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EXPERIMENTAL STUDIES OF ASBESTOSIS

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ASBESTOSIS is a form of pneumonoconiosis resulting from prolonged inhalation of asbestos dust. The name "asbestos," literally "unburnable," is not that of a specific mineral but is a term applied to a number of different minerals whose characteristic feature is a structure composed of long, parallel, flexible fibers. This structure is unique because the fibers are capable of repeated longitudinal subdivision to units of molecular proportions. In length the fibers vary from a few microns to 6 or more inches (15 or more cm.). Some varieties are stiffer than others, but many are sufficiently flexible to be spun into yarn and woven on modified textile machinery.

The asbestos minerals are silicates of variable composition and belong to the serpentine and the amphibole groups. Listed below are the more common varieties.

Amphibole group: actinolite, amosite, amphibole, anthophyllite, crocidolite and tremolite.

Serpentine group: chrysotile.

The bulk of the asbestos of commerce is chrysotile, $3MgO.2SiO_2.2H_2O$, which is mined on this continent principally in the Thetford region of the Province of Quebec, Canada, and in Vermont. Crocidolite and amosite also are used commercially but in much smaller amounts. Chrysotile occurs as veins in serpentine, a mineral of similar chemical composition, which exists in massive form and is made up of microscopic fibers without the parallel orientation characteristic of chrysotile. The massive, bluish black serpentine, which is smooth and soapy to the touch, is traversed by veins of fibrous chrysotile varying in width from a barely perceptible line to 6 (15 cm.) or more inches. The fibers run across the vein and not lengthwise with the formation.

From the Saranac Laboratory of the Edward L. Trudeau Foundation.

This series of studies of asbestosis, initiated at the Saranac Laboratory more than twenty years ago by the late Dr. Leroy U. Gardner, director of the laboratory, was nearly completed at the time of his death in October 1946. Although partial reports and informal reviews of some of the experiments had been given from time to time by Dr. Gardner, this paper presents for the first time a complete survey of the entire experimental investigation.

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in one or more species of animal characteristic tissue changes which are similar to the lesions of human asbestosis (fig. 1). Since the life span of the experimental animal is relatively short, it is not possible to produce the characteristic lesions in animals under conditions identical with the usual industrial environment. Consequently, to obtain a complete evaluation of the tissue response to inhaled particulate and fibrous material, it is necessary to accelerate the reaction by employing higher concentrations of dust than would ordinarily be encountered in industry. While conditions of exposure are thus different, the information yielded by animal experiments is invaluable in furnishing a better understanding of the reaction of the human organism to inhaled asbestos dust.

Experimental Methods

For investigating the tissue reactions of experimental animals to the various asbestos minerals, two types of technic have been employed, namely, the inhalation method and the injection method. In inhalation experiments, groups of animals—up to 100 or more guinea pigs and sometimes smaller numbers of rabbits, cats, dogs, rats or mice—are kept for eight hours a day in a cubical dust room, 8 ft. (2.5 M.) in dimension, in which a cloud of asbestos dust is maintained by a rotating paddle in a dust hopper.[1a] At intervals during the experiment a few animals are killed and the tissues examined to determine the nature and the extent of the dust reaction. Some animals are exposed for periods up to three years. The injection experiments are used to determine in as short a time as possible whether or not a particular dust has a potential capacity to produce inflammatory reaction when in direct contact with tissues of the body. The method involves injecting the dust, either dry or suspended in fluid, into the animal by the intravenous, the intraperitoneal, the intratracheal or another route.

Long term inhalation experiments furnish information on which great reliance is placed when estimating the degree to which a dust might constitute a respiratory hazard to industrial workers. Even though an atmospheric dust may be potentially dangerous, as indicated by injection experiments, only inhalation procedures will reveal whether the dust can be inhaled, pass the natural defense barriers of the body and reach the pulmonary tissue in quantities sufficient to cause damage. Injection methods are useful, however, because they make certain that contact occurs between the dust particles and tissues and because they allow accurate estimation of the dosage and of the potential capacity of that dose to produce reaction. The intratracheal method is particularly valuable when one is dealing with fibrous minerals like asbestos, since it permits observation of the effect of the fibers on pulmonary tissue.

Tissue Susceptibility

Unlike free silica, asbestos does not produce specific effects in all organs of all species of animals. The comparative data presented in table 1 are based on completed observations and therefore differ slightly from a preliminary report.[1b] Fine quartz introduced into various organs

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own essential lining is a low cuboidal type of epithelium but, as their name implies, they actually function in respiration through lateral alveoli distributed along their walls. Either these alveoli or the abrupt change in the character of the lining epithelium, or the small diameter of the respiratory bronchiole, or the combination of all three factors is responsible for retention of the fiber at this site. Only after asbestosis is well established are appreciable numbers of fibers seen in the more peripheral air spaces. Further explanation is required to clarify this observation.

Rate of Tissue Reaction to Asbestos Fibers

The affected tissues react much more rapidly to asbestos than to quartz dust. For example, in rats receiving asbestos fibers by intratracheal injection fibrosis of a characteristic type is visible as early as one month after injection; for quartz dust the latent period is two months or more. Thus, the development of nodular fibrosis due to inhaled silica lags behind the deposition of dust to a greater extent than does the evolution of the diffuse reaction to asbestos. This results in a difference in the degree of progression which follows termination of exposure to dust. For example, on discontinuance of exposure the nodules of silicosis become larger, to a limited extent, for a considerable period of time, whereas the fibrosis of asbestosis increases for only a short time. Subsequently, the asbestotic fibrous tissue contracts; this process often distorts the adjacent pulmonary tissue and may, as a result, progressively interfere with cardiorespiratory function.

Asbestosis Bodies

The peculiar structure known as the asbestosis body or "curious body" is a specific concomitant of asbestosis.[5] The typical body is a golden yellow, beaded or haustrated rod, which may be either straight or curved (fig. 2). Often one or both ends are bulbous like a dumbbell. The bodies vary considerably in length, and dimensions up to 250 microns have been recorded.

It is believed that asbestosis bodies are inhaled fibers on which protein and iron pigment of tissue origin have been deposited.[6] Gloyne [5b] observed reproduction of these bodies in guinea pigs nine months after subcutaneous injection of fibers rendered free of iron. The bodies are abundant in man and in the guinea pig (table 1) but are much larger in the former, probably because the larger-sized air passages admit fibers of greater dimension. In guinea pigs they form after about 70 days

5. Gloyne, S. R.: (*a*) The Formation of the Asbestosis Body in the Lung, Tubercle **12**:398, 1931; (*b*) The Asbestosis Body, Lancet **1**:1351, 1932. (*c*) Gardner and Cummings.[1a]

6. Lynch, K. M., and Smith, W. A.: Asbestosis Bodies in Sputum and Lung, J. A. M. A. **95**:659 (Aug. 30) 1930. Simson, F. W., and Strachan, A. S.: Asbestosis Bodies in the Sputum: A Study of Specimens from 50 Workers in an Asbestos Mill, J. Path. & Bact. **34**:1, 1931. Gardner and Cummings.[1a] Gardner.[1b] Gloyne.[5a, b]

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INHALATION EXPERIMENTS

Four large scale inhalation experiments have been conducted in this laboratory with various forms of asbestos dust. In each of these investigations, more than 160 animals were used, and the experiments were carried on for periods ranging from two to more than five years. The four kinds of asbestos dust employed are designated as King's floats, short fiber, 100 per cent ball-milled, and long fiber asbestos dust.

King's Floats Asbestos Dust

The first inhalation experiment conducted at the Saranac Laboratory with asbestos dust was begun in 1928. Animals inhaled the dust for

TABLE 2.—*Chemical Analysis of Asbestos Dusting Materials*

Type of Asbestos	SiO_2	Fe_2O_3	Al_2O_3	Cr_2O_3	MnO	CaO	MgO	Na_2O	K_2O	CO_2	Ignition Loss	Total
King's floats.....	39.32	8.84		*	*	0.67	35.56	*	*	*	12.74	97.13
Short fiber.....	37.17	9.09	1.40	0.14	0.09	0.85	35.96	0.14	0.20	0.98	14.09	100.11
Long fiber.....	38.40	5.32	0.78	*	0.08	0.31	40.18	0.06	0.06	0.57	14.00	99.76

* Not determined.

TABLE 3.—*Petrographic Analysis of Asbestos Dusting Materials*

King's floats *: The approximate composition, based on particles (except chrysotile) smaller than 10 microns and reported as percentages obtained from particle counts, was chrysotile 14, serpentine 40, magnetite 12, carbonates 18, talc 12, other minerals 4. For chrysotile, fibers up to 200 microns long were included.

Short fiber †: The material, before being ball milled, contained a preponderance of fibrous chrysotile and platy (nonfibrous) serpentine. The approximate composition, by percentage, was chrysotile 17, serpentine 55, magnetite 10, quartz 2, brucite 5, other minerals, including dolomite, actinolite and tremolite, 11.

Long fiber †: The material consisted principally of the fibrous asbestos mineral chrysotile. Shreds of nonseparated fibers 5 to 15 microns in diameter and up to 50 microns in length were present. The approximate composition, by percentage, was chrysotile 75, serpentine 15, magnetite 5, brucite 2, other minerals, among which were calcite and chloritic and micaceous minerals, 3. Only a trace of quartz was observed.

* The analysis of the King's floats asbestos, made by Dr. C. S. Hurlbut Jr., of Harvard University, has been reported elsewhere (Hurlbut, C. S., Jr., and Williams, C. R.: The Mineralogy of Asbestos Dust, J. Indust. Hyg. & Toxicol. 17: 292, 1935).

† For the short fiber asbestos and the long fiber asbestos the petrographic analysis was supplemented with x-ray diffraction examination.

periods up to 33 months. Some guinea pigs with six and nine months' exposure lived for an additional three years after cessation of their exposure. A preliminary report [1a] presented observations after 29 months of exposure. At that time observations covered a period of only 2¼ years and the conclusions as to the ultimate effects of inhaled asbestos dust were provisional. Those conclusions are substantiated by results of the completed study, which is reported as follows.

Composition and Atmospheric Concentration of the Dust.—The dusting material, a commercial variety of asbestos known as King's floats, was composed of short fibers, ranging in length from 1 mm. to 1 micron or less, and of particles which also varied in size. It was obtained from the Thetford, Quebec, plant of the Asbestos Corporation of America, and analyses (tables 2 and 3) reveal that the amount of fibrous chrysotile was only 14 per cent, a rather low value.

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were phagocytosed, and many of them were carried into the wall by migratory cells. Mononuclear leukocytes attracted to the area caused an appreciable thickening of the bronchiolar wall. After 16 months a delicate fibrosis made its appearance. The process evolved gradually, and the number of fine intercellular collagenous fibers steadily increased. As this fibrous deposit contracted, it partially closed and



Fig. 3.—King's floats inhalation experiment: *A*, lung of a guinea pig with 12 months' exposure. It includes a respiratory bronchiole, at the left, branching and becoming an alveolar duct, at the right. Note the accumulation of cells in the wall of the bronchiole and in adjacent alveoli (× 130). *B*, lung of a guinea pig with 28 months' exposure. The field includes a bronchiole, at the center, with peribronchial fibrosis extending into the walls of adjacent alveoli. Note the cuboidal epithelium lining these alveoli. This is the so-called "adenomatoid" appearance (× 200).

distorted the alveoli, and with this change the alveoli became lined with cuboidal cells. The result was an adenoma-like appearance which frequently accompanies

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Asbestosis bodies (fig. 2 *B*), first seen in the lungs of the guinea pigs that had inhaled dust for about two months, became more numerous and more distinctly segmented with increasing exposure.

The reaction produced in guinea pigs exposed for six and nine months did not progress significantly during a subsequent period of 35 and 37 months when the animals lived in a normal atmosphere (fig. 4). Between eight and 11 months after exposure ceased, the cellular reaction in the lung had been completely replaced by thin strands of fibrous tissue. At later periods the scar tissue was less in amount, but in the last animal killed, 37 months after discontinuing dust exposure, some fibrosis was still visible.

Reaction in Guinea Pigs Infected with Tubercle Bacilli at the Onset of Dust Inhalation.—Of the group of 40 guinea pigs infected with attenuated tubercle bacilli, R_1 strain,[9] at the time that dust exposure was begun, 31 died or were killed before the completion of two years of the exposure and were reported in the paper by Gardner and Cummings.[1a] Seventeen of these died from intercurrent pneumonia. Briefly, the results were as follows: Ten revealed some evidence of spread of the tuberculous process (fig. 5 *A*); in 6 of these it was confined to the lungs, and in the other 4 the abdominal viscera also were involved. Extension of the infection was first seen after seven months of dust inhalation; during the next 20 months more than half of the animals showed actively spreading tuberculosis, and in 3 of them small cavities had developed. During the last eight months no animals exhibited any evidence of active infection although in half of them the healed fibrous scars of previous spreads were obvious. The scars were more extensive than is characteristic of either tuberculosis or asbestosis alone.

The nine animals which were still alive after two years of dust exposure were killed at intervals during the following year. In four of them the primary foci of infection were healed with fibrosis and even calcification, and there was no evidence of progression (fig. 5 *B*). In the remaining five the tuberculous foci showed evidence of having previously spread locally; in four of them, by the time of autopsy, the foci were healed, with excessive fibrosis; in the fifth animal there was a generalized chronic tuberculous pneumonia in one lobe, and in the other lobes there were isolated primary tubercles, which were still active but had not spread.

Reaction in Guinea Pigs Infected with Tubercle Bacilli After Establishment of Asbestosis.—Twelve guinea pigs, after inhaling King's floats asbestos dust for 26 months, were infected with tubercle bacilli and then removed to normal air. Six of these animals died within seven weeks, five from intercurrent nontuberculous infection. The remaining six animals were killed at intervals up to 14 months after infection. The subpleural tubercles were no more numerous in the dusted animals than in the nondusted controls, but a considerable number were found in the depths of the lung about foci of asbestosis. The tuberculous component of the combined reaction showed only slight local extension about lesions in the lungs and tracheobronchial lymph nodes. Caseation was found in tubercles 1½ months old, but by 5½ months it had completely disappeared, leaving only scar tissue. Foci of fibrosis still persisted in the last animal, which was killed 14 months after infection.

Reaction in Rabbits.—Rabbits exposed to the asbestos dust for periods up to 19 months showed a foreign body type of reaction of low grade, but no fibrosis. Although their lungs contained particulate elements of the dust, fibers were not present, indicating that the upper respiratory mechanism of the rabbit is adequate to exclude fibrous foreign bodies. Two rabbits, after inhaling dust for six and 19

9. Steenken, W., Jr., and Gardner, L. U.: R_1 Strain of Tubercle Bacillus: Its Dissociation and Virulence of Variants in Normal and Silicotic Guinea Pigs, Am. Rev. Tuberc. **54**:51, 1946.

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their lungs. In a few of the rats, an occasional asbestosis body was discovered, but there was no fibrosis. This phase of the experiment was considered unsuccessful.

Summary and Interpretation of Inhalation Experiment with King's Floats Dust.—The findings in the experiment with King's floats dust can be summarized under two headings:

1. Effect of the inhaled dust on normal animals. The King's floats dust caused a characteristic peribronchiolar fibrosis in guinea pigs but not in rabbits or rats. The fibrosis did not increase significantly in extent after the dust exposure was discontinued.

2. Effect of the inhaled dust on tuberculosis in guinea pigs. In guinea pigs infected with attenuated tubercle bacilli and then placed in the dust room, the results were more variable than is usual in an experiment of this type. A few animals showed no sign of progression of the infection; in most of them there was evidence of temporary progression with subsequent healing; in one animal the tuberculous process remained active to death. In contrast, when guinea pigs after being infected are exposed to quartz dust instead of asbestos dust, the infectious process continues to progress and eventually causes the death of the animals. On the other hand, infected animals exposed to a harmless dust like iron oxide do not show any progression of the infection.[10] Guinea pigs infected with attenuated tubercle bacilli after the termination of two years' asbestos dust exposure did not show progressive disease. The only modification of the infection was in its localization, a few bacilli being retained in the peribronchiolar fibrous tissue, with tubercles forming there in addition to the usual tubercles beneath the pleura.

In view of the variability of the results, the unusual nature of the response and the high proportion of deaths due to intercurrent pneumonia, it is felt that only tentative conclusions as to the influence of asbestos dust on the course of tuberculous infection are justified by this experiment.

Short Fiber Asbestos Dust

Since hazardous dusts like quartz are most effective in producing fibrosis when the particles are 3 microns and less in size, an inhalation experiment was performed to determine whether this condition is true for asbestos dust. It was thought that a short fiber asbestos dust consisting almost entirely of fibers and particles smaller than 3 microns would initiate an accelerated tissue response and produce an advanced reaction in a shorter time than did the King's floats dust, which contained fibers from 1 mm. to 1 micron and less in length as well as much particulate matter.

Composition and Atmospheric Concentration of the Dust.—The dusting material for this experiment was the remains of fibers collected in dust bins of an asbestos-fabricating plant after a carding operation and screened to pass 200 mesh. Since

10. Vorwald, A. J.; Pratt, P. C.; Durkan, T. M.; Delahant, A. B., and Bailey, D. A.: Siderosis: A Benign Pneumoconiosis Due to the Inhalation of Iron Dust, Indust. Med. & Surg. **19**:170, 1950.

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Only after exposures had continued for approximately one year was there an appreciable tendency for dust-containing phagocytes to gather into clumps. By 16 months phagocytes had collected about the walls of a few of the respiratory bronchioles which revealed a little proliferation or infiltration of mononuclear cells. There were also some multinucleated cells, but they were of the inert, foreign body type. At 20 to 24 months the cellular clumps were sometimes quite prominent, and sometimes changes in the epithelium resulted in the adenoma-like or "adenomatoid" appearance (fig. 3 *B*) previously described in the section reviewing the experiment with the King's floats dust. In most of the subsequent members of the series the reaction remained cellular, but a few exhibited pronounced development of fibrous tissue. In these few members of the series the collagen was pale in color and tenuous, with no appearance of being hyalinized. Diffuse chronic pleurisy was present in a few animals without evidence of pulmonary infection. This suggests that pleurisy may be a specific concomitant of asbestosis, but the evidence is not adequate to establish this point. The reaction of the tracheobronchial lymph nodes was more pronounced than in the previous experiment with King's floats asbestos, probably because more fine particles had been transported to the nodes in animals inhaling short fiber asbestos. The nodal reaction was essentially an increase in reticulum, rather than a fibrosis, with the original cells being preserved between the thickened reticular fibers.

TABLE 6.—*Analyses of Lungs of Guinea Pigs After Prolonged Inhalation of Short Fiber Asbestos Dust*

Exposure to Dust, Mo.	Period in Normal Air, Mo.	Amount of Ash, % of Dried Lung	Total SiO_2, % of Dried Lung	Total SiO_2, % of Ash	Tissue Reaction *
Dust Exposure Continuous During Life					
12	0	5.02	0.51	10.23	
		4.58	0.46	10.08	
		5.16	0.54	10.54	±
15	0	5.00	0.49	9.96	
		4.76	0.43	9.00	
		4.95	0.53	10.60	±
20	0	5.86	0.85	14.46	
		6.43	0.90	14.07	2+
24	0	5.42	0.78	14.48	
		5.50	0.78	14.20	3+
30	0	5.35	0.96	17.59	
		6.55	1.27	19.46	4+
34	0	6.06	0.75	12.37	
		6.35	0.96	15.11	4+
Dust Exposure Followed by Prolonged Residence in Normal Air					
20	4	5.16	0.48	9.30	
		5.11	0.36	7.16	2+
20	10	6.11	0.62	10.21	
		3.98	0.34	8.51	3+
20	14	4.77	0.25	5.31	
		5.18	0.26	5.00	
		4.77	0.22	4.60	2+

* The symbols averaging the tissue reaction in each group of guinea pigs represent merely the relative degree of reaction, ranging from ± (questionable) to 4+ (the maximum for this experiment). The relationships apply only within this table and cannot be compared with symbols in other tables.

In the group removed to normal air after 20 months' inhalation of dust, progression of disease was not definitely demonstrated, but neither could it be absolutely disproved, owing to the variability of the response in different animals. The reactions, from mild to severe, occurred sporadically and bore no relationship to the length of time after cessation of exposure. The differences were attributed to variation in individual susceptibility. This view received support from the chemical analyses (table 6), which revealed comparable amounts of ash and silica in lungs with widely different amounts of tissue change. For example, the ash

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alveolar ducts in which the walls of the associated air spaces were very thick, owing to swollen collagen framework. Connective tissue and Foot-Bielschowsky silver preparations revealed complete loss of capillary bed locally. Outside the collagen was a thin layer of epithelial cells. This did not resemble the "adenomatoid" change characteristic of guinea pig asbestosis. Near the lesions the air spaces were filled with phagocytes containing gray to yellow particulate dust and a rare, long, naked asbestos fiber. Careful search failed to reveal even a suggestion of an asbestosis body. Pleurisy was absent. The tracheobronchial nodes showed compact focal collections of monocytic cells at 12 months and, at 20 months, some diffuse thickening of the reticulum. In a few rats there was definite fibrosis along the margins of the node, extending into the mediastinal areolar tissue.

Results of chemical analyses made on the white rats are given in table 7, and the average values have been recorded in table 8 for comparison with similar values for rats inhaling other dusts. It will be noted that the values for asbestos are lower than those for quartz or chert but approximate those for the gypsum-quartz mixture, in which atmospheric agglutination tended to reduce the amount of dust inhaled. This condition prevailed even though the atmospheric concentration of asbestos dust was essentially the same as that of the quartz, was one-half that of the gypsum-quartz mixture and was one-fifth that of the ferruginous chert. Since the values for asbestos are low, it might be inferred that the total quantity of that dust actually inhaled was small or that it had been eliminated from or dissolved within the lungs. Evaluation of these possibilities is not feasible on the basis of the observations derived from this study.

Reaction in Cats.—Twenty cats were used in this inhalation experiment with the short fiber asbestos. Eighteen were kept in the dust room continuously until put to death, the exposure period ranging from one month to nearly 54 months. The other two were removed to normal air after a dust exposure of 31 months; one of these was killed five months, and the other 24 months, later. In general, the tissue response was confined to microscopic foci of fibrosis, which were in the walls of groups of subpleural alveoli rather than in the peribronchiolar areas. In one animal the change was extensive enough to be visualized on gross inspection of the section. Only in the animal with the longest exposure—54 months—did the roentgenogram reveal definitely abnormal shadows. A roentgenogram made after 30 months revealed no abnormality; after 45 months, a faint mottling could be detected throughout both lungs. At autopsy, nine months later, there was only microscopic fibrosis in the subpleural zone plus heavy lymphocytic infiltration about small bronchioles. Asbestosis bodies were rare. On prolonged search a few yellow atypical bodies, smooth and without haustrations, were found in two animals exposed for more than a year.

Reaction in Rabbits.—Eight rabbits were exposed to dust for periods extending from one to more than five years; the last animal was removed from the dust room and left in normal air six months before being killed. There was never enough pulmonary fibrosis to be detected grossly, and there was no chronic adhesive pleurisy. Microscopic evidence of alveolar wall thickening was first detected in one animal after about three years of exposure and was seen in all five animals examined thereafter, including the one removed to normal air. One animal that died of paralysis after nearly four years of exposure exhibited a reaction visible on gross inspection of tissue sections. The possibility of pulmonary infection in this animal could not be excluded. In another animal dying two years later the focal fibrosis was not nearly as obvious or as advanced. Areas of involvement, which were largely visualized because of phagocytic reaction within the air spaces, tended microscopically to become more fibrous with the passage of time, but there was never much encroachment on the lumen of air spaces and the structure of the lung was preserved. Asbestosis bodies were not detected in rabbits that died early in the experiment but were seen in all animals that had been exposed to the dust for more than three years.

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installed, the dust counts were higher, and the over-all average for the remaining 21 months was about 150 million.

Size-frequency studies of atmospheric dust collected inside the animal cages revealed that nearly 99 per cent of the components suspended in the air could be classified as clumps or particles; only about 1 to 1.5 per cent was fibers. One third to one half of the fibers were longer than 10 microns, indicating a concentration of long fibers of about 0.8 million. This figure is about one-half the estimated value of 1.4 million for the short fiber experiment.

Guinea pigs, rats and mice were used in the inhalation experiment with the 100 per cent ball-milled asbestos dust. The results are summarized in table 9.

Reaction in Guinea Pigs.—The experiment was started with 100 guinea pigs. As the dust exposure proceeded, there were 39 accidental deaths, 32 of these being due to pneumonia in an epidemic. The 61 pigs remaining exposed to the dust were killed at intervals during exposure, except for 16 guinea pigs transferred to normal air after 28 months of dusting. For the first year of exposure practically the only reaction to the dust was the presence of scattered phagocytes and an occasional minute asbestosis body. At 16 and 20 months no gross response was visible on the tissue section, but microscopically peribronchiolar foci of inflammatory cells

TABLE 9.—*Summary of Inhalation Experiment with 100 per Cent Ball-Milled Asbestos Dust*

Nature of Experiment	Animals	Maximum Dust Exposure, Mo.	Maximum Survival After Dust Exposure, Mo.	Results
Dust exposure continuous throughout life	84 guinea pigs	24	0	No appreciable pulmonary reaction
	40 rats	20	0	No suggestion of asbestosis
	24 mice	12	0	No suggestion of asbestosis
Dust exposure followed by prolonged residence in normal air	16 guinea pigs	28	12	Fibrosis typical of asbestosis was present 12 mo. after exposure ceased in an amount sufficient to be visible grossly; smaller foci could be seen microscopically at 2 mo. and 8 mo. after termination of exposure

could be seen. At 24 months (fig. 6 *A*) there was still no change large enough to be seen with a hand lens, although microscopic examination revealed cellular accumulations about terminal bronchioles and many more asbestosis bodies, chiefly within cells. The lungs of animals exposed for the full dusting period of 28 months and afterward living in normal air for two months revealed the changes described above and also very slight peribronchiolar fibrosis. For exposed animals living eight months in normal air the findings were similar, but at 12 months three of four animals showed grossly visible characteristic peribronchiolar fibrosis with adenomatoid change (fig. 6 *B*).

The tracheobronchial nodes were essentially normal until exposure had been continued for more than a year and a half. Animals killed at 12 months and at 16 months revealed a few minute collections of phagocytes containing particles but practically no fibers large enough to be recognized as such. After 20 months of exposure many monocytes filled with yellow granules were present. At 30 months there had been a slight increase in reticulum but no fibrosis. No further changes occurred in the nodes. Asbestosis bodies were not seen in the nodes of any of the guinea pigs.

Minute asbestosis bodies were observed in the lungs as early as three months after exposure began, but they did not become numerous until 16 months had elapsed. The bodies were short and practically all were intracellular, although at 20 months some were long enough to project beyond the cell borders. It is

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In view of the high values for silica obtained with the animals exposed to 100 per cent ball-milled dust, it is important to note that their pulmonary response was much less than that of animals exposed for 24 months to the short fiber asbestos in the previous experiment. This again indicates that the biologic activity of asbestos inhaled into the lung is not increased by a reduction in size of the fibers.

Reaction in White Rats and Mice.—In this experiment 40 rats were exposed for periods up to 20 months and 24 mice for periods up to 12 months. In neither species did even a suggestion of asbestosis develop, and reaction was limited to phagocytosis of inhaled particles by widely scattered dust cells which remained free in air spaces or were transported to the tracheobronchial lymph nodes. No asbestosis bodies were found in the rats, but in the mice there were a very few small, nonhaustrated forms within phagocytes.

TABLE 10.—*Analyses of Lungs of Guinea Pigs Exposed to Dust in Inhalation Experiment with 100 per Cent Ball-Milled Asbestos Dust*

Exposure to Dust, Mo.	Period in Normal Air, Mo.	Amt. of Ash, % of Dried Lung	Total SiO_2, % of Dried Lung	Total SiO_2, % of Ash	Tissue Reaction *
Dust Exposure Continuous During Life					
1	0	4.85	0.21	4.28	
		4.30	0.30	7.05	
		4.35	0.24	5.60	0
2	0	4.66	0.23	4.90	
		4.60	0.34	7.40	
		5.05	0.61	12.01	0
3	0	5.60	0.70	12.47	
		5.07	0.32	6.38	
		5.74	0.56	9.77	0
5	0	5.10	0.38	7.51	
		5.08	0.38	7.47	
		5.02	0.39	7.72	0
8	0	4.35	0.52	11.86	0
12	0	5.65	1.25	22.16	
		6.24	1.45	23.28	0
16	0	5.40	1.02	18.96	
		5.01	1.11	21.95	±
20	0	5.65	1.28	22.60	
		5.20	1.28	24.61	±
24	0	6.30	1.85	29.05	
		5.56	1.21	21.70	±
Dust Exposure Followed by Prolonged Residence in Normal Air					
28	2	7.25	1.57	21.63	
		8.67	2.19	25.24	+
28	8	5.25	0.68	12.99	
		5.96	0.87	14.55	+
28	12	6.38	0.84	13.08	
		5.17	0.64	12.41	2+

* The symbols averaging the tissue reaction in each group represent merely the relative degree of reaction, ranging from 0 to ± (questionable) to 2+ (the maximum observed in this experiment). The relationships apply only within this table and cannot be compared with symbols in other tables.

Summary and Interpretation of Inhalation Experiment with 100 per Cent Ball-Milled Asbestos Dust.—The tissue reaction observed in this experiment was not as intense as that in the previous investigation with short fiber asbestos. The reaction was slower in development and less extensive even though more dust accumulated in the lungs. Since there were fewer fibers longer than 3 microns in the material used in this experiment, the results tend to confirm the interpretation made in the summary of the previous short fiber experiment that the reaction is not primarily chemical in nature, and to support the impression that reduction in size of asbestos fibers does not increase the biologic activity of asbestos inhaled into the lung.

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Reaction in Guinea Pigs.—The experiment was started with 100 guinea pigs. After exposure had been carried on for a year, a severe epidemic of pneumonia arose in the dust room and about one third of the animals died or were killed. To replace them, 38 more guinea pigs were added to the surviving group. Histological examination revealed lesions in the lungs after eight months of dust exposure, consisting of cellular connective tissue about the terminal bronchioles (fig. 7 *A*). At 12 months there were adenomatoid changes in the adjacent parenchymal areas, and by the sixteenth month (fig. 7 *B*) definite fibrosis was present in these areas as well as around the bronchioles. The fibrous lesion could be seen macroscopically at 20 months. From this time on the reaction increased in extent and in the amount of collagen, and by the thirty-fourth month, it had fanned out

TABLE 11.—*Size-Frequency of Atmospheric Long Fiber and 100 per Cent Ball-Milled Asbestos Dust Collected Inside Cages*

	Grains, %			Fibers, %			
Type of Asbestos	< 3 Microns	3-10 Microns	> 10 Microns	< 10 Microns	> 10 Microns	Clumps, %	Total
Long fiber.....	65.4	1.1	0.0	25.8	6.7	1.0	100
Ball-milled	90.6	4.8	0.0	0.8	0.6	3.2	100

TABLE 12.—*Summary of Inhalation Experiment with Long Fiber Asbestos Dust*

Nature of Experiment	Animals	Maximum Dust Exposure, Mo.	Maximum Survival After Dust Exposure, Mo.	Results
Dust exposure continuous throughout life	117 guinea pigs	36	0	Definite fibrosis in 16 mo.
	4 cats	42	0	Slowly developing fibrosis first seen at 24 mo.
	20 rats	25	0	Marked peribronchiolar fibrosis first seen at 24 mo.
	20 mice	25	0	Limited reaction; no fibrosis
Dust exposure followed by prolonged residence in normal air	12 guinea pigs	20	14	Clearing of inflammatory reaction and definite contraction of fibrous tissue
	9 guinea pigs	27	9	Clearing of inflammatory reaction and slight contraction of fibrous tissue
	2 cats	18	24	Similar to continuous exposure group; suggestion of progression in one of the two animals

considerably into the parenchyma (fig. 8 *A*). The lesions were rather sharply localized and the extensions from different bronchioles showed no tendency to fuse, even in animals exposed for the maximum period of three years. Although the intrapulmonary reaction sometimes reached the pleura, there was no involvement of that membrane. Emphysema was not detected at any point. Some thickening of the larger bronchi with a chronic inflammatory infiltration was revealed, but it was considered no more than would be produced by a similar period of inhalation of any dust.

In guinea pigs exposed to the dust for 20 months and then removed to normal air, there was a marked tendency for cellular inflammatory reaction to clear. This effect, accompanied by contraction of the fibrous tissue, resulted in a diminishing size of the focal lesions. None of these animals, killed at various periods up to 14 months after exposure, revealed lesions as large as those in the group killed at the end of the 20 month exposure period or those in animals which remained in the dust room for more than 20 months. Fourteen months after dust exposure ceased, the foci in four of the six remaining guinea pigs were so small that they were visible only with a hand lens (fig. 8 *B*).

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began to appear in the medulla, and by the fourteenth month most of the node had been replaced by cellular connective tissue. This picture, which resembled that in early silicosis, persisted to the end of the experiment. Some animals showed, as a variant, heavy sheets of diffusely distributed monocytes and large active giant cells, but there was never any necrosis or hyaline formation. The spindle-shaped

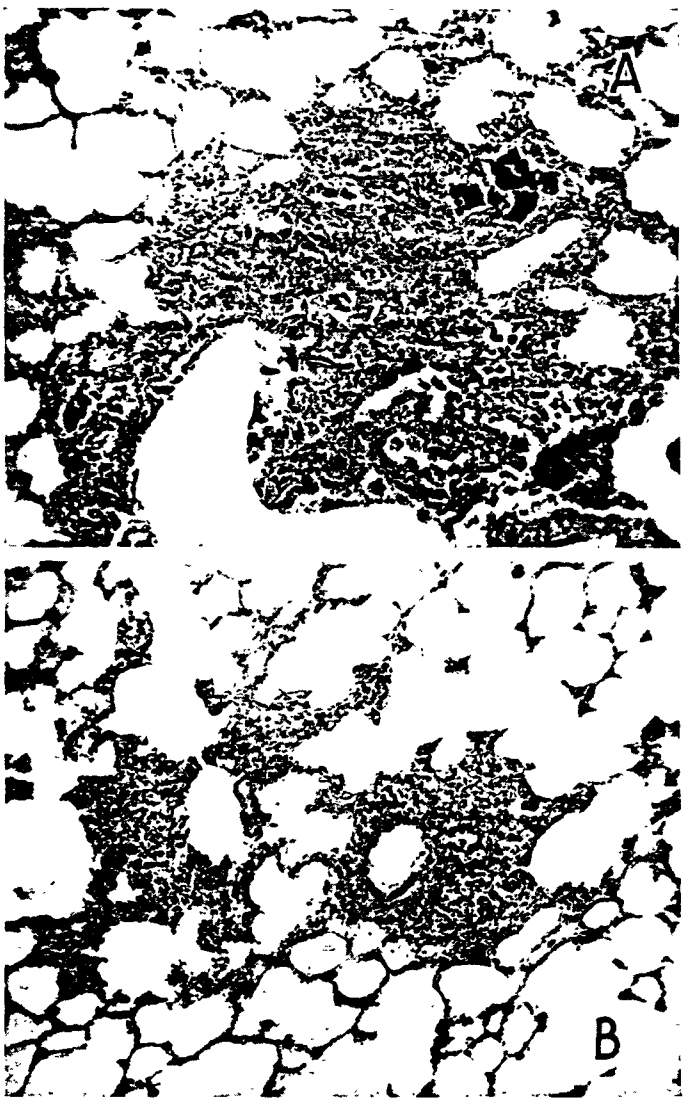


Fig. 8.—Long fiber asbestos inhalation experiment: *A*, lung of a guinea pig with 34 months' dust exposure. A bronchiole is seen at the lower center; the large area above it represents the involvement of alveolar walls. Compare with figure 7 *B* and note the increased extent of reaction (× 200).

B, lung of a guinea pig with 20 months' dust exposure and then 14 months' living in normal air. The reaction is essentially like that shown in figure 7 *B*: The bronchiole at the right center is surrounded by fibrous tissue with adenomatoid change at the right. There is residual scarring in the walls of adjacent alveoli at the left. It is apparent that no progression has occurred (× 200).

new cells were yellowish from fine pigment granules that stained for iron. No fibers or asbestosis bodies were seen.

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pigs of this experiment than in those exposed to either short fiber or ball-milled asbestos, the amount of mineral matter in the lung ash was much less.

Reaction in Cats.—Four cats inhaled the long fiber asbestos dust for periods of 14, 25, 33 and 42 months, respectively, and were immediately killed. Two other cats, after being exposed to dust for 18 months, lived in a normal atmosphere for an additional 24 months. Fourteen months' exposure was sufficient to produce cellular accumulations of phagocytes around terminal bronchioles and peripheral arterioles together with compact collections of similar cells in the tracheobronchial lymph nodes. At that time there were no typical asbestosis bodies, but smooth, pointed, yellow fibers were seen very rarely. With continued exposure, up to 42 months, reaction in the locations noted progressed to the formation of cellular connective tissue which made well defined sheaths about the respiratory bronchioles and arterioles, marked lymphoid hyperplasia and lymphoid infiltration of bronchiolar walls (fig. 9). Typical asbestosis bodies were not formed, although there was

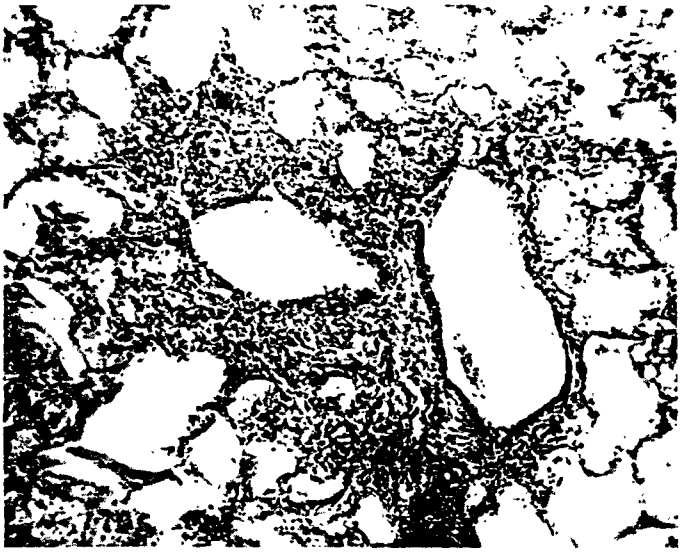


Fig. 9.—Long fiber asbestos inhalation experiment: Lung of a cat with 42 months' dust exposure. Two bronchioles are shown with adjacent cellular reaction and collagen deposition (× 200).

an occasional fiber, smooth, yellow and pointed. Pleurisy was not present. The reaction was similar in location to that in the guinea pigs, but fibrosis was much slower in development. Roentgenograms of cats made after exposure periods of 25, 33 and 42 months, respectively, failed to demonstrate evidence of pulmonary lesions.

Reaction in Rats.—Although 20 rats were placed in the dust room, many died from pneumonia and were not suitable for study. Five animals, of which one was exposed for 19 months and four for 25 months, were free from pulmonary infection and offered a basis for tentative conclusions. In the 19 month animal, the reaction was just beginning. All four animals killed at 25 months showed a well marked peribronchiolar fibrosis. After a long search, only two small, smooth asbestosis bodies were found in the 19 month animal and none was found in the 25 month animal. Thus these animals exhibited fibrosis without asbestosis bodies or fibrosis accompanied by only a very infrequent asbestosis body.

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TABLE 14.—*Comparison of Reactions to Chrysotile and Serpentine Injected Intratracheally*

Dosage: Each animal was given an intratracheal injection of 0.5 cc. of a 5 per cent suspension of the dust. Two weeks later another similar injection was given. Total amount of dust injected was 50 mg.

Animals used: Six groups of 9 guinea pigs each (one group for each type of dust).

Periods at which animals were killed: One or two animals in each group at 1, 2, 6, 8½ and 12 months after last injection.

Preparation of dust: Chrysotile (ball milled) unheated: Ball milled for 1,176 hr., dried and reground in agate mortar.
Chrysotile (ball milled) ignited: Ball milled chrysotile heated for 2 hr. at about 700 C., then ground in agate mortar 2 or 3 min.
Chrysotile (fibrous) unheated: Ground in agate mortar to pass 200 mesh.
Chrysotile (fibrous) ignited: 200-mesh material heated for 2 hr. at about 700 C. No further grinding.
Serpentine (ball milled) unheated: Ball milled for 1,488 hr., dried and reground in agate mortar.
Serpentine (ball milled) ignited: Ball milled serpentine heated for 2 hr. at about 700 C., then ground in agate mortar 2 or 3 min.

Mineral	Size of Dust Particles	Results
Chrysotile (ball milled) unheated	3 microns and less	Grinding destroyed capacity to cause fibrosis. At 1 mo. considerable inflammatory edema and cellular proliferation and localization of dust particles about bronchioles; at 2 mo., only a very slight proliferative reaction; at 6, 8½ and 12 mo., widely scattered small mononuclear phagocytes. At 12 mo., a few microscopic patches of thin alveolar wall thickening with some adenomatoid change in portion of air spaces abutting on thickened bronchi. No asbestosis bodies seen.
Chrysotile (ball milled) ignited	3 microns and less	Reaction limited to large foreign body giant cells without production of fibrous tissue.
Chysotile (fibrous) unheated	20-50 microns approx.	A distinct fibrosis. Reaction localized to connective tissue about terminal bronchioles; little within those tubes. Contraction caused adenomatoid appearance of air spaces given off directly from terminal bronchioles. Reaction area became smaller with progress of time: no new regions involved. No chronic pleurisy even at points abutting intrapulmonary change. At 1 mo. considerable inflammatory edema and foci of cellular proliferation; at 2 mo. well marked cellular proliferation and fibrosis occurring focally about respiratory bronchioles. This reaction developed before asbestosis bodies had formed and was as advanced as that produced by 2 yr. inhalation of asbestos dust. At 6 mo., reaction less extensive than at 2 mo., apparently due to contraction of fibrous tissue; asbestosis bodies were abundant. At 8½ mo., reaction still less extensive, confined to the immediate vicinity of the small terminal bronchioles, where the scar tissue was quite dense and was becoming hyaline in character. Sometimes it even obliterated the bronchiole. Asbestosis bodies had become scarce. At 12 mo., the well developed peribronchial and intrabronchial adenomatoid areas of fibrosis had produced considerable distortion. More peripherally were patches of pneumonitis with eosinophilic infiltration, some of which was being transformed into fibrous tissue. These seemed to be precursors of the localized, diffuse patches of thin alveolar wall fibrosis seen elsewhere.
Chrysotile (fibrous) ignited	20-50 microns approx.	Reaction limited to large foreign body giant cells without proliferation. Heating the fibers, which made them brittle, destroyed their capacity to produce significant reaction.
Serpentine (ball milled) unheated	3 microns and less	Dust relatively inactive. At 1 and 2 mo., simple phagocytosis without proliferation; at 6 mo., no change except possibly lymphoid cell infiltration; at 8½ mo., a slight chronic pneumonitis; at 12 mo., only a little pneumonitis without suggestion of fibrosis.
Serpentine (ball milled) ignited	3 microns and less	Dust relatively inactive. Reaction essentially the same as for unheated serpentine. With ignited serpentine, less tendency for dust to be carried to bronchial nodes.

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The tests were made with long fiber chrysotile, unheated, and with chrysotile that had been ignited to destroy its flexible structure or ball milled to reduce the length of fiber to 3 microns and less. At the same time control tests were made with serpentine, which has the same chemical composition as chrysotile but is nonfibrous. A review of the findings reveals that only the unheated, long fiber chrysotile produced typical peribronchiolar fibrosis and that ball-milled material containing only fibers less than 3 microns in length failed to cause fibrosis (figs. 10 and 11). Fibers subjected to ignition also had lost their capacity to cause serious tissue damage. Ignition produced important changes in the chrysotile fibers, among them being loss of water, an alteration from a flexible to a brittle structure and possibly other changes. Experimental studies concerning this observation will be reported in a separate publication.

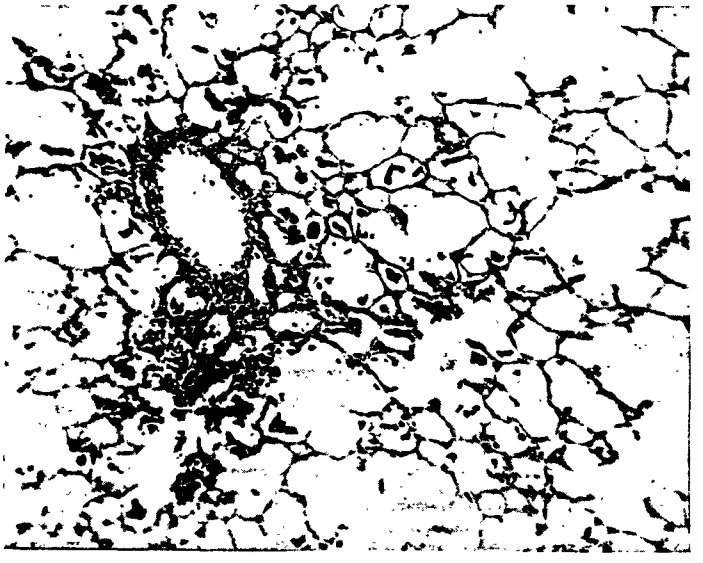


Fig. 11.—Serpentine injection experiment: Lung of a Guinea pig that had received an intratracheal injection of this dust four months before. A bronchiole is shown at the left center. The phagocytic cells exhibit little predilection for the bronchiole and collagen deposition is absent (× 200).

Comparison of Various Long Fiber Dusts.—Some very interesting findings are disclosed by the results of the experiments recorded in table 15. First, all the long fiber asbestos minerals tested, with the exception of anthophyllite, produced typical fibrosis. The characteristic peribronchiolar reaction caused by three representative long fiber asbestos minerals—chrysotile, amosite and crocidolite—is shown in figures 10 *A* and 12. Why anthophyllite behaved differently from the other asbestos minerals is not entirely clear.

Second, with the mineral brucite, which is not a silicate but is a fibrous form of magnesium hydroxide, a characteristic fibrosis like

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that produced by the asbestos minerals was obtained (fig. 13 *A*). Since the brucite used contained only 0.90 per cent silica as an impurity, it is obvious that a siliceous component is not an essential factor in the development of asbestosis.



Fig. 12.—Amosite and crocidolite injection experiments: *A*, lung of a guinea pig four months after an intratracheal injection of amosite. The inflammatory reaction exhibits pronounced accumulation of cells and collagen deposition (× 200). *B*, lung of a guinea pig four months after an intratracheal injection of crocidolite. As in *A*, peribronchiolar accumulation of cells and deposition of collagen are shown (× 200).

Third, no fibrosis resulted from the injection of glass wool fibers (fig. 13 *B*), even though glass wool resembles asbestos in many ways. However, there are fundamental differences. A glass wool fiber 3 microns

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fibrosis. Experimental studies concerning this observation will be reported in a separate publication.

TABLE 16.—*Comparison of Reactions Produced by Long Fiber and Short Fiber Dusts Injected Intratracheally*

Dosage: Two injections of 0.5 cc. of a 5 per cent suspension given two weeks apart. Total dose was 50 mg.

Animals used: Six groups of guinea pigs.

Periods at which animals were killed: 1, 2, 6, 8½ and 12 months after injection.

Mineral	Size of Dust Particles	Results
Chrysotile (Thetford)	Long fiber, 20-50 microns	A distinct fibrosis. Refer to chrysotile (fibrous) unheated in table 14.
	Short fiber, 3 microns and less	No fibrosis. Refer to chrysotile (ball milled) unheated in table 14.
Amosite	Long fiber, 20-50 microns	Typical fibrous endobronchiolitis and peribronchiolitis. Refer to table 15.
	Short fiber, 20 microns and less	Reaction limited to phagocytosis with lymphocytic infiltration of adjacent walls. Short fibers packed inside swollen phagocytes; longer ones free; some coated to form typical asbestosis bodies. At 1 mo. after injection, alveoli contained good-sized giant cells; most phagocytes were within air spaces and had not migrated to walls. At 4 mo., free extracellular fibers had worked themselves into interstitial tissue, where there was extensive proliferation of lymphoid cells and monocytes but no fibrosis. At 8 mo. foreign body reaction with some pneumonitis, no bronchiolitis. Typical asbestosis bodies present.
Crocidolite (Bolivia)	Long fiber, 20-50 microns	Advanced fibrous endobronchiolitis and peribronchiolitis. Refer to table 15.
	Short fiber, 20 microns and less	No fibrosis. At 1 mo., air spaces compressed and largely filled with giant cells packed with dust needles. Walls heavily infiltrated with monocytes and lymphoid cells. At 4 mo., a moderate degree of cellular infiltration of walls; small giant cells packed with dust spicules. At 6 and 8½ mo., masses of giant cells, containing mineral particles, in small bronchi but not in respiratory bronchioles; smaller ones widely scattered in terminal air spaces. Numerous asbestosis bodies. No reaction in connective tissue. No endobronchial proliferation. At 12 mo., many scattered small monocytes packed with dust. No endobronchitis. No peripheral fibrosis. In lymph node, slight reticulosis; no fibrosis.
Anthophyllite	Long fiber, 20-50 microns	Lymphocytic infiltration and giant cells but no definite fibrosis. Refer to table 15.
	Short fiber, 3 microns and less	No fibrosis and practically no asbestosis bodies. At 1 mo., focal collections of dust-filled monocytes and a few giant cells; at 4 mo., some adenomatoid epithelial reaction; at 8 mo., simple pneumonitis with phagocytosis of short fibers; at 12 mo., isolated and sharply localized collections of dust cells inside air spaces about terminal arterioles. Reaction in walls limited to lymphoid cell infiltration. No fibrosis. In lymph node, reaction limited to slight prominence of reticulum.
Tremolite	Long fiber, 20-50 microns	Fibrosis about bronchioles. Refer to table 15.
	Short fiber, 20 microns and less	Simple foreign body reaction. No acute inflammation. No accumulation of dust in or about terminal bronchioles. No endobronchitis. At 1 mo., scattered small giant cells and considerable infiltration of adjacent walls with monocytes and lymphoid cells. At 4 mo., little change except more cellular infiltration of connective tissue. At 8 mo., lymphoid infiltration and thickening of walls about some but not all terminal bronchioles.
Brucite	Long fiber, 20-50 microns	Typical fibrous endobronchiolitis and peribronchiolitis like reaction to asbestos minerals. Refer to table 15.
	Short fiber (made by crushing long fibers with rubber policeman)	Inert type of reaction. At 1 mo. after injection, small monocytes widely scattered through air spaces; focus of atelectasis with lymphoid infiltration of compressed air-space walls. No endobronchial reaction as with chrysotile. At 2 mo., reaction similar to that at 1 mo.; typical asbestosis bodies seen. At 12 mo., small clumps of inactive dust-filled phagocytes; no fibrosis. No reaction in lymph nodes.

Comparison of Long Fiber and Short Fiber Dusts.—With quartz dust it has been demonstrated that the smaller the particles the more

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Vorwald, Durkan and Pratt, "Experimental Studies of Asbestosis" (1951). Reprint with additions, 22 pages
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TABLE 17.—*Summary of Injection Experiments by Intravenous Technic*

Dosage: Total amount of dust was 1.0 Gm., divided into 20 equal doses (each dose was 5 cc. of a 1 per cent suspension) which were given twice a week for 10 weeks.

Mineral	Size of Dust Particles	Rabbits Used	Maximum Survival After Last Injection, Mo.	Results
Chrysotile (Thetford)	3 microns and less (ball milled 192 hr.)	6	..	The rabbits did not tolerate intravenous injections of finely ground chrysotile and 5 of the 6 died after 1 to 5 injections of even diluted suspensions; the other animal died after 27 injections of one-quarter strength suspension (69 days after first injection). Reaction limited to few large giant phagocytes of inactive type in liver, spleen and lungs. No thrombi of dust cells seen in pulmonary capillaries. No definite explanation for fatalities discovered, but material may have been retained in heart, causing local thrombi.
Amosite	3 microns and less (ground in agate mortar)	5	17	Advanced pulmonary infection killed 3 animals at 9, 11 and 17 mo. after last injection, shortening intended duration of experiment and complicating picture. However, rabbits killed earlier (3 and 6 mo.) showed only inert phagocytosis with no progression in the 6 mo. animal. The last two (11 and 17 mo.) were probably the same although focal necrosis of the liver and amyloid of the spleen made interpretation difficult.
Crocidolite	3 microns and less (ground in agate mortar)	4	12	Reaction was that to an inert substance with no change in 12 mo. (Other observations at 3, 4 and 6 mo.) Simple phagocytosis of particles. No tendency to agglomerate and no change in adjacent tissues. Grinding the dust to sizes of 3 microns and under destroyed the fibrous structure of this mineral, and the injected material resembled plates rather than fibers.
Anthophyllite	3 microns and less (ball milled 1,400 hr.)	4	24	Reaction essentially that of an inert mineral. Observations made at 3, 6, 12 and 24 mo. Only suggestion of irritating properties manifested in spleen and lymph nodes, but not liver, of the 24 mo. rabbit. In this animal there had been proliferation of mononuclear and giant cells that was not present in either spleen or lymph node of 12 mo. animal. The absence of associated fibroblastic reaction in these organs and of any change in the liver condition justifies the classification of anthophyllite as an inert silicate. No fibers were retained in lung to demonstrate whether asbestosis bodies would develop.
Tremolite (soda-iron)	3 microns and less (ball milled 140 hr.)	4	19	Reaction essentially that of an inert mineral. Last animal killed showed a little proliferation and lymphocytic infiltration in liver, not seen earlier (at 3, 6 and 12 mo.). No evidence of any activity in lesions in other organs.
Tremolite (soda)	3 microns and less (ball milled 48 hr.)	4	24	An inert foreign body reaction with no change in 24 mo. Observations made at 3, 6, 12 and 24 mo.

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Protective Action of Aluminum Compounds

When colloidal aluminum hydroxide had been added to a suspension of long fiber chrysotile prior to injecting this suspension intratracheally into rats, the aluminum compound did not prevent the irritation of tissue due to chrysotile. If anything, the acute inflammatory response evoked by the injected fibrous mineral was accelerated. One month after the last injection of the dust suspension the bronchiolitis was becoming fibrous. King and his associates also found that aluminum failed to protect pulmonary tissue from the irritation caused by asbestos fibers [11]; in their experiments metallic aluminum was used instead of the hydroxide.

Formation of Asbestosis Bodies

The iron in the coating of the asbestosis body appears to be derived from blood or tissue elements and not, as has been suggested, from the mineral fiber. After two kinds of chrysotile were injected subcutaneously into the groin of a guinea pig—one kind containing 2 per cent and the other 0.2 per cent ferric oxide—the asbestosis bodies were equally numerous at both sites of injection and showed no difference in their reaction to prussian blue, the reagent which stains iron. This finding is in agreement with that of Giroux.[12]

Tissue Reaction to Asbestosis Bodies

Asbestosis bodies recovered from human lung tissue and injected intratracheally into guinea pigs failed to produce a fibrous reaction. The material for injection was obtained by digesting with sodium hypochlorite solution the lung tissue removed at autopsy from an asbestos worker. The asbestosis bodies could be seen in the guinea pigs for at least a year after injection. This experiment shows that the asbestosis body has a rather resistant coating which is not destroyed by moderate hypochlorite treatment, which may be maintained in vivo for a year or longer and which renders the fiber incapable of producing fibrosis. It thus appears that the coating is a protective mechanism. This thought was expressed by Beintker as early as 1934.[13]

THEORY OF IRRITANT ACTION

Two hypotheses have been proposed to explain the tissue irritation and reaction caused by asbestos fibers: the chemical and the mechanical. In the chemical theory, which is based on experience with quartz, it is assumed that the asbestos minerals dissolve in the body fluids and that in this process their bases are leached away to leave silica in a form capable of irritating tissues. According to this hypothesis asbestosis is merely an indirect silicosis. Several facts make the chemical theory untenable: Intratracheal injection of brucite fibers, which had a silica

12. Giroux, M.: Amiantose expérimentale: valeur pathognomonique du "corps d'amiante," Laval méd. **8**:239, 1943.

13. Beintker, E.: Über die Asbestosiskörperchen: Bemerkungen zu der Arbeit von Beger, Virchows Arch. f. path. Anat. **293**:527, 1934.

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dust like quartz but above an inert dust such as iron oxide. In animals infected with attenuated tubercle bacilli, quartz causes the infectious process to progress until the animal dies of tuberculosis. Inert dusts have no effect on the infection, and the lesions usually heal and the disease disappears. Asbestos dust is in a different category. In the experimental investigation, when the fibrous dust was being inhaled during the evolution of the infection, there was spreading of the tuberculous process for a time, but usually the stimulus for continued proliferation of the tubercle bacilli was not sustained, the progression was arrested and healing followed. In guinea pigs infected with attenuated tubercle bacilli after being exposed to asbestos dust for slightly more than two years, progressive disease did not develop. The only modification of the infection was one of localization, a few bacilli being retained in the fibrous terminal bronchioles and forming tubercles there, in addition to the usual foci beneath the pleura. Such tubercles healed in a few months.

Susceptibility to Nontuberculous Infection

There was no specific experiment concerning the effect of inhaled asbestos dust on nontuberculous infection. Intercurrent pneumonia was rather common among animals exposed to asbestos dust, the frequency in guinea pigs exposed in the four inhalation experiments ranging from 16 to 39 per cent. This incidental evidence suggests the possibility of an effect of asbestos dust on nontuberculous infection. Nevertheless, since such epidemics are not uncommon in inhalation experiments with other dusts and even in the colony of normal animals, it is felt that the inhalation of asbestos dust does not exert a significant effect on the susceptibility to nontuberculous pulmonary infection.

Comment and Summary

Owing to the vast amount of data included in this investigation, it seems most convenient to summarize and to state as concisely as possible the various observations which emerged from the experiments and to follow each with a brief résumé of the evidence.

A. Various species of animals, including the guinea pig, the rat and the rabbit, but not the mouse and the dog, develop peribronchiolar fibrosis of the lung similar to human asbestosis after being exposed by inhalation or intratracheal injection to long chrysotile asbestos fibers.

Both inhalation and injection experiments provide ample support for this statement. Figure 8 *A* reveals the cellular fibrosis that occurs in guinea pigs following inhalation of long fiber asbestos; figure 9 shows the fibrosis caused in the cat by inhalation of long fiber asbestos dust. Similar but less extensive fibrosis occurred also in rats and rabbits (table 1). Mice and dogs failed to respond. This variation in response of different species to identical dust exposures is still to be accounted for.

B. Long asbestos fibers are essential in the production of the peribronchiolar fibrosis; short fibers are incapable of producing this reaction.

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lesions, due to contraction of the fibrous tissue. In contrast, an immature tissue response, evidenced primarily by cells with little or no fibrosis, continued to progress. It is assumed that, following attainment of fibrotic maturity, the same process of contraction would ensue as was noted for the mature lesion.

G. The formation of asbestosis bodies represents a coating of the fibers by blood and tissue elements, which results in loss of ability of the fiber to produce fibrosis.

Intratracheal injection of asbestosis bodies failed to produce the typical asbestotic tissue reaction in experimental animals. The cessation of progressive reaction observed soon after exposure terminates may be due to the formation of asbestosis bodies.

H. Aluminum hydroxide failed to neutralize the fibrosing action of the long fiber asbestos.

Aluminum hydroxide added to the suspension of chrysotile asbestos prior to intratracheal injection did not retard or prevent the development of asbestosis in rats.

I. Inhalation of asbestos dust did not alter significantly the final outcome of experimental tuberculosis in two series of guinea pigs exposed to the dust.

The apparently mild influence of asbestos dust is in distinct contrast to the stimulating effect exerted by inhaled quartz on a tuberculous process in the lung. The interpretation must remain tentative, however, since it is based on an investigation limited to two series of guinea pigs exposed to only one kind of asbestos, namely, King's floats: Table 4 shows that when the infection was coincidental with the onset of dust exposure, there was temporary progression of the infectious process, with subsequent healing; when infection was initiated after 26 months of dust exposure, the course of the tuberculosis was not appreciably altered. The latter finding is quite different from our usual experience with quartz dust or with mixed dusts containing quartz, wherein the adverse influence of quartz on a tuberculous infection is manifested most strikingly when infection is initiated after a period of dust exposure, viz., superimposed on a background of established silicosis. As indicated above, this more sensitive test, when applied to asbestos dust, failed to demonstrate that the latter had an adverse influence on a tuberculous infection. The inability of asbestos dust in that experiment to affect unfavorably the tuberculous process furnishes strong support for the interpretation that inhaled asbestos dust has no more than a mildly unfavorable effect on pulmonary tuberculosis.

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