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Pulmonary Tumors in Mice Exposed to Asbestos Dust

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Experimental Study

Since the first case of carcinoma of the lung associated with asbestos was cited by Lynch and Smith in 1935,¹ there have been numerous reports in the literature of similar cases. Authors reviewing the literature describe an incidence of carcinoma of the lung associated with asbestosis ranging from 13% to 16%.²⁻⁴ Individual reports, usually dealing with relatively small numbers of cases, describe an incidence ranging from 7% to 50%.^{3,4} In an editorial in 1955, Hueper⁵ stated that 127 cases of asbestosis carcinoma of the lung were on record and described the occurrence of 114 cases of pulmonary carcinoma in 738 autopsies of asbestosis cases collected from the literature (15%).

Experimental work has not kept pace with the accumulation of clinical data. An increased incidence of pulmonary tumors has been recorded in mice following the subcutaneous injection,⁶ intratracheal injection,⁹ intravenous injection,¹⁰ and inhalation¹¹ of a variety of substances, but only one published statement has been found suggesting the induction of tumors in experimental animals by the inhalation of asbestos.¹²

The purpose of the investigation to be described in this report was to correlate, if possible, the effect of asbestos on the lungs of experimental animals with the suggested carcinogenicity for human subjects.

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Animals.—Mice were selected for this work because the use of inbred strains would eliminate some of the uncertainties inherent in the use of other animals. Furthermore, the published report of Nordmann and Sorge, which has been previously mentioned,¹³ and unpublished data left by Dr. L. U. Gardner,¹⁴ of Sarnac Laboratories, at his death suggested that mice might be suitable test animals. Dr. Gardner mentioned that, of 22 mice inhaling short fiber asbestos for not more than 12 months, 3 (14%) developed lung tumors. Fifty-one mice exposed to other dusts for 10-12 months developed lung tumors in 2%. In both of these studies the number of animals used was small, and the strains employed were not mentioned or apparently varied.

In the study to be described, it was decided to use the F₁ generation offspring of Strain A male mice¹⁵ and Strain C female mice.¹⁶ It was felt that this hybrid strain would possess the genetic purity of the parent stock but would be hardier and hence more suitable for long-term studies.

Strain A breeding stock was obtained from J. J. Bittner, of the University of Minnesota, and Strain C female breeding stock was obtained from Lloyd W. Law, of the National Cancer Institute. As soon as the Roscoe B. Jackson Memorial Laboratories had recovered from the disastrous fire of 1947, all breeding stock was obtained from that source.

ACF₁ animals from the breeding colony were divided into approximately equal groups, and at the age of 2 months one-half were placed in the cages exposed to asbestos dust. The other half were maintained as a control group. Male and female mice were kept in separate cages to avoid breeding in both the control and the dusted groups.

Of the hundreds of mice bred for this work, a great many succumbed to cannibalism, intercurrent infection, or other accidents, but 776 mice were subjected to postmortem examination. Of this number the largest groups were the control and test animals studied at 19 and 24 months of age. The observations in these groups will be emphasized in the discussion which follows.

In order to study larger animals with a tracheo-bronchial tree more comparable to that found in

Fig. 1.—Dusting
equipment for mice:
mouse cage (A), bag
for asbestos (B),
for mixing with
holes directing
dust toward
mouse (C), pulley
support (D), L-shaped
rod (E).

to be mostly irregular pieces of inert granular serpentinite rock. Chrysotile asbestos is the important material in that portion of the substance not passing the 200-mesh screen (Fig. 2). The chemical composition of Canadian chrysotile asbestos, $3\text{MgO} \cdot 2\text{SiO}_2 \cdot 21\text{H}_2\text{O}$, is represented in Table 1 which was supplied by the producers. The data represent high and low limits expressed in percentage.

TABLE 1.—Chemical Analysis of Canadian Chrysotile Asbestos

SiO ₂	MgO	H ₂ O	FeO	Fe ₂ O ₃	Al ₂ O ₃	CaO
52.12-53.36	30.54-33.20	12-15	0.4	1-5	0.2-1.5	0-0.3

The figures in each column represent high and low limits expressed in per cent.

Dust counts were done on numerous occasions, and for extended periods 500,000,000 particles per cubic foot of air were maintained. Counts varied according to time of day, humidity, and other factors, but it was possible to maintain atmospheric concentrations of 150,000,000 to 300,000,000 particles per cubic foot throughout most of the experiment. Dusting equipment was operated for 8 to 12 hours daily for 5 days each week. Samples were collected in water within the mouse cages by means of a standard midjet impinger.

Particle-size determinations on samples of air collected in water from mouse cages in 1950 showed that particles and fibers 10μ in size and over formed 18% to 21% of the total. In 1952 a sample of air collected in 99% isopropyl alcohol and sent to the laboratory of Dr. J. M. Dalla Valle at the Georgia Institute of Technology showed the following distribution of particle sizes: less than 3μ , 15.5%; 3μ - 10μ , 34.0%; 10μ - 20μ , 26.2%, and greater than 20μ , 24.3%.

Results

Control Animals.—At the time that this work was started little was known about AC F₁ mice, and the biologic behavior of these animals was observed with considerable interest. In general, the control colony was maintained without undue difficulty, and most of the animals survived until killed at 19 or 24 months of age. The greatest mortality was in the male group and resulted, apparently, from fighting, but some difficulty was also encountered during the summer months with infectious diseases.

It was originally planned to permit animals to live out a full life span, but exam-

ination, especially microscopically, of animals which were found dead proved very unsatisfactory, and eventually only killed animals were considered in the statistical studies.

The microscopic appearance of the lungs of control animals is shown in Figure 3. Alveolar septa are delicate; alveoli are well expanded, and macrophages, when present, are small and eosinophilic. One or two small collections of lymphoid tissue are frequently present, and inflammatory changes are found but are uncommon. Large foamy macrophages are occasionally found in areas of inflammation.

In Table 2 the occurrence of pulmonary tumors in control mice killed at 19 months of age is recorded. Only those tumors confirmed histologically are included. It should be pointed out that on rare occasions a nodule regarded as a tumor grossly proved to be inflammatory on microscopic examination. On the other hand, tumors were occasionally found on microscopic examination which had not been detected grossly. For practical purposes the results on gross and microscopic examination in the control group were the same.

It is interesting to note that there seems to be a significant difference between the number of male and female control mice

Fig. 3.—Lung of control mouse at 19 months—Mouse 338 (female). Photograph shows a portion of the wall of a bronchiole and related alveoli. Alveolar walls are delicate, and there is a relative absence of intra-alveolar macrophages. $\times 425$.



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TABLE 2.—AC F₁ Hybrid Mice: Controls at Nineteen Months

Sex	No.	Total Mice with Tumors		Mice with Single Tumors		Mice with Multiple Tumors	
		No.	per Cent	No.	per Cent	No.	per Cent
Female.....	119	32	28	26	22	6	5
Male.....	103	48	47	36	35	12	12

The tumors listed are pulmonary adenomas. Only tumors confirmed on microscopic examination are recorded.

bearing pulmonary tumors at 19 months of age. Males developed pulmonary tumors in approximately 47% and females in 28%. However, in a smaller group of mice 20 months and older this ratio was reversed, and, of 43 female controls, 29 developed pulmonary tumors (67%). Of 41 male controls in this age group, 24 developed pulmonary tumors (58%).

The nature of the pulmonary tumors observed in AC F₁ mice has been a matter of considerable interest in this study, and we have not felt warranted in referring to the neoplasms observed as carcinomas. The tumors are basically papillary adenomas, although some are very compact and in some fields do not reveal a papillary appearance. Tumors may be surrounded by small neoplastic masses which we have described as "satellite nodules"; their margins may be sharp or ill-defined, they may extend into the lumen of bronchioles, and they may show very few or many mitotic figures, but we have never found evidence of metastasis. This absence of metastasis is true of all age groups examined, but the largest numbers of mice, as mentioned previously, were in the 19- and the 24-month categories. Some of the tumors do present a relatively malignant histologic appearance, but in 25 consecutive control mice bearing 31 pulmonary tumors, which were studied in great detail, not a single one was classified as malignant, even on a histologic basis.

Other findings of interest in the control animals at 19 months included an abnormality of the reticuloendothelial system in 7%. This entity is characterized by splenic enlargement in most cases and also in some by excessive lymphoid accumulations in the lungs, markedly enlarged lymph

nodes, or hepatomegaly. Blood counts were not done. Four animals, all females, developed extrapulmonary tumors, one of the breast, two of the ovary, and one of the skin. Other breast tumors were seen, but the affected mice did not survive to be killed at 19 months.

Test Animals.—The microscopic appearance of the lung of a mouse dusted for 17

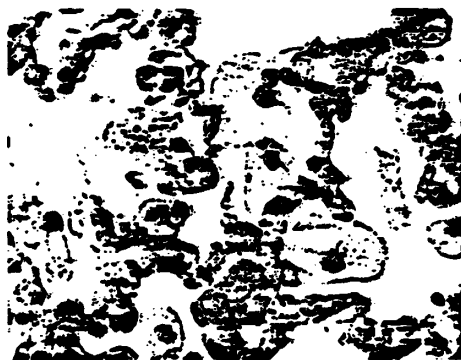


Fig. 4.—Lung of test mouse at 19 months—Mouse B-43 (female). Photograph shows somewhat congested and thickened alveolar walls. Large "dusty" macrophages are prominent. $\times 425$.

Fig. 5.—Lungs of a control animal (Mouse B-38) and of a test mouse (Mouse B-26). Both animals were killed at 19 months of age. In both specimens the hearts are attached and partially hidden by the lungs. The test lungs are larger and heavier and present a mottled gray pleural surface.



B-38



B-26

PULMONARY

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Fig. 6.—Asbestos bodies engulfed by a macrophage. $\times 2000$.

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PULMONARY TUMORS IN MICE EXPOSED TO ASBESTOS DUST

months and killed at the age of 19 months is illustrated in Figure 4. The lungs of dusted animals appeared grayer and larger than normal (Fig. 5). Alveolar walls were thickened; macrophages were a great deal more numerous and were usually foamy and contained a great deal of tiny granular material, "dust." Coarse granular particles of brown material, probably blood pigment, were found in test animals as well as in controls, but larger golden brown particles within macrophages were found only in the dusted animals. These were thought to be

bodies, was also frequently observed in mice. Bodies more than 20μ in length were frequently seen, and the longest recorded length was 40μ . The great majority were shorter than 20μ . Hilar nodes were not noticeably enlarged and apparently were not involved by any specific process related to asbestos. Pulmonary fibrosis did not occur, and squamous metaplasia of bronchial epithelium was not found. Inflammatory changes and bronchiectasis were commoner than in the control animals.

The occurrence of pulmonary tumors in dusted mice killed at 19 months of age is shown in Table 3. Only those tumors confirmed histologically are included.

The microscopic appearance of these tumors was in no significant way different from those described in the control animals. Asbestos bodies were rarely encountered within the substance of the neoplasm.

Comment

Studies referred to previously dealing with human cases of asbestosis strongly suggest that there is an increased incidence of associated carcinoma of the lung. It is important to bear in mind, however, that there are no very large groups of cases, and one is beset with the problem of deciding whether the cases reported are weighted in favor of a high incidence of carcinoma by selection. On the other hand, no consistently high incidence of pulmonary carcinoma has appeared in reports of cases of silicosis. In fact, the incidence of pulmonary carcinoma in silicosis seems to be relatively low, and one writer¹⁷ suggests the possibility that silicosis may protect the patient from the likelihood of carcinoma of the lung.



Fig. 6.—Asbestos body in mouse lung—Mouse 320 (female). Each asbestos body is incompletely engulfed by a large macrophage, and each reveals the typical "stacked coin" appearance. $\times 2000$.

asbestos bodies about small chunks and very short fibers of asbestos. Elongated asbestos bodies (Fig. 6) were found in the lungs of almost every dusted mouse, and 10 to 20 bodies were not infrequently found in one microscopic section. At times one end was globular, but more frequently they were straight or curving golden brown bodies partially engulfed by large "dusty" macrophages. The "stacked coin" appearance, usually described in human asbestos

TABLE 3.—*ACF Hybrid Mice: Test Mice at Nineteen Months*

Sex	Number	Total Mice with Tumors		Mice with Single Tumors		Mice with Multiple Tumors	
		No.	per Cent	No.	per Cent	No.	per Cent
Female	63	23	37	19	25	7	11
Male	64	35	55	19	30	10	16

The tumors listed are pulmonary adenomas; only tumors confirmed on microscope examination are recorded.

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The references which describe animal studies are not regarded as acceptable evidence for the experimental production of asbestosis carcinoma of the lung. Nordmann¹² reports a number of instances and types of epithelial change but found only two pulmonary tumors in 10 mice living to the "cancer age" of 240 days. The number, therefore, is very small, and no suitable control animals are mentioned. Gardner¹³ apparently regarded his observations as merely suggestive. The work was done in a study of pneumoconiosis, and the incidence of naturally occurring pulmonary tumors was not established for the test animals or for the controls.

The animals used in this study, mice, are probably poorly suited for the purpose. The tracheobronchial tree is of small diameter, and the nasopharyngeal filtering apparatus is probably highly efficient. Mice readily form asbestos bodies, but pulmonary fibrosis is not produced. The latter change could be a feature essential to the anticipated epithelial alterations. At least, in studies now in progress in which dogs are used, pulmonary fibrosis is produced and there is a profound associated squamous metaplasia of bronchial epithelium. It is quite possible that future work should be planned about the use of primates. The incidence of newly developing asbestosis in man is fortunately very low at this time, and so the primate best suited for future studies seems to be the monkey.

Asbestos Floats 7-TF-2 is a difficult material with which to work in a climate with high humidity because of its hygroscopic nature. By the use of heat lamps, however, it was possible to keep the storage bin reasonably dry, and all dust counts were certainly far above those in which human asbestosis develops. Even the low counts of 150,000,000 particles per cubic foot are 15 times greater than levels at which human asbestosis is thought to be a danger.

Particle length is a factor in human asbestosis. The quantities of asbestos material of various lengths reaching the lungs

of test mice are not known. It is known, however, that a sufficient quantity of fibrous material reached the mouse lungs to produce asbestos bodies in great numbers and that the same material is capable of producing fibrosis in dogs. If the ability to produce asbestosis, therefore, were associated with carcinogenicity, then Asbestor Floats 7-TF-2 should be capable of producing pulmonary tumors.

If the anticipated life span of a healthy mouse is considered to be 30 months, then the exposure of our animals for periods of 17 and 22 months should correspond to very adequate periods of human exposure. As was mentioned earlier, animals were exposed to the dust at 2 months of age, and the larger groups were killed at 19 and 24 months, respectively.

The results summarized in Table 4 show that there is no striking difference between the total number of control and test animals bearing lung tumors, the figures being approximately 46% for dusted animals and 36% for control animals at 19 months. There are 7 possibilities in 100 that this difference might be due to chance. This difference is not statistically significant.*

There was a considerable occurrence of multiple lung tumors in both the dusted and the control groups. Table 4 shows that this incidence is 18% and 8% for the two groups, respectively. Using the same formula, there are only 0.51 chances in 100 that the difference is a chance difference. This variation is statistically significant. A possible interpretation of this observation is that, given a group of animals with a similar tendency to develop pulmonary tumors, this

*In evaluating the recorded statistics, the standard error is computed on the basis of the following formula:

$$\sqrt{pq(1/N_1 + 1/N_2)}$$

where

p = total percentage occurrence

$q = 1 - p$

N_1 = number in first sample

N_2 = number in second sample

The probability of occurrence of the stated results on the basis of chance is computed from the number of standard errors with Pearl's table.

Group	N
Total Dusted....	18
Total Control....	22

This Table summarizes

tendency is accentuated by asbestos dust, and become evident. It is into the widely held belief that the effect of a susceptible host, then, the more important of host susceptibility.

No appreciable histologic characteristics in the dust. In the absence of the term carcinoma, such neoplasms, specifically, the preformations in the insufficient evidence the occurrence of tumors. The form for multiple foci is a result of at least resistance, noted regardless of logic appearance, regularity of tumor, "infiltration," the cytologic evidence. Enlargement not infrequently pulmonary tumor.

Summary

All 19 mice in the series contained tumors for various lengths.

The largest tumor consisted of 12% after exposure was compared.

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Dust Exposure Incinerators

WILLIAM H. MEGONNELL, M

Introduc

The widespread use of research purposes has been in disposing of radioactive materials for many institutions which may contain experimental animals, embryos, paper used and used planchets. The municipal incinerator is an attractive method for wastes. During the incineration a portion of the radioactive waste is discharged as volatile or particulate combustion gases or is deposited on the interior of the incinerator and the stack. The burning of radioactive wastes with normal incineration may reduce radioactivity. Waste procedures at most of such incinerators provide a satisfactory disposal.

On combustion of radioisotopes in incinerators has indicated that isotopes 132 or 137 remain which is a potential hazard from the incineration and disposal to personnel.

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