C O P Y

THE SARANAC LABORATORY FOR THE STUDY OF TUBERCULOSIS
OF
THE EDWARD L. TRUDEAU FOUNDATION

Post Office Box 551
7 Church Street

SARANAC LAKE, N. Y.

April 19, 1938

Mr. Vandiver Brown
Johns-Manville Corporation
22 East 40th Street
New York City

Dear Mr. Brown:

I am enclosing herewith a summary of our experiments on asbestosis. As the results differ materially from those obtained in the previous experiment I am appending some comparative photographs. In case you wish to make several copies of the report for the various contributors, I am sending along duplicate prints for this purpose.

Trusting that you may not be too disappointed by the negative character of the results, I am, with very best regards

Sincerely yours,

(s) Leroy U. Gardner, M.D.
Director

LUG:CC
Enclosures

Second Progress Report on Experimental Asbestosis

by the Saranac Laboratory, April 18, 1938

The inhalation experiment with asbestos dust has now progressed sufficiently to permit a more detailed discussion. The conditions defining this experiment are briefly summarized by the following data:

The Dust - is predominantly fibrous asbestos (chrysotile) with some serpentine, dolomite, chromite and magnetite. As received from the plant much of it was too coarse to be inhaled. It was therefore reground in a case-hardened steel ball mill until the maximum particle size was 3 microns and a great majority of the fibres were 1 micron or less in diameter. This reground material was too expensive to use in the ordinary 8x8x8 foot dusting chambers and it was so fine that it packed in the dusting hoppers. Therefore a limited amount of unground material was added to it to set up a dust cloud.

Dust Counts are made with a Greenburg-Smith impinger apparatus from time to time. The average concentrations maintained inside the animal cages daily, 6 days a week* are indicated by the follow-

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*As the animals spent their entire lives in the dusting room they were subjected to a considerable but unmeasured concentration of dust during the remaining 24 hours of each day. Enough dust adhered to their bedding so that at night they were exposed to concentrations of the order of 2 million or less particles per cubic foot.

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ing figures:

Particles more than 10 microns 645,000 per cubic foot of air(light fiel

Particles less than 10 microns 86,100,000 per " " " " " "

471,300,000 " " " " " (dark field

The last figure, obtained by counting the particles under dark field illumination, indicates that there were an excess of fines. In most dusts the ratio of dark to light field counts is 2 or 3 to 1; here it is over 5 to 1.

Size Frequency Counts

Particles less than 2 microns	79.4%
Particles 2 to 5 microns	15.0
Particles 5 to 10 microns	3.4
Particles more than 10 microns	2.0

Again the great preponderance of small particles is indicated.

The first report made on May 5, 1937 pointed out that the inhalation of high concentrations of very finely ground asbestos dust had not produced any significant changes in the lungs of guinea pigs, rabbits, white rats or cats during a period of 5 months.

On March 22, 1938 the dust exposures of these animals had been continued for a period of one year. Representatives of each species were killed and their lung tissues were examined microscopically for evidences of asbestosis. The lung tissues of the guinea pigs were also subjected to chemical analysis. The results have been somewhat surprising.

8 Guinea Pigs - failed to show enough evidence of reaction to the dust to be detected on gross examination of their lungs. Even in thin microscopic sections the changes were too slight to be visualized without magnification (see figure 1). When so observed it was found that only the very earliest stages of asbestosis had developed (figure 2). No fibrosis had appeared; the reaction was very largely one of the dust cells which had surrounded and walled off myriads inhaled fibres and particles of dust. Asbestosis bodies, which customarily form about asbestos fibres in the lungs of guinea pigs and human beings had developed but most of them were extremely small, as would be expected in dealing with such fine dust. A moderate number, presumably from the unground dust were much longer and often extracellular.

The lungs of three of these guinea pigs were analysed chemically for their total mineral and silica content. The results obtained are indicated as follows:

	Dry Tissue		Ash
No.	% Ash	% SiO_2	% SiO_2
8	5.02	0.51	10.23
11	4.58	0.46	10.08
12	5.16	0.54	10.54
Normal Controls	5.61	0.10	1.83
SiO_2 Controls	12.02	5.28	43.88

These figures indicate that the total weight of inorganic material in the lungs is not increased even though the sections reveal great numbers of fine particles within the dust cells. This could be explained on the basis of the very small size of the

particles whose combined weight is negligible . That there are appreciable quantities of silica-containing foreign bodies is shown in the other columns; the dried lung tissue contains 4 or 5 times the normal amount of silica and in the ash the silica content is nearly 10 times the normal amount. When compared with the average figures for a group of animals exposed to pure quartz for one year it will be noted that the total amount of ash and the percentage of silica in the asbestos series is relatively low. The results of these chemical analyses seem to indicate that asbestos dust, even when very finely divided is not as readily inhaled as a particulate dust like quartz.

3 Rabbits - Autopsied after one year of exposure show slightly more cellular reaction in the lungs than the guinea pigs. The changes were visible on gross examination of thin microscopic sections but not on inspection of the lungs as they were removed from the body.

Microscopic examination of the sections disclosed considerable accumulations of phagocytic cells about the inhaled dust. The fibres were unchanged and no asbestosis bodies had developed. There was no evidence of fibrosis. A similar result was obtained in a previous experiment on rabbits.

3 White Rats revealed changes comparable to those in the rabbits without the formation of asbestosis bodies. In two other animals killed on the 8th month the changes were similar in character but less intense. Five other rats have died of intercurrent chronic pulmonary infections. They were discarded as their lungs showed little or no inhaled dust. The findings in this species

confirm those obtained in a former experiment.

8 Cats died of infections within the first 3 or 4 months. Their lungs revealed little or no dust or reaction to it. One cat was killed after one year's exposure. Grossly there was no evidence of dust reaction. Microscopic sections showed numerous scattered phagocytes containing minute particles of asbestos. No long fibres or asbestosis bodies could be found.

The injection experiments with 1 to 3 micron particles of the various fibrous silicates and serpentine mentioned in the previous report have uniformly failed to produce scar tissue either in rabbits or in guinea pigs. Anthophyllite, crocidolite, soda tremolite and serpentine have now been observed for one year. Amosite and amphibole for 6 months. The difficulties attendant upon intravenous injection of chrysotile have not been overcome but the attempt has not been abandoned. All of these minerals are without effect upon the extrapulmonary tissues and none have produced asbestosis bodies.

Comments - At first sight the negative character of these inhalation results might seem discouraging for the original objectives are apparently not being attained. Instead of producing pulmonary asbestosis at a rapid rate the reaction is relatively insignificant. A whole year's exposure to a comparatively high concentration of exceedingly fine dust has not produced enough change to be visible without high magnification of microscopic sections.

The results are not only surprising but they challenge explanation. When they are compared with the findings of a previous inhalation experiment on guinea pigs they suggest that the original

hypotheses accepted in planning the present experiment were erroneous. The very fine dust employed for this test has caused much less reaction than the coarser material used in the first one. This result was obtained in spite of the facts that the amount of fibrous material in the new dust was 3 or more times as great as that in the old, and that the atmospheric concentrations maintained were perhaps 10 or 15 times as great.

The factor of <u>particle size</u> is apparently the one responsible for the difference in results. Most of the asbestos fibres in the lungs of the new experiment animals are small enough to be entirely contained within the body of a dust cell. Some, originating from the unground material placed in the dust hopper to facilitate generation of dust are longer. The short intracellular fibres are treated by the lung more or less like particulate dust. Instead of being concentrated about the terminal bronchioles as in the case with longer fibres, they are scattered widely throughout the air spaces. Few establish contact with the pulmonary framework as they are protected by the bodies of the dust cells which surround them. If their action be chemical, not enough are concentrated in any particular focus to expect appreciable reaction. What little effect there has been might readily be due to the limited number of long fibres. It is unfortunate that any unground fibre was added for otherwise it might have been possible to state with certainty that the short fragments caused no fibrosis.

If these early results are substantiated on subsequent observation of the animals exposed for longer periods, it is believed tha the chemical hypothesis of the action of asbestos

will have to be abandoned. In its place it will be proposed that asbestos fibres act as mechanical irritants. Already there is considerable evidence to support such a hypothesis. The particulate serpentine which has the same chemical composition as the fibrous chrysotile has been proved to be incapable of producing a fibrosis in organs other than the lungs. When chrysotile is ground so finely that it approximates serpentine in physical state it loses its capacity to cause fibrosis. If its action were chemical in nature it should become more active as the particle size decreases for under such circumstances relatively more surface should be presented to the cells and fluids of the body. Quartz, whose action has been proved to be physico-chemical, behaves quite differently. The smaller the particles of free SiO_2, the more vigorous and rapid the reaction they promote. Their effects are not restricted to the lungs of certain species but can be elicited in any organ of any animal, bird or fish. In the case of asbestos, on the other hand, irritating properties are only manifested in the lungs, and then only in the lungs of certain species of animals. The smallest fibres, which should theoretically be most active are apparently inert while only the longer ones seem to cause fibrosis. It is inferred therefore that their action is mechanical and that the long stiff fibres are irritating only in the lung because this organ is in active motion during respiration. In sluggishly moving organs like the liver, spleen and subcutaneous tissue the factor of motility is minimized.

The most serious objection to discarding the chemical hypothesis at the present time is the imperfectly known relationship between the asbestosis body and the development of fibrosis. The

asbestosis body must form as the result of chemical reaction. The question which remains unanswered is whether such chemical reaction is also responsible for the development of scar tissue. Some observers are of the opinion that the formation of the bodies is a protective mechanism by which the tissues attempt to coat the irritating asbestos fibre with a smooth non-irritating membrane. Our own experiments and those of others have demonstrated that asbestosis bodies recovered from human lungs by chemical digestion will not produce fibrosis in the subcutaneous tissues of guinea pigs. Their action must also be tested in the lungs before a final answer to this question can be given. The evidence now available suggest that the reaction responsible for the formation of the bodies is not the one producing fibrosis.

But on the other hand, it is known that asbestosis body formation occurs only in tissues which do develop fibrosis in response to local accumulations of asbestos fibres. Thus far only the lungs of guinea pigs, dogs and human beings have responded to the inhalation of asbestos dust by the formation of scar tissue and asbestosis bodies. Injection of asbestos into other organs of guinea pigs produces only occasional bodies and little or no fibrosis. The asbestos corns of the skin in human beings do not contain the bodies. The lungs of species like the rabbit, white rat and cat have shown neither asbestosis bodies nor fibrosis. In their case it will have to be determined whether the lack of reaction is due to more effective upper respiratory protection which precludes the inhalation of effective quantities of asbestos fibres or whether it is due to chemical factors peculiar to these species which prevent reaction with asbestos.

The possibilities are under investigation and if decisive results can be obtained the validity of one or the other hypothesis should be established.

Significance - while the inhalation experiments have failed to produce pulmonary fibrosis at the rapid rate anticipated they are nevertheless instructive. Verification of the proposed mechanical hypothesis and the establishment of a relationship between the length of fibre and the irritating properties of asbestos would explain the hitherto mysterious distribution of asbestosis in various departments of this industry. In the Thetford mills there seems to be little or no disease in spite of heavy atmospheric concentrations of dust. Here the fibrous elements of the dust are largely very short and hence perhaps not irritating. In the English plants it has been stated that the process of "mattress beating" is productive of the greatest amount of asbestosis. This process could readily break many fibres longitudinally, liberating a dust rich in fragments short enough to be inhaled but long enough to be irritating. In the American factories the amount of disease has not always paralleled the atmospheric concentration of dust. More cases have developed after exposures to the "opening" machines than elsewhere. Since opening is a crushing operation it might produce the most effective lenght of fibre.

If these theoretical considerations can be substantiated, they should be of value in planning a program of prevention. Special precautions would then be instituted in departments where fibrous types of dust are generated. In processing mills dealing largely with the extremely fine asbestos powders employed for insulation only sufficient exhaust to conform to accepted standards of good housekeeping would be adequate. Until the hygienic signi-

ficance of fibre length has been established all asbestos dust must still be considered hazardous.

Summary - An inhalation experiment upon guinea pigs, rabbits, white rats and cats using high concentrations of asbestos fibre ground so finely that most of the particles are under 1 micron in maximum diameter has failed to produce evidence of asbestosis in one year's time. The amount and severity of the reaction is not nearly as great as that produced in a previous experiment by the inhalation of 1/10 to 1/15 this concentration of asbestos dust. The only other appreciable difference in the two experiments is the particle size of the dust. These observations together with those from collateral injection experiments suggest that the injury from asbestos may be mechanical in nature and produced only by fibres which exceed an unknown minimum length. If this ultimately proves to be the case it may explain the puzzling absence of asbestosis in certain very dusty parts of the industry. It may also direct attention to the points where dust collection is most important in preventing the development of asbestosis.

To answer the questions that have now been raised it is planned to continue the inhalation of the fine dust for another two years and to perform certain other injection experiments. To reproduce the pathological picture of generalized asbestosis another inhalation experiment with a dust composed of longer fibre will be started within the near future.