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First Interim Report

ASBESTOSIS AND PULMONARY CANCER

to

Quebec Asbestos Mining Association

by

The Saranac Laboratory
of
The Trudeau Foundation
Saranac Lake, New York

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Submitted by:

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I. INTRODUCTION

An experiment has been in progress at The Saranac Laboratory for some 14 months in an attempt to ascertain the influence of inhaled asbestos dust upon the incidence of pulmonary tumors in mice. The purpose of this study is to obtain experimental data for correlation with experience pertaining to pulmonary cancer in industrial workers exposed occupationally to the inhalation of asbestos dust.

Two strains of mice, one susceptible and the other resistant to the development of pulmonary tumors, have been exposed to the asbestos dust; comparable numbers of control animals of each strain have been kept in normal air but under conditions of housing and diet identical with those of the experimental animals. The experiment has been designed to reveal a possible earlier onset of tumors in the exposed animals than in the control group as well as to determine the total incidence of tumors occurring over the entire period of the experiment.

It may be stated here that the evidence obtained reveals no tendency towards an earlier onset of tumors among exposed animals in comparison with control animals. Nevertheless, there does appear to be a trend toward a greater incidence of tumors in the exposed animals than in the control animals. It is important to note, however, that statistical analysis of the data obtained up to the present shows that the differences between the experimental and the control animals are no greater than might be expected to occur by chance alone. The experiment is not completed and it is considered likely that, if the ratios of tumor incidence at the next period when animals

are killed are similar to those for the 14-month interval, the trend which is now noted may finally be shown to be statistically significant because of the larger total number of animals observed.

II. MATERIALS

A. Dusting Material: The dust used in this experiment was received on January 5, 1951, from the Johnson Company Asbestos Mines of Thetford, Quebec, and was given the Saranac Laboratory number MD-136. X-ray diffraction analysis revealed that the material contains a variety of minerals, of which the serpentine group amounted to 70 per cent. Though the particulate mineral serpentine and the fibrous mineral chrysotile yield the same diffraction pattern, petrographic examination showed that there was considerably more chrysotile than serpentine present in the material. Much of the chrysotile occurred in thick unopened bundles, but it was estimated that at least 25 per cent of the chrysotile was present as individual fibers or partially opened bundles. Other components, largely particulate, included a few per cent of magnetite, chromite, brucite, talc, and quartz, and traces of miscellaneous minerals. The quartz content was about 1 to 2 per cent. Of the particulate material at least 85 per cent of the particles were less than 5 microns in size.

Studies of samples of atmospheric dust taken from the animal exposure room revealed, with respect to the length of the chrysotile fibers, that the fibers occurred in the following distribution of sizes:

Fibers less than 5 microns in length	32.3 %
" from 5 to 10 " " "	35.8
" " 10 to 20 " " "	20.7
" " 20 to 50 " " "	8.3
" " 50 to 100 " " "	2.7
	99.8 %

B. Animals: The mice used in this experiment were obtained from the Jackson Memorial Laboratory at Bar Harbor, Maine. That laboratory carries colonies of two different strains of mice, identified as Strain A and Strain C, susceptible to pulmonary tumors. Strain A, under the usual practice of deferring the observation for tumor until the mice approach old age, has a natural incidence of pulmonary tumors that approximates 95 per cent. Strain C, under similar circumstances, has an incidence of the tumors of about 60 per cent. Since in the present experiment it was proposed to study the influence of dust exposure upon the over-all incidence of tumor, Strain A was considered unsuitable for this investigation because any increased incidence above the normal rate of 95 per cent would be difficult to ascertain. Strain C was selected, therefore, because it had a lower incidence of pulmonary tumor and because it offered certain other advantages, among which was the fact that the incidence of mammary carcinoma, in Strain C, which might interfere with interpretation of the pulmonary lesions, was low.

Of the several available strains of mice resistant to the development of pulmonary tumors, the Strain C 57 black was chosen because mice of this strain were readily available in sufficient numbers to permit obtaining a colony of an age and sex ratio comparable to that of the Strain C colony.

III. METHODS

A. Dust Dissemination: The technic devised at The Saranac Laboratory some fifteen years ago for the dissemination of asbestos dust into the air for experimental purposes is being used in the current study. The method consists essentially of mechanical agitation

of the asbestos dust by a rotating paddle in a large hopper filled with asbestos dust to approximately the level of the axle of the paddle. The edges of the paddle are equipped with stiff wire brushes to break up the small balls of asbestos which tend to form. The dusting mechanism is kept in operation inside the 8-foot cubical dust room for 8 hours a day, 5 1/2 days per week. Animals live continuously in the room, which is ventilated with fresh clean air when the dust disseminator is not in operation. Atmospheric samples are collected in the room at regular intervals with a Greenburg-Smith midget impinger and dust counts made by the light field technic with a microprojector. The dust counts here averaged 617 million particles per cubic foot of air. In general, the fibers have represented about 6.6 per cent of the total count, or 53.8 million. Of the total number of fibers, about 11 per cent are longer than 20 microns. It will be recalled that, in regard to the production of asbestosis, the fibers greater than 20 microns in length are of the greatest importance.

B. Animals: At the start of the experiment 260 mice were placed in the dust room; 130 of these were Strain C mice, and 130 were Strain C57 black mice. Of each strain 30 were males and 100 were females. This sex ratio was dictated by space requirements, since male mice tend to fight and must be kept in small groups, while many females may safely be placed in a single cage. The total number of control mice was 340, with a sex distribution similar to that of the exposed groups.

In order to follow the development of tumors and to observe any possible earlier onset of tumors among the exposed mice, experimental animals and control animals were killed at regular intervals after the

beginning of the exposure to dust. The intervals chosen were 2, 4, 6, 10, and 14 months; the next interval has been set for 16 months. At each period 34 experimental and 34 control animals were killed; of each group of 34 animals, 17 were Strain C and 17 were Strain C57 black. In each case, the 17 animals included 4 males and 13 females.

IV. RESULTS

The observations made at each of the periods when animals were killed are presented in table 1. It will be noted that the total number of animals examined in each of the different groups varies slightly. This variation is the result of a few spontaneous deaths in each group. The observations made in such cases have been incorporated in the data for the next period. In many instances animals dying spontaneously have been eaten by their cagemates. It has been necessary to discard these animals completely from the record of experimental observations.

It will be noted that within Strain C there is a higher percentage of tumors among animals exposed to asbestos dust than among the controls. The same situation is true within the Strain C57 black. Obviously, the same condition is true also of the over-all figures combining both strains.

The significance of all these percentage incidence figures has been tested statistically by means of the well-recognized formula:

$$S.E. = \sqrt{\frac{P_1 \cdot Q_1}{N_1} + \frac{P_2 \cdot Q_2}{N_2}}$$ where

S.E. = Standard error of difference in incidence between two groups

N_1 = Total number of animals in the group

P_1 = Per cent of animals with tumor in a specific group of animals

Q_1 = " " " " without tumor in the same specific group

(Thus, $Q_1 = 100 - P_1$)

N_2, P_2, Q_2 } Corresponding symbols for another group of animals

It will be recalled that if an observed difference in percentage incidence between two groups is greater than twice the standard error of that difference, the difference may be considered to be significant and due to factors other than chance. If the observed difference is less than twice the standard error, it is very likely due simply to chance. In table 2 the observed difference in the incidence of pulmonary tumor and the critical value of twice the standard error of difference are compared for the various groups. It will be noted by comparing the values that none of the observations made to date are statistically significant. An example of the method of calculation follows.

Calculation of standard error of difference between over-all incidence of tumors in the control animals and in the experimental mice from data in table 1:

Incidence of tumors: Experimental animals 9.5%

Control animals 5.5

Number of animals observed: Experimental animals 179

Control animals 181

$$S.E. = \sqrt{\frac{9.5 \times 90.5}{179} + \frac{5.5 \times 94.5}{181}} = \sqrt{4.80 + 2.87} = \sqrt{7.67}$$

$$= 2.77$$

$$2 \times S.E. = 5.5$$

Thus a difference up to 5.5 per cent is likely to occur by chance. The observed difference of 4 per cent (9.5 - 5.5 = 4.0) may therefore have occurred simply by chance distribution of animals and is not statistically significant.

V. COMMENT

It will be apparent from a review of table 1 that the experiment is now in its most important phase. The incidence of pulmonary tumors has begun to rise for animals killed at the 14-month period, both for the control animals and for the animals exposed to asbestos dust. The increased incidence is more pronounced for the group exposed to dust, but the rise has occurred at about the same time in both groups. The differences between the two groups, however, as has been noted, are not statistically significant.

The impression is gained from an inspection of table 1 that the large numbers of animals killed early in the experiment are weighting the final figures and detracting from the magnitude of the incidence of tumor. It must be noted that this study has been an experimental one and that it has been necessary to make observations by killing animals for study at regular intervals. It appears that by means of a rather involved statistical procedure the disproportionate weighting of the values by the data of the early observations can be eliminated. This procedure will be carried out after final observations have been made, since it is too laborious an undertaking to be performed at the present stage when the values obtained would be only of academic interest.

It may be noted in passing that if the over-all incidence of tumor among the exposed animals had been only slightly greater—11 per cent rather than 9.5 per cent—the difference between the incidence for the exposed group and for the control group would have been statistically significant.

VI. SUMMARY

An experiment concerning the influence of inhaled asbestos dust upon the incidence of pulmonary tumors in mice has been in progress for 14 months. Analysis of the results of this experiment reveals that thus far (after the animals have been exposed to asbestos dust for 14 months), the dust has not exerted an influence of a degree sufficient to cause statistically significant alterations in the incidence of pulmonary tumors. The final phases of this experiment will be of great importance, since at the most recent period when animals were killed for study the incidence of tumors had increased and the increase seems to be more pronounced among the animals exposed to asbestos dust than among the non-exposed control animals.

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Table 1

Occurrence of Pulmonary Tumors

in Mice Exposed to Asbestos Dust and in Mice Not Exposed to Dust

Strain of mice	Period of observation	Number of mice with tumor in the group exposed to asbestos dust		Number of mice with tumor in the control group not exposed to dust	
	months	Male	Female	Male	Female
Strain C (Tumor susceptible strain of mice)	2	0	0	0	1
	4	0	1	0	0
	6	0	1*	1	0
	10	0	2	0	1
	14	1	7	0	4
			12		7
Number examined:		22	72	20	72
			94		92
Incidence of tumor:		12 ÷ 94 = 12.8%		7 ÷ 92 = 7.6%	
Strain C57 Black (Tumor non-susceptible strain of mice)	2	0	0	0	0
	4	0	0	0	0
	6	0	0	0	0
	10	0	1	0	2
	14	2	2	0	1
			5		3
Number examined:		19	66	20	69
			85		89
Incidence of tumor:		5 ÷ 85 = 5.9%		3 ÷ 89 = 3.4%	
Incidence of tumor in both groups:		17 ÷ 179 = 9.5%		10 ÷ 181 = 5.5%	

* Malignant

Table 2

Difference in Incidence of Pulmonary Tumors

in Mice Exposed to Asbestos Dust and in Mice Not Exposed to Dust

Groups of mice compared	Observed difference in incidence	Twice the standard error of difference	Statistical significance of observed difference
	per cent	per cent	
Strain C asbestos group vs Strain C control group	5.2	8.8	Not significant
Strain C57 black asbestos group vs Strain C57 black control group	2.5	6.5	Not significant
All asbestos groups (both strains) vs All control groups (both strains)	4.0	5.5	Not significant
All asbestos groups at 10 and 14 months vs All control groups at 10 and 14 months	8.3	13.0	Not significant
All asbestos groups (total incidence) vs All groups * (total incidence)	3.1	4.8	Not significant

* The values for all groups (total incidence) are based upon observations for the asbestos and the control groups and also for groups exposed to two other dusts--iron oxide and quartz--in another experiment.