EXPERIMENTAL STUDIES ON ASBESTOS: PART II

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Several clinical reports[1-7] incriminate asbestos dust as a possible factor in the aetiology of mesothelioma and malignant tumours of the lung. In experimental studies no conclusive evidence has been found to support the clinical claims.[8-10] Recently, however, Harrington[11] pointed out that amosite and crocidolite asbestos are rich in primitive oils containing 3:4 benzpyrene and related carcinogens. Although the concentration of these carcinogens in asbestos dust is extremely low compared with that in coal dust, no other reasonable explanation has been offered for the high incidence of cancer in asbestos workers.

In a recent experimental study[10] it was shown that the fibrogenicity of pure, fine, crocidolite particles is extremely low, producing only a grade I fibrosis in the lungs of rats, 220 days after intratracheal administration of 50 mg. of dust. As weathered crocidolite was used, no information regarding the carcinogenic properties of this dust could be obtained. It was therefore decided to reinvestigate the fibrogenic and carcinogenic properties of freshly milled crocidolite.

A long-term experiment was therefore planned to compare the histopathological response of rat lung tissue to natural crocidolite and crocidolite from which the carcinogen was extracted with chloroform. As it was demonstrated previously[10] that a small amount of quartz enhanced the fibrogenicity of pure asbestos in the lung, the influence of quartz added to both the natural and extracted crocidolite samples was also studied.

MATERIALS AND METHODS

Dusts. Milled crocidolite (Cape blue asbestos) was obtained from the North-Western Cape (Kuruman) through the South African Institute for Medical Research, Johannesburg. Microscopic analysis of the dust sample indicated that it consisted of asbestos fibres (0·5 - 200 μ) and unidentified non-asbestos materials.

Twenty grams of the natural crocidolite sample were extracted with chloroform under reflux for 24 hours to eliminate the primitive oils containing benzpyrene. After drying in an oven for 24 hours at 100°C, 3 G of natural and 3 G of the extracted material were weighed out and ground separately in a ball-mill for 6 hours.

Quartz dust was prepared from pure rock crystal, supplied by the Department of Geology, University of Stellenbosch. The size distributions of the particles in the quartz and crocidolite samples are given in Table I.

TABLE I. SIZE DISTRIBUTION OF PARTICLES OF CROCIDOLITE AND QUARTZ

	Crocidolite %	*Quartz* %
$< 5\ \mu$	85	90
$5 - 25\ \mu$	14	10
$> 25\ \mu$	1	0

The following dusts and dust mixtures were used: (1) Natural crocidolite, (2) extracted crocidolite, (3) natural crocidolite + quartz (75:25), and (4) extracted crocidolite + quartz (75:25).

Dust suspension. Samples of 1·25 G were weighed out from each dust and dust-mixture into screw-capped bottles, 25 ml. of 0·9% saline added and the suspensions sterilized by autoclaving for 20 minutes at 15 lb. pressure.

Animals. Specially young albino rats (*Rattus norvegicus*, Wistar Institute) weighing 150 - 175 G were used. Four groups of 20 animals each were injected intratracheally[10] with the respective dust suspension.

The experiments lasted 400 days and 3 rats from each group were killed at 50, 100, 200, 300 and 400 days. A few animals were lost immediately after injection, possibly due to airway obstruction caused by dust aggregates. A few more animals died during the experimental period, and their lungs were discarded after postmortem examination.

Histological technique. The animals were anaesthetized with ether before they were killed, their tracheas exposed and 10 ml. of 15% formol-saline injected via the tracheas into the lungs. The lungs were dissected free and preserved in 15% formol-saline. Blocks of lung tissue (3 mm.) were cut from the left and right lung of each animal in a sagittal plane near the hilum, embedded in wax and sectioned serially (6 μ). Two sections were stained with H & E and another impregnated with silver.[12]

Grading. The grading of the amount and maturity of the fibrosis produced in the lung was done according to Ross *et al.*[13]

RESULTS

Macroscopic examination of the lungs from the different dust groups showed no outstanding differences during the experimental period. The dust distribution between the right and left lungs appears to be rather irregular. Lungs and lobes containing a high concentration of dust were partially or wholly collapsed and could not be inflated by injecting fixation fluid. In such cases the rest of the lung tissue showed extensive compensatory enlargement. In general there was an increase in lung size and weight to such an extent that the normal lung weight was trebled at 300 days. Marked fibrotic areas were visible on the lung surfaces of some animals, and a few pleural lesions containing pus were found. The lungs of other animals remained mostly soft and pliable.

Microscopic Appearances

The cellular response following the injection of asbestos and asbestos-quartz mixtures into the lungs of rats consisted of a typical, severe, foreign-body reaction. Phagocytosis of the fine particles and short asbestos fibres was attained quickly and effectively, and at 50 days almost all of these particles were transported to the vicinity of the bronchioli. The long asbestos fibres were partially ingested by multinuclear giant cells and retained in those loci where they were originally deposited. Initially the tissue reactions to the dust were most prominent in and around the bronchioli and surrounding alveoli and consisted of alveolar wall thickening and a slight, diffuse reticulinosis (Fig. 1).

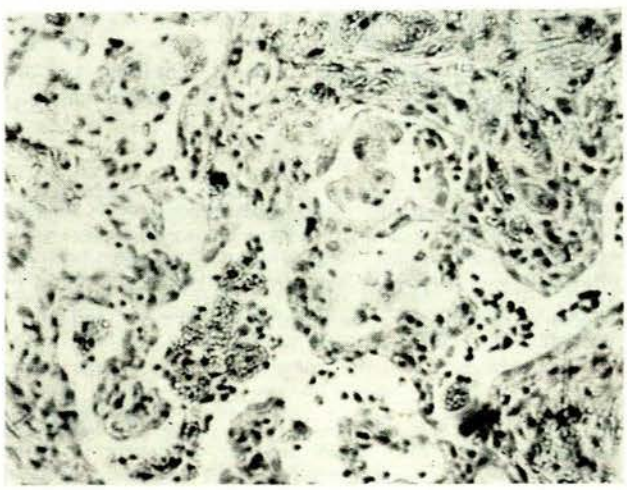

Fig. 1. Lung section showing numerous macrophages loaded with asbestos dust in the vicinity of a bronchiole, with alveolar wall thickening and a diffuse reticulinosis, 50 days after the intratracheal injection of 50 mg. of natural crocidolite (H & E x 150).

The dust fibres which accumulated in the bronchioli and bronchi induced metaplasia of the columnar epithelium and enlargement of the mucous glands with an excessive mucous secretion. Finally, mucous plaques blocked the air passages resulting in lung collapse. Bronchiectasis was found in several cases during the later stages, and finally the bronchial and bronchiolar structure was replaced by massive fibrosis (Fig. 2).

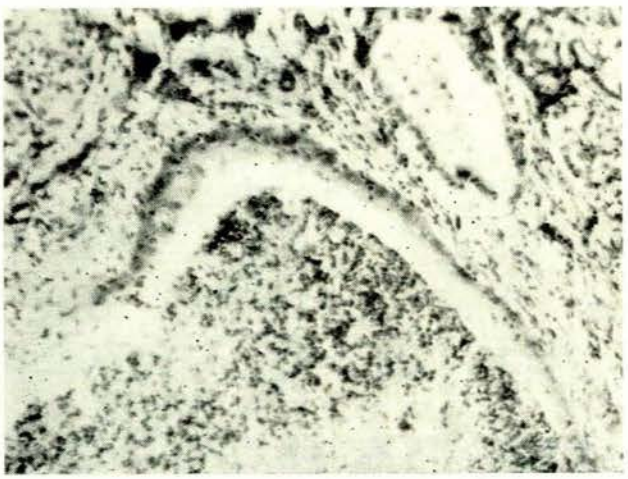

Fig. 2. Lung section showing a bronchiole blocked by a mucous plaque infiltrated with leucocytes and some disintegration of the bronchiolar wall with fibrosis, 200 days after injection of asbestos (H & E x 150).

Lung collapse and consolidation were found in some animals at 50 days and occurred more frequently during the later stages of the experiments in several animals from all the dust groups. In these lungs infection, chronic inflammation and large amounts of dust were prominent features. Undoubtedly, the presence of an infection enhanced the fibrogenicity of asbestos dust.

A prominent phenomenon associated with the cellular response to asbestos was the extensive hyperplasia of the lymphoid tissue of the bronchi which was very marked in cases with lung consolidation. In the asbestos-quartz groups the hyperplasia was characterized by diffusely scattered reticulin nodules with some loosely arranged collagen strands. A diffuse reticulinosis was also present in the hyperplastic lymphoid tissue of the asbestos animals.

The fibrosis produced by all four dusts, although not similar, followed the same trend with time (Table II). Coexistent infection, inflammation and consolidation enhanced the progressive nature of the fibrosis. It was therefore very difficult to assess the fibrogenicity of asbestos only, since infection was present in 75% of the cases. In animals without infection the lesions did not progress beyond grade 2 fibrosis even when a large amount of dust was retained in the lung. The distribution and nature of the lesions in infected lungs were therefore different to those found in the absence of infection. In the presence of an infection massive fibrosis (Fig. 3) predominated, while in its absence diffuse fibrosis was found, localized mostly around the bronchioli.

Neither natural asbestos nor extracted asbestos dusts produced malignant tumours or neoplasms of any kind.

Pleural fibrosis was a quite common finding especially in cases with infection and chronic inflammation. The fibrous changes varied from a thin veiling of the visceral pleura to protruding fibrous nodules.

TABLE II. GRADE (G), AMOUNT (A) AND TOTAL (T) FIBROSIS PRODUCED BY ASBESTOS, EXTRACTED ASBESTOS, ASBESTOS AND QUARTZ, AND EXTRACTED ASBESTOS AND QUARTZ

Days	Group I (asbestos)			Group II (extracted asbestos)			Group III (asbestos + quartz)			Group IV (extracted asbestos + quartz)		
	G	A	T	G	A	T	G	A	T	G	A	T
50	1	2	2	1	2	2	2	1	2	2	2	4L
	2	2	4	2	2	4R	2	3	6R	2	2	4L
	2	2	4R	2	2	4R	2	3	6R	1	2	2
100	1	2	2	2	2	4R	1	3	3	2	4	8L+R
	2	3	6L	2	2	4L	1	3	3	3	2	6L+R
	2	2	4L	1	2	2	2	2	4	3	2	6R
200	3	3	9L	2	3	6R	3	2	6R	2	3	6R
	2	3	6R	3	1	3R	2	3	6L	3	3	9R
	2	3	6L	3	3	9L	3	3	9L	3	2	6L
300	3	3	9R	2	3	6L+R	3	3	9L+R	2	2	4R
	2	3	6R	3	3	9L	3	3	9R	3	3	9L
400	2	2	4	4	4	16L	2	3	6L	3	5	15L+R
	3	2	6L	3	5	15L	1	3	3	2	2	4

L = left lung
R = right lung
L+R = left + right lung
} These lungs showed signs of consolidation with infection.

Fig. 3. Rat lung with infection, showing massive fibrosis (grade V), 400 days after the injection of crocidolite (H & E x 150).

DISCUSSION

In a recent study it was demonstrated that the fibrogenicity of pure, very fine asbestos particles, prepared from isolated and thoroughly cleansed crocidolite fibres, was very low, producing only a peribronchiolar and perivascular reticulinosis in the lungs of rats.[10] In the present experiments, however, it was found that natural crocidolite was much more fibrogenic, especially when accompanied by infection. In the absence of chronic inflammation and infection, the lesions progressed only to a grade 2 fibrosis, and were mainly confined to the peribronchiolar and perivascular tissue.

The factors inducing chronic inflammation, consolidation and infection could not be determined with certainty in the present experiments. Microscopic examination of the milled natural asbestos dust showed that apart from asbestos fibres, crystals of other unidentified minerals as well as quartz were present. It might be possible that some mineral contaminant caused chronic inflammation or that the continuous irritation of the tissue by the relatively long asbestos fibres was primarily responsible. However, when a suspension of short asbestos fibres (80% < 5μ) was administered intratracheally, the incidence of inflammation and infection was higher than in animals injected with quartz of the same size distribution, but never as high as in the present experiments. Whatever the cause might be, these side-effects enhanced the fibrous response due to asbestos dust.

No relationship has been found between the primitive oil content of asbestos dust and the grade and amount of fibrosis produced. The natural dust and the chloroform-extracted dust gave more or less the same fibrous response. The addition of pure quartz to the asbestos dust did not increase the fibrogenicity of the dust mixture, which might indicate that natural asbestos already had an optimal quartz concentration.

Although the visceral pleura was involved in the fibrous response of the lung tissue in some places, no neoplasms were found. Harrington[11] suggested that mesotheliomas might be due to primitive oils in asbestos dust, containing 3:4 benzpyrene, but the present experiments indicated that no malignant tumours were produced by either the natural asbestos dust or the extracted dust. It was doubtful, however, whether the concentration of 3:4 benzpyrene in the primitive oils of asbestos dust was high enough to induce carcinogenesis in the highly resistant rat species.

It appeared in general that the long asbestos fibres, apart from their fibrogenic activity, had a specific deleterious, irritating effect on the columnar epithelium of the bronchioli and bronchi resulting in metaplasia. The mucous glands also become enlarged and were somehow stimulated to excessive mucous production. Mucous plaques, infiltrated by polymorphonuclear cells, eventually blocked the air passages causing collapse of the lung which in turn predisposed to infection and chronic inflammation. Finally, the normal bronchiolar structure and the peribronchiolar lymphoid tissue were obliterated by massive fibrosis. In the presence of these manifestations a very serious condition developed which appeared to be fatal. The high mortality of the animals in the final stages of the experiment might be due to these complications.

SUMMARY

The fibrogenic and carcinogenic properties of suspensions of freshly milled crocidolite, crocidolite plus quartz, chloroform-extracted crocidolite and of extracted crocidolite plus quartz were investigated on the lungs of specially young rats. Each suspension was administered intratracheally (1 ml. containing 50 mg. of dust per ml. normal saline) to 20 rats, and 3 animals of each group were killed at 50, 100, 200, 300 and 400 days after injection of the dust suspensions.

The results indicated that all the dust suspensions induced a very severe pathological reaction which followed the same trend in the lungs of all 4 groups of animals. Neither the primitive oils in natural asbestos, containing 3:4 benzpyrene, nor the addition of a small amount of quartz to natural asbestos dust had any profound effect on developing asbestosis under these experimental conditions.

The incidence of infection, chronic inflammation, lymphoid hyperplasia, bronchiectasis and lung collapse was extremely high in all 4 groups. In the presence of these manifestations

asbestos appeared to be very fibrogenic and produced massive fibrosis (grade V), whereas in their absence only a grade 2 fibrosis developed.

REFERENCES

1. Wagner, J. C. *in* Orenstein, A. **J.**, **ed.** (1959): *Proceedings of the Pneumoconiosis Conference, Johannesburg*, p. 373. London: Churchill.
2. Tobiassen, G. (1955): Acta path. microbiol. scand., suppl. 105, 198.
3. Belloni, G. and Bovo, G. (1957): Acta **med.** Patav., **17**, 367.
4. Godwin, M. C. (1957): Cancer (Philad.), **10**, 298.
5. McCaughey, W. T. (1958): J. Path. Bact., **76**, 517.
6. Wagner, J. C., Sleggs, C. A. and Marchand, P. (1960): Brit. J. Industr. Med., **17**, 260.
7. Lynch, K. M. and Smith, W. A. (1935): Amer. J. Cancer, **24**, 56.
8. Vorwald, A. J. and Karr, J. W. (1938): Amer. **J.** Path., **14**, 49.
9. Vorwald, A. J., Durkan, T. M. and Pratt, P. C. (1951): Arch. Industr. Hyg., **3**, 1.
10. Engelbrecht, F. M. (1964): S. Afr. J. Lab. Clin. Med., **10**, 45.
11. Harrington. J. S. (1962): Nature (Lond.), **193**, 43.
12. Gordon, H. and Sweet, H. H. (1936): Amer. **J.** Path., **12**, 545.
13. Ross, H. F., King, E. **J.**, Yoganathan, M. and Nagelschmidt, G. (1962): Ann. Occup. Hyg., **5**, 149.