the development of emphysema. It is possible that a period long- ten weeks reported above may cythemia. This as well as the de- of cor pulmonale is under investi- his laboratory.

Summary

mature rats receiving intratra- ion of papain and with constrict- developed lesions in the lung hose reported by Gross et al in essential features of the experi- compared to the normal controls ows: (1) elevated functional re- icity, (2) elevated pulmonary 3) reduced pulmonary compli- evated blood carbon dioxide ten- 5) elevated percent of air space al sections.

t was supported by Public Health HE-09532-02 from the National Heart

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Experimental Asbestosis

The Development of Lung Cancer in Rats With Pulmonary Deposits of Chrysotile Asbestos Dust

Paul Gross, MD; Robert T. P. deTreville, MD, DSc; Ethel B. Tolker, BS; Marianne Kaschak, BS; and Mary Ann Babyak, BS, Pittsburgh

DURING a study of experimental asbestosis involving guinea pigs, rats, and hamsters that had inhaled chrysotile dust, it was found that pulmonary cancers had developed in several rats. Spontaneous primary lung cancers, exclusive of lymphoblastomata, have been exceedingly rare in our rats (we have not seen a single spontaneous primary pulmonary carcinoma or fibrosarcoma in about 10,000 rats examined). Therefore, a cause-and-effect relationship between the pulmonary deposition of chrysotile dust and tumor development was suspected. Suspicion increased as the number of malignant lung tumors discovered in rats that died more than 16 months after initiation of the chrysotile exposure increased rapidly.

There has been increasing evidence, mostly epidemiologic, linking long-term exposure to asbestos dust with the development of pulmonary cancer in man.[1,2] Since the development due to the pulmonary deposition of chrysotile asbestos dust of primary lung cancer in an experimental animal has not been reported, the occurrence of malignant lung tumors in our asbestotic rats is being reported and a hypothesis explaining this phenomenon is offered.

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Materials and Methods

Canadian chrysotile asbestos was ball-milled and subsequently hammer-milled, using essentially the method of Holt et al.[3] The principle of this technique is to reflux the comminuted chrysotile through the high-speed hammer-mill so that much of the dust is of ultramicroscopic dimensions (Fig 1). Within less than one year, it became necessary to replace the steel-alloy hammers with new ones because of excessive wear. Also, to maximize the dust concentration in the chamber, the output of a second hammer-mill was added to the inhalation chamber.

The chamber measured 8 × 8 × 8 ft. Initially, it had only one four-inch input opening connecting with the outflow duct of a hammer-mill. After 52 weeks, a second four-inch input for the second hammer-mill was installed. There were three exhaust openings in the chamber. Two of these were 1.5 inches in diameter each. Both led to electrostatic precipitators and then connected with a vacuum pump. The flow of exhaust air through one precipitator was approximately 125 liters per minute whereas the flow through the other precipitator was adjusted accurately to 8 liters per minute. The dust in the latter was carefully weighed at the end of each week to determine the average dust concentration in the chamber during that week. The third exhaust opening in the chamber, ten inches in diameter, led to a ten-inch furnace-type filter and then to an exhaust fan. The latter was used to exhaust the chamber rapidly at the end of each day's exposure. The chamber was actually under a slight, but definite, negative pressure during the dust exposure.

A total of 131 male white rats were exposed to chrysotile dust in the chamber six hours a day, five days a week for a maximum of 62 weeks. Of these 131 animals, 70 had had an intratracheal application of 0.05 ml of 5% aqueous sodium hydroxide, the purpose of which was to impair clearance from the lungs of inhaled dust. (The 70 rats were the survivors from a total of 81 animals that had received the caustic applications intratracheally.) The intratracheal caustic applications were accomplished with the animals under ether anesthesia and with the aid of a self-retaining illuminated speculum that allowed visualization of the vocal chords. A long 18-gauge needle attached to a microliter syringe was used. The end of the needle was plugged, rounded off, and two small openings made on opposite walls about 5 mm from the tip. After expelling the contents of the syringe onto the tracheal wall, the needle was withdrawn with a spiral motion to distribute the caustic as widely as possible.

The caustic-treated animals were introduced into the inhalation chamber at different stages of healing of the tracheal lesions. Some were exposed to the dust immediately; others, one, two, and three weeks after the caustic application. The 61 rats not treated with caustic were also exposed in the chamber to chrysotile dust and were assumed to have normal tracheas. Dust exposures were discontinued after 62 weeks. The survivors were then pastured for the remainder of their lives.

Dust concentrations in the chamber during the first year, with only one mill in operation, ranged from 42 to 106 mg/cu m. During the second year, with both mills in operation, the range was 67 to 146 mg/cu m. The overall average was 86 mg/cu m (based on weekly determinations).

Another group of 64 rats was given intratracheal injections of chrysotile dust obtained from the electrostatic precipitators. The same injection technique was used as that used for intratracheal caustic applications except that the 18-gauge needle had its opening in the tip, the edges of which were rounded off. The dose injected was 3.5 mg suspended in 1 ml water. Of the 64 rats, 14 animals received but one injection; 14 received two; 1 received three; 9, four; and 26, six.

In addition, 30 rats of the same shipment as that of the animals receiving exposure served as laboratory controls and were not treated in any manner. They were housed in a room, remote from the exposure chamber, and were pastured for the remainder of their lives. Another group of 15 rats served as controls for the caustic intratracheal applications. They had survived an intratracheal application of sodium hydroxide but had not been exposed to asbestos dust. Finally, a group of ten rats served as controls for the rats injected intratracheally with

Fig 1.—Electron photomicrograph of chrysotile dust obtained by thermal precipitation from the inhalation chamber.

Table 1.—*Overall Mortality and Lung Cancer Incidence**

Animal Group	No. of Rats	16-Month Survivors		Rats With Lung Cancer*	
		No.	% of Total	No.	% of Survivors
Chrysotile inhalation with intratracheal NaOH	71	31	44	15	48
Chrysotile inhalation without intratracheal NaOH	61	41	67	10	24
Chrysotile, intratracheal	64	19	30	3	16
Laboratory controls (no treatment)	40	25	63	0	0
NaOH controls (no chrysotile)	15	14	93	0	0

* Exclusive of lymphoblastomas (known to occur spontaneously in these rats).

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*nd Lung Cancer Incidence**

	16-Month Survivors		Rats With Lung Cancer*	
No.	% of Total	No.	% of Survivors	
31	44	15	48	
41	67	10	24	
19	30	3	16	
25	63	0	0	
14	93	0	0	

n to occur spontaneously in these

asbestos dust. They had received intratracheal injections of water.

The survivors of the multiple intratracheal chrysotile injections were killed 21 months after the first injection.

The lungs of all rats were distended with 10% formalin solution under a pressure of 12 cm water. Sections were cut at 6μ from blocks of lung embedded in paraffin and stained with hematoxylin and eosin. After photomicrography the sections were decolorized and impregnated with silver according to the method of Gordon and Sweets. Some sections were also incinerated in order to obtain information on the amount and distribution of the chrysotile dust.

Results

The earliest lung cancer was found in a rat that had died 16 months after initiation of chrysotile dust exposure in the inhalation chamber. Calculations of the prevalence of lung tumors were based on the number of animals that live 16 months or longer after having been placed in the inhalation chamber (72 rats) or after they had received the first intratracheal injection (19 rats). Of the 72 surviving rats from the inhalation chamber, 31 had received an intratracheal application of sodium hydroxide and 41 had not been so treated. (These data are shown in Table 1.)

Many lung cancers were microscopic in size but some were visible grossly, the size ranging from 1 to 5 mm in diameter. All tumors were peripheral in location and none originated in a bronchus or bronchiole. The tumors were light gray-tan in color, bulged slightly above the surface, and were covered by a shiny, thin, transparent pleura. The consistency was firm, verging on hardness. On section, the tumor presented a dull, gray, dry, and compact appearance. The cut surface did not appear to retract or bulge. The edges of the tumor were smooth and stood out sharply from the surrounding retracted, moist, generally dark-red lung tissue. There was no evidence of a capsule. Distinguishing these tumors grossly from the lymphoblastomata that occurred spontaneously in our aging rats offered no difficulty. The latter tumors were located in the hilar regions, although some of the larger ones extended to the periphery. They were infiltrating, hence their edges were less sharply defined. The cut surface was glistening gray, tended to bulge slightly and, most important, it tended to be lobulated, each lobule having a tiny hole in the center. The hole was the lumen of either a vessel or a bronchus (or bronchiole), whereas the lobule represented adventitial and periadventitial tumor infiltration.

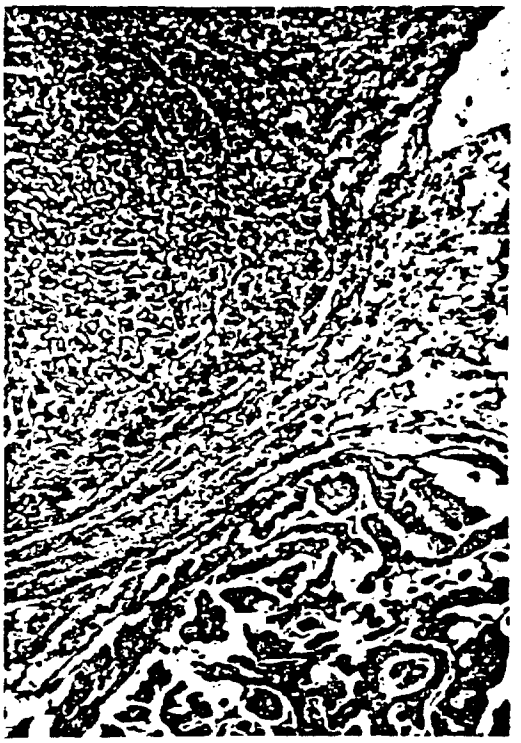

Fig 2.—Adenocarcinoma occupies almost the diagonal upper half of the field whereas the lower portion of the field is occupied by a large papillary adenoma. Neither tumor is encapsulated. Compressed alveolar tissue separates the two tumors. This is from a rat that was exposed to chrysotile dust for 62 weeks beginning immediately after the intratracheal caustic application. The animal died 12 months after removal from the exposure chamber (hematoxylin and eosin, × 40).

Altogether, there were 28 animals with primary malignant tumors of the lung, exclusive of spontaneously occurring pulmonary lymphoblastoma but inclusive of one malignant mesothelioma of the pleura and three questionable, but probable, adenocarcinomata of the lung. The overall prevalence was 31%, based on 91 animals surviving 16 months or longer. Of the 28 rats with malignant pulmonary tumors, 25 had inhaled chrysotile dust, giving this group a cancer prevalence of 35% (based on a co-

Fig 3.—Adventitial and periadventitial invasion by tumor glands about a pulmonary artery in a rat exposed to chrysotile dust for 62 weeks beginning three weeks after the intratracheal caustic application. It died 15½ months after removal from the exposure chamber (hematoxylin and eosin, × 150).

Fig 4.—Example of one of the many perplexing lesions found in the asbestotic rat lungs. The lesion is characterized by a collection of fairly large spaces that have occasional irregular evaginations, lined by deeply staining, hyperplastic cuboidal epithelium. The walls are relatively thick but not collagenized. The lesion resembles an adenoma, but more likely represents adenomatoid metaplasia and hyperplasia. From the same lung as in Fig 3 (hematoxylin and eosin, × 150).

hort of 72); three had been injected intratracheally with the dust, a prevalence of 16% (based on a cohort of 19 rats). Of the 25 animals with primary lung cancer including the mesothelioma in the inhalation group, 15 had received an intratracheal application of lye, giving a cancer prevalence of 48% (based on a cohort of 31 rats treated with lye and surviving 16 or more months). The remaining ten animals with lung cancer that had not had intratracheal lye treatment, represented a cancer prevalence of 24% based on a cohort of 41 rats.

There were 17 rats with certain adenocarcinomata and three animals with questionable but probable adenocarcinoma. Of seven rats with fibrosarcomatous lung tumors, three are also listed with the ones having adenocarcinoma. Of four rats with a pulmonary squamous cell cancer, one also is listed with the 20 having adenocarcinoma. A list of the animals with tumors, their exposure, survival time, and nature of the tumor is given in Table 2.

No lung cancer was seen in any control animal other than the spontaneously occurring lymphoblastoma.

The diagnosis of adenocarcinoma is usually not difficult since a large nodule composed of crowded, diminutive and atypical glands makes recognition of this malignant tumor relatively easy (Fig 2). Often, however, other foci of altered morphology in the same lung or even in the same section lead to less certain conclusions. The diagnosis of some pulmonary lesions is not easy and, in a few instances, was designated as "questionable but probably correct." A fairly common, but fascinating lesion involves regions of acellular, collagenous scarring affecting respiratory bronchioles, their adjoining alveolar ducts, and their evaginating alveoli. In a few

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Table 2.—*Rats With Lung Tumors and Their Survival Time*

Rat No.	Dust Exposure: NaOH*	Dust Exposure: 62 Weeks	Dust Exposure: No. of 3.5-mg Doses	Survival (Mo)	Primary Lung Cancerst: Adeno	Sq Cell	Fibro	Meso	Adenoma
8804	—	+	—	16		+			
8847	—	+	—	16			+		
8742	1	+	—	16			+		
8752	0	+	—	16¼	+				
8761	1	+	—	16¼		+			
9068	0	+	—	23	+				
8747	3	+	—	25¾	+		+		
8781	2	+	—	26	+		+		+
8740	0	+	—	26½	+				+
8834	—	+	—	27¼			+		
8835	—	+	—	27¼	+				
8782	2	+	—	27½			+		
8863	—	+	—	27¾	+		+		+
8767	1	+	—	28				+	
8854	—	+	—	28¼	+	+			
8743	1	+	—	28½	+				
8826	—	+	—	28½	+				
8796	3	+	—	28¾	+				
8768	1	+	—	29½	+				
8811	—	+	—	30¼		+			
8840	—	+	—	31	+				
8783	2	+	—	31¼	+				
10566	3	+	—	32	+				+
10567	3	+	—	32¾	+				
11175	—	+	—	34	+				
9188	—	—	6	21 K‡	+				
9191	—	—	4	21 K	+				
9204	—	—	4	21 K	+				

* Applied intratracheally weeks prior to inhalation exposure. Animals exposed to dust immediately after the caustic application are designated by 0. Those that received no caustic application are designated by —.

† Exclusive of lymphoblastoma, which is a common spontaneous tumor in these aging animals. Adeno, adenocarcinoma; Sq, squamous; Fibro, fibrosarcoma; Meso, mesothelioma.

‡ Killed. All other animals were found dead in their cages.

scarred foci in many lungs, the air spaces are lined by darkly staining cuboidal to columnar epithelium. The combination of distortion of the air spaces with abnormal epithelial lining give the impression of neoplasia. Because the lining epithelium is uniformly regular, well oriented, and devoid of excessive mitotic activity, these foci, if considered as tumors, should be designated as benign, ie, adenomata. But if they are indeed to be considered as adenomata, they are quite different from the spontaneous pulmonary adenomata seen commonly in mice (and rarely in rats and guinea pigs). Although the usual pulmonary adenoma is not circumscribed when small, the larger it grows, the more it tends to compress the surrounding lung tissue and thus develop a pseudocapsule of compressed lung tissue. The adenoma-like foci in the lungs of asbestotic rats are irregular in shape and although of moderate

Table 3.—*Trace-Metal Content of Chrysotile Dust and of Worn Part From Hammer Mill*

Element	Chrysotile: Prior to Hammer Milling, μg/gm Sample	Chrysotile: After Hammer Milling, μg/gm Sample	Chrysotile: After Hammer Milling, Increase, %	Worn Hammer From Mill, μg/gm Sample
Nickel	134.8	245.5	82	2,338
Cobalt	8.8	21.6	145	185
Chromium	114.6	153.3	34	...
Lead	None	37.9	...	3.5

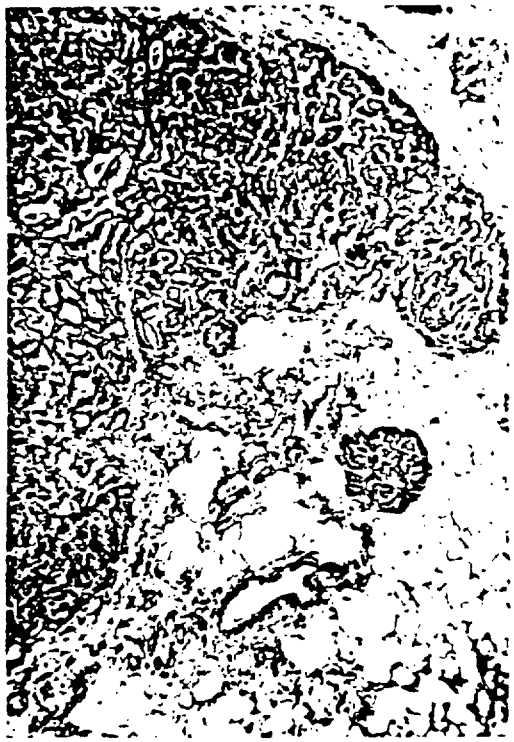

Fig 5.—To the right of this large adenocarcinomatous mass there are two small foci of peribronchiolar adenomatosis. To the right and below center of the field is a smaller nodule of adenocarcinoma in which glandular epithelium is darker than in larger mass. This is from a rat that received six intratracheal injections, each of 3.5 mg chrysotile dust. It was killed 21 months from time of the first injection (hematoxylin and eosin, × 150).

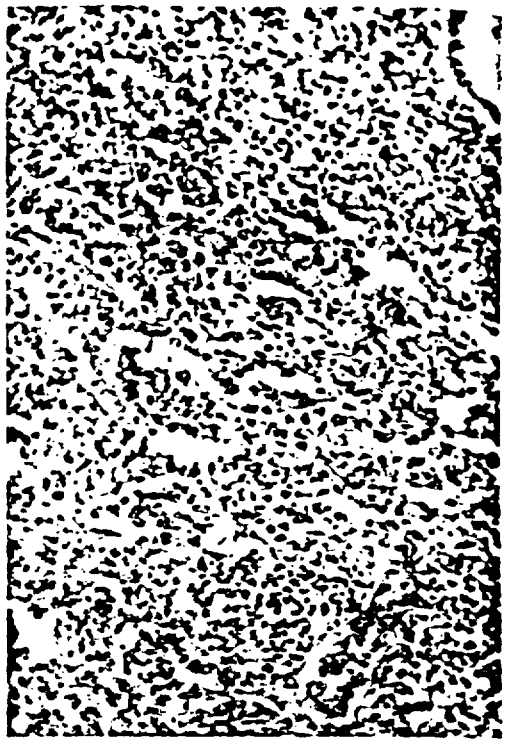

Fig 6.—Small pleomorphic, darkly-staining tumor cells, many of them more or less spindle-shaped, lining the thick walls of slit-like spaces. With silver impregnation the thick walls are recognizable as septal walls. The pleomorphic cells are associated with fine argyrophilic fibers distinct from the thicker septal fibers. This is from a rat exposed 62 weeks to chrysotile dust without an intratracheal caustic application. It died about 11 months after removal from the inhalation chamber (hematoxylin and eosin, × 150).

size, lack even a suggestion of circumscription. Other significant points of difference between adenoma-like foci in asbestotic rat lungs and the usual spontaneous pulmonary adenoma of small laboratory animals are the presence in the latter of crowded glands with minimal intervening stroma, tall columnar epithelial cells with abundant pale cytoplasm, and papillae with delicate stromal stalks. None of these features are found in the adenoma-like lesions of asbestotic rat lungs.

Yet, there are similar foci in which the lining epithelium of the spaces is so crowded as to suggest growth activity beyond that consistent with mere hyperplasia; the epithelium is larger with more abundant cytoplasm; intra-acinar papillae are present, and smaller oval or tubular glandular structures are found within expanses of acellular collagenous stroma. These foci are manifestly neoplastic. Malignancy is evidenced by the presence of numerous irregular small glands and cell nests within broad, densely collagenous scars, forming a picture reminiscent of a scirrhous breast cancer. In other lungs, foci of malignant neoplastic glands may be characterized by cellular atypia such as piling up of nuclei with poorly defined cell boundaries and loss of cellular polarity. Occasionally the diagnosis of malignancy is facilitated by demonstration of epithelial tissue in the adventitia or the periadventitial stroma of larger vessels (Fig 3). Diagnostic problems arise when lesions intermediate between those manifestly neoplastic and those considered as atypical metaplasia are encountered. Such intermediate lesions are fairly common (Fig 4)!

In a number of rats, multiple foci of ade-

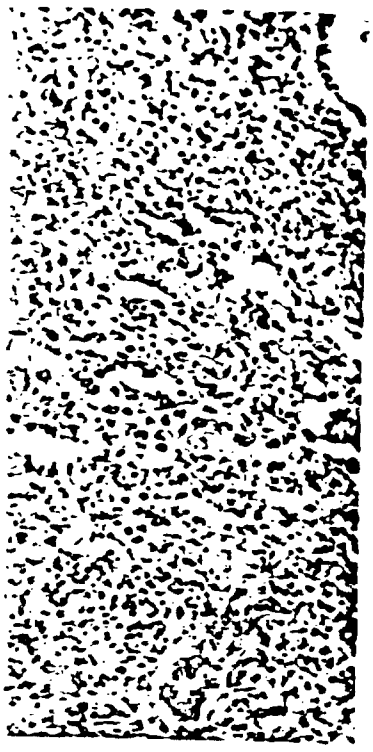

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Fig 7.—This field represents an undifferentiated, large cell sarcoma involving subpleural parenchyma and also extending over the pleural surface. The silver preparation indicates an intimate association of argyrophilic fibers with these cells. From a rat exposed 62 weeks to chrysotile dust, beginning two weeks after an intratracheal caustic application. The animal died 11½ months after removal from the inhalation chamber (hematoxylin and eosin, × 300).

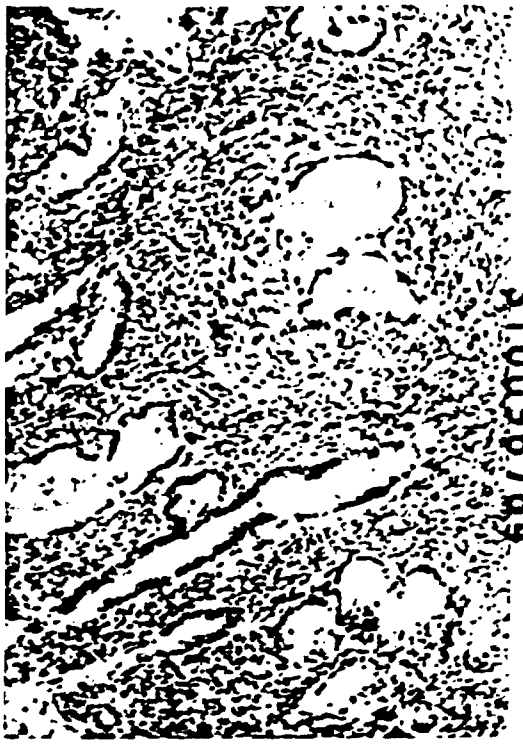

Fig 8.—Combination of adenocarcinoma and fibrosarcoma of the lung. Both tissue elements are well differentiated. Glandular cells secrete mucin whereas stromal cells are spindleshaped and resemble those of endometrium. This field is taken from the lung of the same rat as in Fig 7 (hematoxylin and eosin, × 150).

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nocarcinoma are present in the same section, indicating the multicentric nature of the tumor (Fig 5).

In several animals, a cellular fibrosarcomatous tumor was found in the lungs (Fig 6). On the periphery of such tumors, the air spaces are narrowed, their walls thickened and composed of pleomorphic cells with hyperchromatic nuclei and scanty cytoplasm. Coherent masses of these cells partially fill the air spaces. They are associated with a delicate, argyrophilic supporting stroma. The more substantial stroma of the alveolar septa remains intact. Although superficially resembling the lymphoblastoma that occurs spontaneously in aging rats, these fibrosarcomas differ from the latter by the pleomorphism of their cells, their tendency to respect the alveolar architecture at their periphery, and the presence there of a delicate argyrophilic stroma. In contrast, the spontaneous lymphoblastoma completely destroys the alveolar architecture whether it is of the lymphocyte or the reticulum cell type. A second form of fibrosarcoma was encountered in a lung, with an adenocarcinoma in which the stroma is composed of crowded, fairly uniform vesicular ovoid nuclei and an unusually dense argyrophilic network. In one animal there were foci in which sarcomatous cells resemble decidual cells (Fig 7). Interesting combinations of adenocarcinoma and fibrosarcoma were found in several animals (Fig 8). The fibrosarcoma may be pure or may contain epithelial components (Fig 9).

Perhaps the most interesting of all, because of its rarity, was a pleural mesothelioma in one rat that covered portions of both lungs and the pericardium like a cuirass. The tumor consists of straight and tortuous as well as branching tubular glands seen in longitudinal and cross sections, associated

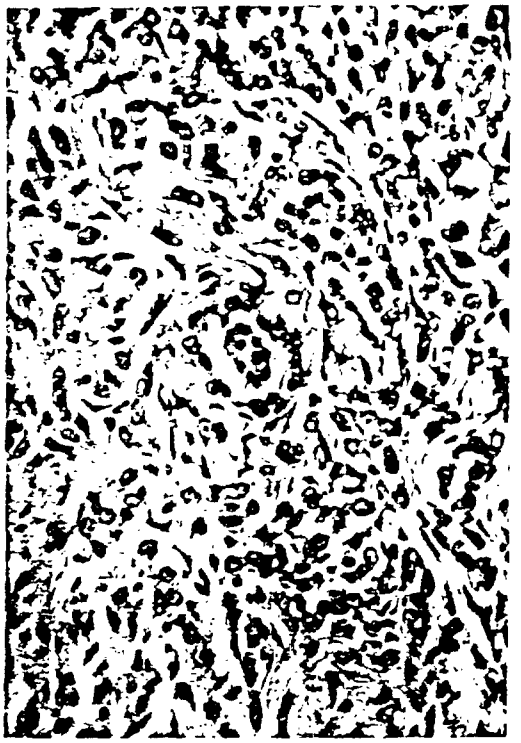

Fig 9.—Another combination of adenocarcinoma and fibrosarcoma of the lung showing a small gland in the center surrounded by well differentiated fibrosarcomatous tissue. This is from a rat exposed to chrysotile dust for 62 weeks and died 13 months after removal from the exposure chamber. It had received no intratracheal caustic application (hematoxylin and eosin, × 300).

Fig 10.—Portion of thick, pleural mesothelial tumor composed of irregular glands embedded in a dense collagenous tissue. Except for a few small superficial foci, the tumor does not invade lung tissue. This tumor was found in a rat exposed to chrysotile dust for 62 weeks beginning one week after an intratracheal caustic application. The animal died 13½ months after removal from the inhalation chamber (PAS stain, × 40).

with a moderately cellular but relatively dense stroma (Fig 10). The glands are lined by sharply delimited, cuboidal to low columnar epithelium with round, uniform-appearing nuclei (Fig 11). The lumen of some of the glands contains PAS-positive material, yet the cytoplasm of the lining cells is PAS-negative. The density of the stroma is best seen in the silver-impregnated section where crisscrossing thick collagen bundles seem to enclose glandular elements. The stromal cells are ovoid in shape and their nuclei of a uniform, moderately dense character. Over the greater portion of the pleural surface, the tumor is sharply confined to the pleura. However, in several foci there is superficial invasion of alveolar tissue. Several mesothelial tumor nodules appear as isolated foci upon an otherwise normal pleura. Whether these are metastases or multicentric primaries is difficult to say. The smallest consists of stroma only and forms a lentiform pleural thickening.

Although the squamous cell carcinomata are not difficult to diagnose (Fig 12), occasional foci of intra-alveolar atypical squamous cell production have some malignant aspects (Fig 13). These have been categorized as probable atypical metaplasia.

Another interesting tumor is a mucinous adenocarcinoma, forming a subpleural mass about 4 mm in diameter. This tumor, also termed "colloid" carcinoma, consists of irregular, mucin-filled cystic spaces that have destroyed and replaced the alveolar architecture (Fig 14). Very few viable tumor cells lining these spaces can be identified (Fig 15). This type of cancer is more commonly seen as a primary tumor in the gastrointestinal tract.

With the exception of one squamous cell carcinoma that metastasized to a satellite

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Fig 11.—Higher magnification of several tubules of the mesothelioma. Cytoplasm of the lining epithelium is PAS-negative although PAS-positive material is present in the lumen (× 300).

Fig 12.—Irregular cords and nests of well-differentiated squamous cells show no keratin formation. Extensive keratinization is present in other fields of this pulmonary squamous cell carcinoma. From a rat exposed to chrysotile asbestos for 62 weeks without caustic treatment. Animal died 14 months after removal from exposure chamber (hematoxylin and eosin, × 150).

lymph node and the possibility that some of the discrete pleural foci of the mesothelioma represent secondary nodules, no evidence of metastasis has been observed in satellite nodes or elsewhere. Examination of other organs in animals with lung cancer failed to demonstrate the presence of a tumor that could have produced a pulmonary metastasis.

These lung cancers have no apparent relation to the degree of asbestotic involvement. Not one tumor-bearing rat had diffuse asbestosis. In all animals the asbestotic involvement was multifocal, with abundant normal, or near-normal alveolar tissue between such foci. Lesions were centered in the proximal portions of the primary lobule.

Comment

The development of lung cancer in rats caused by the pulmonary deposition of chrysotile dust confirms experimentally the cause-and-effect relationship between chrysotile dust exposure and lung cancer. Until this study, such a cause-and-effect relationship was only surmised epidemiologically by a study of workers exposed to chrysotile dust in the British asbestos textile industry[1] (largely prior to 1933). In the United States an epidemiologic study of members of an asbestos workers' union also indicated increased prevalence of lung cancer, but the nature of the dusts to which the workers had been exposed was uncertain.[2]

The present study, with its 31% prevalence of pulmonary cancer, is in striking contrast to results reported by Wagner et al[4] who encountered no lung cancer. It seems unlikely that a difference in susceptibility to lung cancer in the strains of rats used could account for the differences in results. However, there is an excellent probability that a particular quality of chrysotile dust

Fig 13.—Nests of transitional or partially differentiated squamous epithelial cells derived by metaplasia from alveolar lining epithelium. Although there is loss of cellular polarity and nuclei are piled up on one another, latter are uniform in appearance and show no mitotic activity. Squamous cells are confined within limits of alveolar spaces. Lesions such as these are not classified as neoplastic. This is from a rat that had an adenocarcinoma in another portion of lung. This animal had been exposed to chrysotile dust for 62 weeks and had died 16½ months after removal from exposure chamber. There had been no intratracheal caustic application (hematoxylin and eosin, × 150).

Fig 14.—Typical mucinous adenocarcinoma, most commonly primary in gastrointestinal tract but found in lung of rat exposed for 62 weeks to chrysotile dust without a prior intratracheal caustic application. Peripheral (subpleural) nodule composed of irregular mucin-filled spaces that have destroyed and completely replaced alveolar tissue. Rat died 19½ months after removal from inhalation chamber (hematoxylin and eosin, × 40).

deposited in the lungs of our rats was significant in producing lung tumors. The prolonged hammer-milling used to prepare the chrysotile dust in our investigation was not employed in other long-term studies. Wagner et al[4] did not further comminute the dust they used for animal exposures but reported obtaining material directly from industrial dust collecting apparatus. We, therefore, presume, although we are not certain, that the tracemetal content of the latter dust may not have been as high as in the dust to which our animals were exposed (Table 3). The probability exists that factors other than the crystalline hydrated magnesium silicate in chrysotile asbestos dust may account for the severe pathologic response of lung and pleural tissues to this dust. These factors are as follows:

1. The chrysotile dust used in this investigation has a high trace-metal content, considerably higher than it was prior to the hammer-mill treatment (Table 3). The increase in content of trace metals after milling was nickel, 82%; cobalt, 145%; chrome, 34%. Aanlysis of the worn hammer of the hammer mill revealed a nickel content of 2,338μg/gm of sample.

2. There is a disparity in the published prevalence of lung cancer among those who worked in the British asbestos textile industry prior to 1933[1] and that among Canadian chrysotile asbestos miners who worked from 1950 to 1956.[5] Although the workers in the British asbestos textile industry had a tenfold greater risk of developing lung cancer than had the general population,[1] this in-

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Fig 15.—Higher magnification of several of the mucin-filled cysts seen in Fig 14, shows the loss of lining epithelium of these cysts, a feature commonly present in this type of cancer (hematoxylin and eosin, × 150).

creased risk of lung cancer was not found among workers in the Canadian chrysotile industry.[5] Aside from differences in climatic conditions, ethnic backgrounds, density of dust exposure, and possibly also in smoking habits, it appears probable that differences in the dust itself to which these two population groups had been exposed may account for the disparity. For example, at the mines, chrysotile mineral is subjected to considerable crushing contact with high-strength steel alloys to "open" the fibers and remove adherent nonfibrous mineral components prior to shipment to manufacturers. However, considerably more extensive contact with nickel- and chrome-steel alloys, involving more and more of the most finely divided fibers, takes place during this process and during carding as well as spinning, essential processes in the fabrication of asbestos textiles. This, in a manner similar to the hammer-milled asbestos, may cause chrysotile asbestos dust to acquire a higher content of potentially fibrogenic and carcinogenic trace-metals[6] than is present in chrysotile dust at the mine.

3. Preliminary investigations in this laboratory, using synthetic chrysotile prepared by W. T. Granquist, PhD, of Mellon Institute suggest it is biologically "inert" (Gross, P.; deTreville, R. T. P.; and Granquist, W. T. unpublished data). Something other than pure chrysotile crystals may be responsible for the lesions found in human and animal lungs which contain asbestos.

4. There is a uniqueness not only in the association of asbestos dust exposure with the occurrence of mesothelioma but also in the association with lung cancer insofar as there is no relationship between severity of involvement and occurrence of neoplasia. It has not been possible to demonstrate convincingly the presence of the presumed inciting agent at the site of neoplasia. It is very difficult to demonstrate asbestos fibers or asbestos bodies even in non-neoplastic thickened pleurae. If the association of asbestos-dust exposure and neoplasia represents a cause-and-effect relationship—and there is little reason to doubt it—then a logical explanation might be that something accompanying asbestos dust adversely affects tissues located at considerable distances from the site of dust deposition. This could be one or several trace metals that are added to the mineral dust from machinery and become eluted after pulmonary deposition.

Of the trace-metals associated with the asbestos dust used in our study, only nickel has been shown to be capable of producing lung cancer experimentally. Sunderman et al[6] found that rats exposed to nickel carbonyl developed pulmonary squamous cell carcinoma.

The many different types of primary malignant lung tumors in our animals with pulmonary deposits of chrysotile dust and the different types of cells in these tumors bring to mind a concept originated by Waddell[7] and defended by work in this laboratory.[8] This theory holds that the parent cell of the alveolar membrane is multipotential, capable of differentiating either into epithelial or connective tissue. Except for the mesothelioma, all the lung tumors described in this paper are believed to have originated from cells of the alveolar membrane, a uni-

tarian concept in support of which are the peripheral location of all tumors in question and failure to demonstrate a bronchial origin. The thesis that metaplasia of alveolar epithelium to the columnar type represents a so-called "bronchialization," ie, a downgrowth of bronchiolar epithelium, has been greatly weakened by Totten's demonstration that these metaplastic columnar epithelial cells contain osmiophilic inclusions similar to those seen in normal alveolar surface cells, inclusions not found in normal bronchiolar epithelium.[9]

The ability of neoplastic alveolar cells to assume widely different forms was exemplified by the different types of tumor cells observed. The morphology of alveolar tumor cells found in asbestotic rat lungs ranged from squamous to columnar, from partially differentiatd squamous (Fig 13) to fully differentiated (Fig 14), even with much keratin production; and from darkly-staining cuboidal (Fig 4) to the highly differentiated mucin-secreting variety (Fig 8 and 15). The neoplastic connective tissue cell types ranged from undifferentiated small, pleomorphic cells (Fig 6) to large, anaplastic cells (Fig 7); and differentiated types ranged from small spindle cells resembling those of endometrial stroma (Fig 8) to plump, collagen-producing fibroblast-like cells (Fig 9).

The rapidity (within two hours) with which new argyrophilic fibers become demonstrable on an alveolar wall of a sensitized animal when challenged with tuberculin,[10] suggests that cells of the alveolar membrane rather than the exceedingly few and widely scattered interstitial mesenchymal cells of the alveolar interstitium are responsible for these fibers.

In a recent study of pulmonary adenomas of mice, the apparent ability of alveolar membrane cells to produce argyrophilic stromal fibers was described.[8] Finally, the unitarian concept of the origin of these tumors is supported by the observations of previous investigators who noted that many metastases and transplants of mouse adenomas were sarcomatous.[11,12]

The fact that the prevalence of lung cancer among the rats which had intratracheal caustic applications was more than double that in animals not so treated, underlines the importance of the upper respiratory tract clearance mechanism, particularly in eliminating cancerogenic particles.

Attention is also directed to the nature of the asbestotic inflammation in rats. Despite a massive exposure to respirable chrysotile dust for 62 weeks, the asbestotic inflammation was multifocal rather than diffuse, and characterized by poorly cellularized collagen. The presence of the latter and of normal alveolar tissue between the collagenized foci confirms our opinion about healing and nonprogression of chrysotile asbestosis in the rat.[13]

Summary

One hundred and thirty-one rats were exposed to chrysotile asbestos, finely milled, in an exposure chamber for six hours a day, five days a week for a maximum of 62 weeks. Dust concentrations were very heavy, ranging from 42 to 146 mg/cu m (average 86 mg/cu m). About half the animals exposed also had intratracheal application of caustic (lye) to reduce lung clearance and maximize dust retention and pathogenicity. Also, 64 additional animals were given intratracheal injections of chrysotile dust, 14 receiving single doses and the remainder multiple injections of 3.5 mg each. Controls consisted of 30 untreated, unexposed animals and 15 rats treated with caustic but unexposed to asbestos. Designed to study asbestosis, the most important findings of the investigation related to lung tumor production. Other investigators have not been successful in producing lung cancer experimentally in rats with chrysotile. Tumor incidence was high. Of those surviving 16 or more months (ie, after appearance of the first tumor) 31% had primary malignant tumors of the lung. Occurrence in rats treated with lye was 48%, twice the rate of those exposed but not treated with caustic. Malignant pulmonary tumors included adenocarcinomas, fibrosarcomas, and squamous cell cancers as well as a mesothelioma and a mucinous adenocarcinoma. A number of pulmonary lymphoblastomas which occur spontaneously in rats were not counted. Also, atypical metaplasia, not manifestly malignant, was commonly noted. Multicentricity of malignant lesions was observed in several animals. Results of this study sup-

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port the unitarian concept of tumor origin from alveolar cells. Despite extreme exposure conditions, diffuse asbestosis was not seen. Fibrosis observed was multifocal, centered in the proximal portion of primary lobules with normal appearing tissue intervening.

Cancer production with chrysotile in this study appears to involve trace metals introduced during hammer milling which increased nickel, chrome, and cobalt. Previous investigators have not reported subjecting their dusts to hammer milling. The increase in content of trace metals after milling was as follows: nickel, 82%; cobalt, 145%; chrome, 34%. Analysis of the worn hammer of the hammer mill revealed a nickel content of 2,338 μg/gm of sample. This finding, if validated by further studies, should provide an important aid to guide industrial hygiene controls of potential health hazards.

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James M. McNerney participated in the early stages of this investigation.

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SCIENCE VERSUS LIFE

When scientists and engineers undertake to apply science to practical problems, however, they find it impossible to stick to quantitative data and rules of logic. The problems include too many other aspects, and in dealing with them they naturally behave like human beings. And so weary cynics like lawyers and political scientists take a certain malicious pleasure in showing that the natural scientists are led by confidence in their special scientific techniques to try to make judgments on political values or policy decisions, without realizing all the other components of a nonscientific nature that go into those decisions. The lesson is a valid and important one, and needs to be driven home to the general public much more than to the scientists, most of whom know it already even if they do not like for nonscientists to remind them of it.—Price, D.K.: *The Scientific Estate*, Cambridge, Mass: Harvard University Press, 1965, quoted in *Amer Sci* 53:528 (Dec) 1965.