ADSORPTION OF TUBERCULIN BY COAL DUST.

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While investigating the effects of silica dust and coal dust respectively in the production of respiratory disease in coal miners, we have been struck by the sharp contrast between silicotic coal miners in South Wales as compared with silicotic gold miners in South Africa in their respective liability to pulmonary tuberculosis. Coal miners have always been noted for their relatively low tuberculosis mortality, while gold miners and other workers in hard rock, but exempt from simultaneous exposure to coal dust, are conspicuous for their marked liability to fatal pulmonary tuberculosis in late middle age. And yet the recent findings of Cummins and Sladden (1930) and of the Medical Staff of the Welsh National Memorial Association (1930) indicate that both in the pathological, histological and chemical characters of the lungs of Welsh coal miners and in the X-ray appearances observed in long-service colliers of over forty years of age, there is nothing to distinguish them from Rand gold miners suffering from silicosis except the added presence of large amounts of coal dust in the lung tissue of coal miners and their relatively low tuberculosis mortality. This contrast has been discussed by one of us (S.L.C.) in a recent paper (1931) and the suggestion made that the well-known adsorption power of finely divided carbon particles for colloidal substances might be a factor in reducing the liability of the coal miner to pulmonary tuberculosis.

The quantity of "coaly material" accumulating in the lungs of anthracite coal miners is sometimes very large. In the two most marked cases reported by Cummins and Sladden (*loc. cit.*) the coaly material amounted to 119 grm. and 104 grm. respectively, and the average for twenty-two coal miners was 33 grm., whereas in the lungs of five male adults living under industrial conditions but unconnected with coal-mining or trimming, the coaly material in the lungs varied from 0·18 to 1 grm. These figures will serve to show that in coal miners, as opposed to ordinary town dwellers, there is likely to be sufficient coal dust, in a state of very fine division, to determine adsorption effects.

As to the adsorption of the active principle of tubercle bacilli by carbon, while there have been differences of opinion amongst those studying the question, recent observations have established the activity of charcoal in this respect. Long and Seibert (1925) were unable to demonstrate any adsorption

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of the active principle of tuberculin by animal charcoal. Later, however, Boquet, Nègre and Valtis (1928) showed conclusively that a finely divided charcoal, *norit supra*, is very potent in adsorbing from tuberculin the constituent on which its activity depends.

Dorset, Henley and Moskey (1927), too, proved by careful experiments that various adsorbents carrying a negative charge, including charcoal, could largely or completely abolish the activity of tuberculin.

These experiments had been carried out on guinea-pigs and in none of them was coal dust employed, so we decided to investigate the adsorptive power of coal dust and to test the activity of the tuberculin before and after contact with the dust, by the intradermal method in tuberculous patients.

Exp. 1. A small lump of anthracite coal, as used in heating the Laboratory, was ground to a fine powder in a mortar and suspended in normal saline solution, the suspension being sterilised in the autoclave.

Two dilutions of "Old Tuberculin" (No. 1586 B.W. & Co.) were prepared in saline, one of a strength of 1 in 500, the other 1 in 2500. A sample of each dilution was mixed with an equal volume of the suspension of coal dust and the mixtures were left in contact overnight at room temperature. Each tuberculin dilution was, similarly, mixed with an equal volume of sterile normal saline solution and left overnight at room temperature for use as a non-adsorbed "control."

Next day, the carbon-tuberculin mixtures were shaken, centrifuged, and the clear supernatant fluid pipetted off for use. Thus we had available for use two samples of 1 in 1000 tuberculin and two of 1 in 5000 tuberculin, one of each being "adsorbed" and the others "non-adsorbed."

These samples we tested on adult male tuberculous patients, by the intradermal method, sufficient being introduced into the skin to produce a "wheal" about the size of a threepenny piece. The results, read after 48 hours, are recorded in Table I.

Table I. *Exp.* 1.

	Tuberculin 1 in 1000		Tuberculin 1 in 5000	
Patient's initials	Adsorbed Reaction size in mm.	Non-adsorbed Reaction size in mm.	Adsorbed Reaction size in mm.	Non-adsorbed Reaction size in mm.
W.	11	20	—	—
J.	9	20	—	—
Wa.	12	16	—	—
L.	11	15	—	—
M.	7	20	—	—
D.	—	—	0	18
McA.	—	—	0	17
Jn.	—	—	0	18
J.E.	—	—	0	25
R.	—	—	10	30

It will be seen that the amount of active substance in the 1 in 1000 dilution had in all cases been diminished, while it had been completely abolished in four out of the five patients tested with the 1 in 5000 dilution of tuberculin.

Exp. 2. It was decided to repeat the above experiment, approximating more closely to body fluid conditions by making the contact between the coal dust and the tuberculin in a "buffered" solution with a slightly alkaline reaction. This was accomplished by diluting the tuberculin in a solution of 4 per cent. disodium phosphate, the coal dust being suspended in saline as before. The final solutions, both the adsorbed and the non-adsorbed, were as before in tuberculin concentration, but contained 2 per cent. of disodium phosphate.

The adsorbed and non-adsorbed tuberculin solutions were tested on twenty patients as before. The results are recorded in Table II.

Table II. *Exp.* 2.

	Tuberculin 1 in 1000		Tuberculin 1 in 5000	
Patient's initials	Adsorbed Reaction size in mm.	Non-adsorbed Reaction size in mm.	Adsorbed Reaction size in mm.	Non-adsorbed Reaction size in mm.
Cr.	0	18	—	—
R.T.	0	16	—	—
W.	0	23	—	—
Ab.	0	23	—	—
B.	0	17	—	—
G.	0	12	—	—
K.	0	12	—	—
H.	0	12	—	—
H	0	12	—	—
Martin	0	Slight	—	—
A.L.	0	23	—	—
S.R.	—	—	0	0
J.P.	—	—	0	12
W.T.J.	—	—	0	12
H.	—	—	0	20
M.	—	—	0	13
Bu.	—	—	0	16
Wi.	—	—	0	15
Dk.	—	—	0	0
Ja.	—	—	0	15

In this experiment, thanks doubtless to the slightly alkaline reaction, the adsorption sufficed to annul all activity in the tuberculin dilutions exposed to coal dust.

Taken by themselves, Exps. 1 and 2 suggested strongly that anthracite coal dust might be a potent factor in inhibiting or diminishing the activity of the products of growth and disintegration of tubercle bacilli in the tissues. There remained, however, the question whether the massive collections of coal dust in the lungs of miners, situated as they are close to the apices, under the pleura, in the tracheo-bronchial glands and along the peribronchial and periarterial lymphatics, in the areas characteristically liable to tuberculous lesions, might not still be rendered inactive as adsorbents by previous saturation with the colloids of the body fluids.

This consideration raised the question of the extent to which previous saturation with one substance is able to annul the adsorption power of coal dust for another. We decided to make a preliminary test as follows:

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Exp. 3. A thick suspension of anthracite coal dust in saline was prepared and to 3 c.c. of this was added 3 c.c. of fresh human blood serum. The mixture was left at 37° C. for $3\frac{1}{2}$ hours, being shaken up at intervals. The coal dust was then centrifuged down, washed three times in saline, and suspended in the phosphate solution. Tuberculin dilutions of 1 in 1000 and 1 in 5000 were prepared as before by mixing with equal volumes of the serum-saturated coal-dust residue resuspended in phosphate solution; while "control" tuberculin dilutions were made up with phosphate solution alone.

These preparations were left overnight at room temperature and compared by intradermal testing next day after the bulk of the coal dust had been removed by centrifugation. Here, however, a slight difficulty arose, as it proved impossible, with the electric centrifuge used, to get rid of all the coal dust. Contact with serum had apparently so altered things that the dust particles, instead of tending to spontaneous deposit, made a very homogeneous and stable suspension and resisted even fairly prolonged centrifugation.

These intradermal tests proved inconclusive; partly perhaps owing to the imperfect "clearing" of the solutions and partly because the syringe prepared for inoculating the 1 in 5000 "non-adsorbed" dilutions proved defective so that it was not possible to get the quantities correct owing to leakage.

The results of these tests, carried out on eighteen patients, are recorded in Table III.

Table III. *Exp.* 3.

	Tuberculin 1 in 1000		Tuberculin 1 in 5000	
Patient's initials	Adsorbed Reaction size in mm.	Non-adsorbed Reaction size in mm.	Adsorbed Reaction size in mm.	Non-adsorbed Reaction size in mm.
H.J.L.	10	15	—	—
W.M.	0	12	—	—
G.H.	14	14	—	—
H.R.	15	8	—	—
B.J.	8	10	—	—
T.Y.	20	20	—	—
G.C.	6	9	—	—
L.Y.	16	22	—	—
Dn.	12	20	—	—
K.	0	0	—	—
Hy.	12	12	—	—
T.R.	10	14	—	—
M.D.	—	—	0	0
S.P.	—	—	24	20
E.G.H.	—	—	Slight	Slight
L.M.	—	—	12	16
S.L.	—	—	20	12
R.B.	—	—	8	13

Although the results were, on the whole, suggestive of some further adsorption of tuberculin in a majority of the tests, yet there were three anomalous cases in which the "adsorbed" gave a larger reaction than the "non-adsorbed" tuberculin. It is true that two of these were in the 1 in 5000 group, in which the correct quantities of the non-adsorbed tuberculin were not introduced as explained above; but there remained the possibility of some non-specific irritation effect due to imperfect centrifugation and perhaps the retention of some of the serum used in the preliminary adsorption.

The results of Exp. 3 were regarded as inconclusive and as requiring repetition.

Exp. 4. The details of this experiment were identical with those of Exp. 3, except that the serum and coal dust were left in contact for 15 minutes only, the period found sufficient for adsorption by Dorset, Henley and Moskey (1927), instead of for $3\frac{1}{2}$ hours as in Exp. 3. Further, the final products, after tuberculin adsorption, were spun down in a much more powerful centrifuge and were almost completely cleared of suspended carbon, though the same tendency of the carbon to remain in suspension after treatment with serum was noted as in Exp. 3.

The results, in the fourteen female patients tested, are recorded in Table IV.

Table IV. *Exp.* 4.

Patient's initials	Tuberculin 1 in 1000: Adsorbed Reaction size in mm.	Tuberculin 1 in 1000: Non-adsorbed Reaction size in mm.	Tuberculin 1 in 5000: Adsorbed Reaction size in mm.	Tuberculin 1 in 5000: Non-adsorbed Reaction size in mm.
R.P.	20	35	—	—
P.M.P.	15	25	—	—
L.C.	0	20	—	—
M.W.	15	25	—	—
L.M.F.	15	20	—	—
M.F.	20	35	—	—
B.J.	15	30	—	—
M.E.	17	20	—	—
E.P.	—	—	5	12
M.H.	—	—	0	12
K.H.	—	—	0	0
B.C.	—	—	12	20
M.E.	—	—	0	20
D.L.	—	—	0	25

Here the results were invariably indicative of a stronger reaction to the "non-adsorbed" tuberculin. It was quite clear that previous contact with serum had not completely saturated the carbon in respect of its power of adsorbing tuberculin.

In this experiment, the possibility of non-specific effects was excluded by inoculating the supernatant fluid, obtained after washing the carbon free from serum, intradermally into six tuberculous patients, with negative results. It occurred to us, however, that the sharper results of Exp. 4 as compared with Exp. 3 might be due to a less complete adsorption of serum proteids owing to the shorter period, 15 minutes only, allowed for contact between the serum and the coal dust. This question was only capable of solution by a repetition of the test and this is described under Exp. 5. It was thought worth while, however, to estimate the total nitrogen in a sample of the serum-adsorbed coal dust left over from the experiment and to compare it with the total nitrogen in the suspension of coal dust in saline previous to contact with serum. This test, suggested by Mr E. T. Waters, Ph.D., B.Sc., a Biochemist working in the Central Tuberculosis Laboratory with a grant from the Medical Research Council, was executed by him, and the results show, at least, that a significant though small amount of nitrogenous substance had been adsorbed prior to the exposure of the dust to tuberculin. Mr Waters gives his results as follows:

"In order to establish the fact that the coal dust actually adsorbed some of the constituents of the serum under the experimental conditions, and also to obtain an approximate figure of the amounts so adsorbed, if any, it was decided to determine the nitrogen contents of the original coal and of the resulting coal-adsorbate complexes, in the two series of experiments, viz. Exps. 4 and 5.

The various samples were washed free from saline, spun down on the centrifuge, and dried *in vacuo* over phosphorus pentoxide. The nitrogen estimations were carried out by the Kjeldahl method, on amounts of material ranging from 30 to 100 mgm. Several investigators have reported anomalous results in the total nitrogen estimation of coal by the Kjeldahl method. Erratic low results were also obtained by the writer until the preliminary sulphuric acid digestions, carried out as described below, were continued over a prolonged period. Hence the following details are recorded:

The digestions were conducted in small pyrex flasks, employing 2 to 3 c.c. of concentrated sulphuric acid and a small amount of copper sulphate. The mixtures were heated, gently for a few hours then more strongly until they boiled vigorously. This treatment was continued long after the solutions became clear; failure to do so resulted in low nitrogen figures. Thus the digestions were continued for at least 24 hours, and occasionally for 2 days. Consistent nitrogen percentages were then obtained.

Results.

Series 1.

Coal dust only, N = 1·46 %

Coal-adsorbate complex after serum and "1 in 1000" tuberculin, N = 1·56 %

" " "1 in 5000" " N = 1·55 %

Series 2.

Coal only, N = 1·46 %

Coal-adsorbate complex after serum only, N = 1·54 %

" " and "1 in 1000" tuberculin, N = 1·54 %

" " "1 in 5000" " N = 1·54 %

It is to be noted that the nitrogen adsorbed from these tuberculin solutions would hardly reach a detectable amount.

Thus a nitrogen 'difference' of 0·08 per cent. is obtained. Assuming the material adsorbed to be of protein nature and multiplying by the customary factor 6·25, the amount of adsorbed material is approximately 0·5 per cent. of the weight of coal dust used. Treatment with serum in Series 1 took place over a period of 15 minutes at 37° C., that of Series 2 over a period of 24 hours at room temperature. The above analytical figures indicate no increase in adsorption with extended contact with serum, hence it would appear that in the above experiments the free surface of the coal was saturated with respect to serum proteins."

There remained, however, the possibility that a longer period of exposure of the coal dust to the serum might be found, in biological tests, to lead to a more marked saturation of the dust with protein and to a loss of power to adsorb tuberculin. We accordingly repeated Exp. 4 with the necessary modification to throw light on this point.

Exp. 5. Details were as in Exp. 4, but the serum was left in contact with the coal dust overnight at room temperature, and higher dilutions of tuberculin were used. The results are recorded in Table V.

Table V. *Exp.* 5.

	Tuberculin 1 in 5000		Tuberculin 1 in 10,000	
Patient's initials	Adsorbed Reaction size in mm.	Non-adsorbed Reaction size in mm.	Adsorbed Reaction size in mm.	Non-adsorbed Reaction size in mm.
Ga.	10	16	—	—
W.Tr.	14	14	—	—
J.D.	8	14	—	—
G.W.	0	20	—	—
F.C.	0	0	—	—
H.H.	—	—	0	0
W.T.J.	—	—	12	14
T.	—	—	8	14
I.Y.	—	—	5	12
A.	—	—	10	20
T.E.	—	—	0	9

In this test, out of nine patients found capable of reacting to the tuberculin dilutions used, there was evidence of some degree of "adsorption" of the active principle of tuberculin in eight.

The possibility of non-specific effects was again excluded by testing the "washings" from the serum-coal dust mixture intradermally in six patients with completely negative results.

The weight of coal dust per volume of suspension was, in Exps. 4 and 5, 0·5 grm. in 1 c.c. The amount used in the previous experiments was approximately the same.

Discussion.

While our experiments appear to establish the fact that anthracite coal dust is able to adsorb a considerable amount of the active principle of tuberculin, it is to be noted that, limited by what was justifiable in tests on active cases, we worked with very dilute solutions of tuberculin only. Dorset, Henley and Moskey, who used a charcoal, "norite," in their investigations, were able to remove all activity from so strong a concentration of tuberculin as 1 in 20; but it may be assumed that charcoal is a more powerful adsorbent than coal dust.

The chemical analysis described by Mr Waters shows that only a very small amount of serum proteid was adsorbed on to the coal dust, suggesting that the latter is not a very powerful adsorbent. Had charcoal been used, the amount would probably have been greater.

It will be noted that the most complete adsorption of the active principle of tuberculin was attained in Exp. 2, where the contact of fresh coal dust with tuberculin was brought about in a slightly alkaline medium.

The results after previous contact of the coal dust with serum, although proving that the dust still retained a considerable measure of its power to adsorb the active principle of tuberculin, were less sharp and indicated that the exposure to serum had reduced, though not abolished, the adsorption power.

While it is clear that tests of a different kind, involving the production of silico-anthracosis in laboratory animals, are necessary to settle the question whether the power of finely divided coal dust to adsorb the active principle of tubercle bacillary protein is capable of playing a part in the relative exemption of South Wales coal miners from the high incidence of late middle age tuberculosis common in other occupations involving exposure to the dust of hard stone, we think the results of the experiments here described sufficiently suggestive to justify further investigation in this direction.

CONCLUSIONS.

1. Anthracite coal dust is capable of adsorbing the active principle of tuberculin.

2. This power of adsorption is especially well marked when the contact between the coal dust and the tuberculin is brought about in a slightly alkaline medium.

3. Previous saturation with serum proteid, while it appears to diminish, does not annul the power of coal dust to adsorb the active principle of tuberculin.

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