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TRANSACTIONS ТРУДЫ

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EXPERIMENTAL INFECTIVE PNEUMOCONIOSIS*

Quartz and coal-mine dusts were introduced into the lungs of rats by inhalation, in a high concentration, for four months, and then groups of these dusted animals were infected with a virulent strain of human tubercle bacillus (37 Rv). Dusting was continued for a further period of ten months in the case of coal-mine dust. No variation in lung pathology was seen in the quartz groups, although both produced classical silicotic nodules. In the coal-mine dust the infective group showed slightly more fibrosis, diffuse in nature, than either the non-infected or the tuberculous control group. These are preliminary experiments, and the results must be considered as both tentative and not very conclusive. Work is being continued.

The high incidence of pulmonary tuberculosis in trades involving the inhalation of siliceous dusts has long been recognised, and it is now generally accepted that victims of silicosis run a greatly enhanced risk of tuberculosis. An association in the opposite direction is also possible and *Kettle* (1936) believed that tuberculous infection could enhance the fibrogenic action of siliceous dust. Compare *Bellander* (1933), *Gardner* (1934), *Policard* (1939), *Cummins* (1940), *Vorwald, Dworski, Pratt* and *Delahant* (1950).

More recently a similar association has been considered in the case of other dusts of which the most important is coal. A high incidence of pulmonary tuberculosis has been noted in coal miners with massive fibrosis (Cummins, 1940; Belt and Ferris, 1942; Rogers, 1946; Gough, 1947; Fletcher, 1948; Worth and Dickman, 1950; Hasslet, 1951), and here, too, it has been suggested that tuberculous infection may determine the excessive fibrosis that occurs in these cases as compared with the much lesser fibrosis of uncomplicated miners' pneumoconiosis. Unfortunately, it is not possible to demonstrate tuberculosis in all cases of massive fibrosis, and the etiological association remains unproved.

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In view of the great social importance of pneumoconiosis and the uncertainly regarding the role played by tuberculosis in enhancing pulmonary fibrosis, it was considered desirable to re-investigate the problem experimentally in order to determine whether experimental silicosis or experimental anthracosis produces more fibrosis in animals with pulmonary tuberculosis.

The inhalation of dust in dusting cabinets was chosen in preference to intratracheal injection because it approximates more closely to the human hazard, and rats were chosen as an experimental animal because they were known to withstand heavy dusting and because they are relatively resistant to tuberculosis and therefore unlikely to die too soon.

As a preliminary it was necessary to find out the dose of tubercle bacilli that would produce the appropriate degree of infection in rats.

MATERIALS AND METHODS

(1) *Quartz.* The powdered quartz was kindly furnished by Mr. J. W. Wilson of Messrs. Colin Stewart, Ltd.

(2) *Coal Mine Dust.* Most air–borne dusts in coal mines contain about 80% combustible material (coal) and 20% of siliceous ash (mica, kaolin and quartz) and sometimes carbonates; and because it is almost impossible to obtain a sufficient sample of air–borne dust of respirable size from a coal mine, it was decided to make a mixture of freshly powdered coal and finely ground shale, which is the rock accompanying the coal seams in South Wales and which contributes the siliceous matter to the dust in the air of these coal mines. A representative anthracite coal dust was accordingly ground with 20% of shale dust overnight in an electrically driven mortar. In chemical and mineralogical composition this mixture was almost identical with many of the air-borne dusts from South Wales coal mines (Table 1). The anthracite coal was kindly supplied by Dr. B. M. Wright.

(3) *Mycobacterium tuberculosis* (H 37 Rv) was subcultured for three weeks in Proskauer's and Beck's medium. The growth was then filtered off, blotted dry and weighed. Known weights were then emulsified in sterile saline and adjusted to a concentration of 10 mg./ml. Dilutions of this were made to give concentrations of 1.0, 0.1, 0.01, and 0.001 mg./ml.

(4) *Animals.* M. R. C. strain black and white rats of an average weight of 200 g. were used. Twelve were used to test the optimum dose of bacilli, 34 were dusted with quartz and 34 with coal–mine dust, and 18 were injected with the tubercle bacilli as controls.

(5) Two *dusting cabinets.* (WRIGHT, 1950), one for quartz and one for coal, were used. Dry, filtered air from an electric compressor was fed into each of the two cabinets at a rate of 10 litres per minute. The

air was passed through Wright's dusting mechanism (1950), and carried the quartz or mine dust finely dispersed into the respective chamber, to produce in each a dust cloud of uniform concentration, averaging (± 10%) 50,000 particles per ml. of air. Air was drawn through filters from each cabinet by a vacuum pump and carried to the exterior through a tube leading out of a window of the room. Each cabinet was

Table 1

Size Distribution and Analysis of Dusts used for Inhalation

Samples Size in μ.	Quartz Per cent		Coal Mine Dust (80% Anthracite, 20% Shale) Per cent	
	By No.	By Mass	By No.	By Mass
0.32—0.45	27.7	1.0	19.2	0.6
0.45—0.64	28.3	3.3	16.7	1.6
0.64—0.90	21.7	7.0	25.9	6.6
0.90—1.30	12.7	11.4	24.7	17.4
1.30—1.80	6.65	17.0	8.7	17.5
1.80—2.60	2.17	15.6	3.4	19.3
2.60—3.60	0.46	9.5	0.9	14.6
3.60—5.10	0.16	9.2	0.5	22.5
5.10—7.20	0.16	25.9		
Particles counted	646		863	
Estimated surface area $m^2/g.$	1.7		1.8	
Silica solubility (mg./100 ml.)	3.3		0.8	
Ash content (%)	100		22	
Composition (%)	SiO_2 97.1		Coal 78 Kaolin 5 Mica 10 Quarz 6 Carbonates 1	

furnished with a safety hatch, which was designed to open automatically should there be any failure in the supply of air entering through the dust-feed mechanism. The doors and walls of the cabinets contained glass windows, through which the animals could be observed.

(6) *Dusting of Animals.* The animals of the quartz and mine-dust groups were divided at random into two sub–groups containing 17 in each. One sub–group from each was dusted during the day (8 hr.) and the other overnight (16 hr.). After each week the dusting period was changed so that in a fortnight all the animals of each group had equal

numbers of hours of dusting. The animals were exposed to the concentration of 50,000 particles/ml. of air for 5 days a week, except for holidays, Each rat thus had 120 hr of dusting in two weeks.

Two rats died in each group, i. e., 46 and 79 days in quartz and 13 and 72 days in the mine dust. The dusting was temporarily suspended four months after the commencement. By this time each rat had had 750 hours of exposure. One rat from each was then killed. The lungs of the animals, dead and killed, were examined histologically, and the rest of the lung tissue was dried to a constant weight at 105–110° C. for analysis. An amount (50 mg.) of dried lung tissue was examined for silica content and the results obtained are given in Table 2.

Table 2

Mineral content of lungs of rats after four months dusting

Dust	Days of survival	Mode of death	Hours of dusting	SiO_2 in dried lung (%)
Quartz	46	D	315	0.39
	79	D	524	0.46
	120	K	750	0.60
Mine dust	13	D	85	0.04
	72	D	510	0.17
	120	K	750	0.20

From both the histological and chemical analyses it appeared that there was now sufficient quartz, but hardly enough coal mine dust in the lungs of the animals dusted, and it was decided to continue further dusting only in the mine dust group. The remaining 31 rats of both groups were divided into sub–groups of 19 and 12, the former sub–group to be used for intravenous injections of Myco. tuberculosis, and the other for controls. Seven days after the injections, dusting was resumed in the case of the two mine–dust sub–groups. One cabinet was used for the infected animals and the other for the controls. They were dusted for 16 hours a day, 5 days a week except for holidays. Dusting was continued for 10 months more.

(7) *Injection of tubercle bacilli.* Rats were lightly anaesthetized, the right jugular vein exposed and 1 ml. of a suspension of bacilli injected against the blood flow. Groups of 3 rats received each of the following concentrations 0.001, 0.01, 0.1, 1.0 mg./ml. The operation was carried out with aseptic precautions and the wounds were closed with a single suture.

(8) *Injection of the test animals.* From the histological evidence obtained from the above rats, it was found that the proper dose for the rat appeared to be between 0.01 and 0.1 mg. of the organism. So

it was decided, arbitrarily, to use a dose of 0.02 mg.; and a fresh suspension was made to contain this amount per ml. The two dusted groups of animals (quartz and mine dust), 19 in each, were injected intravenously in the same way as described above. One from each group died during the operation, leaving 18 in each. At the same time 18 normal rats were injected to form a control group to the dusted and infected groups.

DURATION OF EXPERIMENTS

Six groups of animals were set up (as explained above).

(1) Preliminary dosage – 12 rats ,3 in each sub–group of tubercle bacilli (0.001, 0.01, 0.1, 1.0 mg.).
(2) Quartz dusted, 4 months (750 hr.) – 12 rats.
(3) Quartz dusted, 4 months (750 hr.) – plus intravenous injection of 0.02 mg. *Myco. tuberculosis* – 19 rats.
(4) Mine–dust, dusted 4 months (750 hr.) – 12 rats.
(5) Mine–dust, dusted 4 months (750 hr.) – plus intravenous injection of 0.02 mg. of *Myco. tuberculosis* – 19 rats.
(6) Intravenous injection of 0.02 mg. of *Myco. tuberculosis* – 18 rats.

The period of killing was decided as follows:

Group 1 – one rat from each sub–group was killed at 4 and 8 weeks and the third one was left till it died, which was 103, 236, 245 days in the 1.0, 0.1, 0.01 mg. doses, respectively. The last one of the 4th sub–group (0.001 mg.) was killed at 250 days.

Animals from groups 3, 5 and 6 were killed at monthly intervals extending the period till 10 months.

Groups 2 and 4 were killed at alternative months as the number of animals in these was smaller than the others.

Three rats, two from quartz dusting and one from the mine dust, were lost by death and cannibalism. Two more rats, one from each of the two groups, died during the intravenous injection and were discarded. As there were a good number of deaths in the tuberculous control group, it was not possible to extend the dusting experiments more than 10 months after the injections of Myco. tuberculosis.

HISTOLOGICAL TECHNIQUE

Routine post–mortems were carried out on both the dead and killed rats and guinea–pigs. The lungs of the dead animals were distended with 10% formol–saline (about 10 ml.) after their removal from the thoracic cavity, since satisfactory replacement could not be obtained otherwise, and in order to study the naked eye appearance of the lungs

and pleura *in situ*. In the case of killed animals the fixative was injected intratracheally after expressing the air out of the lungs, without opening the thoracic cavity.

After preliminary fixation blocks were selected along the long axes of both the lungs at the level of the hilum, to include the hilar lymph nodes and to take maximum representative areas for histology. The fixation was completed in fresh fixative, and blocks were embedded in paraffin wax and sectioned at 5μ. Four serial sections were mounted from each block. The first was stained by Gordon and Sweet's silver impregnation, the second with haematoxylin and eosin, the third with Ziehl Neelsen's carbol–fuchsin for the animals injected with organisms, and the fourth was kept as spare.

PATHOLOGICAL FINDINGS

Sections were examined microscopically, and the pulmonary fibrosis seen in the most advanced lesions in each case was graded according to *Belt and King* (1945). Five grades were recognized: (1) lesions cellular with slight increase of reticulin, (2) coarse, compact reticulin with or without collagen, (3) slightly cellular but mostly collagenous, (4) acellular, fully collagenous, (5) acellular, collagenous and confluent. The pathological gradings so obtained, with the survival days of the animals, died or were killed, and the hours of dusting in inhalation experiments, have been summarized in Tables 3–5.

PRELIMINARY EXPERIMENTS WITH DOSAGE OF MYCO. TUBERCULOSIS

Macroscopically the number of lesions produced by the four different doses (0.001, 0.01, 0.1, 1.0 mg.) of *Myco. tuberculosis* increased with the increase of the dose, although not exactly. 0.001 and 0.01 mg. produced only a few lesions, 0.1 mg. a large number, and they were innumerable in the case of 1.0 mg.

Control quartz. The rat killed 30 days after the stoppage of dusting showed in its lungs small collections of dust cells, within which a few fine reticulin fibrils were occasionally seen (grade 1 fibrosis, sub–minimal, Fig. 1.). The reticulin fibres gradually increased in number and became thicker (grade 1 fibrosis in 90 days). At 150 days the lesions were quite large and irregular, composed mainly of fibroblasts. In some lungs partly acellular areas were seen. With reticulin stain more collagenous fibrosis was noticed (grade 3 fibrosis, minimal, Fig.

Table 3

Assessment of Fibrosis in the Sections of Lungs of Rats Receiving Quartz Dust by Inhalation and Myco. Tuberculosis (0.02 mg) by Intravenous Injection
(Dusting 60,000 particles per ml. of air)

Days of Survival	Hours of Dusting	Quartz		Quartz + T. B.	
		Mode of Death	Grade of Fibrosis*	Mode of Death	Grade of Fibrosis
30	750	K+	1 min.	K+	1 min.
60	750			K	1 min.
90	750	K	1	K	1
120	750			K	2
142	750			D	(—)✳
145	750			D	3 min.
150	750	K+	3	K+	3
180	750			K	3
210	750	K	3	K	3
215	750			D	3 max.
217	750			D	3
240	750	K	3	K	3
247	750			D	(—)✳
256	750			D	3
260	750	D(3)	2, 3, 3		
261	750			D	3 max.
270	750	K	3	K	3 max.
295	750			D	3 max.
300	750	K(3)+	4 early	K+	4

+ Sections from these animals are reproduced in figures.
K = killed; D = died; the figure within brackets indicates number of deaths or killings during stated period.
Max. = maximal; min. = minimal within the indicated group of fibrosis
✳ = Loss of animal due to death and cannibalism.
* Grade of maturity of fibrosis; 1, loose reticulin fibrils with no collagen; 2, compact reticulin with or without a little collagen; 3, somewhat cellular but made up mostly of collagen; 4, wholly composed of collagen fibres and completely acellular.

3.). No further advance of fibrosis was found until 300 days when the lesions were acellular and fully collagenous (grade 4 fibrosis, early, Fig. 5.).

Quartz + Myco. tuberculosis (0.02 mg.). The infected animals showed separately dust lesions, infective lesions and a few combined ones. The progress of the lesions was essentially similar to that in the controls, i. e., grade 1 fibrosis minimum at 30 days (Fig. 2.), grade 3 at 150 days (Fig. 4.) and 4 at 300 days (Fig. 6.). If any difference at all was seen it was within the combined lesions, which were a bit more advanced in the maturity of the fibrosis but remaining within the limit of the indicated grades.

Only a few tiny black spots were seen on the surface of the lungs of the animals, dead and killed during the first 4 months of the dusting period. Microscopically small collections of dust cells were seen at the end of 4 months and some free dust cells were also present. No

Table 4

Assessment of Fibrosis in the Sections of Lungs of Rats Receiving Coal-Mine Dust by Inhalation and Myco. Tuberculosis (0.02 mg) by Intravenous Injection
(Dusting 60,000 particles per ml. of air)

Days of Survival	Hours of Dusting	Coal-Mine Dust		Coal-Mine Dust + T. B.	
		Mode of Death	Grade of Fibrosis*	Mode of Death	Grade of Fibrosis
30	842	K+	0	K+	1 min.
41	970			D	1 min.
60	1194			K	1 min.
90	1530	K	0	K	1 min.
120	1738			K	1 min.
150	1738	K+	0	K+	1
177	1956			D	1
180	1959			K	1
210	2420	K	0	K	2
240	2747	K	0	D	2
240	2747			K	2
270	3018	K	0	K	2
279	3114			D	(—)✳
281	3146	K	0	D(4)	2
300	3386	K(5)+	0	K+	2

+ Sections from these animals are reproduced in figures.
K = killed; D = died; the figure within brackets indicates number of deaths or killings during stated period.
Max. = maximal; min. = minimal within the indicated group of fibrosis
✳ = Loss of animal due to death and cannibalism.
* Grade of maturity of fibrosis; 1, loose reticulin fibrils with no collagen; 2, compact reticulin with or without a little collagen.

increase of reticulin fibres was noted. After the injections with *Myco. tuberculosis* (0.02 mg.) an appreciable difference in lung pathology was noted between the infected and non–infected groups. The non–infected group showed more and more collections of dust particles with the advance of further dusting, but none of the animals in this group showed any increase of reticulin fibres within dust cell aggregations, till the end of the experiment (Table 4). On the other hand

slowly progressive fibrosis, rather diffuse than nodular, was observed in the infected group (grade 3 at 300 days, Table 4). The tracheobronchial lymph glands were black from the beginning. Very little increase in their size was seen in the control group, but they were at least double their normal size four months after the injection of tubercle bacilli.

Control–mine dust. Small discrete collections of dust cells were seen in the sections of lungs of the rat killed 30 days after the renewal of the dusting (Fig. 7). Except for the increase of size and number of such dust cell aggregations no further advance of the lesions was seen in any of the sections examined on 90, 150 (Fig. 9), 210, 240, 270, 281 and 300 days (Fig. 11). A mild degree of emphysema was present in some.

Coal–mine dust + Myco. tuberculosis (0.02 mg.). The same three types of lesions (dust lesions, infective lesions and combined lesions), as were seen in the quartz + infection group of animals, were also seen in the sections of lungs of this group, but more combined lesions were present in these animals than the corresponding quartz plus infection group. Sections examined 30 days after the injection of tubercle bacilli showed a few fine reticulin fibrils within the combined lesions (grade 1 fibrosis, sub–minimal, Fig. 8). No further advance was noticed until 150 days when some increase in number and thickness of reticulin fibres was observed. These were loosely woven (grade 1 fibrosis, Fig. 10). Progress of fibrosis was seen in the sections at 210 days. The reticulin fibres were coarse and compact, and there was slight collagen formation in some (grade 2 fibrosis). All the sections examined from this period to 281 days presented a similar picture. The rat which was killed at 300 days showed more collagenous nodules (grande 2 fibrosis, early, Fig. 12).

T. B. control (0.02 mg.). Owing to death of some of the animals in this group, it was not possible to continue the experiment to the desired period (Table 5). The majority of the animals that died showed tubercular pneumonia in their lungs. The rats killed at monthly intervals showed greyish–white lesions on the surface of their lungs. The lesions remained separate up to 150 days after which they coalesced, and confluent tubercular areas were seen. Microscopically the lesions were composed of mononuclear cells, epithelioid cells and some giant cells. A few reticulin fibrils were seen in some of the lesions at 30 days (grade 1 fibrosis, sub–minimal, Fig. 13). By the 150th day more reticulin fibres had appeared, but they were loosely tangled (grade 1 fibrosis). No further progress of fibrosis was seen in any of the sections examined till 220 days; although the lesions progressed as was evidenced by the appearance of some necrotic areas within the larger ones during this period. The rat that was killed at 270 days showed a little more compact fibrosis with slight collagen formation in some of the lesions (grade 2 fibrosis, minimal, Fig. 14).

Table 5

Assessment of Fibrosis in the Sections of Lungs of Rats Receiving Myco. Tuberculosis (0.02 mg) by Intravenous Injection

Days of Survival	Mode of Death	Grade of Fibrosis*
30	K+	1 min.
60	K	1 min.
90	K	1 min.
115	D	1 min.
120	K	1
137	D	1
148	D	1
150	K+	1
171	D	1
178	D	1
180	K	1
185	D	1
196	D	1
210	K	1
218	D	1
220	D	1 max.
270	K+	2
292	D	1

+ Sections from these animals are reproduced in figures.

K = killed; D = died;

Max. = maximal; min. = minimal within the indicated group of fibrosis

* Grade of maturity of fibrosis; 1, loose reticulin fibrils with no collagen; 2, compact reticulin with or without a little collagen.

DISCUSSION

The clinical and statistical evidence suggest that silica has a specific action on the growth of the tubercle bacillus. Experimental work has, however, supported this to only a limited extent. As both produce similar types of fibrotic lesions, the effect of the one on the other is difficult to evaluate.

Gardner (1930) infected guinea-pigs with the Rl strain of human tubercle bacilli by an inhalation method, and found it disappeared from the lungs after six weeks; but when the infected animals were dusted with powdered quartz, extensive growth of the bacilli occurred in the lungs. In the present series of experiments rats were infected by intravenous injection of B37 Rv human strain of *Myco. tuberculosis*

four months after dusting with quartz and coal–mine dusts. Organisms were demonstrated in the infected groups of animals up to the end of the experimental period (10 months).

Silicotic lesions developed steadily in both the infected and non–infected groups of quartz–dusted animals, but no appreciable difference of fibrous tissue production was observed between the two. In the infected group, only a few combined lesions were seen, within which a small number of bacilli were present. The reason for this is quite obscure, but it may be that the bacilli could not enter the preformed silicotic lesions. Alternatively, the sites of lesion formation in the case of intravenous injection of organisms may not coincide with those produced by inhalation of dust or the tubercle bacilli in the rat lung may not be as fibrogenic as in the human lung.

In the case of coal-mine dust, dusting was continued after the animals were infected. More combined lesions were produced, and some difference in fibrous tissue production was seen between the infected and non–infected groups. The infected group showed larger surface–area involvement, with a greater degree of fibrosis, than did the non–infected or tuberculous control group. It seems probable that the continuation of dusting brought more dust particles into contact with preformed tubercular lesions and thereby promoted both the growth of the tubercle bacillus and the formation of fibrous tissue.

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SADRŽAJ

EKSPERIMENTALNA INFEKTIVNA PNEUMOKONIOZA

Prašina kvarca i ugljenokopna prašina bile su unesene inhalacijom u pluća štakora. Koncentracija je bila visoka, a ekspozicija je trajala 4 mjeseca. Nakon toga su grupe zaprašenih životinja bile inficirane virulentnim sojem humanog tuberkuloznog bacila (H 37 Rv). Kod grupe životinja, koje su bile izvrgnute djelovanju ugljenokopne prašine, nastavljeno je zaprašivanje daljih 10 mjeseci. U kvarcnim grupama nije bilo razlike s obzirom na patologiju pluća, iako je u obje grupe došlo do stvaranja klasičnih silikotičnih čvorova. U grupi životinja, koje su bile izvrgnute ugljenokopnoj prašini, nađeno je nešto više fibroze, koja je bila difuzna, nego bilo u neinficiranoj ili tuberkuloznoj kontrolnoj grupi. Ti eksperimenti su preliminarni i ne treba ih smatrati previše konkluzivnima. Rad se nastavlja.

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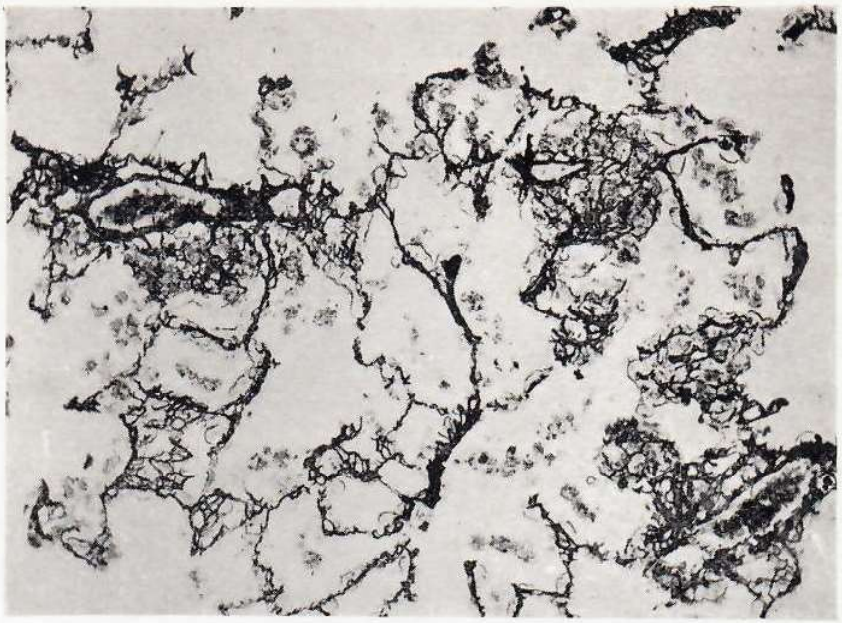

Fig. 1. Rat lung, quartz dusted (4 months, 750 hours) 30 days after the cessation of dusting. Small nodules with a few fine reticulin fibrils (grade 1 fibrosis, subminimal). Silver impregnation.

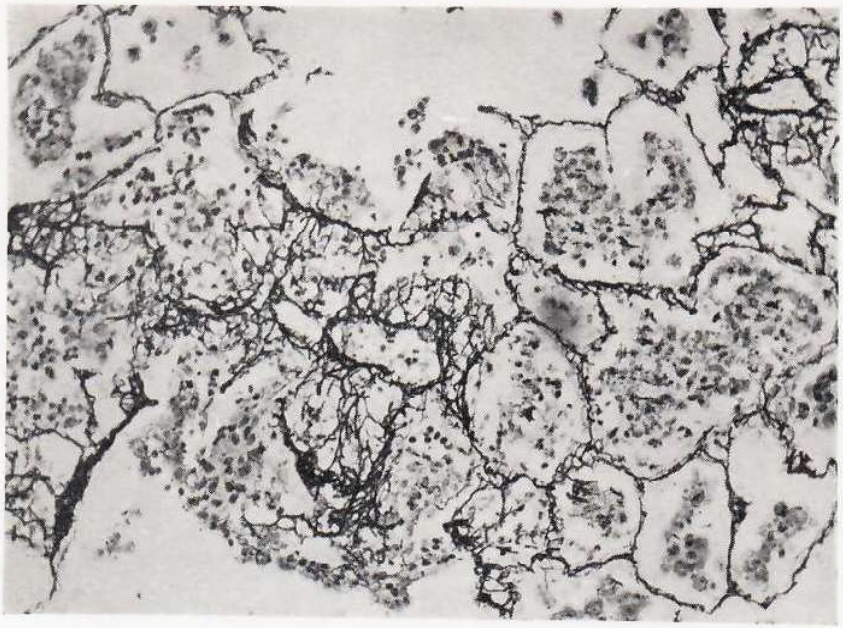

Fig. 2. Rat lung, quartz dusted (4 months, 750 hours) 30 days after the cessation of dusting and injection of 0.02 mg. *Myco. tuberculosis* (I. V.). Combined dust and infective lesion showing loose network of fine reticulin fibres (grade 1 fibrosis, minimal). Silver impregnation X 250.

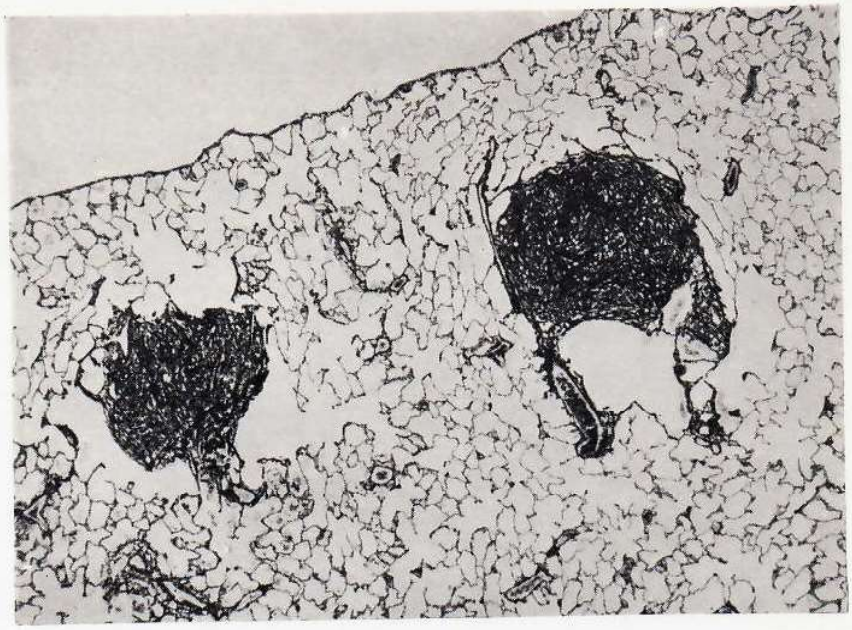

Fig. 3. Rat lung, quartz dusted (4 months, 750 hours) 150 days after the cessation of dusting. Almost collagenous nodules, which were slightly cellular with ordinary nuclear stain (grade 3 fibrosis). Silver impregnation X 65.

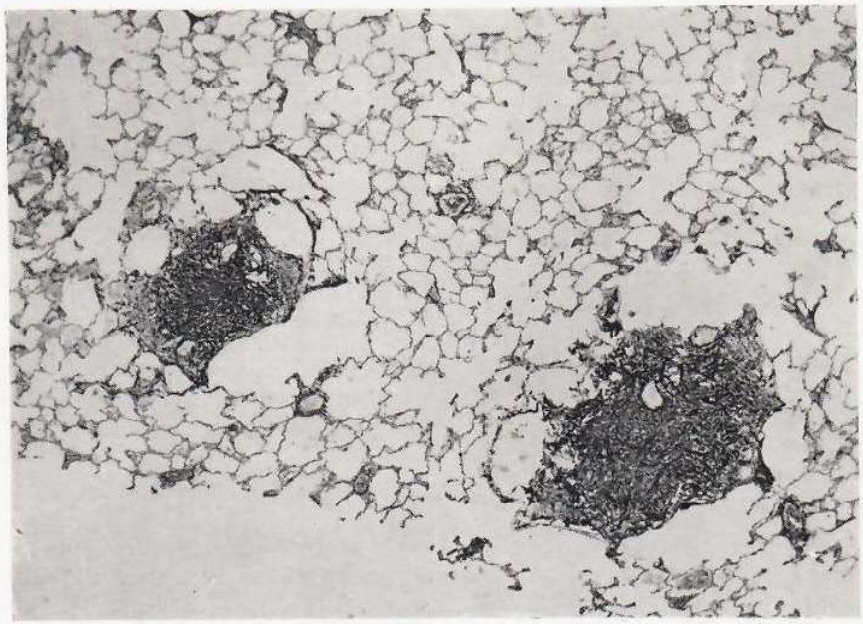

Fig. 4. Rat lung, quartz dusted (4 months, 750 hours) 150 days after the cessation of dusting and injection of 0.02 mg. of *Myco. tuberculosis* (I. V.). Combined dust and infective lesions. Nodules almost collagenous (grade 3 fibrosis).
Silver impregnation X 65.

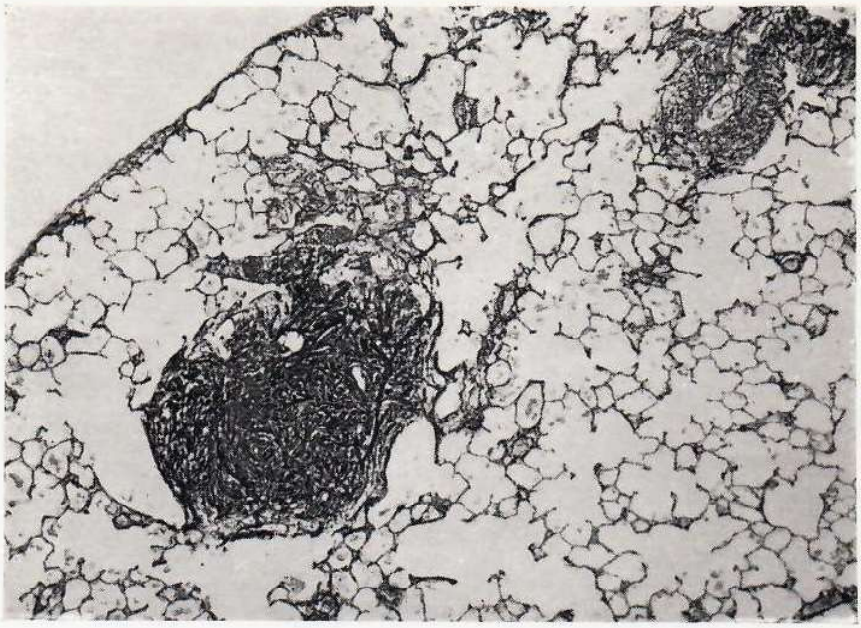

Fig. 5. Rat lung, quartz dusted (4 months, 750 hours) 300 days after the cessation of dusting. Fully collagenous nodule which was completely acellular with ordinary nuclear stain (grade 4 fibrosis, minimal). Silver impregnation.

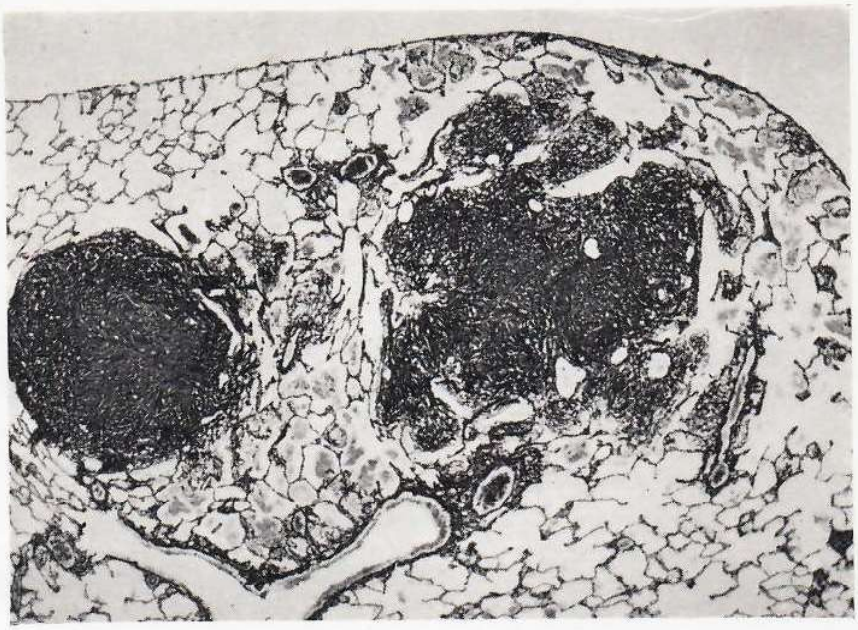

Fig. 6. Rat lung, quartz dusted (4 months, 750 hours) 300 days after the cessation of dusting and infection of 0.02 mg. of *Myco. tuberculosis* (I. V.). Combined dust and infective lesions. Nodules fully collagenous (grade 4 fibrosis).
Silver impregnation X 65.

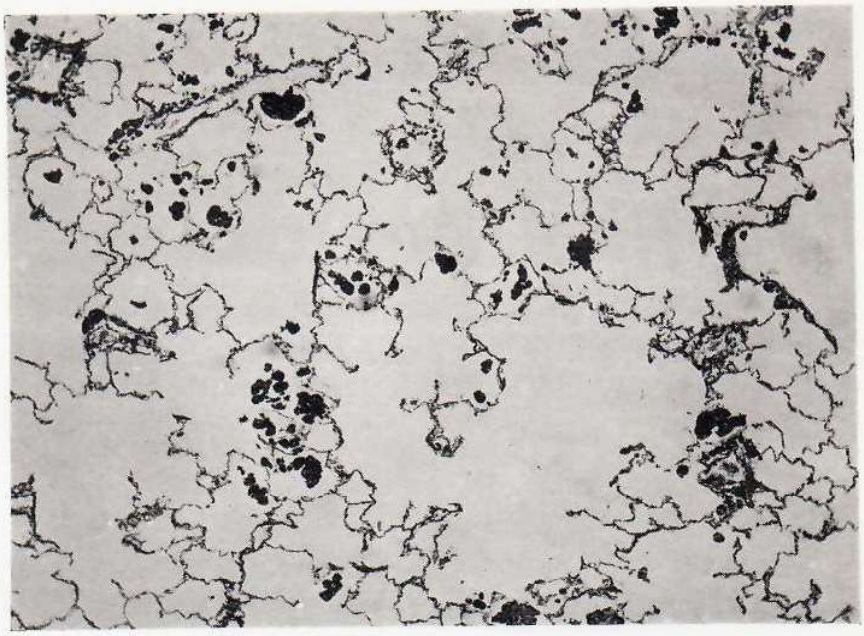

Fig. 7. Rat lung 5 months after the commencement of dusting with coal mine dust (842 hours). Small discrete dust cell collections with no fibrosis. Some emphysema. Silver impregnation X 130.

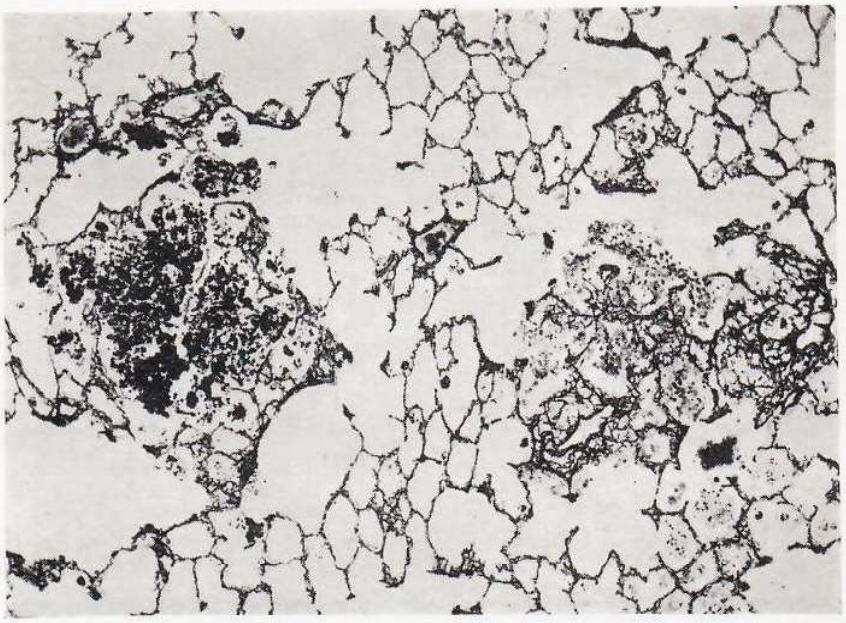

Fig. 8. Rat lung dusted with mine dust (5 months, 842 hours) 30 days after injection of 0.02 mg. *Myco. tubercle* (I. V.) Combined dust and infective lesions with a loose network of fine reticulin fibrils (Grade 1 fibrosis, subminimal). X 145.

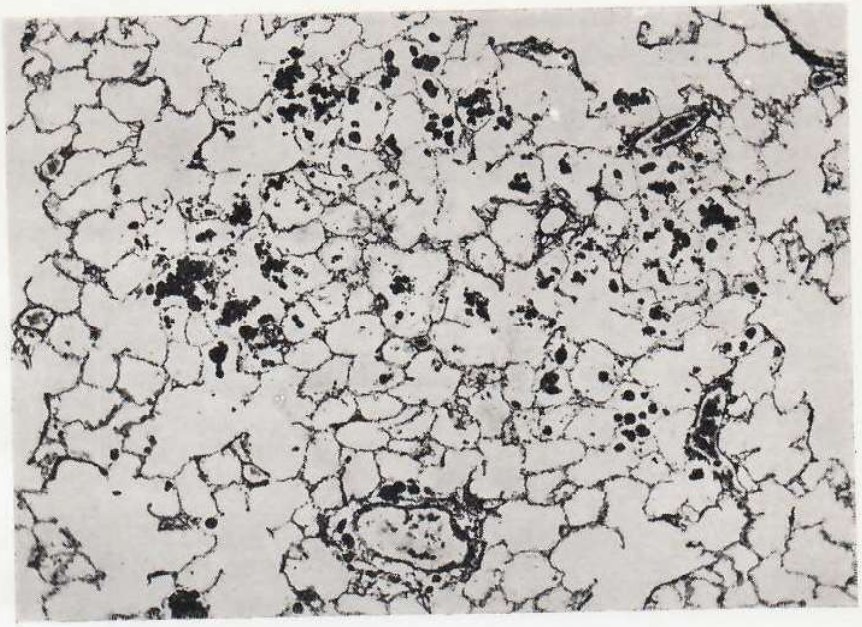

Fig. 9. Rat lung 9 months after the commencement of dusting with coal mine dust (1,738 hours). Small collections of dust cells, no fibrosis. Silver impregnation X 130.

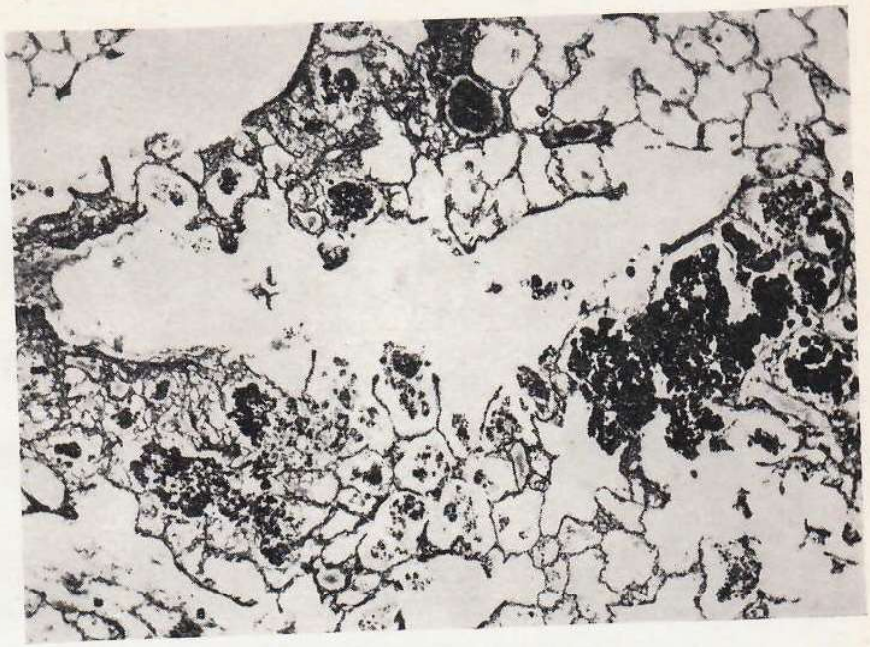

Fig. 10. Rat lung dusted with mine dust (9 months, 1,738 hours) 150 days after the injection of 0.02 mg. of *Myco. tubercle* (I. V.). Combined dust and infective lesions with loose network of fine reticulin fibres (Grade 1 fibrosis), some emphysema. Silver impregnation X 130.

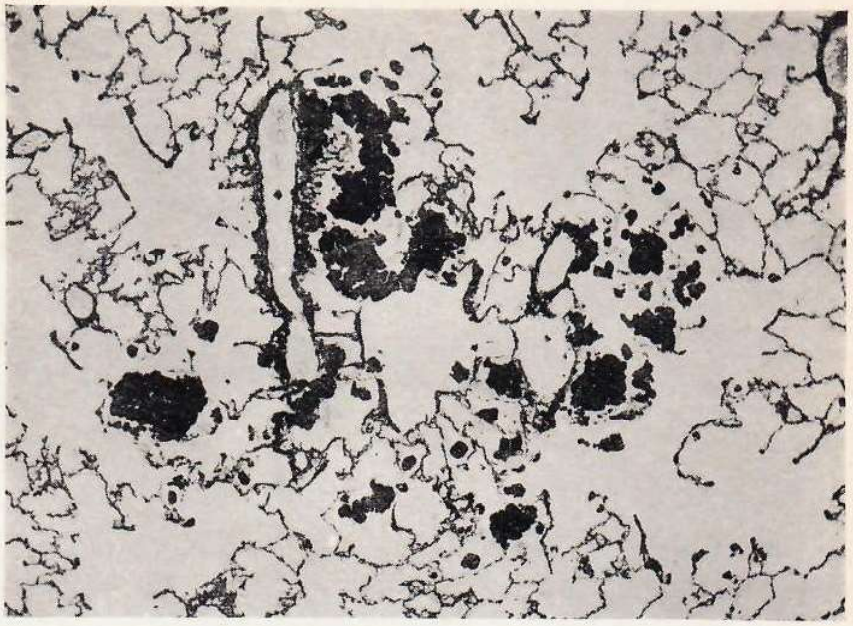

Fig. 11. Rat lung 14 months after the commencement of dusting with coal mine dust (3,386 hours). Dust cells collected into large aggregates with no fibrosis. Some emphysema. X 145.

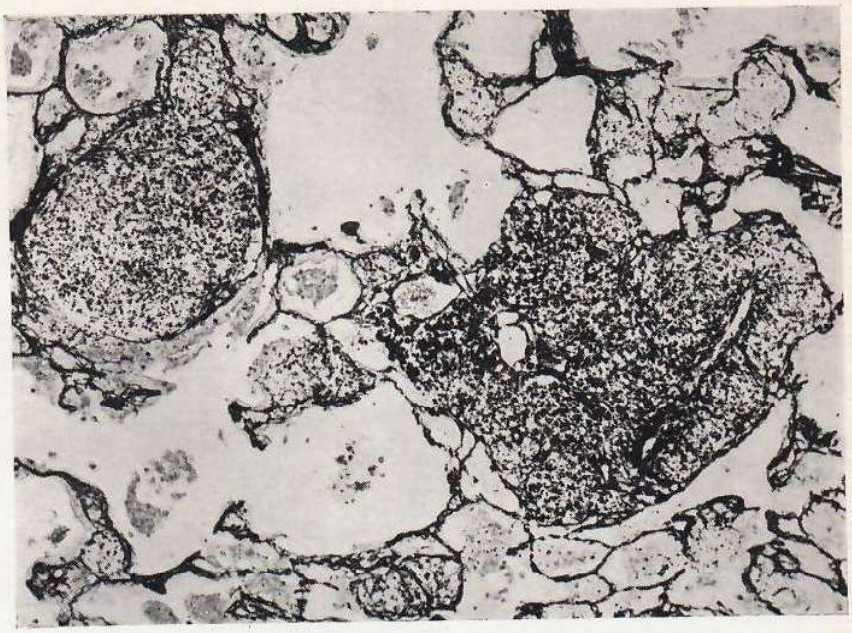

Fig. 12. Rat lung. dusted with mine dust (14 months, 3,386 hours) 300 days after injection of 0.02 mg. of *Myco. tuberculosis* (I. V.) Combined lesions with coarse reticulin fibres and some collagen. Grade 2 fibrosis and some emphysema.

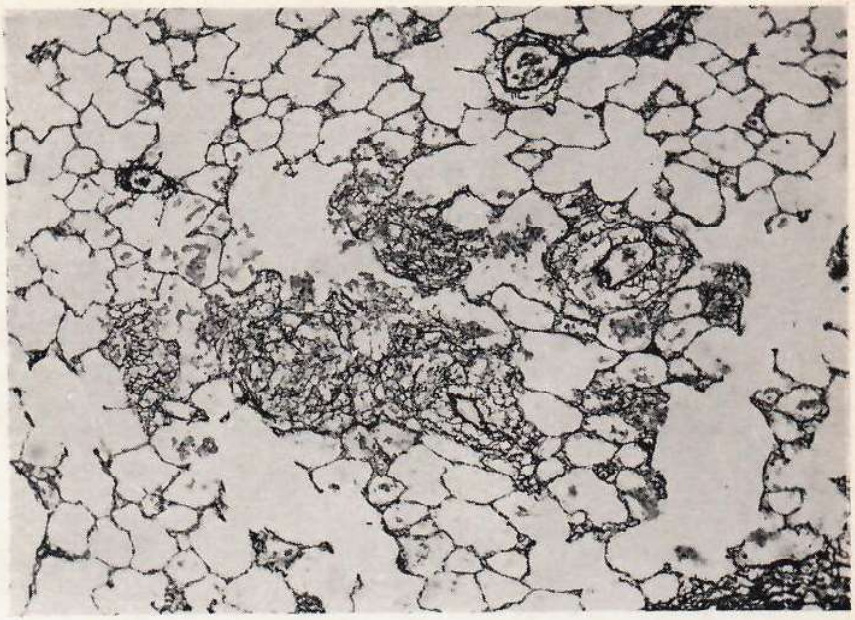

Fig. 13. Rat lung 30 days after injection of 0.02 mg. of *Myco tuberculosis* (I. V.) Nodular lesions with loose network of fine reticulin fibres. (Grade 1 fibrosis minimal). Silver impregnation X 130.

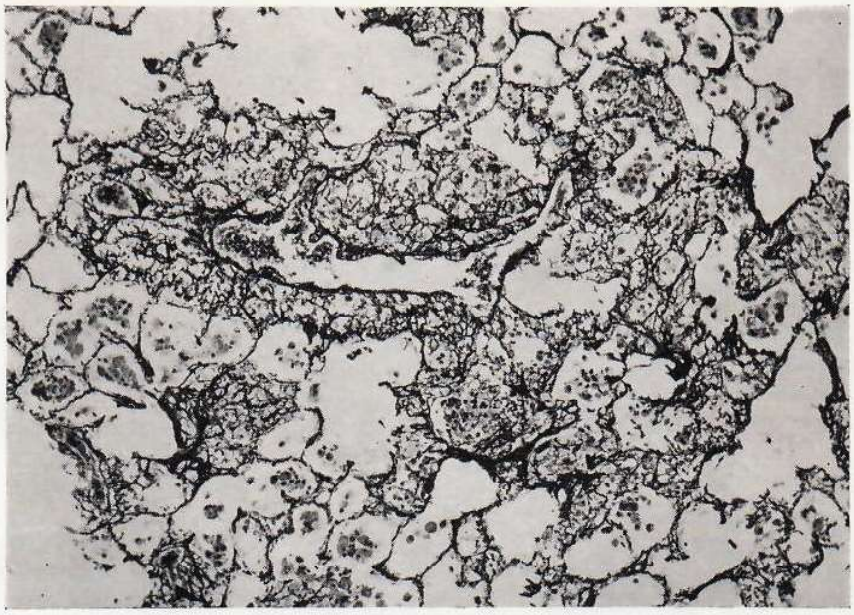

Fig. 14. Rat lung, died 280 days after injection of 0.02 mg. *Myco. tuberculosis.* Diffuse lesions of reticulin, grade 2 fibrosis.