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THE ACTION OF FLINT OF VARIABLE SIZE INJECTED AT CONSTANT WEIGHT AND CONSTANT SURFACE INTO THE LUNGS OF RATS

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The importance of the size of the dust particles concerned in the production of silicosis has been widely appreciated, but the relationship between particle size and intensity of tissue reaction in man is complex. The size of the dust exerts a direct influence upon the number of particles suspended in the air to be inhaled, and on the penetrating power of the particles into the lung. Other factors, such as distribution, weight, flocculation, and aggregation, and variation in the composition of dust in air, depend much on the size of the particles. In the last few years the attention of many workers in this field has been drawn to investigating the range of sizes capable of entering the lung, and the relation of particle size of a particular dust to maximum pathogenicity.

This field has been ably discussed by Hatch and Hemeon (1948) who point out the need for further research to determine the relation between toxicity of quartz and particle size.

As it has been possible to produce definite silicosis in experimental animals within a reasonably short period with free silica dusts, different particle sizes of such dusts might be expected to produce appreciable differences in lung pathology within a convenient experimental time. Some experiments in this field have been carried out by Gardner and Cummins (1933) and Tebbens, Schultz, and Drinker (1945). Gardner and Cummins used intravenous injection into rabbits of quartz of diameter 1–3μ and 6–12μ, and also of aluminium oxide 1–3μ. They found that the fine quartz fraction produced hyaline fibrosis, mainly in the liver, whereas the coarse quartz only gave a foreign body type reaction. The aluminium oxide did not produce any reaction.

Tebbens and his colleagues also used intravenous injection into the ear veins of rabbits of suspensions of 400 mg. quartz of mean sizes 3·3, 1·7, 1·0, and 0·6μ diameter. These produced fibrosis in various organs, particularly the liver and spleen, and the smallest particles were much the most active. It was concluded that

> " Particles of 1 micron and under are the most active and dangerous, not only in their ability to remain suspended in the air, but even of greater importance is their activity once they gain entrance to the deeper recesses and to the body and tissue."

Hatch and Kindsvatter (1947) made dust inhalation experiments on guinea-pigs with quartz below 0·5μ diameter. The dusting period lasted from four to 20 weeks, and the authors calculated that up to 35 mg. per animal could have been retained in the alveolar tissue. Lung sections were illustrated showing fibrosis after 20 weeks, but no detailed pathological description was given. Attention was drawn to the rapidity of the lung changes, and it was claimed that 35 mg. per animal was " strikingly below the dosage commonly employed ".

From these studies it appears that silicosis can be experimentally produced either by inhalation or by injection of silica dusts of a wide range of particle size, the upper limit of which should be less than 10μ. About the lower limit little is known ; it is probably above 0·002μ (c.f. King, 1947 ; Dale and King, 1953) and may be above 0·2μ. To carry out further investigations on the relation of particle size to pathogenicity, and with dusts more accurately

sized than those previously employed, experiments were set up using flint in several fractions covering the range from below 0·5μ to 8μ equivalent diameter.

As we are attempting to develop animal techniques in silicosis research along quantitative lines, injection methods are necessary for such work. Only in this way is it possible to introduce a known dosage directly into the lung tissue. It is recognized that such methods are further removed from industrial conditions in man than are animal inhalation experiments. But a number of complications arising out of maintenance of a dust cloud, the access and/or retention of different amounts of dust in the lungs (see the variable amounts found in inhalation experiments by King, Wright, Ray, and Harrison, 1950), possibly different breathing rates of animals, lung retention characteristics, are all avoided. The experiments were therefore carried out by intratracheal injection. In a first series equal weights of pure flint of five different size doses were injected into the lungs of rats.

The pathogenicity of silica dust is probably a function of the total surface it exposes to the tissues. Two different mechanisms may be involved. Solubility is certainly proportional to the surface and if the fibrogenesis is related to " solubility " then a given dust will act in proportion to its surface. But, if it is not the dissolved silica but the surface itself that acts, perhaps as a catalyst promoting protein degeneration and fibrosis without actually being used up in the process, then surface also is the deciding factor.

Before these relations can be studied in detail it is desirable to get qualitative, and, if possible, quantitative information on the limiting upper and lower sizes that cause such fibrosis and on their different effects on lung tissue.

We, therefore, tested the effects of constant surface area, as well as constant amounts (by weight) in relation to the fineness of particle size. For this purpose the same flint dusts as used in the constant amount experiments were used for a series of tests employing variable amounts of dust to give a constant surface area in each case, with the same wide range of particle sizes.

DESCRIPTION OF SAMPLES

A very pure commercial sample of ground calcined flint was used. It contained by chemical analysis over 99% of silica. After size fractionations were carried out it was found that all fractions, including the finest sample (below 0·5μ), still contained, on an ignited basis, well over 99% of silica. There were five flint samples with the following nominal size limits: under 0·5μ, 0·5–1μ, 1–2μ, 2–4μ, 4–8μ. The size separations for the fractions up to 2μ were done by centrifuging, and for the larger fractions by settling in distilled water without using any dispersing agent. The finest fraction was isolated first, and 30 or more repeated centrifugations were required to remove the particles below 0·5μ equivalent diameter as completely as practicable. The residue was used to isolate the next coarser fraction, and so forth.

Details of the size distribution and chemical composition of the samples are given in Table 1. The nominal sizes of the samples given above refer to equivalent spherical diameters, whereas the measured sizes in Table 1 refer to projected diameters. Insofar as the particles are not spheres the projected diameters are larger. The size distribution of the fraction below 0·5μ was obtained using the electron microscope, and those of the larger fractions using the light microscope. The estimates of the surface areas were derived by calculation from the size counts.

All fractions were examined by *x*-ray diffraction technique. They showed a strong quartz pattern and a medium-strong cristobalite pattern. There was no evidence of any change in the cristobalite/quartz ratio for the different size fractions. The sample of flint of 2 to 4μ size was analysed by a quantitative *x*-ray diffraction method and found to contain approximately 50% of quartz and 35 to 45% of cristobalite. Fig. 1 shows the diffraction patterns of quartz, flint, and cristobalite.

PREPARATION OF THE DUSTS

Amounts of 2 g. of each size fraction were weighed into 100 ml. conical flasks, and well shaken with 60 ml. of sterile physiological saline. These flasks were sterilized by autoclaving (20 min. at 15 lb. pressure) on the day of injection. Thus 1·5 ml. of the dust suspension contained 50 mg. of the individual dust, and this amount was injected into each animal.

The same flint dust was used for the constant surface area experiments. It was calculated that 8 mg. of $<$0·5μ, 17·5 mg. of 0·5–1μ, 42·5 mg. of 1–2 μ, 100 mg. of 2–4 μ, and 199 mg. of 4–8μ dusts had an equivalent surface area (700 cm.^{2}). But as the amounts of the dusts in the last two sizes were too large for a single injection, it was decided to inject all the animals in divided doses, the first four sizes of the dust in two doses, and the last one in four doses, at weekly intervals. Accordingly two lots of 0·2 g., 0·437 g., 1·062 g., and 2·5 g. respectively from the $<$0·5μ, 0·5–1μ, 1–2μ, and 2–4μ dusts, and four lots of 2·488 g. of 4–5μ size, were weighed into screw-cap containers. On the day of the injections one bottle from each size was taken, and well shaken with 25 ml. of sterile physiological saline. The bottles were sterilized by autoclaving. Of the dust suspension,

TABLE 1

SIZE DISTRIBUTION AND ANALYSIS OF FLINT DUSTS

Size (μ)	4–8μ Per cent.		2–4μ Per cent.		1–2μ Per cent.		0·5–1μ Per cent.		<0·5μ Per cent.	
	By No.	By Mass	By No.	By Mass	By No.	By Mass	By No.	By Mass	By No.	By Mass
<0·16	—	—	—	—	—	—	—	—	31·5	2·8
0·16–0·23	—	—	—	—	—	—	2	0·1	29·5	6·6
0·23–0·32	—	—	—	—	—	—	9	0·9	20·4	11·3
0·32–0·45	13	0·005	6	0·01	—	—	29	8·1	9·3	12·9
0·45–0·64	8	0·01	3	0·02	—	—	40	31·5	7·1	31·2
0·64–0·9	10	0·03	4	0·05	—	—	17	39·0	1·8	21·7
0·9–1·3	11	0·1	7	0·22	3	0·4	2·9	18·6	0·4	13·5
1·3–1·8	12	0·3	10	0·9	35	10·9	0·1	1·8	—	—
1·8–2·6	12	0·7	24	6·5	47	42·1	—	—	—	—
2·6–3·6	6	1·2	24	18·4	13·4	33·0	—	—	—	—
3·6–5·1	4	2·2	17	36·7	1·4	9·7	—	—	—	—
5·1–7·2	9	14·3	4·4	26·9	0·2	3·9	—	—	—	—
7·2–10·2	10	45·0	0·6	10·3	—	—	—	—	—	—
10·2–14·4	5	36·1	—	—	—	—	—	—	—	—
Particles counted ..	280		271		365		332		495	
Estimated surface area ($m^2/g.$) ..	0·44		0·69		1·6		4·0		6·7	
SiO_2(%)	98·4		99·2		99·4		99·4		98·8	
Ignition loss (%) ..	0·3		1·8		0·1		0·9		2·2	
Silica "solubility" at "constant weight," i.e., 2% suspensions of the flint dusts (SiO_2 mg./100 ml.)	5·1		8·9		13·1		13·4		13·6	
Silica "solubility" at "constant area," i.e., 7000 $cm.^2$ per 100 ml. Ringer solution	4·1		4·2		4·6		2·1		4·1	
Approximate no. of particles in dose given (mg.) in "constant area" experiment ..	$0·5 \times 10^9$ (199 mg.)		2×10^9 (100 mg.)		10×10^9 (42·5 mg.)		63×10^9 (17·5 mg.)		135×10^9 (8 mg.)	

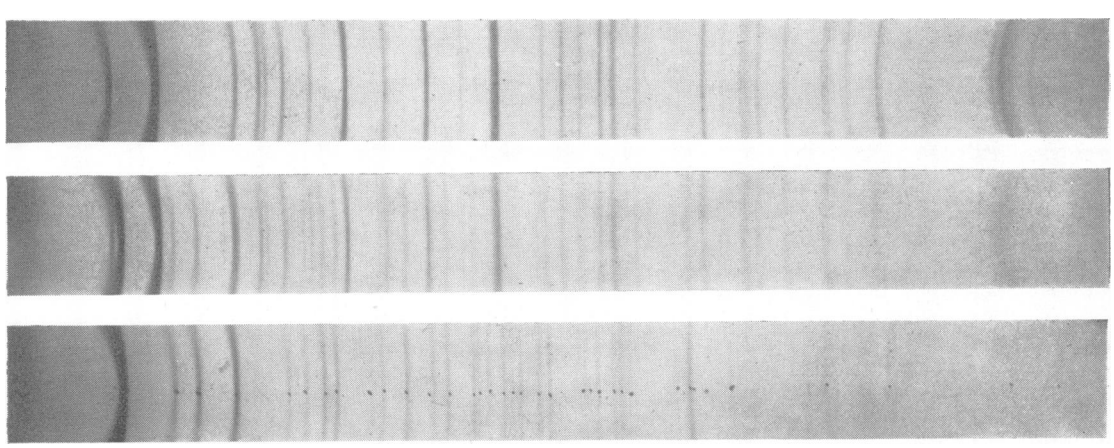


FIG. 1.—Diffraction patterns of quartz, flint, and cristobalite.

0·5 ml. corresponded to 4, 8·75, 21·25, 50, and 50 mg. of <0·5, 0·5–1, 1–2, 2–4, and 4–8μ dusts, respectively. To complete the full dose this amount from each dust was injected twice in the first four and four times in the last case.

When these samples were prepared the size distribution

of the fraction below 0·5μ could not be determined as the electron microscope of the Sheffield laboratory had not yet been installed. Subsequent checks showed that the specific surface of this fraction had been overestimated and the dosage given (8 mg.) actually corresponded to only 520 cm.^{2} per rat.

ANIMALS

Thirty male rats were used for each size of the dust. They were from the Medical Research Council black and white strain, and their average weight was 225 g.

EXPERIMENTAL PROCEDURE

In the constant-amount experiments the dust suspensions were injected into the lungs of the animals intratracheally following the technique of Kettle and Hilton (1938) modified by Belt and King (1945). The rats were anaesthetized lightly with ether, and the trachea was exposed by blunt dissection. The sterile dust suspension was well shaken, and 1·6 ml. (about 0·1 ml. usually remained in the syringe) was withdrawn through the rubber cap, using a 5 ml. syringe to which a short sharp needle (1 in. × 22 gauge) was fitted. To obtain good dispersion of the dust into the lung alveoli the suspension was kept agitated until the time of injection, and to prevent sedimentation within the syringe it was emptied quickly with a certain amount of force into the lungs. A small amount was occasionally regurgitated. The wound was closed by a single suture.

While this technique appeared suitable for constant amounts of dust, the following difficulties were foreseen in its use for injecting the animals for constant-surface-area experiments :—(1) It would probably be more difficult to operate at the same site several times at weekly intervals ; (2) the repeated operations might expose the animals to external infections, and thereby lead to a high mortality rate among them ; (3) occasional regurgitation of the suspension of the dust would make a great variation in the surface area of the injected amount.

To avoid these difficulties it was decided to modify the technique by abolishing the incision and injecting the dust suspension *via* the mouth through a long, blunt needle at the bifurcation of the trachea. The amount of fluid in which the dust was suspended was also kept as small as possible so that the risk of regurgitation would be minimized.

The rats were anaesthetized as before. The tongue was retracted with a small clip. An auroscope fitted with a medium-sized speculum was passed into the mouth as far as the oropharynx. After cleaning the throat of mucus with a swab the two vocal cords were seen running vertically. The cords were lightly touched with 2% "anethaine" solution to produce temporary local anaesthesia. A large, blunted needle (4 in. × 14 gauge) was passed between the cords, keeping the tip of the needle in touch with the anterior wall of the trachea so that it glided over the trachea rings while it was passed forwards, and imparted a vibrating sensation to the needle. When the end of the needle hit the corina it was withdrawn about 2 mm. The sterile dust suspension, kept agitated in a mechanical flask shaker, was withdrawn through the rubber washer, using a 1 ml. syringe to which a short, sharp needle (1¼ in. × 20 gauge) was fitted, and 0·75 ml. was taken, since 0·25 ml. (on the average) remained in the syringe and the large needle, and a little air (about 0·2 ml.) was also drawn into the syringe. The syringe was quickly removed from the short needle, and attached to the large needle in the trachea. The suspension was injected quickly and forcibly. In this way, with the jet of the suspension hitting the corina, the dust runs into both the lungs in approximately equal amounts. The small amount of air in the syringe assists in the shaking to keep the dust in suspension, and also helps to eject the last of the dust suspension from the syringe and through the needle. The needle is kept in position for a second or two and then withdrawn quickly. Immediately after the injection a short period of apnoea of about two to three seconds was noticed, which was followed by a deep breath. No regurgitation of the suspension was seen.

PATHOLOGICAL TECHNIQUE

Routine necropsies were carried out on the rats which died and were killed. The lungs of the killed animals were gently distended by injecting about 10 ml. of 10% formol-saline through the trachea, which was exposed at the neck. Most of the air in the lungs was expressed by gentle pressure on the sides of the thorax before the injection was made. The trachea was tied off, the thoracic cavity opened, and the lungs, with the tied-off portion of the trachea, were removed and placed in the fixative. In the case of the dead ones, the lungs were first removed intact, examined, and then distended gently with the fixative to the normal size, as satisfactory replacement could not be obtained otherwise. After preliminary fixation for four days blocks were selected in the long axes of both the lungs at the level of the hilum to obtain maximum representative areas from each lung and to include the hilar lymph nodes. After complete fixation, the blocks were embedded and cut in paraffin at 5μ. Sets of four serial sections from each lung were taken. The first was stained with Gordon and Sweet's silver impregnation, the second with haematoxylin and eosin, the third was for microincineration, and the fourth was kept as spare and was used for Gram's stain in some cases to exclude infection.

DURATION OF EXPERIMENTS

Forty-one rats from the constant-amount series and five from the constant-surface series were lost by death and cannibalism. The rats were killed at monthly intervals up to a period of one year, and in the constant-surface series up to 500 days. Because of the large number of deaths in the constant-amount series it was difficult to maintain the killing intervals constant all through the experimental period. However, with long enough intervals between killings it was possible to extend the experiments up to one year (Table 2).

In the constant-surface-area series the animals survived better, and after three months two rats from each group were taken at every killing period, up to 500 days, when the experiment was terminated.

TABLE 2

ASSESSMENT OF FIBROSIS IN LUNGS OF RATS PRODUCED BY A CONSTANT AMOUNT (50 mg.) OF DIFFERENT PARTICLE SIZES OF FLINT DUST

Samples	4–8μ		2–4μ		1–2μ		0·5–1μ		<0·5μ	
Days of Survival	Mode of Death	Degree of Fibrosis	Mode of Death	Degree of Fibrosis	Mode of Death	Degree of Fibrosis	Mode of Death	Degree of Fibrosis	Mode of Death	Degree of Fibrosis
1–30	D	(–)	D	(–)	D	(–)	D	0	D	(–)
	D	(–)	D	(–)	K	2	D	(–)	D+	2
	D	0	D	(–)	—	—	D	1	D	2
	D	0	D	0	—	—	D	(–)	D	3
	K+	1	D	0	—	—	K+	2	K	4
	—	—	K+	1 max.*	—	—	—	—	—	—
31–60	D	1	D	(–)	D	1	D	(–)	D	(–)
	D	1	D	(–)	D	1	D	3	D	(–)
	D	(–)	D	(–)	D	(–)	D	(–)	D	(–)
	D	1	D	(–)	D	(–)	D	(–)	K	4
	D	(–)	—	—	D	(–)	D	(–)	—	—
	D	1	—	—	D	(–)	D	(–)	—	—
	D	(–)	—	—	K	3 min.	D	(–)	—	—
61–90	D	(–)	K	1	D	2	K	2	D	(–)
	K	2 min.*	—	—	K	3 min.	—	—	D	(–)
	—	—	—	—	—	—	—	—	D	3
91–150	K+	1	K+	2	D	2	D	4	K	3
	—	—	—	—	D	2	D	4	D	4
	—	—	—	—	K+	3	D	4	D	(–)
	—	—	—	—	—	—	D	4	D	(–)
	—	—	—	—	—	—	D	(–)	D	3
	—	—	—	—	—	—	D	3	K+	5
	—	—	—	—	—	—	D	2	—	—
	—	—	—	—	—	—	D	1	—	—
	—	—	—	—	—	—	K+	3	—	—
150–300	K	1	D	2	D	(–)	D	(–)	K	2
	D	(–)	K	2	D	5	K	2	K	3
	K	2	K	2	D	(–)	K	5	K	3
	D	(–)	D	2	K	4	D	5	K	3
	K	2	K	2	K	3	K	5	—	—
	D	(–)	K	2	K	5	—	—	—	—
	D	2	K	3	K	5	—	—	—	—
	—	—	—	—	K	4	—	—	—	—
301–365	K	2	D	(–)	K	4	K	3	K	2
	K	3	D	5	K	4	K+	5+	K	4
	K	1	K	1	K	1	K	3	K	1
	K	1	K	1	K	5	—	—	K	5
	K	1	K	3	K	1	—	—	K	3
	K	1	K	2	K	3	—	—	K	5
	K+	2+	K	3	K	5	—	—	K+	5+
	K	1	K	3	K+	5+	—	—	K	5
	—	—	K	2	—	—	—	—	—	—
	—	—	K+	3+	—	—	—	—	—	—
	—	—	K	2	—	—	—	—	—	—

+ Sections from these animals are reproduced in figures.
K = killed, D = died. * Max. = maximal, min. = minimal within the indicated group of fibrosis.
(–) Lost through cannibalism

PATHOLOGICAL FINDINGS

Sections were examined microscopically, and the fibrosis present in the most advanced lesions from any pair of lungs has been graded according to Belt and King (1945). The pathological gradings, so obtained, along with the duration of the experiments and the days of survival of the animals, have been summarized in Tables 2 and 3. The basis of the pathological gradings was as follows : grade 1, loose reticulin fibrils with no collagen ; grade 2, compact reticulin with or without some collagen ; grade 3, somewhat cellular, but made up mostly of collagen ; grade 4, completely acellular and fully collagenous ; grade 5, acellular, confluent, and collagenous. It should be noted that in this classification the stages 1 to 4 measure increasing maturity of the silicotic lesions. Progression to stage 5 indicates confluence and depends therefore to some extent on the amount of the lesions, which will depend on the amount of dust introduced as well as on its distribution.

Gross Appearance of the Lungs

In the constant-amount series the appearance of the lungs was very variable within the individual groups. The animals having the largest two sizes of the dust (4–8μ and 2–4μ) showed only small lesions in the lungs. Pin-head, whitish spots were seen discretely scattered over the surface of the lung. No appreciable change was seen till late in

TABLE 3

ASSESSMENT OF FIBROSIS IN SECTIONS OF LUNGS OF RATS PRODUCED BY A CONSTANT SURFACE AREA (700 cm.²) FROM DIFFERENT AMOUNTS OF DIFFERENT PARTICLE SIZES OF FLINT DUST

Size	4–8μ		2–4μ		1–2μ		0·5–1μ		<0·5μ	
Amount	199 mg.		100 mg.		42·5 mg.		17·5 mg.		8 mg.	
Days of Survival	Mode of Death	Degree of Fibrosis	Mode of Death	Degree of Fibrosis	Mode of Death	Degree of Fibrosis	Mode of Death	Degree of Fibrosis	Mode of Death	Degree of Fibrosis
1–30	K	2	K	2	K	1	D	Infective	K	1
	K+	2	K+	3 min.*	K+	3	K	1	K+	3
	—	—	—	—	—	—	K+	3	—	—
31–60	K	2	K	3	K	3	K	3	K	3
61–90	K	2	K	3	K	3 max.*	K	3	K	3 min.
91–120	D	(–)	K	3	K	3	K	3	K	3
	K	2	K	4	K	4 min.	K	3 max.	K	3
121–150	D	(–)	K	4	D	(–)	K	3	D	3
	K	2	K+	4 min.	K	4	—	—	K	3
	K+	2	—	—	K+	4	K+	3 max.	K+	3
151–180	K	2	D	4 min.	D	5	K	3	K	3
	K	2	D	5	K	5	K	3	K	3
	—	—	K	5	K	5	—	—	—	—
	—	—	K	5	—	—	—	—	—	—
181–210	D	2	D	(–)	K	5	K	3	K	3
	D	(–)	D	(–)	K	5	K	3	K	3 min.
	K	2	K	5	—	—	—	—	—	—
	K	2	K	5	—	—	—	—	—	—
211–240	D	2	K	5	K	5	K	4	K	3
	K	2	K	5	K	5	K	4	K	3
	K	3 min.	—	—	—	—	—	—	—	—
241–270	K	3 min.	D	(–)	K	5	K	4	D	(–)
	K	3 min.	K	5	K	5	K	4	K	3
	—	—	K	5	—	—	—	—	K	3
271–300	D	(–)	K	5	D	5	K	4 max.	K	2 max.
	K	2 max.	K+	5	D	(–)	K+	5	K+	4 min.
	K+	3 min.	—	—	K	5	—	—	—	—
	—	—	—	—	K+	5	—	—	—	—
301–365	D	(–)	D	(–)	D	(–)	D	(–)	K	3 max.
	K	3	K	5	D	(–)	D	(–)	K	*a*
	K	*a*	K	*a*	K	5	D	(–)	K	*a*
	—	—	—	—	K	*a*	K	4 max.	—	—
	—	—	—	—	K	*a*	K	*a*	—	—
	—	—	—	—	—	—	K	*a*	—	—
366–400	D	(–)	K	5	K	5	D	(–)	K	3 max.
	K	3	—	—	—	—	D	(–)	—	—
	—	—	—	—	—	—	K	4 max.	—	—
401–450	K	3	D	5	D	5	D	5	K	*a*
	—	—	K	*a*	K	*a*	K	*a*	K	4 min.
451–500	K	3	D	5	—	—	—	—	K	*a*
	—	—	—	—	—	—	—	—	K	*a*
	—	—	—	—	—	—	—	—	K	3 max.
	—	—	—	—	—	—	—	—	K	*a*

\+ Sections from these animals are reproduced in figures.
K = killed, D = died. * Min. = minimal, Max. = maximal within the indicated group of fibrosis.
(–) Lost through cannabilism.
a Used for analysis.

the experiments, when they became slightly larger, about 2 to 3 mm. in diameter. The next two sizes of the dust (1–2μ and 0·5–1μ) produced, within a few weeks, small and discrete lesions, which progressed gradually so that by the eighth month confluence was noted. In the case of the smallest particle size (<0·5μ) the lesions were large and irregular from the beginning. They were about 2 to 4 mm. in diameter and by the fifth month firm and rubbery patches of confluent fibrosis were seen. Pleural involvement was more marked in the groups of animals receiving the smaller sized dust particles.

In the constant-surface series a different picture was produced. Early in the experiment all the groups showed essentially similar lesions, which were large and irregular, about 2 to 4mm. in diameter, and greyish-white. Hardly any change occurred, with time, in the lesions in the 4 to 8μ group, although their number increased. The lesions steadily grew in size in the two groups of animals receiving 2–4μ

and 1–2μ particle-size dust, and by the sixth month coalescence of the nodules was noticed, especially in the posterior aspects of the lungs, the areas being firm and rubbery. In the two groups of animals having the smallest particle-size dust (0·5–1μ and <0·5μ) there was some increase in size of the lesions towards the end of the experiments. The pleura was inflamed in patches in all the groups, more markedly with the smaller sizes of particles.

The posterior aspects of the lungs appeared to be more frequently involved than the anterior. The tracheo-bronchial lymph glands were enlarged to several times their normal size in most of the experimental animals, except with the largest size class. As this finding appeared significant a detailed study of the lymph glands has been undertaken. This will be described in a following paper.

The animals that died within about a month from the beginning of the experiments had dark, partly consolidated lungs, but later in the experiment greyish lesions, either in spots or in streaks, were visible within such dark areas.

Microscopic Appearance of the Lungs

Constant-amount Series.—A large variation in the rate and severity of tissue reaction to equal amounts of the five different sizes of flint dust was seen in the lungs of the experimental animals. Although there were discrepancies within individual groups of animals, still, on the whole, the smaller the particle size of the dust the more severe and quicker was the tissue reaction to it. The largest two sizes of the dust (4–8μ and 2–4μ) were slow to produce a fibrous reaction. Early in the experiment the lesions were essentially similar for both, i.e., a fine focal type of reticulin fibrosis in about 30 days, which was only about grade 1 minimal. As the experiment proceeded there was only a slight increase of fibrosis (grade 2) with the largest particle-size dust (4–8μ); but a gradual progression to a moderate degree of fibrosis (grade 3) was seen in the case of the 2–4μ particles. The next two fractions (1–2μ and 0·5–1μ) produced almost equal changes in the lungs. Although the maximum fibrosis was produced by these dusts, the degree of severity appeared to be delayed when compared with the finest particle size. There was only a mild degree of grade 2 fibrosis in one month, but the nodules progressed steadily and had coalesced in about eight months to grade 5 fibrosis. The smallest particles (<0·5μ) produced, in a month, a severe degree of pulmonary fibrosis (grade 4), and confluence of nodules was seen after only five months.

Constant-surface Series.—In the second (constant surface) series the fibrogenic reaction in the lungs of the rats was most severe within the range of 1 to 4μ particles, less so below 1μ and least with 4–8μ. The earlier reactions were essentially the same (grade 3 fibrosis) with all except the largest particle size, which produced slightly less fibrosis (grade 2). As the experiment proceeded the lesions advanced steadily in the groups of animals receiving 1–2 and 2–4μ particles, so that in 120 days the maximum degree of fibrosis (grade 5) was produced. In the case of 0·5–1μ particles the progress was slower and the maximum degree (grade 5) was not reached till 300 days. No further appreciable change was noticed in the groups of animals injected with the <0·5μ and 4–8μ dusts till the end of the experiments when some increase of fibrosis was seen in both (grade 4 early and grade 3 early respectively).

Detailed Histological Findings

Constant-amount Series.—The series is considered in groups according to particle size.

Flint : 4–8μ (50 *mg.*).—This largest sized flint dust was least pathogenic in the animal lungs. A mild degree of fibrosis (grade 2) was seen at 270 days, and it did not progress any further till the end of one year.

Although the dust cell reaction was quite prominent in the lungs of animals that died within three weeks of the injections, no fibrosis was seen in any. Well defined, rounded aggregations of dust cells were seen in sections at 30 days, but the reticulin fibres were fine and loosely woven (grade 1 fibrosis, Fig. 2). At 87 days the lesions were assessed as grade 2 early ; otherwise the fibrosis remained as grade 1 (Fig. 7) up to 270 days, when the nodules became more fibroblastic. The reticulin fibres within such nodules were coarse and compact, and there was some collagen formation (grade 2 fibrosis). Sections examined at 330 days and 365 days showed similar changes (grade 2 fibrosis, Fig. 12), and some of the rats killed at 365 days had rather less severe lesions.

Flint : 2–4μ (50 *mg.*).—Only a moderate degree of pulmonary fibrosis (grade 3) was produced by this particle size in about a year. Although the earlier reaction was almost the same as with smaller sized particles (grade 1 maximal, 30 days) further progress was much slower.

The lungs of the two rats that died 10 and 24 days after the injections showed good dust cell reaction with small collections of dust cells without any evidence of increase of reticulin fibres within aggregations. The nodules were well formed by

the thirtieth day and a loose network of thick reticulin fibres was seen in some of them (grade 1 fibrosis, maximal, Fig. 3). At 90 days the fibrosis was similar. Sections examined on the 150th day showed larger cellular nodules, the centres of which were closely packed with coarse reticulin fibres, but in the periphery they were loosely woven (grade 2 fibrosis, Fig. 8). The same degree of fibrosis was seen in the sections from all rats which died or were killed between this period and 300 days, when some nodules were found to be slightly cellular and almost collagenous (grade 3 fibrosis). At 330 days the lesions were fewer and less advanced. In the eight rats killed at 365 days grade 3 fibrosis of the lungs was seen in four (Fig. 13) and the rest showed rather less. Pleural surfaces were almost free except for a few tiny patches of thickening.

Flint : 1–2μ (50 *mg.*).—Steadily progressive, classical silicosis was produced in the lungs of experimental animals by this size of flint dust (grade 2 fibrosis at 30 days, grade 3 at 150, and grade 5 at 270 days).

In the sections examined from the lungs of the first rat that was killed, 30 days after the injections, large nodules composed mainly of fibroblasts were seen. Such nodules were tightly packed with coarse reticulin fibres (grade 2 fibrosis, Fig. 4). By the sixtieth day the lesions became partly acellular and more collagenous (grade 3 fibrosis, early). At 150 days they were only slightly cellular and almost fully collagenous (grade 3 fibrosis, Fig. 9). The fibrosis progressed steadily so that by 270 days completely acellular and hyalinized areas were seen. These areas were fully collagenous (grade 5 fibrosis). There was some variation in the lung pathology in this group, especially among the rats that died. Although all the animals after this period did not maintain this degree of fibrosis, some at 365 days showed grade 5 (Fig. 14). There were a few small patches of pleural thickening over the underlying nodules. Lung fields in most of the killed rats were almost clear. Hilar lymph nodes in some cases showed good dust cell reaction.

Flint : 0·5–1μ (50 *mg.*).—The rate of reaction appeared to be about the same as for the previous (1–2μ) fraction, but less rapid than with the following one (<0·5μ). Nodular fibrosis of grade 2 and acellular confluent fibrosis of grade 5 were produced in 30 and 240 days respectively.

One rat which died six days after the injections showed a good dust cell reaction in its lungs. Some dust-laden phagocytes lay within the alveoli. Small dust-cell aggregations were seen in sections at 12 days, within which some fine reticulin fibres were noted (grade 1 fibrosis). Sections examined on the thirtieth day showed larger cellular nodules with coarse reticulin fibres and some collagen (grade 2 fibrosis, Fig. 5). The rats, killed at intervals from this period to the end of one year, showed steady progression of fibrosis, but the reaction was variable. At 150 days partly acellular and almost fully collagenous nodules were seen (grade 3 fibrosis, Fig. 10). By the 240th day there was confluent collagenous fibrosis. This was maintained in the rest of the rats (Fig. 15), except one at 365 days, which showed only grade 3 fibrosis. The pleural involvement and dust cell reaction within hilar lymph nodes were almost the same as in the previous and following groups.

Flint : <0·5μ (50 *mg.*).—Coarse reticulin fibrosis (grade 2) was produced in the lungs of rats in 18 days by this size of the dust. The fibrosis rapidly progressed to the maximum degree (grade 5) by 150 days.

The first rat died 18 days after the injections, and showed in its lungs large stellate cellular nodules and plenty of free dust cells. There was some exudation involving small groups of alveoli. Reticulin fibres within these nodules were thick and closely packed (grade 2 fibrosis, Fig. 6). By the end of the third week the lesions were only slightly cellular and more collagenous. They became completely acellular and hyalinized at 31 days. On reticulin staining such nodules were fully collagenous (grade 4 fibrosis), except in animals at 84 and 94 days, when grade 3 fibrosis was found. Other rats showed similar lung changes up to 150 days, when confluence of hyalinized collagenous areas was found (grade 5 fibrosis, Fig. 11). Some variation in the degree of fibrosis was noted in the lungs of rats that were killed after this period, and until the end of one year, all of them being less than grade 5 fibrosis. However, of the six rats killed on the 365th day, three had grade 5 fibrosis (Fig. 16). The pleura was badly involved in this group. Nearly the whole surface was thickened. Free dust cells were seen all through the experiment, and hilar lymph nodes showed prominent dust cell reaction in some cases.

Constant-surface-area Series.—For the constant-surface-area the grouping by particle is the same as for the constant-amount series.

Flint : 4–8μ (199 *mg.*).—The earliest reaction induced by these maximum-sized particles was as severe and rapid as in the case of the next lower size, i.e., 2–4μ particles (grade 2 at 21 days), but no further progress of the fibrosis was seen till late in the experiment, and then only to grade 3 at 300 to 500 days.

Sections from the lungs of the first rat, killed 21 days after the injections, showed a great number of large cellular nodules, the cells being mainly fibroblasts. Compact reticulation of such nodules, with coarse reticulin fibres and some collagen fibres, was seen in silver-impregnated preparations (grade 2 fibrosis). Plenty of free dust cells were present throughout the lung fields. No further advance of fibrosis was seen in any of the rats, killed or dead, until 240 days (Fig. 17 at 30 days and Fig. 22 at 150 days). One of the two rats killed at 240 days showed in its lungs a few nodules of more advanced fibrosis ; these were slightly cellular and almost collagenous (grade 3 fibrosis, early). Similar pathological changes were seen in sections examined at 270 and 500 days (Fig. 27).

Flint : 2–4μ (100 *mg.*).—This and the next size of dust (1–2μ) produced lung lesions which were very similar in rate and severity (grade 3 at 30 days, 4 at 120, and 5 at 180 days).

The reaction produced at 21 days was a little heavier than in the following groups by the same time. The larger irregular nodules were highly cellular, the cells mainly fibroblasts. The reticulin fibres within these were coarse and compact. Some collagen fibres were seen in a few nodules (grade 2 fibrosis). Plenty of free dust cells, singly or in small groups, were seen in some alveoli and parenchymal tissue. Although the majority of the nodules remained cellular, some were slightly less so, and these were somewhat collagenous at 30 days (grade 3 fibrosis, early, Fig. 18). The changes were similar in sections examined on 60 and 90 days, and from one of two rats killed at 120 days. The other rat at 120 days showed more progressive lung lesions which were completely acellular, hyalinized, and fully collagenous (grade 4 fibrosis). The lesions remained within the maximum limit of grade 4 fibrosis at 150 days (Fig. 23) but they became confluent by 180 days (grade 5 fibrosis). All the rats killed between this period and 500 days showed similar pathological lesions in the lungs (Fig. 28). The three rats which died during this period were lost by death and cannibalism.

Flint : 1–2μ (42·5 *mg.*).—The early reaction was very rapid (as rapid as with the smaller sized particles and more rapidly progressive). Grade 3 fibrosis was reached at 30 days, grade 4 at 120, and grade 5 at 180 days.

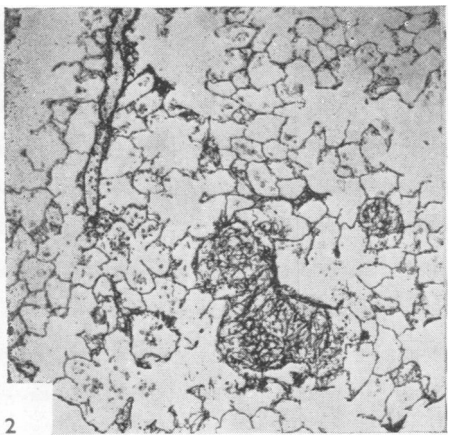
Sections from the lungs of rats at 21 and 30 days showed, respectively, grade 1 fibrosis of loose reticulin meshwork and grade 3 fibrosis of almost completely collagenous nodules (Fig. 19). No further appreciable increase in the degree of fibrosis was seen until after 120 days, when one of the two rats killed showed in the lungs a few acellular, hyalinized, fully collagenous nodules (grade 4 fibrosis, early). By 150 days more nodules became acellular and collagenous (grade 4 fibrosis, Fig. 24). The lesions were confluent in sections examined at 180 days (grade 5 fibrosis), and this grade of fibrosis was maintained in all the rats dead or killed between this period and 450 days (Fig. 29).

Flint : 0·5–1μ (17·5 *mg.*).—The early reaction produced by this size of the dust was about as rapid as in the preceding (larger) and following (finest) particle sizes. The fibrosis progressed slowly to the maximum degree of confluent fibrosis (grade 5) in 300 days.

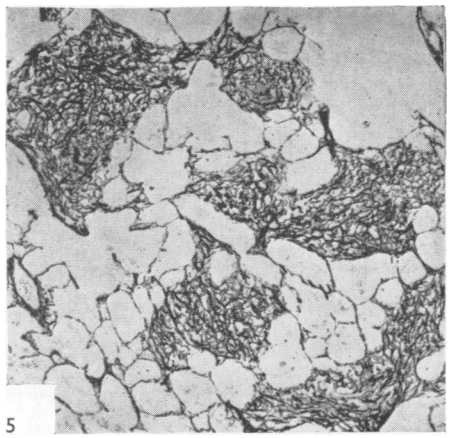
The first rat that died seven days after the injections was excluded from the series, as the lungs were highly infected. Sections from the lungs of the rats killed at 21 days showed grade 1 fibrosis, of loosely woven reticulin fibres ; and all other rats killed from 30 days (Fig. 20) up to 210 days showed collagenous grade 3 fibrosis, except at 120 and 150 days, when the fibrosis was maximum within that grade (Fig. 25). At 240 days completely acellular and hyalinized nodules, which were fully collagenous with reticulin stain, were seen (grade 4 fibrosis). This degree of fibrosis was maintained until 300 days, when confluence of such nodules was noted in one of the two rats killed (grade 5 fibrosis, Fig. 30).

Flint : <0·5μ (8 *mg.*).—A moderately severe degree of pulmonary fibrosis (grade 3) was produced by this dust as early as a month, but it remained absolutely non-progressive till 300 days when some advancement of the lesions was noted (grade 4 fibrosis, early).

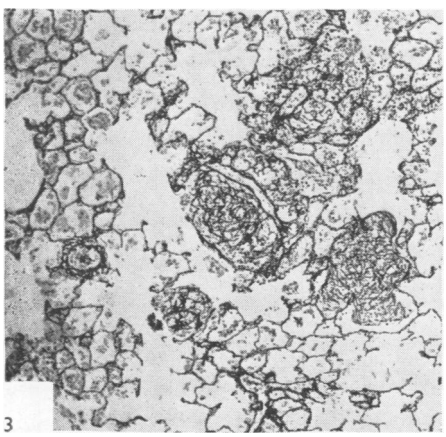
Sections from the rat that was killed three weeks after the injection showed well formed, rounded dust cell aggregations, mainly in the peripheral zone of the lung. Lung fields were clear ; no exudation was present. On silver impregnation fine reticulin fibres of a loose network were seen within such nodules (grade 1 fibrosis). The progression of fibrosis was rapid at this stage. At 30 days the lesions were moderately large and irregular. They were composed mainly of fibroblasts. Some nodules were only slightly cellular. Although the majority of the nodules were closely packed with coarse reticulin fibres with a little collagen formation, the few slightly acellular nodules were almost collagenous (grade 3 fibrosis, Fig. 21). After this period until 270 days very little change in the progress of fibrosis was seen. Most of the nodules, however, matured within the limits of grade 3 fibrosis (Fig. 26). No tissue was taken for histology from



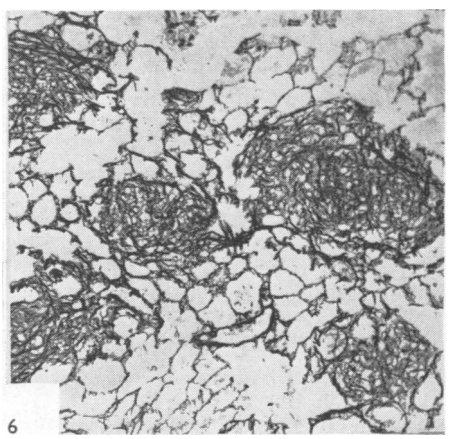
4–8μ



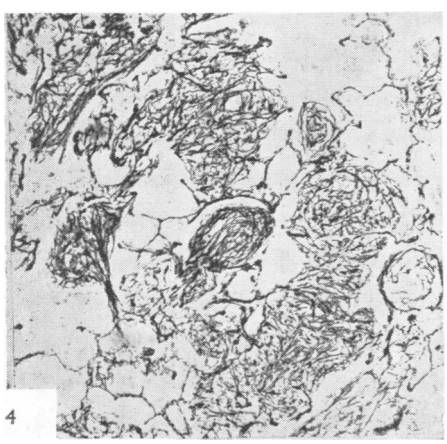
0·5–1μ



2–4μ



<0·5μ



1–2μ

Sections of lungs 30 days after the injection of constant weights (50 mg.) of flint dusts of different sizes. Silver impregnation × 90, showing reticulin nodules.

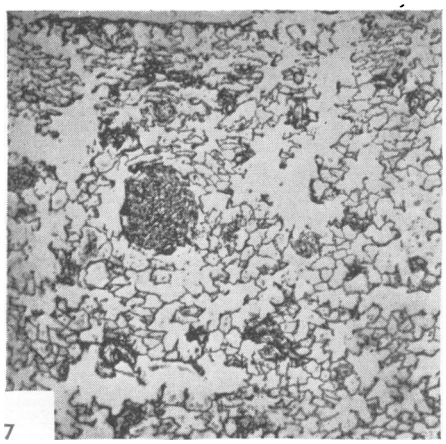
FIG. 2.—Loosely woven fine reticulin fibres. Grade 1 fibrosis.

FIG. 3.—Loose network of thick reticulin fibres. Grade 1 fibrosis maximal.

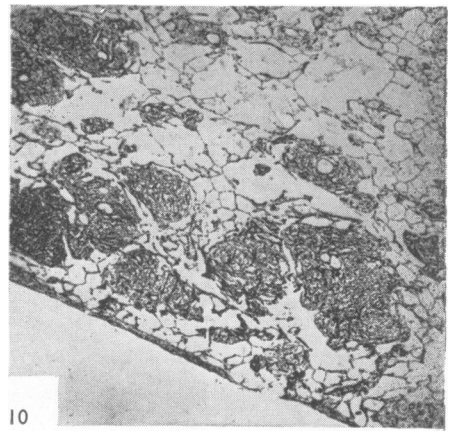
FIG. 4.—Tightly packed coarse reticulin fibres. Grade 2 fibrosis.

FIG. 5.—Coarse reticulin fibres and some collagen. Grade 2 fibrosis.

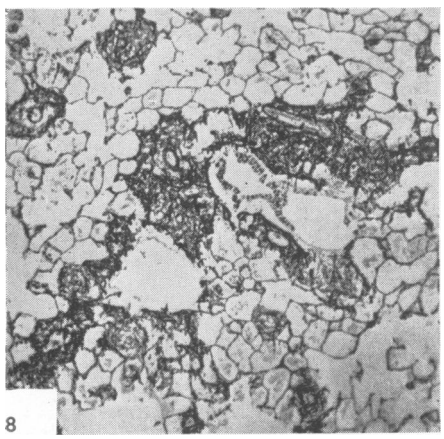
FIG. 6.—Eighteen days. Thick and compact reticulin fibres Grade 2 fibrosis.



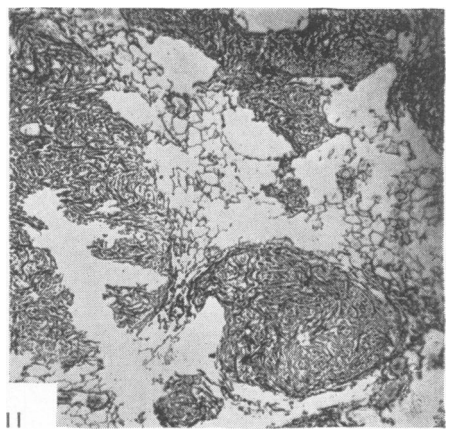
4–8μ



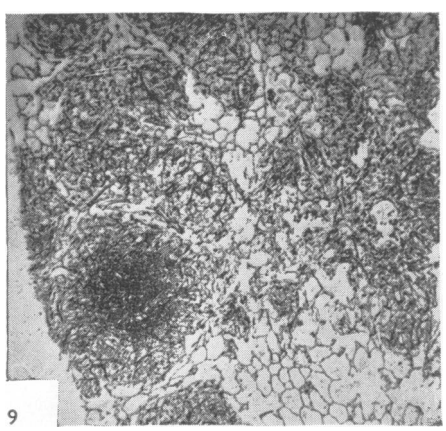
0·5–1μ



2–4μ



<0·5μ



1–2μ

Sections of lungs 150 days after the injection of constant weights (50 mg.) of flint dusts of different sizes. Silver impregnation × 50.

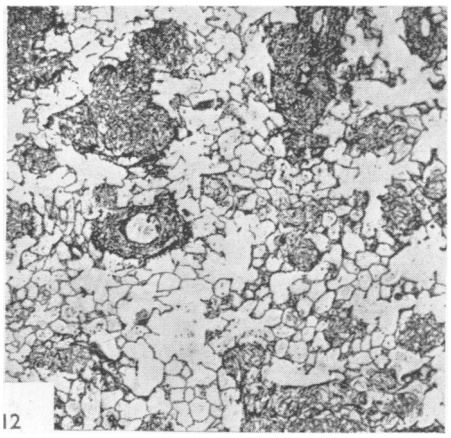
FIG. 7.—Scattered nodules of grade 1 fibrosis.

FIG. 8.—Closely packed reticulin fibres in centres of nodules, but loose at periphery. Grade 2 fibrosis.

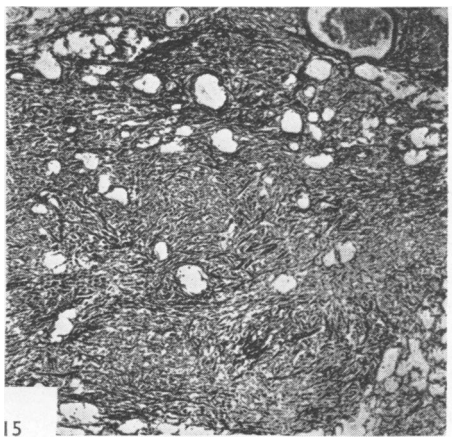
FIG. 9.—Almost fully collagenous nodules, slightly cellular with ordinary nuclear stain. Grade 3 fibrosis.

FIG. 10.—Almost fully collagenous nodules, partly acellular. Grade 3 fibrosis.

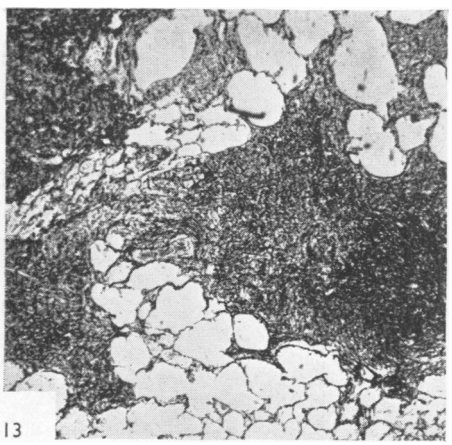
FIG. 11.—Confluent collagenous fibrosis of grade 5.



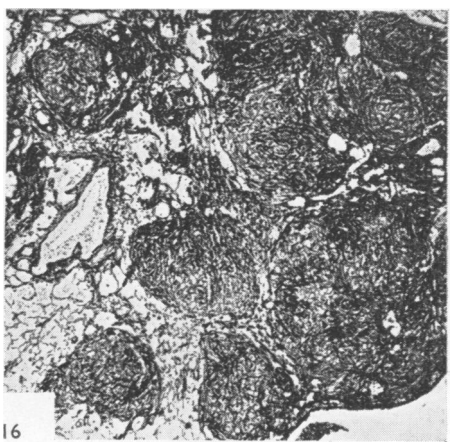
4–8μ



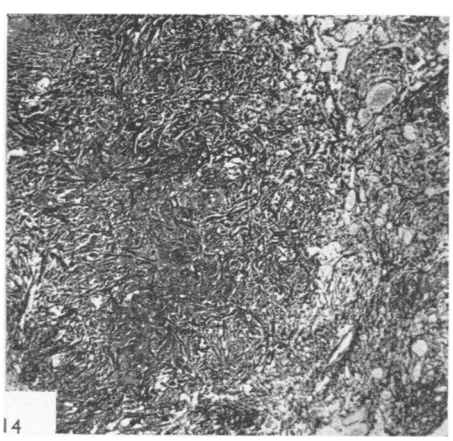
0·5–1μ



2–4μ



<0·5μ



1–2μ

Sections of lungs 365 days after the injection of constant weights (50 mg.) of flint dusts of different sizes. Silver impregnation × 40.

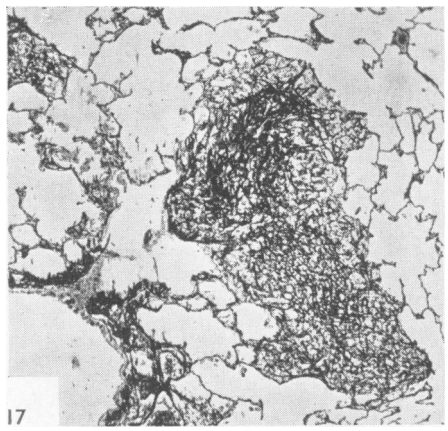
FIG. 12.—Nodules with coarse and compact reticulin fibres and some collagen fibres. Grade 2 fibrosis.

FIG. 13.—Almost fully collagenous nodules, slightly cellular with ordinary nuclear stain. Grade 3 fibrosis.

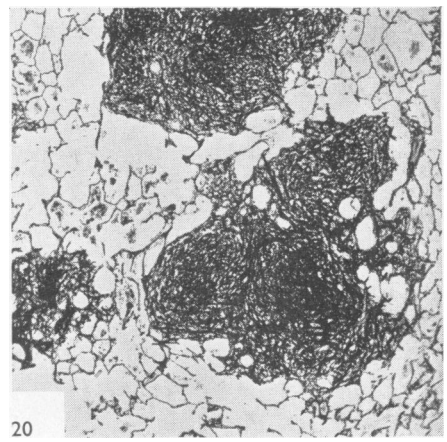
FIG. 14.—Confluent collagenous fibrosis of grade 5.

FIG. 15.—Confluent collagenous fibrosis of grade 5.

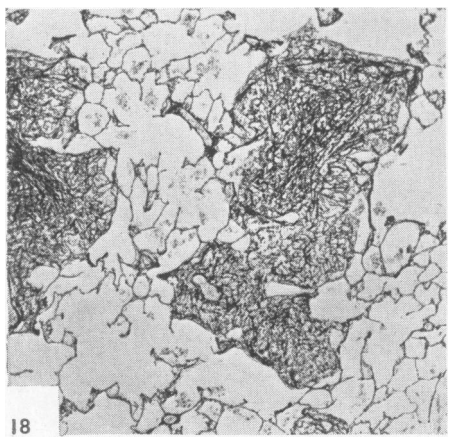
FIG. 16.—Confluent collagenous fibrosis of grade 5.



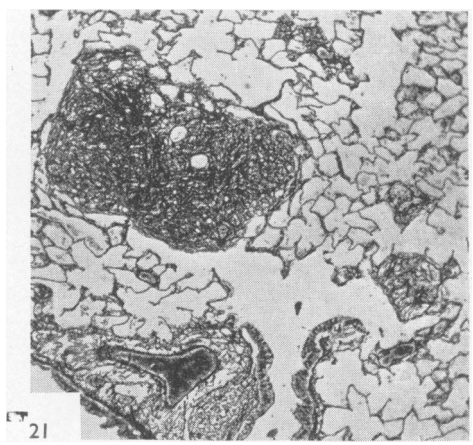
4–8μ



0·5–1μ



2–4μ



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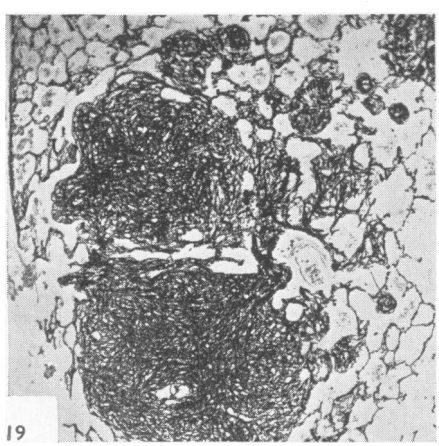


1–2μ

Sections of lungs 30 days after the injection of constant "areas" (700 cm.²) of flint dust of different sizes, given in amounts calculated to yield the same area of particle surface. Silver impregnation × 66.

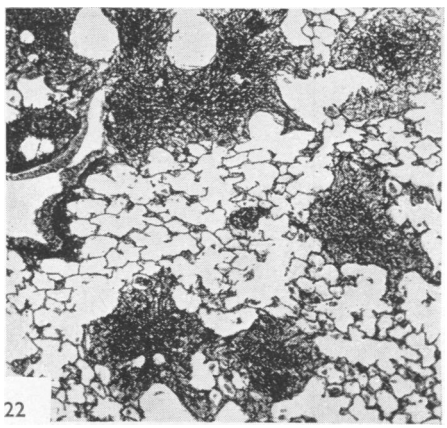
FIG. 17.—199 mg. of 4–8 μ. Nodules with coarse and compact reticulin fibres and some collagen. Grade 2 fibrosis.

FIG. 18.—100 mg. of 2–4 μ. Somewhat collagenous nodules, partly acellular with ordinary nuclear stain. Grade 3 fibrosis, early.

FIG. 19.—42·5 mg. of 1–2 μ. Almost fully collagenous nodules, slightly cellular. Grade 3 fibrosis, maximum.

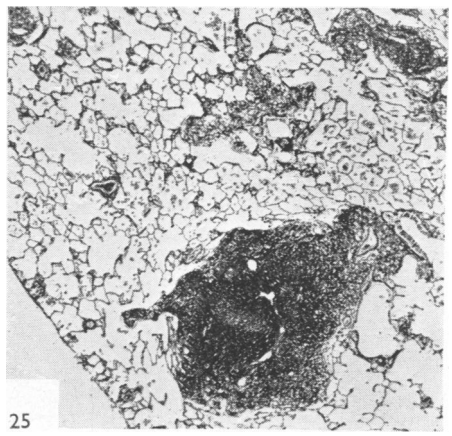
FIG. 20.—17·5 mg. of 0·5–1 μ. Almost fully collagenous nodules, slightly cellular. Grade 3 fibrosis, maximum.

FIG. 21.—8 mg. of < 0·5 μ. Almost collagenous nodules, slightly cellular. Grade 3 fibrosis.



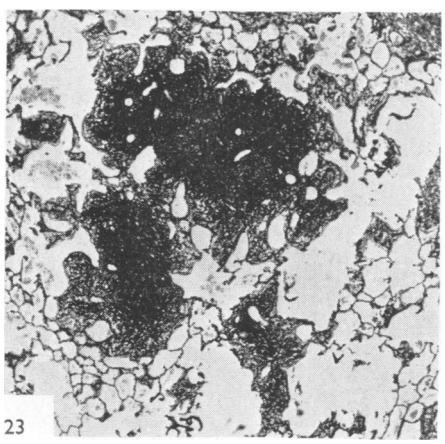
22

4–8μ



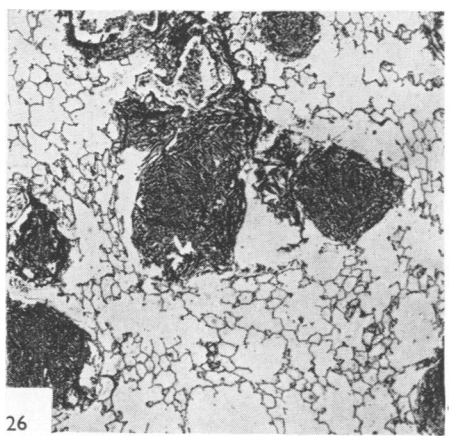
25

0·5–1μ



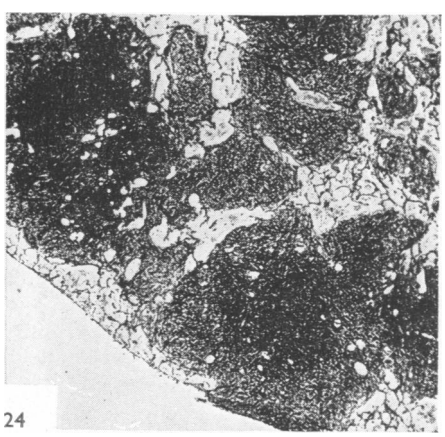
23

2–4μ



26

<0·5μ



24

1–2μ

Sections of lungs 150 days after the injection of constant "areas" (700 cm.²) of flint dust of different sizes, given in amounts calculated to yield the same area of particle surface. Silver impregnation × 40.

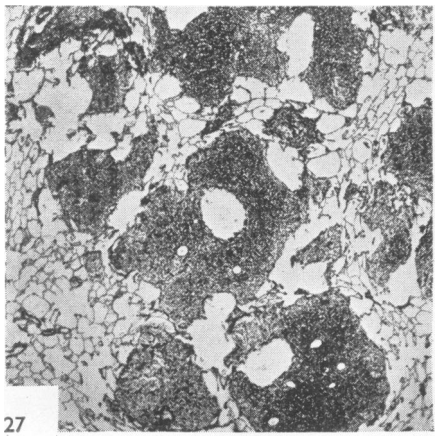
FIG. 22.—199 mg. of 4–8 μ. Nodules of grade 2 fibrosis.

FIG. 23.—100 mg. of 2–4 μ. Fully collagenous nodules, completely acellular with ordinary nuclear stain. Grade 4 fibrosis, minimal.

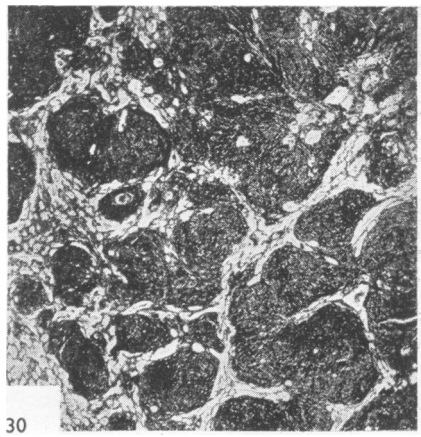
FIG. 24.—42·5 mg. of 1–2 μ. Fully collagenous nodules, completely acellular. Grade 4 fibrosis.

FIG. 25.—17·5 mg. of 0·5–1 μ. Almost collagenous nodules, slightly cellular, Grade 3 fibrosis, maximal.

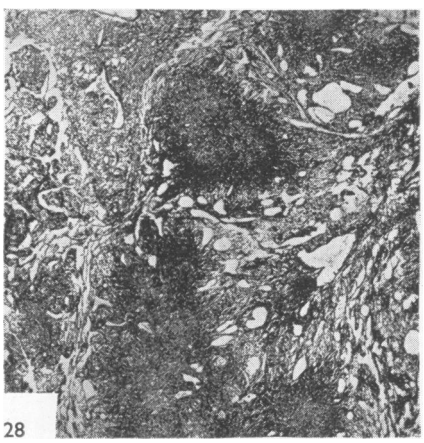
FIG. 26.—8 mg. of < 0·5 μ. Almost collagenous nodules of grade 3 fibrosis.



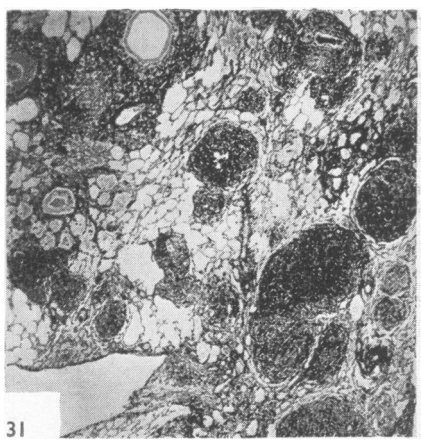
4–8μ



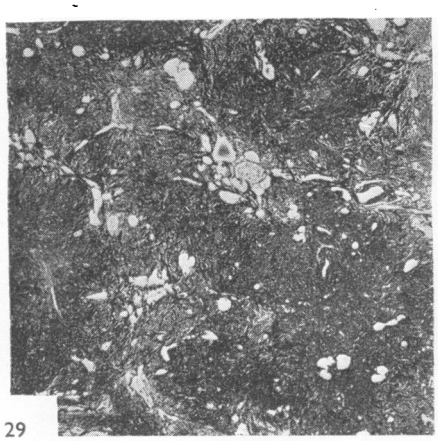
0·5–1μ



2–4μ



<0·5μ



1–2μ

Sections of lungs 300 days after the injection of constant "areas" (700 cm.²) of flint dust of different sizes, given in amounts calculated to yield the same area of particle surface. Silver impregnation × 30.

FIG. 27.—199 mg. of 4–8 μ. Almost collagenous nodules, partly acellular with ordinary nuclear stain. Grade 3 fibrosis, early. (× 50).

FIG. 28.—100 mg. of 2–4 μ. Complete collagenous fibrosis of grade 5.

FIG. 29.—42·5 mg. of 1–2 μ. Confluent collagenous fibrosis of grade 5.

FIG. 30.—17·5 mg. of 0·5–1 μ. Confluent collagenous fibrosis of grade 5.

FIG. 31.—8 mg. of < 0·5 μ. Fully collagenous nodules, completely acellular. Grade 4 fibrosis early.

one rat that died at 244 days, owing to almost complete replacement of its lungs by abscess formation. At 300 days sections from one of the two rats that were killed showed rather less marked fibrosis than previous ones, but a few completely acellular and fully collagenous nodules were present in the lung sections from the other rat. They were assessed respectively as grade 2 maximal and grade 4 early fibrosis (Fig. 31). Between 350 and 500 days three rats showed stage 3 or 4 minimum.

DISCUSSION

The object of the present work was to get as precise information as possible on the effect of size and surface area on the grade of fibrosis produced in the lungs of rats in injection experiments. There should be a lower size limit somewhere between 1 and 0·01μ (Nagelschmidt, 1949), and it was thought that it might be possible to reach it experimentally. Also there was no information on what the reaction would be if samples representing equal surface areas, but composed of different size classes, were injected.

In theory it should be possible to make similar experiments on the basis of equal particle numbers. However, it would not be possible in practice to cover such a wide range of sizes. To give equal particle numbers each succeeding fraction in the present flint series would have to be given at about one tenth the weight of the next larger fraction.

Preparation of samples is a major difficulty in such work. In any grinding process quartz gets contaminated, and quartz sands usually contain clay as impurities. Both types of contamination will accumulate in the finest size fractions. For this reason calcined flint was used in the present experiment. This flint consisted of quartz and cristobalite, but recent work by ourselves (1953) has shown that these two modifications of silica have very similar fibrogenic properties.

Assessment of fibrosis is ideally based on maturity of the lesions and total amount of fibrosis. In the present study only the degree of fibrosis classified into five stages as outlined above has been used. It is possible to measure fibrosed areas on tissue sections, but the uneven development of fibrosis throughout the lung makes such measurements valueless unless a very large number of sections is made. It may be possible to develop in the future biochemical or radiographic methods to assess the total amount of fibrosis per lung.

Constant-amount Series (50 mg. Flint per Rat)

The main results can be summarized as follows :

Flint Size (μ)	Silica Solubility (mg./100 ml.)	Surface per Rat (cm.²)	Maximum Stage of Fibrosis	Reached after Months	Minimum Time (Months) to Give Stage			
					2	3	4	5
4–8	5·1	170	2	9	9	—	—	—
2–4	8·9	350	3	10	5	10	—	—
1–2	13·1	750	5	9	1	5	7	9
0·5–1	13·4	2,000	5	7	1	2	3	7
<0·5	13·6	3,000	5	5	<1	<1	1	5

There is a regular increase in activity as the particle size decreases and the differences are large. The smaller the mean size of flint the faster or more advanced is the tissue reaction, if equal weights are injected.

Constant-surface Series

The same samples given on an equal surface basis gave more homogeneous results when 700 cm.² of flint per rat gave different fibrosis stages after the following number of months :

Flint Size (μ)	Weight of Dust per Rat (mg.)	Minimum Time (Months) to Give Fibrosis Stage		
		3	4	5
4–8	199	8	—	—
2–4	100	1	4	6
1–2	42	1	4	5
0·5–1	17·5	1	7	10
<0·5*	8	1	10	—

* This sample represents 520 cm.².

The largest fraction only just reached stage 3 and the smallest fraction stage 4. Thus this series seems to demonstrate that there is an optimum fibrogenic size in the region of 1 to 2μ under our experimental conditions.

At the time the injections were made no facilities were available to measure the surface area of the fraction below 0·5μ, and its surface area had been over-estimated. The 8 mg. injected actually represented only 520 cm.² and this dose failed to produce stage 5 (confluent) fibrosis. This may suggest that a minimum weight, as well as a minimum of silica surface, is required to produce stage 5, which, as explained above, depends on number and size of lesions as well as on their maturity. Because of phagocytosis and dissolution of particles one might say that by the time stage 5 should have been reached there was insufficient silica left to produce it. It may be possible to check this hypothesis by analysing the parts of the rat lungs not used for sectioning. It must be remembered that a larger dose of the same fraction produced stage 5 in only five months.

If the failure of the fraction below 0·5μ. to produce stage 5 fibrosis can perhaps be explained on the ground that the absolute amount was insufficient, there is no simple explanation for the failure of the largest fraction to go beyond stage 3. There was

no difference in the ease of phagocytosis as far as could be judged from the sections, and no other obvious explanation can be seen. Some light may be thrown on this by further work on the lymph nodes.

Assessment of the results is complicated by the fact that the animal technique was changed from single-dose intratracheal injection through an incision in the neck for the "constant-amount" series to multiple dose peroral intratracheal injection for the "constant-surface" series. The fraction 1–2μ, which occurred at similar weights in both series, shows that the oral technique gave fibrosis more quickly. This is confirmed by the results of the two neighbouring fractions. The slower fibrosis and more irregular results obtained in the "constant-amount" experiments may have been due to regurgitation of variable amounts of dust through the nose, which often occurs with this technique immediately after injection, whereas the oral procedure is almost never followed by loss of dust through the nostrils.

The numbers of particles received by the animals in the different groups were grossly different. In the constant-surface experiment, despite the decreasing weight of dust used with decreasing size, the numbers of particles greatly increased. The rats receiving 199 mg. of 4–8μ flint got about 500 million particles, those receiving 42 mg. of 1–2μ got 10,000 million, and those receiving the 8 mg. of the smallest size (<0·5μ) got 135,000 million. It might be contended that the largest-size dust (4–8μ) failed to produce severe silicosis because of the small number of particles (if 500 million can be considered small), in spite of the large mass of dust, equal surface, and equal "solubility". But this can certainly not be claimed in respect of the smallest-size (<0·5μ) with which less severe fibrosis, and less of it, resulted than with the 1–2μ dust, despite there being enormously more particles, though of less weight but the same surface area and same "solubility". As far as the constant surface experiments are concerned, therefore, we feel justified in concluding that the finest particles of flint dust have been less pathogenic than those in the 1–2μ range which appears to be the most dangerous size in producing lung fibrosis, under the conditions described.

These results support the conception that fibrogenesis is closely related to the silica surface, but they also point to there being a range of maximally fibrogenic sizes. It is hoped to determine in further experiments whether the decrease in pathogenesis of the smallest size classes is also found when the same flint fractions are given at higher surface levels.

SUMMARY

Graded flint samples between <0·5 and 8μ were given to rats by intratracheal injection on the basis of equal weights (50 mg. per rat), and also on the basis of equal surfaces (700 cm.^{2} per rat).

With equal weights the fibrogenic activity increased considerably with decreasing size. Whereas the largest fraction only gave stage 2 fibrosis in nine months, the smallest one gave stage 5 in five months.

With equal surfaces the largest fraction went only to stage 3 and the smallest fraction to stage 4. The optimum fibrogenic size of flint particles in this type of experiment was between 1 and 2μ equivalent diameter. Possible explanations for the falling off at both size ends are discussed.

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