INFECTIVE PNEUMOCONIOSIS

I. THE INFLUENCE OF DEAD TUBERCLE BACILLI (B.C.G.) ON THE DUST LESIONS PRODUCED BY ANTHRACITE, COAL-MINE DUST, AND KAOLIN IN THE LUNGS OF RATS AND GUINEA-PIGS

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The close association between silicosis and tubercular infection has long been recognized. In South Africa, Mavrogordato (1926) and Strachan and Simson (1930) concluded that infection played an important part in the development of the disease. Policard (1933) was of the opinion that practically all cases of silicosis were complicated by tuberculosis. Kettle and Archer (1933) observed that infective silicosis was more common in Great Britain than simple silicosis. By infective silicosis was meant "the modification in a dusty lung of the reaction of a chronic low-grade infection, frequently tuberculosis, in the direction of increased fibrosis", a definition first used by Simson, Strachan, and Irvine (1931). Gye and Kettle (1922) showed that tubercle bacilli proliferated readily in necrotic lesions produced in mice by the inoculation of silica into the interstitial tissues. Kettle (1927) proved that the proliferation of the bacilli was not dependent on the presence of necrotic tissue, but on the actual presence of silica in the lesion. Other workers found that in experimental animals attenuated or dead tubercle bacilli produced proliferating lesions in the presence of silica (Gardner, 1929; Cummins, 1940; Vorwald and Delahant, 1938; Vorwald, Dworski, Pratt, and Delahant, 1950). Gardner (1930) showed that healed tubercular foci which had been produced originally by a low virulence strain were reactivated as a result of exposing the animals to silica dust inhalation. This capacity for new growth on the part of the organisms was not due to an increase in their virulence, since subinoculation from these animals did not produce anything other than the lesions characteristic of the strain. Policard (1939) and Sen (1939) also demonstrated the reactivation of latent tuberculous infections in animals in the

presence of silica. Kettle (1932) found that living tubercle bacilli, introduced into the general circulation of mice, proliferated in interstitial lesions previously produced by inoculation with silica, siliceous dust, shale and kaolin, while no growth of bacilli was seen in lesions produced by inert substances such as iron, carbon, and aluminium. By a combination of dust and infection Kettle (1934) produced a degree of fibrosis which could not be obtained from the action of either factor alone. He introduced into the lungs of guinea-pigs a suspension of killed tubercle bacilli from an avirulent strain (B.C.G.) with and without kaolin dust. The usual active infection which results from the presence of live organisms was thus replaced by a transient one which, while merely accelerating the pneumoconiotic process, would not dominate the histological picture.

Several workers have reported a high incidence of tuberculosis among coal-miners whose lungs show the condition known as progressive massive fibrosis (Cummins, 1931; Belt and Ferris, 1942; Rogers, 1946; Gough, 1947; Fletcher, 1948). Tubercle bacilli have been demonstrated in the lesions in 38.5% of such cases by James (1948; 1954). Davies and Mann (1949) and Mann (1951) showed the relation between tuberculosis and the massive fibrosis of coal pneumoconiosis radiologically. Although the organism has not been demonstrated after death in all cases of progressive massive fibrosis, many workers in this field believe that the tubercle bacillus is the chief causative agent. Experimentally, Cummins (1940) injected coal dust and quartz with and without dead tubercle bacilli intratracheally in rabbits and found that the reactions produced in the lungs by the combined action of dust and organisms were more severe

and extensive than those produced by dust alone. But the published results of biological experiments appeared to us to be not very conclusive, and it was, therefore, decided to conduct further animal experiments in order to study the effect of tubercle bacilli and dust in the lungs. Since coal dust and coal-mine dust are of great importance from the social and industrial points of view, it seemed desirable to use these; and kaolin was also included in order to compare the results with those obtained by Kettle in his experiments in 1934.

Experiments Planned

In order to study the effect of various dusts on tuberculous infection in the lungs of animals over a long period of time, it was thought better to use a species of animal that is known to be more resistant to this infection rather than one which is more susceptible. Rats are highly resistant to tuberculosis, and they are commonly used in pneumoconiosis research. In order to compare the results with Kettle's work (1934), a parallel series of experiments was set up with guinea-pigs.

Six groups of rats and six groups of guinea-pigs were injected with the following: (1) B.C.G. (heat-killed), (2) "mine-dust", (3) "mine-dust" + B.C.G., (4) anthracite + B.C.G., (5) kaolin, (6) kaolin + B.C.G.

Various workers have shown that anthracite coal does not produce any permanent lesions in the lungs of experimental animals, even after long periods of time, grade 1 fibrosis rarely being attained (e.g., Belt and King, 1945; Ray, King, and Harrison, 1951; Wright, 1951). Therefore it was deemed unnecessary to set up a control experiment with anthracite alone. Moreover, a control experiment with "anthracite coal-mine dust" (containing some siliceous matter as well as the anthracite) was being included.

Description of Samples of Dust and Organisms

Mycobacterium tuberculosis—B.C.G.—The strain of B.C.G. was kindly supplied by Dr. H. J. Benstead, Central Public Health Laboratory, Colindale. It was from the standard Copenhagen strain, a 12-day culture on Lowenstein-Jensen's medium.

Anthracite.—This sample from South Wales was turbine ground and kindly prepared by Dr. B. M. Wright. Its ash content was 2.4% and its size distribution is given in Table 1.

"Mine-dust".—Because of the difficulty of obtaining sufficient airborne coal-mine dust it

TABLE 1
SIZE DISTRIBUTION AND ANALYSIS OF DUSTS USED FOR INJECTIONS

Samples (Size in μ)	Anthracite		"Mine-Dust" (80% Anthracite + 20% Shale)		Kaolin	
	Per cent.		Per cent.		Per cent.	
	By No.	By Mass	By No.	By Mass	By No.	By Mass
0.16-0.23	—	—	—	—	3	0.08
0.23-0.32	—	—	—	—	18.9	1.32
0.32-0.45	—	—	19.2	0.6	38.2	7.9
0.45-0.64	—	—	16.7	1.6	16.8	9.8
0.64-0.90	37.7	1.0	25.9	6.6	12.3	20.5
0.90-1.30	14.7	1.1	24.7	17.4	9.5	43.6
1.30-1.80	11.7	2.4	8.7	17.5	1.3	16.8
1.80-2.60	16.9	9.6	3.4	19.3	—	—
2.60-3.60	9.9	15.9	0.9	14.6	—	—
3.60-5.10	6.5	29.4	0.5	22.5	—	—
5.10-7.20	0.6	33.3	—	—	—	—
7.20-10.20	0.2	7.3	—	—	—	—
Particles counted	390		863		640	
Estimated surface area ($m^2/g.$)	1.2		1.8		—	
Silica solubility (mg./100 ml.; King, 1945) ..	0.2		0.9		1.1	
Ash content (380°) (%) ..	2.4		22		99	
Composition (%)						
Coal ..	97.6		78		—	
Kaolin ..	1		5		77	
Mica ..	$\frac{1}{2}$		10		12	
Quartz ..	$\frac{1}{2}$		6		8	
Carbonates ..	$\frac{1}{2}$		1		(hydrated silica)	

was decided to prepare a synthetic mixture of powdered coal and shale which would closely approximate coal-mine dust in composition. The sample contained 80% anthracite coal (turbine-ground as above) + 20% shale. The shale (Ammanford B) was likewise from a South Wales anthracite mine, from a seam overlying the coal measure. It contained 31% quartz, 51% mica, 6% kaolin, and 7% carbonates, and was found by Belt and King (1945) to be only minimally fibrogenic in animals.

Kaolin.—The sample originally used by Kettle in 1932 and 1934 was still available (British Drug Houses, labelled "Society of Leather Trades Chemists' Specification"). It was mostly crystalline, partly amorphous, and consisted mostly of aluminium silicate.

The size distribution and analyses of these dusts are given in Table 1. Because the shale dust was of smaller size than the anthracite, the size distribution of the mixture ("mine-dust") was slightly lower than that of the anthracite.

Preparation of Suspensions for Injection

Myco. tuberculosis—B.C.G.—The strain of B.C.G. was sub-cultured on glycerine nutrient agar medium. A fine granular suspension of the organisms was killed by autoclaving for 20 min. at 13 lb. pressure. (Sterility tests in Lowenstein-Jensen medium ensured the effectiveness of autoclaving.) Just before the injections were made this was added to the various dust suspensions, or sterile physiological saline for controls, in such volumes as to make the final concentration of B.C.G. 2 mg./ml. of suspension when introduced into the lungs of the experimental animals.

Dust Suspensions.—Of each of the dusts (anthracite, "mine-dust", and kaolin), 2.5 g. was weighed into 4 oz. screw-cap bottles. Cream-free milk (about 3%) was added to help to keep the particles in suspension. Sterile physiological saline was then added to the bottles so that the suspension contained 100 mg. of dust per ml. The bottles were then sterilized by autoclaving (20 min. at 15 lb. pressure).

To those suspensions which were to be injected in combination with killed B.C.G. the previously sterilized B.C.G. suspension was added and intimately mixed immediately before the injections were made. The volume of B.C.G. suspension added was calculated to a concentration of 2 mg. B.C.G./ml. of the final mixture. Thus the individual suspensions contained 100 mg. of the particular dust and/or 2 mg. of B.C.G. per ml.

The dose of dust and organisms injected varied in rats and guinea-pigs, depending on their body weight, and was as follows :—

Body Weight of Animal (g.)	Volume of Suspension (ml.)	Amount of Dust (mg.)	Amount of B.C.G. (mg.)
200	0.5	50	1.0
300	0.75	75	1.5
400	1.0	100	2.0
500	1.25	125	2.5
600	1.50	150	3.0
700	1.75	175	3.5
800 and over	2.0	200	4.0

Animals

Twenty rats were used in each of the six groups. They were all of the male sex and were from the black-and-white hooded variety of the Medical Research Council strain. Their average weight was 300 g.

Eight guinea-pigs, four male and four female, were used in each of the six groups of experiments. Their body weights varied from 300 to 800 g. : they were equally distributed in all the groups.

Experimental Procedure

The anaesthetized animals (ether) were injected by the intratracheal route *via* the mouth (Kettle and Hilton, 1932 ; modified by King, Mohanty, Harrison, and Nagelschmidt, 1953). No dust was regurgitated. No immediate post-operative deaths occurred in any of the groups.

Duration of Experiments

The experimental animals were killed at monthly intervals in the case of rats and the experiments extended up to a period of 500 days ; guinea-pigs at longer intervals to 365 days.

Histopathological Technique

Routine necropsies were carried out on all the animals which were killed at regular intervals, as well as on any that were found dead. The lungs were fixed in 10% formol-saline and blocks taken along their long axes through the hilum to include the hilar lymph glands. The blocks were then treated with fresh fixative, dehydrated, impregnated with paraffin wax, embedded, and sectioned at 5 μ .

Serial sections were stained by Gordon and Sweets' (1936) silver impregnation, with haematoxylin and eosin, and with Van Gieson's stain. In addition, the sections from the lungs of animals which had been injected with B.C.G. were stained with Ziehl-Neelsen's carbol-fuchsin, and also with phenol auramine and the sections examined under fluorescent light in order to study the distribution of the organisms in the lesions.

Pathological Findings

Sections from the lungs were studied microscopically, and the fibrosis seen in the most advanced lesions in each case was graded according to Belt and King (1945). The five grades of fibrosis recognized are as follows : grade 1, loose reticulin fibrils, 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 collagenous.

The pathological gradings obtained, together with the number of days of survival of the animals and their mode of death, are summarized in Tables 2 and 3.

The macroscopic and microscopic appearances in the two species of animals were very similar, except in the case of the animals which had been given kaolin + killed B.C.G., where, histologically,

TABLE 2

ASSESSMENT OF FIBROSIS IN SECTIONS OF LUNGS OF RATS AFTER INTRATRACHEAL INJECTION OF DIFFERENT DUSTS, ORGANISMS, OR BOTH

Days of Survival	B.C.G.		" Mine-dust "		" Mine-dust " + B.C.G.		Anthracite + B.C.G.		Kaolin		Kaolin + B.C.G.	
	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis
30	Killed*	1 min.	Killed*	0	Killed* Died	1	Killed*	1	Killed*	1 min.	Killed*	1
60	Killed	1 min.	Killed	0	Killed	1	Killed	1	Killed	1 min.	Killed	1
90	Killed	1 min.	Killed Died	0	Killed	1	Killed	1	Killed	1 min.	Killed	1
120	Killed	1 min.	Killed	0	Killed	1	Killed	1	Killed	1 min.	Killed	1
150	Killed*	1 min.	Killed Died	0	Killed	1	Killed	1	Killed	1 min.	Killed	1 max.
180	Killed	1 min.	Killed*	0	Killed*	2 min.	Killed	1 max.	Killed*	1 min.	Killed*	3 max.
210	Killed (2)	1 min.	Killed (2)	0	Killed (2)	2 min.	Killed (2)	1 max.	Killed (2)	1 min.	Killed (2)	3
240	Killed	1 min.	Killed	0	Died Killed	2 min.	Died Killed*	2 max.	Killed	1 min.	Killed	2
270	Killed (2)	1 min.	Killed (2)	0	Killed (2)	2 min.	Killed (2)	2 max.	Killed (2)	1 min.	Killed (2)	2
300	Killed* Died	1 min.	Killed	0	Killed	2 min.	Killed	2	Killed	1 min.	Killed	2
330	Killed	1 min.	Killed	0	Killed	2 min.	Killed	2	Killed	1 min.	Killed	2
365	Killed	1 min.	Killed	0	Killed*	2 max.	Killed	2	Killed	1 min.	Killed	2
400	Killed	1 min.	Killed	0	Killed	1	Killed Died	1	Killed Died (2)	1 min.	Killed	1 min.
450	Killed	1 min.	Killed	0	Killed	1	Killed	1	Killed	1 min.	Killed	1 min.
477	—	—	—	—	Died*	1 min.	—	—	—	—	—	—
500	Killed (2)	1 min.	Killed* Died	1	—	—	Killed* Killed	1 min.	Killed (3) Killed*	1 min.	Killed (2) Killed*	1 min.

TABLE 3

ASSESSMENT OF FIBROSIS IN SECTIONS OF LUNGS OF GUINEA-PIGS AFTER INTRATRACHEAL INJECTION OF DIFFERENT DUSTS, ORGANISMS, OR BOTH

Days of Survival	B.C.G.		" Mine-dust "		" Mine-dust " + B.C.G.		Anthracite + B.C.G.		Kaolin		Kaolin + B.C.G.	
	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis
30	Killed*	1 min.	Killed* Dead	0	Killed*	1	Killed*	1	Killed*	1 min.	Killed* Dead	1 max.
90	Killed	1 min.	Killed	0	Killed	1	Killed	1	Killed	1 min.	Killed	1 max.
120	Killed	1 min.	Killed	0	Killed	1 max.	Killed	1	Killed	1 min.	Killed	1 max.
180	Killed*	1 min.	Killed	0	Killed	2 min.	Killed	2 min.	Killed	1	Killed*	2 max.
240	Killed	1 min.	Killed*	0	Killed*	2 max.	Killed*	2 max.	Killed*	1	Killed	2
300	Killed	1 min.	Killed	0	Killed	2	Killed Dead	2 min.	Killed (2)	1	Killed	2
365	Killed*	1 min.	Killed*	0	Killed*	1 min.	Killed*	1	Killed*	1	Killed*	1
500	Killed	0	—	—	Killed	1 min.	—	—	—	—	—	—

*Sections from these animals are reproduced in the figures.

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

Numbers in brackets indicate the numbers of animals killed or found dead.

the reaction in rats seemed to be slightly more marked than that in guinea-pigs.

Macroscopic Appearance in Rats and Guinea-pigs

Macroscopically there was no difference between the lungs of rats and guinea-pigs in the various series, and they are described together. The lesions appeared somewhat larger in the groups of animals which received dust and organisms than in those which had the dust or the organisms alone.

B.C.G.—Evenly distributed, discrete, whitish lesions, 1 to 2 mm. in diameter, were seen in the lungs from 30 days onwards. They were slightly larger at 240 days, being about 4 mm. in diameter; later they diminished in size and number and at 500 days they were small and scanty.

"Mine-dust".—In both rats' and guinea-pigs' lungs the dust was distributed diffusely. In some cases, subpleural collections of dust were seen on the dorsal aspects of the lungs.

"Mine-dust" + B.C.G.—On the lung surfaces there were collections of dust, around which in the early stages were greyish lesions about 1 to 2 mm. in diameter. These lesions increased in size during the course of the experiment and persisted till its end at 500 days.

Anthracite + B.C.G.—The appearances were very similar to those found in the lungs of animals receiving "mine-dust" + B.C.G.

Kaolin.—Small, whitish collections of dust were seen distributed diffusely throughout the lungs of both rats and guinea-pigs. There was no change in the macroscopic appearance throughout the experiment.

Kaolin + B.C.G.—From 30 days onwards there were white areas over the lung surfaces which were larger than those seen in the previous group. From about 210 days there were definite firm, white areas on the lungs, resembling fibrosis. Later still, towards the termination of the experiment, the appearances were very similar to those seen with kaolin alone, except that the lesions were larger.

The tracheo-bronchial lymph nodes were enlarged to several times the normal in all these groups, and were black in animals receiving coal.

Microscopic Appearances

The histological lesions in the lungs of rats and guinea-pigs in the various groups of experiments

were very similar in the type of reaction. "Mine-dust" did not produce any fibrosis in either rats or guinea-pigs; B.C.G. alone and kaolin alone produced mild fibrosis (grade 1); while "mine-dust" + B.C.G., anthracite + B.C.G., and kaolin + B.C.G. produced well marked fibrosis (grades 2 and 3 respectively in rats and grade 2 in guinea-pigs). The lesions reached a maximum at about the same period in all cases and then decreased in severity towards the end of the experiments. The demonstration of acid-fast organisms in the case of guinea-pigs was difficult; they were seen in animals killed early in the experiment, but only in small numbers, or not at all, during the last 100 to 150 days.

Rats

B.C.G.—At 30 days there were many nodular lesions, about 100 to 200 μ in diameter, throughout the lung fields. They were composed mainly of mononuclear cells with some bacilli in the centre. On reticulin staining there appeared to be a loose network of fine reticulin fibres within these nodular lesions (grade 1 fibrosis, Fig. 1). Thereafter, the lesions showed a slight increase in size (200 to 300 μ) but there was no evidence of increase in reticulin up to 150 days (Fig. 2). After this period there appeared to be a diminution in the size of the lesions (100 to 150 μ). The fibrosis still remained the same as at first and at 300 days was grade 1 (Fig. 3). From this time onwards the lesions seemed to be reduced, both in size and in number, and at 500 days only an occasional small cellular lesion could be seen. Acid-fast bacilli were, however, demonstrable in such lesions, although they were reduced in number compared with the earlier stages.

"Mine-dust".—In the early stages the dust appeared to be distributed diffusely throughout both lungs. At 30 days there were plenty of dust particles lying free within the alveoli. In some areas, dust cells were collected into small aggregations but there was no evidence of fibrosis (Fig. 4). Between this time and 180 days the dust cells began to collect into definite nodules, leaving the intervening lung clear. At 180 days the dust cell collections had enlarged to a certain extent, without the appearance of any fibrosis (Fig. 5). Between 180 days and the termination of the experiment at 500 days, the dust appeared to become more tightly packed together into focal aggregations with just enough reticulin fibres around them to hold the dust together. There was no further fibrosis (Fig. 6).

"Mine-dust" + B.C.G.—At 30 days there were some dust cells lying free within the lung alveoli,

Rat lungs after the injection of 1.5 mg. of dead B.C.G. Silver impregnation (80).

FIG. 1.—30 days. Nodular lesions. Loose network of reticulin fibres. Grade 1 fibrosis.

FIG. 2.—150 days. Nodular lesion with loosely woven reticulin fibres. Grade 1 fibrosis.

FIG. 3.—300 days. Small nodular lesion with loose reticulin network. Grade 1 fibrosis.

Injection of 75 mg. of "mine-dust". Silver impregnation (60).

FIG. 4.—30 days. Diffuse distribution of dust particles with an occasional small aggregation. No fibrosis.

FIG. 5.—180 days. Dust collected into dense aggregations without any fibrosis.

FIG. 6.—500 days. Dust tightly packed into focal aggregations with a few reticulin fibres around them, but no fibrosis.

while others were aggregated to form nodular lesions. These lesions were composed of dust cells together with many epithelioid cells and some acid-fast organisms, and seemed to be due to the combined action of the dust and organisms (Fig. 7). Within such lesions were loosely arranged fine reticulin fibres (grade 1 fibrosis; Fig. 8). By 120 days the lesions had increased in size, but there was very little increase in fibrosis, the lesions remaining within the maximum limits of grade 1 fibrosis. At 180 days, they appeared to have advanced and the reticulin fibres were thicker and becoming compact (grade 2 fibrosis, minimal; Fig. 9). No further progress was noted in the lesions till 365 days, when the reticulin appeared to be very thick and compact (grade 2 fibrosis, maximal; Fig. 10). After this the lesions gradually diminished in number and severity. The nodules were smaller and the reticulin fibres, which had been thick and compact earlier, were finer and loosely arranged and, near the end of the experiment (477 days) only a few small nodules were seen. At this stage a few collections of dust with mild thickening of the alveolar walls were seen but no definite fibrosis (Fig. 11).

With fluorescent light the microscopic preparations examined for organisms showed about eight in an area of $2 \times \frac{3}{4}$ cm. of lung at the period when the fibrosis was at its maximum, and two only in the same area, approximately, at 500 days.

Anthracite + B.C.G.—The lesions produced by anthracite coal and B.C.G. in the lungs of rats were very similar to those produced by "mine-dust" and B.C.G. In the early stages (30 days) there were cellular lesions composed mainly of epithelioid cells, dust cells, and acid-fast organisms (Fig. 12). Within these lesions there was a slight increase of reticulin fibres (grade 1 fibrosis, minimal; Fig. 13). The lesions remained unchanged till 150 days, when there was thickening of the reticulin fibres in them and the fibrosis reached a fully developed grade 1. By 240 days the cellular lesions were larger, more numerous, and showed thick and compact reticulin (grade 2 fibrosis, maximal; Fig. 14). There was no appreciable change from this time till 400 days. At this stage the lesions were somewhat smaller and less numerous, and at 500 days there were only scattered lesions with a few reticulin fibres (grade 1 fibrosis; Fig. 15).

Examination of sections stained with auramine revealed under fluorescent light about eight bacilli in an area $2 \times \frac{1}{2}$ cm. of lung tissue at 180 days, and only three bacilli in an area 3×1 cm. at 300 days.

Kaolin.—At 30 days this dust produced a diffuse exudative reaction with numerous giant cells containing dust particles. The cells tended to collect together but did not form compact nodules. A few fine reticulin fibres were seen within these collections (grade 1 fibrosis, minimal; Fig. 16). The lesions did not appear to progress and the fibrosis still remained grade 1 minimal (Figs. 17 and 18).

Kaolin + B.C.G.—The lesions resulting from the introduction of kaolin dust and B.C.G. were very different from those produced by the dust alone. At 30 days there was the same diffuse infiltration of the lungs, with large numbers of giant cells containing engulfed dust particles and collected together in some areas to form nodules, within which were acid-fast organisms and loose reticulin fibres (grade 1 fibrosis; Fig. 19). With the advance of time, however, the lesions showed a definite increase of reticulin. There was generalized thickening of the alveolar walls, and at 180 days portions of the lung showed some consolidation due to dust-laden cells. Some bacilli were demonstrable in these areas, and there were many thick compact bundles of collagen fibres (grade 3 fibrosis; Fig. 20). The fibrosis remained at this stage till 270 days, when the lesions began to show signs of regressing, and at 500 days there were only a few nodular lesions present together with mononuclear cells distributed throughout the lungs and some alveolar fibrosis in the form of a reticular network (Fig. 21).

Auramine-stained sections examined with fluorescent light revealed about seven bacilli in an area $2 \times \frac{1}{2}$ cm. of lung tissue at the period when the fibrosis was most marked, and only about three bacilli in an area 3×1 cm. at the end of the experiment.

Guinea-pigs

The histological lesions produced by dust, organisms, and the combined action of the two in the lungs of guinea-pigs were similar to those produced in the lungs of rats by the same agents. The different degrees of susceptibility of the two animal species to the infecting organism did not seem to affect the lesions.

B.C.G.—At 30 days there were numerous cellular lesions about 100 to 200 μ in diameter. A few fine, loosely arranged reticulin fibres were seen in some of the lesions (grade 1 fibrosis, minimal; Fig. 22). Within the next six months the lesions enlarged to 200 to 300 μ , and at 180 days there was no visible increase in the reticulin fibres within the

Rat lungs after the injection of 75 mg. of "mine-dust" and 1.5 mg. of dead B.C.G.

FIG. 7.—30 days. Nodular lesion composed of dust cells with epithelioid cells, due to the combined action of dust and organisms. Haematoxylin and eosin (55).

FIG. 8.—30 days. Same area as Fig. 7. Combined dust and infective lesion with loose network of fine reticulin fibrosis. Grade 1 fibrosis. Silver impregnation (60).

FIG. 9.—180 days. Combined dust and infective lesion with thick reticulin fibres. Grade 2 fibrosis, minimal. Silver impregnation (80).

FIG. 10.—365 days. Combined dust and infective lesion with thick compact reticulin fibres. Grade 2 fibrosis, maximal. Silver impregnation (80).

FIG. 11.—477 days. Collections of dust with mild thickening of the surrounding alveolar walls, but no fibrosis. Silver impregnation (80).

Rat lungs after the injection of 75 mg. of anthracite coal and 1.5 mg. of dead B.C.G.

FIG. 12.—30 days. Nodular lesions composed mainly of dust cells and epitheloid cells due to the combined action of dust and organisms. Haematoxylin and eosin (60).

FIG. 14.—240 days. Nodular lesions, due to dust and organisms, showing thick and compact reticulin. Grade 2 fibrosis, maximal. Silver (80).

FIG. 13.—30 days. Same area as Fig. 12. Combined dust and infective lesions with a few fine reticulin fibres. Grade 1 fibrosis, minimal. Silver impregnation (80).

FIG. 15.—500 days. Combined dust and infective lesion, much less severe than at 240 days, showing a few fine reticulin fibres. Grade 1 fibrosis. Silver (80).

lesions (Fig. 23). From this time onwards they diminished in size (100 to 200 μ) and at 365 days were seen as small cellular areas with a few reticulin fibres (Fig. 24). They persisted even up to 500 days although they were few and small.

“**Mine-dust**”.—Numerous dust particles were seen distributed diffusely throughout the lung fields within the alveoli and in the interstitial tissue at 30 days (Fig. 25). By 150 days dust cells had

aggregated to form irregular nodules. There was no development of reticulin fibres within such nodules (Fig. 26). At 365 days the dust collections appeared compact but there was no fibrosis at all (Fig. 27).

“**Mine-dust**” + B.C.G.—At 30 days there were small nodular lesions composed of dust cells and epitheloid cells together with a loose network of fine reticulin fibres in them (grade 1 fibrosis ;

Rat lungs after the injection of 75 mg. of kaolin dust. Silver impregnation (60).

FIG. 16.—30 days. Commencing aggregation of dust particles. (Diffuse exudative reaction with numerous dust-containing giant cells.) A few fine reticulin fibres within the aggregations. Grade 1 fibrosis, minimal.

FIG. 17.—180 days. Nodular collections (of dust-containing giant cells). A few reticulin fibres arranged loosely within the nodules. Grade 1 fibrosis, minimal.

FIG. 18.—500 days. Nodular lesions with loose reticulin network. Grade 1 fibrosis, minimal.

Injection of 75 mg. of kaolin dust and 1.5 mg. of dead B.C.G. Silver impregnation.

FIG. 19.—30 days. Combined dust and infective lesions with loose network of reticulin fibres. Grade 1 fibrosis (60).

FIG. 20.—180 days. Diffuse lesions, due to the combined action of dust and organisms, with thick and compact collagen (grade 3 fibrosis). Generalized thickening of alveolar walls in the neighbourhood of the lesions (65).

FIG. 21.—500 days. Few reticulin fibres within nodules and some alveolar fibrosis in the form of a reticular network. Reaction much milder than at 180 days (70).

Guinea-pig lungs after the injection of 3 mg. of dead B.C.G. Silver impregnation (60).

FIG. 22.—30 days. Nodular lesions with a few fine reticulin fibres. Grade 1 fibrosis, minimal.

FIG. 23.—180 days. Nodular lesions with a loose reticulin network. Grade 1 fibrosis.

FIG. 24.—365 days. Cellular lesions, smaller in size than at 180 days, and with a few fine reticulin fibres. Grade 1 fibrosis.

Injection of 150 mg. of "mine-dust". Silver impregnation (60).

FIG. 25.—30 days. Dust diffusely distributed throughout lungs. No evidence of increased reticulin.

FIG. 26.—240 days. Small collections of dust in irregular nodules, without any fibrosis.

FIG. 27.—365 days. Dust collected into compact nodules with scanty reticulin fibres, but no definite fibrosis.

Fig. 28). The lesions progressed, and by 240 days the nodules had enlarged and the reticulin fibres were thick and compact, with a small amount of collagen (grade 2 fibrosis; Fig. 29). From this time till 300 days there was no progress, and after that the lesions seemed to regress; by 365 days there were only a few cellular collections with scanty reticulin fibres (grade 1 fibrosis, subminimal; Fig. 30), and at 500 days the picture was no different.

Anthracite + B.C.G.—Histologically the lesions were similar to those seen in the previous group. At 30 days there were small focal accumulations of dust cells and epithelioid cells in the form of nodules (Fig. 31), which contained a loose network of reticulin fibres (grade 1 fibrosis). At 180 days there were many, some of them with grade 2 fibrosis (minimal). By 240 days the nodules were larger and the reticulin fibres compact, with some collagen (grade 2 fibrosis, maximal; Fig. 32). After this the lesions gradually diminished in size and at 365 days there were only a few with some reticulin fibres in them (Fig. 33).

Kaolin.—At 30 days a large number of foreign-body giant cells containing dust particles were seen distributed throughout the lung fields (Fig. 34). A loose reticulin network was demonstrable in some of these areas, as well as among localized collections of giant cells (grade 1 fibrosis, minimal; Fig. 34). From this period up to 240 days there was very little change in the lesions, with the exception of a slight increase in reticulin fibres (Fig. 35). The lesions were, on the whole, diffuse, and remained so from the beginning of the experiment to its termination at 365 days (grade 1 fibrosis; Fig. 36).

Kaolin + B.C.G.—At 30 days there were large portions of the lung packed with giant cells containing dust particles. In certain areas there were nodular lesions composed of these giant cells, mononuclears, and epithelioid cells. Some fine strands of reticulin were seen in the affected parts of the lung (grade 1 fibrosis; Fig. 37). During the next five months the lesions advanced in severity and the histological appearances were markedly different in every respect from those produced by kaolin alone at the same period of time. Areas of necrosis were seen in some of the lesions and parts of the lung were solid with dust cells. By 180 days there was coarse reticular fibrosis of the alveolar walls in the affected regions and thick compact reticulin, together with some collagen within the actual lesions (grade 2 fibrosis, maximum; Fig. 38) whereas in the lesions produced

by kaolin alone at this same period there was nothing more than a fine network of reticulin. Thereafter, the lesions decreased in severity, and at 365 days only a few fine reticulin fibres remained together with some mild alveolar fibrosis (Fig. 39).

Discussion

It is apparent from the foregoing results that the influence of anthracite, "mine-dust", and kaolin on avirulent tuberculous "infection"* produced by dead B.C.G. in the lungs of rats and guinea-pigs is such that the lesions are aggravated by them. The combined action of any one of the dusts and dead B.C.G. produced more marked lesions in the lungs than any of these substances alone in the same period of time. "Mine-dust" did not produce any permanent pulmonary lesions, and kaolin and B.C.G. alone produced mild reticulosis only, while the combination of anthracite dust or "mine-dust" with B.C.G. produced definite fibrotic lesions (grade 2) and kaolin and B.C.G. together produced collagenous lesions of grade 3 severity. Dust and dead tubercle bacilli influence each other in such a way that one enhances the lesions produced by the other. There were small differences in the size distributions of the dusts used, but these appeared to have little or no effect on the results produced.

A striking feature in all the experiments where dust and organisms were injected simultaneously is the regularity with which the severe reactions present in the lungs at one stage gradually disappeared during the later stages of the experiment. In all cases the lesions became more pronounced during the mid-period of the experiment and the maximum fibrosis was noted between 180 and 240 days, after which the lesions showed signs of regression till, at the termination of the experiment at 365 or 500 days, no fibrosis was evident. It would seem from these findings that fibrous tissue is capable of undergoing partial resolution spontaneously in certain cases. Collagen fibres which were plentiful at the peak period when the fibrosis was at the maximum were no longer found in any of the lesions examined in the later stages of the experiment. The fact that this was repeatedly found in every experiment involving the use of dust and organisms together is of significance and cannot be dismissed as a chance occurrence. It is not possible to offer a wholly satisfactory explanation for this partial resolution. It may be due to the disappearance of one of the stimuli which were in

* "Infection" is usually employed in connexion with invasion by living organisms. It is used here because of lack of a better term. Living bacilli are being used in present experiments to be described in a future paper.

Guinea-pig lungs after the injection of 150 mg. of "mine-dust" and 3 mg. of dead B.C.G. Silver impregnation (60).

FIG. 28.—30 days. Nodular lesions with fine reticulin fibres. Grade 1 fibrosis.

FIG. 29.—240 days. Combined dust and infective lesions with thick and compact reticulin fibres and some collagen. Grade 2 fibrosis.

FIG. 30.—365 days. Nodular lesions, due to dust and infection with only a few reticulin fibres arranged loosely within them. Grade 1 fibrosis, minimal.

Injection of 150 mg. of anthracite coal and 3 mg. of dead B.C.G. (60).

FIG. 31.—30 days. Nodular lesions produced by dust and organisms and composed of dust cells and epitheloid cells. Overlying pleura shows inflammatory thickening. Haematoxylin and eosin.

FIG. 32.—240 days. Lesions due to combined dust and organisms and composed of thick and compact reticulin fibres, together with some collagen. Grade 2 fibrosis, maximal. Silver.

FIG. 33.—365 days. Lesions much smaller and less severe than at 240 days. Only occasional reticulin fibres. Silver.

Guinea-pig lungs after the injection of 125 mg. of kaolin. Silver impregnation (60).

FIG. 34.—30 days. Diffuse exudative reaction with many dust-containing giant cells. Loose network of reticulin fibres among some collections of cells. Grade 1 fibrosis, minimal.

FIG. 35.—240 days. Fine reticulin fibres in giant cell collections. Grade 1 fibrosis.

FIG. 36.—365 days. No increase of reticulin in the lesions beyond that seen at 240 days. Grade 1 fibrosis.

Injection of 125 mg. of kaolin and 2.5 mg. of dead B.C.G. Silver impregnation. (60).

FIG. 37.—30 days. Large areas of lung show combined dust and infective lesions in which are loosely arranged strands of reticulin. Grade 1 fibrosis, maximal.

FIG. 38.—180 days. Lesions show numerous thick strands of reticulin arranged in compact manner. Grade 2 fibrosis, maximal. Coarse reticular fibrosis of alveolar walls in neighbourhood of lesions. Mild degree of emphysema.

FIG. 39.—365 days. Lesions less severe than at 180 days. Loose reticulin network. Grade 1 fibrosis. Mild reticulosis of alveolar walls.

the form of dead bacilli themselves or the toxic substances which they contain. There was, in fact, some evidence that the bacilli were being removed from the lungs during the later stages of the experiment. Examinations of lesions for organisms (under fluorescent light) during the period when the fibrosis was maximum, and again when partial resolution had taken place, revealed a definite difference in the number present. Even at the time when the lesions were severe bacilli were scanty, being only about six to ten in the whole lung field examined (approximately 3 sq. cm.); but towards the end of the experiments, when partial resolution had taken place, the numbers were still further reduced, being about two to four in a lung field of approximately the same areas as before. This evidence that bacilli were disappearing from the lungs agrees with Kettle's (1934) findings. Kettle (1934) produced collagenous lesions very similar to the infective silicotic nodules seen in human beings. The maximum fibrosis was found at 112 days and was still present at 141 days. No descriptions were given of lesions beyond this period. It is possible that if his experiments with dust and organisms had been continued for a longer period results similar to ours might have been obtained, i.e., partial resolution of lesions. With dead B.C.G. alone he found that the organisms "disappeared altogether in the later stages of the experiment", but no definite time is mentioned. In our experiments a very few organisms were demonstrable in rats even as late as 500 days after injection. The experiments of Cummins (1940) also were conducted up to a period of 180 days only, and it is possible that had they been continued longer the lesions might have resolved.

Summary

The influence of anthracite, "mine-dust", and kaolin on avirulent tuberculous "infection" produced by dead bacillus Calmette-Guérin (B.C.G.) in the lungs of rats and guinea-pigs has been studied by the intratracheal injection of these substances alone and in combination. The reactions produced by the combinations of the substances were similar in the two species of animals and did not seem to have been affected by their different degrees of susceptibility to tuberculosis.

Bacillus Calmette-Guérin alone produced mild lesions in rats and guinea-pigs; they appeared at

30 days and persisted till 500 days and 300 days respectively, although at this stage they were scanty and smaller in size. "Mine-dust" did not produce any pulmonary fibrosis in as long a period of time as 500 days. "Mine-dust" and B.C.G. produced definite fibrosis which reached its maximum (grade 2) at 365 days in rats and 240 days in guinea-pigs; thereafter the lesions underwent partial resolution. Anthracite dust and B.C.G. produced lesions essentially similar to the foregoing, which reached the maximum severity (grade 2) between 180 and 240 days in both species and then resolved partially. With kaolin and B.C.G. the reaction was more marked in rats than in guinea-pigs. In both, the maximum fibrosis (grade 3 and grade 2 respectively) was reached at 180 days, the lesions diminishing in severity beyond that period.

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