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Acute Bronchoconstriction Induced by Cotton Dust: Dose-Related Responses to Endotoxin and Other Dust Factors

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Fifty-four healthy humans, selected for their acute airway responsiveness to cotton dust, had spirometric tests immediately before and after 6 hours of exposure to card-generated cotton dust from seven different cottons (of several grades and growing regions). During exposures, we measured airborne concentrations of viable fungi and bacteria (total and gram negative), vertically elutriated gravimetric dust, and vertically elutriated endotoxin. Correlation between each of these five exposure indices and exposure-related acute changes in forced expiratory volume in 1 s showed a statistically significant relationship between all of the indices except concentration of viable fungi. Of the other four indices, endotoxin was the most highly correlated ($r = -0.94$; $p < 0.00001$), and gravimetric dust was the least correlated ($r = -0.34$; $p < 0.05$). These findings suggest that gram-negative endotoxin may play a major role in the acute pulmonary response to inhaled cotton dust.

SEVERAL HYPOTHESES have been proposed about the cause of byssinosis, an occupational respiratory disorder caused by the inhalation of cotton, flax, or hemp dust. Operators of textile mills in the early 19th century claimed that the behavioral excesses of the weekend caused their workers' characteristic complaints on Monday of chest tightness, shortness of breath, and cough (1). However, more recent epidemiologic studies have convincingly related the disorder to dust exposure at the workplace (2, 3). Cotton dust is a complex mixture of organic and inorganic components, and the agent(s) responsible for byssinosis remains unknown. Chemical compounds that are endogenous to the cotton plant (4, 5), as well as various microorganisms and their constituents that are natural contaminants of the cotton plant (6, 7), have been implicated as the active component(s) of cotton dust.

To limit the occurrence of byssinosis, current occupational health practice in the United States includes limiting the concentration of gravimetrically measured lint-free cotton dust in the work areas of textile mills (8). Current permissible exposure limits reflect the nonuniform composition of cotton dust in textile mills: 0.200 mg/m^3 for yarn preparation areas and 0.750 mg/m^3 for weaving areas of mills. Many other reports have shown that cotton dust, either naturally (9-11) or as a result of treatment (12, 13), is not uniformly potent.

To better define the particular characteristics of cotton dust that are related to its acute pulmonary toxicity, we measured acute ventilatory changes in human volunteers exposed on separate occasions to dust from several different commercially available cottons. During the exposures, we sampled the air for concentrations of gravimetric dust, viable bacteria and fungi, and endotoxin. We then analyzed the data for quantitative relationships between acute pulmonary function responses of the volunteers and each of the exposure indices.

Materials and Methods

SELECTION OF HUMAN VOLUNTEERS

All spirometry and environmental sample collection was done at the Cotton Quality Research Station of the U.S. Department of Agriculture in Clemson, South Carolina. Adult volunteers, aged 18 to 65, were solicited from the general public in the area. After receiving an explanation of the study and consenting to participate, each volunteer was given a standardized questionnaire and performed spirometry using standard techniques (*see* below). On the basis of the initial spirometry and questionnaire results, 119 of 606 volunteers were excluded from further participation because they had one or more of the following characteristics: history of asthma, chronic bronchitis,

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or dyspnea when walking with peers; other significant medical conditions precluding safe participation; frequent use of medications or current occupational exposure known to affect airway response; or a forced expiratory volume in 1 second (FEV_1) of less than 80% of the predicted value (14), using a 0.85 correction factor for blacks.

The remaining 487 persons underwent further selection on the basis of their ventilatory response to inhaled cotton dust. All persons had four 6-hour exposures, two with airborne, card-generated, standard cotton (Mississippi-grown strict low middling) dust at approximately 1.00 mg/m^3 and two with clean air (see below for details of dust generation and control). Exposures were scheduled so that each person had one clean air and one dust exposure during each of 2 successive weeks, and at least 2 complete days between any two of the four exposures. Due to space limitations in the exposure facilities, persons were exposed in subgroups of approximately 25 to 30 persons per room per exposure.

Ventilatory function was measured by spirometry (*see* below) just before the volunteers entered the exposure room and after 6 hours in the room. For each person, the change in FEV_1 (Δ FEV_1) occuring over each 6-hour exposure was expressed as a percentage as follows: $([(FEV_1 \text{ after exposure}) - (FEV_1 \text{ before exposure})]/(FEV_1 \text{ before exposure})) \times 100 = \Delta FEV_1(\%)$.

To protect very susceptible persons, 3 persons with reductions in FEV_1 of greater than 30% on their first dust exposure were excluded. Others withdrew voluntarily, leaving 421 persons who completed the four screening exposures. Of 164 who had a greater than 5% decrease in FEV_1 on exposure to cotton dust compared with their response to clean air ([mean change in $FEV_1(\%)$ for clean room exposures] − [mean change in $FEV_1(\%)$ for cotton dust exposures] > 5%), 90 were selected to have additional screening exposures.

Spirometry was done before and after 10 further exposures to standard cotton dust at 0.40 $\pm$ 0.14 (SD) mg/m^3 and 8 exposures to clean air (see below for details of exposure schedule). During this selection period 17 persons voluntarily withdrew, and 12 others who showed the least mean response to cotton dust were excluded, leaving 61 subjects for the study exposures. Spirometric data from 54 persons who were present for all dust exposures were analyzed. Of these persons, 21 were males; 48, whites; 29, current smokers; and 21, had never smoked. Their mean age was 34.5 years (range, 18 to 63), and 28 had worked in a cotton textile mill in the past.

EXPERIMENTAL EXPOSURES

Selection of Experimental Cottons: Five bales each of two different cotton grades (strict low middling and strict low middling spotted) grown in three different areas (Mississippi Delta, Texas High Plains, and California San Joaquin Valley) were purchased from commercial sources. On the basis of laboratory tests of bulk cotton samples for viable microbiologic content, endotoxin contamination, and fiber properties, two typical bales from each growing area-grade combination were selected for use. In addition, two bales of typical California strict middling cotton were obtained, making a total of seven experimental cottons.

Dust Generation and Control: To simulate exposure in a textile mill, each of the selected cottons was independently processed on carding machines under typical commercial production conditions. The cards were located in two experimental card rooms with independent atmospheric controls. Ambient outside air was filtered, conditioned to 24° C and 55% relative humidity, and passed through the card rooms at an airflow rate of 650 ft^3/min (18.4 m^3/min). Airborne dust generated in each card room was partially exhausted to a separate exposure room. The dust concentration in each exposure room was maintained at the desired level by adjusting the proportion of card room exhaust air entering the exposure rooms. The card rooms, exposure rooms, and connecting air ducts were thoroughly cleaned after each exposure.

To assure relative constancy of dust exposure, monitoring was done by a continuous aerosol monitoring system (ppm, Inc., Knoxville, Tennessee), and gravimetric vertical elutriator dust was measured approximately hourly with eight elutriators, one in each quadrant of each exposure room. The time-weighted average dust concentration in each room was calculated for each 6-hour exposure from the vertical elutriator measurements for that room.

Exposures: All exposures were 6 hours long, occurred at the same time of day, and were scheduled so that dust exposures occurred on Mondays and Fridays and clean air exposures occurred on Wednesdays. Volunteers and spirometry technicians were not informed about which, if any, cotton was being processed on any particular day. Exposure to dust from each experimental cotton was replicated. Sequencing of exposures was done randomly, except that replicate exposures were not allowed to occur consecutively. Because one of the exposures was prematurely terminated (*see* below), an additional exposure to that cotton was done. Data from the prematurely terminated exposure were not used for the correlation analyses.

Spirometric Measurements: Spirometry was done before and after all exposures. For each person at each session, at least 5 maximal expiratory maneuvers were recorded with a waterless spirometer (Model 840; Ohio Medical Products, Madison, Wisconsin) directly interfaced with an oscilloscope and digital computer (LSI-11; Digital Equipment Corporation, Maynard, Massachusetts). Flow-volume curves, as well as FEV_1 and forced vital capacity (FVC) values corrected for BTPS (body temperature, pressure, saturated with water vapor) were displayed and recorded as each forced expiratory maneuver was done. All spirometric tests met the quality control recommendations of the American Thoracic Society (15). Each of the two subgroups of persons was assigned to a spirometer operated by one technician throughout the course of the study. Electrical and volume calibration checks were made at the beginning and end of each session, both before and after exposures.

Ventilatory indices were determined for each person at each spirometry session as follows: FVC and FEV_1 were determined by the highest values regardless of the curve on which each occurred. Maximal flow at 75% of expired vital capacity. (FEF_{75}) was determined by the maximum envelope method after aligning flow-volume curves at total lung capacity.

MEASUREMENTS OF EXPOSURE INDICES

Endotoxin Analyses: Vertical elutriator filters were analyzed for endotoxin contamination after approximately 4 hours of sampling during each exposure. Each filter (5-μm pore size, 37-mm-diameter, polyvinyl chloride) (VM-1; Gelman Sciences, Inc., Ann Arbor, Michigan) had been labeled by number, and the laboratory technician was not told which experimental dust, if any, was on them.

Sterile, nonpyrogenic plasticware was used throughout the laboratory analysis, as described previously (16). Dust in each filter was extracted separately in 10 mL of sterile nonpyrogenic water (Travenol Laboratories, Inc., Deerfield, Illinois) by rocking at room temperature for 60 minutes. The fluid was centrifuged at 1000 *g* for 10 minutes, and the supernatant fluids were separated. Five-millilitre aliquots from the extracts of the four filters from each collection period from each exposure room were combined for the assay. The content of gram-negative bacterial endotoxin was measured in duplicate by a spectrophotometric modification of the limulus amebocyte lysate gel test (Pyrostat; Millipore Corporation, Bedford, Massachusetts). Sample results were analyzed by linear regression, compared to a standard curve, and reported as nanograms of U.S. Reference Endotoxin.

This technique, which has become our standard method for comparing endotoxin content of various airborne dusts, may underestimate the total endotoxin content due to the likelihood of some endotoxin remaining with the filter or undissolved dust particles, or both. However, the technique has generally yielded reproducible results in replicate trials with card-generated dust from different cottons (17).

A mean value of endotoxin per milligram of vertically elutriated dust was determined for each experimental cotton on the basis of endotoxin analysis and gravimetric weights of all avail-

able filters. To determine an exposure value in terms of endotoxin per cubic metre, the nanogram-per-milligram value for the appropriate experimental cotton was multiplied by the time-weighted average concentration of vertical elutriated dust (in mg/m^3) for that particular exposure.

Viable Organisms: Airborne viable microbe counts were made with Andersen six-stage viable air samplers (Andersen 2000, Inc., Atlanta, Georgia) (18). Samplers were placed upright at positions corresponding to locations of the vertical elutriators, and plates were incubated within 5 hours of exposure.

For total bacteria counts, cotton extract agar with the following composition was used: $(NH_4)_3PO_4$, 0.5 g/L of distilled water; K_2HPO_4, 0.5 g/L; $MgSO_47H_2O$, 0.2 g/L; NaCl, 1.0 g/L; yeast extract (Difco Laboratories, Detroit, Michigan), 0.5 g/L; glucose, 5.0 g/L; and agar, 20.0 g/L. Cotton extract was added, and the volume brought up to one litre with distilled water. The cotton extract was obtained by soaking 10 g of unwashed cotton in 1000 mL of distilled water at room temperature for 0.5 hours, hand expressing the liquid, and filtering it through several layers of cheesecloth. To inhibit fungi, cyclohexamide (Sigma Chemical Co., St. Louis, Missouri) was added to cooled (55° C) media to achieve a final concentration of 50 $\mu g/mL$.

Unlike trypticase soy agar, half-strength nutrient agar, or yeast sucrose agar, cotton extract agar supported small discreet colonies rather than confluent mucoid colonies. In addition, it supported good growth of all the major bacteria that were previously isolated (19) from cotton on trypticase soy agar.

For gram-negative bacteria, gram-negative selection agar with the following composition was used: peptone, 5.0 g/L of distilled water; glucose, 1.0 g/L; K_2HPO_4, 0.03 g/L; sodium lauryl sulfate, 0.5 g/L; bromthymol blue, 0.03 g/L; and agar, 20.0 g/L. Cyclohexamide was added, as described above.

Both cotton extract agar and gram-negative selection agar were found in preliminary tests to support growth of the following bacteria: *Acinetobacter calcoaceticus* vars. *anitratum* and *lwoffii, Agrobacterium* species, *Enterobacter agglomerans, E. cloacae, E. hafniae, E. sakazaki, E. aerogenes, Flavobacterium* species, *Klebsiella pneumoniae, K. ozaenae, Pseudomonas cepacia, P. fluorescens, P. lemoignei, P. putida, P. stutzeri, P. syringae,* and Centers for Disease Control category 5e-2 isolates. Bacteria that do not grow on gram-negative selection agar but do grow on cotton extract agar are *Bacillus* species, *Cytophaga johnsoniae, Micrococcus* species, *Streptomyces* species, and *Xanthomonas campestris* var. *malvacearum.*

For fungi, glucose yeast extract agar with the following constituents was used: glucose, 5.0 g/L of distilled water; yeast extract, 5.0 g/L; peptone, 5.0 g/L; agar, 20.0 g/L; and gentamicin sulfate (Sigma Chemical Co.), 0.1 g/L. All media were autoclaved to achieve sterility.

The mean concentration of airborne microbial particles for each combination of growing area, grade, and microbial group was based on 16 samples. Two air samples were collected simultaneously on cotton extract agar and glucose yeast extract agar at two different times in each exposure room for all experimental dust conditions. For total bacteria and fungi, counts were made directly on these sample plates. Particle counts of gram-negative bacteria were made after replicate plating from cotton extract agar plates to gram-negative selection agar plates after 24 hours of incubation.

The sample times used (from 0.5 to 5.0 minutes for dust and 1 hour for clean room) were based on results from preliminary tests and were adjusted to reflect anticipated levels of airborne microbes. All air-sample plates were incubated at 25° C, and colonies were counted at 24 hours on cotton extract agar and gram-negative selection agar and at 48 hours on glucose yeast extract agar. A mean value of colony-forming units per milligram of vertically elutriated dust was determined for each experimental cotton dust on the basis of Andersen sampler colony counts and gravimetric weights of vertical elutriator filters. To determine exposure values in terms of colony-forming units per cubic metre, the colony-forming unit per milligram value for the appropriate experimental cotton was multiplied by the time-weighted average vertical elutriated dust concentration (mg/m^3) for each exposure.

ANALYSIS

For the initial analysis, each person's ventilatory function changes and the gravimetric dust concentrations for each exposure were considered. Simple linear regression analysis was done for each experimental cotton using the following linear model (which analysis of residuals indicated was appropriate): mean Δ ventilatory function parameter(%) = intercept + $\text{constant}_d \times$ gravimetric dust concentration. All results from clean room exposures were included in the regressions for each different type of cotton dust. The slopes (dust coefficients, constant_d, in the above model) of the regression lines specific for particular types of cotton dust were tested for statistically significant differences from zero (that is, no dust effect) using one-sided *t*-tests.

Additionally, linear regression analyses were similarly done using data from all experimental cottons simultaneously. The dependent variable was the change in FEV_1, and the independent variables were gravimetric elutriated dust, endotoxin, and viable microbial concentrations measured during exposures. Because the 2 subgroups of persons were separately exposed in the two rooms to slightly different dust concentrations, the figures show the mean ($\pm$ SE) change in FEV_1 for each subgroup at each dose to which it was exposed.

Results

Mean ($\pm$ SE) forced expiratory volume in 1 second (FEV_1) responses for each subgroup of persons to each dose of each cotton exposure are shown in Table 1. On the basis of gravimetric elutriated dust, a wide range of dose-response slopes for acute pulmonary function effects was measured using the experimental cottons (Table 2). Dust from California strict middling cotton caused no measureable effect on forced vital capacity (FVC), FEV_1, or maximal flow at 75% of expired vital capacity (FEF_{75}). At the other extreme, dust generated by carding Mississippi strict low middling spotted cotton caused marked acute reductions in all three spirometric indices, with the greatest effect on the flow parameters. An initial exposure to this dust, at a concentration slightly exceeding 0.50 mg/m^3, was prematurely terminated after only 4 hours due to unusually marked symptoms among the volunteers. Nearly all complained of chest tightness, cough, shortness of breath, or all of these. Many developed sore throats and stuffy noses, and a few had muscle or headaches after 4 hours. A few reported transient chills or fever occurring several hours after exposure had ended. Mean change in FEV_1 for the subgroups at the end of this 4-hour exposure was -11.4% ($\pm$ 1.2%) for subgroup I at a concentration of 0.56 mg/m^3 and -11.3% ($\pm$ 1.4%) for subgroup II at 0.52 mg/m^3.

Concentrations of gravimetric elutriated cotton dust and the mean acute FEV_1 responses measured for each of the exposures to all of the various experimental cotton dusts are shown in Figure 1A. These two variables, gravimetric elutriated dust concentration and FEV_1 response, are significantly correlated but show obvious scatter ($r = -0.34$; $p < 0.05$).

Correlation coefficients for individual exposure indices with acute FEV_1 responses are given in Table 3. Viable fungi were not correlated with the FEV_1 response ($r = -0.14$; $p > 0.3$), but total bacteria and, even more so, gram-negative bacteria were more highly correlated with FEV_1 response than was the gravimetric dust index.

Table 1. Dose-Related Responses in Forced Expiratory Volume in One Second of Persons Exposed to Dust from Various Cottons*

Cotton Type, Subgroup	Dust Concentration†	Exposures	Change in FEV_1
	mg/m³	*n*	*%*
California strict middling			
I	0.38	1	−1.5 ± 0.6
I	0.48	1	−0.9 ± 0.5
II	0.30	1	−0.1 ± 0.6
II	0.51	1	+0.7 ± 0.7
California strict low middling			
I	0.48	1	−1.3 ± 0.5
I	0.49	1	−2.8 ± 0.6
II	0.50	1	−2.2 ± 0.6
II	0.53	1	−1.1 ± 0.7
California strict low middling, spotted			
I	0.53	2	−2.0 ± 0.5
II	0.52	1	−1.4 ± 0.7
II	0.53	1	−1.7 ± 0.6
Texas strict low middling			
I	0.52	2	−1.7 ± 0.5
II	0.47	1	−1.5 ± 0.7
II	0.55	1	−1.7 ± 0.8
Texas strict low middling, spotted			
I	0.45	1	−3.0 ± 0.7
I	0.47	1	−3.8 ± 0.9
II	0.43	1	−1.9 ± 0.6
II	0.47	1	−2.7 ± 0.9
Mississippi strict low middling			
I	0.48	1	−1.3 ± 0.6
I	0.50	1	−2.5 ± 0.5
I	0.48	2	−1.7 ± 0.4
Mississippi strict low middling, spotted			
I	0.24	1	−5.9 ± 1.0
I	0.45	1	−9.6 ± 1.3
II	0.26	1	−8.7 ± 1.3
II	0.42	1	−9.1 ± 1.1
None (clean air)			
I	0.03	6	−0.5 ± 0.3
I	0.04	1	−0.3 ± 0.5
II	0.02	1	−0.5 ± 0.5
II	0.03	5	−0.1 ± 0.3
II	0.04	1	+0.1 ± 0.6

* Both subgroups I and II comprised 27 healthy human volunteers. FEV_1 = forced expiratory volume in 1 second (value given is mean ± SE).
† Vertically elutriated airborne dust concentration.

The highest correlation was seen between the endotoxin exposure and acute FEV_1 response ($r = -0.94$; $p < 0.00001$), as shown in Figure 1B. Four points in both figures represent mean FEV_1 decrements of more than 8% and appear to be statistical outliers. Even when these outlying points are removed from the regression analysis, endotoxin remains the exposure index most highly correlated with the FEV_1 response. ($r = -0.84$; $p < 0.0001$).

Discussion

Byssinosis is an important occupational respiratory problem for which a specific etiologic agent remains unidentified. Previous reports have suggested that all cotton dust does not have an equivalent acute respiratory potency (2, 3, 9, 10, 11). We took advantage of this naturally occurring variability in potency to measure associations between individual components of the dust and the degree of acute ventilatory response to the inhalation of cotton dust from different cottons. Descriptions of such associations have implications for monitoring and controlling occupational exposures, as well as for guiding further research to identify the active agent(s) in cotton dust.

This study was experimental, but the cottons used, method of dust generation, and schedule of exposures were designed to simulate actual mill conditions. The bales of cotton used were all commercially available for use by textile mills. Exposures took place at a yarn production facility where dust was generated by actual carding machines. Moreover, these exposures were scheduled to be nearly as long as a typical work shift in the mills, and dust exposures were separated by at least 2 days to simulate the classic occurrence of acute byssinosis in cotton mill workers on return to work after the weekend. The observed acute reversible obstructive ventilatory responses to cotton dust were qualitatively and quantitatively characteristic of those occurring in acute byssinosis. We thus believe that our experimental system is a valid model for the ventilatory changes of acute byssinosis and have used virtually the same system of exposures to cotton dust in previous studies (20, 21; PETSONK EL, OLENCHOCK SA, CASTELLAN RM, et al. Unpublished observations.)

Our results suggest that some substance(s) associated with the presence of gram-negative organisms, perhaps endotoxin, is involved in the pathogenesis of byssinosis. Observational and experimental data reported by others are also consistent with these observations. Pernis and colleagues (22) have observed that rabbits failed to respond to the second of two inhalation exposures to purified endotoxin given on consecutive days; they noted that this phenomenon was reminiscent of the "Monday effect" characteristic of byssinosis. Rylander and colleagues (23) have reported that acute FEV_1 decrements on Monday among card room workers correlated better with an exposure index incorporating the number of gram-negative bacteria contaminating bale cotton than with level of vertical elutriated cotton dust alone. Cinkotai and Whitaker (24) have reported a good correlation between airborne gram-negative bacteria and prevalence of byssinotic symptoms among workers in cotton spinning mills.

Previous work done by us or our associates has yielded variable results on the correlation of endotoxin with the acute human bronchoconstrictor activity of cotton dust (20, 25, 26; PETSONK EL, OLENCHOCK SA, CASTELLAN RM, et al. Unpublished observations.) However, unlike the present study, none of these previous investigations assayed vertically elutriated cotton dust for endotoxin. Either bulk cotton fiber or total airborne dust was analyzed for endotoxin content. Thus, these earlier methods of quantifying exposures measured endotoxin on physical particles, some of which were too large to penetrate the human upper airways and deposit on airway walls. This difference could explain some previously reported poor correlations between endotoxin exposure and acute ventilatory response. Another possible explanation for past variable results, as cited in some of those reports, is that endotoxin is not a uniform substance. Endotoxins from various gram-negative organisms may have very different

Table 2. Slopes of Dose-Response Regressions for Individual Cottons Using Vertically Elutriated Gravimetric Dust as an Exposure Index*

Cotton Type	Change in FVC	Change in FEV_1	Change in FEF_{75}
	% per mg/m³ of dust		
California strict middling	−0.9†	−0.4†	+4.0†
California strict low middling	−2.3‡	−3.4§	−18.0§
California strict low middling spotted	−2.7§	−3.1§	−13.2‡
Texas strict low middling	−2.3§	−2.9§	−18.4§
Texas strict low middling spotted	−3.6§	−6.2§	−27.3§
Mississippi strict low middling	−3.1§	−3.5§	−20.1§
Mississippi strict low middling spotted	−15.9§	−24.4§	−88.6§

* Regressions based on spirometric responses of 54 persons. FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second; FEF_{75} = maximal flow at 75% of expired vital capacity.
† p = not significant.
‡ $p < 0.01$.
§ $p < 0.0001$.

toxicities, both quantitatively and qualitatively (27, 28). The limulus amebocyte lysate gel assay may not reflect all these differences.

In our previous studies, we have found that washing cotton generally results in marked reduction of endotoxin content and bronchoconstrictor potency (25, 26; PETSONK EL, OLENCHOCK SA, CASTELLAN RM, et al. Unpublished observations.) Because washing removes many other substances in addition to endotoxin, this finding alone has limited implications in terms of causal role for endotoxin. However, at the very least, endotoxin measurement may prove to be a useful indicator of the effectiveness of washing (16, 26).

Evidence against endotoxin being the causal agent of acute byssinosis has been presented by Buck and Schachter (29). By measuring human ventilatory response to short-term inhalation of chemically fractionated extracts of cotton dust, they found that quantitative endotoxin concentrations in various fractions did not correlate with the acute bronchoconstrictor activity. Further investigation is obviously needed to explain these differences. Clues to the etiology and pathogenesis likely exist in the qualitative and quantitative differences in ventilatory responses seen when extracts, instead of actual mill dust, are inhaled.

Information on the natural contamination of cotton by microorganisms has been available for some time and was recently reviewed (19). New studies (3) have confirmed earlier work (31) showing that the number of gram-negative organisms on parts of field cotton plants rise sharply soon after the first frost. Frost-killed cotton is rarely harvested in the San Joaquin Valley, and recent surveys of the microbiologic quality of the U.S. cotton crop (32, 33) have indicated that relatively low levels of gram-negative bacteria are consistently present on cotton from the San Joaquin Valley, compared with levels on cotton from other areas of the cotton belt. This difference in the gram-negative bacteria level may relate to the generally less

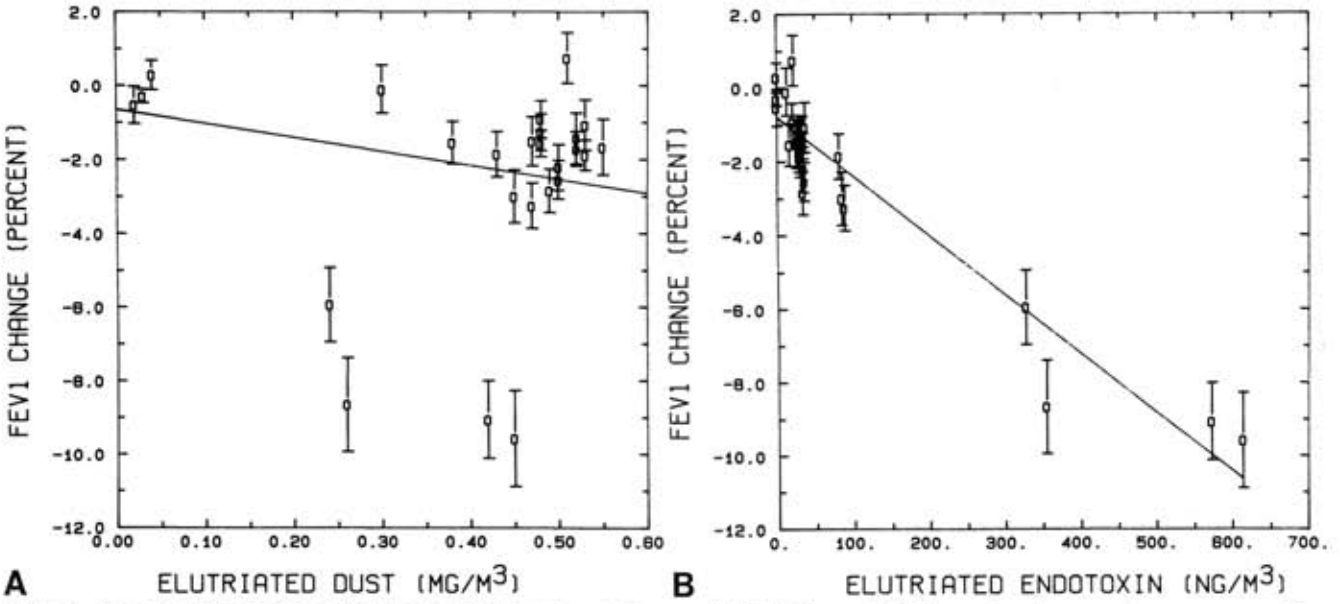

Figure 1. The percentage of change in forced expiratory volume in 1 second (FEV_1) (mean ± SE) for each subgroup of persons exposed for 6 hours to a clean room and to dust from various cottons (54 persons in two equal-sized subgroups; 7 different cottons). **A.** Plot of change in FEV_1(%) versus vertically elutriated airborne gravimetric dust concentration (mg/m³). **B.** Plot of change in FEV_1(%) versus vertically elutriated airborne endotoxin concentration (ng/m³).

Table 3. Correlation of Mean Changes in Forced Expiratory Volume in One Second with Individual Exposure Indices

Exposure Index	r	p
Elutriated dust (in mg/m^3)	−0.34	< 0.05
Total bacteria (in colony-forming units/m^3)	−0.71	< 0.00001
Gram-negative bacteria (in colony-forming units/m^3)	−0.91	< 0.00001
Fungi (in colony-forming units/m^3)	−0.14	Not significant
Elutriated endotoxin (in ng/m^3)	−0.94	< 0.00001

marked human airway response to dust from California-grown cotton noted previously (PETSONK EL, OLENCHOCK SA, CASTELLAN RM, et al. Unpublished observations.) and, at least among strict low middling spotted cottons, to dust from the California-grown cotton in this study.

On the basis of data from a study using humans exposed to card-generated dust from several different cottons, Rylander and Haglind (34) have calculated a threshold value of approximately 100 ng of elutriated endotoxin per cubic metre, below which no FEV_1 reduction occurs. On the contrary, data in our Figure 1B do not show evidence for the existence of an endotoxin exposure threshold. Rylander and Haglind (34) used only 11 persons, who were not selected on the basis of FEV_1 responsiveness to cotton dust, and they exposed these persons for only 4-hour periods. Thus, their FEV_1 response variable was probably less sensitive, and their statistical power was lower than ours.

It is noteworthy that one exposure to the cotton dust most highly contaminated with microoganisms and endotoxin resulted in such marked complaints by the volunteers that the test was prematurely stopped. During this exposure, which had a time-weighted average dust concentration slightly in excess of 0.50 mg/m^3, the volunteers were exposed to the greatest endotoxin concentration of the series of exposures—approximately 700 ng > m^3. A considerable number of the persons' symptoms were consistent with mill fever, a condition that is usually considered a separate entity from acute byssinosis and that has been thought to be caused by inhalation of endotoxin (35). A widespread and severe outbreak of mill fever occurred in rural mattress-makers when they used cotton that was highly contaminated with gram-negative bacteria (11). Mill fever may represent the high-dose end of a spectrum of acute reactions to cotton dust inhalation, including acute byssinosis. However, our study did not directly attempt to discern a relationship between mill fever and other responses to cotton dust, a relationship that is complicated by factors such as schedule of exposure and the development of tolerance to various effects of cotton dust. On the contrary, our study investigated responses to temporally isolated exposures, with at least 2 nonexposure days intervening, and was so designed to limit the otherwise expected variability in acute ventilatory response related to the development of tolerance in cotton-dust responsive persons exposed on consecutive days to cotton dust (36).

The implications of our findings for the control and monitoring of workplace exposures to cotton dust are apparent. On the basis of the best available epidemiologic evidence, in 1978, the Occupational Safety and Health Administration established a health standard incorporating a permissible exposure level for textile mills based on the gravimetric concentration of vertically elutriated airborne cotton dust (8). Our findings clearly indicate that at least in terms of the acute bronchoconstrictor effect, cotton dust as it might be generated in textile mills is not uniformly potent on a basis of gravimetric elutriated dust. Thus, a more appropriate exposure index could be developed on which to base a health standard. Vertically elutriated airborne endotoxin concentration may represent such an index. However, a inexpensive and rapid, but quantitatively accurate and reproducible, assay for endotoxin is needed. More importantly, because we limited our study to a group of healthy persons specifically selected for their FEV_1 responsiveness to cotton dust, additional investigation is needed to better characterize endotoxin dose-response relationships relevant to all workers in the cotton industry.

Use of tradenames is for identification only and does not imply endorsement by the U.S. Government or its agencies.

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