

Exposure to cotton dust in an experimental cardroom

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ABSTRACT Changes in respiratory function (FEV_1) and polymorphonuclear neutrophils (PMN) on nasal epithelium were studied in 68 students and 39 cotton mill workers in an experimental cardroom. The exposure was characterised by the vertical elutriator dust and endotoxin levels. A dose related decrease was found for FEV_1 which was more pronounced in smoking workers. The thresholds for no FEV_1 reaction were 0.58 mg/m^3 dust and $0.17 \text{ } \mu\text{g/m}^3$ endotoxin for students and 0.43 mg/m^3 and $0.08 \text{ } \mu\text{g/m}^3$ for smoking workers. The PMN increased in most of the experiments but no dose response relationship could be shown. In experiments with smoking workers or when washed cotton was carded a better correlation was obtained between FEV_1 decrease and endotoxin levels than for dust levels.

Byssinosis in the cotton and flax industry is generally related to the amount of airborne dust although the causative agent is not the cotton fibre itself but a contaminant thereon.¹ Dose response relationships have been presented by several researchers and used as the basis for standards.²

A closer relationship has been shown between chest tightness and airborne Gram-negative bacteria^{3,4} and between the decrease in forced expiratory volume in one second (FEV_1) and the number of Gram-negative bacteria in the processed cotton.⁵ The effect has been related to bacterial lipopolysaccharides (endotoxin) present in the outer cell wall of Gram-negative bacteria.⁶ In some studies a larger reaction has been found among smokers compared with non-smokers at the same exposure levels.²

To explore further the reactions among previously unexposed subjects and cotton mill workers after exposure to cotton dust studies were undertaken in an experimental cardroom where the dust level could be varied. Workers from cotton mills and students not previously exposed to cotton dust were used as test subjects. FEV_1 was measured before and after a four hour working session which for the cotton mill workers took place on a Monday. The number of polymorphonuclear leukocytes (PMN) in the nasal secretion was determined. Information on subjective symptoms such as chest tightness and fever was obtained from questionnaires.

Material and methods

The study was performed in an experimental cardroom measuring $6.4 \times 5.3 \times 2.8 \text{ m}$. A carding machine without a protective cover was situated in the middle of the room and cotton laps were processed. The dust levels in the room could be varied by adjusting the ventilation.

In the experiments United States middle grade bale cotton was used. For some of the experiments washed cotton was prepared. The washing procedure consisted of flushing water over a web of cotton on a rayon manufacturing line whereafter the cotton was dried. The maximum temperature during the process was 66°C .

The exposure in the cardroom was determined using three vertical elutriators⁷ placed on different sides of the carding machine. The filters collecting respirable dust were weighed on a semimicro balance (Sartorius 2474) using an alpha-radiating source to prevent static electricity. The mean value from two or three filters was calculated and expressed as mg/m^3 .

After weighing, the elutriator filters were shaken in 10 ml of pyrogene free water, which was serially diluted. The amount of airborne endotoxin was determined using the Limulus amoebocyte lysate (LAL) technique.⁸ Then 0.1 ml of Limulus lysate (Cape Cod Associates) was added to 0.1 ml portions of dilutions of dust filter water extracts. The last dilution giving a stable clot was read as the *Escherichia coli* endotoxin equivalent concentration. Dilutions were also prepared with commercial *E coli* endotoxin (*E coli* 026-B26 Difco) to assess the

accuracy of the LAL reference standard. These values were always found to agree closely with the stated values. The amount of endotoxin in the water extract of the dust filter was related to the amount of air drawn through the filter. The mean value from two or three filters was calculated and reported as μg of endotoxin/ m^3 of air.

Female and male cotton mill workers, with or without a history of byssinosis (grade $\frac{1}{2}$ or 1) according to Schilling's classification, were taken to the experimental cardroom on a Monday morning. All but one of the workers had a FEV_1 above 80% of the predicted value⁹ before work.

Non-smoking university students of both sexes were used as subjects not previously exposed to cotton dust. Those with asthma or allergic symptoms were not allowed to participate. All but one of the students had a baseline FEV_1 above 80% of the predicted value.⁹ Three experiments with 15 students entailed no exposure to cotton dust to obtain baseline values for the FEV_1 change over a time period equal to the exposure time in the cardroom. The workers were divided into non-smokers and smokers. Table 1 shows the age and time of cotton dust exposure in the different groups.

Before carding, the FEV_1 was determined using a Collins survey water spirometer with a computer (Eagle One). The forced expirations were followed on an XY-recorder. The measurements were supervised by experienced technicians and calibrations were made daily. At least two and usually several more satisfactory forced expirations were obtained. The single largest FEV_1 was expressed as a percentage of the predicted value.⁹

The subjects worked in the cardroom for about four hours after which the measurements were repeated. The difference between the percentages before and after carding was calculated for each subject.

The number of PMN on the surface of the nasal epithelium was determined by inserting a medical cotton swab into one nostril, drawing it along the nasal wall, and suspending the secretion in saline. A sample was stained and counted in a Bürker chamber. The relation between the number of PMN and the number of epithelial cells was determined

and the difference between values before and after carding was calculated.

Questionnaires were used to register the presence of cough, chest tightness, and mill fever at the end of the exposure and later in the evening.

For statistical analyses, Pitman's test was used in combination with a pooling procedure testing the relation between two variables while a third factor remained constant.¹⁰ In certain tests the Mann-Whitney U-test was used. All tests were two-tailed and $p < 0.05$ was accepted as the level of significance. To describe the relation between FEV_1 changes and dust or endotoxin exposures, linear regressions were calculated using natural logarithmic transformation of the dose as the independent and the FEV_1 values as the dependent variable.

Results

The study comprises 68 students and 39 cotton mill workers participating in 23 cardroom experiments. The vertical elutriator dust levels in the experiments ranged from 0.46 to 6.87 mg/m^3 and the airborne endotoxin levels ranged from 0.08 to 12.06 $\mu\text{g}/\text{m}^3$. The correlation between the vertical elutriator dust and airborne endotoxin was high ($r_{xy} = 0.77$, $p < 0.001$).

The average FEV_1 change among 15 non-exposed students was -0.3 ($\text{SD} \pm 2.86$) with individual changes from +8% to -3%. The relation between the individual FEV_1 decrease over the shift and exposure was significant for both dust ($r_{xy} = 0.31$, $p < 0.01$) and endotoxin ($r_{xy} = 0.25$, $p < 0.01$).

At a given exposure level, certain individuals showed a pronounced decrease in FEV_1 and others had no reaction at all. To minimise the individual variations, average ΔFEV_1 values were calculated for groups consisting of three or more subjects. Three groups of workers did not fulfil this requirement and the results from 11 workers were omitted. These averages were calculated for students, non-smoking workers, and smoking workers. The results from these calculations are shown in tables 2 and 3 and illustrated in figs 1 and 2. It can be seen that the reactions among non-smoking students and non-smoking workers differed from those among smoking workers.

There was no significant correlation between the exposure data and the group average ΔFEV_1 in the 20 cardroom experiments but if smoking habits were kept as a constant variable the correlations between the average ΔFEV_1 and dust and endotoxin did become significant, $p < 0.01$ and $p < 0.05$ respectively. The correlation between dust and average ΔFEV_1 for the students was $r_{xy} = -0.86$ ($p < 0.001$)

Table 1 Age and time of cotton dust exposure among students and non-smoking and smoking workers (mean and range)

	Students	Workers	
	(n = 68)	Non-smoking (n = 16)	Smoking (n = 23)
Age (y)	26 (18-44)	49 (27-63)	44 (22-64)
Time of cotton dust exposure	0	21 (7-38)	18 (4-42)

Table 2 Exposure data and group average ΔFEV_1 and PMN-index over the shift for non-smoking students. Dust and endotoxin exposure represent mean values from two or three vertical elutriators

Dust (mg/m ³)		Endotoxin (μ g/m ³)		ΔFEV_1 % No	Mean	SD	Δ PMN-index No	Δ PMN-index	
Mean	SD	Mean	SD					Mean	SD
0.63	0.30	0.50	0.21	7	-0.3	2.3	7	1.8	4.2
1.71	0.52	2.74	0.10	6	-5.2	5.1	5	0.3	4.8
1.51	0.34	0.68	0.02	5	-5.4	4.0	5	-0.6	1.7
3.18	0.17	3.90	0.12	6	-9.8	5.8	6	1.1	1.2
4.02	0.18	3.60	0.17	6	-7.3	3.9	6	0.2	3.1
2.42	0.20	0.50	0.01	5	-9.0	5.8	5	0.3	1.7
0.73	0.23	0.61	0.26	3	-2.7	2.9	3	—	—
1.19	0.10	1.82	0.06	3	0.0	1.0	3	—	—
0.77	0.32	0.76	0.28	5	-3.4	2.5	5	0.4	2.1
1.71	0.27	0.60	0.24	4	-2.8	3.3	2	-3.4	2.0
1.79	0.11	0.61	0.26	6	-2.3	3.7	3	-0.8	0.6
6.87	0.19	12.06	3.93	6	-12.8	6.9	2	0.8	1.0
1.30	0.16	1.95	0.10	6	-4.0	1.8	0	—	—

— Indicates results not available.

Table 3 Exposure data and group average ΔFEV_1 and PMN-index over the shift for workers. Dust and endotoxin exposure represent mean values from two or three vertical elutriators

Dust (mg/m ³)		Endotoxin (μ g/m ³)		ΔFEV_1 % No	Mean	SD	Δ PMN-index No	Δ PMN-index	
Mean	SD	Mean	SD					Mean	SD
<i>Non-smokers</i>									
0.66	0.22	0.93	0.31	3	-2.7	0.6	3	0.7	1.0
1.80	0.55	2.6	1.6	4	-2.5	8.2	4	0.2	0.7
2.31	0.71	1.63	1.62	4	-10.0	1.8	4	1.4	1.5
<i>Smokers</i>									
0.87	0.07	0.73	0.01	3	-20.3	26.4	0	—	—
1.35	0.16	0.73	0.01	3	-14.3	3.2	0	—	—
0.61	0.46	0.24	0.22	6	-2.7	3.5	4	1.1	2.0
0.58	0.06	0.08	0.03	5	-3.2	4.3	5	0.2	0.9

— Indicates results not available.

and between endotoxin and ΔFEV_1 , $r_{xy} = -0.65$ ($p < 0.02$).

Regarding smoking workers, the FEV_1 reactions were more pronounced at the same levels of dust and endotoxin than the reaction among non-smoking workers and students. The correlation between the dust levels and the group average FEV_1 decrease was $r_{xy} = -0.72$ ($p > 0.05$) and between endotoxin and the group average FEV_1 decrease $r_{xy} = -0.86$ ($p > 0.05$). The increased reaction was

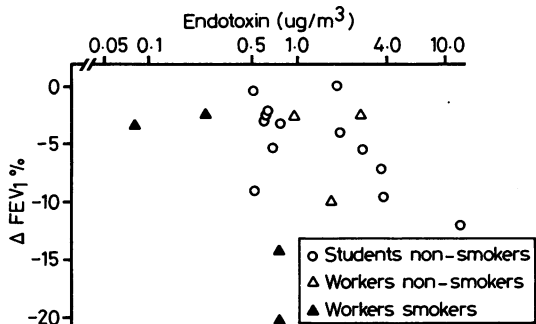


Fig 1 Relation between group average FEV_1 change over shift and endotoxin exposure.

mostly caused by a few individuals in each group who showed pronounced reactions. Even if those participants were excluded, however, the reactions among the remaining smokers were more pronounced than among non-smokers.

Table 4 shows the amounts of airborne dust and

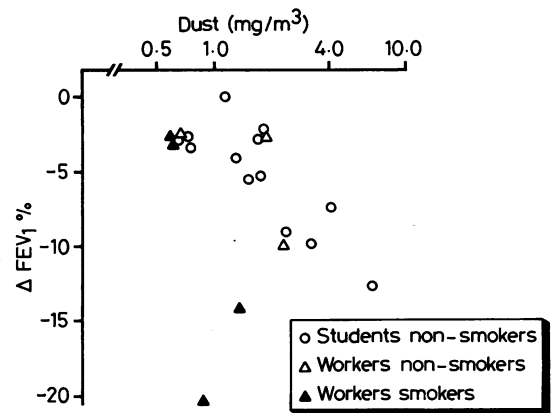


Fig 2 Relation between group average FEV_1 change over shift and dust exposure.

Table 4 Airborne dust (mg/m^3) and endotoxin ($\mu\text{g}/\text{m}^3$) levels at 0%, 2.5%, and 5% decrease in group average ΔFEV_1 over shift

Average FEV_1 change	Non-smoking		Smoking			
	Students (n = 13)		Students + workers (n = 16)		Workers (n = 4)	
	Dust	Endotoxin	Dust	Endotoxin	Dust	Endotoxin
+0	0.58	0.17	0.54	0.16	0.43	0.08
-2.5	0.99	0.48	0.94	0.47	0.50	0.11
-5	1.69	1.35	1.63	1.38	0.58	0.15
Regression equations						
Students:	$y = -4.70 \times -2.53$		$r_{xy} = -0.86$ dust		$p < 0.001$	
	$y = -2.42 \times -4.28$		$r_{xy} = -0.65$ endotoxin		$p < 0.02$	
Students + non-smoking workers	$y = -4.56 \times -2.78$		$r_{xy} = -0.82$ dust		$p < 0.001$	
	$y = -2.32 \times -4.25$		$r_{xy} = -0.58$ endotoxin		$p < 0.02$	
Smoking workers	$y = -15.90 \times -13.62$		$r_{xy} = -0.72$ dust		$p > 0.05$	
	$y = -7.01 \times -18.15$		$r_{xy} = -0.86$ endotoxin		$p > 0.05$	

Table 5 Exposure data and group average ΔFEV_1 and PMN-index over the shift in workers exposed to normal and washed cotton in two cardroom experiments. Dust and endotoxin represent mean values from two or three vertical elutriators

	Dust (mg/m^3)		Endotoxin ($\mu\text{g}/\text{m}^3$)		ΔFEV_1 %		
	Mean	SD	Mean	SD	No	Mean	SD
Non-smokers							
Washed	0.38	0.09	0.03	0.007	3	-2.3	4.2
Normal	0.66	0.22	0.93	0.31	3	-2.7	0.6
Smokers							
Washed	0.71	0.14	0.14	0.00	3	-13.3	5.1
Normal	0.87	0.07	0.73	0.01	3	-20.3	26.4
Washed	2.06	0.08	0.18	0.01	3	-8.3	3.2
Normal	1.35	0.16	0.73	0.01	3	-14.3	3.2

endotoxin where no reduction in FEV_1 occurred and where the average decrease was 2.5% and 5%. The exposure threshold level for a zero reaction was about the same for students and smoking workers with respect to dust but for airborne endotoxin the threshold for smoking workers was lower than for students. The same pattern regarding endotoxin was found for a 2.5% or 5% reduction of FEV_1 .

Table 5 shows the results from the experiments with washed cotton. When the smoking workers carded normal cotton, the dust level was about the same or lower than when they carded washed cotton. The endotoxin levels were about 80% lower when washed cotton was carded and the group average FEV_1 reactions was also lower.

The correlations between the change in FEV_1 and the PMN-index and between the change in PMN-index and exposure data were not significant. Nevertheless, the PMN-index decreased in the experiments where no exposure took place whereas it increased in seven of the ten experiments with exposure to cotton dust.

The correlation between endotoxin exposure and a fever reaction later the same evening was $r_{xy} = 0.60$ ($p > 0.05$) taking into account only eight cardroom experiments where 80% of the students that

took part gave an answer in the questionnaire. In the three cardroom experiments where the endotoxin exposure levels were above $3 \mu\text{g}/\text{m}^3$, 80-100% of the students reported fever in the evening and they all reported symptoms of chest tightness or cough at the end to the cardroom experiment.

Discussion

The exposure in these experiments was expressed using vertical elutriator filters that sample the respirable portion of the dust as opposed to the total dust or total dust less fly.⁷ The dose levels were expressed as the average of two or three vertical elutriator samples in different parts of the cardroom. A better description of the dose could have been obtained by using personal samplers with cyclones or vertical elutriators, but this equipment was not available when the experiments started. As the subjects moved around in the cardroom during the four hour working period, the stationary vertical elutriator values were considered to give a sufficiently precise description of the individual's dose.

A dose response relationship was shown between the average FEV_1 decrement over the working shift and the amount of airborne dust or endotoxin. The

threshold for a 5% decrease in FEV₁ among non-smoking students and workers was about 1.63 mg dust/m³ and for smoking workers 0.58 mg/m³. Boehlecke exposed a group of 30 subjects to different types of cotton with mean dust concentrations ranging from 0.04 to 0.98 mg/m³.¹¹ The subjects were selected from those who reacted with at least a 5% change in FEV₁ when exposed to 1 mg/m³ of respirable cotton dust. The mean decrease in FEV₁ in the 11 experiments reported ranged between 1% and 2% of the baseline value. The threshold exposure giving a mean decrease in FEV₁ of about 5% could be estimated from the fitted regression equations in Boehlecke's study to be 0.76 mg/m³. The discrepancy in threshold levels between this study and the present investigation regarding the value for a 5% FEV₁ reduction among subjects not previously exposed to cotton dust is probably due to the populations studied. Boehlecke's study was made with subjects selected because of their airway reactivity. The present investigation was made with subjects without selection regarding airway reactivity.

Merchant *et al* found a 5% decrease in FEV₁ at an exposure level of 0.62 mg/m³ (measured with a cyclone separator described by Lynch) in 12 cotton workers exposed to strict low middling cotton in an exposure chamber.¹² Among these workers seven were smokers, and the group was selected because they had exhibited decreased expiratory flow rates during cotton dust exposures in earlier studies. The exposure value corresponding to a 5% decrease in that study is similar to the value for workers in the present study.

The observations on smoking workers in the present study comprise only four data points and the conclusion regarding a different dose response relationship is not strong. Nevertheless, a larger decrease in FEV₁ was observed among the groups of smoking workers than among the non-smoking workers or the students at the same exposure levels. The large average decrease in smoking groups was partly caused by certain individuals with a large reaction. Among non-smoking workers these extreme reactions were not observed. Similar differences between individuals have been observed in another study where smoking cotton mill workers are exposed to dust from cotton from different geographic locations (R Rylander *et al*, submitted for publication). This suggests that in certain individuals cigarette smoke may predispose to a more pronounced airway reaction to cotton dust exposure.

The conclusion agrees with results from earlier studies, in which a stronger reaction was found among smokers than among non-smokers. Merchant *et al* fitted dose response equations to data obtained from a study on cotton mill workers in

North Carolina.² Different relationships between dust and FEV₁ changes were found for smokers and non-smokers. In a group of cotton workers subjected to detailed respiratory function testing before and after the shift indications of larger acute and chronic effects were found among smokers.¹³

The presence among the students of chest tightness at high exposure levels shows that this reaction does not require a previous exposure to cotton dust over long periods. It is possible that the reaction threshold may be successively lowered with time among certain individuals. The mechanism behind this is not known.

In this study no dose response relationship was found between the change in PMN-index over the working shift and the amount of vertical elutriator dust or endotoxin. Compared with the findings when no exposure to cotton dust took place, an increase in PMN on the nasal epithelium was found in seven of the ten experiments with students and in all experiments with workers. In this respect the findings agree with previous observations by Merchant *et al*.¹² The dose response relationship that has been found for animal exposure¹⁴ could not be shown. The PMN-index evaluated with the method used here thus lacks the precision required to make it suitable as a response criterion for people exposed to cotton dust.

A strong correlation was found between levels of airborne dust and endotoxin. The experimental set up therefore does not lend itself to evaluate whether dust or endotoxin is the best dose descriptor. The results from the group with smoking workers or the experiment with washed cotton indicates that the FEV₁ response was more strongly related to endotoxin. The washing procedure could, however, have removed other possible byssinosis producing agents from the cotton.

The presence of fever among certain of the students in the evening is similar to the "mill fever" reported among newly employed cotton workers. This is caused by the endotoxin present in the cotton dust.

The results from the experiments with washed cotton agree with previous findings by Merchant *et al*¹⁵ who measured pulmonary reactions in cardroom workers who were carding cotton that had been treated in different ways. In those experiments, however, the dust level in the cardrooms was reduced to one quarter of the original value when washed cotton was used. The results thus cannot be used to determine whether the washing procedure selectively removed a particular agent from the cotton. The same deficiency is present in another report on the effect of washing cotton.¹⁶ To explore further the role of endotoxin for changes in pulmo-

nary respiratory function, experiments should be undertaken in which subjects are exposed to different types of cotton with varying contents of endotoxin. Such studies are under way.

The experiments were performed with funds from Cotton Incorporated (agreement 77/342) and the Swedish Work Environment fund (contract 78/132). The statistical analyses were kindly performed by A Odén, Gothenburg, and Dr E Römer, Institute for Biological Research, Cologne.

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