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PART 2. TISSUE BURDENS: WHAT DO THEY TELL US?

Analysis of Asbestos Fibers in Lung Parenchyma, Pleural Plaques, and Mesothelioma Tissues of North American Insulation Workers[a]

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It has been well established that asbestos exposure produces various neoplastic and fibroplastic diseases including lung cancer, malignant mesothelioma, pulmonary asbestosis, pleural fibrosis, and pleural hyaline plaques. Epidemiologically, a high incidence of other diseases, such as cancers of the larynx, stomach, colon, esophagus and kidney, has also been reported among asbestos insulation workers.[1]

A linear dose–response relation for the induction of asbestos-related diseases has been postulated. Although both the occupational history and chest X-ray and histopathologic findings, including identification of ferruginous bodies (FBs), have been used for the estimation of the degree of cumulative asbestos exposure, counting of the number of intrapulmonary fibers by electron microscopy has recently been utilized for the same purpose. In addition to the number of asbestos fibers, the types and dimensions of inhaled asbestos fibers have recently been considered as important in evaluating asbestos dose. Asbestos tissue burden studies by analytical electron microscopy have concomitantly raised two questions: whether inhaled asbestos fibers are cleared from the lung and whether the types of inhaled fibers are the same in both extrapulmonary and intrapulmonary sites. Studies have reported that chrysotile clears at faster rates than the amphiboles do from the lungs of asbestos workers exposed to admixtures of several fiber types. While the fibrotic pleura and/or hyaline plaques of these workers were found to contain mainly chrysotile,[2,3] the converse was true for the lung parenchyma. Dodson *et al.* have reported that short (≤5 microns) chrysotile fibers were predominantly translocated into the pleura and the regional lymph nodes from exshipyard workers' lungs exposed to both chrysotile and amphibole asbestos.[4]

We have undertaken a tissue burden study of tissues of asbestos insulation workers to explore these questions by examining the types, numbers, and sizes of asbestos fibers in the lungs, and comparing the findings in extrapulmonary sites.

[a] This work was partially supported by NIEHS Center Grant No. ES00928 in the U.S.A. and also by Grants in Aid for Scientific Research No. 03202133, Ministry of Education, Japan.

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EXHIBIT
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Insulation workers whose findings we report are known to have been occupationally exposed to materials containing chrysotile (mainly Canadian chrysotile) and amosite (South African amosite). Mortality data for the cohort from which our specimens were derived have been reported by Selikoff and his associates.[1] All information related to pathological diagnoses, occupational and cigarette smoking histories, and clinical summaries of each of the insulation workers were available to us. Our purposes were (*a*) to elucidate the types, quantities, and size distributions of asbestos fibers in lung parenchyma, pleura (fibrotic and mesotheliomatous), and peritoneum (mesotheliomatous); (*b*) to determine the qualitative and quantitative differences between fibers found in the tissues described above; and (*c*) to evaluate both the clearance and translocation of intrapulmonary asbestos. Such knowledge would better permit a better understanding as to whether the inhaled fibers are commonly translocated from the lung to the pleura and/or the peritoneum, and which types and sizes of fibers have increased potential to translocate.

MATERIAL AND METHODS

Material

The 33 human tissue samples from 13 insulation workers in the United States and Canada were prepared for analysis of asbestos bodies and asbestos fiber content as well as mineralogical characteristics. These cases were derived from a large prospective study of asbestos insulation workers.[1]

The 13 cases included 3 of asbestosis, 3 of asbestosis associated with lung cancer, 2 of pleural mesothelioma, and 5 of peritoneal mesothelioma. Demographic data and histopathological features are listed in TABLE 1. Pathological diagnoses were validated by one of the authors (Y.S.) It was noteworthy that, histopathologically, pulmonary fibrosis was seen in all but a single case (case 7). The duration from exposure to death was mostly over 30 years. Onset of asbestos exposure was mostly in the 1930s and 1940s, although in two cases the subjects started work in the 1950s.

Three samples, consisting of the lung parenchyma, pleural plaque and tumor tissues of either mesothelioma or lung cancer were available in six cases, including two cases of asbestosis associated with lung cancer and four of mesothelioma. In the other seven cases, paired samples of lung parenchyma and pleural plaque or tumor of lung or peritoneum were available. Details are listed in TABLE 2.

Digestion Procedure

Small pieces (approximately 1 cm^3 in volume) of wet tissue were used for analysis. Both wet and dry (dried in an air bath at about 100°C for a few hours) samples were weighed. The mean weights of the individual tissue samples were about 1 gram in their wet state and 0.1–0.2 gram dry. The dried tissue samples were allowed to react with laboratory bleach (Clean 99-K200®: a combined solution of 30% sodium hypochlorite, 4% potassium hydroxide, and an anionic surface-active agent) at 60°C for 3–6 hours. The digested solutions were centrifuged at 11,000 rpm for 30 minutes and the supernatant discarded. Distilled water was added, and the centrifugation process repeated. After repeating the process twice, distilled water was again added and the solution weakly sonicated. The suspen-

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TABLE 1. History of Asbestos Exposure and Pathologic Features in 13 Insulation Workers

Case No.	Age at Death and Sex	Asbestos Exposure: Smoking History[a]	Asbestos Exposure: Age and Calendar Year at Onset	Asbestos Exposure: Duration[b]	Diagnosis[c]	Pathologic Features[d]
1	67, M	No inf.[e]	25 ('41)	42	Asb	IF (s)
2	54, M	No inf.	21 ('52)	34	Asb	IF (m)
3	58, M	40 PY	21 ('47)	37	Asb	IF (m), PC
4	63, M	100 PY	20 ('39)	44	Asb + LC	IF (s), Sc-C
5	62, M	15 PY	20 ('43)	41	Asb + LC	IF (s), Ad-C
6	70, M	25 PY	31 ('40)	40	Asb + LC	IF (s), Lc-C
7	52, M	No inf.	18 ('43)	34	Pl. Meso	IF (n)
8	67, M	No inf.	24 ('39)	44	Pl. Meso	IF (s)
9	55, M	Ex-smoker	27 ('48)	29	Pe. Meso	IF (m)
10	56, M	35 PY	24 ('50)	32	Pe. Meso	IF (s)
11	62, M	No inf.	24 ('36)	38	Pe, Meso	IF (s)
12	71, M	Non-smoker	18 ('30)	53	Pe. Meso	IF (m)
13	45, M	40 PY	19 ('54)	27	Pe. Meso	IF (s)

[a] PY = Pack-years.
[b] Years from first exposure to death.
[c] Asb = asbestosis; LC = lung cancer; Pl = pleural, Pe = peritoneal; Meso = mesothelioma.
[d] IF = interstitial fibrosis; (s) = severe; (m) = moderate; (n) = none; PC = pancreatic cancer, Ad-C = adenocarcinoma; Lc-C = large-cell carcinoma, Sc-C = small cell carcinoma
[e] No inf. = not known.

TABLE 2. Type and Number of Asbestos Fibers in Lung Parenchyma, Pleural Plaques, and Tumor Tissues of Insulation Workers

Case No.	Site[a]	Disease[b]	Asbestos Body[c]	Asbestos Fibers[d]					
				Chry	Amos	Croc	Anth	Tr/Ac	D.L.[e]
1	L	Asb	8707	136	156	1.28	<DL	<DL	1.28
	P		<DL = 2.47	85.0	1.55	<DL	<DL	<DL	1.55
2	L	Asb	815	196	63.2	<DL	3.50	<DL	1.80
	P		1.53	64.3	0.80	<DL	<DL	<DL	0.80
3	L	Asb	3.72	27.6	7.27	<DL	<DL	<DL	0.48
	P		<DL = 4.09	19.3	<DL	<DL	<DL	<DL	0.36
4	L	Asb + LC	497	18.2	74.7	1.92	<DL	<DL	0.96
	T		412	4.03	131	6.45	<DL	<DL	0.81
5	L	Asb + LC	1856	51.4	89.2	<DL	<DL	<DL	1.51
	P		<DL = 5.67	39.7	<DL	<DL	<DL	<DL	0.71
	T		<DL = 2.02	71.1	3.16	<DL	<DL	<DL	1.58
6	L	Asb + LC	671	67.1	86.6	5.30	<DL	<DL	0.88
	P		9.26	89.7	5.06	<DL	<DL	<DL	0.72
	T		579	50.8	129	5.08	<DL	<DL	1.70
7	L	Pl. Meso	1697	28.3	125	<DL	2.83	<DL	2.83
	P		2.20	12.1	1.29	<DL	<DL	<DL	0.16
8	L	Pl. Meso	489	28.6	194	<DL	3.00	3.00	1.50
	P		6.18	39.2	0.60	<DL	<DL	<DL	0.60

[illegible]	L	Asb + LC	497	18.2	74.7	1.92	<DL	<DL	0.96
	T		412	4.03	131	6.45	<DL	<DL	0.81
5	L	Asb + LC	1856	1.4	89.2	<DL	<DL	<DL	1.51
	P		<DL = 5.67	39.7	<DL	<DL	<DL	<DL	0.71
	T		<DL = 2.02	71.1	3.16	<DL	<DL	<DL	1.58
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	P		9.26	89.7	5.06	<DL	<DL	<DL	0.72
	T		579	50.8	129	5.08	<DL	<DL	1.70
7	L	Pl. Meso	1697	28.3	125	<DL	2.83	<DL	2.83
	P		2.20	12.1	1.29	<DL	<DL	<DL	0.16
8	L	Pl. Meso	489	28.6	194	<DL	3.00	3.00	1.50
	P		6.18	39.2	0.60	<DL	<DL	<DL	0.60
	T		<DL = 0.06	62.1	<DL	<DL	<DL	<DL	1.27
9	L	Pe. Meso	366	24.0	126	7.57	<DL	<DL	1.26
	P		<DL = 1.38	36.3	6.34	<DL	<DL	<DL	0.58
	T		<DL = 1.43	14.8	<DL	<DL	<DL	<DL	0.76
10	L	Pe. Meso	6499	111	282	25.6	<DL	<DL	2.13
	P		17.4	31.8	6.81	<DL	<DL	<DL	0.76
	T		<DL = 0.28	16.5	0.52	<DL	<DL	<DL	0.17
11	L	Pe. Meso	226	25.5	120	<DL	<DL	<DL	0.77
	P		<DL = 1.90	29.4	1.80	<DL	<DL	<DL	0.60
	T		1.07	12.6	1.76	<DL	<DL	<DL	0.44
12	L	Pe. Meso	2753	91.9	213	86.4	<DL	3.68	1.84
	T		0.38	50.1	1.79	<DL	<DL	<DL	0.60
	T		0.05	43.7	<DL	<DL	<DL	<DL	0.48
13	L	Pe. Meso	10570	15.0	415	11.3	<DL	11.3	3.75
	T		10.9	89.6	14.2	<DL	<DL	<DL	1.42

[a] L = lung; P = plaque, T = tumor.
[b] Asb = asbestosis; LC = lung cancer; Pl = pleural; Pe = peritoneal; Meso = mesothelioma.
[c] × 10^3/gram (dry tissue).
[d] × 10^6/gram (dry tissue). Chry = chrysotile; Amos = amosite; Croc = crocidolite; Anth = anthophyllite; Tr/Ac = tremolite/actinolite.
[e] DL = detection limit; <DL = under detection limit (no fiber detected).

sion was made up precisely to 50 ml with distilled water and transferred into a clean screw-top glass container. All solutions used in this preparation were prefiltered using a membrane filter to eliminate fiber contamination.

An aliquot (volume 5–25 ml) from each digested solution was filtered through a 25-mm Millipore membrane filter (pore size 0.45 μm). The filter was washed well with distilled water and allowed to dry. The filter was fixed on a glass slide with acetone vapor to make a transparent slide specimen, and was ashed in a plasma low-temperature asher. Ferruginous bodies (FBs) were then counted by polarized light microscopy (PLM) at 250×. 200 FBs or 50 fields were counted and the concentrations of FBs (in FBs per gram dry tissue) calculated for each sample. After the FBs were counted, the sample area on the slide was "corner-affixed" with a cellophane glue tape and covered by 8% polyvinyl alcohol (PVA) solution and dried overnight at room temperature or for 2–3 hours at 45°C. The dried PVA film was removed and turned over and fixed on the glass slide by cellophane tape. After relatively heavy carbon evaporation on the PVA film, it was floated on a hot water bath for several hours to dissolve the PVA film. Small pieces of floating carbon film, still holding mineral particles, were taken up by 200-mesh nickel TEM grids. Generally, 10 to 20 specimen grids were made. Details of this new quantitative carbon extraction (QCE) method will be reported elsewhere.

Analytical Procedure

Randomly selected grid squares were scanned for fibers with an analytical transmission electron microscope (TEM) at ×20,000 to ×30,000 direct magnification. Fibers were differentiated into five types (chrysotile, amosite, crocidolite, anthophyllite and tremolite/actinolite) utilizing several criteria: fiber morphology (as seen on the fluorescence screen with a light microscope [×10] attached to the TEM), chemical composition determined by energy dispersive X-ray spectra and, sometimes, lattice geometry as shown from the electron diffraction patterns. All asbestos fibers were sized for length and diameter directly on the fluorescence screen, and all asbestos fibers 0.2 μm in length or longer were counted. A fiber, [redacted] ratio. The data were entered into a computer to calculate the concentrations (fibers/gram dry tissue), size distributions, and some statistical features. Except for the lung cancer tumors of Cases 4, 5, and 6, the average detection limits for lung samples and plaque and tumor samples, respectively, were 1.6×10^6 f/g dry tissue (ranging from 0.5 to 3.8×10^6 f/g dry tissue) and 0.7×10^6 f/g dry tissue.

RESULTS

The concentrations of asbestos bodies (ABs) and asbestos fibers found in lung parenchyma, pleural plaques, and tumor tissues of the 13 cases are listed in TABLE 2.

Asbestos Bodies

A considerable number of ferruginous bodies (FBs) were found in the lungs of all 13 cases. A limited number of these FBs were studied by analytical TEM. This revealed that most of the core fibers were amosite; rarely, chrysotile or nonasbes-

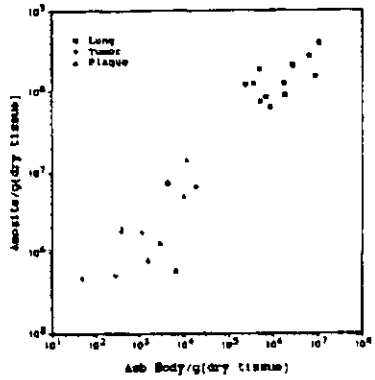

FIGURE 1. Comparative concentrations of amosite fibers and asbestos bodies in lung parenchyma, pleural plaques, and tumor tissues.

tos fibers were found. Therefore, almost all FBs seen by PLM were considered ABs, with cores of amosite fibers, and FBs counted by PLM were expressed as ABs in TABLE 2. There was good correlation between the concentrations of amosite fibers counted by TEM and ABs counted by PLM, as shown in FIGURE 1. The mean concentration of ABs was 1.69×10^6 per gram dry lung. ABs were also found in the pleural plaque and the mesotheliomatous tissues by PLM, but in very low concentrations compared with those in the lung parenchyma (mean concentrations were about 5×10^3 and 5×10^2 per dry tissue, respectively). These concentrations were only about 0.1% of those in the lung.

Types and Numbers of Asbestos Fibers in Lung Parenchyma

The types and numbers of asbestos fibers detected in the different sites of each of the 13 cases are summarized in TABLE 2 and FIGURE 2 in conjunction with the diseases involved. In the lung parenchyma, chrysotile and amosite were found in all cases as the major asbestos types. The concentrations of chrysotile were from 15×10^6 to 196×10^6 fibers per gram dry lung, with a mean value of 63.1×10^6 fibers per gram dry lung, and those of amosite from 7.27×10^6 to 415×10^6, with a mean value of 150×10^6. Amphibole asbestos other than amosite was seen in some cases in the lung: crocidolite fibers were present in seven of the 13 cases, but at much lower concentrations (mean value 11.4×10^6 f/g dry lung) compared with either chrysotile and amosite (TABLES 2 and 3). A small number of anthophyllite

TABLE 3. Type and Concentration of Asbestos Fibers Observed in the Lung Parenchyma of 13 Insulation Workers

	Chrysotile	Amosite	Crocidolite	Anthophyllite	Tremolite
Number of cases in which asbestos fibers were observed	13 (100%)	13 (100%)	7 (54%)	3 (23%)	3 (23%)
Concentration*	63.1 (±53.5)	150.2 (±102.6)	11.4 (±22.6)	1.86 (±1.05)	2.45 (±2.71)

* Mean values expressed as $\times 10^6$ fibers/gram (dry tissue) with detection limits (i.e., not detected) approximately $1.61 \pm 0.86 \times 10^6$ f/g (dry tissue).

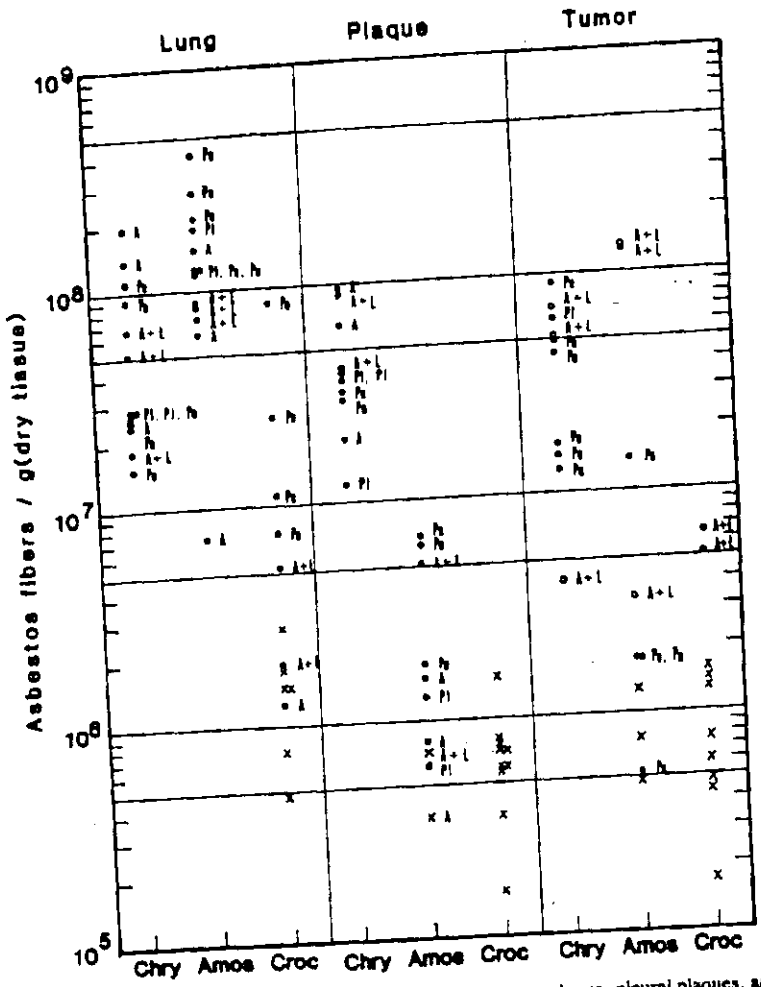

FIGURE 2. Concentrations of asbestos fibers in the lung parenchyma, pleural plaques, and tumor tissues in specimens from 13 asbestos insulation workers. A = asbestosis; A + L = asbestosis with lung cancer; Pl = pleural mesothelioma; Pe = peritoneal mesothelioma; *open circle* = lung cancer tissue, x = not detected (indicating the detection limit).

fibers were seen in three cases (mean value 1.9×10^6 f/g dry lung) and tremolite/actinolite fibers were identified in another three cases, again in very small numbers (mean value 2.5×10^6 f/g dry lung). These findings indicate that the insulation workers studied here had been mostly exposed to dusts containing admixtures of chrysotile and amosite. The number of amosite fibers detected in the lungs were

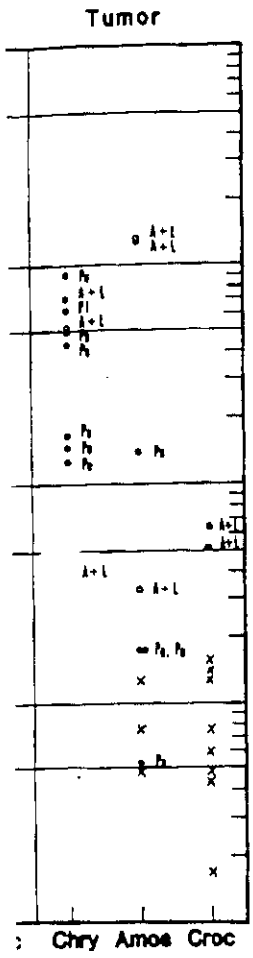

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TABLE 4. The Concentrations[a] of Asbestos Fibers Observed in Lung Parenchyma, Pleural Plaques, and Mesothelioma Tissue in 13 Insulation Workers

	Chrysotile	Amosite	Crocidolite	Anthophyllite	Tremolite
Lung (n = 13)	63.1 (±53.5)	150.2 (±102.6)	11.4 (±22.6)	1.86 (±1.05)	2.45 (±2.72)
Plaque (n = 10)	44.6 (±25.0)	2.53 (±2.39)	n.d.[b]	n.d.	n.d.
Tumor (n = 7)	49.4 (±26.0)	3.3 (±4.53)	n.d.	n.d.	n.d.
			[c]	n.d.	n.d.

[a] Mean values expressed as × 10^6 fibers/gram (dry tissue).
[b] n.d. = under detection limit value of 0.68 ± 0.34 × 10^6 f/g (dry tissue).
[c] 6.45 × 10^6 and 5.08 × 10^6 f/g (dry tissue) of crocidolite were found in two lung cancer specimens (Cases 4 and 6).

usually higher than those of chrysotile in each of the 13 insulation workers. The mean ratio of the concentrations of amosite and chrysotile fibers detected in the lungs of the workers was 4.8, as shown in FIGURE 3a and TABLE 5.

Types and Numbers of Asbestos Fibers in the Pleural Plaques and Mesotheliomatous Tissue

A remarkable finding in this study was that chrysotile fibers were found in pleural plaques and mesothelioma tumor in concentrations similar to those seen in the lung parenchyma (FIG. 2 and TABLE 4). On the other hand, amosite, while abundant in the lung parenchyma, was [illegible] in the pleura and in the tumor (FIG. 2 and TABLE 4). In TABLE 5 and FIGURE 3a, the ratios of the concentrations of amosite to chrysotile in plaques and tumors were about 0.06. FIGURE 3b shows the ratios of the concentrations of chrysotile and amosite in the pleural plaques to those in the lungs, and also in mesothelioma tissues compared to those in the lungs. It can be clearly seen that the concentrations of amosite in both pleural plaques and mesothelioma tissue are markedly lower compared with chrysotile, unlike the comparable ratios in the lung. FIGURE 4a-c shows the concentrations by site for each case. Except for lung cancer (Case 4 and Case 6), the concentration of amosite fibers was clearly reduced in both the pleural plaques and the mesothelioma tissues, again unlike the concentration of chrysotile fibers, which did not decrease. Indeed, in some cases, it actually increased (Cases 5, 8, and 13).

TABLE 5. Ratio of the Concentration of Amosite to That of Chrysotile in Tissue Samples of 13 Insulation Workers by Site

Site	Amosite/Chrysotile	Range
Lung (n = 13)	4.81 ± 6.88	0.26–27.67
Plaque (n = 10)	0.07 ± 0.07	0.013–0.214
Tumor (n = 7)[a]	0.07 ± 0.06	0.011–0.159

[a] Except for lung cancer tissue (Cases 4T, 5T and 6T in TABLE 2).

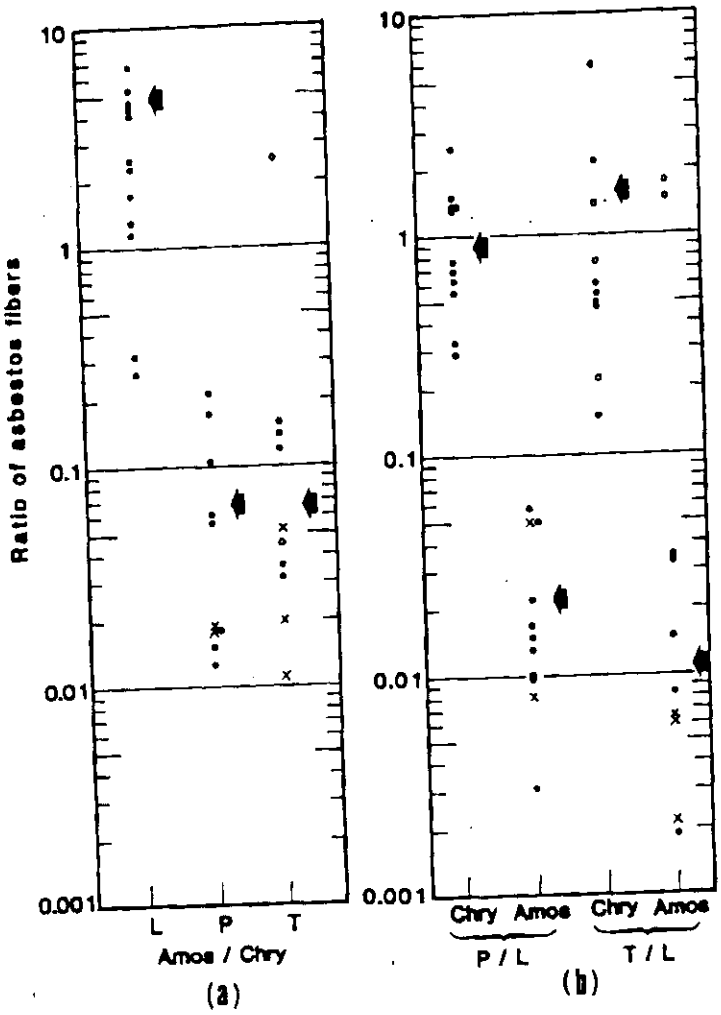
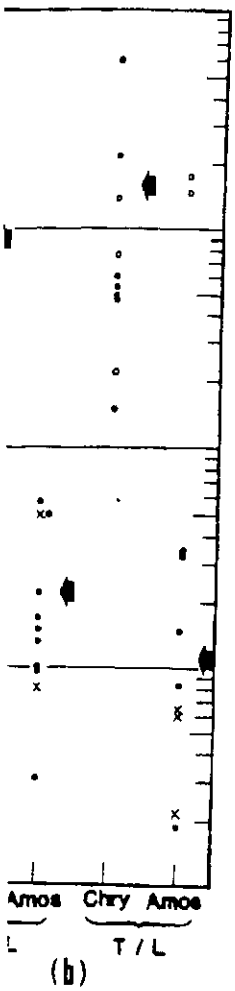


FIGURE 3. (a) Ratio of concentration of amosite to that of chrysotile in the lung, plaques, and tumor tissue in 13 asbestos insulation workers. (b) Ratios of concentrations of chrysotile in plaques and tumor to those in the lung; and, similarly, those of amosite in plaques and tumor to those in the lung. *open circle* = lung cancer tissue; x = not detected; *arrow* = geometric mean.



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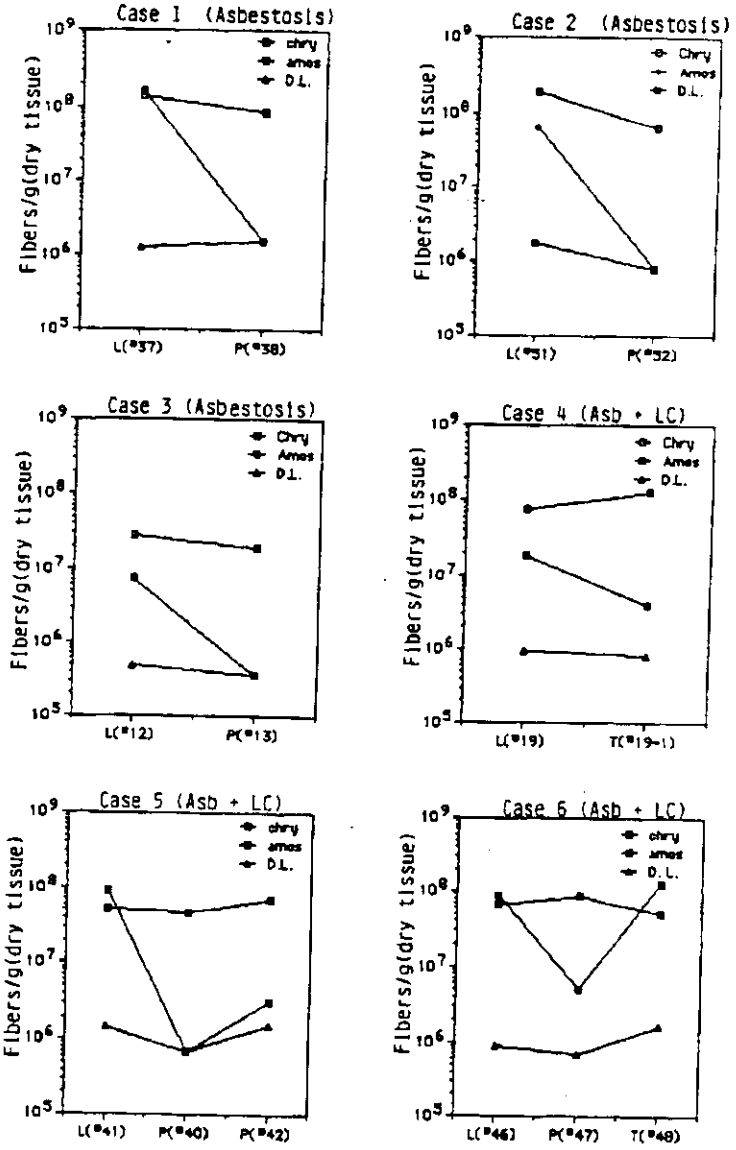

FIGURE 4. (*Legend is on p. 39.*)

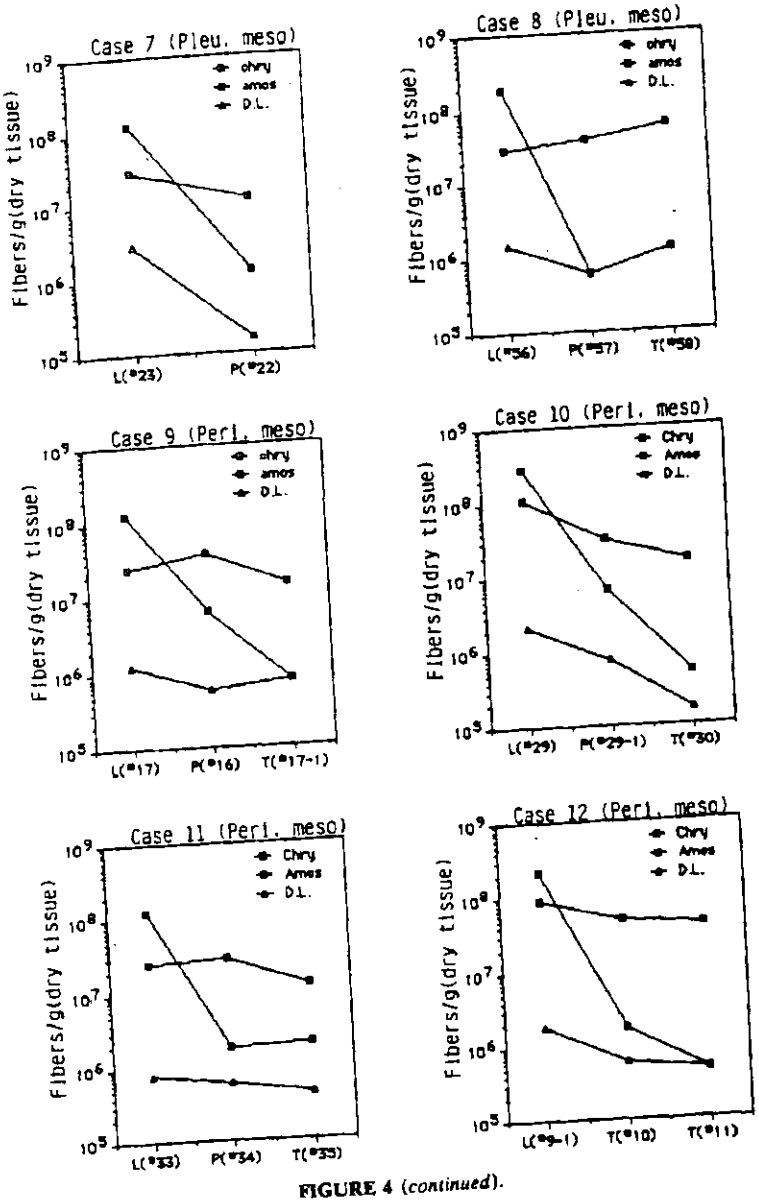

FIGURE 4 (*continued*).

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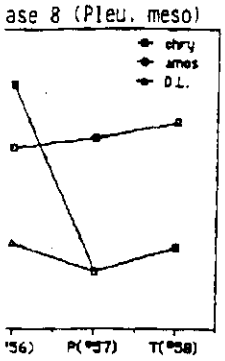
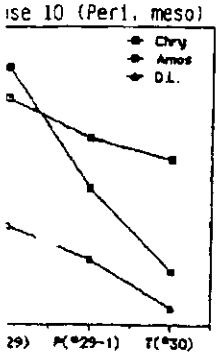
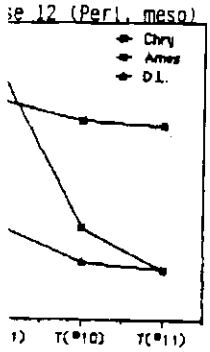






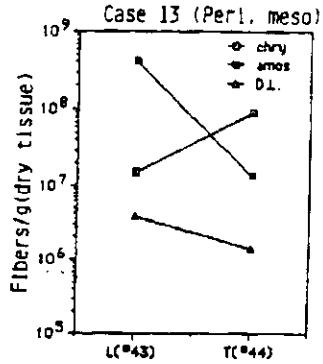


FIGURE 4. Concentration of asbestos fibers by site in each case. L = lung parenchyma; P = pleural plaque; T = tumor tissue, such as lung cancer, pleural, or peritoneal mesothelioma; D.L. = detection limit.

Comparison of Fiber Dimension (Length and Diameter) in the Three Sites

All asbestos fibers found by analytical TEM were measured for length and width and plotted on a scattergraph by site for each case. Some typical cases, for example, Case 6 (asbestosis and lung cancer), Case 8 (pleural mesothelioma), and Case 11 (peritoneal mesothelioma), are shown in FIGURE 5a-c. The cumulative percentages of undersize, by length and width, were then plotted against the length and width in log scale as shown in FIGURE 6a-c. Size data for chrysotile and amosite found in each site in these three cases are listed in TABLE 6. In the lung parenchyma, the geometric mean length of chrysotile was about 1.0 μm and, that

TABLE 6. Dimensions of Asbestos Fibers Found in Lung, Plaques, and Tumor Tissue of Insulation Workers

		Length (μm)						Width (μm)	
		Chrysotile		Amosite		Chrysotile		Amosite	
Case No.	Site	Mg	(δg)	Mg	(δg)	Mg	(δg)	Mg	(δg)
Case 6	Lung	1.1	(2.2)	2.3	(3.5)	0.03	(1.3)	0.10	(1.6)
	Plaque	1.1	(2.2)	1.8	(2.2)*	0.02	(1.5)	0.08	(7.5)
	Tumor (LC)	1.2	(2.4)	2.0	(4.0)	0.04	(1.2)	0.08	(2.5)
Case 8	Lung	1.0	(3.0)	3.3	(2.9)	0.02	(1.2)	0.13	(2.3)*
	Plaque	0.8	(3.2)		—		0.03	(1.7)	—
	Tumor (Pl. meso)	1.4	(3.1)		—		0.03	(1.3)	—
Case 11	Lung	1.0	(2.5)	2.8	(2.9)	0.03	(1.9)	0.09	(2.6)*
	Plaque	1.0	(3.0)	2.5	(4.0)	0.04	(1.4)	0.15	(2.1)*
	Tumor (Pe. meso)	0.7	(3.3)	4.3	(2.3)	0.03	(1.4)	0.15	(1.5)*

NOTE: Mg = geometric mean; δg = geometric standard deviation; LC = lung cancer; Pl. meso = pleural mesothelioma; Pe. meso = peritoneal mesothelioma.

* Number of detected fibers is too small.

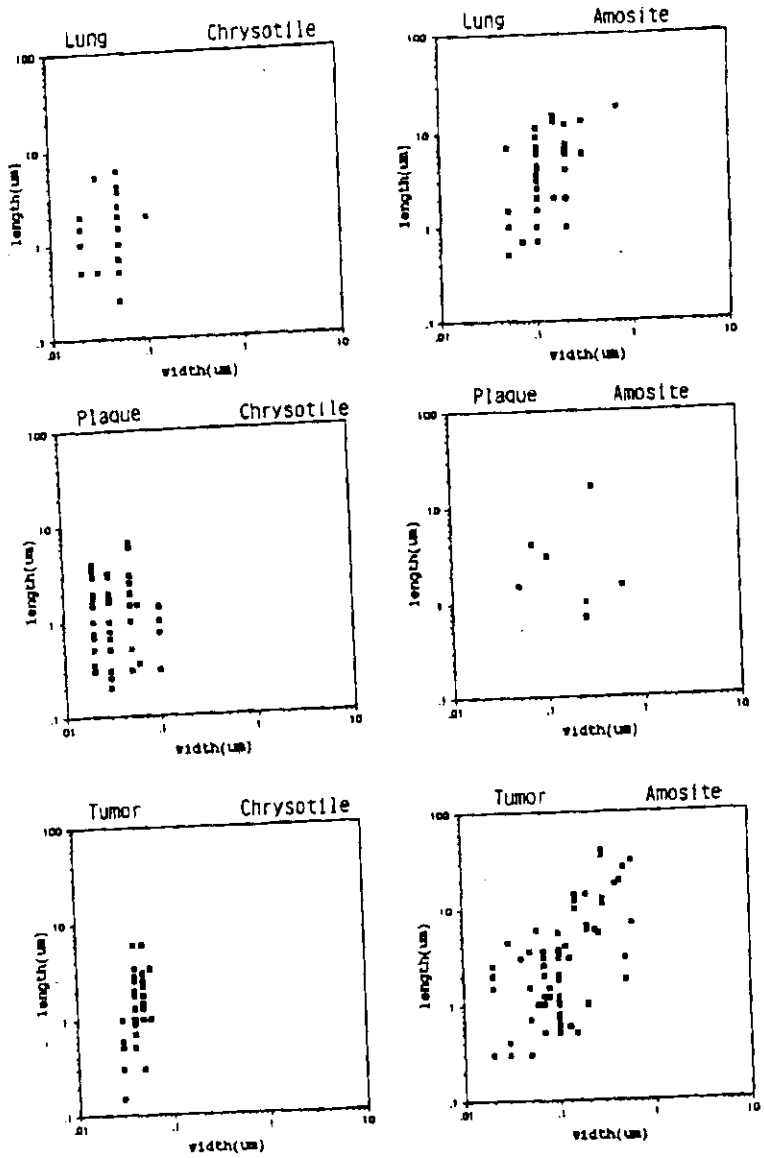
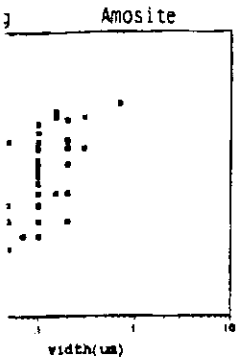
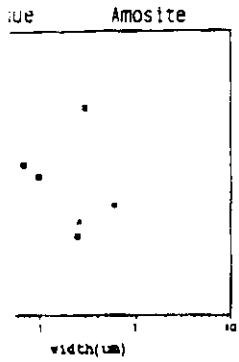
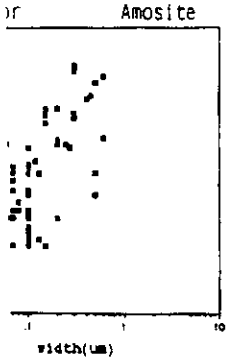


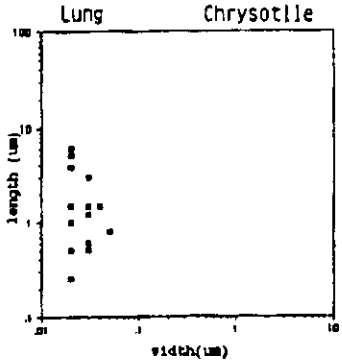
FIGURE 5a. Length and width of chrysotile and amosite fibers found in pulmonary parenchyma, pleural plaques, and tumor tissues in Case 6 (asbestosis with lung cancer).

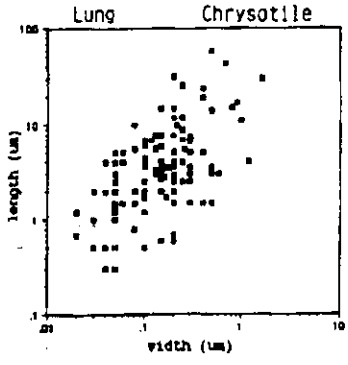


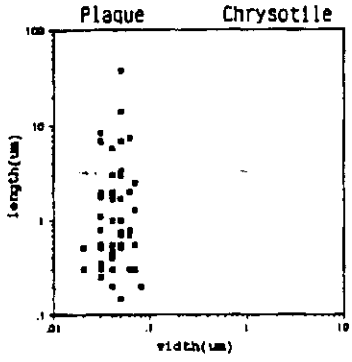




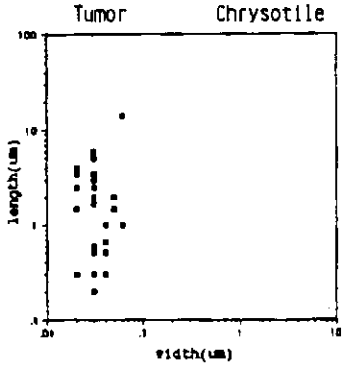
found in pulmonary paren-
s with lung cancer).





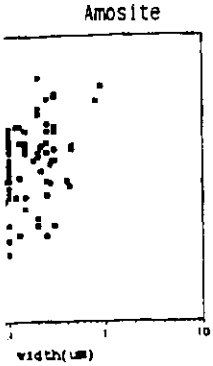


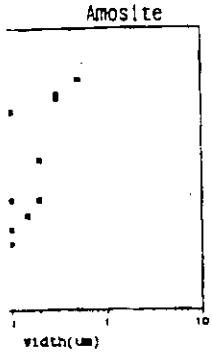
Plaque Amosite
not detected



Tumor Amosite
not detected

FIGURE 5b. Length and width of chrysotile and amosite fibers found in pulmonary parenchyma, pleural plaques, and tumor tissues in Case 8 (pleural mesothelioma).





found in pulmonary paren-
mesothelioma).

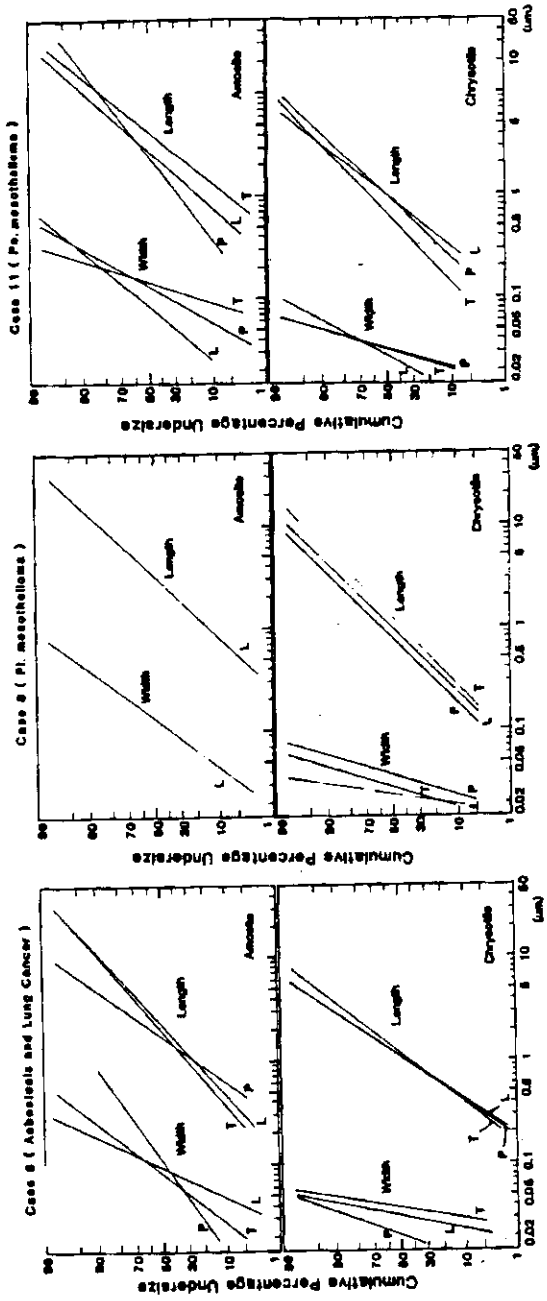


FIGURE 6. Cumulative percentage of undersize expressed by length and width in log scale in Cases 6 (*left*), 8 (*middle*), and 11 (*right*).

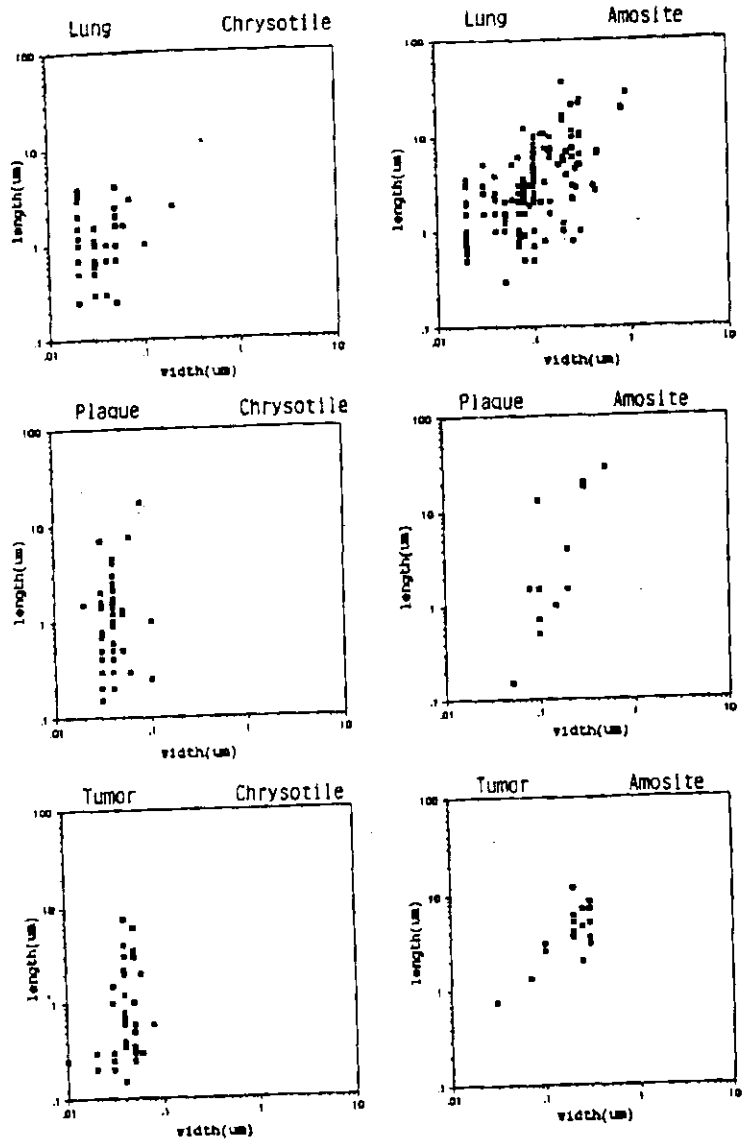


FIGURE 5c. Length and width of chrysotile and amosite fibers found in pulmonary parenchyma, pleural plaques and tumor tissues in Case 11 (peritoneal mesothelioma).

of amosite, 2.8 μm. The geometric mean widths of chrysotile and amosite in the lung parenchyma were also very different from each other—about 0.03 μm and 0.11 μm, respectively. Neither the mean length nor width of amosite and chrysotile seem to be changed in extrapulmonary sites, such as pleural plaques and tumors, compared with those in the lung parenchyma. Although these data are subject to considerable statistical variability, particularly because the number of fibers enumerated was limited, the relationships are noteworthy. We were struck by the fact that the size distributions of chrysotile found in the lung and in extrapulmonary sites did not show systematic differences, for example, shorter or thinner as we went from lung (L) to pleural plaques (P) to tumor (T), but rather were similar in the three sites (FIG. 6a-c). This tendency was also seen in the size distributions of amosite in each site (FIG. 6a-c).

Long fibers, such as those longer than 10 μm, were sometimes found even in pleural plaques and tumors, as shown in FIG. 3b (chrysotile in the plaque and tumor) and in FIG. 5a and c (chrysotile in the plaque and amosite in the plaque and tumor).

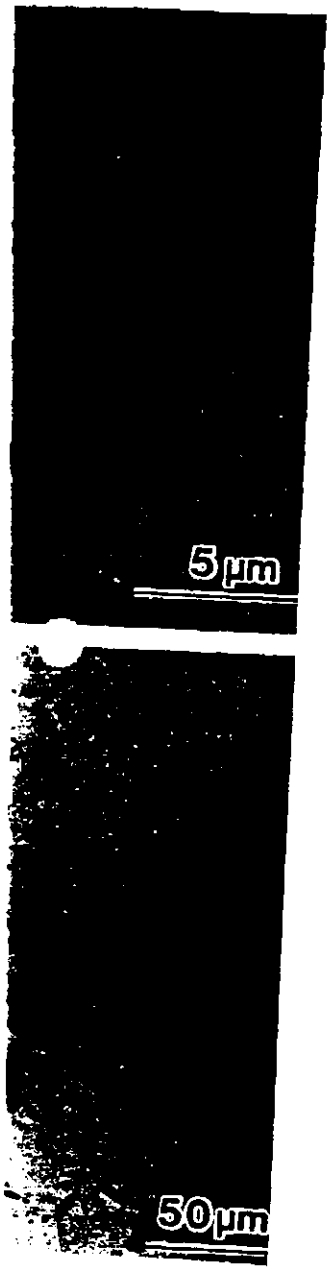
FIGURE 7a depicts the asbestos fibers seen by TEM in lung tissue from Case 13, which contained a large number of asbestos bodies and amosite fibers. The same sample was studied by PLM (FIG. 7b). FBs(=ABs) and fibers in the pleural plaque and mesothelioma tissue observed by PLM are shown in FIGURE 8a-d. In the pleural plaque of Case 10, a long amosite fiber approximately 82 μm in length and ABs (62 μm and 45 μm in length) were also found (FIG. 8a and b). In the peritoneal mesotheliomatous tissue of Case 12, a long chrysotile fiber approximately 176 μm in length (FIG. 8c) and a fine AB (12 μm in length) were observed (FIG. 8d).

DISCUSSION

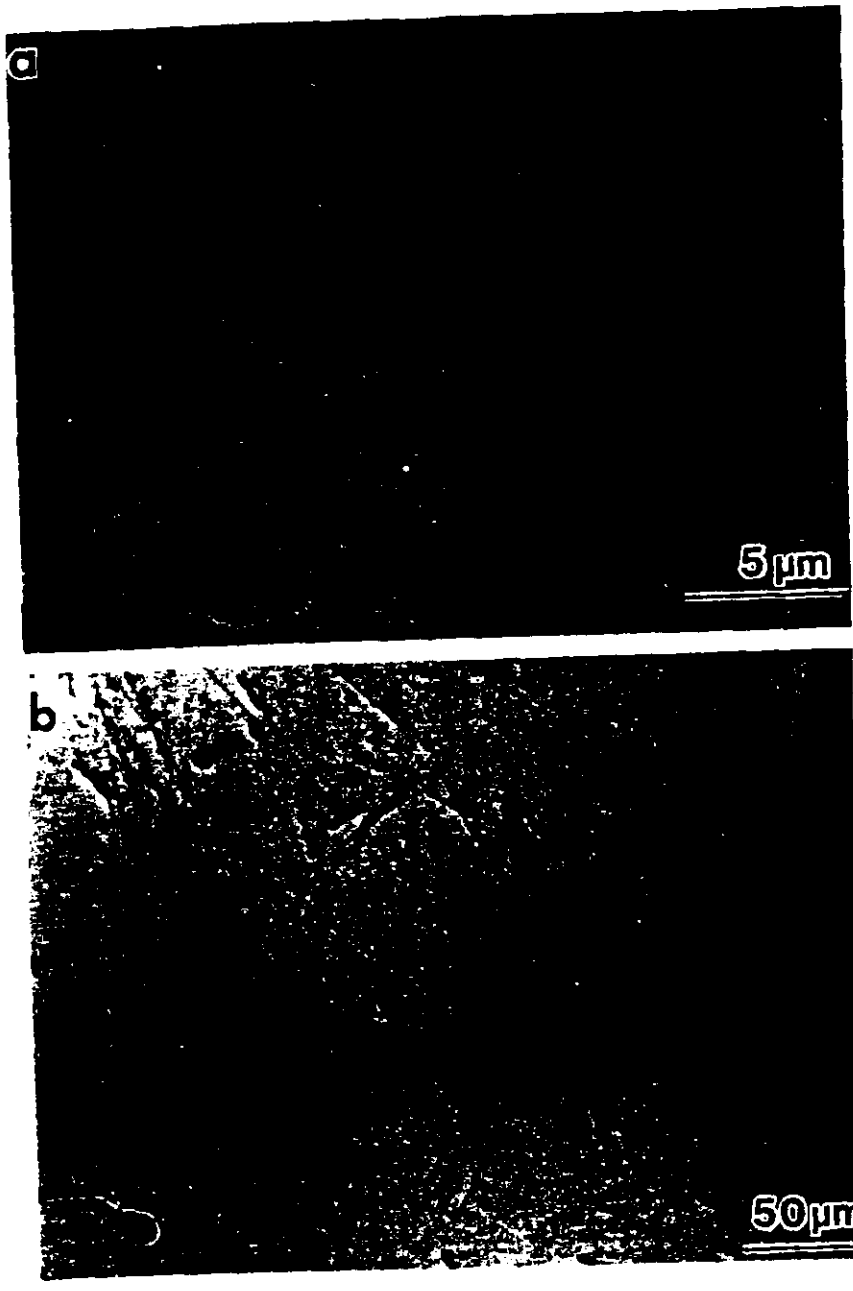
North American insulation workers are known to have been occupationally exposed to materials containing chrysotile (mainly Canadian chrysotile) and amosite (South African amosite) in an approximate ratio of 6 : 4.[1] Our asbestos tissue burden study of the 13 cases confirmed the epidemiological understanding that the workers had been exposed during their work to a mixed dust of chrysotile and amosite, often of considerable intensity.

The concentration ratios of chrysotile and amosite fibers in their lungs were different, however, from the ratios in the exposure estimates (that is, the number of amosite fibers in the lungs was generally higher than that of chrysotile). More chrysotile than amosite was inhaled; more amosite than chrysotile was retained in the lung parenchyma. On the other hand, large numbers of chrysotile fibers were detected in the extrapulmonary sites, such as in the pleural plaques and in pleural and peritoneal mesotheliomatous tissues. Here, the levels were similar to those in the lungs. In some cases, chrysotile fibers in the pleural plaques and/or mesotheliomatous tissues were even slightly more numerous compared to the levels in the lungs (Cases 5, 6, 8, 9, 11, and 13). In contrast, the numbers of amosite fibers in these extrapulmonary sites were very much lower than the levels in the lungs.

It is of interest that, in addition to the findings in the present study, one of the authors (Y.S.) has also detected asbestos fibers in the "sugar coat" (*Zuckerguss*,



◀ **FIGURE 7.** Asbestos fibers and asbestos bodies in the lung (Case 13) observed by (a) TEM and (b) PLM.


a
5 μm
b
50 μm

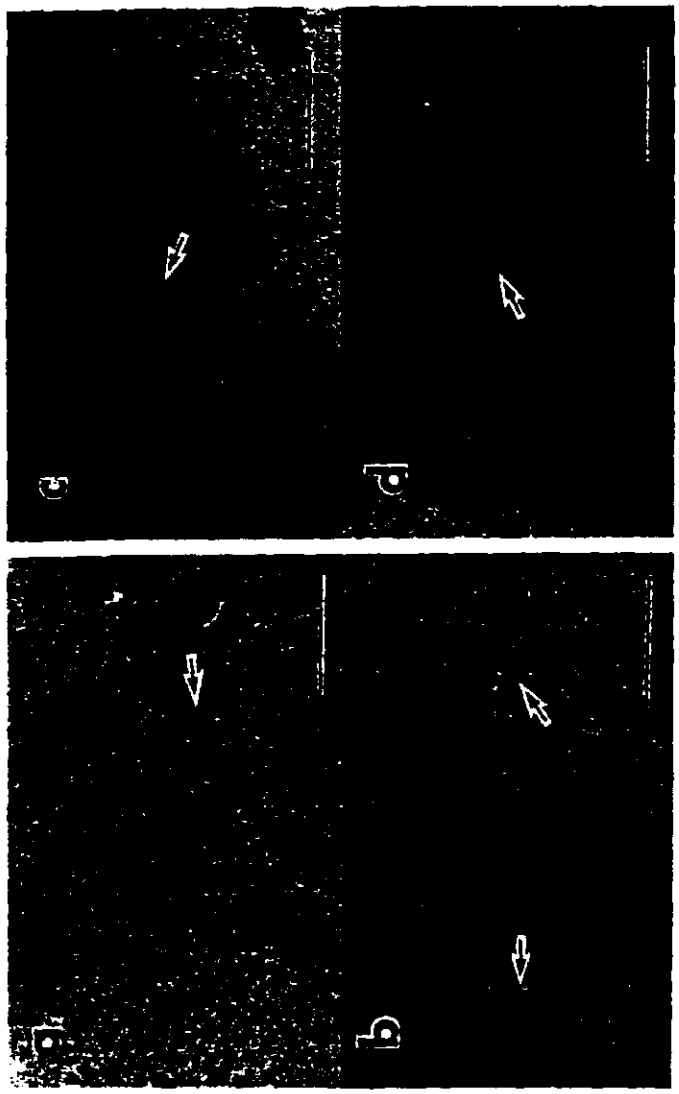


FIGURE 8. Asbestos fibers and ferruginous bodies (= asbestos bodies) in (a and b) a pleural plaque (Case 10), and (c and d) mesotheliomatous tissue (Case 12) observed by PLM. Scale: 50 μ in length.

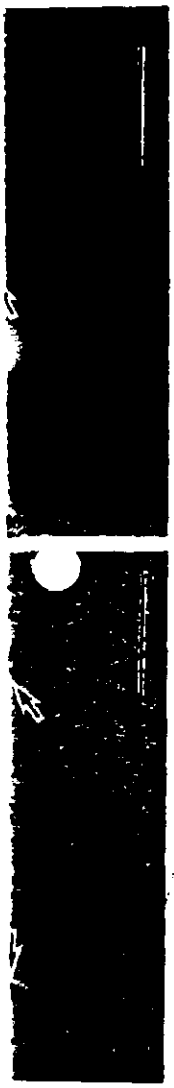


FIGURE 8. Asbestos fibers and ferruginous bodies (= asbestos bodies) in (a and b) a pleural plaque (Case 10), and (c and d) mesotheliomatous tissue (Case 12) observed by PLM. Scale: 50 μ in length.

a type of peritoneal fibrosis, histologically identical to that of hyaline plaques) of the hepatic and splenic capsules (part of the peritoneum). The specimens were derived from persons who had been occupationally exposed to both chrysotile and amphibole asbestos.[5] Proportionally, chrysotile fibers dominated in number in the "sugar coats", although smaller numbers of amphiboles were also detected.

These results imply that chrysotile fibers have high mobility and are translocated from the lung to extrapulmonary sites. Consequently, this potential may constitute one reason for finding that the number of chrysotile fibers in the lungs will be found to be less than the amount of chrysotile actually inhaled. Moreover, the concentration of chrysotile fibers detected in the lung will not necessarily be proportional to exposure. On the other hand, the number of amphibole fibers in the lung will be approximately proportional to the exposure level because they tend to remain in the lung.

Asbestos fibers are known to be durable and not easily digested or dissolved once inhaled. It has also been reported that some are cleared from the lung during the course of several years, and that the clearance rate is greater for chrysotile than for amphiboles.[6] Some chrysotile fibers would have disappeared from the lung by chemical dissolution, and some by physical removal. There are several possible routes for the removal of particulate matter from the lung, such as by ciliary motion and the mucus production of broncho-bronchiolar cells, by pulmonary lymphatics to the hilar lymph nodes, by the blood stream to many organs, and by accumulation in the visceral pleura.[7,8]

The fate of asbestos fibers, after clearance from the lung, however, has not been completely explored. If the fibers are totally cleared from the lung to outside the host's body, then the risk of asbestos-related diseases will likely be considerably less, even if early processes of multi-stage steps for the development of asbestos-related pulmonary diseases, asbestosis and bronchogenic carcinoma, may have been initiated before fiber elimination. However, such an optimistic assumption cannot be entirely accepted, since the translocation of intrapulmonary asbestos fibers (particularly chrysotile) from the lung into the parietal pleura has been strongly suggested by asbestos tissue burden studies done by LeBouffant *et al.*[2,4] and Sébastien *et al.*[3] The former found numerous short chrysotile fibers in hyaline pleural plaques; simultaneously, amphiboles were more common in the lung. The latter also detected asbestos fibers in both the lung and the fibrotic parietal pleura in a patient with asbestosis who had been exposed to both chrysotile and amphiboles. They reported that long amphibole fibers predominated in the lung, while short chrysotile fibers were exclusively seen in the fibrotic parietal pleura. Thus, these earlier studies concluded that the distribution of fiber types depended on fiber size, the mean length of the fibers being greater in the lung and visceral pleura than in the parietal pleura, particularly in the case of amphibole fibers.

Our present study has, to a considerable extent, confirmed the results of earlier investigations, but has also shown differences as well. For any given fiber type, the fibers found in extrapulmonary sites, such as pleural plaques and mesotheliomatous tissues, showed the same size distributions as those of fibers in the lungs (that is, fibers in the pleura were neither shorter nor thinner than those in the lung parenchyma). This could be seen for both chrysotile and amosite. The mean width was very different, however, in that chrysotile fibers were consistently thinner than amosite fibers. Presumably, fiber bundles of inhaled chrysotile can be easily separated into thin unit-fibrils in the lung, whereas amphiboles do not cleave so readily into thin fibrils. It was also noteworthy that long (>50 μm) and thick (>1 μm) asbestos fibers were rare in the lung, suggesting that such fibers do not easily reach the lung parenchyma through the respiratory tract.

Hillerdal[8] reviewed many earlier studies concerned with relocation of inhaled asbestos and compiled interesting information: slow migration of asbestos and other dust towards the pleura is well known ("pleural drift"). There is a constant exchange of fluids and soluble matter in the pleural cavity; even in a resting dog, 17 percent of the plasma volume passes through the pleural cavity each day[10]; in an artificial chest, fluid dripped from the lung surface with every exhalation.[11] It was also reported that the parietal lymphatics were probably of greater importance with regard to pleural drainage: the clearance of particles in the pleural effusion might be executed by the lymphatic system via stomata in the parietal pleura.[12] These data suggest that thin fibers, such as unit-fibrils of chrysotile, leave the lung and pass through the pleural cavity, as does fluid, to reach the parietal pleura.

According to our observation and earlier data, we consider that thin fibers (i.e., under 0.1 μm in width) can easily pass through the pleura and accumulate in the pleural cavity. The length of fibers would not be an important factor in translocation because even a very long fiber would be able to reach the parietal pleura if sufficiently thin. From the view point of aerodynamics, Timbrell[13] has emphasized that an inhaled fiber could penetrate the lung as long as its diameter was smaller than 3 μm, even if its length might exceed 200 μm.

Many researchers have found a marked discrepancy between extensive exposure to chrysotile at the workplace and the lack of increased concentration of chrysotile fibers in the lung parenchyma of individual workers.[14,15] This discrepancy could be explained by the results presented here (that is, that thin chrysotile fibers have high motility in the host's body compared with considerably thicker amphibole fibers).

It is generally accepted that all asbestos types can induce human malignant mesothelioma, although its incidence is said to be different among the several types of asbestos, with crocidolite associated with the greatest hazard.[16] On the other hand, in animal experiments all asbestos types induce tumors without marked differences in incidence when the size distributions of the fibers are similar.[18,19]

Despite the absence of high concentrations of chrysotile fibers in the lung, significant accumulation of chrysotile fibers in pleural and peritoneal tissues should be considered a potentially important factor in the induction of human malignant mesothelioma.

It has been reported that commercially available Canadian chrysotile, the fiber widely utilized in U.S. insulation materials, was contaminated with tremolite. Asbestos fibers in the lungs of Canadian chrysotile miners and millers with mesothelioma were predominantly tremolite, suggesting that tremolite may have played a significant role in the induction of the neoplasms.[14]

Although the asbestos insulation workers studied by us were exposed to Canadian chrysotile together with South African amosite, tremolite was nevertheless rare in their lungs and no tremolite was detected in either the hyaline plaques or in mesotheliomatous tissue. Consequently, unlike the situation with Canadian chrysotile miners and millers, tremolite would not seem to be an etiologic factor in the induction of malignant mesothelioma in these insulation workers.

It is known that, as with bronchogenic carcinoma and malignant mesothelioma, the incidence of a number of other cancers originating in the larynx, esophagus, stomach, colon and the kidney is higher among asbestos insulation workers than in the general population.[1] It would be of interest to know whether asbestos fibers are detected in significant numbers in these tissues, and if so, whether their types and dimensions are similar to those seen in pleural hyaline plaques and mesotheliomatous tissues.

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Although, generally, the number of FBs observed is only a fraction of the total number of fibers inhaled, that fraction tends to be constant, and thus most routine histological examinations for asbestos disease have utilized FB counts to estimate fiber level inhaled. The constraints of this technique, which include the necessity for sufficient experience on the part of the microscopist to recognize uncoated fibers, are compensated for by its simplicity. We have also counted FBs in this study. The mean concentration in the lungs of the 13 insulation workers was about 1.7×10^6 FBs per gram dry lung, similar to the concentrations found in workers exposed to crocidolite in British gas-mask factories.[20]

The AB concentrations found in this study correlate well with the amosite fiber concentrations in both lungs and in extrapulmonary sites, as shown in FIGURE 1. As Davis *et al.*[21] have emphasized, most of the ABs in human lungs can be shown to have been formed on amphibole fibers. This has led to suggestions that the coating of chrysotile is rare and that enumeration of ABs is not useful as a measure of asbestos exposure, if only chrysotile exposure has occurred.

In this study, extrapulmonary AB concentrations were considerably lower than those in the lungs, in spite of the presence of numerous concomitant chrysotile fibers. This observation is consistent with the likelihood that coating of chrysotile fibers is rare.

Detection of FBs in pleural plaques and other organs has been reported in earlier studies.[22–25] It is not yet resolved as to whether the ABs found in extrapulmonary sites have been translocated there from the lung or formed in place.

In the present study, the ratio of AB (i.e., FB) to amosite fiber concentrations in the lung parenchyma was about 1 to 100 (i.e., 1%), whereas the ratio in pleural plaques and/or mesotheliomatous tissue was approximately 1 to 1000 (i.e., 0.1%). This difference may perhaps be explained in one of two ways: that ABs have a lower tendency to translocate from the lung parenchyma to extrapulmonary sites compared with amosite fibers, or that the ability to form ABs is less in the pleural or peritoneal tissues than in lung parenchyma, even if formation of ABs can occur in these extrapulmonary sites.

For tissue digestion, it is important to recover all asbestos fibers and asbestos bodies in the sample without loss and to free them from interfering debris. A number of methods have been investigated for this purpose. Currently, however, there is no perfect method. If we use acids after tissue digestion, we can obtain clear specimens for ATEM analysis. However, chrysotile, especially fine chrysotile, is easily soluble in acids and may disappear, whereas amphiboles are strongly resistant. In this study, with this in mind, we used a modified bleach digestion and succeeded in having clear TEM fields. Consequently, even thin fibers of chrysotile showing extremely low contrast in the fields could be easily seen and counted.

CONCLUSIONS

The results of our tissue burden studies of samples from 13 North American insulation workers can be summarized: Asbestos fibers and asbestos bodies were investigated in three sites—lung (non-neoplastic lung parenchyma), parietal pleura (hyaline plaques), and tumor tissue (lung cancer, and pleural and peritoneal mesothelioma). In all the lung parenchyma and lung cancer sites, chrysotile and amosite fibers were found in high concentrations, whereas other amphibole asbestos fibers were small in number or rare. In the pleural plaques and the pleural and peritoneal mesothelioma tissues, amosite fibers were much fewer in number, while chrysotile fibers were seen in numbers similar to those in the lung. The

numerical concentrations of amosite fibers and of ABs were well correlated. No significant differences in the size distribution of asbestos fibers of each type were seen in the different sites. However, the mean widths of chrysotile fibers were thinner than those of amosite fibers in all sites. It was also noteworthy that long (>50 μm) and thick (>1 μm) asbestos fibers were rare in the lung.

From these results, we have drawn the following conclusions: Translocation of inhaled asbestos fibers from the lung to other organs, such as the pleura and the peritoneum, occurs frequently among asbestos insulation workers, although the route of translocation has not been completely elucidated. In insulation workers (occupationally exposed to asbestos, primarily chrysotile and amosite), chrysotile, more than amosite, seemed to be more readily cleared from the lung to accumulate in extrapulmonary tissues. The size distributions of chrysotile fibers detected in the three sites were not different, but the chrysotile fibers were thinner than amosite. Width of fiber seemed to be an important factor in dissemination to extrapulmonary sites. Chrysotile fibers translocated from the lung were not later eliminated, and may play an important role in the induction of either malignant mesothelioma and/or hyaline plaques, since the asbestos fibers detected in both were mainly chrysotile.

Tremolite fibers were very rarely present in the lungs and were not detected in the hyaline plaque and mesotheliomatous tissues of the asbestos insulation workers who had been exposed to Canadian chrysotile as one of the major asbestos types. This suggests that tremolite did not contribute to the induction of malignant mesothelioma in these workers. It will be interesting to determine whether asbestos-related cancers other than bronchogenic carcinoma and malignant mesothelioma (such as cancers of the larynx, esophagus, stomach, colon, or kidney) are induced by the translocated chrysotile fibers.

SUMMARY

Asbestos fibers and ferruginous bodies (FBs) in lung parenchyma, lung cancer tissues, pleural plaques, and pleural and peritoneal mesothelioma tissues from 13 North American insulation workers were analyzed and quantified using an analytical transmission electron microscope and a polarized microscope. Diseases from which these workers suffered included asbestosis, lung cancer, and mesothelioma. They had been occupationally exposed to materials containing chrysotile and amosite; their pathological diagnoses, occupational and cigarette smoking histories, and clinical summaries have been reported. Large numbers of FBs were found in the lungs and small numbers found in extrapulmonary sites. Most of the FBs had cores of amosite fibers. In all instances, lung parenchyma and lung cancer tissues showed chrysotile and amosite fibers in high concentrations (63.1×10^6 and 150.2×10^6 fibers/g dry tissue as mean values, respectively). Crocidolite fibers were seen in seven of the 13 cases, but in much smaller numbers. Other amphiboles were rarely found. In pleural plaques and in pleural and peritoneal mesothelioma tissues, amosite fibers were markedly fewer in number, whereas chrysotile fibers were seen in similar numbers as in the lungs. No significant differences in the size distribution of asbestos fibers were seen in the different sites. However, the mean widths of chrysotile fibers were thinner than those of amosite fibers. These results strongly suggest that translocation of inhaled asbestos fibers from the lung to other tissues, such as the pleura and the peritoneum, occurs frequently, and that chrysotile may be more actively translocated from the

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lung, compared to amosite or amphibole asbestos. The likelihood of translocation seems to be strongly related to the thinness of the fibers. Translocated chrysotile fibers may play an important role in the induction of either malignant mesothelioma and/or hyaline plaques, since the asbestos fibers detected in both these sites were mainly chrysotile.

ACKNOWLEDGMENTS

We express sincere thanks to Dr. I. J. Selikoff, Professor Emeritus, Mount Sinai School of Medicine, for providing the material and encouragement. We also thank R. Ashley, A. Calderaro, S. R. Yuen, and T. Okada for their assistance.

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