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# .lmonary Tumors in Mice Exposed to Asbestos Dust

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ince the first case of carcinoma of the associated with asbestos was cited by ch and Smith in 1935,[1] there have been rous reports in the literature of sim- ases. Authors reviewing the literature ribe an incidence of carcinoma of the associated with asbestosis ranging from to 16%.[2-4] Individual reports, usually g with relatively small numbers of describe an incidence ranging from to 50%.[5,6] In an editorial in 1955, per[7] stated that 127 cases of asbestosis oma of the lung were on record and ibed the occurrence of 114 cases of monary carcinoma in 738 autopsies of tosis cases collected from the literature ).

perimental work has not kept pace with cumulation of clinical data. An in- ed incidence of pulmonary tumors been recorded in mice following the taneous injection,[8] intratracheal in- on,[9] intravenous injection,[10] and inhala- [11] of a variety of substances, but only published statement has been found esting the induction of tumors in ex- imental animals by the inhalation of stos.[12]

he purpose of the investigation to be ribed in this report was to correlate, if ible, the effect of asbestos on the lungs xperimental animals with the suggested inogenicity for human subjects.

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## Experimental Study

*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 Furthermore, the published report of Norumann and Sorge, which has been previously mentioned,[10] and unpublished data left by Dr. L. U. Gardner,[13] of Saranac 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 $F_1$ generation offspring of Strain A male mice[14] and Strain C female mice.[15] 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.

$ACF_1$ 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 tracheobronchial tree more comparable to that found in



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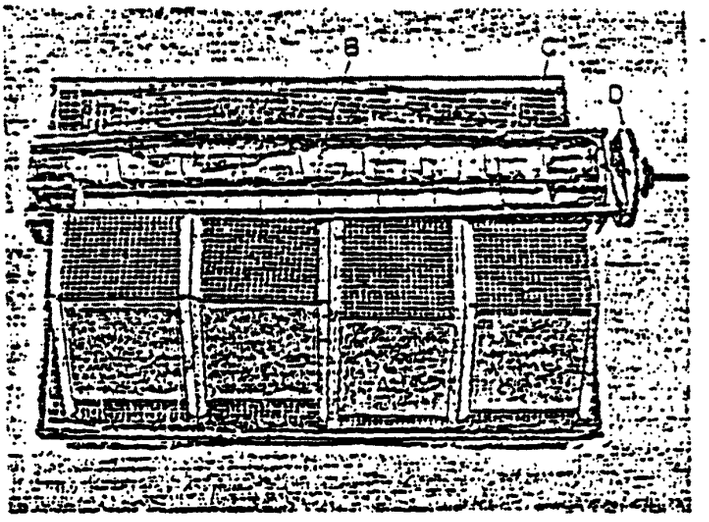



Fig. 1.—Dusting apparatus for mice: (*A*) mouse cage; (*B*), trough for asbestos; (*C*), copper tubing with minute holes directing compressed air toward cages and (*D*), pulley used to rotate L-shaped wire paddles.

man, dogs have also been subjected to dusting with asbestos. The results of this study will form the basis of another report.

Methods and Materials.—Test animals were kept in wire cages on racks in an especially constructed room. Usually 10 or fewer animals were placed in each cage. In the early stages of the work a single metal bin, with a paddle wheel near the bottom, was loaded with asbestos to provide a cloud of dust for the entire room. Later a trough filled with asbestos was run behind each row of cages (Fig. 1), the asbestos was stirred by a series of small rotating metal agitators, and dust was blown toward the cages by multiple fine streams of compressed air. Most of the test animals were dusted in this manner because heavier concentrations could be obtained.

The asbestos used in this work was generously supplied, as was much of the information concerning its composition, by the manufacturers. This material is referred to commercially as Asbestos Floats 7-TF-2. It is described as a "very fine float with little fibrous content" but was selected because of its similarity to "King's Floats," used in other asbestos studies,[4] and because no material with a greater fibrous content in the 10μ-100μ length was available. Kenneth W. Smith, M.D., provided the information that 35.5% of Asbestos Floats 7-TF-2 passes a 200-mesh wet screen. This large proportion of the dust appears



Fig. 2.—Asbestos Floats 7-TF-2. Photograph shows that portion of the material which failed to pass through a 200-mesh wet screen. × 100.



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e mostly irregular pieces of inert granular ntine rock. Chrysotile asbestos is the im- nt material in that portion of the substance passing the 200-mesh screen (Fig. 2). The ical composition of Canadian chrysotile as- s, $3MgO{\cdot}2SiO_2{\cdot}2H_2O$, is represented in Table ich was supplied by the producers. The data esent high and low limits expressed in per- ge.

TABLE 1.—*Chemical Analysis of Canadian Chrysotile Asbestos*

| | MgO | $H_2O$ | FeO | $Fe_2O_3$ | $Al_2O_3$ | CaO |
|---|---|---|---|---|---|---|
| % | 39.34-43.00 | 12-15 | 0-6 | 1-5 | 0.2-1.5 | 0-0.3 |

Figures in each column represent high and low limits ered in per cent.

t counts were done on numerous occasions, for extended periods 500,000,000 particles per foot of air were maintained. Counts varied rding to time of day, humidity, and other ors, but it was possible to maintain atmospheric ntrations of 150,000,000 to 300,000,000 par- per cubic foot throughout most of the ex- ent. Dusting equipment was operated for .2 hours daily for 5 days each week. Sam- were collected in water within the mouse by means of a standard midget impinger.

rticle-size determinations on samples of air ted in water from mouse cages in 1950 showed particles and fibers 10$\mu$ in size and over ed 18% to 21% of the total. In 1952 a of air collected in 99% isopropyl alcohol ent to the laboratory of Dr. J. M. Dalla at the Georgia Institute of Technology ed the following distribution of particle less than 3$\mu$, 15.5%; 3$\mu$-10$\mu$, 34.0%; 10$\mu$-26.2%, and greater than 20$\mu$, 24.3%.

## Results

*Control Animals.*—At the time that this k was started little was known about F, mice, and the biologic behavior of e animals was observed with consider- interest. In general, the control colony maintained without undue difficulty, most of the animals survived until killed 19 or 24 months of age. The greatest rtality was in the male group and re- d, apparently, from fighting, but some culty was also encountered during the mer months with infectious diseases.

was originally planned to permit ani- s 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. ×425.





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

| Sex | No. | Total Mice with Tumors, No. | Total Mice with Tumors, per Cent | Mice with Single Tumors, No. | Mice with Single Tumors, per Cent | Mice with Multiple Tumors, No. | Mice with Multiple Tumors, 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 the 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 successive 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.

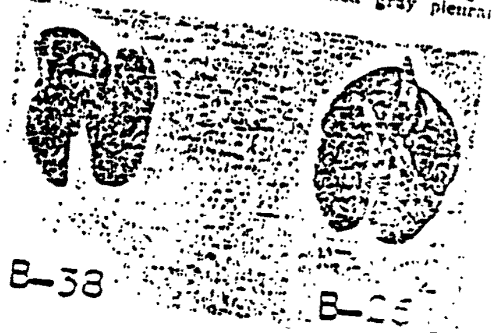
Other findings of interest in the control mice at 19 months included an abnormality of the reticuloendothelial system [illegible]. This entity is characterized by splenic enlargement in most cases and also by excessive lymphoid accumulation in the lungs, markedly enlarged lymph nodes, or hepatomegaly. 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-45 (female). Photograph shows somewhat congested and thickened alveolar walls. Large "dusty" macrophages are prominent. ×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.






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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. Nordmann [12] 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. Gardner [13] 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 however, that a sufficient quantity o material reached the mouse lungs to asbestos bodies in great numbers : the same material is capable of pr fibrosis in dogs. If the ability to asbestosis, therefore, were associat carcinogenicity, then Asbestor Float. 2 should be capable of producing pul tumors.

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

The results summarized in Table 4 that there is no striking difference be the total number of control and test a. bearing lung tumors, the figures beir proximately 46% for dusted animal 36% for control animals at 19 m There are 7 possibilities in 100 tha difference might be due to chance. difference is not statistically significa

There was a considerable occurren multiple lung tumors in both the duste the control groups. Table 4 shows tha incidence is 18% and 8% for the groups, respectively. Using the same fo la, there are only 0.31 chances in 100 the difference is a chance difference. variation is statistically significant. A ble interpretation of this observation is given a group of animals with a s. tendency to develop pulmonary tumors

* In evaluating the recorded statistics, the ard error is computed on the basis of the fo ing 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 ... test on the basis of chance is computed f... the ber of standard errors with Pearl's



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TABLE 4.—AC $F_1$ Hybrid Mice: Summary

| Group | No. | Total Mice with Tumors. | | Mice with Single Tumors. | | Mice with Multiple Tumors. | |
|---|---|---|---|---|---|---|---|
| | | No. | per Cent | No. | per Cent | No. | per Cent |
| Dusted..... | 127 | 58 | 46 | 35 | 28 | 23 | 18 |
| Control..... | 222. | 80 | 36 | 62 | 28 | 18 | 8 |

. Table summarizes the findings described in Tables 2 and 3, and all tumors listed were confirmed on microscopic examination.

...ncy is accentuated by the inhalation of ...s dust, and multicentric foci of origin ... evident. Such an interpretation fits ...e widely held belief that one require- ...for the effectiveness of a carcinogen is ...eptible host. The weaker the carcino- ...e more important becomes the factor ...t susceptibility.

... appreciable difference is noted in the ...gic characteristics of pulmonary tu- ...in the dusted and the control groups. ... absence of any evidence of metastasis ...m carcinoma should be applied to ...neoplasms with great caution. Spe- ..., the presence of small neoplastic ...ons in the vicinity of larger ones is ...ent evidence of malignancy, as is ...rrence of invasion of bronchial lu- ... The former is more likely evidence ...ultiple foci of origin, and the latter ...result of growth in the direction of ...esistance. Both findings may be ... regardless of the histologic or cyto- ...ppearance of the primary lesion. Ir- ...ity of tumor margins, the appearance ...nitration," is similarly unrelated to ...tologic evidence of possible malig- ... Enlarged lymph nodes are encoun- ...not infrequently, but metastasis from ...onary tumors has not been found.

## Summary and Conclusion

... $F_1$ mice were exposed to dusty atmos- ...s containing Asbestos Floats 7-TF-2 ...arious lengths of time.

...e largest single experimental group ...sted of 127 animals killed at 19 months ... exposure for 17 months. This group ...compared with 222 control animals.

Primary lung tumors were demonstrated microscopically in 46% of the dusted mice and in 36% of the control animals.

Multiple pulmonary tumors were demonstrated in 18% of the dusted animals and in 8% of the control animals.

No significant histologic or cytologic difference was observed between tumors in the control and the dusted groups.

No clear-cut proof of malignancy was found in any of the lung tumors studied.

We have been unable to demonstrate any proof of the carcinogenicity of asbestos under the conditions of this experiment, but the increased incidence of multiple lung tumors in dusted animals must be regarded as a possible accentuation by the inhalation of asbestos dust of an existing tendency to develop lung tumors.

Future experiments with asbestos should include the use of larger animals, probably monkeys.

The equivocal nature of this report does not answer the questions raised by accumulating clinical evidence that asbestosis is strongly associated with, and therefore a possible cause of, pulmonary carcinoma in man.

In the choice of experimental animals very helpful advice was supplied by H. B. Andervont, Walter Heston, and Lloyd W. Law, of the National Cancer Institute. C. D. Coleman, Building Superintendent at the Medical College of South Carolina, provided valuable assistance in designing and maintaining the mechanical equipment. Advice and assistance in the technique of dust counting were provided by W. G. Crosby, of the South Carolina State Board of Health, and Dr. L. M. Petrie, of the Georgia State Board of Health. Further assistance was rendered by Robert A. Brown and Miss Ethyl McMinn, of the Department of Medical Illustration, Medical College of South Carolina.



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