

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.²⁻⁹

The pathological evidence associating these tumours with asbestos appeared to be inconclusive. Wagner,¹ 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.¹⁰ Vorwald *et al.*¹¹ found that ordinary industrial asbestos dust and long fibres from which the small particles had been separated gave peribronchiolar fibrosis. With dust particles 3μ or less there was little or no reaction in experimental animals. Behrens¹² 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 %
> 5μ	20	10
< 5μ	80	90
< 2μ	40	50
< 1μ	30	30

MATERIALS AND METHODS

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

Department of Geology, University of Stellenbosch. Long 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 of

TABLE II. CHEMICAL ANALYSIS OF CROCIDOLITE (from Vermaas¹³)

SiO ₂	51.94	CaO	0.19
Al ₂ O ₃	0.20	Na ₂ O	6.07
Fe ₂ O ₃	18.64	K ₂ O	0.04
FeO	19.39	H ₂ O+	0.04
MgO	1.37	H ₂ O-	2.58
		Total	100.73

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

Animals. Albino rats (*Rattus norvegicus*, Wistar Institute) weighing 150-175 G. were used. Two groups of 15 animals each were injected intratracheally with suspensions 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. 1-G. samples of each of the dusts were weighed out in screw-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 sloping dissecting board ($\pm 45^\circ$), the tongue retracted with a clip, a speculum introduced through the mouth into the throat, and the head drawn forward and downward until the chords were visible. A 16-gauge blunt hollow needle, 6 inches long, was then inserted into the trachea; a syringe containing 1 ml. of suspension and 1 ml. of air was quickly attached and the suspension injected forcibly into the lungs.

Duration of experiment. The experiments lasted 220 days and one rat out of each group was killed at 20-day 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 15% formol-saline injected into the lungs by inserting a syringe needle into the trachea. The tracheas were then tied off, the animals autopsied, and the unopened lungs preserved in 15% formol-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 6μ thickness were cut from each block. Of these, two were stained with haematoxylin and eosin and another impregnated 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 Ross *et al.*,¹⁵ as follows:

Grade of fibrosis	Amount of fibrosis
1. Loose reticulin, no collagen	Very few nodules
2. Compact reticulin with or without collagen	Few nodules
3. Somewhat cellular but made up mostly of collagen	Moderate number of nodules
4. Wholly composed of collagen fibres and virtually acellular	Many silicotic nodules
5. Acellular, collagenous, confluent	Large areas involved

RESULTS

Macroscopic Appearances

Macroscopic examination of the lungs of the animals in the asbestos and quartz-asbestos groups showed no outstanding differences over the first 100 days. The dust distribution between the two lungs tended to be irregular, and more dust apparently entered the left lung than the right lung. Foci of dust were seen on the surface of the lungs, but no fibrotic changes could be observed.

After 100 days the lungs of the quartz-asbestos animals became firmer and also larger in size compared to the lungs of the asbestos group. Areas of fibrosis, particularly on the dorsal aspects of the left lungs, were seen while in the asbestos group the lungs remained soft with only a few minute lesions visible on the pleural surfaces.

Microscopic Appearances

The grade of fibrosis and the amount of fibrosis produced by asbestos and an asbestos-quartz admixture in the lungs of rats over a period of 220 days are given in Table III.

TABLE III. GRADE AND AMOUNT OF FIBROSIS PRODUCED BY ASBESTOS AND AN ASBESTOS-QUARTZ ADMIXTURE IN THE LUNGS OF RATS

Days of survival	Asbestos			Asbestos and Quartz		
	Grade of fibrosis	Amount of fibrosis	Total fibrosis	Grade of fibrosis	Amount of fibrosis	Total fibrosis
20	1	2	2*	1	3	3
40	1	2	2*	1	3	3
60	1	3	3*	1	4	4
80	2	4	8*	1	3	3
100	1	3	3	1	2	2
120	1	3	3	2	3	6
140	1	3	3*	2	3	6
160	2	1	2*	2	4	8
180	1	3	3*	3	3	9
200	2	2	4*	3	3	9
220	1	3	3	3	3	9*

*Indicates cases with infection.

The pathological changes caused by these two dust samples followed more or less the same pattern over the first 100 days after the intratracheal administration of the dusts. Some differences were observed concerning the cell types involved, the rate of phagocytosis of the dusts, and the sites of tissue reaction, as well as the incidence of infection. On the introduction of both dusts a typical foreign-body reaction was found. The reaction in the quartz-asbestos group was more severe and numerous mononuclear cells and leukocytes were mobilized, much more so than in the lungs of the asbestos group, where most of the asbestos particles were phagocytosed within 80 days and relatively limited cell destruction occurred compared to the asbestos-quartz group. In the latter group some asbestos and quartz particles were found lying free in the alveoli up to 100 days.

Asbestos dust alone was readily ingested by the macrophages and transported to the vicinity of the small bronchioli, where big aggregates of dust eventually accumu-

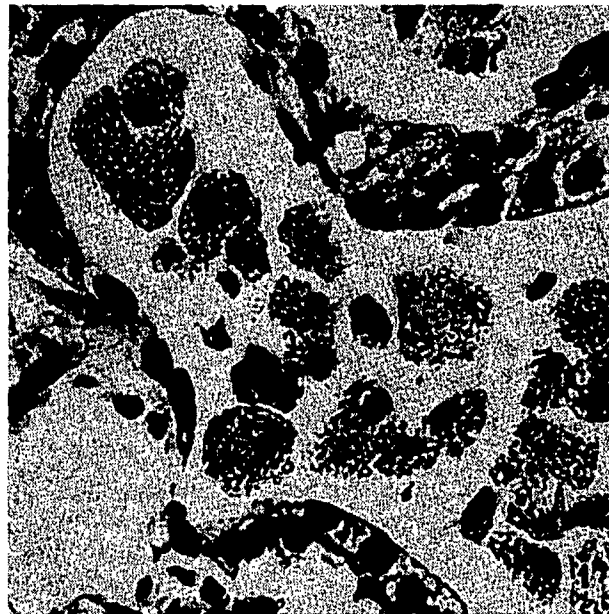


Fig. 1. Lung section showing numerous macrophages loaded with asbestos particles in the vicinity of a bronchiole, with some alveolar wall thickening, 60 days after the intratracheal injection of 50 mg. of pure asbestos dust. (H. & E. $\times 450$.)

lated. The dust lesions in the asbestos lungs were therefore primarily encountered around the bronchioli and to a lesser extent in lung areas with a relatively high concentration of dust. These lung areas were well marked by alveolar wall thickening and the accumulation of numerous macrophages and giant cells, loaded with asbestos particles (Fig. 1). The quartz-asbestos mixture, on the other hand, provoked a general and widespread tissue reaction in which the bronchioli, blood vessels and alveolar walls were involved. It appeared as if the presence of a small concentration of quartz interfered with the phagocytosis of the dust to such an extent that a diffuse fibrosis resulted.

Hyperplasia of the lymph nodes of the lung was uncommon in the asbestos group except in cases with infection. In the quartz-asbestos lungs the nodes appeared to be hyperplastic and infiltrated with fibrous tissue.

The incidence of lung infection in animals injected with pure asbestos tended to be higher than in those which received the asbestos-quartz mixture. From the histological sections it was observed that the bronchioli of several lungs injected with asbestos dust were completely blocked with mucus and infiltrated by leukocytes, macrophages, and mononuclear cells. Dust lesions in these lung areas, isolated from the respiratory tree, showed a marked increase in collagen content and progressed to grade-2 fibrosis (Fig. 2). In animals without infection the progression of the lesions was relatively slow and they reached only grade-1 fibrosis after 220 days (Fig. 3).

In the quartz-asbestos group an acute infection was

found in only one animal and the fibrotic changes in this group could therefore not be attributed to infection. Although the progression of the lesions in this group was

produced in this period (Fig. 4), compared to a grade-1, in animals without infection, in the asbestos group.

No malignant tumours or any neoplasms of the pleura

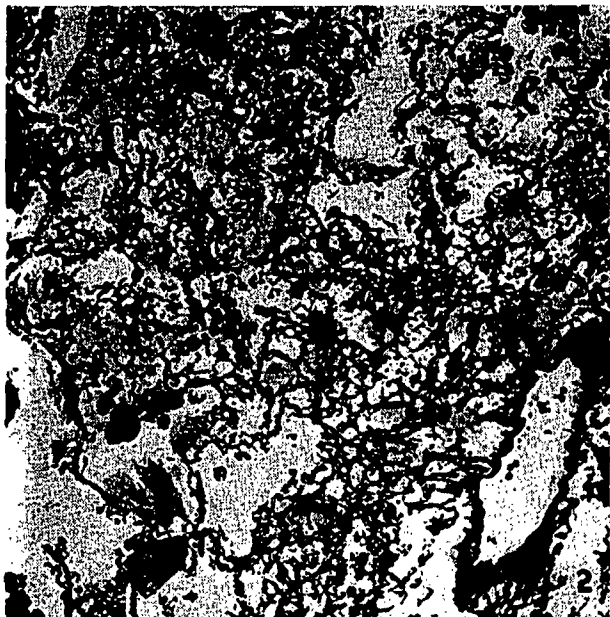


Fig. 2. Rat lung with infection, 200 days after the injection of 50 mg. of asbestos dust, showing grade-2 fibrosis. (Silver impregnation $\times 150$.)

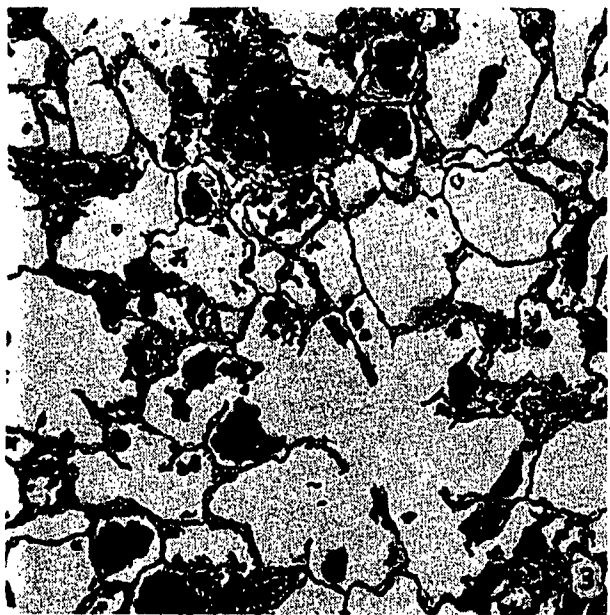


Fig. 3. Rat lung without infection, 220 days after the injection of 50 mg. of asbestos dust, showing grade-1 fibrosis, dust aggregates, and alveolar wall thickening. (Silver impregnation $\times 150$.)

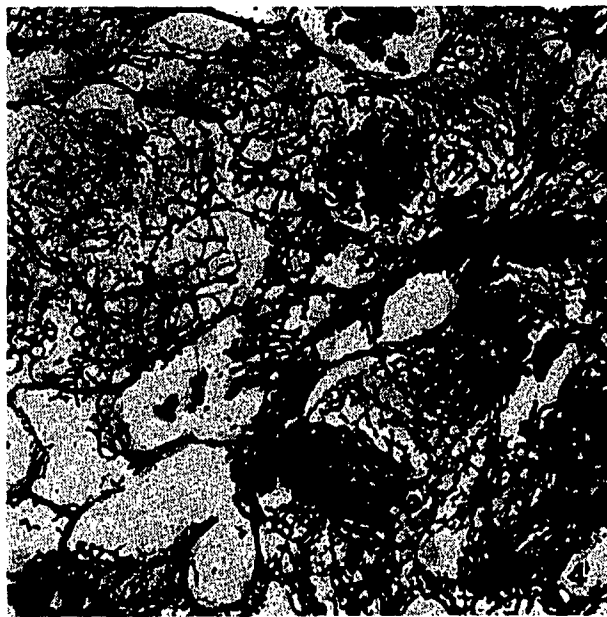


Fig. 4. Rat lung section, 200 days after the injection of dust consisting of 12.5 mg. quartz + 37.5 mg. asbestos, showing massive grade-3 fibrosis. (Silver impregnation $\times 150$.)

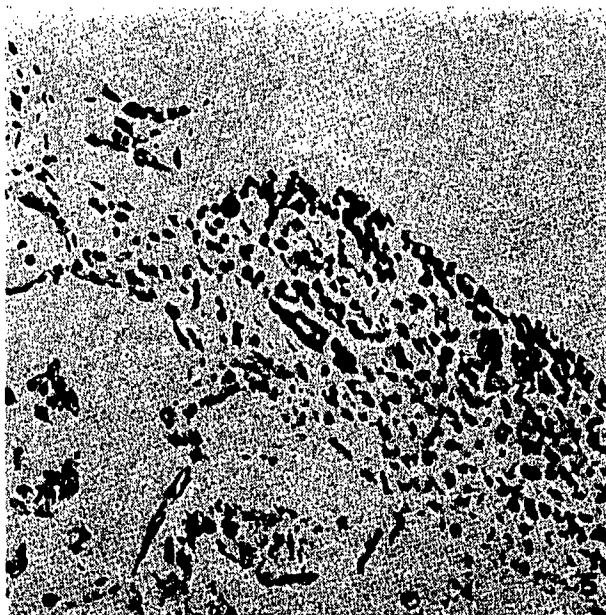


Fig. 5. Part of lung section, 180 days after injection of a quartz-asbestos mixture, showing pleural thickening with mononuclear-cell infiltration. (H. & E. $\times 150$.)

exceptionally slow up to 100 days, a marked increase in collagen was found thereafter, till the termination of the experiment at 220 days. An advanced grade-3 fibrosis was

observed, although pleural thickening was found in a few cases with infection and in most animals injected with the quartz-asbestos mixture (Fig. 5).

DISCUSSION

The proliferation of reticulin in the lungs of rats injected with fine asbestos particles and an asbestos-quartz admixture, was found to differ significantly in the later stages of the experiment. In the asbestos-quartz group a progressive increase of reticulin and collagen in the dust lesions was observed, resulting terminally in the production of compact, partially acellular, collagenous nodules. In the asbestos group progression of the initial reticulin was limited and it ended with only slight increase in compactness, with or without a few fine collagen fibres in cases with acute infection.

The present results demonstrate that fine asbestos particles are of a very low toxicity to lung tissue and produce only peribronchiolar, and to a lesser degree perivascular, reticulin networks. Even in cases with acute infections, fibrogenesis did not progress beyond grade-2 fibrosis. The typical asbestosis of humans could not be induced with pure fine asbestos dust in the lungs of rats. However, the addition of silica dust (12.5 mg.) to the same asbestos dust (37.5 mg.) led to massive fibrosis (advanced grade-3). Both the maturity and the amount of fibrosis seemed excessive when compared to the lesions caused by equivalent amounts of the individual dusts. The microscopic pattern of the pathological changes appeared to be very similar to those described by Gloyne¹⁶ and Lynch and Cannon¹⁷ in humans. Bouser *et al.*¹⁸ concluded that the common factor in the lungs of miners of asbestos and iron-ore is silica and it is suggested that this may be the carcinogenic agent, causing pulmonary fibrosis, which precedes the initiation of the malignant process. Doll⁹ also pointed out that the risk of lung cancer in asbestos workers was of the order of 10 times that experienced by other men. Probably this risk was greater before 1933 and has become progressively less during recent years as the duration of employment under the old dusty conditions has decreased.

The fact that no malignant tumours have been produced by either crocidolite asbestos or a quartz-asbestos admixture might be due to the very high resistance of the rat species to neoplasms, as well as to the limited duration of the present experiments. However, it appeared that free silica enhanced the pathogenicity of asbestos dust and that in the presence of silica some pleural changes have been produced, although not of a malignant nature.

Recently it was pointed out by Harrington¹⁹ that oils containing 3.4 benzpyrene and related substances occur in crocidolite and amosite. The significance of the association between asbestos fibres and hydrocarbons on the one hand and the production of malignant neoplasms on the other hand could not be assessed from the present experiments,

because weathered crocidolite has been used, in which the hydrocarbon content might have been extremely low. However, it is doubtful whether the concentration of 3.4 benzpyrene in virgin crocidolite would be high enough to produce neoplasms, seeing that coal dust with a relatively higher concentration appeared to be inert.

The present results support the conclusions of Bouser *et al.*¹⁸ that silica in combination with asbestos may be extremely dangerous.

SUMMARY

The pathogenicity of pure, very fine crocidolite particles and of a mixture of crocidolite and quartz was investigated after intratracheal injection of 50 mg. of these dusts into the lungs of rats. The proliferation of reticulin due to these two dusts respectively was found to differ significantly.

The crocidolite particles elicited an initial reticulosis that became slightly more compact, with or without collagen fibres, especially in cases with acute infections. It appeared that exposure only to asbestos dust predisposed to acute respiratory infections.

The asbestos-quartz admixture gave a progressive increase in reticulin and collagen, resulting in partially acellular collagenous nodules (grade-3 fibrosis). The histological picture of the pathological changes appeared to be very similar to those observed in human asbestosis. Asbestos dust in combination with a small amount of free silica should be regarded as extremely dangerous.

No malignant tumours have been produced in the experimental period by either asbestos or the asbestos-quartz admixture. Some non-malignant pleural changes were observed in animals of the asbestos group with infection, and in animals of the asbestos-quartz group.

REFERENCES

1. Wagner, J. C. in Orenstein, A. J. ed. (1959): *Proceedings of the Pneumoconiosis Conference, Johannesburg*, p. 373. London: Churchill.
2. Tobiasen, 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., Siegas, 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. Doll, R. (1955): *Brit. J. Industr. Med.*, 12, 81.
9. Cartier, P. (1952): *Arch. Industr. Hyg.*, 5, 262.
10. Vorwald, A. J. and Karr, J. W. (1938): *Amer. J. Path.*, 14, 49.
11. Vorwald, A. J., Durkan, T. M. and Pratt, P. C. (1951): *Arch. Industr. Hyg.*, 3, 1.
12. Behrens, W. (1951): *Schweiz. Z. Path.*, 14, 275.
13. Vermaas, F. H. S. (1952): *Trans. Geol. Soc. Sth. Afr.*, 55, 199.
14. Gordon, H. and Sweet, H. H. (1936): *Amer. J. Path.*, 12, 545.
15. Ross, H. F., King, E. J., Yoganathan, M. and Nagelschmidt, G. (1962): *Ann. Occup. Med.*, 5, 149.
16. Gloyne, S. R. (1951): *Lancet*, 1, 840.
17. Lynch, K. M. and Cannon, W. M. (1948): *Dis. Chest*, 14, 874.
18. Bouser, G. M., Faulds, J. S. and Stewart, M. J. (1955): *Amer. J. Clin. Path.*, 25, 126.
19. Harrington, J. S. (1962): *Nature (Lond.)*, 193, 43.

A CASE OF PERSISTENT THYMUS DIVERTICULA IN THE NECK

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The persistence of stalks of thymic tissue in the neck, demonstrating the embryological origin of the thymus gland from the entoderm of the third pharyngeal pouch, is undoubtedly a rare condition. In the course of 30 years' experience of teaching anatomy this is the first instance of such a condition that I have seen in the

dissecting room. Descriptions of such a congenital abnormality are absent in many standard anatomical and embryological textbooks, and only a few references could be found. In Braus' *Anatomie des Menschen* (1924)¹ there is a schematic and hypothetical drawing of the neck and upper thoracic region in the adult, with the various