FIBROSIS IN RATS' LUNGS PRODUCED BY RADIOACTIVE MINE DUST*

B. F. THIART, B.Sc. (Physical Educ.), B.Sc., and F. M. ENGELBRECHT, D.Sc.

Department of Physiology, University of Stellenbosch

Jones,[1] after having examined the mineral residues of 29 silicotic lungs, concluded that sericite (the hydrated silicate of aluminium and potassium) rather than free silica (quartz) was the main cause of silicosis. He also maintained that the gold-bearing quartz conglomerate (banket) of the Transvaal, South Africa, was one of the most notoriously dangerous rocks with respect to its silicotic effect. The gold-bearing quartz of the Kolar Gold Fields, India, although it contained more quartz than the South African rock, was found less pathogenic. Jones[1] ascribed the greater fibrotic activity of the

* Paper presented at 2nd anniversary of the Stellenbosch Medical School, Bellville, 5 September 1958.

South African rock to the presence of acicular fibres of sericite. These fibres were absent from the Kolar quartz or occurred very rarely.

Many authors (Fallon and Banting,[8] Lemon and Higgins,[10] Simson and Strachan,[11] and Cummins[7]) investigated the fibrotic activity of sericite with quite contradictory results. The general consensus of opinion, however, is that of the two, free silica is the more dangerous component (Mavrogordato[4]). No acceptable explanation has yet been offered for the extremely dangerous silicotic effect of the S.A. gold bearing rock and it occurred to us that the presence of radioactive material in the S.A. rock might be a possible contributing factor in the pathogenesis of silicotic fibrosis.

Animal experiments were therefore planned to examine the fibrotic response caused by radioactive and non-radioactive samples of mine dust.

MATERIAL

All the dust samples used in this experiment, except snowit, were modifications of an original dust sample obtained from one of the gold mines on the Witwatersrand, Transvaal, South Africa, which also produces uranium. The radioactivity of the samples was determined by means of a labgear model D4019/W scintillation counter with a D4019/L discriminator-unit set at 15 volts. The output pulses were counted with a Philips electronic counter. One-g. samples were used and the counting time was one minute. Silica determinations were also carried out according to the method described by Stacy and King.[5] The following samples were used:

1. R_{153}. Original mine dust with 153 output pulses per minute and a silica content of 84%.

2. R_{502}. This sample was prepared from the R_{153} dust by sedimentation in distilled water and subsequent centrifuging of the supernatant suspension. The very fine dust obtained by this procedure gave 502 pulses per minute and contained 66% of silica.

3. R_0. Non-radioactive dust was prepared from sample R_{153} by treating it with acid to remove its radioactivity. (Snell and Snell.[6]) The silica content of this R_0 dust was 86%.

4. R_{300}. This sample was prepared by mixing equal weights of R_{502} and R_0 and gave 300 pulses per minute. It contained 76% of silica.

5. Snowit (Belgian quartz). It contained 94% of silica by chemical analysis and showed no radioactivity.

All the fractions except R_{502} were ground in an electrically driven agate mortar for at least 24 hours. The particle-size distribution of the samples was determined microscopically (Table I).

TABLE I. SIZE DISTRIBUTION OF DUST PARTICLES AND SILICA CONTENT

	Snowit	R_{502}	R_{300}	R_{153}	R_0
$>10\mu$	1%	3·3%	2·1%	0·5%	0·8%
5-10μ	4%	18·8%	9·5%	1%	1·7%
$< 5\mu$	95%	77·9%	88·4%	98·5%	97·5%
% Silica	94	66	76	84	86

Preparation of Dust Suspensions for Injection

Of each sample 2 g. were accurately weighed out into screw-capped bottles, 40 ml. of sterile physiological saline added to each and then sterilized in a boiling water bath for 30 minutes. In all the samples 1 ml. of dust suspension corresponded to 50 mg. of the individual dust, and each animal received this amount.

Animals. Five groups of 20 rats (*Rattus norvegicus*, Wistar Institute) were injected with one each of the 5 samples in doses of 50 mg. of dust per animal. The intratracheal-injection technique of King *et al.*[2] was used.

Duration of experiments. The experiments lasted 320 days and the rats were killed at regular intervals as indicated in Table II. Owing to a few deaths duplicate killings could not be performed in all cases. The lungs of the animals that died were discarded after post-mortem examination.

TABLE II. DAYS OF SURVIVAL AND GRADE OF FIBROSIS

Days	*Snowit*	R_{502}	R_{300}	R_{153}	R_0
30	3	2	1	1	1
60	3	2	1	1	1
90	4	2	2	2	1
120	4	2	2	2	1
150	4	2	2	2	1
180	4	3	3	2	2
210	5	3	3	2	3
240	5	3	3	3	3
280	5	4	3	3	3
320	5	4	4	3	3

Histopathological technique. The lungs were fixed in 10% neutral formol saline and suitable blocks selected along their long axes at the level of the hilum. Serial sections were made at 6 μ and were stained with haematoxylin and eosin and by silver impregnation (Gordon and Sweet[3]). The sections were examined and the pulmonary fibrosis graded according to King *et al.*[12] Five grades of fibrosis were recognized: Grade I, loose reticulin fibres with no collagen; Grade II, compact reticulin with or without a little collagen; Grade III, somewhat cellular but made up mostly of collagen; Grade IV, wholly composed of collagen fibres and completely acellular; and Grade V, acellular, collagenous and confluent. The gradings and days of survival are summarized in Table II.

PATHOLOGICAL FINDINGS

Macroscopic appearance of the lungs. In comparing the gross appearances of the lungs, R_{153} and R_0 samples appeared to be the least harmful; the R_{300} and R_{502} fractions were more pathogenic and snowit the most pathogenic. Greyish irregular dust foci were visible subpleurally in all animals killed. The size of these lesions increased steadily and became firmer in consistency, especially in the Snowit, R_{502} and R_{300} groups. The hilar lymph nodes gradually increased in size, the increase being the greatest in the snowit group.

Microscopic study of the lungs. The pulmonary changes caused by the various dust modifications varied in rate and severity. In the snowit rats fibrosis developed more rapidly than in the other groups and by 30 days Grade III fibrosis was obtained. The lesions progressed fairly rapidly and by 210 days Grade V fibrosis was reached.

The various mine dust modifications produced more or less the classical lung pathology of experimental silicosis, but fibrosis developed more slowly than in the snowit group. The R_{502} and R_{300} samples were more pathogenic than samples R_0 and R_{153}. They produced an advanced Grade III fibrosis by 180 days, while the R_0 and R_{153} groups only reached Grade II. Photomicrographs of the lesions are shown in Figs. 1-5.

From the sections examined it also appeared that relatively more cell destruction occurred in the lungs of the R_{502} and R_{300} groups than in the other groups especially during the early stages of the experiment. The fibrosis was more diffusely scattered throughout the lungs, and not mainly nodular in character as in the snowit group.

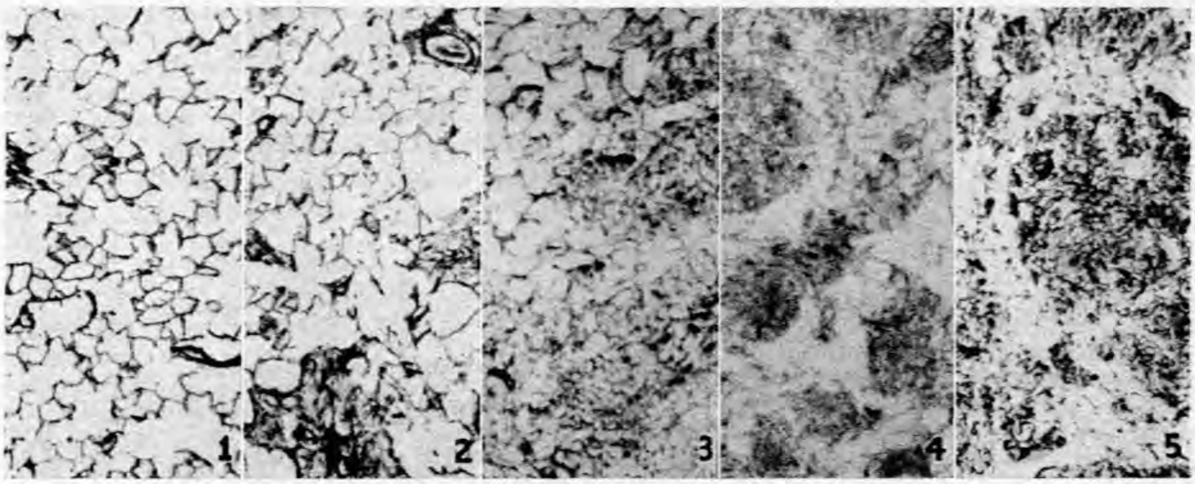

Fig. 1. Rat lung, 150 days after the injection of 50 mg. non-radioactive mine dust (R_0) with a silica content of 86% showing early Grade I fibrosis. Silver impregnation. (X 200). *Fig.* 2. Rat lung, 150 days after the injection of 50 mg. radioactive mine dust (R_{153}) with a silica content of 84% showing early Grade II fibrosis. Silver impregnation. (X 200). *Fig.* 3. Rat lung, 150 days after the injection of 50 mg. radioactive mine dust (R_{300}) with a silica content of 76% showing Grade II fibrosis. Silver impregnation. (X 200). *Fig.* 4. Rat lung, 150 days after the injection of 50 mg. radioactive mine dust (R_{502}) with a silica content of 66% showing an advanced Grade II fibrosis. Silver impregnation. (X 200). *Fig.* 5. Rat lung, 150 days after the injection of 50 mg. snowit with a silica content of 94% showing Grade IV fibrosis. Silver impregnation. (X 200).

Hyperplasia of the peribronchial lymphoid tissue was a common feature of all the sections examined, and in advanced fibrotic nodules scattered collections of lymphocytes were observed near the blood vessels and bronchial tubes.

Another prominent feature was the presence of needle-shaped, probably crystalline, structures in the lungs of the rats injected with R_{502} and R_{300}. In the R_0 and snowit groups these structures could not be detected while in the R_{153} group only a few 'crystals' were seen in some of the sections. These 'crystals' were especially abundant in the acellular nodules of the R_{502} and R_{300} series, but were also observed in some of the macrophages, in some alveoli and in a few loosely arranged cellular fibrotic nodules. There was a conspicuous new formation of canals in some of the advanced fibrotic areas caused by R_{502}, R_{300} and snowit samples.

DISCUSSION

In the present investigation snowit served as a control and it produced more fibrosis than the mine dust samples. No correlation could be established between the silica content of the different dust samples and the grade of fibrosis produced during the first 150 days of the experiment. Although sample R_{502} contained only 66% silica, it was more pathogenic than the original dust sample R_{153} which contained 84% silica. This finding suggests the presence of a possible fibrogenetic factor other than the silica content.

Needle-shaped structures were observed in the lung sections of the rats injected with samples R_{502}, R_{300} and R_{153}. In the R_{502} and R_{300} these structures occurred in abundance, especially in the fibrotic nodules and to a minor extent in the alveoli and macrophages. No such structures could be detected in the snowit and R_0 groups. According to Jones[1] the South African gold-bearing rock contains many acicular fibres which he regarded as sericite and from which he concluded that sericite was the main cause of fibrosis. Fallon and Banting,[8] Lemon and Higgins,[10] and Cummins[7], however, found sericite to be much less fibrogenetic than quartz. The high pathogenicity of the R_{502} and R_{300} samples could therefore not only be ascribed to their possible sericite content.

King, Gilchrist and Rae[9] found that acid treatment of sericite increased its fibrogenetic properties. In spite of acid pre-treatment of Sample R_0 to remove its radioactivity, it produced less fibrosis than R_{153} over the initial 150 days. (R_0 was prepared from R_{153} by acid treatment.) The fibrogenetic activity of sample R_{153}, therefore, probably depended on its radioactivity.

It is interesting to note that there was no correlation between silica content and grade of fibrosis during the early stage of the experiment. At this stage the grade of fibrosis produced corresponded to the degree of radioactivity of the various dust samples. This appeared to be very important especially when the composition of sample R_{502} is considered. It consisted of very fine particles obtained from the original dust R_{153} (see material). This fine dust (R_{502}) contained more radioactive material and less silica and produced more fibrosis than the original. This observation might explain the extremely high pathogenicity of the Witwatersrand mine dust in the following way:

Although the natural mine dust (R_{153}) produced only slightly more fibrosis than the non-radioactive R_0 sample, it is possible that those working underground are in fact exposed to a dust with a much higher radioactivity than natural mine dust, (R_{153}). The finer the dust particle the longer it will remain airborne and the better it will be inhaled. According to Orenstein[13] we are justified in regarding with some scepticism the ability of water to remove from the air the dangerous very fine particles. Since these dust particles have the highest radioactivity the inhaled dust will be more radioactive than natural mine dust, (R_{153}).

CONCLUSION AND SUMMARY

The fibrogenetic action on the lungs of rats of different radioactive mine-dust samples was investigated. In the presence of radioactive material no correlation was found

between the silica content of the samples and the grade of fibrosis produced. During the early stages of the experiment the samples containing radioactive material caused a greater fibrosis than the non-radioactive mine dust sample. During this stage the grade of fibrosis produced corresponded to the degree of radioactivity of the samples. In the later stages no marked difference was observed. It is suggested that the presence of radioactive material in the Witwatersrand mine dust contributes to its exceptional fibrogenetic activity.

We are indebted to the South African Council for Scientific and Industrial Research for a grant to defray part of the expenses of this work.

REFERENCES

1. Jones, W. R. (1933): J. Hyg. (Lond.), **33**, 307.
2. King, E. J., Mohanty, G. P., Harrison, C. V. and Nagelschmidt, G. (1953): Brit. J. Industr. Med., **10**, 76.
3. Gordon, H. and Sweet, H. H. Jr. (1936): Amer. J. Path., **12**, 545.
4. Mavrogordato, A. (1940): J. Chem. Soc. S. Afr., **40**, 326.
5. Stacy, B. D. and King, E. J. (1954): Brit. J. Industr. Med., **11**, 192.
6. Snell, F. D. and Snell, C. T. (1937): *Colorometric Methods of Analysis*: 3rd ed., Vol. II, p. 490. London: Chapman and Hall.
7. Cummins, S. L. (1937): J. Exp. Med., **49**, 163.
8. Fallon, J. T. and Banting, F. G. (1935): Canad. Med. Assoc., J. 33, 407.
9. King, E. J., Gilchrist, M. and Rae, M. V. (1947): J. Path. Bact., **59**, 324.
10. Lemon, W. S. and Higgins, G. M. (1935): Amer. Rev. Tuberc., 32, 243.
11. Simson, F. W. and Strachan, A. S. (1935): S. Afr. Inst. Med. Res. Publications, **6**, 367.
12. King, E. J., Harrison, C. V., Mohanty, G. P. and Nagelschmidt, G. (1955): J. Path. Bact., **69**, 81.
13. Orenstein, A. J. (1957): S. Afr. Med. J., **31**, 797.