THE ACTION OF VARIABLE AMOUNTS OF QUARTZ ON THE LUNGS OF RATS

THE EXTENT OF PATHOLOGICAL CHANGE IN RELATION TO THE AMOUNT INJECTED

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It is generally agreed that once a foreign body has come to rest in the lung alveoli the chief mechanism of its elimination is the lymphatic system. Foreign bodies are first engulfed by the phagocytes and these migrate along the walls of the air spaces until they reach the nearest lymph vessels, from which they ultimately reach the tracheo-bronchial lymph nodes. Therefore, small amounts of quartz which the lung can eliminate through its lymphatic system into the lymph nodes should not produce silicotic lesions in the lung parenchyma, but might cause them in the nodes, where most of the silica will ultimately be trapped.

Larger quantities of quartz, however, might be expected to overload the lymphatic drainage system, and, being retained in the lung, produce silicosis there. To test the truth of this assertion we have administered 2 mg., 5 mg., 10 mg., 30 mg. and 75 mg. doses of quartz of small particle size by intratracheal injection.

The sample of quartz used was from a commercial source.* On analysis it was found to contain 97.1% of SiO_2 . (The balance consisted of H_2O , Ca, Fe, and Mg carbonates.)

The size distribution of the particles, determined by the method of Nagelschmidt (1943), is shown in Table 1.

The silica solubility was determined by the procedure described by King (1945, 1947), and was found to be 3.4 mg. SiO_2 /100 ml. (buffered Ringer solution (pH 7.4) for 48 hours at 37°C.).

Method

The rats used were the black and white piebald variety (Medical Research Council strain). For each group the appropriate dose of quartz was suspended in a standard volume of 1.5 ml. because this has been found

TABLE 1
SIZE DISTRIBUTION OF POWDERED QUARTZ

Size (μ)	Percentage of Quartz Particles	
	By Number	By Mass
Under 0.25	7.7	0.04
" 0.5	28.9	0.8
" 1.0	67.1	12
" 1.5	83.6	20
" 2	91.9	31
" 3	98.0	54
" 4	99.0	62
" 5	99.5	70
" 6.8	100.0	100

to be well tolerated by rats of 150 to 200 g. weight. Milk, 3%, was added to help to hold the dust in suspension. The suspensions were sterilized by heating in a boiling-water bath for about 10 minutes.

The rats were anaesthetized lightly, and the trachea was exposed by dissection. The 1.5 ml. of quartz suspension was then injected intratracheally, using Kettle and Hilton's (1932) method. During the procedure both the flask and the syringe into which the suspensions were drawn were kept agitated to prevent sedimentation. The injection was done quickly and with a certain amount of force to disperse the dust well into the lung alveoli. The wound was then closed by a single suture. There was no death due to the operation.

Pathological Technique

The lungs of the rats that died or were killed were removed after insufflation to normal size with 10% formol saline. Fixation, imbedding, sectioning, and histological examination were carried out according to the technique of Belt and King (1945). Due to cannibalism and untimely deaths, it was not possible to obtain exactly equal days of survival in the several experiments. But, as far as has been possible, sufficient intervals have been maintained between killings to study the progress of the lesions on a comparative basis.

* Messrs. Colin Stewart, Ltd.

In assessing the degree of pathological change, four grades of fibrosis have been recognized: grade 1, loose reticulin fibrils with no collagen; grade 2, compact reticulin with or without a little collagen; grade 3, somewhat cellular but made up mostly of collagen; grade 4, wholly composed of collagen fibres and completely acellular; and grade 5, acellular, collagenous, and confluent. The details of the individual experiments are set out in Table 2.

TABLE 2

ASSESSMENT OF FIBROSIS AND AVERAGE MAXIMUM SIZE OF NODULES IN SECTIONS OF LUNGS OF RATS RECEIVING QUARTZ

Days of Survival	Size of Nodules (μ)	Grade of Fibrosis
2 mg. Quartz		
22	—	0
50	0	0
77	0	0
103	0	0
105	0	0+
183	0	0
293	0	0
295	0	0
365	0	0
} Fibrosis in lymph node } Some degree of peri- } vascular and peri- } bronchial fibrosis, and } fibrosis in lymph nodes		
5 mg. Quartz		
14	0	0
15	0	0
134	186	1 (minimal)
168	186	1
182	372	2
331	362	3
407	372	2
10 mg. Quartz		
7	0	0
27	0	0
30	0	0
101	141	1 (minimal)
123	160	2
154	186	2
162	223	2
165	234	2
316	316	2
30 mg. Quartz		
66	112	1 (minimal)
94	234	1
116	272	2
164	502	3
283	458	4
350	577	4
351	564	4
75 mg. Quartz		
68	260	1
121	551	3
131	595	4
207	558	5
240	949	5
302	1135	5

Pathological Findings

Quartz 2 mg.—This amount did not produce any lesion until 105 days when some fibrosis was seen in the tracheo-bronchial lymph nodes. No fibrosis was seen in the lung parenchyma of rats killed, for example, at 183 days, but there was some perivascular and peribronchial fibrosis. These changes were seen again in rats killed after 295 days, and there was some fine reticulinosiis of isolated alveoli, but this was too mild to be included under grade 1 fibrosis.

Microincineration followed by hydrochloric acid proved unsatisfactory for finding the very small amount of quartz present in these lungs. However, it was possible to recognize quartz by dark ground examination of the sections stained with haematoxylin and eosin and incinerated sections which had not been treated with hydrochloric acid. Using these methods quartz could be found in the lungs from the second to the 365th day; but it was not possible to tell, except very roughly, whether the amount diminished during the course of the experiment. The impression was gained that there was more in the early sections than in the later, but this impression cannot be considered quantitatively.

Quartz 5 mg.—Dust cell reaction was marked in rats killed at 14 and 15 days, but there was no reticulinosiis. After 134 days isolated foci of reticulinosiis were seen with grade 1 (minimal) fibrosis. A rat killed after 168 days showed typical grade 1 fibrosis (Fig. 1). On the 182nd day nodules with grade 2 fibrosis were seen. One rat that died on the 331st day had well developed nodules with grade 3 fibrosis. But the rat that lived to 407 days showed only grade 2 fibrosis. In this and subsequent groups the lymph nodes were always involved when there was fibrosis of the lungs.

Quartz 10 mg.—No fibrosis was seen in the lungs of rats killed or dying in the first 30 days, although dust cell reactions remained well marked. On the 101st day areas of reticulinosiis with grade 1 (minimal) fibrosis were present. Nodules with grade 2 fibrosis were seen in the lungs of rats after the 123rd day. This grade 2 fibrosis continued till 316 days (Fig. 2).

Quartz 30 mg.—Grade 1 fibrosis was present in the lungs of rats killed on the 66th and 94th days. On the 116th day the character had changed to grade 2, and on the 164th day to grade 3. The lungs of rats examined on or after 283 days showed grade 4 fibrosis (Fig. 3).

FIG. 1.—Rat lung, 168 days after a dose of 5 mg. of quartz, showing grade 1 fibrosis. There is a localized lesion consisting of a loose network of fine reticulin fibrils without any collagen. (Silver impregnation $\times 190$).
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FIG. 2.—Rat lung, 316 days after a dose of 10 mg. of quartz, showing grade 2 fibrosis. The lesion contains a dense network of coarse reticulin fibrils, some of which are thick enough to give the staining reactions of collagen. (Silver impregnation $\times 190$).
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Quartz 75 mg.—A rat that was killed on the 68th day showed grade 1 fibrosis. Lungs of rats examined on the 121st day showed grade 3 fibrosis. On the 131st day the lungs had acellular and collagenous nodules of grade 4 fibrosis. This changed to grade 5 from 207 days onwards (Fig. 4).

To summarize : 2 mg. of quartz did not produce any lesion in the lung, but caused fibrosis of the nodes. On the other hand 5 mg. of quartz produced grade 1 (minimal) fibrosis in 134 days, as opposed to 101 days with 10 mg. of quartz. Grade 2 fibrosis was reached with 5 and 10 mg. of quartz in 182 and 123 days respectively ; and in both experiments this grade 2 fibrosis was not exceeded during 300 days (except one grade 3 with 5 mg. at 331 days). Grade 1 fibrosis was produced in 66 and 68 days by 30 mg. and 75 mg. of quartz, but with 30 mg. the fibrosis was minimal. The 30 and 75 mg. of quartz produced grade 3 fibrosis in 164 days and 121 days, and grade 4 and 5 in 283 and 207 days respectively. In both these cases the grade remained constant after 300 days, although with the higher concentration (75 mg.) the nodules became more confluent and massive in character.

Although the highest grade of fibrosis seen in the lungs has been mentioned, it should be pointed out that in animals receiving the larger amounts of quartz the lung sections after 300 days showed all grades of nodules.

To help to assess the pathogenic action of the various amounts of quartz quantitatively, the diameters of fully matured nodules were measured in each case. The results, with the grades of fibrosis, are given in Table 2. The final figure of the size of the nodules was obtained by measuring the diameters of the three largest nodules in two directions at right angles to each other and then taking the mean average figure of the three. The measurement was made with the help of a micro-millimetre scale. Photomicrographs of sections illustrating typical findings with the different amounts of quartz are given in the figures.

Discussion

These experiments are a repetition and extension of preliminary work already described (King, Harrison, and Ray, 1949), where 5 mg. of quartz appeared to be at least partly cleared from the lungs in many of the animals used, and even 10 mg. in some, and consequently produced little or no fibrosis. Because the results were not entirely uniform, the present experiments were planned on the basis of a wider range of quartz dosage. They well illustrate the disturbance of the mechanism of the physiological disposal of foreign matter from the lungs and the stages of development of animal

silicosis. It is probable that, in the sequence of events, impairment of the drainage system of the lung constitutes the first stage in the development of silicosis.

Small amounts of quartz, namely 2 mg., are easily cleared from the lungs by the phagocytes, but are ultimately trapped in the tracheo-bronchial lymph nodes, where the progress of fibrosis is more rapid than in the lung parenchyma. With small amounts of quartz, therefore, fibrotic nodules first appear in the lymph nodes. The first irritative and proliferative reactions in the lung proper are seen round the vessels and bronchi, which are rich in lymph vessels, as was evident with 2 mg. of quartz at 183 days.

Larger amounts of quartz upset the mechanism of dust dispersal by impairing the lymph movement, and ultimately lymph stasis sets in within the lung itself. This gives a better chance for the accumulation of particles in the lung. With still larger amounts of quartz the whole cleaning mechanism breaks down, and more silica remains in the lung and thereby sets up inflammatory changes with the formation of dust granulomas and connective tissue proliferation in the lung tissue. This is the beginning of true silicosis, and is characterized by discrete nodules distributed throughout the lung.

The rate and severity with which quartz acts varies with the amount. With quartz of the same particle size, the larger the amount injected the greater is the area of lung tissue involved, and so also the nature or grade of fibrosis, when the same time factor is taken into consideration. The maximum size of nodules produced by 2, 5, 10, 30, and 75 mg. of quartz over 300 days are 0 μ , 372 μ , 316 μ , 577 μ , and 1135 μ respectively.

The grades of fibrosis are 0, 2, 2, 4, and 5 respectively. (With 5 mg. of quartz grade 3 fibrosis was seen in one animal at 362 days ; this was probably due to a high concentration of quartz at one place, caused by unequal dispersal of the dust in the lung tissue.) With 5 and 10 mg. of quartz the difference is not so marked as with higher doses where it is quite obvious. It appears, therefore, that the tissue reactions in animals are more or less proportional to the concentration of quartz in the lung.

Summary

Small amounts of quartz (2 mg. in this experiment with rats) are mostly cleared from the lung and produce fibrosis in the lymph nodes only. With larger amounts the lymphatic drainage is insufficient to remove the dust from the lung, where it remains to produce its characteristic reaction.

The pathological response of the lungs to quartz varies with the amount : with 2 mg. no fibrosis was

FIG. 3.—Rat lung, 350 days after a dose of 30 mg. of quartz, showing grade 4 fibrosis. The nodule consists of tangled collagen fibres, and with ordinary nuclear stains was practically acellular. (Silver impregnation $\times 190$).

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FIG. 4.—Rat lung, 302 days after a dose of 75 mg. of quartz, showing grade 5 fibrosis. Several grade 4 nodules have coalesced to form this large mass of dense fibrosis. (Silver impregnation $\times 80$).

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found in the lung, but fibrosis was found in the lymph nodes which were always involved where larger amounts of quartz were used. In the lungs, with 5 and 10 mg., nodules of compact reticulin were formed ; 30 mg. produced collagenous nodules ; and with 75 mg. the nodules were both collagenous and confluent.

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REFERENCES

- Belt, T. H., and King, E. J. (1945). *Spec. Rep. Ser. med. Res. Coun. Lond.* No. 250, p. 29.
 Kettle, E. H., and Hilton, R. (1932). *Lancet*, 1, 1190.
 King, E. J. (1945). *M.R.C. Spec. Rep. Ser. med. Res. Coun. Lond.* No. 250.
 — (1947). *Occup. Med.*, 4, 26.
 —, Harrison, C. V., and Ray, S. C. (1949). *Proc. 9th Int. Congr. industr. Med.*, p. 666. London.
 Nagelschmidt, G. (1943). *Spec. Rep. Ser. med. Res. Coun. Lond.* No. 244, p. 99.