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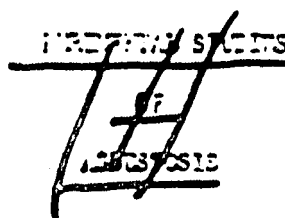
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ABSTRACT

Asthenosis is a pulmonary disease caused by the inhalation of asbestos dust. In animals it is characterized by a peribronchiolar fibrosis which ^{physiologically} 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 filamentous 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 ^{does not} alter significantly the course of experimental tuberculosis in guinea pigs. The asthenosis body, which is a specific concomitant of asthenosis 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.



2. Introduction

Asbestosis is a form of pneumoconiosis resulting from prolonged inhalation of asbestos dust. The name asbestos, literally "unburnable," is not that of a particular 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 six or more inches. Some varieties are stiffer than others but many are sufficiently flexible to be spun into yarn and woven on modified textile machinery.

3. Asbestos Minerals

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

Amphibole Group

Anthophyllite	(Mg, Fe) silicate
Actinolite	(Mg, Fe, Al) silicate
Amphibole	(Ca, Mg, Fe, Al, Na, H) silicate
Tremolite	(Ca, Mg) silicate
Actinolite	(Ca, Mg, Fe) silicate
Crocidolite	(Na, Fe) silicate + Fe silicate

Corundum Group

Cryptite	Mg silicate (hydrous)
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The bulk of the asbestos of commerce is chrysotile, Mg₃Si₂O₅(OH)₄, which is mined in the English region of the Province of Quebec. Crocidolite and anthophyllite are also used commercially but in much smaller amounts. Chrysotile occurs as veins in serpentines, a mineral similar in chemical composition to chrysotile but which occurs in massive form and is made up of interlocking fibers without the parallel orientation characteristic of chrysotile. The massive blue black serpentine, which is smooth and easy 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 veins and are lengthwise with the formation.

Attention is directed to the mineral brookite, MgSiO₃, which is often found in the same formations with serpentines 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 submicroscopic minerals, brookite is not a silicate and for this reason it has been a valuable tool in an experimental combination of the action of fibrous minerals upon lung tissues.

2. Experimental Asbestos

For many years studies have been carried out by the Bureau Laboratory in an investigation of the cause, nature and development of asbestosis. The present report is devoted to Experimental Asbestos. 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 Asbestososis and will cover the histological aspects of asbestos dust have been exposed to asbestos dust in an industrial environment.

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 tissues 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.

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 asbestiform dust, even though potentially dangerous, can be inhaled, pass the natural defense barriers and reach the pulmonary tissues in quantities sufficient to cause damage can be determined only by inhalation experiments. Inhalation 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 tissues.

6. Species Susceptibility

Unlike free silica, asbestos does not exert its specific effect in all organs 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 tadpoles will produce silicotic nodules. However, similar injections of long or short fiber asbestos have resulted in a fibrous reaction in the lung ^{cat, dog, rabbit, rat, guinea pig, and chick} but not in other organs of the guinea pig, rabbit, cat and chick. The long and short asbestos ^{is not to be considered for} is of

7. Particular Characteristics of Asbestos

Experiment has demonstrated that most of the particulate matter inhaled into the lungs of man and animal is 10 microns or less in median 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 500 microns in length have been found in the terminal air spaces of human lungs. In small laboratory animals exposed to asbestos dust the median length of fiber found in the lung rarely exceeds 50 microns. Not every kind of fibrous material is inhaled with equal readiness; for example,

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 lung; 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 asbestososis 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. Evidence 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, the recently-inhaled

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.

9. Subcutaneous Bodies

The parallel structures known as the subcutaneous body or cutaneous body is a special development of subcutaneous. The typical body is a golden-yellow, beaded or beaded red 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 subcutaneous 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-colored air tubes admit fibers of greater dimension. In rats, 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 subcutaneous body prevents damage to the tissue by the fiber.

The above report, however, was slightly changed.

2. MATERIALS EXAMINED

Four comprehensive inhalation experiments have been conducted at the Bureau 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 Blue's Fibers, Chert Fiber, 300 per cent ball-milled, and Long-fiber asbestos dust.

2.7
2/9

IX
INHALATION EXPERIMENT WITH "AIR" AS AEROSOL DUST

The first inhalation experiment conducted at the German Laboratory with asbestos dust was begun in 1928. Animals inhaled the dust for periods up to nearly three years and even 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 January, 1931 issue of THE JOURNAL OF INDUSTRIAL MEDICINE. At that time observations covered a period of only 3-1/2 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 starting material was a commercial variety of asbestos dust known

as King's Flints and was composed of short fibers and particles of vermiculite also. It was obtained from the Bedford, Quebec plant of the asbestos corporation of America. *(See page 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000)*

13. The dust composition (Table 2) reveals that the amount of fibers suspended 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 6.8 million particles per cubic foot of air. An appreciable number of large particles (or fibers) also were present, as

SPERMATOPHYTES OF NORTHWESTERN CANADA. VI. Isolates of Sphagnum, Cladonia, L. V., and Cladonia, D.S. J. Isol. No., 13: 65-81, 97-114, 1971.

shown by an average count of 0.6 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 new light-field method, and 1.6 million for particles larger than 10 microns.

15. Reaction in Animals to Inhaled "King's Flints" Asbestos Dust. Results of the investigation, briefly summarized in Table 3, show that inhalation of King's Flint 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 32 months developed a characteristic fibrosis occurring in cervical 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. Proliferation 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 fibrosis

28. Preparation. The reaction produced in exposed films gave did not proceed sufficiently during a subsequent period of 37 months when the contents lived in a normal atmosphere. Between 8 and 22 months after exposure ceased, the collimator reactions had been completely replaced by dead elements of silver chloride. Observed still longer, the over time decreased in amount but in the last element sacrificed, 37 months after elementizing that exposure, some elements was still visible.

29. Exposure conditions with pure substance. Of the group of 60 films also indicated with silver

chloride substance handled (By silver) 22 died or were sacrificed before two years of dark exposure and were reported in the paper by Gardner and Cunningham mentioned

consisted of extensions of the original salivary. No areas were not involved.

Apparently, the tubular fibers were caught in the posterior-like strands that are given off from the lateral walls of the respiratory bronchioles. These they were phagocytosed and many of them were carried into the wall by migratory cells. The tubular structures extended to the area around an approximately spherical of the bronchial wall. After its entrance a delicate fibrous end the appearance. The process involved so gradually that tubular division of fibrous strands would rarely be discovered; nevertheless, the number of fine intercellular collagenous fibers (fibers) steadily increased. As with fibrous condensation, it partially enclosed the alveoli, and with this encroachment the lung epithelium assumed its original embedded form. The result was the dense-like appearance that will be described in future plus intubating alveolar cavity. The larger arteries were not only so were thickening of the walls of the air spaces, largely due to an increase in the amount of fibrous. The fibrous tissue always remained collagenous and never changed the histological character of alveoli.

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 primary 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 these 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 infected with tubercle bacilli and then removed to normal air. The subpleural tubercles in the dusted animals were

so 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 11 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, as 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.

24. Summary and Interpretation of Inhalation Experiment with King's Floate Dust.

The findings in the experiment with King's Floate dust can be summarized under three headings.

A. Effect of the inhaled dust on normal animals. The King's Floate 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 calcite 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 tubercle bacillus infection on the reaction to inhaled dust.

Infection complicating tuberculosis 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.

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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 floate asbestos, which contained fibers from 1 mm. 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 Norville

plant of the Johns-Norville Corporation. It was the remains of fibers collected in dust bins after a curving 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 volume 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

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 83 million to 182 million. The average of counts was 130 million for the first year of the experiment, 134 million for the second year and 140 million for the third year.

29. Disto-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

short-fiber asbestos was essentially the same as that already observed in the experiment with King's flake 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 pig.

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 contained capillaries. In the tracheobronchial lymph nodes the reaction was more pronounced in this experiment than in the previous one with King's flake 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 primary infection was present in a few animals.

33. Progression. In the 11-12 months following the cessation of 20 months' exposure to dust, progression of disease was not definitely demonstrated but not. 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 0 and one as 2+. Among the 13 removed from dust the findings were variable: in 2 the reaction was 2; in 4 it was 0; 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 after revealed comparable amounts of ash and silica in lungs with widely different amounts of fibrous change. For example in ash and silica values were quite similar for three animals in dust exposure of 20 months and three in normal air 11-12 months, yet the fibrous reaction for one animal was 4+, for another, 0, and for the third, only 2.

34. Atherosclerotic Bodies. The formation of atherosclerotic bodies was at first extremely limited. After 5 months' exposure only a very few short body could be found, usually inside of cells. Around the finest intercellular particles there was yellow deposits having the same color as the atherosclerotic body. Exposure of one year had permitted an accumulation of many larger fibers about which the atherosclerotic-body coating developed. Most of these were still short enough to be partially or entirely within phagocytic cells. By the 10th month and thereafter, they were comparatively numerous although still rare in comparison with the findings in the King's fiasco experiment.

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 6 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 asbestotic 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 tissues and Foot-Moleschowski 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 asbestotic body. The tracheobronchial nodes showed compact focal collections of mononuclear cells at 12 months and, at 30 months, some diffuse thickening of the peritonsils. In a few rats there was definite fibrosis along the margins of the nodes 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.

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 4-1/2 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 4 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 39-1/4 months the picture was negative; after 65 months a faint mottling could be detected throughout both lungs. At autopsy, 8 months later, there was only microscopic

fibrosis in the pleurae, some plus heavy lymphocytic infiltration about small bronchioles.

10. Asbestosis Bodies. On prolonged search a few yellow atypical asbestotic bodies, smooth and without haustriations, were found in two animals exposed for more than a year.

11. 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.

12. 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, tended 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.

13. 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.

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 accelerated 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.

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~~Interpretation experiment with 100 Ppc Corp. Lally-Lilly 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 effect of the more abundant fine dust. Consequently, another inhalation experiment was started in which no unground material was used.

45. 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.

Due to the tendency of the material to form small spheres 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 spheres 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 22 months of the experiment.

47. 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.

48. 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 106 million. The average of counts for the second year was 163 million and for the third year 145 million.

49. The size-frequency of the components of atmospheric dust collected inside the animal cages with the electrostatic apparatus is reported in Table 11. Two series 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 longer fibers was released into the air.

50. 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.

51. Guinea Pigs. The experiment was started with 100 guinea pigs. As the dust exposure proceeded, there were 39 accidental deaths, 32 of pneumonia in an epidemic. After 28 months of dusting the 16 surviving guinea pigs were transferred to normal air.

52. Rate and Type of Reaction. 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 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 had been retained in the lung.

53. Progression. The lungs of animals exposed for the full dusting period (7 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 12 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 reticular 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 30 months and 24 mice for periods up to 12

months. Neither species developed over a suggestion of asbestosis and reaction was limited to phagocytosis of isolated 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 non-hausted forms within phagocytes.

In 22 mouse lungs sectioned there were 3 instances of pulmonary anisocytosis (11%).

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 54 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.

LONG FIBER ASBESTOS DUST.

~~5. Inhalation Experiment with Long-Fiber Asbestos Dust.~~

After article: Inhalation short-fiber asbestos dust for more than a year had failed to develop significant reaction, the hypothesis that asbestos is produced by the mechanical irritation of long fibers was given added support.

Since the King's Flaming 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 Merrill plant of the Johns-Merrill Corporation. Samples of several varieties of asbestos dust were first submitted to the Bureau Laboratory for examination and one kind, identified as lot D, which was low in magnesite and chrysotile and had a fibrous content estimated to be about 75 per cent, was collected as most suitable. Steel wire brushes were fastened to the inside surfaces 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 spectrographic analyses given in Table 14.

It appears that this material was a much purer form of asbestos 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 dust in Table 15.

61. The first 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

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, rats, mice and monkeys 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 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, 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 lesions could be seen macroscopically at 30 months. From this time on the reaction increased in extent and in the amount of collagen and

by the 12th month, it had spread 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 attained a stage of hyalinisation suggestive of silicosis.

A moderate individual variation occurred among the exposed 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 15) disclosed that although the tissue 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.

66. Regression. 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 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 hard 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 8th month patches of cellular connective tissues began to appear in the medulla and by the 11th 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 connective tissue and large active giant cells but there was never any necrosis or hyaline formation. The spindle-shaped cells were yellowish in color from fine pigment granules that stained for iron. No fibers or asbestos bodies were seen.

68. Asbestos Bodies. Although asbestos 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 asbestos bodies were only moderately numerous.

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 asbestos 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 tissues which made wall-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 asbestos 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.

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 adenomas 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 animal content in the lungs. Furthermore, a typical peribronchiolar fibrosis was produced

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in case 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.

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~~EXPERIMENTAL~~ NOVII. 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 advanced fibrosis in extra-pulmonary tissues, 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 bronchi, from which it flows into the lungs.

79. Comparison of Fibrous and Non-Fibrous Dusts.

ability of asbestos to

produce fibrosis varies in its fibrous character, the series of injection experiments reported in Table 29 were performed. The tests were made with embedded long-fiber chrysotile and with chrysotile that had been ignited to destroy its

ible structure or being killed by sodium hypochlorite or other strong oxidizing agents. At the same time covered with a thin layer of sodium carbonate, which has the same chemical composition as the soda ash in the mineral. A review of the findings reveals that only the untreated long-fiber asbestos produced fibrosis. Fibers subjected to rupture or sheared by ball-milling had lost their capacity to cause serious damage. Rupture produced structural changes in the chrysotile fibers, among them being loss of water, an alteration from a flexible to a brittle structure and possibly other changes.

80. Comparison of Various Long-Fiber Data. Some very interesting findings are disclosed by the results of

the experiments included in Table 27. 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, if of 8 animals died of pneumonia within the first two months of the experiment and the remaining animals were sacrificed at 2, 8 and 12 months; thus observations were not made at the optimum periods of 2 and 4 months. Second, with the mineral brucite, which is not a silicate but is a fibrous

form of magnesian hydroxide, a characteristic fibrous form that of the asbestos minerals was obtained. Since the brucite used contained only 0.30 per cent silica (as an impurity), it is obvious that a siliceous component is not an essential factor in the development of asbestos.

Third, no fibroids 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 impart to the fiber a high degree of flexibility. It would seem that this structure and the associated flexibility are

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

61. Comparison of Long-Fiber and Short-Fiber Dusts. With quartz dust it has

been demonstrated that the smaller the particle, the more intense is the fibrosis reaction, and that there is little reaction to particles larger than 3 microns in diameter. In the case of asbestos, however, the reverse is true and apparently only long fibers have any specific effect. This is confirmed by the data of Table 21, in which a series of tests with fibrous minerals is reported. When the injected dust consisted of fibers 20 to 50 microns long, all the minerals tested (except anthophyllite, as noted in Section 60) produced a fibrosis; when the material was prepared by first grinding the fibrous dust until the length of fibers was reduced to 20 microns and less (or, in some cases, 3 microns and less), none of the injected minerals caused fibrosis.

22. Experiments Using Interference Techniques.

The experiments, described in Table 22, in which the interference method of injection was employed, show that the asbestos minerals are far different from quartz in their action on glass. It has been repeatedly demonstrated that various injections of quartz particles, minerals and types in diameter will cause a typical glass reaction with the development of ^{hydrolytic} fibrosis, an extraordinary effect, such as the linear and columnar asbestos minerals, however, on interminable injections generally produce only an hour type of reaction, as is revealed by the results given in the table. The reason for the early deaths in the experiment with chrysotile particles is not clear; it may have been caused by cells and liberated by the finely-ground mineral.

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.

Shaver 6/16/61
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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 Asbestos Bodies.

The iron in the coating of the asbestos 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 Fe₂O₃ - the asbestos 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.

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LXXVII. THEORY OF IRRITANT ACTION OF ASBESTOS MINERALS

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 ferro-cilic fibers, which had a silica content of only 0.90 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

hydroxide neutralises 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; ^{Probably}
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, would produce 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 extrapulmonary organs.

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LXXXIII. COMPLICATIONS ⁶

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 pul-
monary infection was studied was the first inhalation experiment, carried on with
"King's Flinto" dust. It is, perhaps, unfortunate that infection studies were
not made in the other inhalation experiments also.

90. Susceptibility to Tuberculous Infection. The development of a tuber-

culous 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.

97. Susceptibility to Non-tuberculous Infection. There was no pointed experiment concerning the effect of inhaled asbestos dust on non-tuberculous infection. Interventive periods among animals exposed to asbestos dust was rather common, the frequency in guinea pigs exposed in the four inhalation experiments ranging from 16 to 57 per

cent. This incidental evidence supports 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 several animals, it is felt that the inhalation of asbestos dust does not exert a significant effect on the susceptibility to non-tuberculous pulmonary infection.

9a. Neoplasia.

C. C. ... Kline
Coiled between the inhalation of asbestos dust and tumors of the lung.
No specific experiment was conducted to determine whether the inhalation of asbestos favors the development of neoplastic disease, but certain observations on this subject were recorded in the outline of the proposed monograph on asbestosis submitted by the late Dr. L. U. Gardner in February 1943. In it he called attention to the high incidence of lung cancer among mice inhaling long-fiber asbestos. In his experimental notes, however, he referred to these lesions as adenomas.

There is an important distinction between adenoma and cancer which should be made clear. A cancer is a tumor, or neoplasm, capable of local invasion and destruction of tissue, which can distribute cells through the lymphatic or blood stream to produce isolated foci, from which new tumors develop. This phenomenon of dissemination is known as metastasis and any tumor which exhibits it is a malignant growth, of which cancer is one type. An adenoma, on the other hand, is a so-called benign or non-malignant tumor (neoplasm) which may or may not be capable of local invasion but which does not metastasize.

In order to clarify the exact nature of these lesions the pathological material is being carefully examined. Since it is felt desirable to have the benefit of Doctor Verneid's judgment, a review of the data on this subject is being postponed until after his return from Europe. Rather than delay the entire report, further discussion will be reserved for a supplement to be issued later.

With the view of making this report it seems most convenient to state the conclusions derived from the investigation and, when necessary, follow each one with a brief review of the evidence.

- C. 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 conclusion. Figures 5 and 6 show the reaction to two different kinds of asbestos mineral.

- B. The mode of action ^{is mechanical} ~~is primarily~~ mechanical rather than chemical in nature.

The evidence is given in section XXIV. Figures 1, 2, 3, 4 and 7 illustrate the important points. The fibrous filamented structure of asbestos ~~is essential~~ ^{plays} an essential part in the irritating action, since the solid fibers of glass wool do not produce fibrosis (see Figure 6).

- C. Short asbestos fibers do not produce fibrosis.

^{Statement} The ~~conclusion~~ ^{statement} is implied in the evidence mentioned in paragraph B above. Experiments which further support this finding ~~are reported~~ ^{are reported} in Tables 21 and 23.

- D. Typical fibrosis can be produced by an atmospheric suspension of asbestos dust containing only an extremely small proportion of long fibers.

In the inhalation experiment with 100 per cent ball-milled asbestos dust a typical, though delayed, fibrosis was

obtained from Table 1, at least 1 per cent of the asbestos dust consisted of fibers longer than 10 microns, as is shown in Table 1'. In contrast, the intratracheal injection experiment with fine asbestos dust containing no long fibers failed to produce fibrosis, (see Table 1').

3. Inhalation of asbestos dust ~~apparently~~ ^{may} does not alter significantly the ~~course of experimental tuberculosis in guinea pigs~~ ^{in guinea pigs}.

~~This conclusion~~ ^{conclusion} is tentative since the evidence on which it is based does not conform with our usual experience. Reference to Table 2 will show that when infection was coincident with dust exposure there was temporary progression of the disease with subsequent healing; when infection was initiated after 2 3/4 months of dust exposure the course of the tuberculous disease was not appreciably altered. In contrast, it has been observed in experiments with mixed dusts containing quartz that if there is a slight progression of the tuberculosis when infection and dust exposure are coincident, this effect is more marked (instead of less, as with asbestos) when infection is initiated after a period of dust exposure.

This conclusion concerning the effect of inhaled asbestos dust on tuberculosis seems justified because in the more sensitive test (infection initiated after a period of dust exposure) there was no appreciable increase in susceptibility to the tuberculous infection. However, since the findings in the asbestos study do not conform with previous experience, and

since the dusting material used in the single experiment dealing with infection contained only a relatively small amount of fibrous asbestos, it is recommended that a further investigation of this phase of asbestos be considered.

7. The formation of asbestos bodies ^{by the action of asbestos} represents a coating of the fibers and ^{the loss of the ability to produce fibrosis.} is due to the formation of asbestos bodies.

Intra-tracheal injection of asbestos bodies failed to produce the typical tissue reaction (see section 36).

The cessation of progressive reaction to inhaled

asbestos dust soon after exposure terminates may be due to the formation of asbestos bodies.

The formation of asbestos bodies is a result of the action of asbestos on the lungs. The formation of asbestos bodies is a result of the action of asbestos on the lungs. The formation of asbestos bodies is a result of the action of asbestos on the lungs.

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