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Factors Affecting the Development of Mesotheliomas Experimentally Induced With Asbestos, Fibrous Glass and Related Minerals[1]

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SUMMARY

Seventeen potential carcinogens (three types of asbestos in seven forms, six types of fibrous glass, two types of silica and two types of metal particles) were applied directly to the pleura of rats on a fibrous glass vehicle. In a 2 year period ~~[illegible]~~, amosite, chrysotile, and three different specimens of crocidolite yielded equally high incidences of pleural mesotheliomas. Hand-milled crocidolite ore not exposed to extraneous oils or metallic milling yielded dose-related tumor responses comparable to those of conventionally milled asbestoses. Neither pulverized fragments of the steel mill nor nickel metal at doses exceeding potential contaminating levels yielded tumors. The intact fibrous glass vehicle did not induce mesotheliomas nor did the absence of the glass vehicle alter the incidence of crocidolite induced tumors. However, when the fibrous glass vehicle and two other types of fibrous glass of relatively large diameter were reduced to smaller fibrous fragments and applied to the pleura a small number of mesotheliomas were observed. Similarly, one of two samples of non-fibrous silica yielded a single mesothelioma. Two forms of an especially fine fibrous glass, further reduced by milling to approach the size of asbestos particles, induced moderately high incidences of mesotheliomas. Notably, the crocidolite that induced a high incidence of mesotheliomas, induced fewer mesotheliomas when reduced to a much finer form by excessive milling. These observations suggest that the carcinogenicity of asbestos and glass is related to structural features rather than acquired or intrinsic physicochemical properties.

Introduction

Mesotheliomas of the pleura and antecedent fibrous plaques, comparable to those resulting from asbestos exposure in man, can be induced readily in the rat and hamster by direct intrapleural application of asbestos (1-8). Such experiments not only attest to the carcinogenicity of asbestos but offer an excellent means of investigating those carcinogenic mechanisms inherent to the asbestos fiber.

Three years ago we began a series of experiments based on initial investigations which suggested that quantitative data could be obtained if treated rats were observed for two years (7). Open thoracotomy was employed of apply asbestos on gelatin-saturated glass pledgets directly to the pleura. The glass pledgets acted as a scaffold to uniformly distribute and retain asbestos over a large area of the pleura, and in the absence of asbestos acted as an irritant that could serve as a control on the specificity of the carcinogenic response. In themselves, the glass pledgets do not appear to be carcinogenic, nor do they appear to increase the incidence of asbestos-induced mesotheliomas. However, we have continued to use the pledgets as a vehicle for the sake of convenience and accuracy. The results of 28 experiments which demonstrate the remarkable carcinogenicity of asbestos and offer some insight into the nature of this process are reported here.

Methods

Twelve hundred weaning, pen-bred, pathogen-free, female Osborne-Mendel rats, a strain noted for hardiness and tolerance to surgical procedures, were house five to a metal hanging cage and fed conventional laboratory chow and water ad lib. Test materials were implanted on the pleura when rats were between 11 and 16 weeks of age, with the exception of two groups treated between 42 and 47 weeks of age. All groups were observed daily for two years, and survivors were killed during the 25th month after treatment. Detailed gross necropsy findings were recorded on 97% of the rats and histologic sections were examined from the implant site and all other grossly abnormal tissues. Selected neoplasms were stained with PAS, toluidine blue, mucicarmine, and Hale's stain for acid mucopolysaccharides, before and after digestion, with diastase and hyaluronidase. Concurrently, 256 untreated rats were killed in groups of 50 at 6-month intervals during the 2-1/2 years. These controls, like several thousand previously studied in this laboratory had no primary intrathoracic neoplasms other than a rare thymic lymphoma and a distribution of neoplasms outside the chest essentially like that of the experimental groups.

In our colonies, the average lifespan of this strain of rats is less than three years; consequently, mortality from disease other than mesothelioma was high during the latter half of the experiment. Periodic epidemics of acute pneumonitis were readily controlled by oral tetracycline therapy; however, the partially compromised function of the treated left lung greatly increased mortality in rats that had contralateral pneumonitis. During the second year of life the mean incidence of mammary neoplasms in this strain is approximately 36%. Mammary tumors were surgically removed, care being taken through histologic examination to insure that those of the left thorax did not represent invasion from underlying pleural neoplasms. Neoplasms of the adrenal cortex and genital organs are also common during the second year of life. Because these and other diseases represent a significant mortality factor, notation of significant lesions as well as time of death of each rat is recorded on the text-figures.

The method of introducing the test material to the pleura was described previously (7). Thin pledgets of autoclaved coarse fibrous glass measuring approximately 30 X 20 X 2 mm were trimmed to weigh 45 mgms. The test material at appropriate dosage was suspended in warm 10% gelatin and, during continous agitation, 1.5 ml aliquots were spread over the surface of each glass pledget. Control pledgets were treated with either gelatin alone or additional glass. All pledgets were allowed to harden at 4°C. The treated surface of a pledget was placed directly against the visceral pleura of the left lung through a 3 cm rib-spreading thoracotomy in rats anesthetized with open-drop ether. The incision was closed in layers to insure against leakage to subcutaneous sites. Post-operative mortality was less than 3%.

TABLE 1.--*The Seventeen Implanted Materials Distributed into Particle Size Ranges and Ranked by Overall Pleural Neoplasm Incidence*

		A.--Distribution of 1000 Consecutively Counted Particles Visible at 1000 X																
Overall Incidence Pleural Neoplasms (%)		75	67	65	61	60	58	20-32	18	12	4-6	6	4	4	0	0	0	0
Particle Dimensions (μ) Diameter x Length		J.C. WAGNER CROC.	HAND-MILLED CROC. ORE	BALL MILLED CROC. ORE	UICC SRAS CROC.	UICC SRAS AMOSITE	UICC SRAS CHRYS.	PART. PULV. UICC CROC.	PART. PULV. AAA-C GLASS	PART. PULV. AAA-U GLASS	PART. PULV. COARSE GLASS	CAB-O-SIL SiO_2	PART. PULV. PYREX GLASS	PART. PULV. GLASS WOOL	SILICA SOOT SiO_2	WHOLE COARSE GLASS‡	NICKEL METAL FRAG-MENTS	STEEL MILL FRAG-MENTS
1-2.5	1-2.5*	640	559	598	442	363	466	840	396	291	330	86	120	346	949		187	126
	>2.5-5	219	87	126	272	339	280	103	276	263	43		4	41				
	>5-10	80	138	89	169	165	135	45	144	189	18			30				
	>10-20	20	63	57	55	69	54	12	58	64	26			31				
	>20-40	5	34	20	25	31	40		21	40	19			13				
	>40-80	4	20	16	8	11	8		8	10	24					16		
	>80-160	1	15	8	1	3	3		14	4	12			2		16		
	>160-320	3	2		3	3	1		2	6	15					344‡		
2.5-5	2.5-5*	6	48	58	6	8	9		26	56	195	593	465	287	21		286	144
	>5-10	4				1			16	32	15			10				
	>10-20	3	1		2	3			8	8	2			45				
	>20-40	3	1		2	2	1		8	16	21			22				
	>40-80				1				3	6	16			3		8		
	>80-160	2	3		3				3	2	23							
	>160-320		2	1	1	1				2	30			2		288‡		
>5-10	>5-10*	5	16	16	1	3	3		6	1	41	138	225	110	15		361	373
	>10-20								2	2	19		36					
	>20-40												51	13				
	>40-80				1				4	4	5		38	3				
	>80-160				1				4	4	11		7					
	>160-320	1		1					3		24					232‡		
>10-20	>10-20*	2	8	3							35	149	45	37	12		146	150
	>20-40												7					27
	>40-80													2				
	>80-160										5			3				
	>160-320	1		1							20					64‡		
>20-40	>20-40*	2	3	2							1	34	2		3		20	167
	>40-80																	43
	>80-160																	
	>160-320															32‡		
		B.--Estimated Number of Particles in Each Size Range / 0.1 ug**																
1-2.5	1-2.5*	181	157	133	578	896	2135	10966	188	183	6.6	3.0	9.6	58	420		2.3	0.3

	" >10-20	5.7	18	13	72	170	247	157	28	40	0.5			5.2				
	" >20-40	1.4	9.6	4.4	34	77	183		10	25	0.4			2.2				
	" >40-80	1.1	3.6	4.0	11	27	37		3.8	6.3	0.5							
	" >80-160	0.3	4.2	1.8	1.3	7.4	14		6.7	2.5	0.2			0.3				
	" >160-320	0.8	0.6		3.9	7.4	4.6		1.0	3.8	0.3							
>2.5-5	>2.5-5*	1.7	14	13	7.9	20	41		12	35	3.9	23	37	48	9.3		3.4	0.3
	" >5-10	1.1				2.5			7.6	20	0.3			1.7				
	" >10-20	0.8	0.3		2.6	2.5			3.8	5.0	0.1			7.5				
	" >20-40	0.8	0.3		2.6	4.9	4.6		3.8	10	0.4			3.7				
	" >40-80				1.3				1.4	3.8	0.3			0.5				
	" >80-160	0.3	0.8		3.9				1.4	1.3	0.5							
	" >160-320		0.6	0.2	1.3	2.5				1.3	0.6			0.3				
>5-10	>5-10*	1.4	4.5	3.6	1.3	7.4	14		2.9	0.6	0.8	5.3	18	18	6.6		4.3	0.9
	" >10-20								1.0	1.3	0.4		2.9					
	" >20-40												4.1	2.2				
	" >40-80				1.3				1.9	2.5	0.1		3.0	0.5				
	" >80-160				1.3				1.9	2.5	0.2		0.6					
	" >160-320	0.3		0.7					1.4		1.5							
>10-20	>10-20*	0.6	2.3	0.7							0.7	5.7	3.6	6.2	5.3		1.7	0.4
	" >20-40												0.6					0.1
	" >40-80													0.3				
	" >80-160										0.1			0.5				
	" >160-320	0.3		0.2							0.4							
>20-40	>20-40*	0.6	0.8	0.4							0.1	1.3	0.2		1.9		0.2	0.4
	" >40-80																	0.1
	" >80-160																	
	" >160-320																	
	Total fibrous particles/0.1ug	98	103	72	713	1546	2391	2089	273	411	8	0	12	37	0		0	1
	Total non-fibrous particles/0.1ug	186	178	151	588	924	2190	10966	203	219	12	38	69	131	443		12	2
	**Estimated micro-fibers averaging 3.75μ in length/0.1ug	6189	5584	5863	6099	6278	7854	3147	1127	1679	249	0	207	312	0		0	11
	Overall incidence pleural neoplasms (%)	75	67	65	61	60	58	20-32	18	12	4-6	6	4	4	0		0	0

*Non-fibrous compartments (non-fibrous asbestos particles most often represent mixtures of non-asbestiform minerals and clumps of submicroscopic fibrils)

**$p = \frac{np}{vd}(10^5)$ where np = number of particles counted in a given size range
v = total volume of the 1000 particles counted, in cubic microns assuming that the particles are cylinders and in each size range have volumes of a normal distribution, d = density

***Assuming that all fibers fragment in vivo to mean lengths of 3.75μ and that all asbestos fibers fragment longitudinally to mean diameters of 1.25μ

‡Most [illegible] particles exceeded lengths of 320μ

Materials

Seventeen materials differing in composition or struction were applied to the pleura. Physical and chemical data on the asbestoses have been reported in detail (9-12); data on other materials were often sparse. In each glass and asbestos sample, particles varied from submicroscopic to macroscopic dimension in both length and diameter. Assessing this range quantitatively is a problem not yet solved, but to obtain a simple estimate of size distribution, aliquots of the materials that had been applied to the pledgets were suspended in water or formvar, air-dried on glass slides, and photographed at 250 and 1000 magnifications (Figs. 1-18). From the 1000 X photographs, 1000 consecutive particles were counted and assigned to 30 ranges of dimension (table 1). Similar preparations impressed on formvar-coated copper grids were examined in an AEI-EM6B electron microscope.

Characterization of the test samples have been obtained from information or analyses made in this laboratory or supplied by manufacturers and processors.*

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Coarse Coated Fibrous Glass Vehicle: The glass pledgets used in all experiments were from a single source of flexible, thermal insulation fibrous glass of the type used in building construction. They consisted of meshworks of long intertwining strands of borosilicate glass coated with a heat cured phenol-formaldehyde resin. They contained in addition to SiO_2 the oxides noted in table 2. The strands were of diameters 3- to 10-fold that of the asbestoses with a mean diameter of 6.5 μ and a few fibers in both the < 1 μ and > 25 μ range. The counts indicated in table 1 are skewed by the predominant large fibers that were complexly intertwined and exceeded lengths of 320 μ. The great length of the fibers made it impractical to assess the number of whole fibers per unit weight with accuracy.

UICC Standard Reference Samples of Asbestos (Crocidolite, Amosite, Chrysotile A): The International Union Against Cancer on the recommendation of a working group in 1964 established Standard Reference Asbestos Samples (SRAS) of the major types of asbestos prepared from as pure a parent material as possible (9,10). Three of these samples, crocidolite, amosite and chrysotile A are included in the studies. The UICC-SRAS crocidolite served as a baseline for comparative studies with the other materials. The samples were received from the Pneumoconiosis Unit, Llandough Hospital, Penarth, Wales in polythene plastic bags and were stored in glass containers. All three samples have been characterized both physically and chemically in previous reports (11, 12). Our own measurements on the samples coincide with the reported range; however, different parameters of measurement were used in table 1.

South African Crocidolite (Wagner Preparation)--This sample, obtained from Dr. J. C. Wagner, is fine milled crocidolite from a single source of Northwest Cape Blue asbestos. It was originally used to determine carcinogenicity in the rat by Wagner et al. and Harington, et al. (1, 2, 5, 6) and later by our laboratory (7). Physical and chemical properties have been documented and coincide with the measurements in table 1 (13-15).

UICC-SRAS Crocidolite (partially pulverized)--To treat asbestos in a manner similar to that of some glass samples, the UICC-SRAS crocidolite was partially pulverized in a Spex model 5000 stainless-steel ball mill (Spex Industries, Inc., Metuchen, N. J.), operating at 3200 rpm for 1-1/2 minutes, using 15 mgm loads in 2 ml chambers. The resultant differences in particle size and reduction to particles, that by optical standards appeared non-fibrous (i.e., dimensional ratios of 3:1), are indicated in table 1. X-ray diffraction analysis of samples of this crocidolite after comparable pulverization was carried out by both the National Bureau of Standards and by the Johns-Manville Research and Engineering Center. The results of these analyses indicated a persistence in the diffraction lines characteristic of crocidolite, although areas beneath selected peaks were reduced. Electron microscopic examination indicated that the non-fibrous particles by optical standards were essentially clumps of submicroscopic fibrils (fig. 8), consequently the changes in the X-ray diffraction pattern most probably represented changes in fiber size rather than loss in structure.

Table 2. Minor Mineral Oxide Content of the Four Glasses Analyzed by Emission Spectroscopy After Ignition at 1000°F.*

Percent.	Al_2O_3	B_2O_3	CaO	MgO	Fe_2O_3	Na_2O	TiO_2
Coarse Fibrous Glass (Vehicle)	4.0	8.0	6.4	3.1	0.36	14.0	0.06
Fibrous Pyrex Glass	3.6	5.5	14.0	2.4	0.62	13.0	0.05
Old Glass Wool	8.5	-	32.0	9.5	0.80	0.55	0.60
AAA Fine Fibrous Glass	11.0	7.2	17.0	5.4	0.60	1.0	0.46

* Analyses were performed by personnel of the Johns-Manville Research and Engineering Ctr. and the Owens-Corning Fiberglas Corp.

Virgin Crocidolite Ore, Hand Milled.--This material was obtained as a single hand cobbed ore specimen measuring approximately 8 X 8 X 10 cm through the courtesy of Mr. L. N. Kuyper, of Cape Asbestos South Africa (Pty.) Ltd. Much of the stratified fibrous vein was separated from extraneous minerals by hand; then, using the two halves of the remaining fibrous surface as a grinding media, the fibers were ground by hand to a fibrous quality that resembled that of the UICC-SRAS crocidolite.

Virgin Crocidolite Ore, Ball Milled.--Other portions of the stratified fibrous vein from the above ore sample were subjected to brief fragmentation in the Spex mill until, again, they were reduced to a quality comparable to the UICC-SRAS crocidolite.

Spex Stainless-Steel Mill Fragments.--A stainless-steel chamber of the type used in our milling process was reduced to minute fragments with a carbide steel lathe, and these fragments were further reduced in size by prolonged 30-minute pulverization in the mill. Most particles ranged in size from 2.5 to 40 μ. This nickel-chrome steel was not analyzed for trace elements.

Metallic nickel fragments.--The nickel was purified precipitate from disintegration of nickel carbonyl, composed of 2 to 100 μ aggregates of particles that rarely exceeded 1 μ in diameter. By electron microscopy, these particles possessed sharply angulated spine-like projections over their surface. The sample originally obtained from the International Nickel Company, Toronto, by Dr. W. C. Hueper has been reported to yield local sarcomas in the rat at a cumulative intrapleural dose of 300 mgms (16).

Other Glasses.--The coarse fibrous glass that served as a vehicle, and four other types of fibrous glasses, were tested for carcinogenicity after reduction to fiber lengths, roughly of 1-20 μ, comparable to those of asbestos by brief milling in the Spex mill. Table 1 and figures 9-16 indicate the distribution of fiber sizes and particularly the quantity of such materials that were reduced to non-fibrous particles by the milling. While it was difficult to satisfy ourselves that sufficient material could be examined electron microscopically to make valid quantitative assessments of the finest fibers it was apparent that fibers less than 0.5 μ in diameter were present in all partially pulverized glasses and were particularly abundant in the AAA fibrous glass. Table 2 indicates the mineral content of these glasses exclusive of the major silicate component and other features prior to milling are indicated below.

Fibrous pyrex brand glass wool is a commercial product used in coarse filtration procedures. It consisted of long silky fibers, 5 to 12 μ in diameter coated with a binder of undetermined composition.

Old glass wool is an obsolete loose commercial fiber, popular several decades ago as house insulation. The fibrous glass was notably irritating for the skin and consisted of abundant short fibers ranging widely from 1 to 35 μ in diameter with many hook-ended fibers. It had the typical glass wool mineral oxide content indicated in table 2.

AAA fibrous glass was an especially fine fibrous glass that was tested because it tended to approach the fiber diameters of asbestos. The mean diameter of whole fibers was 0.22 μ with a range of .06 to 3 μ. Two forms of this glass were tested after partial pulverization to reduce fiber length: uncoated glass designated AAA-u and identical glass coated with a urea-formaldehyde resin designated AAA-c. The mineral oxide content and size range of the two after partial pulverization were essentially identical (tables 1 and 2).

Silicon Dioxide.-- Two forms of purified especially fine particulate SiO_2 (silica soot and Cab-O-Sil) were tested. Both samples were prepared by flame hydrolysis of silica tetrachloride and were more than 99.9% pure. A considerable difference in particle size was apparent between the two types of silica at the optical level (table 1, figs. 12 and 15). However, of more significance at the submicroscopic level, these particles were composed of agglutinated clumps of minute spheres that ranged in size from 5+15 nm for silica soot and 50-150 nm for Cab-O-Sil.

Results

Analysis of tumor yields:- The results of 28 experiments, each employing 30 rats, are shown in text-figures 1-7. The text-figures are arranged in identical format for comparing data, and are sufficiently detailed to permit alternative methods of calculation. Since the earliest mesothelioma was observed 54 weeks after treatment, we arbitrarily considered only those rats that survived the first year after treatment as the effective number. The over-all incidence of mesotheliomas in this effective number is recorded in the heading of each text-figure. In general, deaths from other causes during the second year were sufficiently comparable to make direct tumor incidence comparisons valid. Within each text-figure the space below the baseline represents rats dead during the first year. Above this line the solid area represents rats dead with mesothelioma, and the outlined area, rats dead from other causes. Numbers at the base of each text-figure tabulate this information by week. The extent of pleural fibrosis at the implant site was assessed in all rats that died during the second year on a 1-4 $\frac{1}{4}$ scale and the predominent value is indicated at the lower right of each text-figure. The major lesions noted in each rat at death are indicated by an alphabetic code within the text-figure. Each alphabetic character represents an organ system, with upper case letters indicating neoplasms and lower case letters representing other lesions, primarily of inflammation or degeneration;

A - adrenal, B - bone, C - cutaneous tissues, D - muscle, E - pancreas, F - peritoneum, G - digestive tract, H - liver, I - EENT, J - nervous system, L - respiratory tract, M - mammary glands, O - genitalia, P - pituitary, Q - thymus, R - lymphoreticular system, S - spleen, T - thyroid, U - urinary system, V - vascular system, W - salivary glands, X - no lesions, Y - generalized disease, Z - lost or unaccounted.

Text-figure 1 a-d compare the incidence of mesotheliomas after a maximum dose level of 40 mgms for four different types of asbestos. The earliest neoplasm in the entire series of experiments was detected in the amosite group during the 54th week. Two further tumors were detected in this group before the 68th week, suggesting an earlier onset of neoplasms with amosite than with chrysotile or the crocidolites--a phenomenon also apparent in Wagner's experiments (2,17). However, during all comparable periods of time in the last three-quarters of the second year, the cumulative incidence of deaths with mesotheliomas from the four types of asbestos was not significantly different, and the final incidences of pleural neoplasms resulting from the three UICC asbestoses were remarkable similar (15/25, 15/26, 14/23). The final 15/20 incidence for the Wagner crocidolite does not exceed this range sufficiently to indicate a statistically significant difference in tumor response. It was considered that this high incidence-range might represent a maximum tumor response and that, for comparative purposes, 40 mgms might represent an excessive dose. But this seems unlikely since half this dose of crocidolite in a subsequent experiment showed an appreciable reduction in tumor incidence. The extent of fibrosis at the implant site was uniformly high in all four groups and served as a basis for comparisons to follow. One rare neoplasm, a generalized lymphosarcoma not involving the pleural site, was encountered in the chrysotile group.

Text-figures 2a-d illustrate the response with UICC-SRAS crocidolite in graded doses from 20 to 1 mgm. With the data from text-figure 1c on the 40 mgm dose, it was clear that a simple dose response relationship existed both for the apparent time of tumor onset and final incidence of pleural neoplasms. From these data, a dose of approximately 23 mgms was calculated by graphic probit analysis to yield a two-year 50% tumor incidence if deaths from other causes remained constant (18). Fibrosis less extensive than that at 40 mgms was present in all rats at doses of 10 and 20 mgms, and appreciably lesser degrees of fibrosis occurred at lower doses. In these groups, four relatively rare neoplasms were noted: an osteogenic sarcoma of the skull, two hemangiomas of the spleen, and a peritoneal lipoma. These experiments, along with the controls in text-figure 3, served as a standard of reference for the experiments to follow.

Text-figures 3a-c illustrate 3 controls for the previous and subsequent experiments in which the pledgets of large fibered glass laden with gelatin alone were employed. In the three experiments, none of the 90 rats had pleural neoplasms. However, only 58 rats survived long enough to serve as valid treated controls; thus incidences of mesotheliomas less than 3/30 rats in comparable experiments could conceivably result from the vehicle alone (19). The distribution of all neoplasms in these groups was essentially like that in several hundred untreated controls, unusual neoplasms remaining as isolated examples. Although initial inflammatory reactions to the pledgets appeared to be as vigorous as to pledgets saturated with asbestos, the subsequent histological changes indicated far less fibrous proliferation than with 1 mgm of crocidolite. In rats killed at the end of these experiments only minute macroscopic residues of glass and scar tissue could be detected.

Text-figure 3d represents the single converse control of the 3 experiments above in determining the potential effect that the glass pledget might have on neoplasms induced with asbestos. A median dose of 10 mgm, UICC-SRAS crocidolite was suspended in physiologic saline and delivered to the left pleura through an open thoracotomy without the glass vehicle. We expected less tumors because asbestos injected in this manner tended to pool in the costophrenic angle and elicited less fibrosis than when applied with the glass vehicle. However, comparing this result with that of text-figure 2b, it is evident that, although the neoplasms tended to be lethal somewhat later in the glass vehicle group, the final incidence in the two experiments (9/21 vs. 11/27) (indicated that the glass neither enhanced nor suppressed the induction of mesotheliomas by asbestos.)

Text-figures 4a-c illustrate the results of applying crocidolitedifferent from the UICC-SRAS crocidolitein that it was from a single specimen of northwest cape crocidoliteore that was milled in our laboratory using only the ore itself as a milling media without exposure to extrinsic oil or metal. After hand milling to a consistency like that of the UICC-SRAS crocidolite, serial doses were applied. The incidence of mesotheliomas at levels of 40 and 20 mgms was virtually identical to that of the UICC-SRAS crocidolite, and at the 1 mgm level no significant difference in response was evident (2/25 vs 4/30) ($p > 0.5$). A graphic probit analysis of dose response over this range indicated that a 25 mgm dose would yield a two year 50% tumor incidence compared with the 23 mgm 50% tumor dose for UICC-SRAS crocidolite (18). The extent of pleural fibrosis correlated with dose and closely matched that of comparable doses of the UICC-SRAS crocidolite and again no exceptional deviation in the incidence of neoplasms outside the thorax was noted.

The control experiment, text-figure 4d, illustrates the result of implanting 40 mgms of the same crocidolite ore after milling in a stainless steel ball mill. Here again the 15/23 incidence of pleural neoplasms was virtually identical with both the 14/23 incidence from UICC-SRAS crocidolite (text-fig. 1c) and more importantly with the 18/27 incidence from the crocidolite ore milled by hand (text-fig. 4a). Both implant site fibrosis and extra-thoracic tumor incidence followed that of the two previous crocidolite tests. These indications that mill contamination was not a factor in asbestos carcinogenesis were further supported by the experiments illustrated in text-figure 5.

The experiments in text-figures 5a-b explored the carcinogenic potential of nickel chrome steel, the prime metallic contaminant in the milling process and nickel, the most carcinogenic constituent of the mill. Pulverized particles of nickel-chromesteel from a mill similar to that which was used to process the crocidolite ore yielded no mesotheliomas at a dose equal to that of the highest level of asbestos used. Pure nickel metal at similar high levels was excessively toxic; all rats were dead with hemorrhagic pneumonitis within 60 days. Consequently, a 1 mgm dose, still far in excess of potential nickel contamination from the mill, was employed. Toxicity of the nickel even at this level was high in the first year, but no pleural neoplasms developed in the group of 18 rats which had a survival rate during the second year comparable to that of the asbestos treated groups. The experiments in text-figures 5c-d are relevant to testing the carcinogenic potential of silicon, the predominant constituent of both asbestos and glass. Silicon dioxide in the form of smooth surfaced spheres of submicroscopic size was used in both experiments. The result of these two experiments was a single mesothelioma developing in the early part of the second year. All four of these experiments with non-fibrous materials resulted in a minimum of pleural fibrosis, although the degree of fibrosis seemed greater with the steel fragments than with the other materials. In the nickel treated group two relatively rare tumors, a pituitary adenoma and a carcinoma of the kidney, were noted.

The subsequent experiments tabulated in text-figures 6 and 7 concern the role that the physical characteristics of asbestos might play in carcinogenesis. Text-figure 6 illustrates our first attempts to examine non-asbestaformfibers of a size similar to asbestos. The large fibered glass that we had used as a vehicle was reduced to short fibers by brief milling in a stainless-steel ball mill and implanted in two groups of rats. The first group was young rats as in the other experiments, but the second group was 40-week-old rats that were used to test age-related susceptibility. Two further experiments (text-figs. 6c-d) were done on fibrous glasses of slightly smaller diameters, the highly irritating old glass wool and pyrex fibrous glass. The results of all four experiments were essentially alike in that only single pleural neoplasms occurred in each of the four experiments, the degree of pleural fibrosis was minimal, and unusual neoplasms occurred only rarely. Although no tumors had been noted with the intact fibrous glass vehicle, the occurrence of four mesotheliomas in these experiments clearly indicated that fibrous glass could be carcinogenic if reduced to short fibrous lengths. However, the low incidence of mesotheliomas, whether the four experiments were considered separately or collectively (4/91), was not sufficient to conclude that the partially pulverized glasses had a higher potential for tumor induction than the intact glass.

Text-figures 7a-b illustrate two further experiments carried out in the same way after partial pulverization, but employing fibrous glasses of much finer diameter than the vehicle glass. As noted in the materials section and table 1, these two samples were identical except for a coating of urea-formaldehyde resin on the latter. The range in fiber diameters after pulverization closely approached that of medium sized asbestos fibers. The result of each experiment was a small but significant number of mesotheliomas, 3/26 and 5/28. These incidences if taken together were sufficient to indicate a statistically significant increase in incidence over both the intact coarse glass vehicle (8/54 vs 0/58) or the combined groups of partially pulverized glass of more coarse dimension (8/54 vs 4/91). The extent of pleural fibrosis was also greater in these groups than the groups treated with coarse fibrous glasses.

Text-figures 7c-d represent a final set of experiments designed as controls on the experiments with partially pulverized glass. Groups of both young and old rats were implanted with UICC-SRAS crocidolite that had been treated in the same manner as the partially pulverized glass. That is, the UICC-milled crocidolite was further pulverized in our ball mill for 1-1/2 minutes. Gross effects of the additional milling were not apparent; however, subsequent microscopic examination indicated appreciable reduction in particle size (table 1). The result of both of these experiments was a reduction in the number of pleural neoplasms and the extent of pleural fibrosis as compared with the four groups treated with crocidolite milled conventionally. Since this difference seemed of particular importance a series of statistical calculations comparing the differences were done. Although data from the experiment with 40-week-old rats suggested a reduced incidence, no rats of comparable age had been treated with conventionally milled crocidolite. To compensate for this difference and the associated increased mortality from extraneous causes, a cumulative mortality rate from mesotheliomas corrected for extraneous deaths was plotted for the two groups of rats treated with partially pulverized UICC-SRAS crocidolite (text-figs. 7c-d) and for the four more conventionally milled crocidolites (text-figs. 1c, 1d, 4a, and 4d) by the method of

Pilgrim and Dowd (20). Text-figure 8 clearly indicates the consistent mesothelioma incidence from the conventionally milled crocidolites, and the consistent reduction in mesothelioma incidence throughout the course of the two experiments with crocidolite subject to prolonged milling. A chi square test of comparison of the overall incidence of mesotheliomas between the single group of young rats treated with partially pulverized crocidolite (text-fig. 7c) and each of the four groups of comparable age treated with conventionally milled crocidolite (text-figs. 1c, 1d, 4a, and 4d) indicates the probability of a valid difference to be 95% when calculated for two of the four groups and more than 99% for the other two groups, or for the four groups combined.

Considerations of Fiber Size in Relation to Tumor Yield.--The results of the experiments in text-figure 7 pointing to differences in tumor response relevant to particle size or structure motivated an assessment of the size distribution of fibers of the 17 implanted materials. The wide range in dimensions in any one of the 17 materials made a precise tabulation of the size of individual particles a formidible task. Consequently, the assignment of 1000 consecutively counted particles to 30 ranges of dimension was a compromise which gave us a fair sampling of size distribution in the optical range. This distribution is tabulated in table 1, Part A. The preparations that were counted are illustrated at low magnification in figures 1-18. Extending these ranges to particles of submicroscopic size proved futile because of the limited sample size that could be evaluated by electron microscopy. However, our impressions can be summarized as follows: In all asbestos and glass samples, fibers (i.e., particles with ratios of diameter to length of 1:3 or greater) extended nearly to the limits of electron microscopic resolution. Fibers less than 1.0μ in diameter were far more numerous in asbestos samples than in glass samples. Both the large and small clumps of asbestos that appeared non-fibrous (glass) in the optical range were composed of clumps of microfibrils in the range of 0.05-0.2 μ X 0.6-2.0 μ (Fig. 8). In contrast, non-fibrous glass particles were irregular solid masses. Neither the silica nor metal samples contained fibrous particles. Nickel particles were irregular, sharply angulated spiney crystal; both types of silica were smooth surfaced spheres.

Type

Since dose was determined by weight, we further derived an estimate of the number of particles in each dimensional range per unit weight by taking density into consideration. Part B of table I represents this estimate, assuming that the fibrous particles were cylinders and that the particles in each dimensional range were distributed uniformly about the mean volume for each range. In the ranges of large sized particles, particularly those with few particles, these assumptions were liable to error. Furthermore, the whole fibrous glass used as a vehicle could not be treated this way since the length of most particles exceeded limits that could be measured accurately. But despite this defect in methodology, we felt that such estimates might be revealing if only broad differences in carcinogenic response and particle distribution were compared.

Considered in this way, 3 of the 4 conventionally milled crocidolite preparations with equally high tumor incidences had nearly comparable distributions of particles. The one deviation was UICC-SRAS crocidolite which had fewer large particles and a consequent excess of particles in the finer ranges because of more complete milling. Similarly, the UICC-SRAS amosite and chrysotile preparations with comparably high tumor incidences, contained few particles of large size, but here the number of small particles per unit weight was far greater because of the lesser density of these asbestoses when compared with crocidolite. From this data we could conclude either that fibrous particles in excess of 5 μ in diameter were not essential to the induction of tumors or that these large fibers were reduced to finer fibers in vivo so that the ultimate distribution of fibers would be the same.

The critical comparison seemed to be between the excessively milled, partially pulverized crocidolite with its reduced incidence of tumors and the conventionally milled crocidolites with their high tumor incidences. Table 1, Part B indicates that prolonged milling had pulverized the crocidolite until no particles were greater than 2.5 μ in diameter or 20 μ in length. Slightly more than half of the sample by weight (84% of the number of particles) consisted of minute clumps not exceeding 2.5 μ in either dimension that were non-fibrous by optical standards. However, these clumps, like those occurring less frequently in other asbestos samples, were composed largely of submicroscopic fibrils (figs. 7 and 8). Since the number of these submicroscopic fibrils far exceeded those in the conventionally milled crocidolite samples it was reasonably to assume that they were less carcinogenic than larger fibers and served to reduce tumor incidence by simply diluting the dose. Presumably, the 20-32% tumor incidences resulting from this sample were related to those particles which retained their fibrous structure in the optical range. Interestingly, the three dimensional ranges of fibers represented in this low tumor yield sample, each contained more fibers than their counterparts in the samples that yielded high incidences of mesotheliomas. Furthermore, if one totalled all of the fibers in the low-tumor-yield partially pulverized crocidolite, they numbered more than the totalled fibers in the high-tumor-yield crocidolites. Thus, it was evident that the incidence of mesotheliomas did not relate directly to either the number of fibers in a particular dimensional range or the total number of

fibers implanted. However, this reasoning did not consider the fate of the particles after implantation. Asbestos fibers are composed of bundles of microfibers which readily separate through fragmentation of the fiber. Touch preparations from the lesions suggested that fragmentation frequently occurred in vivo. It, therefore, seemed reasonable to consider the number of fibers present in the rat if all fibers had been reduced to a lesser dimension.

Reduction of all particles to their ultimate submicroscopic fibrillar dimension would, of course, result in equal numbers of the low-tumor-yield submicroscopic fibrils in all groups. But eliminating these submicroscopic fibrils from the calculations on the assumption that their minute size rendered them inert yielded different results. The smallest fibers that could be recognized by light microscopy and counted were those with diameters of approximately 1.25 μ and consequent lengths of 3.75 μ -- the approximate mean dimension of our smallest category of optically visible fibers. Converting all of the particles in excess of 1.25 X 3.75 μ to this microfiber dimension and excluding all fibrils and particles of lesser dimension resulted in the calculation at the bottom of table 1. The potential number of microfibers per unit weight was roughly equal for not only the four high-tumor-incidence conventionally milled crocidolite samples, but also the high-tumor-incidence amosite and chrysotile samples as well. Conversely, the low-tumor-incidence partially pulverized crocidolite sample, diluted by the submicroscopic fibril clumps of lesser dimension, could yield only half as many microfibers as the high-tumor-incidence groups. The same reasoning was then used to consider the glass samples. We assumed that glass fibers could fragment transversely as easily as asbestos, but since glass was not composed of bundles of microfibers, longitudinal fragmentation would be a rare event. Consequently, only transverse fragmentation of all fibers to lengths of 3.75 μ was calculated for the glass samples.

For the two fine glass samples that induced 12-18% mesothelIomas the total number of calculated microfibers was approximately half that of the partially pulverized crocidolite that yielded 20-32% mesothelIomas, and approximately one fourth to one fifth that of the conventionally milled asbestos that yield 58 to 75% mespthelIomas. The three coarse fibrous glass samples that yielded negligible numbers of tumors had comparably low numbers of calculated microfibers that totalled less than a fifth the number of microfibers in the fine glasses and less than 5% the number of microfibers in the high-tumor-incidence asbestos samples. The microfibers in the whole glass vehicle could be assumed to be less than those in the milled coarse glass unless the process of fragmentation was perfectly efficient.

Thus, mesothelioma incidences for both the asbestoses and glasses correlated reasonably well with the number of microfibers in the range of 1.25 X 3.75 μ that theoretically could result from the fragmentation of larger fibers in vivo. Whether the induction of mesotheliomas is indeed closely related to this relatively narrow median range of fiber sizes would seem amenable to further investigations.

Morphologic Observations: The primary response of the pleura to all of the asbestos implants was a vigorous granulomatous reaction ending in the formation of dense fibrous coats firmly adherent to the visceral pleura and pericardium. This fibrous tissue closely invested residual particulate matter and was composed of avascular, interlacing, immature spindle cells and abundant collagen interspersed with foci of hyalin and liquifactive necrosis (figs. 19,20). Pleural fibrosis in each rat was assessed, grossly and histologically, as extensive, moderate, or slight. Using this simple evaluation it was evident that for each group the extend of fibrosis roughly correlated with the incidence of pleural neoplasms not only in the high and low tumor incidence groups, but also in the medium tumor incidence groups treated with low doses of asbestos or fine pulverized glass. One of the purposes of the glass as a control was to determine whether pleural cell proliferation in the milieu of an inflammatory effusion might act as an in vivo cell culture with a high potential for non-specific neoplastic transformation. Ideally, one would expect such a control to yield a reactive fibrosis as extensive as that of the asbestos, however this was not the case. In all of the experiments that yielded few or no mesotheliomas, irrespective of the type of material used, fibrosis was negligible. Reactive sites in these groups had an abundance of mononuclear leukocytes, foreign body giant cells, and capillary vessels, but a paucity of collagen and connective tissue (fig. 21). The dense fibrous plaques which characterized the primary reaction to asbestos and pulverized fine glass had many of the qualities of neoplasia (fig. 20), but true neoplastic

development by our criteria was distinguished by abrupt conversion of the proliferating collagenous connective tissue to masses of closely packed, atypical disoriented cells that extended beyond the residual foreign particulates and the reactive fibrosis to them. In general, abundant mitoses suggested a rapidly lethal course for these neoplasms. However, this may not have been the case since the data indicate that a large proportion of the neoplasms became evident only when the rats were killed at the end of the experiment (text-figs. 1-7). The latter neoplasms were not structurally different from those that killed rats earlier in the experiment, and since only 13% of the neoplasms metastasized or invaded structures adjacent to the thorax (table 3), the most reasonable explanation is that most neoplasms were relatively slow-growing and remained confined to local sites for long periods of time. It is perhaps significant that a similar slow course often characterizes the mesotheliomas of man (21).

The histologic characteristics of the 170 neoplasms were not unlike those previously described (2,7). None could be conceived as arising from tissues other than the reactive pleural mesenchyme, but interesting variations in structure were evident (table 3). The dominant cells had either a spindle or pleomorphic structure sometimes intermixed - a phenomenon common to most non-epithelial neoplasms in the rat. Additionally, some contained giant multinucleate cells, other osteogenic foci, and a few had tubulopapillary patterns suggesting surface covering features of mesothelium (figs. 22-26). Efforts to identify hyaluronic acid were equivocal in selected neoplasms, although some contained neutral and acid mucopolysaccharides. The 14 neoplasms with foci of osteogenesis appear to be unique to our strain of rats. There was no apparent difference in structural types between tumors induced by asbestos and tumor induced by glass. The single tumor induced by silica was a simple fibrogenic spindle cell sarcoma.

Table 3.--Structural types of the 170 mesothelliomas induced by asbestos, glass and silica.

	Asbestos	Glass & Silica
Spindle cell types:		
Fibrogenic	105 (13)*	10 (1)
Osteogenic	12	2 (1)
Giant cell	9 (2)	
Pleomorphic cell types:		
Medullary	23 (3)	1
Tubulopapillary	8 (2)	
Total	157 (20)	13 (2)

*Figures in parentheses are those neoplasms that metastasized or invaded structures outside the thorax.

DISCUSSION

The present experiments were not designed to examine all parameters of the potential carcinogenic hazard of asbestos or glass to man. In fact, the direct application of our results to the problems in man would be unwise since the method of application and the high doses employed are remote from the usual exposure of man to fibers. However, the means by which these materials exert their carcinogenic effect is a critical problem and the present data along with that of other reports seem adequate to develop preliminary conclusions.

The present studies confirm that conventionally-milled asbestoses have a high intrinsic capacity for inducing neoplasms and that this capacity is virtually equal regardless of the type of asbestos or its source. Furthermore, fibrous glass when reduced to a distribution of sizes approaching that of conventionally milled asbestos is also intrinsically carcinogenic. This capacity to induce neoplasms can be related to (1) contamination by carcinogenic hydrocarbons and metals, (2) the carcinogenic constituents of the firbrous mineral itself, or (3) the structural character of the fiber.

Both natural contaminating oils and minerals and those introduced in the milling and packaging of asbestos have been investigated as causes of asbestos carcinogenicity (2, 6, 14, 23, 24, 25). These studies indicate that asbestos from which oils have been rigorously extracted is as carcinogenic as the non-extracted asbestos, and that these contaminating hydrocarbons are not present in amounts sufficient to account for asbestos carcinogenicity. Experiments reported here concern the role that contaminating metals might play, an hypothesis suggested by the findings of other investigators (13, 14, 16, 26, 27). Such metals retain a carcinogenic potential of their own, but they seem unlikely causes of asbestos carcinogenicity because of the minute amounts involved. The present experiments demonstrate that at levels far exceeding those that might contaminate asbestos, neither finely particulate nickel metal nor nickel-chrome steel are sufficiently carcinogenic to account for the mesotheliomas induced in the rat with asbestos. This observation would not exclude a synergistic or catalytic action of contaminating metals. But such an hypothesis is not supported by the experiments which show that handcobbed crocidolite ore, milled without exposure to metal induces numbers of mesotheliomas at graded dose levels equal to the numbers of mesotheliomas induced either by the same ore milled in a steel ball-mill or by mine-milled UICC-SRAS crocidolite.

These experiments would not exclude the potential carcinogenicity of the variety of heavy metals or other complex constituents of the asbestos or glass fiber itself. Yet, with the exception of silicon there are remarkable differences in the composition of asbestoses of different types and these differences do not seem to alter the intrinsic carcinogenicity of the asbestos fiber as indicated in our experiments or those reported previously (2, 3, 6, 12, 13). Silica, the major constituent of both asbestos and glass proved to be relatively inert in our experiments. However, this result must be reconciled with the high incidence of intrathoracic reticulum cell sarcomas induced with an alkaline washed silica-sand by Wagner et al (2, 4, 6). Wagner emphasized that his silica-induced tumors were unlike those induced with asbestos and perhaps were the result of a different mechanism of induction. It is probable that the absence of such neoplasms in our experiments is related to differences in the physical structure of the silicas employed by us and by Wagner. Because we were interested in testing the smallest particle of silica, we used silicas prepared by flame hydrolysis. These silicas were composed of agglutinated clumps of particles 5-150 nm in diameter that were spherical in shape. Neither type caused the extensive reactive fibrosis characteristic of freshly fractured quartz silica - a quality that may be relevant to fibrogenesis and in turn to carcinogenesis (28). Further evidence against the idea that intrinsic constituents

of the fiber are responsible for the neoplasms are the experiments with partially pulverized crocidolite. Prolonged milling not only reduced the fibers to clumps of submicroscopic fibrils, but also exposed this residue to additional metal contamination from the mill. The resultant loss in carcinogenicity implies that the structural integrity of the asbestos fiber above that of the finest submicroscopic fibril is essential to carcinogenicity, and that neither contaminants nor elemental components are likely factors in this process.

If the physical structure of asbestos rather than its chemical constituents are critical to carcinogenicity, then similar particles, if sufficiently durable, should induce similar tumors. On this reasoning rested our attempts to reduce both coarse and fine fibered glass to a range of sizes comparable to those of milled asbestos. Our attempts to reduce 3 types of coarse fibered glass resulted in a range of particle sizes that spanned the range of asbestos, but quantitatively had far more large fibers and, consequently, few of the minute fibers so abundant in asbestos. In contrast to the intact fibrous glass all four experiments with partially fragmented coarse glass yielded single mesotheliomas, implicating this fragmented fibrous glass as a carcinogen, but these experiments were sufficient to show that reduction in size was the causative factor. However, the idea that fiber size is critical was supported by the experiments with the partially fragmented AAA-glasses of especially fine diameter, for here both the incidence of neoplasms and the range in fibrous particle sizes more closely matched that of the asbestoses. Furthermore, by assuming that larger fibers tended to be reduced in vivo to a narrow range of sizes just above the limit of optical resolution, it was possible to correlate the number of microfibers potentially present in vivo with the incidence of mesotheliomas, irrespective of the type of asbestos or glass employed.

The simplest explanation that can be derived thus far is that the intrinsic carcinogenicity of asbestos and glass is not related to any contaminating or inherent physicochemical quality, but rather to its fibrous structure perhaps in a narrow range of sizes. This conclusion is not new for Bryon and Bischoff argue that asbestos carcinogenicity is simply another form of "solid state carcinogenesis", the key factor being the formation of a unique avascular fibrous tissue in response to chemically inert materials (28). These experiments offer no challenge to this argument for it was our impression that the incidence of mesotheliomas correlated well with the extent of fibrous tissue in the lesions just as they did with numbers of microfibers potentially present in the lesions.

All of the experiments reported here require further confirmation and controls. Of particular significance in examining our hypothesis will be the testing of both glass and asbestos in narrow, selective ranges of fiber size and totally reduced to non-fibrous form. Additionally, the experiments indicate that similar microfibers of other types of durable material, particularly those unrelated chemically to asbestos or glass, should be tested.

TABLE I.—The Asbestos Implanted Materials Distributed into Particle Size Ranges and Ranked by Overall Pleural Neoplasm Incidence

A.—Distribution of 1000 Consecutively Counted Particles Visible at 1000 X

Overall Incidence Pleural Neoplasms (%)		75	67	45	61	60	50	30-32	18	12	4-6	6	4	4	0	0	0	0
Particle Dimensions (µ) Diameter x Length		J.C. [illegible] Conc.	[illegible] Conc. [illegible]	[illegible] Conc. [illegible]	[illegible] Conc.	[illegible]	[illegible] Conc.	Part. Poly. [illegible] Conc.	Part. Poly. [illegible] Glass	Part. Poly. [illegible] Glass	Part. Poly. [illegible] Glass	Car-B. Sil. [illegible]	Part. Poly. [illegible] Glass	Part. Poly. Glass Wool	Silica Dust SiO_2	[illegible] Glass[*]	[illegible] Metal Frag-ments	[illegible] Frag-ments
≥2.5	≤2.5[*]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	330	[illegible]	130	[illegible]	[illegible]		187	[illegible]
"	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		6	[illegible]				
"	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			[illegible]				
"	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			[illegible]				
"	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]			[illegible]				
"	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]					[illegible]		
"	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]			[illegible]		[illegible]		
"	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]					[illegible]		
>2.5-5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
>5-10	[illegible]	5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	35		[illegible]	[illegible]
>10-20	[illegible]	[illegible]	[illegible]	[illegible]							[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
>20-40	[illegible]	[illegible]	[illegible]	[illegible]							[illegible]	[illegible]	[illegible]		[illegible]		[illegible]	[illegible]

B.—Estimated Number of Particles in Each Size Range / 0.1[illegible][**]

≥2.5	≤2.5[*]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
>2.5-5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
>5-10	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
>10-20	[illegible]	[illegible]	[illegible]	[illegible]							[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
>20-40	[illegible]	[illegible]	[illegible]	[illegible]							[illegible]	[illegible]	[illegible]		[illegible]		[illegible]	[illegible]
Total fibrous particles/0.1[illegible]		[illegible]	[illegible]	72	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0	[illegible]	[illegible]	[illegible]	0		0	[illegible]
Total non-fibrous particles/0.1[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
[***]Estimated micro-fibers averaging [illegible] in length/0.1[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0		0	[illegible]
Overall incidence pleural neoplasms (%)		75	67	45	61	60	50	30-32	18	12	4-6	6	4	4	0		0	0

[*] Non-fibrous [illegible] (nonfibrous asbestos particles [illegible] represent mixtures of [illegible] and [illegible] of [illegible])

[**] [illegible] where [illegible] = number of particles counted in a given size range; [illegible] = total volume of the 1000 particles counted, in cubic microns, assuming that the particles are cylinders and in each size range have volumes of a normal distribution; [illegible] = density

[***] Assuming that all fibers fragment [illegible] to mean lengths of [illegible] and that all asbestos fibers fragment longitudinally to mean diameters of [illegible]

[illegible] particles [illegible] lengths of [illegible] by [illegible]

EXPERIMENT	TEST MATERIAL PREPARATION	SOURCE OF TEST MATERIAL	DOSE	SURGERY DATE	SACRIFICE DATE
CROCIDOLITE ASBESTOS					
Unopened fibers, Cape Blue cobbed ore	None	Cape Asbestos South Africa (Pty.) Limited	40 mg	5/27/70-6/1/70	6/1/72
Fully pulverized UICC Crocidolite	Pulverized 10 minutes in Spex mill	UICC	40 mg	3/17/71	3/17/7
2nd Series UICC Crocidolite - 40 mg	None	UICC	40 mg	4/8/71	4/8/73
2nd Series UICC Crocidolite - 20 mg	None	UICC	20 mg	4/9/71	4/9/73
2nd Series UICC Crocidolite partially pulverized	Pulverized in Spex mill 3 minutes	UICC	40 mg	4/16/71	4/16/7
Microfibrillar fraction UICC Crocidolite	Sedimentation and centrifugation in both water and alcohol	UICC	40 mg	5/4/71	5/4/73
Finely ground Crocidolite 4106-22-1B	Pulverized in Spex mill with plastic balls 10 hours	Johns-Manville	40 mg	5/5/71	5/5/73
Crocidolite, long, fine >10μ x .5μ # 4106-22-4	Cape Blue crude fiber sheared in Waring blendor, screened in special bottle	Johns-Manville	40 mg	5/7/71	5/7/73
Crocidolite, short, fine <5μ x .5μ #4106-25-2	Cape Blue crude fiber sheared in Waring blendor, screened in special bottle	Johns-Manville	40 mg	5/6/71	5/6/73
Macrofiber fraction UICC Crocidolite	Sedimentation and screening in water and detergent	UICC	40 mg		

Received from General Yudkin Friday, June 11, 1971 who in turn received it from Ed Datgen.

CHRYSOTILE ASBESTOS					
Air-cleaned Jeffrey Chrysotile - 6D20	None	Johns-Manville	40 mg	7/21-22/70	7/22/72
Air-cleaned Jeffrey Chrysotile - 6D20	None	Johns-Manville	20 mg	7/31/70	7/31/72
Air-cleaned Jeffrey Chrysotile - 6D20	None	Johns-Manville	10 mg	8/2/70	8/2/72
Air-cleaned Jeffrey Chrysotile - 6D20	None	Johns-Manville	5 mg	8/3-4/70	8/4/72
Air-cleaned Jeffrey Chrysotile - 6D20 fully pulverized	Pulverized in Spex mill for 10 minutes	Johns-Manville	40 mg	11/23/70	11/23/72
Cobbed crude Chrysotile ore from Jeffrey mines	None	Jeffrey mines, Asbestos, Que. Canada	40 mg	4/13/71	4/13/73
Air-cleaned Jeffrey Chrysotile - 6D20 partially pulverized	Pulverized in Spex mill for 3 minutes	Johns-Manville	40 mg		

EXPERIMENT	TEST MATERIAL PREPARATION	SOURCE OF TEST MATERIAL	DOSE	SURGERY DATE	SACRIFIC DATE
FIBROUS GLASS					
Whole fiber uncoated fine fiberglas	None	Owens-Corning	40 mg	4/14/70	4/14/72
Glass fibers $<5\mu \times 1\mu$	Sheared in Waring blendor, sedimented, and pulverized in Spex mill	Johns-Manville	40 mg	8/31/70	8/31/72
Glass fibers $<5\mu \times 3\mu$	Sheared in Waring blendor, sedimented, and pulverized in Spex mill	Johns-Manville	40 mg	9/6/70	9/672
Glass fibers $>10\mu \times 1\mu$	Sheared in Waring blendor and sedimented twice, filtered.	Johns-Manville	40 mg	9/13/70	9/13/72
Glass fibers $>10\mu \times 3\mu$	Sheared in Waring blendor and sedimented twice, filtered	Johns-Manville	40 mg	9/19/70	9/19/72
Code 106 Micro-fiber glass	None	Johns-Manville	40 mg	12/1/70	12/1/72
Sheared uncoated fine fiberglas - 40 mg	Sheared in Waring blendor 10 minutes	Owens-Corning	40 mg	12/29-30/70	12/30/72
Sheared uncoated fine fiberglas - 80 mg	Sheared in Waring blendor 10 minutes	Owens-Corning	80 mg	12/22-23/70	12/23/72
Sheared uncoated fine fiberglas - 160 mg	Sheared in Waring blendor 10 minutes	Owens-Corning	160 mg	1/15/71	1/15/73
Glass fibers, long, fine #4106-27-5 Code 100 micro-fiber glass (475 glass)	Sheared in Waring blendor, centrifuged in Sharples super-centrifuge, screened	Johns-Manville	40 mg	5/13/71	5/13/73
Fully pulverized fine uncoated fiberglas	Pulverized 10 minutes in Spex mill	Owens-Corning	40 mg		
Partially pulverized fine uncoated fiberglas	Pulverized 2 minutes in Spex mill	Owens-Corning	40 mg		

EXPERIMENT	TEST MATERIAL PREPARATION	SOURCE OF TEST MATERIAL	DOSE	SURGERY DATE	SACRIFICE DATE
RELATED FIBROUS AND NON-FIBROUS MATERIALS					
Antigorite	Pulverized in Spex mill 1½ minutes	Smithsonian Institution	40 mg	7/6-13/70	7/13/72
Aluminum oxide whiskers	None	Artech Corp.	40 mg	9/29-30-70	9/30-72
Aluminum oxide whiskers	None	Artech Corp.	20 mg	10/12/70	10/12/7
Aluminum oxide whiskers	None	Artech Corp.	10 mg	10/19/70	10/19/7
Aluminum oxide whiskers	None	Artech Corp.	5 mg	10/20/70	10/20/7
Linde Corp. Alumina 0.3μ	None	Artech Corp.	40 mg	12/18/70	12/18/7
Attapulgite #4418-100-1 Fuller's earth	Paste form, 18.3% solids, originally obtained from Engelhard Research Labs.	Johns-Manville	40 mg	5/12/71	5/12/7
Tungsten carbide whiskers	None Originally from Suwa Seikosha Co., Ltd., Japan	Metals & Methods, Ltd.	40 mg	5/17-24/71	5/24/7