TISSUE REACTIONS PRODUCED BY CALCIUM FLUORIDE IN THE LUNGS OF RATS

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In a previous paper (King, Mohanty, Harrison, and Nagelschmidt, 1953), we showed that removal of an amorphous "high-solubility layer" from the surface of quartz particles with hydrofluoric acid appeared to enhance their fibrosis-producing capacity rather than to diminish it. Although the HF-treated quartz had been repeatedly washed, and even electro-dialyzed, it had at least as much, and usually more, fibrogenic capacity than the original quartz powder. We considered that this might be due to traces of hydrofluoric acid retained on the quartz particles. We thought it would be interesting, therefore, to test the effect of a relatively insoluble fluoride in the lungs of similar animals.

Lung changes have been reported by Luton and Champeix (1951) in men working with calcium fluoride in France. Their radiographic changes were described as typical of pneumoconiosis, and, indeed, similar to silicosis. The disease had evolved very rapidly, six of the 19 cases having developed it in less than two years. In another group of workers, severe lung disease was also found, but the radiographic shadows were atypical and different from those of silicosis. The dust inhaled by the first group of workers contained 60% of silica with 36% calcium fluoride and small amounts of aluminium and iron oxides. The second group inhaled dust which was mainly calcium fluoride (92-96%) with only 3-5% silica. Luton, Champeix, and Faure (1953) considered that calcium fluoride may by itself injure the lung tissue and produce lesions, and that it certainly enhances the action of silica, resulting in a severe and rapidly-developing form of pneumoconiosis.

Policard and Collet (1953) injected calcium fluoride intraperitoneally into rats, and found that after one to two months it gave a marked fibrotic reaction in the peritoneum, even more severe than that produced by quartz, but that, in contrast to quartz, it caused only an insignificant reaction in the lymph glands.

Experimental

Water-clear colourless crystals of calcium fluoride were ground to a fine powder and water-sedimented to give a 0.5-2 μ fraction. The particle size distribution of the sample is given in Table 1. It contained less than 0.1% of silica, and had a solubility in water at room temperature of 1.6 mg. CaF₂ per 100 ml.

TABLE 1
PARTICLE SIZE DISTRIBUTION AND ANALYSIS OF CALCIUM FLUORIDE DUST

Size (μ)	Percentage by Number	Percentage by Mass
0.04 - 0.06	14.5	—
0.7 - 0.8	8.4	—
0.9 - 0.11	8.1	—
0.12 - 0.16	6.6	—
0.17 - 0.23	5.5	0.2
0.24 - 0.32	7.6	0.5
0.33 - 0.45	9.0	1.7
0.46 - 0.64	15.0	8.2
0.65 - 0.9	15.4	23.1
1.0 - 1.3	7.9	32.9
1.4 - 1.8	1.6	18.9
1.9 - 2.6	0.4	14.5

Dvs* 0.98

MMD† 1.0

σ ‡ 1.6

* Dvs = volume surface diameter, $\frac{\sum nd^3}{\sum nd^2}$.

† MMD = Mass median diameter:

Size at 50% mass on cumulative size distribution curve.

‡ σ = geometrical standard deviation:

ratio of diameters having 50% and 16.5% mass or having 83.5% and 50% mass. These are 1.7 and 1.5, mean 1.6.

A group of 22 male rats of the black and white Medical Research Council strain (average weight 200 g.) was injected intratracheally with 50 mg. each of calcium fluoride. The animals were killed at regular intervals up to one year and the lung pathology studied. The collagen content of the lungs was determined in order to compare the values obtained with those from a group of rats injected intratracheally with 50 mg. of quartz (Stacy and King, 1954). The lymph glands were dissected and weighed.

The preparation of the dust suspension for injection was as follows: A sample of the dust, 1.5 g., was weighed into a glass tissue grinder, and 30 ml. of sterile physiological saline added. The dust particles dispersed easily.

The suspension was transferred to a bottle, sterilized by autoclaving for 20 minutes and then placed on a mechanical shaker until the time of injection.

The animals were lightly anaesthetized with ether, and were injected intratracheally with 1 ml. of the suspension (containing 50 mg. CaF_2). The injections were made, as described in the previous paper (King *et al.*, 1953), through a long blunt needle inserted through the mouth and down the trachea as far as the carina.

The fate of the rats, whether they died or were killed, and the duration of the experiment are shown in Table 2.

TABLE 2
PATHOLOGICAL CHANGES IN LUNGS OF RATS GIVEN
50 MG. CALCIUM FLUORIDE

Days of Survival	Mode of Death	Grade of Fibrosis
30	Killed (2)	2
39	Died (2)	2
41	Died (1)	2 max.
42	Died (1)	2 max.
70	Killed (2)	2 max.
81	Died (1)	3
90	Killed (2)	3*
91	Died (1)	2
105	Died (1)	2
150	Killed (1)	2
180	Killed (1)	2
207	Died (1)	—
210	Killed (1)	2
275	Killed (1)	2
300	Killed (1)	2
330	Killed (1)	4*
365	Killed (1)	3

* Sections reproduced in figures.

Numbers in brackets indicate number dead or killed.

Max. = maximum within the indicated grade of fibrosis

Grades of fibrosis: (1) lesions cellular, loose network of reticulin
(2) cellular, thick compact reticulin, with or without some collagen
(3) slightly cellular, mainly collagen
(4) completely acellular and wholly collagenous
(5) lesions acellular, collagenous and confluent

Most of the deaths took place within the first 90 days. Two animals were killed each month until this time, thereafter one animal.

The techniques of removing the lungs, fixation, embedding, and sectioning were as previously described (King *et al.*, 1953). Blocks were selected through the long axes of both lungs at the level of the hilum and sections were stained with haematoxylin and eosin, impregnated with silver for reticulin and collagen, and micro-incinerated to reveal the distribution of the dust.

Collagen analyses were performed on the dried powdered remnants of each pair of lungs, to which were added the remainders of the blocks and their trimmings after suitably de-waxing with benzene. Results have been expressed as milligrams of collagen contained in each pair of lungs. These figures are comparable, and are substantially correct, since only very small proportions of the lungs were lost to analysis by inclusion in the sections.

Results

Subpleural collections of dust were seen as greyish-white foci, situated chiefly over the dorsal aspect of the lungs, early in the experiment. The

hilar lymph nodes were enlarged to four or five times normal size. By 40 days small areas of fibrosis were seen scattered over both lungs. By 90 days they were 2 to 3mm. in diameter, and were hard. The animals which died during the first 90 days showed evidence of bronchopneumonic consolidation, widespread necrosis, and appearances simulating an acute pulmonary infection; but Gram-staining did not reveal the presence of organisms. The lymph nodes were greatly increased in size and were about six times normal at 110 days. From this time onward there appeared to be a regression in the size of the lymph nodes, and at one year they were two or three times the normal size and very firm. Average weights of the nodes are given in Table 3 together with those of control rats, (a) without dust and (b) with 50 mg. of quartz.

TABLE 3
FRESH WEIGHTS OF LYMPH NODES IN CaF_2 EXPERIMENT
COMPARED WITH WEIGHTS WITH DOSE OF 50 MG. QUARTZ
AND WITHOUT DUST

Time after Injection (days)	Node Weight (mg.)		
	CaF_2	Quartz	No Dust
0-90	260 (12)*	240 (9)	32 (8)
90-210	180 (4)	660 (12)	35 (14)
270-365	80 (4)	1,100 (3)	—

*Numbers in brackets are number of rats.

Microscopically, there appeared to be a good dust cell response by 30 days and a marked reaction in the peribronchial lymphoid tissue. Small discrete lesions of irregular shape were mainly perivascular or peribronchial. But a large proportion of the dust was lying free in clumps in the alveoli. At both 30 and 40 days the lesions were seen to contain a compact network of reticulin with some collagen. Besides the fibrotic lesions some of the lungs showed evidence of patchy consolidation, with widespread necrosis and infiltration with acute inflammatory cells. No microorganisms could be found. This condition was particularly evident in the animals that died, and we have assumed that it was brought about by the dust itself. At 60 days the nodular lesions were still scanty, cellular, and discrete. At 90 days the reticulin network was largely replaced by collagen fibres, thick and compact (Fig. 1). (The fibrosis produced by a similar dose of flint is shown in Fig. 2.) The very high concentration of dust within the lesions is shown in Fig. 3. Animals which died at 101 and 105 days showed marked necrosis and acute inflammatory cell infiltration, in addition to the fibrotic nodules, but no microorganisms were demonstrated. Up to 330 days there was an increase in the number of nodules, but the maturity of their fibrosis was not increased, except in one animal, where they were fully collagenous although smaller

FIG. 1.—Compact, mainly collagenous nodules, in a rat lung, 90 days after 50 mg. calcium fluoride, Grade 3 fibrosis, Silver impregnation, $\times 76$.

FIG. 2.—For comparison, rat lung 90 days after 50 mg. of flint. Grade 4 fibrosis.

FIG. 3.—Serial section to Fig. 1. Dark-ground illumination, showing dense, centrally deposited dust collections within the nodules. Micro-incinerated preparation, $\times 76$.

FIG. 4.—Small, compact, wholly collagenous nodules, 330 days after 50 mg. calcium fluoride. Grade 4 fibrosis. Silver impregnation, $\times 76$.

and fewer (Fig. 4). In the one animal remaining alive at 365 days they were likewise fewer and smaller, and they were less collagenous than in the 330-day-old animal, but in addition there were many thick bands of collagen in both lungs which were not aggregated together to form nodules.

The animals killed at 180, 300, and 365 days showed, in addition to the fibrotic nodular lesions, evidence of bronchiectasis with some bronchiectatic abscesses, septal thickening, destruction of lung tissue, and emphysema. The mode of death and histological assessment are given in Table 2.

The collagen analyses of these animals injected with 50 mg. of calcium fluoride were compared with the figures obtained by Stacy and King (1954) with a similar group of rats injected with 50 mg. quartz. The results are contained in Table 4. Up to 90 days

TABLE 4
COLLAGEN CONTENT OF LUNGS OF RATS INJECTED WITH CALCIUM FLUORIDE AND WITH QUARTZ*

Duration of Experiment (days)	Total Collagen in Both Lungs (mg.)		
	Calcium Fluoride in Rats	Quartz In Rats	Control Rats
30	18	12	16
34	20	—	—
40	13	—	18
42	14	—	—
60	31	39	17
81	34	—	16
90	33	42	—
91	27	—	—
105	28	—	21
120	—	94	21
150	36	141	26
180	68	90	21
210	36	115	24
240	—	400	24
270	—	146	—
275	39	—	—
300	37	247	—
330	34	151	—
365	32	158	—

*We are indebted to Dr. Brian Stacy and Dr. F. M. Englebrecht for these results.

there was not much difference, but thereafter the amount of collagen which formed in the calcium fluoride rats was very much less than that in the rats injected with quartz.

Discussion

In this experiment calcium fluoride had a marked toxic effect on the cells. Up to 120 days there was evidence of widespread aseptic necrosis of the lung

tissue, associated with large amounts of dust lying free and within macrophage cells. Although there was certainly some fibrosis, the fibrotic nodular lesions were not nearly as prominent a feature as had been anticipated from the description of Policard and Collet (1953) of their results with the intraperitoneal injection of calcium fluoride. Injected intratracheally into the lungs, we found calcium fluoride very much less fibrogenic than quartz, although much more toxic to cells. Whether the minute traces of fluoride which may have remained on the HF-treated quartz, used in our previous experiment, had anything to do with the greater pathogenicity which it showed over the parent quartz from which it was prepared, remains in doubt. Recent experiments with quartz etched with HF and with NaOH showed similar enhanced fibrogenic activity and make this unlikely (Englebrecht, Yoganathan, King, and Nagelschmidt (1958). It might, however, be interesting to test the effect on the lungs of giving calcium fluoride and quartz simultaneously, in view of the very rapidly developing pneumoconiosis found by Luton and Champeix (1951) in workers in a fluoride mine who inhaled a mixed dust consisting of silica and calcium fluoride.

Summary

Calcium fluoride dust of 1μ mean diameter was injected into the lungs of rats. It had a severe toxic effect on the cells, especially during the early stages, leading to extensive necrosis. The fibrogenic capacity of calcium fluoride was much less marked than that of quartz, as seen histologically and as determined by chemical analysis of the collagen content of the lungs.

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