An evaluation of effect of airborne dust from a cotton mill on the guinea-pig ileum with reference to byssinosis¹

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Cinkotai, F. F. and Franklin, D. W. (1975). *British Journal of Industrial Medicine*, 32, 239-243. **An evaluation of the effect of airborne dust from a cotton mill on the guinea-pig ileum with reference to byssinosis.** The effect of airborne dust on the guinea-pig ileum was studied. Tyrode extracts of airborne dust collected freshly in the cardroom of a cotton mill, and extracts of air pollutant samples drawn on the roof of the mill and of the local town hall were all found to induce the guinea-pig ileum to contract when applied in a tissue-bath. However, the force of contraction with air pollutants was rather greater than that with the cardroom dust. Considering the variables involved, the ileum response to the cardroom dust may have been due to ordinary air pollutants which constitute a significant part of the dust. It is concluded that this pharmacological phenomenon is probably not relevant in the context of byssinosis.

Davenport and Paton (1962), and Nicholls (1962) have shown that aqueous extracts of cotton mill dust cause smooth muscles such as guinea-pig ileum and trachea, rat stomach strip and duodenum, and human bronchial muscles to contract. It has been suggested that this activity of the dust is possibly linked with its pathogenic property to bring about byssinosis and may be a useful index of the pathogenicity of the dust. Recently however, Berry *et al.* (1973) found that extracts of airborne dust drawn from a mill processing man-made fibre affect the guinea-pig ileum nearly as much as do those of cotton mill dust, suggesting that this pharmacological activity may not be relevant to the aetiology of byssinosis. The study presented here has been initially undertaken to evaluate the effect of airborne dust on the guinea-pig ileum as a possible test of the capacity of the dust to induce byssinosis.

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Procedure

Sampling of airborne dust

Dust samples were obtained from one of the coarse cotton mills of Courtaulds Limited at Shaw, Lancashire. The samples were drawn at 1.5 m height in the centre of the floor of a shop occupied by 30 high-speed cards, eight tandem cards, and numerous draw-frames and speed-frames. The carding machines were fitted with Shirley Pressure Point System dust control equipment. Air pollution samples were obtained on the roof of the mill as well as on the roof of Shaw Town Hall half a mile away. Sampling was carried out by means of a portable dust sampler (Rotheroe and Mitchell Ltd., model L60) and a seven-stage cascade impactor (Sciotech Corporation, USA, model CS-1-6A). The Rotheroe and Mitchell sampler collected total dust less 'fly' and consisted simply of a pump and filter capable of sampling the atmosphere at the rate of 60 litres per minute. The sampling head was placed into a 22 cm × 27 cm cage made of wire gauze with a 2 mm mesh to exclude fly.

The cascade impactor sampled at the rate of 12.7 litres per minute. The particles sampled were deposited according to size (Stoke's diameter) on six circular plates

smeared lightly with Vaseline and a glass-fibre filter. Plate 1 collected mostly the particles with aerodynamic diameter greater than $16\text{ }\mu\text{m}$; plate 2 collected the 8 to $16\text{ }\mu\text{m}$ particles; plate 3, 4 to $8\text{ }\mu\text{m}$; plate 4, 2 to $4\text{ }\mu\text{m}$; plate 5, 1 to $2\text{ }\mu\text{m}$; plate 6, 0.5 to $1\text{ }\mu\text{m}$; and the filter collected the particles smaller than $0.5\text{ }\mu\text{m}$. The air sample was drawn through a wire gauze of 2 mm mesh to exclude fly. Particles on the various plates were examined under light and scanning electron microscopes.

Extraction of dust samples with Tyrode solution

In preparation for the guinea-pig ileum test the dust samples were extracted with Tyrode solution. The Rotheroe Mitchell samples (total dust less fly) together with the glass-fibre paper carrier were ground for 15 minutes with acid-washed sand and Tyrode solution in a ceramic pestle and mortar. The suspension was centrifuged and the supernatant extract was used for testing. The extract solution was characterized by the ratio of the weight of dust to the volume of Tyrode solution (mg/ml) used. The cascade impactor samples collected on Vaseline layers were in some instances washed before extraction with petroleum ether to remove the Vaseline and in others the sample and Vaseline were extracted together. A control solution was prepared with each test solution.

Measurement of effect of dust extracts on guinea-pig ileum under tension

A piece of guinea-pig ileum about 1–2 cm long was prepared freshly as described before (Department of Pharmacology, University of Edinburgh, 1970). One end of the ileum was tied to a glass hook with cotton thread and was lowered into a tissue bath of 2 or 10 ml in volume containing aerated Tyrode solution. The other end was then tied to a strain gauge and the tension in the ileum was set at 500 mg by raising the gauge on the rack. The output of the strain gauge was recorded on a pen-recorder. The temperature of the bath was maintained at 37°C by circulating water in its jacket from a water tank regulated at that temperature. Measured doses of the test solution were either added to the Tyrode solution in the bath or dripped down the ileum preparation with the bath empty, as described by Gaddum (1953). Resultant changes in the ileum tension were sensed by the strain gauge and recorded on the pen-recorder, the deflections on which were calibrated in milligrams. Between measurements the preparation was twice washed by emptying and immediately refilling the bath from a reservoir of Tyrode solution kept at 37°C .

Before the dust extracts were tested, the contraction of the ileum preparation was calibrated with histamine acid phosphate (HAP) solution added to the Tyrode solution in the bath or dripped down the preparation with the bath empty, as was appropriate. The HAP concentrations applied to the ilea ranged between 2 ng/ml and 200 ng/ml. A curve of HAP concentration (ng/ml) versus ileum response (percentage of maximum) was plotted for each preparation. The sensitivity of the preparation was characterized by the HAP concentration that induced 50% of the maximum tension ($\text{HD}_{50\%}$). On the basis of the calibration curves the dust extract responses (contractions) were adjusted for differing ileum sensitivities by expressing the tension changes (ΔF) in terms of HAP

concentrations which would have induced the changes, and finding the tension changes (ΔF_s) that these concentrations would have caused on a typical ileum preparation (with a sensitivity of 140 ng/ml $\text{HD}_{50\%}$). The tension changes per 10 mg/ml dust concentration were approximately calculated by adding the term 1350 ($1 - \log_{10} C$) to the response. In doing so, the concentration (C)—response (ΔF) relationship was taken to be $\Delta F = 1350 (1 + \log_{10} C)$, which in fact is a rough approximation of the actual curve. No adjustments were made in cases of ileum relaxation.

Results

Microscopy of the impactor fractions

The results of the examination of dust samples under light and scanning electron microscopes did not basically differ from those of earlier investigators. Cotton fibres were seen on plates 1, 2, and 3, plant fragments and vegetable debris probably on all plates, mineral particles on plates 3 and 4, spheres and possible fragments of fungal colonies on plates 4, 5 (Fig. 1), and 6, and pollution particles on plates 5 and 6. Many particles on plates 4, 5, and 6 appeared to be greater than $10\text{ }\mu\text{m}$ in diameter. Culture studies indicated that many of the spheres were fungal spores. Viable bacteria were not detected but bacterial spores were.

FIG. 1. An electron micrograph of particles collected on plate 5 of the cascade impactor ($\times 1000$).

TABLE 1
RESPONSE OF ILEUM PREPARATIONS TO TYRODE AND HISTAMINE ACID PHOSPHATE (HAP) SOLUTIONS

Solution applied	Number of guinea-pigs	Number of measurements	Average response (mg)	Range (min. value-max. value in mg)
Tyrode	1	9	20	-30 to +60
	14	36	20	-30 to +70
HAP solution 10 ng/ml	1	11	1300	1030-1660
	20	20	1120	360-1780
HAP solution inducing maximum tension	1	7	3040	2750-3250
	15	15	2750	1680-4370

Stability and sensitivity of the ileum preparations used

The ileum preparations were found to respond to Tyrode solution with a small tension change of -30 mg to +60 mg force (Table 1). When 10 ng/ml HAP solution was applied in a 2-ml bath, as was usually the case, the ileum taken from one guinea-pig contracted with a force of 100 mg to 1 660 mg and the ilea from 20 others with 360 mg to 1 780 mg (Table 1). A contraction with 2 750 mg force was on average the maximum with these preparations. The sensitivity of the preparation to histamine solutions depended to some extent on whether the solution was added to the organ bath containing the Tyrode solution or was dripped down the preparation (superfused) with the bath empty. Curves of HAP concentration (ng/ml) versus ileum response (as a percentage of maximum) with histamine solutions added to the Tyrode baths or superfused are shown (Fig. 2).

Effect of dust extracts and control solutions on ileum preparations

The Tyrode extracts of cardroom dust in 1 mg/ml

FIG. 2. The dose-response relationship for histamine acid phosphate (HAP) solutions on guinea-pig ileum.

to 10 mg/ml concentrations caused the ileum preparations to contract on average with 1 256 mg force and the control solutions with 201 mg (Table 2). Extracts of the outside dust from the roof of the mill and the local town hall in 5 mg/ml to 10 mg/ml concentrations brought about on average a contraction of 1 518 mg, and the corresponding controls 106 mg. When the responses were expressed as those of a standard ileum (with $HD_{50\%} = 140$ ng/ml) per 10 mg/ml dust concentration, the above average became 1 370 mg for the cardroom dust and 2 131 mg for the outside dust.

The extracts of old cardroom dust (Table 3) that was kept standing for 160 to 185 days after collection but before extraction and a further 1 to 42 days before testing caused the ileum to relax from its base tension of 500 mg on average by 186 mg ($\Delta F = -186$ mg). The corresponding controls induced on average a contraction of 15 mg force (Table 3). Tests on the impactor fractions were inconclusive.

Discussion

The abundance of regular spheres on the lower impaction plates as revealed by microscopy was striking (Fig. 1). They were probably mostly fungal spores, and judging from the weights of the fractions they may have contributed up to 15% of the total airborne mass sampled. The apparently large particles observed in these fractions had low aerodynamical diameter, probably because of light sponge-like structure. These may have been plant debris or fragments of fungal colonies.

As the size of the dust samples was restricted by the capacity of our sampling instruments, the number of ileum tests carried out had to be kept to a minimum. Because of this and because of the rather high variability of ileum response the dust extracts were tested repeatedly, usually at a single concentration value rather than over a range of concentrations.

TABLE 2
RESPONSE OF ILEUM PREPARATIONS TO EXTRACTS OF CARDROOM DUST AND POLLUTION PARTICLES

Source of dust sample	Inside mill								Outside mill						
	Card room							Mean	Mill roof				Shaw Town Hall		Mean
Date of sample collection	6-11 Nov 1972							—	2 Apr 73	1 Mar 73				30 Aug 73	—
Period between collection and extraction (days)	2	2	2	2	2	2	19	18	—	1	1	1	1	1	—
Period between extraction and testing (days)	0	0	0	0	3	13	42	1	—	0	0	0	0	0	—
Extraction concentration (C in mg/ml)	10	5	2.5	1.0	10	10	10	10	—	5	10	10	10	10	—
Ileum sensitivity in HD ₅₀ % (ng/ml)	130	130	130	130	120	220	90	220	—	140	240	130	140	320	320
Ileum response to control solution (ΔF in mg)	200	200	200	200	200	450	20	140	201	0	430	100	—	0	0
Ileum response to dust extract															
Tension change (ΔF in mg)	1860	1270	850	540	1590	1870	1110	960	1256	240	2020	1430	2250	1890	1280
HAP conc. equivalent to ΔF (HDe in ng/ml)	12.7	9.6	9.0	1.3	9.0	21	10	9.0	—	1.0	86	50	37	64	35
Tension change (ΔF s)															
HDe on ileum of 140 ng/ml HD ₅₀ % sensitivity	1260	1060	1000	250	1000	1750	1070	1000	—	220	2680	2450	2250	2570	2210
ΔF s + 1350 (1 - log C)	1260	1470	1810	1600	1000	1750	1070	1000	1370	625	2680	2450	2250	2570	2210

TABLE 3
RESPONSE OF ILEUM PREPARATIONS TO EXTRACTS OF 'OLD' CARDROOM DUST

Source of dust sample	Cardroom										
Date of sample collection	31 Jan-2 Feb 1972							2-4 Feb 72	7-9 Mar 72	26-31 Oct 73	Mean
Period between collection and extraction (days)	185	185	185	185	185	185	185	183	160	170	170
Period between extraction and testing (days)	22	26	29	34	34	42	34	11	1	2	—
Extract concentration (C in mg/ml)	0.5	0.5	0.5	0.5	1.0	1.0	0.5	2.5	10	10	—
Ileum sensitivity (HD ₅₀ % in ng/ml)	110	130	150	120	120	130	120	110	110	150	—
Ileum response to control solution (mg)	0	0	0	40	-40	-40	40	0	60	90	15
Ileum response to dust extract (mg)	-110	-110	-70	-220	-430	-150	-250	-160	-190	-170	-186

Ileum responses indicated that extracts of the aerosol polluting the Lancashire air caused the ileum to contract more than did the extracts of airborne dust collected in the cardroom of this particular mill. Considering the variabilities involved, the ileum response to the cardroom dust could have been brought about purely by ordinary air pollutants which constitute up to 20% of the airborne dust in the mills. Thus the ileum contraction brought about by extracts of cardroom dust may have had nothing to do with particles that

derive from the cotton spinning process, and based on this a test to assess the capacity of the dust to cause byssinosis may not be possible. This conclusion is entirely consistent with the earlier findings of Berry *et al.* (1973).

It is interesting that samples of airborne dust which were left standing for several months before extraction and testing caused the ileum to relax rather than to contract. It points to the pit-falls of using in this or any other tests samples which are not freshly drawn from airborne dust.

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