THE ADSORBENT EFFECTS OF VARIOUS DUSTS ON DILUTED "OLD TUBERCULIN"

By Prof. S. L. CUMMINS and Dr ENID M. WILLIAMS

From the Tuberculosis Department, Welsh National School of Medicine, Cardiff

One of us (S. L. C.), with C. Weatherall and E. T. Waters, published in 1931 the results of some experiments on the adsorption of the active principle of tuberculin by the dust of anthracite coal and showed that the activity of a diluted tuberculin was greatly diminished after contact with pulverized anthracite. In view of this fact, we made the suggestion that some such adsorbent action of autotuberculin by the finely divided coal-dust retained in the lung tissues of silico-anthracotic coal-miners might, perhaps, explain the relative freedom of these latter from the progressive pulmonary tuberculosis so commonly noted as a sequel to silicosis in gold-miners and others exposed to silicious dust free from admixture with coal-dust. These experiments have since been repeated by us with similar results and we, therefore, planned to try out the adsorption effects of other dusts liable to be inhaled in the course of coal-mining in South Wales.

Source of the anthracite and fusain dusts tested

In 1936, one of us (S. L. C.) had the pleasure of discussing this problem with Dr R. Lessing, a chemist engaged in investigating problems connected with coal constitution and the preparation and utilization of coal. Dr Lessing suggested that it might be worth while to take up the question of the adsorption activity of some of the constituents of coal-dust and kindly offered to supply us with material for further experiments on these lines. From him we received, on 15. iv. 36, two dusts, No. 9531 "smaller than 25 microns", a finely divided dust separated by elutriation from a picked lump of anthracite after grinding, and No. 9538 "smaller than 25 microns", separated likewise from ground "fusain" picked from an anthracite seam. The latter must not be regarded as pure fusain but as a portion of an anthracite seam rich in this component. (For definition of fusain see Stopes (1919).) North (1926) says: "The fusain layers in a piece of coal are planes of weakness along which the coal readily breaks and, owing to its friable character, the fusain is quickly reduced to the condition of fine dust, so that naturally formed coal-dust is richer in fusain than the unbroken coal."

Dr Lessing's sample of fusain enabled us to attempt to compare it with his anthracite dust of similar particle size submitted at the same time.

HUMAN TEST GROUP

For some years, we had been engaged in the occasional estimation of the tuberculin sensitivity of patients of the "dementia precox" type at the Cardiff Mental Hospital with the kind consent and assistance of the late Medical Superintendent, Dr P. K. McCowan, and his staff. These serial tests seemed to offer an opportunity for comparison between the reactions induced by standard tuberculin dilutions and similar dilutions after contact with the dusts under test. The necessary arrangements for a fresh group of patients having been made, we started to prepare our tuberculin dilutions, ensuring that every manipulative process to which the "adsorbed" samples were exposed should also be applied to the non-adsorbed tuberculin "control" except, of course, the actual addition of the dusts. The steps taken were as follows.

ADSORPTION TECHNIQUE

A gram of each dust was weighed out into a "shaking bottle"; a tuberculin solution of 1 part of M.R.C. (Douglas) Old Tuberculin, double strength, in 2000 parts of borate-buffer solution (A. T. Glenny *et al.* 1928), was prepared and 10 c.c. added to each dust in its shaking bottle. Another volume of 10 c.c. of tuberculin solution was transferred to a third dust-free shaking bottle to ensure that the non-adsorbed tuberculin should be strictly comparable. All three bottles were then closed with rubber stoppers and "shaken" for 5 min. in an International centrifuge (shaking head) machine. The dust mixtures, which had tended to flocculate when mixed by hand, were thus reduced to homogeneous suspensions. All these shaking bottles were now placed in the 37° C. incubator for 30 min. and afterwards left on the bench at room temperature overnight. It will be noted that the tuberculin, 1 part in 2000 of "double strength" M.R.C. tuberculin, is the equivalent of 1 part in 1000 of standard "old tuberculin" and is so referred to in the reports and tables to follow. The reaction of the tuberculin solution before distribution to the "shaking bottles" was found to be 7·8 *p*H to phenol red; a suitable reaction as shown in the previous paper (1931) already referred to.

It was found that the dust in the suspensions tended overnight to separate into two layers, one aggregated towards the surface of the fluid and the other sinking to the bottom. Between these dust aggregations was a zone of clear fluid and it was, therefore, possible to tap the clear zone by means of a fine pipette passed down through the surface layer. The relatively clear fluids were again centrifuged to get rid of residual dust particles, which were noted as being more numerous after fusain contact than after contact with anthracite dust; and all three tuberculin solutions, the anthracite-adsorbed, the fusain-adsorbed, and the non-adsorbed, were now autoclaved to ensure sterility.

Tuberculin tests

With these three tuberculins of 1 : 1000 strength (O.T. standard) twenty cases of dementia precox and five cases of pulmonary tuberculosis were tested by the Mantoux method, 0·1 c.c. being inoculated intradermally. All except one anergic tuberculosis case gave sharp reactions to the non-adsorbed tuberculins. To the two adsorbed tuberculins, all were completely negative save for one slight and equivocal reaction to the fusain-adsorbed fluid (see Table I A). It was now decided to try the effect of these dusts on contact with stronger tuberculin and so the above experiment was repeated with tuberculin dilutions of 1 : 100, the dusts being weighed, shaken and incubated with the tuberculin as before and the clear fluid pipetted off, centrifuged and autoclaved as above described. Only one difference is to be noted. To avoid severe reactions, the strength of the non-adsorbed tuberculin was kept at 1 : 1000. These preparations were tested on thirteen mental cases. All except one reacted to the non-adsorbed 1 : 1000 tuberculin. None reacted to the adsorbed 1 : 100 tuberculin, whether adsorbed with "whole" anthracite or with fusain (see Table I B).

This complete removal of activity from a tuberculin-concentration as strong as 1 : 100 strikes us as remarkable and indicates a decidedly more active adsorption effect with Dr Lessing's dust than had been recorded for our locally pulverized anthracite in the previous paper above referred to, in which, while a 1 : 5000 dilution was always completely inactivated, a strength of 1 : 1000 was often left with some, though reduced, power of evoking reaction after "adsorption" with the dust. Dr Lessing agrees with us in attributing this improvement to the smaller particle size in his specially pulverized and elutriated dusts (see Table II B).

To test still further the quantity of tuberculin which could be adsorbed by anthracite and fusain dusts, a third experiment was carried out, 1 g. of each dust being added to 10 c.c. of a 1 : 10 tuberculin dilution. Of the twelve mental patients tested, all reacted violently to both the adsorbed tuberculins, the reactions markedly exceeding those to the 1 : 1000 non-adsorbed "control" (see Table I C).

It had been proved in the second experiment that a concentration of 1 : 100 was completely inactivated. In the third, the tuberculin was ten times as strong and; even if several hundredths were inactivated, there would still have remained sufficient active tuberculin to cause an intense reaction. For easy reference, four typical cases from each of the three experiments are tabulated below (see Table I) in order to show the order of size of reaction, in terms of "redness" and "oedematous area", in each series.

Adsorption tests with other dusts

With these findings as to the adsorption power of anthracite and fusain dust at our disposal, we decided to try out the effects of certain other dusts

Table I. *Anthracite and fusain dust*

Tuberculin reactions

	Patient's initials	Non-adsorbed 1 : 1000		Anthracite adsorbed 1 : 1000		Fusain adsorbed 1 : 1000	
		Redness	Oedematous	Redness	Oedematous	Redness	Oedematous
(A)	W. W.	16 × 20	16 × 20	—	—	—	—
	A. G.	18 × 21	11 × 11	—	—	—	—
	J. W.	11 × 9	11 × 9	—	—	—	—
	B. E.	12 × 11	12 × 11	—	—	—	—
		Non-adsorbed 1 : 1000		Anthracite adsorbed 1 : 100		Fusain adsorbed 1 : 100	
		Redness	Oedematous	Redness	Oedematous	Redness	Oedematous
(B)	J. D.	25 × 20	7 × 7	—	—	—	—
	C. W. M.	30 × 30	10 × 10	—	—	—	—
	V. C. M.	25 × 25	12 × 12	—	—	—	—
	J. L.	45 × 40	10 × 10	—	—	—	—
		Non-adsorbed 1 : 1000		Anthracite adsorbed 1 : 10		Fusain adsorbed 1 : 10	
		Redness	Oedematous	Redness	Oedematous	Redness	Oedematous
(C)	J. R. L.	10 × 10	10 × 10	40 × 40	35 × 35	40 × 45	25 × 20
	A. J. A.	21 × 20	18 × 18	50 × 40	30 × 30	60 × 50	35 × 35
	B. A.	19 × 23	18 × 20	60 × 50	28 × 30	55 × 70	25 × 30
	W. P.	17 × 17	15 × 15	45 × 40	30 × 25	60 × 60	25 × 25

likely to be encountered in mine air. We had received from Dr W. R. Jones, for the purpose of some other experiments, a supply of finely pulverized quartz dust and a sample of sericite dust of comparable particle size. Dr Jones had described these dusts as having been crushed to the fineness of 90 mesh per inch (aperture $140\,\mu$).

All the steps described above for the adsorption of tuberculin by the coal-dusts were repeated with the quartz and sericite dusts. These dusts, like the coal fractions, tended to separate into an upper and lower layer on standing after the preliminary shaking. The fluid from the quartz-tuberculin suspension was opalescent, while that from the sericite had a faint green-grey colour. Both cleared well on centrifuging.

The results of the "adsorbed" and "non-adsorbed" tuberculin tests are given in Table II A, in which, for convenience, only six out of the twelve tests are given to avoid repetition.

Table II A. *Quartz and sericite*

Tuberculin reactions

Patient's initials	Non-adsorbed 1 : 1000		Quartz adsorbed 1 : 1000		Sericite adsorbed 1 : 1000	
	Redness	Oedematous	Redness	Oedematous	Redness	Oedematous
C. M.	37 × 37	21 × 16	30 × 33	21 × 25	8 × 8	8 × 8
J. S.	40 × 33	20 × 18	30 × 28	18 × 19	—	—
J. H.	20 × 20	16 × 16	18 × 16	12 × 12	—	—
A. D. G.	40 × 40	20 × 20	15 × 15	12 × 12	—	—
S. N.	16 × 16	13 × 14	5 × 6	5 × 6	—	—
F. G.	—	—	—	—	—	—

From the findings recorded in this table it is clear that the quartz dust had hardly any adsorption power for 1 : 1000 tuberculin, whereas the sericite was extremely active in adsorbing the tuberculin in that dilution. This may, perhaps, be explicable in terms of the actual "surface" of particles exposed in the fluid. From microscopic observation, the impression is gained that 1 g. of quartz shaken up with 10 c.c. of fluid represents far fewer particles than 1 g. of sericite similarly suspended. It may be, too, that the one variety of dust is more easily "wetted" than the other; or it may be a question of differences in solubility of quartz and sericite. The facts are recorded for what they are worth and the explanation must wait for the verdict of the physicists.

To obtain a quantitative idea of the adsorption power of sericite and, at the same time, to try whether a rather coarser anthracite dust than that previously used was or was not as active as the finely divided first sample, a further experiment was made, on lines similar to those previously described, a dilution of 1 : 100 "old tuberculin" being adsorbed with the same sericite dust as before and also with a relatively coarse anthracite dust provided by Dr Lessing and described by him as No. 9531 "ground anthracite <64 microns >25 microns". As no more male patients were available, the tests were carried out on twelve female mental cases, not all of "dementia precox" type. The results are given in Table II B in which six typical results are set forth.

Table II B. *Anthracite* $<64\,\mu>25\,\mu$ *and sericite*

Patient's initials	Tuberculin reactions: Non-adsorbed 1 : 1000		Anthracite adsorbed 1 : 100		Sericite adsorbed 1 : 100	
	Redness	Oedematous	Redness	Oedematous	Redness	Oedematous
V. G.	62 × 44	21 × 15	60 × 34	20 × 12	35 × 21	9 × 8
D. J.	35 × 45	15 × 12	50 × 48	15 × 22	20 × 21	9 × 9
M. B.	25 × 28	11 × 9	25 × 25	11 × 12	12 × 11	12 × 11
E. G.	32 × 38	7 × 10	—	—	34 × 35	20 × 15
A. G.	42 × 45	17 × 19	—	—	56 × 60	30 × 28
A. F.	25 × 20	10 × 12	—	—	32 × 24	10 × 15

From this test we learn that sericite fails to remove the activity of 1 : 100 tuberculin. We learn, too, that the coarser anthracite, while a more active adsorbent than sericite, is less active than the finer anthracite dust first tested, a fact which supports the view that particle size is probably of importance in this connexion.

Sandstone and shale dust, Ammanford Colliery

Dr Lessing has, since, been so kind as to furnish us with still other dusts of South Wales origin for further tests. Two of these dusts, "Sample no. 8870", sandstone, Ammanford Colliery, fraction $<20\,\mu$, and "Sample no. 8871", shale, Ammanford Colliery, fraction $<20\,\mu$, were treated as before, 1 g. of each being mixed, shaken and left in contact with 10 c.c. of 1 : 1000 tuberculin as described previously; and the adsorbed and non-adsorbed solutions were tested on twelve male patients. There is no need to tabulate the results as both dusts completely removed all activity from 1 : 1000 old tuberculin, while the non-adsorbed 1 : 1000 tuberculin gave a "positive" to every test.

CHALCEDONY AND "PENNANT" DUST

There were still two dusts in my possession and only a few patients on whom they might be tried. We decided to amplify our field by testing the two remaining dusts with 1 : 1000 tuberculin rather than to go on to stronger tuberculin concentrations. These dusts were as follows: Dr Lessing's third sample, No. 8851, sandstone, Pennant, South Wales, and a chalcedony dust kindly handed to me in 1931 by Prof. J. Bayley Butler from some silica works in Ireland.

Table III. *Chalcedony dust and "Pennant" dust*

Patient's initials	Tuberculin reactions: Non-adsorbed 1 : 1000		Chalcedony adsorbed 1 : 1000		Pennant adsorbed 1 : 1000	
	Redness	Oedematous	Redness	Oedematous	Redness	Oedematous
C. M.	35 × 30	20 × 18	—	—	—	—
S. O.	—	—	—	—	—	—
S. N.	25 × 30	8 × 10	—	—	—	—
A. G.	11 × 10	11 × 10	—	—	—	—
W. C. J.	34 × 28	16 × 18	14 × 12	14 × 12	—	—
T. S.	—	—	—	—	—	—
J. A.	8 × 7	8 × 7	—	—	—	—
C. C.	38 × 35	12 × 15	—	—	5 × 6	—
V. C. M.	17 × 21	11 × 10	8 × 6	—	—	—
J. B.	22 × 22	22 × 22	—	—	—	—
C. E.	32 × 27	14 × 16	7 × 9	7 × 9	—	—
M. D.	34 × 30	12 × 15	—	—	—	—

It was found that the chalcedony dust failed to adsorb completely the active fraction of 1 : 1000 tuberculin in three out of twelve tests, while Pennant dust failed in one only, two of the twelve patients being negative to the non-adsorbed tuberculin. It is strange, in view of the absence of adsorption power of the pure quartz dust sent me by Dr W. R. Jones, that both the Ammanford and the Pennant sandstone dusts, rich in quartz, should prove relatively active in adsorption power. It may be that the presence of a certain proportion of very finely divided and, therefore, very active mineral component along with the silica provides the explanation.

DISCUSSION

Since our last publication (S. L. C.) on the adsorption of tuberculin by coal-dust, several investigations into the lung diseases of old retired or disabled coal-miners in South Wales have lent support to the suggestion that the tuberculophile action of inhaled silica dust may be favourably modified by the previous or simultaneous inhalation of coal-dust. The study of old and retired coal-miners by one of us (Enid Williams (1933), Univ. of Wales Press

Board, Cardiff) brought to light six sputum-positive persons out of 100 cases examined, in five of whom the existence of tuberculosis had not been suspected prior to the investigation. Dr P. K. Sen (1937), in a further study of 100 South Wales coal-miners suffering from pneumonoconiosis, isolated tubercle bacilli from the sputum of no less than twelve, the majority being men in whom the disease had not been previously recognized. Both Williams and Sen found reason to suspect tuberculosis in about a quarter of the total number examined, though the final proof of a positive sputum was wanting in many of them; and both called attention to the absence or diminished severity of constitutional symptoms in these cases, suggesting that some factor had neutralized or inhibited the toxic action of the tuberculous infection.

Seeing that the outstanding mineralogical difference between the lung content of silicotic gold-miners and those of coal-miners exposed to silicious dust is the marked accumulation of coal-dust in the latter, it seems not unreasonable to associate the low incidence of clinically recognizable tuberculosis in coal-miners with the pulmonary accumulation of coal-dust.

Coal-dust particles and tubercle bacilli are transported in the same vehicles, the phagocytes, and carried along the same channels, the lymphatics; and so the accumulation of dust particles must coincide with the deposition of tubercle bacilli in the lung tissue. The close approximation of the source of tuberculous toxin to the adsorbent dust may, therefore, be assumed to occur.

In the adsorption experiments above described, all reaction to 1 mg. of tuberculin—0·1 c.c. of a 1 : 100 tuberculin—was completely annulled for "sensitive" subjects by adsorption with 1 cg. of anthracite or fusain dust—0·1 c.c. of a suspension of 1 g. of dust in 10 c.c. of fluid. At a rough estimate, 1 mg. of tuberculo-proteid, the active principle of tuberculin, can be obtained from 50 mg. of dried tubercle bacilli, the equivalent of about 100,000 million bacilli.

It may be assumed that the "leaking" out of soluble toxin from tuberculous foci is a slow process and that only small quantities pass out from deposits of tubercle bacilli into the tissues at any given time. The amount of "coaly material" accumulation in silico-anthracotic lungs is relatively enormous, between 30 and 40 g. as an average, rising to over 100 g. in marked cases (Cummins & Sladden, 1930). There is, therefore, *no quantitative* objection to the "adsorption of toxin" theory.

Further, it may now be suggested, in the light of our new experiments, that if, as we believe, the adsorption of the toxic factor of implanted tubercle bacilli plays a part in retarding or preventing the spread of pulmonary tuberculosis in anthraco-silicotic coal-miners, the presence of sericite and shale dust may often supplement the more powerful action of coal-dust in this direction. It is possible, too, that the retardation of the average age of tuberculosis mortality noted even in uncomplicated silicosis (Beattie, 1916) may, in part, depend upon some degree of adsorption of tuberculo-toxin by the quartz dust itself since, even with the relatively coarse quartz dust used in our experiments, the dimensions of the "reactions" to 1 : 1000 tuberculin were diminished as compared with the control; while the much finer "Pennant" and "chalcedony" dusts were still more active, whether by virtue of their quartz content or through the presence of some associated mineral.

These experiments may also have a bearing on the interaction between the different constituents of a mixed dust when inhaled into the lungs. Emmons & Wilcox (1937) have recently revived the theory of J. S. Haldane that certain minerals, notably shale, exercise a protective influence in the lung on those exposed to the silicosis hazard, and they attempt to explain "protective influence" on a basis of electrical charge and flocculation.

Sweany (1936) suggests that the progress of silicosis itself, as opposed to associated tuberculosis, may be "greatly retarded by coal-dust". It is possible that the more adsorbent minerals in a mixed dust may adsorb the toxic silica sol alleged to be produced by surface solution of crystalline silica in the body fluids. Briscoe *et al.* in a still more recent paper (1937) write as follows: "Our experiments also indicate that the solubility of siliceous dusts may be profoundly affected by the presence of extraneous substances. For example, the apparent 'solubility' of finely divided silica (quartz) is greatly reduced (even to one-tenth of the usual figure) by simple admixture with an equal weight of finely divided sugar charcoal, anthracite or bituminous coal." The fact remains, however, that typical silicosis can and often does develop in coal-miners who have worked in hard stone as well as in coal, which suggests that the diminution of solubility of the silica, if real, is insufficient to eliminate the silicosis risk. All these considerations point to the need for further work on the "adsorption" potency of the various dusts produced in industry.

CONCLUSIONS

1. Further experiments confirm our previous work on the adsorption power of finely divided anthracite dust for the active part of tuberculin. Evidence is produced that the adsorption power increases as the size of the dust particles is reduced.

2. Fusain dust of comparable size is as active in this respect as the whole anthracite dust within the limits of our experiments.

3. Sericite dust is a much more active adsorbent of tuberculin than quartz dust of similar dimensions.

4. Shale and sandstone dust from the Ammanford Colliery, and also Pennant sandstone and chalcedony dust to a rather less extent, adsorb tuberculin with considerable activity.

5. The suggestion is made that the relatively low morbidity, toxicity and mortality from tuberculosis noted in coal-miners exposed to silicious dust is due to the adsorption of tuberculous toxin by finely divided coal-dust, inhaled along with the dust from hard stone.

REFERENCES

Beattie, J. M. (1916). *J. State Med.* **24**, 33.

Briscoe, H. V. A., Matthews, J. W., Holt, P. F. & Sanderson, P. M. (1937). *Bull. Inst. Mining Metall.* No. 391.

Cummins & Sladden (1930). *J. Path. Bact.* **33**, 1095.

Cummins, S. L., Weatherall, C. & Waters, E. T. (1931). *J. Hyg., Camb.*, **31**, 464.

Emmons, R. C. & Wilcox, Ray (1937). *Amer. Min.* **22**, 257.

Glenny, A. T., Pope, C. G. & Waddington, H. (1928). *J. Path. Bact.* **31**, 133.

North, F. J. (1926). *Coal and the Coal Fields.* London: Humphrey Milford and Co.

Sen, P. K. (1937). *J. industr. Hyg.* **19**, 225.

Stopes, M. (1919). No. 1. *Proc. roy. Soc.*, B, **90**, 470.

Sweany, H. C. (1936). *J. Amer. med. Ass.* **106**, 1959.

Williams, Enid M. (1933). *Health of Old and Retired Coal-Miners in South Wales.* Univ. of Wales Press Board.

(*MS. received for publication* 14. vi. 1938.—Ed.)