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Airway Responses to the Inhalation of Cotton Dust and Cotton Bract Extracts

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Key Words
Airway hyperresponsiveness · Byssinosis · Cotton bract · Cotton dust

Abstract
Background: Exposure to dust in the cotton industry is associated with respiratory dysfunction. Healthy subjects challenged with cotton bract extract (CBE) develop transient airway hyperresponsiveness. CBE, a major component of cotton dust, is potentially an important agent for studying byssinosis. ***Objectives:*** To compare airway responses to cotton dust extract (CDE) and CBE in healthy subjects. ***Methods:*** In 21 healthy, non-smoking subjects we compared the effects of CBE and CDE in a double-blind random order, following a 10-min aerosol inhalation. The response to methacholine (MCh) 2 h following CBE or CDE was measured. Lung function was recorded using maximal (MEFV) and partial expiratory flow volume (PEFV) curves, measuring MEF at 60% of baseline vital capacity below total lung capacity [$MEF_{40\%}$(P)] on the PEFV curve. Responders were subjects who developed a 20% or greater fall in $MEF_{40\%}$(P) following extract challenge. Endotoxin levels were low for CBE (5.71 EU/mg) and CDE (31.88 EU/mg). ***Results:*** There were 18 responders to CBE and 17 responders to CDE. The average maximal falls in $MEF_{40\%}$(P) were 70 ± 4.9 and 70 ± 4.4% of baseline (nonsignificant) following CBE and CDE, respectively. All subjects enhanced their MCh response following CBE or CDE. The MCh dose which reduced $MEF_{40\%}$(P) by 40% was identical for CBE and CDE (1.3 μg/ml). ***Conclusions:*** We conclude that CBE and CDE exert similar physiologic effects.

Introduction

Byssinosis is a chronic airway disease associated with the inhalation of cotton and other textile dusts [1]. It is recognized as a worldwide occupational lung disease and remains a problem in the United States despite federally mandated regulations [2]. Acute manifestations of the disease are tightness in the chest, bronchospasm, and shortness of breath. These responses occur early in the workweek, following a weekend without exposure, in a pattern described as 'Monday dyspnea' (grade 0.5 or 1 byssinosis in the Schilling classification). Clinically, the onset of this response typically occurs after several years of exposure to dust in the industry. This temporal pattern of symptoms distinguishes byssinosis from other occupational airway diseases. Findings of 'Monday dyspnea' may progress to chronic bronchitis with permanent lung function changes and chronic, debilitating airflow obstruction [3, 4].

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Based on airway challenge observations, McKerrow et al. [5] suggested in 1958 that cotton dust contains a pharmacological agent responsible for airway constriction. From subsequent studies, Bouhuys et al. [6] confirmed that the acute byssinotic response was elicited by aerosol inhalation of an aqueous dust extract, mediated by a water-soluble agent or agents. This group further demonstrated that not only textile workers but also healthy nonworkers were susceptible to the bronchoconstrictor effects of cotton dust, and that these responses were of similar magnitude. Thus, the acute reaction is not dependent on prior exposure or presentation. The experimental application of cotton dust to lung tissue in vitro was found to induce the release of histamine and other mediators [7]. Nicholls et al. [8] systematically studied components of the cotton boll for the capacity to cause histamine release. Among the parts of the plant tested were pericarps (fruit capsules), bracts and fibers. Of these, only the bract extract had consistent histamine-releasing properties. Neither extract of the pericarps nor that of the cotton fiber itself induced airway constriction in studies in humans [9].

Cotton bracts are leaf-like structures surrounding the stem of the cotton boll. They are friable and cling to cotton fibers after ginning [10]. Bract was found by Morey et al. [11] to be the major trash component in raw baled cotton, with a mean value of 43.2% of total trash by weight. Investigators have considered bract a possible source of agents capable of inducing airway obstruction in byssinosis because of its high content in cotton dust and its ability to induce symptoms in challenged subjects. However, a direct comparison of the bronchoconstrictor effect of cotton bract extract (CBE) with a similarly prepared extract of cotton dust has not previously been carried out.

To test the hypothesis that CBE and cotton dust extract (CDE) have similar effects, we undertook a comparison of the physiologic effects of CBE and similarly prepared CDE in 21 healthy subjects.

Methods

Study Design

A double-blind, randomized study was performed involving airway provocation with either CBE or CDE. On study day 1, subjects were evaluated for suitability in the study based on standard history, physical examination and baseline lung function test results. Subjects underwent a methacholine (MCh) inhalation challenge (MIC) on this initial screening day. On study day 2, at least 48 h later, subjects underwent an inhalation challenge with either CBE or CDE. The extract used on study day 2 was selected in a blinded, random order. Pulmonary function studies were performed before and at 15-min intervals for 120 min following challenge. An MIC study was performed 2 h after the extract challenge. Subjects returned at least 7 days later on study day 3 for challenge with the extract not used on study day 2. The post-challenge sequence of lung function tests and the MIC were repeated. Testing was carried out at the same time of day for each subject.

Study Participants

Entrance criteria for participation in this study included the absence of chronic medical illness including asthma or other respiratory disease. Smokers were excluded, as was anyone with any previous exposure to cotton or textile dust. Twenty-one healthy, non-smoking adults participated. These subjects were recruited through local advertisement, and were mostly members and students at the Mount Sinai Medical Center or members of the local community. Baseline lung function was expressed in percent of predicted using the prediction equations of Schoenberg et al. [12]. The mean age of the subjects was 35 ± 2 years. There were 14 male and 7 female subjects; 11 were Caucasian, 6 were African-American, 3 were Hispanic and 1 was Asian. Average lung function (mean ± SE) was normal [forced vital capacity (FVC) = 99 ± 5% of predicted; forced expiratory volume in 1 s (FEV_1) = 105 ± 5% of predicted; $FEV_1/FVC = 0.90 \pm 0.06$, and maximal expiratory flow at 50% ($MEF_{50\%}$) = 88 ± 6% of predicted]. The protocol was approved by the Institutional Review Board of the Mount Sinai Medical Center, and all subjects gave informed consent. Subjects were instructed not to jog or bicycle to the test center, to refrain from beverages containing caffeine and not to use large doses of vitamin C.

Preparation of CBE and CDE

Cotton bracts were collected from West Texas cotton fields. Cotton dust was obtained from Cotton Incorporated (DB1/D8) and the National Institute for Occupational Safety and Health. CBE and CDE were prepared and purified by identical processes as described by Buck and Bouhuys [13, 14] and Buck et al. [15].

In brief, dried bracts were initially pulverized and mixed in a 0.01 *M* phosphate solution buffered to 7.4 ± 2. This suspension was initially dialyzed and then subjected to centrifugation for 10 min at 16,000 *g*. The precipitate was discarded, and further purification was obtained by filtration through a 0.45-μm filter; by precipitation, polymeric material was removed by the addition of methanol, negatively charged components were removed by DEAE-Sephacel chromatography, and lacinilenes were removed with ether extraction. A single large quantity of CBE was prepared in order to assure the delivery of similar concentrations of active agent with each challenge, and freeze-dried and stored for re-constitution at a later date. The pH of the reconstituted bract and dust solutions was between 5.5 and 6.0, and the osmolarity of the preparation averaged 200 mosm.

Cotton dust obtained from Cotton Incorporated (DB1/DS) was provided to us by the National Institute for Occupational Safety and Health.

It is assumed that a 70-kg textile worker at rest inhales 15 m^3 of air over a work shift (5 l/min). This level of ventilation can rise to 90 m^3 of air over a work shift under conditions of mild-to-moderate effort. At the current US dust standard of 200 $\mu g/m^3$, a worker at rest over a work shift might be anticipated to inhale 3 mg of dust at rest and 18 mg of dust under conditions of moderate work. If approximately 500 μl of CBE are inhaled with our dosimeter chal-

lenge, then it follows that approximately 15 mg of CBE are delivered during an average challenge. This is not an unreasonable simulation of the natural challenge that occurs in workers over an average active work shift. For workers exposed to higher concentrations of cotton dust, such as those in developing countries [16], the standard challenge would represent conditions experienced at rest or under minimal effort.

Pulmonary Function Testing

Lung function was measured using partial expiratory flow-volume (PEFV) and MEF volume (MEFV) curves [17]. Subjects inspired to approximately 70% of vital capacity and then forcefully exhaled to residual volume. Next, the subjects inspired to total lung capacity (TLC) and again exhaled forcefully to residual volume. These maneuvers generated the PEFV and MEFV curves [18]. Reproducibility criteria for pulmonary function measurements were those established in the guidelines of the American Thoracic Society on spirometry [19].

A 1-second timer permitted identification of FEV_1. From these data, FVC and PEF were determined. Instantaneous MEF on both PEFV and MEFV curves were measured at the lung volume 60% of the FVC below TLC, as $MEF_{40\%}$(P) and $MEF_{40\%}$, respectively.

Baseline function measured before challenge with cotton or methacholine consisted of three flow-volume maneuvers; data for each parameter were averaged. After challenge, pulmonary function measurements also consisted of groups of three flow-volume maneuvers separated by 1 min; the data for each parameter were averaged, and the average was expressed as a percentage of the baseline value to determine the status as a reactor or nonreactor.

The PEFV curve is of special interest in studies of airway constrictor agents because it measures expiratory flow rates during forceful expiration from a volume less than TLC. A deep inspiration to TLC may induce bronchodilation and thus obscure the effect of airway constriction in response to challenge [17, 18]. TLC served as a reference point for comparing flows before and after experimental inhalations. Studies have shown that TLC remains constant after bronchoconstriction in normal subjects [20–22].

MCh Inhalation Challenge

Subjects were challenged with progressive concentrations (0, 1, 10, 25, 50, 100 and 200 mg) of Provocholine (MCh chloride, Roche Laboratories, Nutley, N.J., USA) prepared in normal saline. An aerosol was generated using a DeVilbiss Model 45 nebulizer powered by compressed air and driven through a dosimeter designed to provide 0.6 s of nebulizer flow for each inspiration. Five consecutive inhalations under conditions of tidal breathing were performed with each dose of MCh. Lung function was measured before the first dose of MCh and following each of the consecutive challenges. The study was terminated if the maximal dose was given or if a 20% decrement in FEV_1 occurred.

CBE/CDE Challenge

Thirty milligrams of freeze-dried CBE were dissolved per milliliter of distilled water in a volume of 5 ml. The reconstituted CBE/CDE was aerosolized by the same nebulizer system [23] as MCh, and was inhaled over approximately 10 min for a total of 120 breaths. Lung function (MEFV and PEFV curves) was measured before challenge and at 15-min intervals following CBE/CDE extract.

Responder status was defined as a 20% or greater fall in $MEF_{40\%}$(P) following CBE challenge.

Endotoxin and Chemotactic Peptide Determination

Endotoxin content of CBE and CDE was measured using the Limulus amebocyte lysate assay [24]. The level of chemotactic peptide (n-formyl-methionyl-leucyl-phenylalanine, FMLP), a bacterial cell wall component, was determined by a modification of an HPLC protocol described previously [25, 26]. Briefly, lyophilized extracts were extracted in methanol (50 mg/0.5 ml) for 4 h at 25°C. The methanol extract was passed through a 0.2-μm filter. This material was placed on a 10 μm, 3.9 mm × 15 cm uBondapak column. FMLP and oxidized FMLP were eluted from the column with 25/75 (vol/vol) acetonitrile/0.1 *M* phosphoric acid (pH = 3) at 1 ml/min and absorbance read at 190 min.

Statistical Analysis

All data were expressed as the mean ± 1 SE. The means at different time points and concentrations were compared by one-way analysis of variance, and individual time points or concentrations were compared by the standard paired t test using the Bonferroni criteria where appropriate. The statistical package Statview (Abacus Concepts, Berkeley, Calif., USA) was used.

We estimated that with 20 subjects there would be an 80% power to detect a difference of 20% in the pulmonary function parameters measured at the 0.05 level of significance [27].

Because of the possibility of an effect due to the order in which CBE or CDE are administered (e.g. lingering bronchoconstrictor effect, learning effect for pulmonary function tests), we employed a crossover design in this study. We used the method of Wallenstein and Fisher [28] to analyze possible interactions. For the MCh studies we limited our analysis to the following doses: control (PBS), and 1 and 10 mg because of the significant dropout of subjects at higher doses (following extract) due to bronchoconstriction.

Results

Baseline Lung Function and Subject Characteristics

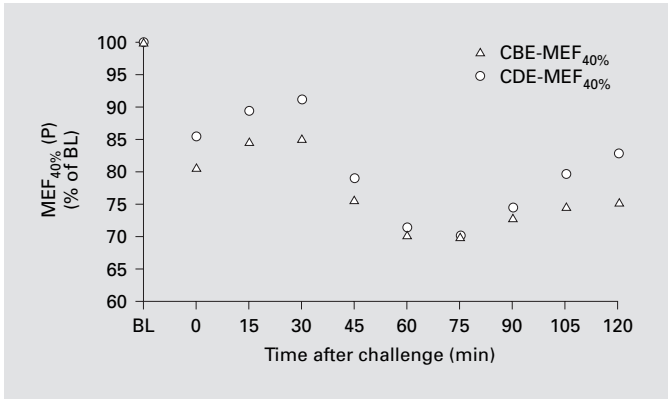
All 21 subjects completed the study. No subject suffered from any chronic medical illness as determined by a clinical questionnaire and a physical examination by a physician. Histories of respiratory illness as well as workplace or domestic exposures to dusts and fumes were absent.

Characterization of the Extracts

Endotoxin levels were 5.71 EU/mg for CBE and 31.88 EU/mg for CDE; oxFMLP levels were 90.2 μg/mg for CBE and 0.2 μg/mg for CDE. While CDE demonstrated a slightly higher endotoxin content than CBE, and CBE contained more FMLP, all levels were low compared to usual biologic specimens. The average amount of CBE solution aerosolized per challenge was 2.5 ± 0.2 g; the average amount of CDE solution was 2.4 ± 0.3 g (NS).

Ventilatory Response to CBE and CDE

There were 18 responders to CBE and 17 responders to CDE. All CBE responders but 1 were CDE responders.



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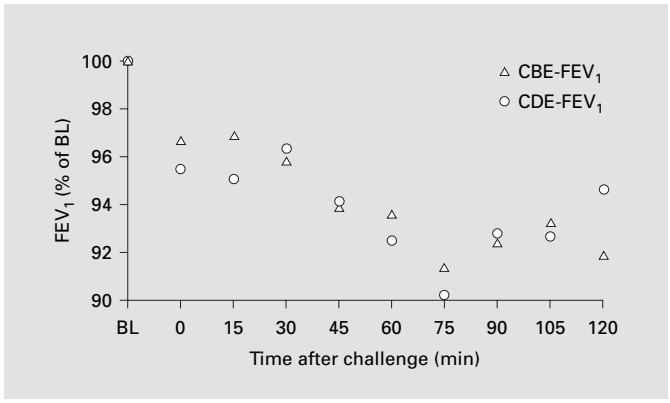

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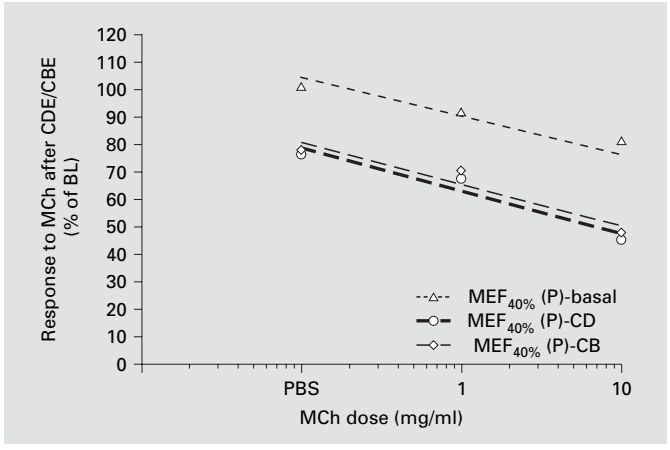
Fig. 1, 2. Response to CBE and CDE following a 120-breath challenge with each of these agents followed up for 2 h after challenge. Lung function is expressed as $MEF_{40\%}(P)$ (**1**) and FEV_1 (**2**) measured as a percentage of baseline (BL). Each point represents the mean of 21 subjects.



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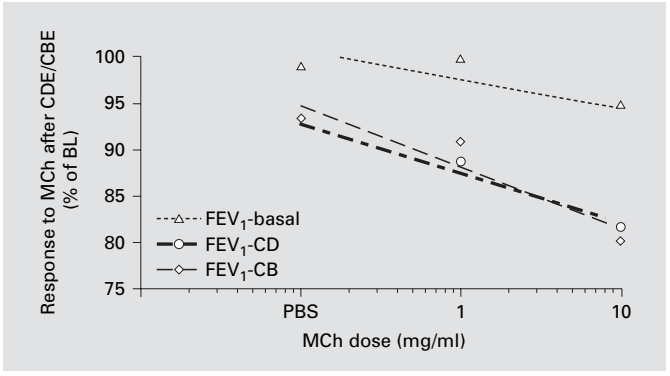

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Fig. 3, 4. MCh dose-response on the 3 protocol days: the screening day (basal), CDE challenge day (CD), and CBE day (CB). Lung function is expressed as $MEF_{40\%}(P)$ (**3**) and FEV_1 (**4**) measured as a percent of baseline (BL; before challenge). Each point is the mean of 21 subjects. The MCh dose is represented on a logarithmic scale and extrapolated curves are log-linear regression fits.

The average changes in $MEF_{40\%}(P)$ over time following extract inhalation are shown in figure 1. The average maximal response to CBE was a fall in $MEF_{40\%}(P)$ to 70 ± 4.9% of the baseline compared to 70 ± 4.4% of baseline for CDE (nonsignificant). In figure 2, we display the same analysis for FEV_1 (note the difference in scale). Both challenges show a progressive reduction in lung function, achieving a maximum drop 75 min after challenge. An initial constriction occurred in the first 15 min following challenge. This transient early response may represent the effect of endogenous mediators present in the cotton dust or released from airway cells upon challenge [29, 30]. The responses to CDE and CBE are similar. This was initially studied using ANOVA and pairwise comparisons of each lung function measurement at each time point and revealed no statistically significant differences between both groups. In order to rule out a significant carryover effect (i.e. the effect of the order in which the treatments are given), we performed an ANOVA for crossover design. As would be expected from figures 1 and 2, a significant effect over time is demonstrated for the CDE and CBE challenges. All the other actions or interactions show no differences, suggesting that, in fact, there is no difference between the response to CDE or CBE.

The duration of the post-challenge follow-up of 2 h clearly demonstrates the subacute nature of this response. After 2 h, the response gradually subsides and there was no late-phase response demonstrated.

Response to MCh Provocation

None of the subjects were hyperresponsive to MCh provocation (defined as a $PD_{20}FEV_1$ of ≤ 8 mg/ml) prior to CBE or CDE challenges. None of the subjects reduced their FEV_1 by more than 20% following challenge with 100 g of MCh.

All subjects, both responders and non-responders, enhanced their MCh response following CBE or CDE. The $PD_{40}\,MEF_{40\%}(P)$ was identical for CBE and CDE (1.3 mg/ml). Figures 3 and 4 illustrate the average MCh response at baseline and following the dust challenges for $MEF_{40\%}(P)$ and FEV_1. No significant differences existed between the post-CBE and post-CDE MCh responses, although both were significantly increased compared to baseline.

Our analysis of the carryover effect for the post-CBE and post-CDE MCh challenge showed no difference for the $MEF_{40\%}(P)$ measurement (with the exception of an isolated dose × day effect). In particular, there was no carryover or agent effect. On the other hand, an analysis of the carryover effect for the post-CBE and post-CDE MCh challenge as measured by FEV_1 does show a significant carryover effect.

Discussion

This study demonstrates that the inhalation of aerosolized CBE results in a nearly identical effect on lung function as the inhalation of similarly prepared CDE. For many years, we have used this bract extract as a surrogate for cotton dust in clinical and in vitro studies of byssinosis. It has been appreciated at least since the studies of Bouhuys et al. [6] that, of all the macroscopic components of the harvested cotton plant, bract alone contains a bronchoconstricting element. That this component of the plant is a plausible agent involved in byssinosis was shown by the study by Morey et al. [11] indicating that bract makes up a significant proportion of cotton dust. Our findings complement and extend these earlier studies by showing that the physiologic response to CBE is essentially the same as that to CDE. In particular, no difference in lung function parameter [$MEF_{40\%}(P)$ and FEV_1] changes are seen following challenge with CBE and CDE. Our crossover analysis confirms and broadens this observation of similar response to CBE and CDE by controlling for the possibility of an effect due to the order in which the agents are administered.

We also demonstrate that the inhalation of aerosols of both extracts increases airway responsiveness to MCh in an almost identical manner. Witek et al. [31] have shown that aerosol inhalation of CBE produces a mild and transient increase in MCh responsiveness. Our results confirm that CBE enhances MCh responsiveness and that this effect is similar in timing and degree to that of CDE, as measured by the provocation dose of MCh which causes a 40% decrease in $MEF_{40\%}(P)$, the $PD_{40}\,MEF_{40\%}(P)$. Our crossover analysis in this case did indicate a potential difference in the response to MCh as measured by FEV_1 following the two agents. In particular, the response to MCh appeared to be enhanced by the use of CBE on the 1st challenge day. Our analysis of demographic parameters, comparing the characteristics of those receiving CBE on day 1 and CDE on day 1, showed no differences except for a predominance of non-white subjects in the CBE group compared to the CDE group [8/14 (57%) vs. 2/7 (29%)]. This could suggest a possible effect of race on the response to cotton extracts. We subsequently analyzed baseline responsiveness to MCh (measured on the screening day) between whites and non-whites. We found no difference between whites and non-whites (white: n = 11, intercept = 99.2, slope = –0.8; non-white; n = 10: intercept = 6.9; slope = –1.1). We conclude that either a real difference exists between the response to cotton extracts based on the order of challenge, and this may possibly be related to race, although baseline demographics do not support this, or, more likely, the difference results from the large number of comparisons analyzed and is a random occurrence.

It has been postulated that airway hyperresponsiveness, induced by environmental agents, plays a central role in the pathogenesis of chronic airway disease. Hyperresponsiveness to pharmacological agents is considered to be a manifestation of underlying airway inflammation, and has been used to link irritants causing acute effects with chronic airway disease. Hence the finding that both CDE and CBE cause similar enhancement of airway responsiveness validates our previous suggestions that airway hyperresponsiveness following CBE suggests a role of this agent in chronic lung disease and byssinosis in textile workers. Additionally, our results continue to demonstrate that healthy, non-smoking, naïve (never before exposed) subjects can develop acute symptoms from a single exposure to cotton waste products.

Our findings may support the hypothesis that chronic occupational diseases, including byssinosis, are caused by repeated injury from small doses of an agent during con-

tinued workplace exposure. In 1974, Bouhuys [32] proposed as a working hypothesis that repeated microinsults to the lungs on each exposure to the toxic component of cotton dust would have a cumulative damaging effect by a mechanism of damage that was not yet known. Subsequent studies on byssinosis have demonstrated the inflammatory potential of cotton bracts for airways [31] and skin [30]. The 'Dutch hypothesis' of chronic obstructive lung disease proposes that airway hyperreactivity, resulting from an inflammatory response to an inhaled substance, increases the risk of irreversible obstruction and chronic disease [31].

While our investigation only studied single acute responses to cotton extracts, epidemiologic findings associating acute, across-shift changes with chronic manifestations of the disease suggest that our findings have relevance for the occurrence of more chronic effects. It remains nevertheless to be seen whether the acute effects that we report do in fact reflect the findings of 'Monday dyspnea' with its characteristic tachyphylaxis as the workweek progresses. The current study is consistent with the hypothesis that the mechanism of disease progression, induced by cotton dust, results from transient, repeated inflammatory insults by the bract component of cotton dust.

Our challenge method exposed volunteers to amounts of dust and bract commonly inhaled over an 8-hour shift. Because the challenge only lasted 10–15 min, the concentration of the inhaled solution was much higher than that experienced in the mill. This method has nevertheless been useful in studying the inflammatory effects of these agents [13–15].

Other investigators have used an inhalational model to deliver extracts of other organic products such as corn, grain, and soybean, in order to characterize the mechanisms of acute disease in volunteers [34, 35]. Clapp at al. [34] delivered aqueous solutions of corn dust extract and buffered saline to volunteer grain handlers without a history of asthma or atopy. Bronchoalveolar lavage specimens demonstrated a neutrophilic alveolitis with enhanced cytokine production and release in the lower respiratory tract following inhalation of this corn dust extract, but not after saline inhalation. After corn dust inhalation, subjects also developed fever and peripheral leukocytosis, with physiological manifestations of acute airflow obstruction, suggestive of an acute inflammatory response that was not dependent on preexisting asthma or atopy. Similarly, Cooper at al. [36] administered aqueous CBE to healthy human subjects, and demonstrated both polymorphonuclear leukocytes (PMNs) and chemotactic factors for PMNs in fluid recovered from bronchoalveolar lavage fluid. In addition, the levels of both PMNs and chemotactic factors were correlated with the degree of bronchoconstriction induced by CBE. Both complement activation and chemotactic factor synthesis were considered crucial to the inflammatory response. Taken together, these findings suggest a unique inflammatory mechanism for this category of organic dusts, which does not require previous sensitization.

In our study, the endotoxin concentration measured was low in both extracts (compared to levels of 5,000 EU/mg commonly found in mill dust), and it is thus unlikely that this agent explains the physiologic effects seen in this study. In acute human challenge studies using many cotton extract preparations, Buck at al. [15] failed to document a dose-response relationship with endotoxin. Studies by Ayars at al. [40] on the toxicity of cotton mill dust extract, green bract extract and field-dried bract extract, and their components, tannins and endotoxin, to rat and human pneumocytes failed to correlate endotoxin levels with pneumocyte injury.

By contrast, in epidemiological studies [37, 38] and in the experimental cardroom [39], endotoxin levels in cotton dust and cumulative endotoxin exposures have been related to the prevalence of byssinosis as well as to physiologic and biologic changes. In an interesting study by Jagielo et al. [41] comparing the airway challenge effects of an extract of corn dust and lipopolysaccharides, the authors concluded that the presence of lipopolysaccharides strongly influences the constrictor and inflammatory effect of the corn extract. These results could suggest that endotoxin works synergistically with an extract component to enhance airway inflammation; alternatively, it may be that endotoxin is a co-variable with other inflammatory cotton dust components in field studies [42].

We have demonstrated that CBE and CDE cause indistinguishable physiologic effects when inhaled by naïve, healthy, non-smoking subjects. These same individuals develop similar airway hyperresponsiveness following exposure to both preparations. These findings strongly suggest that the active agent responsible for acute byssinosis is associated with a component or components of the cotton bract. CBE can be used for further clinical studies to elucidate the disease mechanism of byssinosis and to suggest strategies for promoting worker safety.

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