

EXPERIMENTAL STUDIES ON ASBESTOS

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Since the establishment of the Pneumoconiosis Research Unit in Johannesburg in 1954, investigations have been undertaken to ascertain whether pulmonary lesions and complications following the inhalation of crocidolite and amosite dusts were similar to those associated with exposure to chrysotile dust.¹ The results indicated that crocidolite and amosite dusts essentially caused an interstitial fibrosis; this was seen to be peribronchiolar and perivascular in distribution during the early stages, and in the later stages the lesions became diffuse, with alveolar wall thickening and peribronchiolar and perivascular fibrosis.

Other clinical investigations suggest that asbestos may be implicated in the development of malignant tumours of the pleurae, and primary neoplasms of this nature have been described by many authors in recent years.^{2,3}

The pathological evidence associating these tumours with asbestos appeared to be inconclusive. Wagner,⁴ however, reported that 47 cases of mesothelioma had been identified in South Africa up to the end of June 1960. In 45 of these a possible association with exposure to crocidolite from the Northern Cape has been established. Whether the primary factor in the development of these tumours is exposure to asbestos dust, or to a contaminant of it, is as yet not known. According to Wagner, one would expect similar cases from the Lydenburg district, since the nature of the rock, ore and fibre, are almost identical with those of the Northern Cape.

Attempts to produce tumours in experimental animals by exposing them to an atmosphere of asbestos dust or to intratracheal injection of asbestos have been disappointing.⁵ Vorwald *et al.*⁶ found that ordinary industrial asbestos dust and long fibres from which the small particles had been separated gave peribronchiolar fibrosis. With dust particles 3 μ or less there was little or no reaction in experimental animals. Behrens⁷ also reported that pure chrysotile asbestos injected intraperitoneally into mice and intratracheally into rats produced only a non-specific fibrosis that might be due to a foreign-body reaction. At present no evidence is available regarding the pathogenicity of crocidolite in experimental animals, although clinical reports incriminate crocidolite as a possible aetiological factor in the development of malignant tumours in the lung of humans. The present preliminary study was therefore planned to investigate the pathogenicity of crocidolite and a mixture of crocidolite and quartz in the lungs of rats.

TABLE I. SIZE DISTRIBUTION OF PARTICLES OF CROCIDOLITE AND QUARTZ

	Crocidolite	Quartz
5 μ	20	10
3 μ	80	90
2 μ	40	30
1 μ	30	30

MATERIALS AND METHOD

Dust. Samples of very pure rock crystal (quartz) and crocidolite were obtained from the

Department of Geology, University of Stellenbosch. The asbestos fibres were isolated, cut up as finely as possible and ground for several days. The rock crystal was crushed and also ground in an agate ball mill. The size distribution of the particles is given in Table I. A mixture

TABLE II. CHEMICAL ANALYSIS OF CROCIDOLITE (from Vermaak¹³)

SiO ₂	51.94	CaO	0.11
Al ₂ O ₃	0.20	Na ₂ O	6.07
Fe ₂ O ₃	18.64	K ₂ O	0.0
FeO	19.39	H ₂ O	0.0
MgO	1.37	H ₂ O	2.31
		Total	100.72

quartz and asbestos was prepared in the proportion 25:75. The chemical composition of the crocidolite asbestos is given in Table II.

Animals. Albino rats (*Rattus norvegicus*, Wistar line) weighing 150-175 G. were used. Two groups of animals each were injected intratracheally with suspension of asbestos and a mixture of quartz and asbestos, respectively.

Dust suspensions. The dusts were suspended in 0.9% saline in the proportion of 50 mg. of dust per 1 ml. Samples of each of the dusts were weighed out in 25 ml. capped bottles, 20 ml. of saline added, and the suspension sterilized by autoclaving for 20 minutes at 15 lb. pressure. The suspensions were shaken before use.

Injection of dusts. The animals were lightly anaesthetized with ether. They were tied on their backs on a skin dissecting board (1:45), the tongue retracted with a speculum introduced through the mouth into the throat and the head drawn forward and downward, until chords were visible. A 16-gauge Hunt hollow needle 1 inch long, was then inserted into the trachea; a syringe containing 1 ml. of suspension and 1 ml. of air quickly attached and the suspension injected forcibly into the lungs.

Duration of experiment. The experiments lasted 28 days and one rat out of each group was killed at 20 intervals. A few animals died and had to be discarded.

Histological technique. The animals were anaesthetized with ether, the trachea exposed, and 10 ml. of formal-saline injected into the lungs by inserting a syringe into the trachea. The tracheas were then tied and the animals autopsied, and the unopened lungs preserved in 15% formal-saline. After fixation, the lungs were sectioned in a sagittal plane near the hilum and whole lung blocks were embedded in paraffin wax. Serial sections of thickness were cut from each block. Of these, two stained with haematoxylin and eosin and another imbedded with silver (Gordon and Sweet¹⁴). The remaining sections were kept as spares.

Grading. The grading of the maturity and severity of the lesions was done according to the new system of grading as follows:

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EXHIBIT

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