THE INTRA-TRACHEAL INOCULATION OF ANTHRACITE DUST MIXED WITH DEAD HUMAN TUBERCLE BACILLI INTO RABBITS

BY S. LYLE CUMMINS, C.B., C.M.G., M.D.
Welsh National School of Medicine, Cardiff

(With Plate I)

IN 1931 Cummins, Weatherall & Waters investigated the adsorption of tuberculin by anthracite dust and the subject was further investigated by Cummins & Williams in 1938. After the publication by Kettle (1934) of his observation that the intra-tracheal inoculation of kaolin dust mixed with dead tubercle bacilli gave rise to a much greater reaction than did the inoculation of kaolin alone, the result of the mixture being comparable to an early silicotic lesion, the author, thinking that this effect might have been due to adsorption of the products of the bacilli on to the dust, tried the same experiment with a number of other dusts, met with in mines, such as those of silica, sericite and anthracite. He obtained comparable results with all of them, though of different degrees according to the dust used (Cummins, 1940*b*).

It was clearly important to follow up this new work, especially with anthracite dust and dead human tubercle bacilli, as these constituents produced lesions very similar to the well-known lesions sometimes found in miners who had worked for long periods in anthracite mines. Although good evidence of the absence of similar consolidation following the inoculation of dead human tubercle bacilli alone had been produced, it was thought well to prove this by direct experiment. Though only a small number of animals was used, these experiments confirm and extend the previous observations.

EXPERIMENTS. SERIES I

Anthracite dust composed, as before, of particles 25μ or less in size, was furnished through the kindness of Dr R. Lessing. Three rabbits were used, two being inoculated intra-tracheally with 0·2 g. of anthracite dust mixed with 0·2 c.c. of a thickish emulsion of dead human tubercle bacilli, the emulsion being made up to 1 c.c. by the addition of sterile saline.

A third rabbit was inoculated with 0·2 g. of anthracite dust in 1 c.c. of saline.

The mixtures were given according to Kettle's technique and introduced into the exposed trachea by means of a sterile syringe.

Results

After four months, the rabbits were killed and the lungs examined. Unfortunately, in one of the two rabbits inoculated with dust and dead tubercle bacilli, the mixture was coughed upwards and very little reached the lungs. Although the lungs showed a few small areas which were suggestive, the animal was not included in the records.

In the second rabbit the whole of the mixture had passed into the right lung, and the upper, middle and lower lobes all showed very marked lesions of a characteristic appearance (Pl. I, fig. 1). These lesions consisted of black areas, which, on section, were seen to consist of masses of epithelioid and 'giant' cells surrounding anthracite dust that had fallen into the alveoli. These lesions were strictly confined to the sites of anthracite deposit and were completely absent from the surrounding lung, which was quite healthy. Only some of the smaller anthracite particles had been phagocytosed and a few of these had been carried by the cells to the peri-bronchial lymphatic glands (Pl. I, fig. 3). The dust in the glands was surrounded by epithelioid cells. Very similar lesions are found in human glands, but owing, perhaps, to being of long standing, with more fibrosis.

In the third rabbit the anthracite dust, which had been given alone, had reached the three lobes of the right side and had formed characteristic dust areas (Pl. I, fig. 2), completely different from those found in the animal to which the mixture of dust and tubercle bacilli had been given. The anthracite had been gathered into large masses in and around which there were few phagocytes and no giant cells.

Experiments. Series II

This series of experiments was designed to ascertain whether dead tubercle bacilli alone can cause lesions in any way resembling those above described.

Six rabbits were each given, intra-tracheally by Kettle's technique, 0·3 c.c. of a thickish emulsion of dead human tubercle bacilli, killed by heating to 90° C. for half an hour, the volume of the inoculum being made up to 1 c.c. with saline.

One rabbit died of diarrhœa, due to an ancillary infection, three days after the inoculation. The lungs showed no marked reaction, but numerous tubercle bacilli were demonstrated in them.

Two rabbits were killed after a month, and the results of the post-mortem examinations of the lungs were as follows: In the lungs of one a few scattered tubercle bacilli were seen in monocytes but otherwise the lungs seemed normal. In the lungs of the second after long search some abnormal collections of monocytes were found and in some of these cells acid-fast dots, possibly the remains of tubercle bacilli, were noticed. There were no accumulations com-

parable to the great masses of cells seen after inoculation of anthracite dust mixed with dead bacilli. The other three rabbits, which remained in very good health, were killed after four months. Though the peri-bronchial lymphatic tissues were, perhaps, rather increased, and some collections of cells were suggestive of recent infection, the lungs were free from tuberculous lesions, and no tubercle bacilli were found in them.

These experiments showed that dead human tubercle bacilli in the numbers used failed to produce lesions in, and were rapidly removed from, the lungs of the rabbits.

Discussion

As was suggested by previous work (Cummins, 1940*a*, *b*) these experiments show that the collections of cells around anthracite dust mixed with dead human tubercle bacilli are quite different in nature from those that occur around dead tubercle bacilli alone or anthracite dust alone. In the last two cases the absence of any large lesions in the lungs is very noticeable, whereas in lungs exposed to anthracite dust mixed with dead tubercle bacilli the areas marked out by the collections of epithelioid and giant cells are extremely large and well marked. It might be thought that such lesions are due to an absorption of auto-tuberculin on to the dust (Cummins & Williams, 1938), but my previous experiments showed that 'old' tuberculin and anthracite dust introduced into the lungs of rabbits gave rise to no such collections. It is, of course, well known that the fats, lipoids and phosphatides of the tubercle bacillus are responsible for the endothelial and giant cell accumulations in the lesions caused by living tubercle bacilli (Sabin, Smithburn & Thomas, 1935), but there is as yet no evidence that these constituents are adsorbed on to carbon particles. It appears probable that these fatty and lipoid materials in tubercle bacilli may be held by some substance attached to the tuberculin portion which is, as has been demonstrated, adsorbed by the dust, or that there may be an adsorption, by the dust, of these constituents as well. The results of further experiments with fatty and lipoid elements must be awaited before any assertion is made.

Conclusions

The intra-tracheal inoculation of anthracite dust mixed with dead human tubercle bacilli has been shown, as previously (Cummins, 1940*a*), to result in great accumulations of epithelioid cells and giant cells around the dust collections in the lungs of rabbits.

It has also been shown that neither the intra-tracheal inoculation of anthracite dust alone nor the inoculation of dead human tubercle bacilli alone are able to bring about such cell accumulations.

My thanks are due to Prof. J. B. Duguid for his permission to carry out these experiments in the Pathology Department at Cardiff, to Dr Enid Williams for much valuable help, to Dr Lessing for the dust samples, and to Mr J. P. Napper and Mr G. R. Armstrong whose assistance was freely given during the course of these experiments.

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EXPLANATION OF PLATE I

Fig. 1. The right side of the figure shows a lesion produced by the intra-tracheal inoculation of anthracite dust mixed with dead human tubercle bacilli in the lung of a rabbit. ×700.

Fig. 2. The figure shows an accumulation of anthracite dust in the lung of a rabbit after intra-tracheal inoculation with anthracite dust alone. ×700.

Fig 3. Anthracite dust particles in a peri-bronchial lymphatic gland after intra-tracheal inoculation with anthracite dust mixed with dead human tubercle bacilli. ×700.

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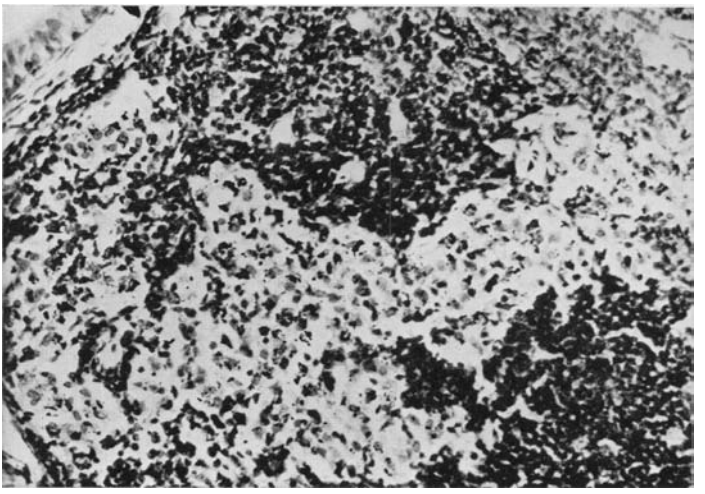

Fig. 1.

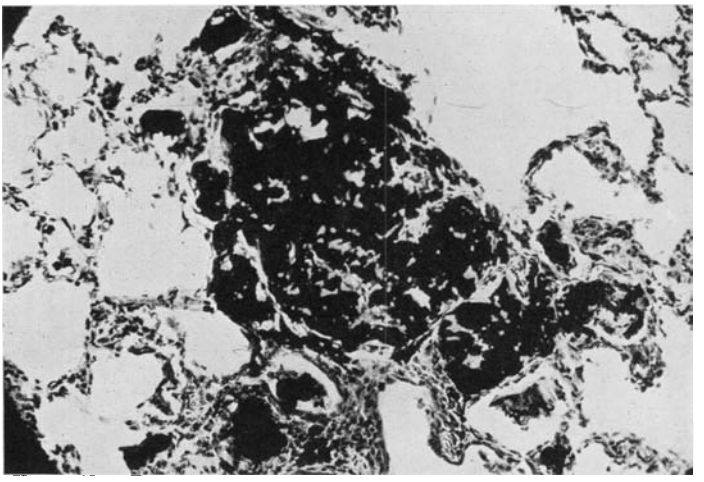

Fig. 2.

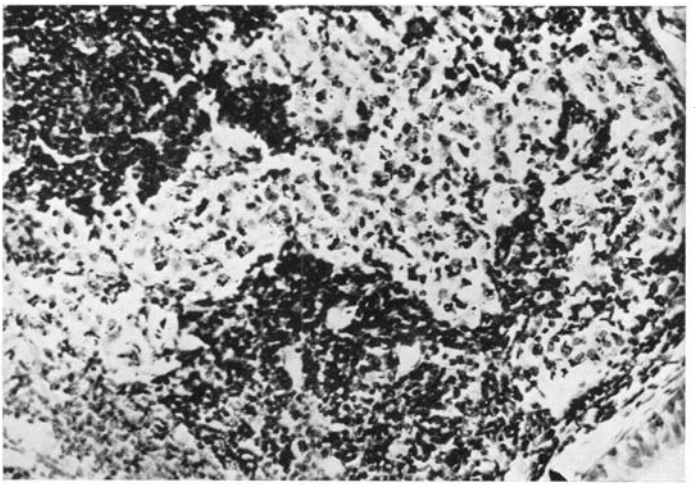

Fig. 3.

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