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Experimental Studies

by

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Report to the

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ABSTRACT

Asbestosis is a pulmonary disease caused by the inhalation of asbestos dust. In animals it is characterized by a peribronchiolar fibrosis which seems to be the result of mechanical, rather than chemical, irritation of the tissue by asbestos fibers. Only the long fibers produce a typical reaction; short fibers are relatively inert. The filamented structure of the fibers is an essential factor in the mechanism of irritation. A characteristic tissue response can be produced by non-siliceous as well as siliceous fibrous minerals. Inhalation of asbestos dust apparently does not alter significantly the course of experimental tuberculosis in guinea pigs. The asbestosis body, which is a specific concomitant of asbestosis and forms soon after the entrance of the asbestos fiber into the lung, is believed to prevent further damage to the tissue by the fiber and thus to limit progression of the reaction when exposure ceases. Aluminum does not exert a protective action against the tissue irritation of asbestos fibers as it does against that of quartz particles.

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The bulk of the asbestos of commerce is chrysotile, $3MgO.2SiO_2.2H_2O$, which is mined in the Thetford region of the Province of Quebec. Crocidolite and amosite are also used commercially but in much smaller amounts. Chrysotile occurs as veins in serpentine, a mineral similar in chemical composition to chrysotile but which exists in massive form and is made up of microscopic fibers without the parallel orientation characteristic of chrysotile. The massive blue 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 six or more inches. The fibers run across the vein and not lengthwise with the formation.

Attention is directed to the mineral brucite, $MgO.H_2O$, which is often found in the same formations with serpentine and chrysotile and may be fibrous in structure. It has no commercial value at present because its fibers are not sufficiently flexible to be used in textiles but they are capable of repeated longitudinal subdivision. Unlike other asbestiform minerals, brucite is not a silicate and for this reason it has been a valuable tool in an experimental evaluation of the action of fibrous minerals upon lung tissue.

I. EXPERIMENTAL ASBESTOSIS

For many years studies have been carried on by the Saranac Laboratory in an investigation of the cause, nature and development of asbestosis. The present report is devoted to Experimental Asbestosis. In it are described the animal experiments with various kinds of asbestos dust. Another report, to be prepared and issued later, will be concerned with Human Asbestosis and will cover the health aspects of workers who have been exposed to asbestos dust in an industrial environment.

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Although asbestosis in man is a chronic disease which requires years to develop, it is possible to reproduce in one or more species of animal characteristic tissue changes which are similar to the lesions of human asbestosis. Since the life-span of the experimental animal is relatively short, it is not possible to develop the characteristic lesions in animals under the usual industrial conditions. 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 experiments with animals is invaluable in furnishing a better understanding of the reaction of the human organism to inhaled asbestos dust.

5. Experimental Methods

For investigating the biological reaction of the experimental animal to the various asbestos minerals, two types of technique 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, eight feet in dimension, in which a cloud of asbestos dust is maintained. At intervals during the experiment, a few animals are sacrificed and the tissue examined to determine the nature and extent of the dust reaction. Some animals are exposed for periods up to three years. The injection experiments, in which the dust, either dry or suspended in fluid, is introduced into the animal by the intravenous, intraperitoneal or intratracheal procedure, are used to determine whether or not a particular dust has a potential capacity to produce tissue reaction.

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Long-term inhalation experiments furnish information upon which great reliance is placed when estimating the degree to which a dust might be hazardous to industrial workers. Whether or not atmospheric dust, even though potentially dangerous, can be inhaled, pass the natural defense barriers and reach the pulmonary tissue in quantities sufficient to cause damage can be determined only by inhalation procedures. Injection experiments are useful because in them contact between the dust particles and tissues is assured and the potential capacity of the dust to produce reaction can be estimated accurately. When dealing with fibrous minerals like asbestos, the intratracheal method is valuable since it permits observing the effect of the fibers on pulmonary tissue.

6. Species Susceptibility

Unlike free silica, asbestos does not exert its specific effect in all species of all species of animal (Table 1). Injection of fine quartz into various organs of the guinea pig, rabbit, rat, cat, dog, chicken and even cattle will produce silicotic nodules. However, similar injections of long or short fiber asbestos have resulted in a fibrous reaction in the lung and, to a lesser extent, in the peritoneum but not in other organs.

7. Peculiar Characteristics of Asbestos

Experience has demonstrated that most of the particles either inhaled into the lungs of man and animal is 10 microns or less in maximum diameter. Larger particles apparently are excluded by the protective mechanism of the upper respiratory tract. In the case of fibrous materials, however, this restriction does not apply and fibers 100 and even 200 microns in length have been found in the terminal air spaces of human lungs. In small laboratory animals exposed to asbestos dust the maximum length of fiber found in the lung rarely exceeds 60 microns. Not every kind of fibrous material is inhaled with equal readiness; for example,

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the synthetic fibers of glass wool apparently are too inflexible to pass easily through the nose, pharynx, trachea and bronchi and seldom reach the terminal bronchioles and alveoli.

Inhaled particulate matter comes to rest throughout the terminal air spaces (alveolar duct, atria, alveoli) in all parts of the lungs; inhaled asbestos fibers are first retained in the respiratory bronchioles. These very small tubes are immediately distal to bronchioles lined by ciliated epithelium. Their own essential lining is a low cuboidal type of epithelium but, as their name implies, they actually function in respiration through lateral alveoli given off as pouches along their walls. Either these pouches, or the abrupt change in the character of the lining epithelium, or the decrease in diameter of the tube, or perhaps the combination of all three factors is responsible for local retention of the inhaled fiber. Only after asbestosis is well established are appreciable numbers of fibers carried into the more peripheral air spaces.

3. Rate of Tissue Reaction to Asbestos Fibers

The rate of tissue reaction to asbestos is much more rapid than to an active dust like quartz. Evidences of tissue response appear as soon as fibers have localized in sufficient concentration in specific areas. In rats receiving asbestos fibers by intratracheal injection this evidence is visible as early as two weeks after injection; for quartz dust the latent period might be two months or more.

The behavior of the tissue reaction to inhaled dust after the termination of exposure is not the same in silicosis as in asbestosis. In silicosis, the young nodules become larger; in asbestosis, young scar tissue, that may have formed, contracts and becomes more dense but the area of involvement decreases in size. If exposure to asbestos dust is terminated after a brief period, of recently-inhaled

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fibers in the lung may cause the fibrous tissue response to continue for a short time, until the fibers have been coated. This progression is of only a slight degree and of little significance.

5. Asbestosis Bodies

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

It is believed that asbestosis bodies are due to a deposit of protein and iron pigment upon the surface of inhaled fibers. In guinea pigs they form after about 60 days of contact with the tissue. They are abundant in man and the guinea pig (see Table 1) but are much larger in the former, probably because the larger-sized air tubes admit fibers of greater dimension. In cats, rabbits and mice there is an atypical coating of a few of the fibers after much longer residence in the lungs. In rats and dogs no bodies could be discovered. Although the evidence is incomplete, it appears that the formation of the asbestosis body prevents damage to the tissue by the fiber.

V. INHALATION EXPERIMENTS

Four comprehensive inhalation experiments have been conducted at the Saranac 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 2 to more than 5 years. The four kinds of asbestos dust employed are identified as King's floats, short fiber, 100 per cent ball-milled, and long-fiber asbestos dust.

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11. Inhalation Experiment with "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 periods up to nearly three years and some guinea pigs lived for about four years after their first exposure to dust. A preliminary report giving observations after 29 months of exposure appeared in the February, 1931 issue of THE JOURNAL OF INDUSTRIAL HYGIENE.* At that time observations covered a period of only 2-1/2 years and the conclusions as to the ultimate effects of inhaled asbestos dust were provisional. Results of the completed study show that most of the conclusions drawn in the preliminary report were substantiated. A complete review of this experiment follows.

12. The dusting material was a commercial variety of asbestos dust known as King's floats and was composed of short fibers and particles of variable size. It was obtained from the Thetford, Quebec plant of the Asbestos Corporation of America.

13. The dust composition (Table 2) reveals that the amount of fibrous constituents was only 14 per cent, a rather low value. However, there was sufficient fibrous material to produce a characteristic fibrosis.

14. The dust concentration at first was quite low and for impinger samples taken soon after the experiment was started, the average light field count by the standard technique was only 6.0 million particles per cubic foot of air. An appreciable number of large particles (non fibrous) also were present, as

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*STUDIES ON EXPERIMENTAL PNEUMOCONIOSIS. II. Inhalation of Asbestos Dust., Gardner, L.U., and Cummings, D.E. J. Ind. Hyg., 13. (2-Sec. B)-41, 1931.

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shown by an average count of 0.8 million for particles greater than 10 microns. After the inhalation experiment had been under way for about two years, the speed of the rotating paddle in the dusting machine was increased and for the remaining 9 or 10 months of the experiment considerably more dust was dispersed into the atmosphere. Average dust counts for impinger samples collected after this change were 53.7 million for the usual light-field method, and 1.6 million for particles larger than 10 microns.

15. Reaction in Animals to Inhaled "King's Floats" Asbestos Dust. Results of the investigation, briefly summarized in table 2, show that inhalation of King's floats asbestos dust produced a typical peribronchiolar fibrosis in guinea pigs but not in rabbits or rats.

16. Guinea Pigs. Seven groups of guinea pigs were used. In three groups the effect of a continuous and of an interrupted dust exposure was studied; in two other groups the relationship between infection and dust exposure was investigated. The remaining two groups were infection controls.

17. Rate and Type of Reaction. Guinea pigs inhaling this dust for periods up to 11 months developed a characteristic fibrosis occurring in conical patches about the respiratory bronchioles. During this exposure the peripheral alveoli were not involved. The particulate elements in the dust were transported to the lymphatic system where they caused no significant reaction; the fibrous elements remained fixed at the site of original localization and were seldom detected in the lymphoid tissue. Pleurisy and fibrosis in the septa were observed only when infection complicated the process.

After exposure of approximately a year, a small amount of cellular reaction had been produced about many respiratory bronchioles. As more dust was inhaled, it continued to accumulate in the same location and later stages of the disease

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consisted of extensions of the original lesions. New areas were not involved.

Apparently, the inhaled fibers were caught in the pocket-like alveoli that are given off from the lateral walls of the respiratory bronchioles. There they were phagocytized and many of them were carried into the wall by migratory cells. Mononuclear leucocytes attracted to the area caused an appreciable thickening of the bronchial wall. After 16 months a delicate fibrosis made its appearance. The process evolved so gradually that mitotic division of fibroblasts could rarely be discovered; nevertheless, the number of fine intercellular collagenous fibers (fibrosis) steadily increased. As this fibrosis contracted, it partially closed the alveoli, and with this obliteration the lining epithelium assumed its embryonic cuboidal form. The result was the adenoma-like appearance that Miller described in guinea pigs inhaling silicon carbide. The longer exposures resulted only in more thickening of the walls of the air spaces, largely due to an increase in the amount of fibrosis. The fibrous tissue always remained cellular and never showed the hyalinization characteristic of silicosis.

17. Progression. The reaction produced in exposed guinea pigs did not progress significantly during a subsequent period of 37 months when the animals lived in a normal atmosphere. In groups I and II months after exposure ceased, the cellular reaction had been completely replaced by thin strands of fibrous tissue. Observed still later, the scar tissue decreased in amount but in the last animal sacrificed, 37 months after discontinuing dust exposure, some fibrosis was still visible.

18. Infection Coincident with Dust Inhalation. Of the group of 40 guinea pigs infected with attenuated tubercle bacilli (H_{37} strain) 31 died or were sacrificed before ten years of dust exposure and were reported in the paper by Gardner and Cummings mentioned

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above. Seventeen of these died from intercurrent pneumonia. Briefly, the results were as follows: 10 revealed some evidence of spread of the tuberculous process; in 6 of these it was confined to the lungs and in the other 4 the abdominal viscera also were involved. Usually a slight local extension of the tuberculous infection had occurred but subsequent healing had resulted in fibrosis of both the pulmonary lesions and the secondary lesions in other organs. The healed pulmonary lesions showed more fibrosis than is characteristic of either tuberculosis or asbestosis alone.

The 9 animals which were still alive after two years of dust exposure were sacrificed at intervals during the following year. In 4 of them the primary foci of infection had healed with fibrosis and even calcification and there was no evidence of progression. In the other 5 the tuberculous foci showed evidence of having previously spread locally: in 4 of them it had healed, by the time of autopsy, with excessive fibrosis; in the other animal there was a generalized chronic tuberculous pneumonia in one lobe and isolated primary tubercles, which were still active but had not spread in the other lobes.

Evidence of extension of the infection was first seen after 7 months of dust inhalation; during the next 20 months more than half of the animals showed an actively spreading tuberculosis and in 3 of them small cavities had developed. During the last 8 months no animals exhibited any evidence of active infection although in half of them the healed fibrous scars of previous extensions were obvious. Sixty per cent of the guinea pigs with spreading pulmonary tuberculosis showed tuberculosis of the spleen and liver.

20. Infection Superimposed Upon an Established Asbestosis. Twelve guinea pigs, after inhaling asbestos dust for nearly 26 months, were inoculated with tubercle bacilli and then removed to normal air. The subpleural tubercles in the derived animals were

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no more numerous than in non-dusted controls, but a considerable number were found in the depths of the lung about foci of asbestosis. The reaction to infection showed only slight local extension about the original sites in the lungs and tracheobronchial lymph nodes. The abdominal viscera were involved in only one animal. Caseation was found in tubercles 1-1/2 months old but by 5-1/2 months it had completely disappeared, leaving only scar tissue. The latter still persisted in the last animal, which was killed 21 months after infection.

21. Asbestosis Bodies. Moderate numbers of asbestosis bodies occurred in the lungs of the guinea pigs, becoming more numerous and more distinctly segmented in later months.

22. Rabbits. Rabbits exposed to the asbestos dust for periods up to 19 months developed a low-grade foreign-body type of reaction 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 6 and 18 months, lived in normal air for more than two years. At autopsy neither animal showed any evidence of cellular reaction or fibrosis in the terminal bronchioles nor were there any asbestosis bodies.

23. Rats. All the white rats had acquired an infection, resulting in the formation of pulmonary abscesses, before they came to autopsy. Apparently, so much heavy mucus obstructed their bronchi that very few fibers could have entered their lungs. In a few of the rats, an occasional asbestosis body was discovered but there was no fibrosis.

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21. 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 three headings.

A. 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 progress after the dust exposure was discontinued and the guinea pigs transferred to normal air.

B. 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; in most of them there was evidence of temporary progression with subsequent healing; in one animal there was continuous progression to death. In contrast, when guinea pigs, after being infected, are exposed to quartz instead of asbestos dust, the infectious process continues to progress and eventually causes the death of the animals. On the other hand, exposure of infected animals to a harmless dust like whiting or gypsum does not lead to any progression of the infection. Guinea pigs infected with attenuated tubercle bacilli following the termination of about two years' exposure to asbestos dust did not develop progressive disease. The only modification of the infection was in its localization, a few bacilli being retained in the fibrous terminal bronchioles and forming tubercles there in addition to the usual foci beneath the pleura.

In view of this variability, the unusual nature of the response and the high proportion of deaths from intercurrent pneumonia it is felt that definite conclusions as to the influence of this dust on the course of tuberculous infection are not justified.

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C. Effect of tuberculous infection on the reaction to inhaled dust.

Infection complicating asbestosis accentuated the tendency of the dust to produce fibrous tissue. This change occurred whether or not the bacterial lesion was in contact with the area of dust reaction.

25. Inhalation Experiment with 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 carried on to determine whether this condition is true also for asbestos dust. It was thought that by using a short-fiber asbestos dust consisting almost entirely of fibers and particles smaller than 3 microns an accelerated tissue response might be initiated and an advanced reaction obtained in a short time. The previous inhalation experiment with King's floats asbestos, which contained fibers from 1 cm. to 1 micron or less in length as well as a great deal of particulate matter and which produced a typical peribronchiolar fibrosis in exposed guinea pigs, served as a basis of comparison.

26. The dusting material for this experiment was forwarded from the Manville plant of the Johns-Manville Corporation. It was the remains of fibers collected in dust bins after a carding operation and screened to pass 200 mesh. Since the material as received contained many long fibers, it was ground in a steel ball mill to reduce practically all the particles to 3 microns or less in size. When used alone in a standard dusting machine, this finely-ground asbestos tended to pack in the hopper and it became necessary to mix one volume of the unground material with three volumes of the ground to generate a satisfactory dust cloud. The addition of the small quantity of unground asbestos was unfortunate because it confused the interpretation of results. Probably the minor amount of reaction that developed was due to the long fibers in the mixture although the

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data of this experiment do not prove the point.

27. The composition of the short-fiber asbestos as received is disclosed by the chemical and petrographic analyses given in Table 4. Samples taken before and after grinding yielded about the same values on analysis, indicating that there was no contamination from the mill or loss of water content.

28. The dust concentration varied somewhat during the experiment and light field counts for atmospheric samples collected inside the animal cages with the impinger apparatus ranged from 85 million to 172 million. The average of counts was 130 million for the first year of the experiment, 135 million for the second year and 140 million for the third year.

29. Size-frequency measurements of air-floated dust from inside the cages at a magnification of 1300X revealed a great preponderance of fine particles (Table 5). Nearly 90 per cent of the particles seen were smaller than 3 microns.

30. Reaction in Animals to Inhaled Short-Fiber Asbestos Dust. Four species of animals - guinea pigs, white rats, cats and rabbits - were used in this experiment. The results of the dust exposure, which are summarized in Table 6, will be considered more in detail below.

31. Guinea Pigs. Eighty guinea pigs were originally placed in the dust room but 21 of them were later eliminated from the experiment and killed because of enlarged lymph nodes. Of the other 59 animals, 46 remained in the dust room until they were sacrificed or died from natural causes and 13, after being exposed to dust for 20 months, were transferred to a normal atmosphere.

32. Rate and Type of Reaction. The type of tissue reaction to the inhaled

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short-fiber asbestos was essentially the same as that already observed in the experiment with King's floats asbestos. The rate of reaction also was approximately the same but the extent of involvement with the short-fiber dust was very much less and after 16 to 24 months of exposure only a very few small foci of reaction, which generally required microscopic examination for detection, were produced in the guinea pigs.

Until exposures had continued for approximately one year, there was little tendency for dust-containing phagocytes to collect into clumps. By 16 months phagocytes had begun to collect about the walls of a few of the respiratory bronchioles with a little proliferation or infiltration of mononuclear cells in these walls. There were also some multinucleated cells but they were always of the inert foreign-body type. At 20 to 24 months the cellular clumps were sometimes quite marked and sometimes changes in the epithelium resulted in the adenoma-like or "adenomatoid" appearance previously described in Section 17. In most of the subsequent members of the series, the reaction remained cellular in type. In a few, however, fibrous elements dominated the picture. In the latter case, the collagen was pale in color and tenuous with no heavy swollen hyalinization. As in the rats described below, the alveolar walls might be made up of a band of collagen supporting a layer of epithelium, but with no combined capillaries. In the tracheobronchial lymph nodes the reaction was more pronounced in this experiment than in the previous one with King's floats asbestos, probably because of the transportation of an excess of fine particles to the nodes in animals inhaling short-fiber asbestos. The reaction was essentially an increase in reticulum, rather than a fibrosis, with preservation of the original cells between the thickened reticular fibers. Diffuse chronic pleurisy without evidence of pulmonary infection was present in a few animals.

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33. Progression. In the 13-3/4 months following the cessation of 20 months' exposure to 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. At the end of the dust exposure of 20 months two pigs were read as + and one as 2+. Among the 13 removed from dust the findings were variable: in 2 the reaction was ±; in 4 it was +; in 3 it was 3+; in 3 it was 4+; and in one animal it was 5+. It is quite possible that those with the most marked changes had already developed more reaction than the remainder by the time exposure ceased. Since the more severe reactions 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 7), which often revealed comparable amounts of ash and silica in lungs with widely different amounts of tissue change. For example, the ash and silica values were quite similar for three animals in dust 20 months and then in normal air 13-3/4 months, yet the tissue reaction for one animal was 4+; for another, +; and for the third, only ±.

34. Asbestosis Bodies. The formation of asbestosis bodies was at first extremely limited. After 9 months' exposure only a very rare short body could be found, usually devoid of cells. Around the finest intracellular particles there were yellow deposits having the same color as the asbestosis body. Exposure of one year had permitted an accumulation of many longer fibers about which the asbestosis-body coating developed. Most of these were still short enough to be partially or entirely within phagocytic cells. By the 20th month and thereafter, they were comparatively numerous although still rare in comparison with the findings in the King's Floats experiment.

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35. White Rats. Seventy-three white rats were exposed to atmospheric short-fiber asbestos dust for periods up to 32 months. Sacrificings during the first 10 months were made bimonthly and for the remainder of the experiment at less frequent intervals.

36. Rate and Type of Reaction. The dust cells until 8 months were widely scattered and existed in foci only sporadically. Reaction was limited to occasional slight thickenings of the septa about small accumulations of dust cells. In a few rats at 10 months, there was a suggestion of early fibrosis but the change was so slight that it would probably be overlooked without the clump of dust cells to attract attention to the area. Only 10 animals were exposed from 12 to 32 months. In each of them the lungs showed minute patches of well-defined fibrosis distributed like that of asbestosis but without asbestosis bodies. The lesions, visible only at a magnification of 150 diameters or more, consisted of patches along alveolar ducts in which the walls of the air spaces were very thick, due 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. No pleurisy was present. 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. 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 and extending into the mediastinal areolar tissue. Compared with the response to active dusts like quartz and chert the reaction to short-fiber asbestos was negligible.

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Results of chemical analyses made on the white rats are given in Table 8 and the average values have been tabulated in Table 9 for comparison with similar values for rats inhaling other dusts. The concentration of atmospheric particles to which the animals were exposed was approximately the same for asbestos and quartz; for the gypsum-quartz mixture, it was about twice as high and for chert five times as high. It will be noted that the percentages for asbestos are lower than those for quartz or chert but are similar to those for the gypsum-quartz mixture, in which atmospheric agglutination tended to reduce the amount of dust inhaled. It might be inferred that the total quantity of asbestos dust inhaled was low or that it had been eliminated from or dissolved within the lungs. In the present state of our knowledge evaluation of these hypotheses is not possible.

37. Cats. Twenty cats were used in this inhalation experiment with the short-fiber asbestos. Eighteen were kept in the dust room until death, the exposure period ranging from one month to nearly 1-1/2 years, and two, after a dust exposure of 31-1/2 months, were removed to normal air. One of these was sacrificed 5 months, and the other 24 months, later.

38. Rate and Type of Reaction. The reaction was essentially that to an inert dust, even after more than 1 years of exposure. The tissue response in this species was confined to microscopic foci of fibrosis 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.

39. X-Ray Changes. Only in the animal with the longest exposure did the X-ray reveal definitely abnormal shadows. After 29-3/4 months the picture was negative; after 45 months a faint mottling could be detected throughout both lungs. At autopsy, 8 months later, there was only microscopic

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fibrosis in the subpleural zone plus heavy lymphocytic infiltration about small bronchioles.

4C. Asbestosis Bodies. On prolonged search a few yellow atypical asbestosis bodies, smooth and without haustrations, were found in two animals exposed for more than a year.

4I. 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 6 months before being sacrificed.

4J. Rate and Type of Reaction. There was never enough fibrosis to be detected grossly and there was no chronic adhesive pleurisy. Microscopic evidence of alveolar wall thickening was first detected after about 3 years of exposure and was seen in all five animals examined thereafter. In one animal that died of paralysis after nearly four years of exposure the reaction was extensive enough to be visible on gross inspection of tissue sections. The possibility of pulmonary infection in this animal could not be excluded. However, 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, seemed microscopically to become more fibrous with the passage of time but there was never much encroachment upon the lumen of air spaces and the architecture of the lung was preserved.

4J3. Asbestosis Bodies. Asbestos 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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44. Summary and Interpretation.

The original purpose of the experiment was to evaluate the chemical theory of the pathogenesis of asbestosis. It was felt that if the tissue reaction to asbestos were chemical in origin an accelerated or accentuated response would result from exposure to finely-divided asbestos, as is the case with quartz. This experiment, in which the reaction was slower and less extensive than with King's floats, indicates that the reaction probably is not primarily chemical in nature.

Of the four species exposed in this experiment only the guinea pig and rat reacted with characteristic peribronchiolar fibrosis. The cat reacted with atypical sub-pleural fibrosis and in the rabbit the fibrosis which occurred could not be positively attributed to the dust because of a strong possibility of pulmonary infection.

45. Inhalation Experiment with 100 Per Cent Ball-Milled Asbestos Dust.

In the inhalation experiment with short-fiber asbestos dust a small quantity of unground asbestos was mixed with ground material in order to produce a suitable dust cloud. When evidence of a dust reaction appeared in the guinea pigs during the experiment, it was not clear whether this was a tissue response to the small number of long fibers in the unground asbestos or was a delayed action of the more abundant fine dust. Consequently, another inhalation experiment was started in which no unground material was used.

46. The dusting material was the ground short-fiber asbestos used in the previous inhalation experiment but no unground material was mixed with it. To obtain a sufficient amount of atmospheric dust, the design of the dusting apparatus was changed to an open type of hopper and fresh dust was added daily.

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Owing to the tendency of the material to form small spherules which prevented much of the fibrous portion from floating out of the hopper, the dispersal of the dust was not entirely satisfactory and after 7 months of operation, the dusting machine was reconverted to its original design. To prevent "pilling" or the formation of spherules of asbestos, steel wire brushes were attached to the inside surface of the hopper and to the rotating paddle. This arrangement gave satisfactory results and was used for the remaining 11 months of the experiment.

17. The composition of the raw material (short-fiber asbestos) and of atmospheric dust liberated from the ball-milled product in the dusting machine is given in Table 10. These values are based upon petrographic study and X-ray diffraction analysis. The atmospheric sample was collected with an electrostatic precipitator after wire brushes had been installed in the dusting machine. Previous to this, the chrysotile content of the air-suspended material was undoubtedly less than the 15 per cent value given in Table 10. In an interim report, it was stated that the air-borne dust contained about 5 per cent of chrysotile before the wire brushes were used and up to 6 per cent afterwards, but these values were probably low. Quantitative estimates on ball-milled asbestos dust may be somewhat inaccurate because it is difficult to determine how much of a dust sample is fibrous chrysotile and how much is non-fibrous serpentine.

18. The dust concentration for the first 7 months of the experiment was about 100 million particles per cubic foot of air. After the wire brushes had been installed, the dust counts were a little higher and the overall average for the first year was 116 million. The average of counts for the second year was 163 million and for the third year 145 million.

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19. The size-frequency of the components of atmospheric dust collected inside the animal cages with the electrostatic apparatus is reported in Table 11. Two samples were taken, one before the wire brushes were installed and one after. It will be noted that after the wire brushes were in use a greater proportion of very fine particles and also of larger fibers was released into the air.

20. Reaction in Animals to Inhaled 100 Per Cent Ball-Milled Asbestos Dust. 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 12.

21. Guinea Pigs. The experiment was started with 111 guinea pigs. At the end [illegible] there were 39 accidental deaths, 32 of pneumonia in an epizootic. After 20 months of exposing the 11 surviving guinea pigs were transferred to normal air.

22. Rats and Mice at Death. For the first year or two the rats [illegible] the only gross change in the lungs was the presence of scattered phagocytes and a occasional minute arteriosclerotic body. At 16 and 18 months no gross changes are visible on the lungs section and microscopically peribronchiolar foci of inflammatory cells could be seen. At 24 months 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.

Chemical analyses of the lungs (Table 13) reveal that in spite of the limited tissue reaction considerable dust has been retained in the lung.

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53. Progression. The lungs of animals exposed for the full dusting period (28 months) and then living in normal air for 2 months revealed the changes described above and also very slight peribronchiolar fibrosis. After 8 months in normal air the findings were similar but at 12 months 3 of 4 animals showed grossly-visible characteristic peribronchiolar fibrosis with adenomatoid change.

54. Lymph Node Involvement. The tracheobronchial nodes were essentially negative until exposure had been continued for more than a year and a half. Animals sacrificed at 18 months and 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.

55. Asbestosis Bodies. Minute asbestosis bodies were observed as early as 3 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 important to note that in the later months of exposure there was a distinct increase in the number of long fibers (up to 70 microns in length) in the lungs and that after exposure ceased characteristic long asbestosis bodies were seen.

56. 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

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months. Neither species developed even a suggestion of asbestosis and reaction was limited to phagocytosis of inhaled particles by thinly-scattered dust cells which remained free in air spaces or were transported to the tracheobronchial lymphnodes. No asbestosis bodies were found in the rats but in the mice there were a very few small non-haustrated forms within phagocytes.

In 21 mouse lungs sectioned there were 3 instances of pulmonary adenoma (14%).

57. Summary and Interpretation.

The tissue reactions observed in this experiment were much less extensive and slower in development than in the previous investigation with short-fiber asbestos. Since presumably there were fewer fibers longer than 3 microns in the material used in this experiment, the results tend to confirm the interpretation made in Section 44 of the short-fiber experiment that the reaction probably is not primarily chemical in nature.

The finding of long asbestosis bodies in animals inhaling the ball-milled material is an example of the difficulty of completely eliminating long fibers from an asbestos preparation.

In regard to progression of reaction after removal from dust, which was observed in this experiment but not in the others, the following interpretation is offered: When the reaction is well-developed at the termination of exposure, the contraction of the fibrous tissue would obscure any possible progression. In this experiment, however, since only the earliest stage of reaction was present at the time of removal from dust, its subsequent progress was apparent. It should be noted that the degree of progression was so slight that it can have little, if any, practical significance.

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58. Inhalation Experiment with Long-fiber Asbestos Dust.

After animals inhaling short-fiber asbestos dust for more than a year had failed to develop significant reaction, the hypothesis that asbestosis is produced by the mechanical irritation of long fibers was given added support. Since the King's floats asbestos used in the first inhalation experiment had a rather low content of fibrous chrysotile and contained considerable serpentine and other impurities, it was decided to conduct a new inhalation experiment with a purer form of chrysotile which would be richer in long fibers.

59. The dusting material employed in this investigation was obtained from the Manville plant of the Johns-Manville Corporation. Samples of several varieties of asbestos dust were first submitted to the Saranac Laboratory for examination and one kind, identified as lot D, which was low in magnetite and chromite and had a fibrous content estimated to be about 75 per cent, was selected as most suitable. Steel wire brushes were fastened to the inside surface of the hopper and to the rotating paddle in order to open up the bundles of asbestos and liberate more fibers into the atmosphere.

60. The composition of the long-fiber asbestos used in this experiment is indicated by the chemical and petrographic analyses given in Table 14. It appears that this material was a much purer form of chrysotile than the short-fiber dust used in other experiments. This is borne out by comparing the approximate analyses of the long-fiber and short-fiber dusts in Table 15.

61. The dust concentration as revealed by impinger samples taken inside the animal cages was much lower than the concentration for the experiments with short-fiber or ball-milled dust. For the first year of the experiment with long-fiber asbestos the average of the light field counts was 12 million; for

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the second year, 48 million; for the third year, 39 million; and for the fourth year, 43 million. Examination of the impinger samples with dark field illumination disclosed that many fine particles less than one micron in size accompanied the larger particles and dark field counts were, on the average, about 5 or 6 times larger than the light field counts.

62. The size-frequency of atmospheric samples of the long-fiber asbestos dust and of the ball-milled dust is shown in Table 16. Both samples were collected with the electrostatic precipitator. It will be noted that there was far more fibrous material in the long-fiber dust.

63. Reaction in Animals to Inhaled Long-Fiber Asbestos Dust. Guinea pigs, cats, rats and mice were employed in the inhalation experiment with long-fiber asbestos. Results of the experiment, summarized in Table 17, are described in greater detail below.

64. Guinea Pigs. The experiment was started with 200 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, 30 more guinea pigs were added to the surviving group in the dust room.

65. Rate and Type of Reaction. Histological examination revealed grossly visible lesions in the lungs after 6 months of exposure to dust, consisting of cellular infiltration about the terminal bronchioles. At 12 months, there were adenomatoid changes in the air spaces and by the 16th month a definite fibrosis was present in these areas in half the animals. 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

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by the 24th month, it had fanned out into the parenchyma. 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 (3 years). Although the intra-pulmonary reaction sometimes reached the pleura, there was no involvement of that membrane. No emphysema was visible at any point. Some thickening of the larger bronchi with a chronic inflammatory infiltration was revealed, but it probably was no more than would be produced by a similar exposure to any dust. For the first 5 months the phagocytes consisted of monocytes or very small giant cells; later, giant cell formation was more prominent. After 16 months the giant cells were large, filled with yellowish-brown pigment and sometimes vacuolated. An occasional animal showed an admixture of polymorphonuclear leukocytes and, in guinea pigs exposed for a considerable period, eosinophiles. The reaction was at first entirely cellular but by 16 months fibrous tissue formation was definite. However, it never exhibited a stage of hyalinization suggestive of silicosis.

A moderate individual variation occurred among the animals, both in the rate of developing lesions and in the stage of development attained at the end of exposure.

Analyses of the lungs (Table 10) disclosed that although the fibrous response was much greater in these guinea pigs than in those exposed to either short-fiber or ball-milled asbestos, the amount of mineral matter in the lung ash was less.

56. Progression. 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. More

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of these animals, killed at various periods up to 14 months after exposure, revealed lesions as large as those in the group sacrificed 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.

Reaction in the group exposed for 27 months and then transferred to a normal atmosphere was quite similar to the response in the 20-month exposure animals mentioned above. However, small foci were always visible on gross inspection of sections of all guinea pigs of the 27-month series but in no instance was there evidence of extension of the reaction.

67. Lymph Node Involvement. Reaction in the tracheobronchial lymph nodes was first visible at the third month of exposure. At the 5th month patches of cellular connective tissue began to appear in the medulla and by the 14th 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, as a variant, showed heavy sheets of diffuse monocytes and large active giant cells but there was never any necrosis or hyaline formation. The spindle-shaped new cells were yellowish in color from fine pigment granules that stained for iron. No fibers or asbestosis bodies were seen.

68. Asbestosis Bodies. Although asbestosis bodies were seen as early as one month after exposure began, they were rare and hard to find. At 5 months more were visible, chiefly coiled inside giant cells, and at 8 months many bodies were free in connective tissue. They became fairly abundant as exposure progressed although in some later animals the asbestosis bodies were only moderately numerous.

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69. Cats. Four cats inhaled the long-fiber asbestos dust for periods of 14, 25, 33 and 42 months, respectively, and were immediately sacrificed. Two other cats, after being exposed to dust for 18 months, lived in a normal atmosphere for an additional 24 months.

70. Rate and Type of Reaction. Exposure for 14 months 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. The bronchiolar epithelium was low and flattened, giving the tubes a smooth contour. Typical asbestosis bodies were not formed although there was an occasional yellow, smooth, pointed fiber. No pleurisy was present. The reaction was similar in location to that in the guinea pigs, but fibrosis was much slower in development and had not reached the same degree of maturity.

71. X-ray Changes. Roentgenograms of three cats were made after exposure periods of 25, 33 and 42 months, but tissue changes were not dense enough to be seen on an X-ray film.

72. 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 offer a basis for conclusions.

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73. Rate and Type of Reaction. All four animals sacrificed at 25 months showed a well-marked peribronchiolar fibrosis. In the 19-month animal, reaction was just beginning. Asbestosis bodies were practically absent at both 19 and 25 months although two small smooth bodies were found in the 19-month animal after a long search. Thus, these animals exhibited fibrosis without asbestosis bodies.

74. Mice. Out of 20 white mice used in this experiment, 11 lived a year or more in dust and died or were killed without showing an appreciable degree of pulmonary infection.

75. Rate and Type of Reaction. Reaction was limited to phagocytosis by mononuclear cells. Usually these were widely scattered through the air spaces; a limited number were grouped about the terminal bronchioles producing some thickening of their walls. There was no suggestion of fibrosis. The striking feature of the experiment was that 9 out of the 11 mice (82 per cent) exposed to dust for a year or more showed pulmonary tumors, usually adenomatous in type. These lesions did not contain dust or asbestosis bodies.

Numerous asbestosis bodies were observed in animals killed late in the experiment. Thus, these animals exhibited asbestosis bodies without fibrosis.

76. Summary and Interpretation.

The purpose of this experiment was to evaluate the importance of long fibers in the tissue response to inhaled asbestos. The results indicate strongly that long fibers are chiefly responsible for the reaction. Thus, in guinea pigs reaction developed earlier and became more extensive than in previous experiments in spite of a smaller concentration of atmospheric dust and a lower mineral content in the lungs. Furthermore, a typical peribronchiolar fibrosis was produced

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in cats although in a previous experiment with short-fiber dust it did not develop in this species.

The cause of the cellular fibrosis in the lymph nodes of the guinea pigs is not clear. It did not occur in other inhalation experiments with asbestos.

LXXVII. INJECTION EXPERIMENTS

In order to determine to what extent the various fibrous minerals possess the capacity to produce tissue damage, numerous injection experiments were performed. In these experiments guinea pigs and rabbits were used and the mineral dust was injected by the intratracheal, intraperitoneal and intravenous techniques. For the purpose of simplification the findings in each series of tests have been condensed and reported in tables, to which reference will be made later.

78. Experiments Using Intratracheal Technique.

Since the asbestos minerals do not cause a typical adenoid fibrosis in extra-pulmonary tissue, the intratracheal technique is the preferred way of introducing fibrous dust into the experimental animal. In this method the dust suspension is injected by means of a special needle or catheter deep into the trachea, from which it flows into the lungs.

79. Comparison of Fibrous and Non-Fibrous Dusts. To demonstrate that the ability of asbestos to produce fibrosis resides in its fibrous character, the series of injection experiments reported in Table 19 were performed. The tests were made with unheated long-fiber chrysotile and with chrysotile that had been ignited to destroy its

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flexible structure or ball-milled to reduce the length of fiber to 3 microns or less. At the same time control tests were made with compounds, which has the same chemical composition as chrysotile but is non-fibrous. A review of the findings reveals that only the unburned long-fiber chrysotile produced fibrosis. Fibers subjected to ignition or shortened by ball-milling had lost their capacity to cause serious tissue damage. Ignition produced fundamental changes in the chrysotile fibers, among them being loss of water, an alteration from a flexible to a brittle structure and possibly other changes.

30. Comparison of Various Long-Fiber Dusts. Four very interesting findings were obtained by the results of the experiments included in Table 2. First, all the long-fiber asbestos minerals tested, with the exception of anthophyllite, produced a typical fibrosis. It is not entirely clear why anthophyllite behaved differently from the other asbestos minerals. Unfortunately, 7 of 8 animals died of pneumonia within the first two weeks of the experiment and the remaining animals were sacrificed at 1, 3 and 11 months; thus observations were not made at the optimum period of 3 and 6 months.

Second, with the mineral crocidite, which is not a chrysotile but is a fibrous form of magnesium hydroxide, a characteristic fibrosis like that of the other mineals was obtained. Since the crocidite used contained only 5 % iron and silicon (as an impurity), it is obvious that a siliceous component is not an essential factor in the development of asbestosis.

Third, no fibrosis resulted from the injection of glass wool fibers, even though glass wool resembles asbestos in some ways. There are fundamental differences, however. A glass wool fiber 3 microns in diameter is a solid rod and, in short lengths, is fairly rigid, while an asbestos fiber of the same diameter is a bundle of extremely fine filaments which imparts to the fiber a high degree of flexibility. It would seem that this structure and the associated flexibility are

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important factors governing the capacity of a mineral to produce peribronchial fibrosis.

81. Comparison of Long-Fiber and Short-Fiber Dusts. With quartz dust it has been demonstrated the smaller the particles, the more intense is the tissue reaction, and that is little reaction to particles larger than 3 microns in diameter. In the ca of asbestos, however, the reverse is true and apparently only long fibers hav any specific effect. This is confirmed by the data of Table 21, in which a s of tests with fibrous minerals is reported. When the injected dust consists fibers 20 to 50 microns long, all the minerals tested (except anthophyllite, noted in Section 80) produced a fibrosis; when the material was prepared by grinding the fibrous dust until the length of fibers was reduced to 20 micro less (or, in some cases, 3 microns and less), none of the injected mineral caused fibrosis.

82. Experiments Using Intravenous Technique.

The experiments, described in Table 22, in which the intravenous metho injection was employed, show that the asbestos minerals are far different f quartz in their action on tissue. It has been repeatedly demonstrated that venous injection of quartz particles 3 microns and less in diameter will ca typical tissue reaction with the development of fibrosis in extrapulmonary such as the liver and spleen. Asbestos minerals, however, on intravenous i generally produce only an inert type of reaction, as is revealed by the re given in the table. The reason for the early deaths in the experiment with tile particles is not clear; it may have been caused by silicic acid liber the finely-ground mineral.

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83. Experiments Using Intraperitoneal Technique.

The results of injection experiments with the intraperitoneal technique are given in Table 23. It will be noted that the long-fiber dusts produced a fibrous reaction while dusts composed of particles 3 microns and less in size caused only an inert type of response. These experiments indicate also that the fibrosis initiated by the irritation of asbestos fibers is not restricted to the lungs, as was formerly assumed, but can be produced in the peritoneum as well.

LXXXIV. OTHER EXPERIMENTS WITH ASBESTOS MINERALS

A number of additional experiments were conducted to throw more light on specific phases of the asbestosis problem.

85. Protective Action of Aluminum Compounds.

Intratracheal injection of a suspension of long-fiber chrysotile to which colloidal aluminum hydroxide had been added revealed that the addition of the aluminum compound did not prevent the tissue irritation produced by chrysotile. If anything, the acute inflammatory response to the injected fibrous mineral was accelerated. One month after the last injection of the dust suspension the bronchiolitis was becoming fibrous.

86. 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. Following subcutaneous injection of two kinds of chrysotile into the groin of guinea pigs - one kind containing 2 per cent and the other 0.2 per cent Fe_2O_3 - the asbestosis bodies were equally numerous at both sites of injection.

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An attempt to produce asbestosis bodies in guinea pigs by implantation of three silk bags containing fibrous chrysotile was unsuccessful. One bag planted subcutaneously in the abdominal wall disappeared; the other two bags, placed in the peritoneal cavity, produced a little foreign body reaction but no asbestosis bodies in a year.

Intratracheal injection into guinea pigs of asbestosis bodies recovered from human lung tissue failed to produce the typical tissue reaction to asbestos fibers. The injected material was obtained by digesting with sodium hypochlorite solution 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 and may be maintained in vivo for a year or longer.

LXXXII
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 upon 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 would be merely an indirect silicosis. Several facts make the chemical theory untenable: (1) Intratracheal injections of brucite fibers, which had a silica content of only 0.80 per cent, caused a typical fibrosis like that produced by the asbestos minerals; (2) free-silica particles increase in potency as the particle size becomes less, but asbestos fibers shorter than about 10 to 20 microns are relatively innocuous; (3) aluminum

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hydroxide neutralizes the irritating effect of quartz but not of asbestos; (4) serpentine has the same chemical composition as long-fiber chrysotile but it does not produce the same kind of tissue reaction; (5) there is a wide range in the chemical composition of the minerals which do cause asbestosis (see Table 24). In view of this evidence it seems more likely that asbestosis is caused by an unusual mechanical irritation from long asbestos fibers; this irritation being related to the peculiar filamented structure of the fiber and the associated flexibility, which are possessed by no other foreign body. For example, ignition of chrysotile fibers changed their structure and made them inert while the same fibers, before being heated, produced fibrosis (see Table 19). Further support for the theory of mechanical irritation is that asbestosis occurs in an organ of high mobility - the lung - and that a fibrous reaction can be produced by injection of asbestos fibers into the peritoneum, where there is also a degree of mobility, but not in other extra-pulmonary organs.

LXXXVIII
C O M P L I C A T I O N S

The experimental investigation with asbestos minerals was concerned primarily with the effect of the dust on normal tissue but some attention was given to other phases, such as susceptibility to infection and occurrence of malignancy.

89. Infection.

The only experiment in which the effect of inhaled asbestos dust on a pulmonary infection was studied was the first inhalation experiment, carried on with "King's floats" dust. It is, perhaps, unfortunate that infection studies were not made in the other inhalation experiments also.

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90. Susceptibility to Tuberculous Infection. The development of a tuberculous process initiated at the beginning of exposure to asbestos dust, and also of an infection superimposed upon an established asbestosis, was described in Sections 19 and 20 of this report. It will be noted that asbestos, when classified according to the effect of a dust on tuberculous infection, would be placed below an active dust like quartz but above inert dusts, such as calcite and gypsum. In animals infected with attenuated tubercle bacilli, quartz will cause the infectious process to progress until the animal dies of tuberculosis. Inert dusts will have no effect on the infection and the lesions will usually heal and the disease disappear. Asbestos dust is in a different category. When the fibrous dust was being inhaled during the evolution of the infection, there was a 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 following the completion of nearly three years of exposure to asbestos dust, 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 and there was nothing to suggest any influence on the course of the disease.

91. Susceptibility to Non-Tuberculous Infection. There was no pointed experiment concerning the effect of inhaled asbestos dust on non-tuberculous infection. Intercurrent pneumonia among animals exposed to asbestos dust was rather common, the frequency in guinea pigs exposed in the four inhalation experiments ranging from 16 to 39 per

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cent. This incidental evidence suggests the possibility of an effect of asbestos dust on non-tuberculous 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 asbestod dust does not exert a significant effect on the susceptibility to non-tuberculous pulmonary infection.

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S U M M A R Y

Owing to the vast amount of data included in this report it seems most convenient to state as precisely as possible the various points which emerged from the investigations and, when necessary, follow each with a brief resume of the evidence.

A. Various forms of asbestos fibers produce a peribronchiolar fibrosis of the lungs of guinea pigs, rats, cats and rabbits but not of mice and dogs.

Both inhalation and injection experiments provide ample support for this statement. Figures 5 and 6 show the reaction to two different kinds of asbestos mineral in the guinea pig lung. Similar but less extensive fibrosis occurred also in rats, cats 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. The mode of action of the asbestos fiber is primarily mechanical rather than chemical in nature.

The evidence for this is given in Section LXXXVII. Figures 1, 2, 3, 4 and 7 illustrate the important points. The fibrous filamented structure of asbestos plays an essential part in the irritating action, since the solid fibers of glass wool do not produce fibrosis (see Figure 8).

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

Experimental evidence discloses that fibrosis develops following the injection of asbestos fibers between 20 and 50 microns in length, but not following injection of particles less than 20 microns, (Tables 21 and 23). This shows that the capacity to produce fibrosis develops somewhere between 20 and 50 microns.

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Pointed studies have not been carried out to determine the upper limit of effective fiber length. It appears, however, that that limit will be determined by the inhalability of the fiber.

D. Typical experimental asbestotic fibrosis was produced by the inhalation of an atmospheric suspension containing an average of 138 million particles per cubic foot of air of which only 0.6 per cent consisted of fibers longer than 10 microns.

In the inhalation experiment with 100 per cent ball-milled asbestos dust containing 0.6 per cent of fibers longer than 10 microns (Section 45 to 57 inclusive) a typical fibrosis was obtained (see Table 12). The evidence presented shows at least that an atmospheric concentration of asbestos dust containing 0.8 million (0.6% x 138,000,000) fibers greater than 10 micronsper cubic foot of air is capable of producing experimental asbestosis. The actual lower limit of concentration of long fibers necessary to produce asbestosis cannot be established on the evidence available.

E. The duration of exposure required to develop the pulmonary reaction to inhaled asbestos dust is inversely proportional to the concentration of long fibers in the atmosphere, viz. as the concentration is increased, the reaction develops in shorter time.

Evidence for this statement is presented in the inhalation experiment with Long-fiber Asbestos Dust (Sections 58 to 76 inclusive). Study of the size-frequency of the long-fiber dust discloses that it contained 6.7 per cent of fibers greater than 10 microns in length (Table 16). The lungs of animals exposed to this dust revealed that the pulmonary reaction developed in approximately one-half the exposure time required for its development in animals subjected to the ball-milled dust, which contained only 0.6 per cent of long fibers.

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F. Established experimental asbestosis ceases to progress on discontinuing exposure to the dust.

Evidence shows in fact that on discontinuing exposure there was an appreciable clearing of the mature pulmonary lesions due to contraction of the fibrotic reaction. In contrast, an immature tissue response consisting primarily of 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 (Section 57).

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 (Section 86). The cessation of progressive reaction to inhaled asbestos dust 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 (Section 85).

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

This interpretation is in distinct contrast to the stimulating effect of inhaled quartz upon a tuberculous process in the lung. It must remain tentative, however, since it is based upon evidence limited to two series of guinea pigs exposed to only one kind of asbestos, namely "King's floats" (Section 19-20, 90). Reference to Table 3 shows that when infection was coincident with onset of dust exposure, there was temporary progression of the infectious process with subsequent healing; when infection was initiated after 25-3/4 months of dust exposure the course of the tuberculosis was not

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appreciably altered. This latter finding is in marked contrast with our usual experience involving quartz dusts or mixed dusts containing quartz wherein the adverse influence of quartz upon a tuberculous infection is most strikingly manifested where infection is initiated after a period of dust exposure, viz. superimposed upon a background of established silicosis. As indicated above, application of this more sensitive test to asbestos dust failed to demonstrate that such dust had an adverse influence upon a tuberculous infection. Thus, there is strong evidence in support of the interpretation. However, it is recommended that further investigation be undertaken to establish this point.

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Table 1

REACTION TO LONG-FIBER CHRYSOTILE

IN LUNGS OF MAN AND OTHER SPECIES OF ANIMAL

Species	Mode of Exposure	Fibrosis	Asbestosis Bodies
Man	Inhalation	4+	4+
Guinea Pig	Inhalation and injection	2+	3+
Rabbit	" " "	+	±
Cat	" " "	+	±
White Mouse	Inhalation	0	±
White Rat	Inhalation and injection	+	0
Dog	Injection	0	0

± Very small occasional asbestosis bodies.

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