Tracor-Northern pulse analyzer (Tracor Northern, Inc., Middleton, Wisconsin) and computer interface. One to three grids were examined per case, and the entire grid scanned at 2000x.

Asbestos bodies were not found by this technique in cases with less than 19 asbestos bodies (AB)/g lung tissue (Table 1), and there was insufficient tissue for electron microscopic studies in those cases with counts higher than 19 AB/g seen at our institution prior to 1975, (e.g., Cases 5, 6, 20, and 21).

Fiber length and diameter measurements were obtained for 50 standard fibers, and compared with the length and core diameter of 50 asbestos bodies (37 from patients with pleural mesothelioma and 13 from patients with asbestosis). EDXA was performed on 90 asbestos body cores (59 from patients with pleural mesothelioma and 31 from patients with asbestosis) employing a tilt angle of 37°, accelerating voltage of 60 KV, and collection time of 100 seconds. This was compared with EDXA data from 20 fibers of each of the five UICC asbestos types (amosite, crocidolite, anthophyllite, and chrysotile A and B) as well as 20 fibers of tremolite

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TABLE 2. Distribution of Metastases in 24 Cases of Malignant Mesothelioma (Pleural and Peritoneal)

Metastatic site	No. of cases*
Lymph nodes	16 (4)
Lungs	10
Liver	8 (1)
Adrenal glands	7
Pericardium & epicardium	5
Kidneys	4
Bone	3
Peritoneum	2
Pancreas	2
Pleura,† omentum, mesentery, brain, stomach, gastrointestinal tract, gallbladder, trachea, thyroid, chest wall & skeletal muscle	1 ea

* Represents total number of cases with metastases to given sites, with number of peritoneal mesothelioma cases indicated in parentheses.

† Peritoneal mesothelioma with pleura metastases.

(provided by the Geology Department of Rice University, Houston, Texas) and 20 fibers of commercial fibrous glass (provided by Owens-Corning Co.). SAED patterns were obtained from 12 asbestos body cores and 14 standard asbestos fibers.

Analysis of EDXA Data

The relative peak heights for seven elements—magnesium (Mg), silicon (Si), calcium (Ca), sodium (Na), manganese (Mn), iron (Fe), and aluminum (Al)—were measured for each standard fiber and each asbestos body core analyzed. Values for the relative peak-height percentage of these seven elements were plotted on a Gibbs triangular coordinate diagram.²⁴ In addition, the asbestos body cores were further classified using a discriminate function analysis technique based on the data from the 140 standard fibers. The method is similar to that described by Millette and McFarren²⁵ for the classification of amphiboles, but has been extended to include chrysotile and fiberglass as well.

Results

The age, sex, occupational data, diagnosis, time from initial exposure to asbestos to presentation with asbestos-

TABLE 3. Distribution of Histologic Types of Malignant Mesothelioma in 24 Patients With and Without Occupational Asbestos Exposure*

Histologic type	With asbestos exposure	Without asbestos exposure
Tubulopapillary	3 (1)	5 (3)
Pleomorphic (solid) epithelial	1	2
Sarcomatous	2	3 (1)
Mixed	5 (1)	3

* No. represents total number of cases with given histologic type, with number of peritoneal mesothelioma cases indicated in parentheses.

related disease (latency), and AB/g lung tissue (wet weight) for the mesothelioma and asbestosis cases are shown in Table 1. The mean age for the 25 mesothelioma patients is 60.8 years, and the group includes 21 men and four women.

Occupational Data

Eleven of the 24 individuals with malignant mesothelioma had a history of occupational exposure to asbestos. Three were pipefitters or insulators (Cases 1, 3, and 20), three had been employed in shipyards (Cases 4–6), three had a history of asbestos exposure while working in oil or chemical refineries (Cases 7–9), and one was employed in a plant which manufactured asbestos products (Case 2). One individual (Case 12) was a chemical engineer who was exposed to asbestos while developing insulation for boilers. The duration of employment in an asbestos-related occupation for these 11 patients ranged from 2–40 years. The mean latent period from initial exposure until the detection of mesothelioma was 29 years for ten of these workers (unknown for Case 6). The occupation for the four patients with asbestosis but without mesothelioma (Cases 26–29) included insulation employing asbestos materials (three cases) and guard for an asbestos plant (one case); the duration of employment ranged from 7–18 years, and the mean latent period was over 30 years. None of the other 14 patients with mesothelioma (including the one individual (Case 19) with a localized fibrous tumor of the pleura) had an identifiable exposure to asbestos.

Pathologic Findings

Autopsies were performed in 23 cases of mesothelioma, and in two cases material from surgical resection (Cases 8 and 19) was available for study. Metastases were present in 21 of 23 cases at autopsy, with a distribution as shown in Table 2. In addition, metastases to regional nodes were present in the lobectomy specimen of Case 8; this patient died approximately one year after surgery; an autopsy was not performed. The distribution of histologic types of mesothelioma for individuals with and without asbestos exposure is shown in Table 3; there is no significant difference in distribution of tumor types between these two groups ($P = 0.72$). The patient with a localized fibrous tumor of the pleura (Case 19) had a 15 cm ovoid well circumscribed mass attached posteriorly to the right lower lobe. There was no evidence of metastatic disease, and he is alive and well seven months postlobectomy.

Quantitation of Asbestos Bodies

The distribution of asbestos body counts for the patients with asbestosis and mesothelioma as compared to

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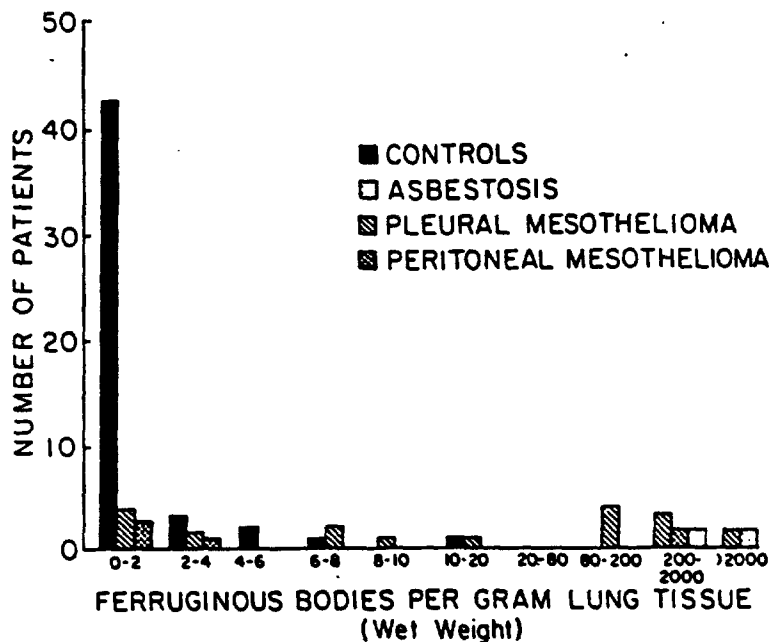
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FIG. 1. Bar diagram showing distribution of ferruginous (asbestos) body counts per gram of wet formalin-fixed lung tissue for mesothelioma and asbestosis cases as compared to controls.



the control population is shown in Figure 1. Eleven patients with mesothelioma had lung parenchymal asbestos body counts ranging from 80–29,700 AB/g; all but one of these individuals was employed in an occupation with known exposure to asbestos (complete historical information not available for Case 21). Fourteen patients with mesothelioma had asbestos body counts of <1–19 AB/g; only one of these individuals (Case 12) had identifiable exposure to asbestos. The mean asbestos body counts for the three groups were: asbestosis, 10,900 AB/g; mesothelioma, 2330 AB/g; and control popula-

tion, 1.2 AB/g. Asbestos bodies were found in the lungs of 92% of individuals in the control population.

Electron Microscopic Studies

Electron microscopic studies of asbestos bodies show electron dense central fibers, or "cores", with discontinuous coated areas, consisting of iron-protein-mucopolysaccharide material deposited by macrophages.²⁶ Uncoated areas, as shown in Figure 2, are suitable for EDXA and SAED studies. The results of EDXA analysis

FIG. 2. Transmission electron micrograph of typical asbestos body from Patient 2. Bare areas (arrows) between the irregular masses of iron-protein coating represent the core fiber and are suitable for EDXA and SAED analysis (×9800).



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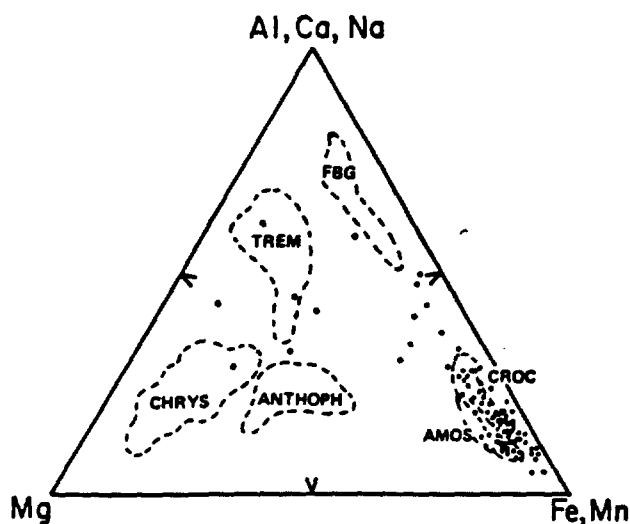


FIG. 3. Gibbs triangular coordinate diagram showing relative elemental composition for several types of fibrous minerals (broken lines), based on analysis of 20 standard fibers of each type: Amos: amosite; CROC: crocidolite; Anthoph: anthophyllite; Trem: tremolite; Chrys: chrysotile A and B; FBG: fiberglass. Dots represent analyses of 90 individual asbestos body cores from patients with mesothelioma (59 cores) and asbestosis (31 cores).

for elemental composition of 90 asbestos body cores and 140 standard fibers are plotted on the Gibbs triangular coordinate diagram in Figure 3. The majority of the asbestos body cores (80/90) fall within the area occupied by amosite and crocidolite standard fibers. Two asbestos body cores are located in the area occupied by the tremolite standards, and one within the chrysotile area. The relative percentage of four major elements (Mg, Si, Ca, and Fe) based on peak height measurements for the 90 asbestos body cores as well as the standard fibers are shown in Table 4. Classification by discriminate function analysis showed that 80 of 90 cores were most probably amosite or crocidolite, four each were tremolite or anthophyllite, and two were chrysotile. SAED studies showed that the asbestos body core had a crystalline-lattice structure compatible with that of amphibole asbestos for all 12 cores for which a diffraction pattern was obtained (Figs. 4A and 4B).

The core diameter for 37 asbestos bodies from patients with mesothelioma as measured from scanning-transmission electron micrographs was $0.41 \pm 0.21 \mu$, and for 13 bodies from patients with asbestosis was $0.27 \pm 0.13 \mu$ (mean $\pm$ 1 SD). The diameter for 39 UICC standard fibers was $0.60 \pm 0.32 \mu$, for three tremolite fibers $1.24 \pm 0.46 \mu$, and for eight fiberglass fibers $1.38 \pm 1.15 \mu$. The mean length for the asbestos bodies was $34.15 \pm 19.90 \mu$ as compared to $18.03 \pm 11.44 \mu$ for the 50 standard fibers.

Discussion

The clinical and pathologic features of the cases of mesothelioma in the current study are similar to those reported in other series.^{2-4,27} the majority of the patients are men (83% in our series) in the sixth or seventh decade of life; pleural mesotheliomas are seen more frequently than peritoneal (75 versus 25%); and epithelial types are more common than sarcomatous or mixed types (54, 21, and 25%, respectively). Histochemical studies (Alcian blue $\pm$ hyaluronidase and PAS $\pm$ diastase) gave variable results: stromal Alcian blue positive material was sensitive to hyaluronidase, whereas intraepithelial Alcian blue positive material was either partially sensitive or resistant to hyaluronidase. However, none of our cases contained PAS-positive material which was diastase resistant. Thus, we believe that while histochemical findings may supplement the gross and microscopic features in the diagnosis of mesothelioma, they are not, in themselves, reliable independent diagnostic criteria. Asbestos body counts in the pulmonary parenchyma of mesothelioma patients with a history of asbestos exposure but without asbestosis, (Cases 3-9, 20, 21) gave a mean count of 244 AB/g lung (wet formalin-fixed weight). The mean count for all malignant mesothelioma cases without asbestosis (Cases 3-18, 20-25) was 90 AB/g lung. The relatively low count (3.8 AB/g) in the single case of localized fibrous tumor of the pleura is consistent with the failure to correlate the occurrence of this neoplasm with exposure to asbestos.^{23,28}

The percentage of patients with malignant mesothelioma of the pleura or peritoneum from whom a history of substantial asbestos exposure has been obtained has varied widely from one study to another.^{3,4,27} A history of asbestos exposure was obtained in 46% of our cases of malignant mesothelioma by direct telephone interview with living relatives. This figure correlates well with that obtained in a previous large epidemiologic study of malignant mesothelioma in North America,²⁹ in which just under one half of the men (but only 5% of women) with malignant mesothelioma had an identifiable occupational exposure to asbestos. Although Cochrane and Webster³ have emphasized the hazards inherent in obtaining histories from family members or fellow workers, it seems likely that our cases with a negative history of asbestos exposure were correctly classified as such, since all but one (Case 21) of the 13 malignant mesothelioma patients from whom a history of asbestos exposure was not obtained had asbestos body counts in the lung which overlapped with the counts obtained from the control population (Fig. 1). Similarly, all but one (Case 12) of the 11 malignant mesothelioma cases

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TABLE 4. Relative Percentage Elemental Composition of Cores of 90 Asbestos Bodies from Patients with Pleural Mesothelioma*

Elements N‡	Ref. Std.†	Cores								
		Case 1 (10)	Case 2 (20)	Case 4 (10)	Case 8 (2)	Case 9 (10)	Case 10 (7)	Case 26 (10)	Case 27 (11)	Case 29 (10)

Amosite-crocidolite (40)										
Mg	4.26 ± 0.77	4.17	4.70	5.40	1.31	(6) 5.66	(1) 4.02	3.73	5.10	4.02
Si	45.98 ± 5.17	28.89	32.05	36.17	29.33	43.92	34.65	36.20	32.78	40.62
Ca	4.01 ± 1.98	3.96	5.70	8.10	8.91	5.94	9.09	9.18	6.10	4.81
Fe	45.75 ± 5.83	62.97	57.54	50.32	60.46	44.48	52.25	50.88	56.02	50.55
Anthophyllite (20)										
N						(1)	(3)			
Mg	20.51 ± 2.17					2.38	7.95			
Si	62.72 ± 3.25					70.11	57.11			
Ca	4.49 ± 2.32					12.43	16.27			
Fe	12.28 ± 2.43					15.08	18.67			
Tremolite (20)										
N						(1)	(3)			
Mg	16.29 ± 2.84					15.90	19.27			
Si	57.97 ± 5.59					58.07	65.62			
Ca	21.94 ± 7.50					22.97	8.53			
Fe	3.80 ± 2.21					3.06	6.58			
Chrysotile (40)										
N						(2)§				
Mg	32.52 ± 4.97					29.94	2.26			
Si	56.18 ± 5.53					50.96	91.45			
Ca	4.80 ± 2.78					8.92	2.75			
Fe	6.51 ± 2.98					10.19	3.54			

* Includes data from six patients with mesothelioma (Cases 1, 2, 4, 8, 9, 10), 2 with asbestosis and lung cancer (Cases 26, 29) and one with asbestosis (Case 27).

† Reference standards show mean ± 1 SD for number of fibers indicated in parenthesis.

‡ N (numbers in parenthesis under case no.) indicate number of fibers analyzed per case.

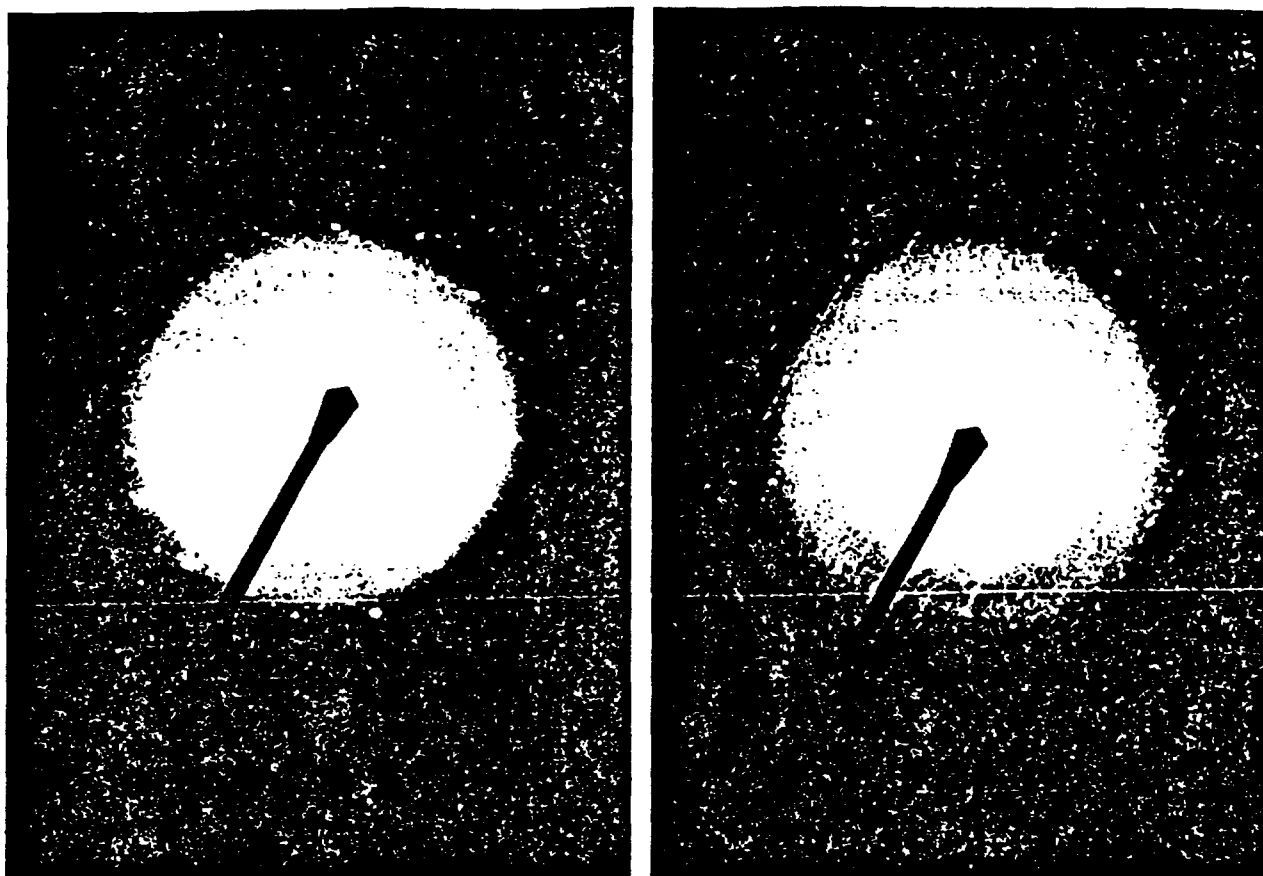
§ Data for these two fibers shown separately because one fiber showed considerable *in vivo* leaching of Mg.

from whom a positive history of asbestos exposure was obtained had asbestos body counts far greater than those seen in the control population. Of the patients with mesothelioma, the two individuals (Cases 1 and 2) who also had pulmonary interstitial fibrosis (asbestosis) had the highest pulmonary asbestos body counts. There was no difference in the morphologic features of mesotheliomas from patients with occupational asbestos exposure as compared to those without occupational exposure (Table 3).

Several possible alternatives should be considered in attempting to explain the occurrence of mesotheliomas in patients with no occupational exposure to asbestos and low asbestos body counts (<20 AB/gm) in the lung. One hypothesis is that there is no threshold level of asbestos exposure below which mesotheliomas will not occur; hence a certain small percentage of susceptible individuals will develop mesothelioma in any industrialized society in which there is ubiquitous (albeit low level) asbestos contamination of the environment.³⁰ Evidence in support of an inherited susceptibility for development

of malignant mesothelioma has recently been presented in a report of familial clustering of five cases of malignant mesothelioma (three peritoneal and two pleural mesotheliomas).³¹

A second possibility is that those individuals with low asbestos body counts were subjected to undetected occupational or environmental exposure to submicroscopic asbestos fibrils or to chrysotile asbestos, which does not readily form asbestos bodies.³² However, studies by Ashcroft and Heppleston³³ have shown that the ratio of asbestos bodies to uncoated asbestos fibers (as determined by phase contrast microscopy) and to submicroscopic fibers (enumerated by electron microscopy) remains rather constant. Thus asbestos bodies are found in the presence of larger numbers of uncoated and submicroscopic fibers. In addition, various studies have cast doubt on the likelihood that short fiber (<5 μ in length) and submicroscopic asbestos particles *per se* are either fibrogenic or carcinogenic,³⁴ although an isolated case of pulmonary interstitial fibrosis with large numbers of submicroscopic chrysotile fibers in the lungs has been



FIGS. 4A AND 4B. Selected area electron diffraction (SAED) patterns obtained from a U.I.C.C. amosite standard fiber (A, left) and an asbestos body core (B, right) from Patient 2 show identical layer line spacings as measured from photograph negatives. The pattern of each is typical of amphibole asbestos.

described.³⁵ The study by Morgan and Holmes¹⁶ demonstrated that in patients with mesothelioma it is the long amphibole fibers which tend to accumulate in lung parenchyma, and it is these fibers which have the greatest likelihood of becoming coated, (*i.e.*, asbestos bodies). Conversely, in individuals from the general population with low asbestos body counts and no evidence of asbestos-related disease, large numbers of short ($<5 \mu$) chrysotile fibers may be identified.³⁶ These data are consistent with the experimental findings of Stanton³⁷ that long thin fibers are the most efficient in the induction of mesotheliomas.

A third alternative is that factors other than asbestos may be involved in the pathogenesis of mesothelioma.² Case 17 in our series was a patient who had been treated for tuberculosis by thoracic plombage with instillation of leucite spheres 20 years prior to the diagnosis of mesothelioma of the ipsilateral pleural cavity; this patient had very low levels of asbestos bodies in the lung. In addition, an excessive number of our patients with me-

sothelioma (29%) were employed in the petrochemical or oil refinery industry, or in engineering jobs involving site visits at a refinery or chemical plant (the percentage of the population employed by these industries is 2% for Harris County and the Greater Houston area, and 6-18% for adjacent counties on the Gulf Coast³⁸). However, Lillis *et al.*³⁹ have pointed out the risk of asbestos exposure among certain employees of chemical plants and oil refineries,⁴⁰ and have described pleural thickening and radiographic pulmonary opacities in a substantial percentage of these workers (35-40%), although cases of mesothelioma have not yet been identified. In our series, two of the oil refinery workers had occupations with probable asbestos exposure: one (Case 7) was a welder for 17 years, and the other (Case 11) was a former laborer and superintendent at a steel mill for six years. In addition, two of the chemical workers had histories of occupational asbestos exposure: one (Case 8) as an insulation worker in the 1940s and 1950s, and one (Case 12) was exposed to asbestos while developing in-

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sulation for boilers. In one instance (Case 25), the patient's spouse was a chemical worker who did pipefitting and boiler work for 1½ years.

It is interesting to note that the seven workers employed by an oil refinery or chemical company (Cases 7-9, 11, 12, 14, and 16) had relatively low pulmonary asbestos body counts (<150 AB/g lung). This suggests a fourth alternative explanation for the development of mesothelioma in patients with low-dose asbestos exposure, and that is a synergistic effect between asbestos and some as yet unidentified environmental factor. The synergistic effect between asbestos and cigarette smoking in the pathogenesis of bronchogenic carcinoma is well recognized,⁴¹ although cigarette smoking has not been identified as a risk factor in the development of mesotheliomas.⁴ There is some experimental evidence that asbestos may act synergistically with radiation or chemical carcinogens in the induction of mesothelioma.⁴² Further investigation is required to clarify the role of additional possible etiologic factors in the pathogenesis of mesothelioma.

Electron microscopic and microanalytical studies on asbestos bodies from several of our cases confirmed the findings of previous investigators that practically all ferruginous bodies isolated from the general population⁴³⁻⁴⁵ and the few previously isolated from patients with mesothelioma^{5,15,17} are nucleated on asbestos cores, the majority of which cores are amphiboles. We did not identify any type of fibrous dust other than asbestos in the small number of mesothelioma cases which we examined by EDXA and SAED. Interestingly, in Case 10, six of seven cores examined were anthophyllite or tremolite, which are known contaminants of talc.⁴⁶ However, we could not confirm a history of exposure to talc in this 84-year-old woman. Churg and Warnock previously noted that asbestos bodies with anthophyllite or tremolite cores were isolated from women much more frequently than from men,⁴⁵ and suggested contamination of talc by these fiber types as a possible source. The asbestos body length and core diameter data reported by these investigators⁴⁵ are in close agreement with similar measurements of the 50 asbestos bodies described in the present study.

Langer and associates⁴⁷ have described leaching of Mg from chrysotile fibers *in vivo*, which was also noted in the two chrysotile cores identified in our study, and in the four anthophyllite fibers as well (Table 4). In addition, the iron content of the asbestos body cores was in general greater than that of the corresponding standard fibers. The effects of these ionic shifts and their role in the pathogenesis of asbestos related diseases are currently poorly understood and require further investigation.⁴⁸ Although there was considerable overlap in the

amosite and crocidolite regions on the Gibbs triangular coordinate diagram (Fig. 3), better separation of these two types was obtained by discriminate function analysis. However, three of the 40 known amosite and crocidolite fibers were misclassified by this latter technique, so we did not attempt to further separate the unknown fibers which were classified as amosite or crocidolite by computer analysis, since the increased iron content of coated fibers would likely magnify this error. The percentage calcium peak height in the present study (Table 4) was greater than that reported previously by other investigators⁴⁵ because we did not correct for background counts.

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