THE EARLY EFFECTS OF CHRYSOTILE ASBESTOS DUST ON THE RAT LUNG

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PLATES VII–XI

The name asbestos is used to cover a group of fibrous mineral silicates. Those of commercial importance include chrysotile, a hydrated magnesium silicate, approximately $3MgO.2SiO_2.2H_2O$, amosite $3MgO.11FeO.16SiO_2.2H_2O$ and crocidolite or blue asbestos, an iron sodium silicate approximately $Na_2O.3FeO.Fe_2O_3.8SiO_2.H_2O$. The compact masses of fibres occur between layers of other rocks. There is sometimes mineral matter of other types between the fibres which, in the case of chrysotile, may be serpentine, a hydrated magnesium silicate chemically identical with chrysotile but having a foliate form.

Chrysotile asbestos has been shown by electron microscopy to have a tubular structure (Whittaker, 1956; Maser, Rice and Klug, 1960), with concentric layers of siloxan lattice and down the fibre a central hole, which contains platy fragments, probably unrolled or shattered pieces of the chrysotile tubes. The finest fibres have a diameter of about 0·02 μ.

Asbestos dust was first implicated as a cause of pneumoconiosis at the beginning of this century, but until some 30 yr later very few cases had been described. For some years there was a heavy incidence but, as a result of the introduction of rigorous precautions in the factories, the number of cases has fallen very considerably.

As now seen in workers in asbestos textile processes, asbestosis is a chronic disease; it may take 15 or 20 yr to develop. According to Knox and Beattie (1954*a* and *b*) the degree of fibrosis is related to the time that has elapsed since the first exposure to the dust rather than to the mineral content of the lungs. Wyers (quoted by Perry, 1947) considered that the disease took a more acute form when the dust concentrations were very high.

A characteristic of the disease in man is the presence in the lungs of numerous "asbestos bodies", which consist of fibres of asbestos, or altered asbestos, coated with protein. Beattie (1961) has suggested that the rapid breakdown of these bodies, often many years after the dust was inhaled, may immediately precede a phase of rapid fibrogenesis.

Experimental studies on animals were made, particularly by Stewart (1930), by Vorwald Durkan and Pratt (1951) who administered dust by inhalation and by intratracheal injection, and by King, Clegg and Rae (1946), who injected suspensions of dust into the trachea of rabbits. In certain cases and with certain varieties of asbestos, fibrosis was induced in the lungs of these animals. In some cases asbestos bodies also were observed. Vorwald *et al.* (1951) described differences in the response of different species to asbestos dust. Guinea-pigs showed definite fibrosis and asbestos bodies, rats fibrosis but no bodies, mice bodies but no fibrosis, and rabbits slight fibrosis but no bodies; rabbits gave no response. The results were, to some extent, anomalous, but they were interpreted by Vorwald *et al.* as meaning that asbestos is fibrogenic because of its fibrous nature—a result at variance with

the observations of other workers who have studied asbestosis in man. Vorwald *et al.* believed that fibrosis was not produced by particles shorter than 20 μ, but King *et al.* produced an interstitial fibrosis with particles as short as 2·5 μ.

Earlier workers had no fully satisfactory method of producing asbestos suitable for administration to animals by inhalation. In Gardner's laboratories opened asbestos was stirred in a hopper by wooden paddles. King *et al.* used microtome sectioned fibres of graded sizes for intratracheal injection. Some publications do not describe the nature of the dust that was administered.

In order to obtain more information on this problem we have subjected rats to relatively pure chrysotile dust produced in a dust generator that gave a dust of consistent particle size.

Materials and methods

The dust generator. The Asbestos Dust Generator, Mark II (fig. 1), a modification of the instrument of Holt and Young (1959–60), utilises the same hammer mill that was described in that publication, but the mill motor is now arranged to behave as a centrifugal pump so that the charge, instead of remaining in the mill, is forced from the circumference of the mill housing through a length of plastic tubing back into the centre of the mill. As a result of the considerable reduction in mill duty the very sensitive method of feed used in the earlier apparatus can be eliminated and replaced by a steady feed from a hopper at a pre-set rate. The apparatus, which produces a reasonably consistent dust cloud from asbestos fibre and maintains it for some weeks at an almost constant concentration, is described in detail in an appendix to this paper.

Asbestos dust. The air of factories where asbestos is used contains, beside asbestos fibres and dust derived from clothing and the abrasion of floors, platy particles, mostly of serpentine, which become freed from between the asbestos fibres; these are lighter and remain in suspension longer than the asbestos fibres. Six random samples taken from the air of an asbestos weaving shed showed that between 8 and 14 per cent. of the particles were asbestos fibres.

Some samples produced in our dust generator contained particles which appeared to be non-fibrous, particularly when the mill was overloaded. These are formed by the crushing of fibres, and electron microscopy reveals that they are composed of masses of very fine fibres. The process of formation is indicated by the flattened end of the fibre marked in fig. 3. When the mill is running normally, the proportion of non-fibrous dust particles produced by the Dust Generator Mark II is very small. The length and thickness of the fibres produced have a very wide range. Photomicrographs and electron micrographs of the dust (fig. 2) indicate that it contains numerous small particles, many of which are too small to be seen in the optical microscope. Apparently there is no method of expressing the concentration of such heterogeneous dust in absolute terms; dust levels in our dust chambers were therefore followed with a monitor that measured in arbitrary units light scattered by the particles. However, to give some indication of the character of the dust cloud some thermal precipitator samples were taken and counts were made differentiating according to length of fibre. There were approximately 5000 particles per ml. of air, and the differential count gave the following values:

Size range (μ)	<1	1–2	2–5	5–10	10–20	>20
Count (as per cent. of total)	24	36	24	10	4	2

Inhalation experiment. Twenty-four rats were exposed to a dust cloud containing chrysotile asbestos particles for a total period of approximately

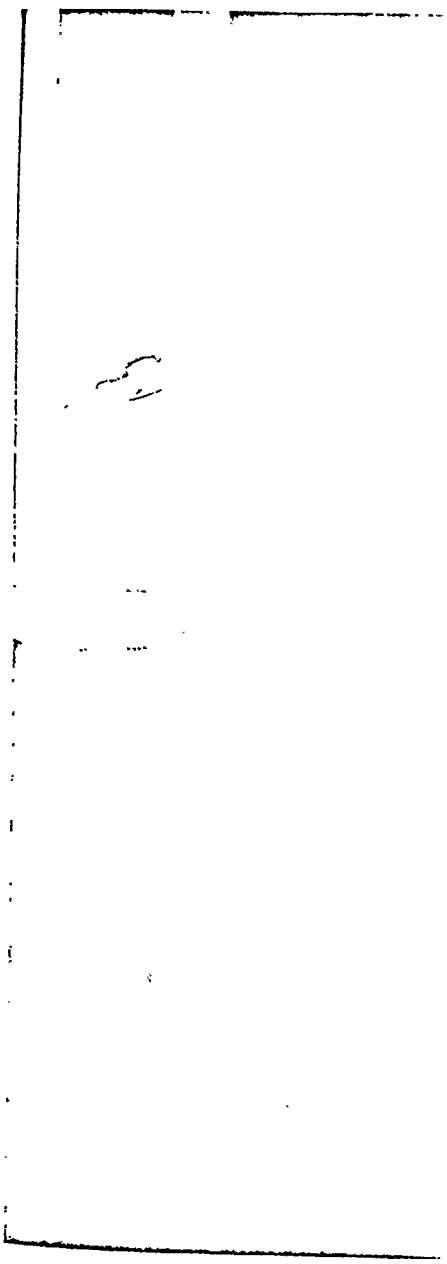

FIG. 1.—Apparatus for maintaining

100 hr. over 30 days. Rats were killed at intervals, and their lungs were fixed in formol-saline. As a routine, paraffin sections were stained with haematoxylin and eosin, and Van Gieson's stain, and by Gordon and Sweets' silver impregnation method. A few sections were stained for iron and fat, and some by the periodic acid-Schiff method.

Controls. As naturally occurring inflammatory lesions are frequent in the lungs of rats, many untreated control animals of all ages have been examined.

RESULTS

The lungs of a rat killed on the 14th day of inhalation showed surprisingly little evidence of dust. Only after careful search was dust noted at all, and there is no sign of the generalised dust pneumonia that follows the inhalation of silica dust under similar circumstances. There are, however, a few sharply defined focal inflammatory lesions (figs. 4 and 5), most frequently in the walls of a terminal bronchiole but occasionally in the perivascular tissue of a small blood vessel in the wall of an alveolar duct (fig. 5). Although these lesions could not be more than 14 days old if they had been generated by asbestos dust, a delicate collagen capsule had already formed and a reticulin net could be demonstrated within the focus.

The most striking features of these lesions are multinucleated giant cells of irregular shape, which often send cytoplasmic processes between the other cells of the focus. The many nuclei are arranged around the periphery of the cell or bunched at one or both ends and the cytoplasm is almost invariably vacuolated and foamy (figs. 4 and 6). The giant cells occupy the greater part of some lesions, giving the appearance of a syncytium, and shadow cells can be found in some of the vacuoles, suggesting phagocytosis by the giant cells (fig. 6).

Other cells of the focus were round cells of lymphocytic type or larger cells of monocytic type, some of which contained brownish dust particles in their cytoplasm. Round the periphery were flattened cells of fibroblastic type and the whole focus was sometimes enclosed by a delicate strand of collagen. Very few asbestos fibres were visible, but occasionally a short glistening fibre up to 5 μ in length was found between the cells or in the cytoplasm. Dark-ground illumination revealed more dust particles than transmitted light (fig. 7), but even so no large amount of dust was to be found. Although in the optical microscope this intracellular dust appeared to be non-fibrous, Dr J. M. Davis reported that electron micrographs revealed that the particles were closely packed fibres.

In addition to the focal lesions, dust cells (figs. 4 and 7) were widely but thinly distributed throughout the lung, and there were occasional lobules whose alveoli were filled with lightly dusted macrophages (fig. 8). Some of the alveolar walls were thickened by cellular exudate in which collagen was appearing—the early stage of an organising interstitial pneumonia.

No asbestos bodies were found and there was no evidence of

dust in the lymphoid tissue within the lung or in the tracheal lymph-glands.

The lungs of 5 rats killed 39 days after the cessation of dusting showed little dust. The same sharply defined nodules are present in relation to the terminal bronchioles, but the giant cells are becoming less evident (fig. 9), whilst round cells, fibroblasts and collagen are more prominent. Some of the terminal bronchioles are plugged with cellular exudate (fig. 10), mostly foamy macrophages, between which a few reticulin fibres are demonstrable. Isolated pneumonic lobules are now frequent, some of the lobules served by affected bronchioles having their alveoli filled with foamy macrophages; many of these are multinucleated, and a few contain a slight sprinkling of fine brown dust in the cytoplasm (fig. 11). Reticulin is present between the cells and frequently seals the alveolar duct from the surrounding alveoli. Cellular exudate thickens the alveolar walls adjacent to these pneumonic areas, and patchy interstitial pneumonia is present in the intervening lung. Again no lesions were found in the lymphoid tissue and no asbestos bodies could be identified. This is the picture of a patchy organising lobular pneumonia associated with a more diffuse chronic, organising interstitial pneumonia with early fibrosis in the alveolar walls.

Rats were killed at convenient intervals up to a year to follow the subsequent changes in their lungs. The rats showed a slowly progressing fibrosis of the lungs of an extent astonishing in view of the small amount of foreign matter that could be identified. Fig. 12 shows a typical lesion 364 days after the first administration of dust to the rat. At no stage were asbestos bodies identified; fibres were few and far between and never more than 10 μ in length. Interstitial cell infiltration and fibrosis were widespread.

Frozen sections from the lungs of one of the rats killed on the 40th day show no fat in the vacuoles of the giant cells. Sections from rats killed on the 14th day and on the 69th day show material stained by periodic acid-Schiff reagent in many of the macrophages and in the giant cells of the focal lesions.

In another experiment 25 rats were exposed in the dust tunnel to asbestos dust for only 68 hr during a period of 45 days. One rat killed at the end of the dusting period showed giant-cell systems in the lungs. The remaining rats were killed after 167 days without further dusting. Dust was now found in the lungs in small amounts and there were lesions similar to those previously described. These lesions are smaller, much less obvious and only rarely show giant cells. There are in addition small foci where the alveolar walls are thickened by infiltrating cells. Collagen is demonstrable in the focal lesions and in the alveolar walls. The subsequent history of these rats is similar to that described above.

Lesions similar to those described were never found in the control rats.

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Discussion

The rat lung reacted rapidly to chrysotile dust—more rapidly than is observed in similar experiments with silica dust. The concentration of asbestos particles in the inhaled air was high compared with that encountered in industry, but a more important difference was the very small particle size. Our dust mechanism produced a cloud in which more than 80 per cent. of the fibres were shorter than 5 μ. The lung was thus presented with particles small enough to reach the alveoli and to be ingested directly by phagocytes.

Within 14 days characteristic lesions had developed, but few dust particles could be detected in these by transmitted light; dark-ground illumination revealed many more, indicating the small size of most of the dust. Subsequently, increasing amounts of collagen were apparent in the focal lesions and cellular infiltration of the alveolar walls with later development of collagen gradually extended throughout the whole lung. This feature is reminiscent of the finding of Beattie and Knox (1961) that the degree of fibrosis is related to the lapse of time rather than the amount of dust present. No asbestos bodies were found in our animals.

There is no generally accepted explanation of the ability of asbestos to produce fibrosis. Vorwald *et al.* (1951) considered that the fibres produced damage by their mechanical action and concluded (p. 42) that " Inhalation experiments with asbestos dust suggest, and intratracheal injection experiments confirm, that peribronchiolar fibrosis is produced by asbestos fibers between 20 and 50 microns in length but not by particles shorter than 20 microns ". Their analyses indicated, however, that the animals that received " short fiber " dust inhaled mainly serpentine and little asbestos. All we would conclude from their inhalation experiments is that serpentine is less likely to produce fibrosis than is chrysotile, and we do not consider that this result is at variance with our own.

In our experiments the rats inhaled dust made from a Rhodesian chrysotile of high purity. The dust contained few particles other than asbestos particles and even very small particles that appeared to be non-fibrous when viewed in the optical microscope proved to be bundles of very short fibres when they were examined by electron microscopy. A careful search of the histological sections of the lungs revealed very few asbestos fibres longer than about 2 μ; the majority of the particles were in phagocytes; they had evidently evoked an immediate reaction and fibrosis progressed rapidly.

We conclude that chrysotile particles less than 2 μ in length can produce peribronchiolar fibrosis in the rat. The virtual absence of particles longer than a few microns in the rats' lungs when they were undoubtedly present in the air-borne dust indicates that the respiratory system of the rat can eliminate longer fibres from the inspired air. The fact that particles were mostly small enough to be taken up by

phagocytes may explain the absence of asbestos bodies in the tissues.

The early onset of the fibrotic reaction calls for comment. Although in our experiment fibrosis appeared within 14 days of the initial inhalation of the dust, Vorwald *et al.* reported " a suggestion of early fibrosis " in rats that had inhaled " short fiber " dust for 10 mth and minute foci of well-defined fibrosis after 12–32 mth. In rats that had inhaled " long fiber " dust there was a well-marked peribronchiolar fibrosis after 25 mth. This suggests that the dust administered by Vorwald *et al.* differed in either its quantity or quality from that which we used.

Beattie and Knox (1961) found no correlation between the severity of the disease and the mineral content of the lungs in a series of 50 cases of human asbestosis; the severity of the disease appeared to be related rather to the duration of the exposure to dust (exposure time) plus the time interval between the last known exposure and death (survival time). There was evidence, too, that the asbestos fibres were breaking up in the lungs. Beattie and Knox concluded that " when fragmentation occurs the development of asbestotic changes is likely to be progressive ". On this evidence the small asbestos particles are ultimately the main fibrogenic agent and this suggests that gross fibrosis follows only when the particles are small enough to be phagocytosed.

The following sequence would then fit the evidence. The asbestos worker inhales a mixed dust and may retain fibres of any length below perhaps 100 μ. Particles in the lung below about 5 μ are taken up by phagocytes and, if the dust concentration is not too high, they may be moved to the lymph-glands. Presumably a high concentration of these small particles will induce rapid fibrosis of the type seen in our rats. This may correspond to the acute type of asbestosis seen in men in the early part of the century before dust was adequately controlled in the asbestos works. Wyers observed that this acute form had been replaced by the more chronic type that occurs today.

The longer asbestos fibres become coated with protein. The asbestos bodies remain in the lung and accumulate over the years. After some years, perhaps because of changes in the *p*H (Clark and Holt, 1961), they begin to disintegrate, producing a very large number of asbestos fragments which are collected into phagocytes. There follows a fibrotic process which is similar to that produced by the heavy doses of short asbestos fibres.

It is possible that, in the conditions that prevail in modern factories, the short asbestos fibres are of minor importance in the production of asbestosis in normal people. When the survival time reaches 10–15 yr, however, and asbestos bodies may be disintegrating to produce large numbers of small particles, the inhalation of small particles of asbestos dust will then add to the significant load and increase the embarrassment.

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This hypothesis assumes that in modern factory conditions the small particles of asbestos that initiate rapid fibrosis in man are ultimately provided by the breakdown of asbestos bodies. To judge from some experiments in which rats inhaled asbestos dust for only 68 hr, there is another possibility. Although the amount of dust in the lungs of these rats was small and the early response was slight, a fibrosis later developed, which followed the same course as that initiated by larger amounts of dust and progressed continuously. It is conceivable that in human cases also a steadily progressing fibrosis may be initiated by the small particles, which does not under present conditions become significant for a number of years, though infection may increase its severity. It is evident that a study of the effects on the lung of very small amounts of small chrysotile particles may clarify this.

SUMMARY

Chrysotile dust of small particle size ($<3\ \mu$) rapidly produces fibrotic lesions in the lungs when inhaled by rats. It is suggested that asbestos is fibrogenic only when it can be ingested by phagocytes. The rapid fibrosis that can occur some years after asbestos has been inhaled by man may be due to the breakdown into large numbers of small fragments of fibres; these are then phagocytosed. Alternatively the small particles originally inhaled may act slowly and their effect may be enhanced by a superimposed infection.

An apparatus that maintains an atmosphere containing small asbestos fibres at nearly constant concentration is described.

This paper describes part of a programme of research in progress under the auspices of the Asbestosis Research Council. The authors are indebted to Mr R. A. Jerrome and Mr G. Pollard for technical assistance.

Appendix

Dust generator, Mark II

General description (see fig. 13). A Micro Hammer Mill (Glen Creston) is suspended with the motor spindle vertical by three adjustable nylon cords. A length of Perspex tube 4 in. (10 cm.) in diameter forms a vertical extension to the mill casing. It is attached to the hinged lid of the mill and connected by a thin rubber sleeve to a tube of similar material extending into an elutriator which leads into an experimental dusting tunnel.

The normal radial mill outlet is obstructed and replaced by a tangential thin-walled metal tube. When the mill is in operation, asbestos is ejected through this tube into a plastic pipe of 1 in. (2·5 cm.) bore and 4 ft (120 cm.) length which returns it to the centre of the mill. This pipe enters the mill extension tube obliquely and its free end then passes through a metal sleeve whose attitude can be adjusted externally to ensure that the returning asbestos is accurately projected through a 1 in. diameter aperture in the mill cover and directly on to the axis of hammer rotation.

Prevention of clogging. At the lower end of the Perspex mill extension is fitted a polished steel metal funnel that directs newly fed as well as stray pieces of asbestos into the centre of the mill and thus reduces the tendency to build up and

clog. As an additional deterrent a small weight attached eccentrically to the mill motor spindle impresses a 100 cycles per sec. oscillation on the suspended unit.

Feed system. The mill is fed with opened chrysotile, supplied from an industrial source, contained in a graduated vertical glass tube of 4 mm. diameter serving as a hopper. This tube joins a horizontal length of similar tubing through which the asbestos is conveyed by a slowly rotating wire spiral of 3 cm. diameter, to be ejected at a point in the elutriator chamber immediately above the mill motor. The pitch of the spiral increases progressively from 2 cm. beneath the hopper to 6 cm. at the outlet; this provides more even feeding. The spiral is driven by a small

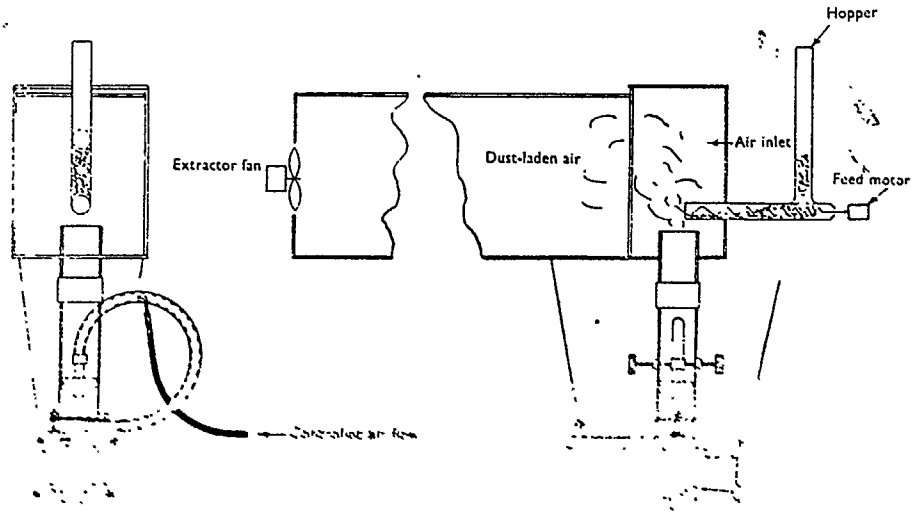

FIG. 13.—Diagram of apparatus for maintaining a dust cloud of asbestos particles. $\times \frac{1}{10}$.

(5 watt) synchronous motor (Everett Edgcumbe) at 4 r.p.hr. If a reduction in the average rate of feed is required, it is effected by intermittently interrupting the motor current with a Simmerstat (Sunvic Controls) energy regulator.

[illegible] and are carried into the dusting tunnel by air drawn by an extractor fan from outside the apparatus over the elutriator chamber. Alteration of the volume of air entering the chamber provides a method for regulating the dust concentration. The rate of flow is measured by a rotameter.

Operation. When a minimum of work is to be done on the charge, the rate of feed of asbestos is set to correspond with the rate of its removal by the scavenging airflow so that a minimum of asbestos is in circulation. Alternatively, if maximum work is to be done on the charge, the tangential mill outlet is obstructed so that the asbestos remains in the mill and does not circulate through the plastic tube. The feed rate is then adjusted to maintain a given motor load current to correspond with a set rate of scavenging airflow.

Protective device. Should the temperature of the motor casing exceed 80° C a bi-metal switch (Motor Guard, Otter Controls) interrupts the current to the mill motor.

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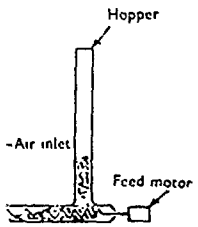

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