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ASBESTOS DUST DEPOSITION AND RETENTION IN RATS

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Introduction

In previous animal dusting experiments histological studies have established differences in the fibrogenic properties of various types of asbestos (Wagner, 1963 and elsewhere in this monograph). Experiences with human cases of pleural mesothelioma have indicated that these have occurred mainly in association with the inhalation of crocidolite, but in a current research project the intrapleural inoculation of a variety of dusts — chrysotile, crocidolite, amosite, and silica — into rats has induced pleural mesotheliomas. The amount of dust inoculated, 20 mg., appeared by histological examination to be greatly in excess of the amount found in the majority of human cases of pleural mesothelioma.

In an attempt to clarify the position a series of dusting experiments was planned, and for this it was desirable to produce an experimental method by which equal amounts of any asbestos dust could be deposited in the lungs of rats by an inhalation method. Additionally it was necessary to ascertain whether the various types of asbestos were retained in the lungs to the same extent and had a similar histological distribution. With these objectives a method was devised for producing the dust clouds and a preliminary experiment carried out.

Methods

Materials

Fifty-seven male and 58 female standard Wistar Albino rats were available for the experiment, and these were randomly allocated on a statistical basis into 5 groups. Four of the groups each containing 22 male and 12 female rats were exposed to airborne clouds of chrysotile, crocidolite, amosite, or powdered glass; the first group was not exposed to dust, and the rats making up this group were used as controls. The powdered glass was included as an inert nonfibrous dust for comparative purposes.

Rhodesian chrysotile and South African crocidolite and amosite were obtained in the most finely ground commercial grade, and each was further ground in a microhammer mill to break down any compressed lumps.

Dust Cloud Production

The airborne dust clouds were generated by redispersing the ground asbestos, and the method is indicated schematically in FIGURE 1. The as-

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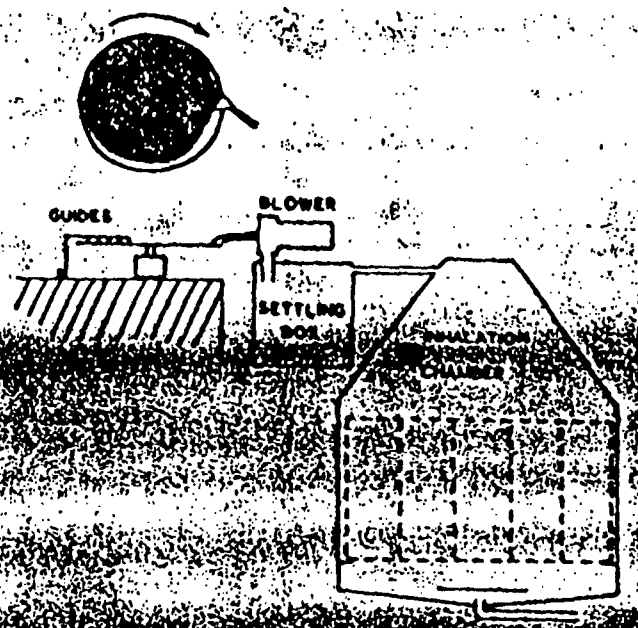

FIGURE 1. Asbestos dust inhalation unit.

bestos was loaded as an even deposit on the aluminum plate, shown in plan and elevation, which was rotated at the rate of three revolutions per hour. Guides suspended above the plate maintained a regular flow of dust to the periphery where it was picked up at the extended inlet of a smaller blower, which delivered it to the settling box. The dust aggregates were deposited here, and the fine dust was carried into the inhalation chamber. For the powdered glass a Wright Dust Feed Mechanism (Wright, 1950) was used to produce the dust cloud. The cage in the inhalation chamber was subdivided into 25 compartments so that each rat occupied an individual cage.

Dust Cloud Measurements

Gravimetric dust sampling was used to measure the concentrations of the airborne dust clouds, and measurements of the total dust, including particles of all sizes, were made by drawing air at a steady rate through a paper filter. To measure the concentrations of the dust which, by virtue of its free-falling speed, could reach the alveolar regions of the lung, a Gravimetric Thermal Precipitator (Wright, 1953) fitted with a horizontal

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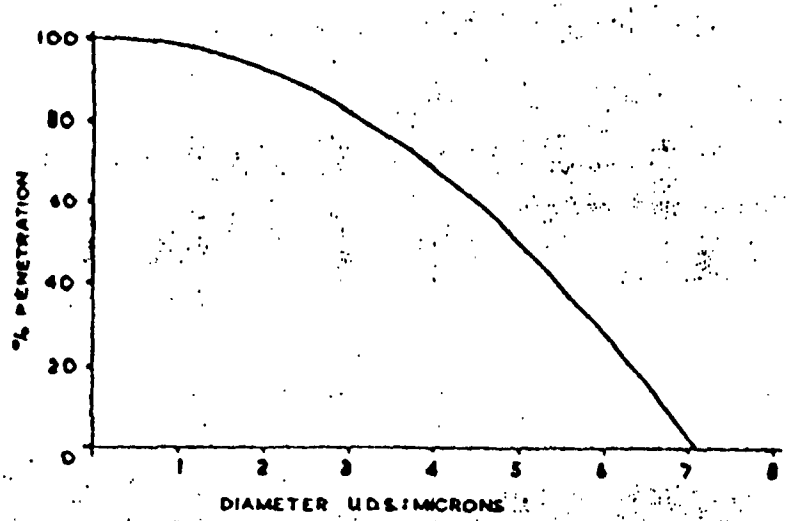

FIGURE 2. Size selection characteristic of horizontal elutriator.

elutriator was used. The design size selective characteristic of the elutriator is shown in FIGURE 2. The free-falling speed of a particle is dependent on its size, shape, and density and is conveniently expressed in terms of the sphere of unit density having the same falling speed. Particles with falling speeds greater than that of a 7.1 micron unit density sphere would fail to penetrate the elutriator and so were excluded from the collected sample. A previous experiment with an airborne cloud of glass spheres had shown that particles larger than this are very rarely found in the rat lung (Cartwright & Skidmore, 1964). The sampler was placed immediately below the vacant cage compartment and sampled continuously during each exposure period. A few samples were also obtained in each inhalation chamber with a Long Running Thermal Precipitator, which incorporates a similar elutriator, to determine the particle length distributions.

Duration of Exposure and Survival

The rats were exposed to the dust clouds for 7½ hours per day, on 5 days of each week over a period of 6 weeks. At the end of each day's exposure the rats were transferred from the inhalation chambers to larger cages in a clean atmosphere keeping the rats exposed to each dust cloud separated from the others. One day after the last day of exposure randomly selected groups of 4 male and 4 female rats exposed to each dust cloud

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were killed with chloroform. Similarly selected groups of rats were killed 29 and 58 days after the last day of exposure.

Lung Isolation and Analysis

Following the method of Mottura (personal communication), the trachea was tied off before opening the thorax and removing the lungs which were then fixed in normal saline. After four days a sagittal slice was taken from the left lung for histological examination. Both lungs, including all the material except for three histological sections, were then available for the determination of the dust content.

It was expected that the weight of dust retained in the rat lung would be of the order of 5 mg. and since all the types of asbestos contain appreciable amounts of silica, the lung silica content was determined using a colorimetric method (King *et al.* 1955). The weights of dust were calculated by reference to the similarly determined silica content of each of the dusts collected by the size-selective samplers.

To assist in the interpretation of the results the dust was extracted from the lungs of two of the rats exposed to each dust cloud using a formamide digestion technique. This dust was completely dispersed for microscopical examination.

RESULTS

The rats were randomly allocated into the 5 groups, and each rat was weighed and given an identification mark. The average weight of the male rats was 181 gm. and 180 gm. respectively, with no significant difference between the dust groups. The four inhalation units were set up and the dust cloud generators adjusted to give approximately the same concentration as measured by the Gravimetric Thermal Precipitator (G.T.P.). The rats were exposed for 30 daily dusting sessions over a period of 6 weeks during which time 2 rats died of bronchiectasis. The samples of total and respirable dust, obtained over the whole period of exposure,

TABLE 2

DUST CONCENTRATIONS

Dust	mg./m³			
	Chrysotile	Crocidolite	Amosite	Glass
Total Dust	66.6	49.5	43.6	71.5
Std. Deviation	20.6	13.1	13.3	15.0
Respirable Dust	36.1	38.8	31.0	31.0
Std. Deviation	6.3	7.7	12.5	6.0

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were evaluated at the end of each day. The average dust concentrations, together with the standard deviations about the average values, are given in TABLE 1. The length distributions obtained from the Long Running Thermal Precipitator samples are shown in FIGURE 3. Since all the particles penetrated the elutriator it follows that their free-falling speeds would not prevent their penetration to the alveolar regions of the lung.

Four male and four female rats from each dust group were killed 1, 29, and 58 days after the last day of exposure and their lungs extracted by the method previously described. Histological sections were obtained from the left lung of each rat.

Histology

Examination of the histological sections from the animals exposed to crocidolite and amosite dust showed similar features in the animals killed

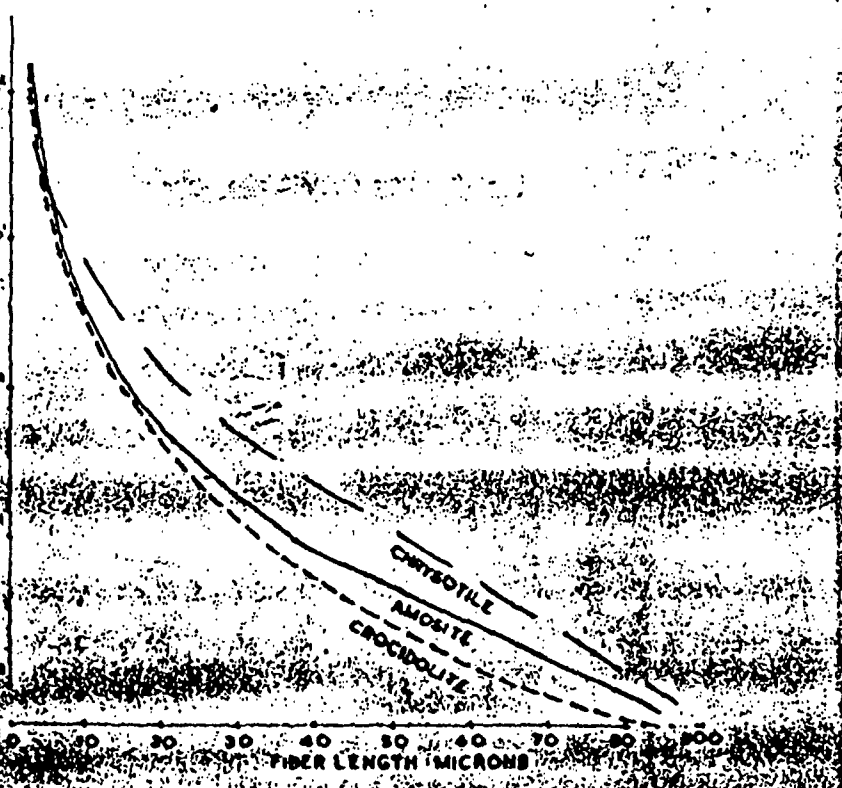

FIGURE 3. Length distributions of airborne asbestos dusts.

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24 hours after the final exposure. Recognizable fibers and dust particles were observed in alveolar phagocytes that were concentrated in the alveoli arising directly from the respiratory bronchioles. The majority of the fibers were within the phagocytes, but the larger ones projected out from them. A few phagocytes were observed lying free in the large bronchioles and bronchi.

Sections from the animals killed on the 29th and 58th day after exposure showed the phagocytes in the same distribution with progressive organization in the alveoli; some of the phagocytes had fused to form giant cells.

In the sections from the rats exposed to chrysotile and killed 24 hours after exposure, phagocytes were seen in similar distribution to those previously described, but in striking contrast only occasional small fragments of fiber were present in these cells. In the rats killed 29 days after exposure these phagocytes had become large, foamy, and vacuolated, and only very occasional fragments of fiber were seen. By the 58th day, although the organization was similar to that seen in the animals exposed to crocidolite and amosite, no evidence of the dust was observed.

In the lungs of the animals exposed to powdered glass, the distribution of the dust containing phagocytes seen in the sections from the rats killed 24 hours after the end of exposure was much more widespread. Phagocytes heavily laden with dust were seen scattered through the airspaces. By the 29th day these phagocytes were mainly concentrated in the alveoli arising from the respiratory bronchioles, and by the 58th day these alveoli were packed with phagocytes. There was little evidence of organization even in this group.

Lung Dust Analysis

The quantitative assessment of the weight of dust in the rat lungs was

TABLE 2.
WEIGHTS OF DUSTS IN RAT LUNGS

Days after exposure to dust	Sex	Mean Weight of Dust in Lungs ± Std. Error of mean, Mg.			
		Chrysotile	Crocidolite	Amosite	Glass
1	M	0.98 ± 0.06	3.98 ± 0.39	4.10 ± 0.28	8.04 ± 0.81
	F	0.58 ± 0.12	3.90 ± 0.61	3.26 ± 0.32	5.60 ± 0.56
29	M	0.33 ± 0.07	3.34 ± 0.09	3.32 ± 0.37	5.60 ± 0.20
	F	0.37 ± 0.15	2.80 ± 0.41	2.78 ± 0.16	4.30 ± 0.18
58	M	0.18 ± 0.02	1.76 ± 0.31	2.88 ± 0.62	6.60 ± 1.37
	F	0.15 ± 0.03	1.76 ± 0.18	2.18 ± 0.16	3.80 ± 0.49

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made by first ashing each lung and determining the silica content colorimetrically. The weights of dust were calculated by reference to the silica content of the airborne dusts, similarly determined, after allowing for the silica content of unexposed rat lungs. The values are presented in TABLE 2.

The silica content of the size-selected dust samples collected from the airborne clouds of chrysotile, crocidolite, amosite, and powdered glass were 40, 50, 51, and 78 per cent, respectively, and the lungs of the unexposed control rats contained 0.11 mg. silica (range 0.05 – 0.15 mg.). There were small differences between the airborne dust concentrations. The sampling rate of the G.T.P. was 100 ml./min., which approximates the minute volume of the rat, and the net uptake of the dusts over the exposure period can usefully be compared by expressing the weight of dust found in the lungs of the rats killed one day after the last exposure as a percentage of the cumulative weight of dust collected by the G.T.P. The comparative uptakes of the four ducts are shown in TABLE 3. The uptake of the chrysotile is only about one-third that of the crocidolite and amosite; the uptake of the powdered glass is twice as great.

TABLE 3.
THE UPTAKE OF DUSTS IN THE RAT LUNGS

Dust	Chrysotile	Crocidolite	Amosite	Glass
G.T.P. mg.	36.4	38.9	41.8	43.4
Lung mg.	0.83	2.0	2.7	6.8
$\frac{\text{Lung}}{\text{G.T.P.}} \times 100$	2.3	5.1	6.5	15.8

The uptake of dust is the end product of continuous deposition and elimination of dust during the inhalation period. To determine the comparative elimination rates the average weights of the dusts found in the lungs of the rats, male and female, after the three survival times have been plotted on logarithmic-linear coordinates in FIGURE 3. The elimination curves are approximately exponential, and the slope of the weighted regression line for each dust gives the elimination rate. The slopes for [illegible] amosite, and crocidolite [illegible] are not significantly different at the 5 per cent level of significance. The slope of the line for chrysotile is −0.26, practically three times greater. The differences in the weight uptake of the three asbestos dusts in the lung at the end of the period of exposure can thus be explained by reference to the elimination rate, and the weights of dust found are quite compatible [illegible]

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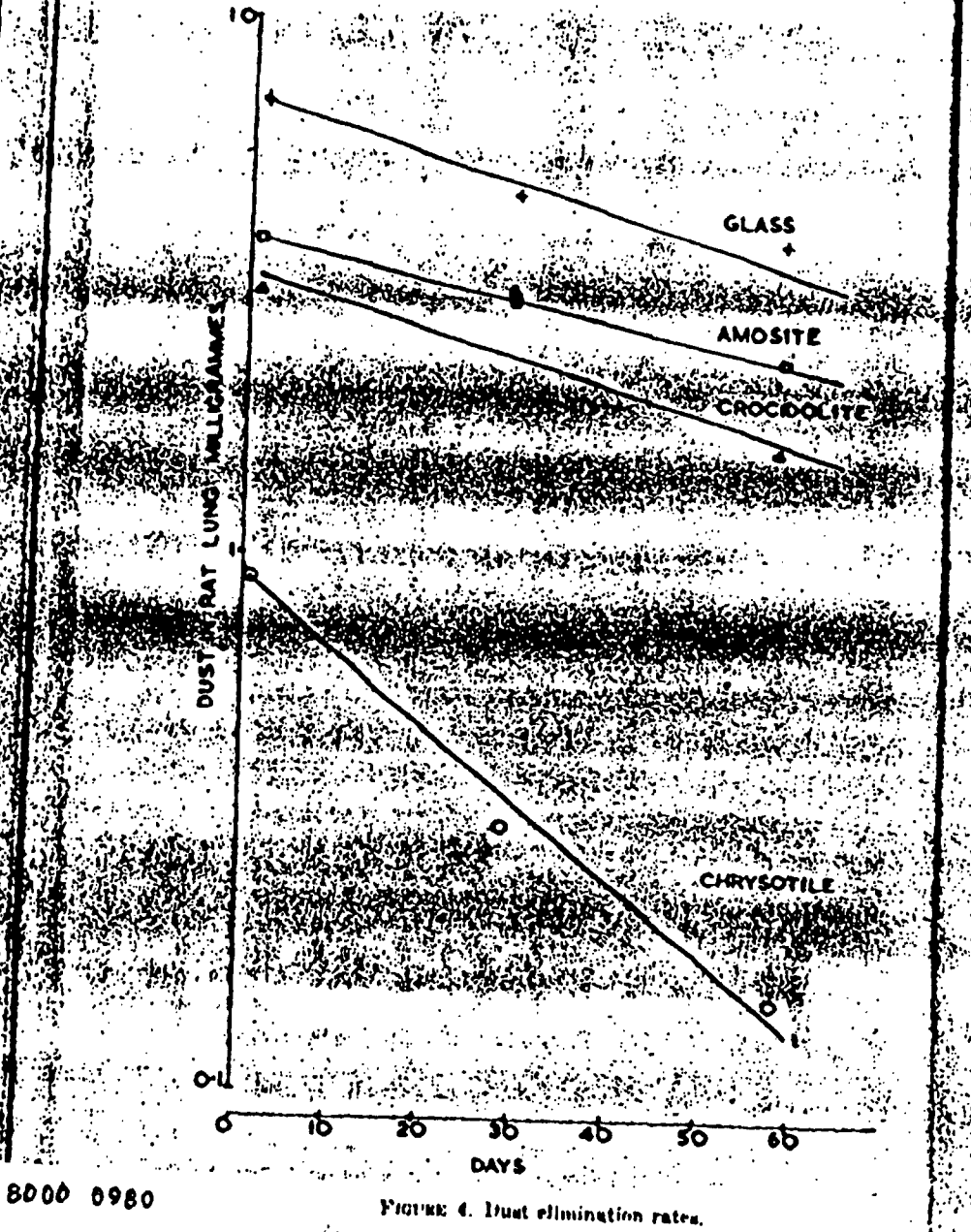

FIGURE 4. Dust elimination rates.

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with depositions of the dust directly related to the weight of dust collected by the size-selective sampler.

Thoroughly dispersed samples of dusts extracted from the lungs of rats killed one day after exposure and of the dusts collected by the G.T.P.'s were prepared by stirring a small amount of dust in a viscous solution of 5% nitrocellulose in amyl acetate and examined microscopically. The size distributions of the dust extracted from the lungs and the G.T.P. dust from the powdered glass chamber were very similar, both containing particles with projected diameters up to 10 microns, but from the crocidolite and amosite chambers a marked difference was observed between the lung and sampled dusts. The dust from the samplers contained fibers up to 250 microns long but fibers longer than 50 microns could not be found in that extracted from the lungs.

The size selection of the horizontal elutriator takes no account of the shapes of particles except in so far as they affect the free-falling speed. The difference in the uptakes of the compact glass dust and of the fibrous asbestos dust clearly indicates the higher efficiency of the upper respiratory tract in preventing the penetration of the longer fibers to the alveolar regions.

Discussion

A method of producing asbestos dust clouds has been devised and an animal inhalation experiment carried out to test it. Observations on the histological distribution of the dusts in the lungs of the rats have shown that the dusts tend to accumulate in the alveoli arising directly from the respiratory bronchioles. The weights of the asbestos dusts found in the lungs of the rats at the end of exposure were found to differ considerably, but the subsequently determined elimination rates indicated that the weights of dust deposited were in a constant ratio with the weights of dust collected by the size-selective sampler. To deposit an equal amount of a non-fibrous dust, allowable must be made for the reduced efficiency of the upper respiratory tract in preventing the penetration of the compact dust particles to the alveolar regions. The elimination rate of Rhodesian chrysotile has been found to be three times greater than that of the amosite and crocidolite, which suggests an explanation for the previously observed reduced fibrogenicity of this dust. The reason for the difference in the elimination rate remains to be determined.

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