Bronchial Responsiveness after Inhalation of Cotton Bract Extract[1-3]

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SUMMARY This study examined nonspecific airway responsiveness to methacholine (MC) after inhalation of cotton bract extract (CBE). In a randomized double-blind, crossover trial, 13 healthy volunteers underwent an MC inhalation challenge test prior to inhalation of CBE and normal saline solution (NSS) aerosol sham as well as 2, 8, 24, and 168 h (7 days) later. The response parameter was the concentration of MC required to induce a 25% decrement in the maximal expiratory flow at 40% of the vital capacity below total lung capacity on the partial expiratory flow-volume curve ($PC_{25}MEF_{40\%}(P)$). Five of 13 subjects demonstrated a ventilatory response to CBE with a 20% or larger decrement in the $MEF_{40\%}(P)$; no subject demonstrated such change with NSS. For the group, the maximal decrement in $MEF_{40\%}(P)$ was to 76.5 ± 20.3% of baseline (mean ± SD), occurring approximately 60 to 90 min after provocation, whereas the largest decrement after normal saline was to 88 ± 10.6% of baseline, occurring immediately after inhalation. Changes in airway responsiveness to MC were transient. For example, the $PC_{25}MEF_{40\%}(P)$ for the group (mean ± SD) was 51.3 ± 41.1 mg/ml at baseline and 25.8 ± 30.3 and 52.2 ± 57.3 mg/ml at 2 and 8 h. After a pre-sham baseline of 50.4 ± 43.2 mg/ml, $PC_{25}MEF_{40\%}(P)$ was 57.6 ± 83.8 and 153.8 ± 148 mg/ml at 2 and 8 h. Repeated measures ANOVA on these acute, same-day changes (i.e., 2 and 8 h after provocation) demonstrated a statistically significant effect of CBE on airway responsiveness ($p = 0.048$). These data demonstrate that inhalation of CBE, in addition to bronchospasm, causes a transient increase in airway responsiveness.

AM REV RESPIR DIS 1988; 138:1579-1583

Introduction

Byssinosis is an occupational lung disease associated with the inhalation of cotton and other textile dusts (1, 2). Cotton processing is by far the largest industry whose workers are at risk of byssinosis. In the United States, the population at risk has been estimated at 200,000 cotton workers and 5,000 flax workers (3).

In the early phases of byssinosis, acute reversible symptoms such as wheezing, chest tightness, and shortness of breath accompany reversible changes in lung function (4). "Monday dyspnea" is the term used to describe these symptoms that occur on the first day back at work after an absence. The disease may progress to a stage at which symptoms are present throughout the work week and may eventually result in severe respiratory disability (5). This has been estimated to occur in 7% of older cotton textile workers (6). While the acute phase of the disease has been characterized by across-shift changes in lung function and the chronic phase by the presence of chronic bronchitis and/or abnormal lung function levels, the transition between the two is poorly understood.

Recent studies suggest that airway hyperresponsiveness plays a role in the accelerated loss of lung function in patients with chronic airflow obstruction (7-10). Additionally, it has been shown that airway inflammation is related to the development of hyperreactivity (11, 12). Because the inflammatory potential of cotton bracts has been demonstrated for human airways (13) as well as skin (14), we postulated that airway challenge with cotton bract extract would cause airway hyperresponsiveness and that this effect might be important in understanding the natural history of byssinosis.

Methods

Study Design

A double-blind, random, crossover study involving airway provocation with either cotton bract extract (CBE) or normal saline solution (NSS) was carried out (figure 1). Prior to provocation testing, subjects reported to the laboratory for an explanation of the study and a brief medical examination and questionnaire.

On the first test day, a methacholine inhalation challenge (MIC) was performed. On a subsequent day, approximately 24 to 48 h later, subjects underwent randomly assigned provocation with either CBE or NSS. Pulmonary function was measured every 15 min for 120 min, and an MIC was carried out. Subjects returned for MIC tests that evening (8 h later), the next morning (24 h later), and 1 wk later. This sequence of MIC tests was repeated for the remaining provocation (i.e., CBE or NSS), approximately 1 wk after completion of the first series. Testing for both series was carried out at the same time of day in all subjects.

Subject Volunteers

Thirteen adults, mostly staff members and students at the Mount Sinai Medical Center, agreed to participate in the study. All but one subject were nonsmokers. The protocol was approved by the Institutional Review Board of the Medical Center, and all subjects gave informed consent.

Entrance criteria for participation included the absence of chronic medical illness including asthma or other respiratory disease and an age between 18 and 35 yr. Subjects were instructed not to jog or bicycle to the laboratory and to refrain on the study days from consumption of beverages containing caffeine or large doses of vitamin C.

Inhalation Challenges

Methacholine solutions were prepared in normal saline solution and then stored at 4° C until use when the vials were warmed to room temperature. Cumulative dose response was obtained by inhalation of 1, 10, 20, 40, 80, 160, and 320 mg/ml.

The aerosol was generated by a modification of the technique described by Chai and colleagues (15). A DeVilbiss Model No. 45

(Received in original form March 21, 1988 and in revised form June 13, 1988)

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[2] Supported by Grant No. 1 R03 OHO 2265-01 from the National Institute of Occupational Safety and Health of the Centers for Disease Control.

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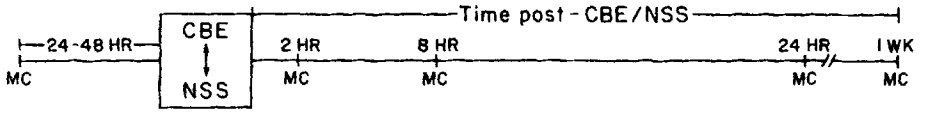

Fig. 1. Schematic representation of protocol for bronchial challenge. Each subject completed the series twice, with the order of CBE or NSS exposure randomized.

nebulizer was attached to a dosimeter driven by compressed air at a pressure of 20 psig. Inspiration by the subject produced a negative mouth pressure that was sensed by a low pressure sensor/relay. This signal opens the nebulizer flow and carrier gas valves. Nebulizer flow is timed to stop after 0.6 s; the carrier gas flow continues for approximately 3 s to permit the subject to complete an inspiration. Inspiration began at functional residual capacity and continued to total lung capacity. Exhalation was passive and normal. The maneuver was repeated five times. One milliliter of test solution was placed in the nebulizer. The output of the nebulizer was 5.5 µl per breath, equalling 27.5 µl per given dose.

Provocative challenges with cotton bract extract and normal saline involved 120 consecutive inhalations of solution and were also delivered with the same nebulizer and dosimeter system.

Preparation of Cotton Bract Extract

Aqueous extracts of cotton bracts (collected from Texas cotton fields just before harvest) were prepared with pyrogen-free water by Dr. Marion G. Buck of Yale University (16). The clear brown supernatant obtained after centrifugation of the portion of the bracts that could be squeezed through cheesecloth was sterilized by passage through a 0.45-µm filter. To this sterile filtrate, methanol (7:1, vol/vol) was added, and precipitated material was removed by centrifugation, producing a clear brownish extract. A single large quantity of bract extract was prepared in this manner at the onset of this experiment. This extract was freeze-dried for storage purposes and reconstituted at a concentration of 30 mg/ml for inhalation challenges. This approach was used to assure delivery of similar concentrations of active agent with each challenge. The pH of the dissolved bract solution was between 5.5 and 6.0, and the osmolarity approximately 200 milliosmoles. The CBE was essentially endotoxin-free at 1.3 ng/ml; normal saline was endotoxin-free.

The workplace relevance of the delivered concentration can be estimated by assuming that a 70-kg textile worker ventilates 15 m^3 of air over a workshift (17). As reported by Hammad and coworkers (18), cotton dust levels can range from 0.28 to 6.36 mg/m^3 of air as measured with vertical elutriators in southern United States mills. Over 8 h, the dust burden can range between approximately 4 and 95 mg. Assuming that 50% of the particles are in the respirable range (17), pulmonary deposition can range from approximately 2 to 48 mg/shift. If approximately 660 µl are delivered at the mouth, then it follows that 20 mg of cotton bract extract is inhaled. Although not all industrial dust may be from the bract portion of the cotton, it is reasonable to assume that the clinical challenge in naive volunteers given over 10 to 15 min is equivalent to the dose inhaled over a workshift.

Pulmonary Function Testing

Pulmonary function was measured using partial expiratory flow-volume (PEFV) and maximal expiratory flow volume (MEFV) curves. A pneumotach integrator system was used, and flow was plotted against volume (19). The maneuver performed consisted of inspiring initially to approximately 70% of vital capacity and then expiring forcefully to residual volume. The subject next inspired to total lung capacity and once again expired forcefully to residual volume. These maneuvers generated the PEFV and MEFV curves (20). A 1-s timer permitted identification of the forced expiratory volume in one second (FEV_1). From these data, forced vital capacity (FVC), FEV_1, and peak expiratory flow (PEF) were determined. Maximal expiratory flows on both MEFV and PEFV curves were measured at 60% of the control vital capacity below total lung capacity, $MEF_{40\%}$ and $MEF_{40\%}(P)$, respectively (figure 2).

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 status as a reactor or nonreactor.

Because airway caliber is known to change after the deep inspiration required for MEFV curves, it may alter the airway constrictor response. The PEFV curve is of special usefulness in studies of airway constrictor agents because it avoids a previous inspiration to TLC. To compare PEFV curves before and after drugs or environmental agents such as methacholine and CBE, one needs a reference volume. Residual volume (RV) is not suitable because it often increases during airway obstruction and is not constant. TLC is more suitable because it remains constant unless bronchoconstriction is severe. As noted by Bouhuys (21), if a subject inspires to TLC directly after the PEFV maneuver, TLC can be established as a reference volume point on the recording. Therefore, we routinely superimpose MEFV curves on PEFV curves, using TLC as a common reference point (figure 2). Several studies have confirmed that TLC does not change after airway narrowing in normal subjects (20, 22-24).

The primary response parameter in this in-

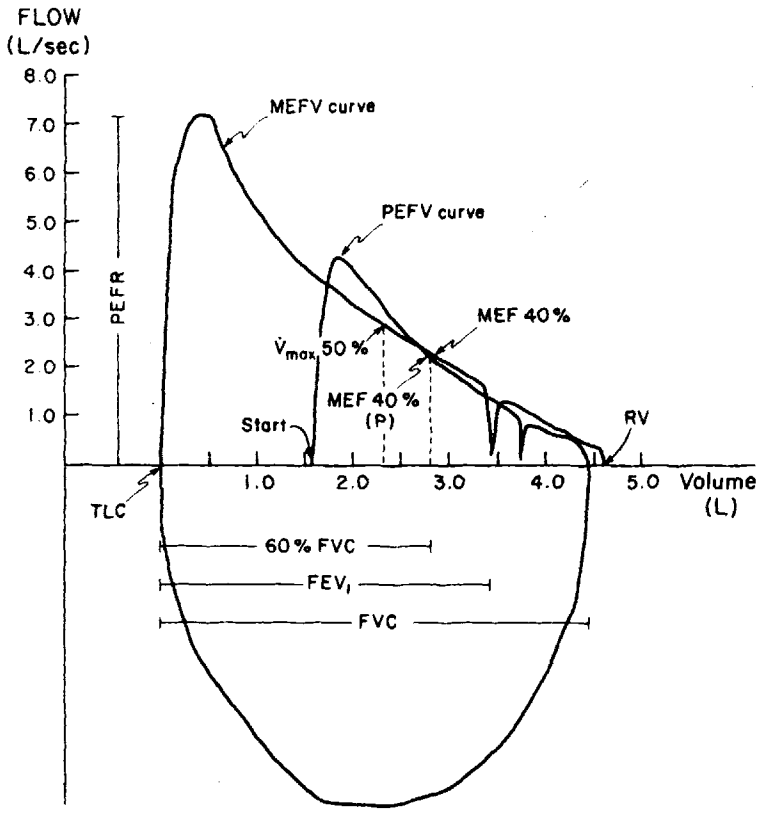

Fig. 2. Typical partial and maximal expiratory flow-volume curve.

TABLE 1

ANTHROPOMETRIC DATA AND BASELINE LUNG FUNCTION*

Subject No.	Sex	Race†	Age (yr)	FVC			FEV_1			$MEF_{50\%}$			PC_{25} (mg/ml)
				O	P	%	O	P	%	O	P	%	
1	M	W	25	4.4	4.1	85.8	3.8	4.1	92.5	4.2	5.0	83.5	82.3
2	M	W	29	4.3	4.9	88.5	3.5	3.8	91.9	2.7	4.6	58.3	113.1
3	M	W	32	5.1	5.0	101.2	4.0	4.0	100.0	4.5	4.8	94.5	17.4
4	M	W	31	5.4	4.7	114.4	4.0	3.7	109.0	2.9	4.5	65.0	4.7
5	M	B	24	3.7	4.1	90.9	3.0	3.6	88.8	2.9	4.6	62.4	56.7
6	M	L	27	4.6	5.3	87.1	3.7	4.2	88.5	3.7	5.0	73.9	40.8
7	F	W	32	3.9	4.0	97.3	3.4	3.2	105.9	4.4	4.0	109.2	87.7
8	F	W	24	5.1	3.9	132.1	4.3	3.3	131.5	4.5	4.2	108.4	113.2
9	M	L	35	4.8	4.6	103.5	4.1	3.6	112.6	4.8	4.4	108.6	25.2
10	F	L	33	3.8	3.4	110.1	2.9	2.8	102.5	3.1	3.9	80.3	6.4
11	F	W	22	3.4	3.1	110.8	3.1	2.7	116.5	3.6	3.7	96.3	23.5
12	M	W	23	6.0	5.5	108.5	4.5	4.5	99.6	3.5	5.5	63.2	20.0
13	M	W	24	5.6	5.4	102.9	4.7	4.4	105.6	4.2	5.5	76.5	1.6
Mean			27.8	4.62	4.54	102.8	3.77	3.67	103.5	3.77	4.58	83.8	45.6
SD			4.4	0.80	0.77	13.1	0.57	.57	12.0	0.71	0.58	18.5	40.7

* Expressed as observed (O), predicted (P), and percentage of predicted (%) based on the equations of Schoenberg and colleagues (27).
† W = white; B = black; L = Latino.

vestigation is the concentration of methacholine required to induce a 25% reduction in the baseline $MEF_{40\%}$(P). This variable is termed $PC_{25}MEF_{40}$(P). This parameter is obtained by linear interpolation of the delivered doses. Because PC_{25} values were not normally distributed due to skewness, log transformations were carried out on all methacholine data before carrying out statistical tests. Because differences from baseline in log PC_{25} resulted in negative numbers in instances where values were less than one, all data presentation and analysis were performed on variables that had the constant of 1.0 added (i.e., log (PC_{25}) + 1).

Pulmonary function data were abstracted in a blinded fashion from original flow-volume tracings and recorded and stored on computer tapes. Repeated measures analysis of variance was carried out with an IBM 370 computer using the GLM program of the SAS statistical package (25). In this analysis, there are two repeated factors: provocation (i.e., CBE versus NSS) and time of methacholine challenge (i.e., 2, 8, 24, and 168 h after provocation). The dependent variable was the log (PC_{25}) + 1. The assumption of equal variances and equal covariances (e.g., sphericity) was checked prior to using this analysis; this is an assumption not made with multivariate tests (26).

Results

Baseline Lung Function and Subject Characteristics

All 13 volunteers completed the study. No subject suffered from any chronic medical illness as determined by a clinical questionnaire and a physical examination by a physician. Although none were taking therapeutic medicines, two female subjects were taking oral contraceptives. Histories of respiratory illness as well as workplace or domestic exposures to dusts and fumes were absent. One male subject (Subject 7) was a smoker with a 7-pack-year history. None of the subjects were considered to have atopy on the basis of a history of hay fever, eczema, or allergies to food or medication.

At the start of the study, all the subjects had been free of symptoms of upper respiratory infection for at least 4 wk. Only one subject developed upper respiratory symptoms when questioned each day over the course of the study. This occurred 1 wk after normal saline provocation and did not result in a significant change in bronchial reactivity as compared to the previous methacholine challenge.

Table 1 lists anthropometric data and baseline lung function expressed as percentage of predicted values based on the equations of Schoenberg and associates (27). There were nine men and four women, with ages ranging from 22 to 33 yr. Baseline lung function was normal for the group, with no subject exhibiting an abnormal FVC or FEV_1. There were five subjects with a baseline $MEF_{50\%}$ below 75% of their predicted value, but no subject had any apparent signs of pulmonary disease. All denied asthma or a history of respiratory disease.

Ventilatory Response to Cotton Bract Extract

The mean ventilatory responses to cotton bract extract and normal saline solution expressed as absolute values and as a percentage of baseline $MEF_{40\%}$(P) are listed in table 2. For the group, the mean maximal fall in $MEF_{40\%}$(P) was 23.5%, occurring at the 75 min measurement after inhalation. For normal saline, the mean maximal drop was only 12.2%, occurring at the 1 min measurement after inhalation. The pattern of lung function response is illustrated for the group in figure 3.

The effect of the CBE on baseline lung function per se was not found to alter subsequent methacholine tests (i.e., 2 h post-CBE challenge), as an improving trend in $MEF_{40\%}$(P) was noted after the maximal decrements. Comparison of the pre-CBE baseline $MEF_{40\%}$(P) to the pre-MC baseline $MEF_{40\%}$(P) showed no significant difference by paired t test ($p = 0.19$). Additionally, there was no correlation between the 2-h change in PC_{25} and the maximal decrease in $MEF_{40\%}$(P) after CBE ($r = 0.004$).

Response to Methacholine Provocation

A summary of $PC_{25}MEF_{40\%}$(P) measurements made at baseline and after CBE and NSS over time are provided in table 3. The largest decrement from baseline occurred for the group at 2 h after CBE inhalation. The patterns of bronchial responsiveness after CBE and sham provocations are illustrated for the group in figure 4. The decrements 2 h after provocations were not dependent on which agent (CBE versus NSS) was administered first in the randomization.

Repeated measures analysis of variance on the acute, same-day changes (i.e., 2 and 8 h after provocation) resulted in a statistically significant effect of CBE on airway responsiveness as compared to NSS (df 1,12; $F = 4.83$; $p = 0.048$). When all time points were used in the repeated measures analysis (i.e., all four time points after provocation), a statistically

TABLE 2

MEAN ABSOLUTE AND PERCENT CHANGES IN $MEF_{40\%}(P)$ (L/s) AFTER CBE AND NSS*

			Time in Minutes									
		BL	1	15	30	45	60	75	90	105	120	BL/MC
Absolute												
CBE	Mean	2.61	2.37	2.59	2.55	2.42	2.10	1.99	2.12	2.24	2.30	2.45
	SD	0.67	0.56	0.66	0.71	0.67	0.63	0.68	0.63	.57	0.75	0.68
NSS	Mean	2.77	2.42	2.55	2.67	2.67	2.78	2.73	2.74	2.78	2.65	2.77
	SD	0.63	0.57	0.62	0.68	0.61	0.77	0.73	0.68	0.77	0.68	0.71
Percent												
CBE	Mean	100	91.85	100.38	98.46	93.15	80.38	76.46	81.46	86.38	88.00	94.54
	SD	00	10.70	14.29	15.93	17.98	14.92	20.27	16.55	10.74	17.36	15.75
NSS	Mean	100	87.85	92.92	96.62	97.23	99.77	98.46	99.15	100.15	96.46	100.23
	SD	00	10.56	11.69	13.14	11.69	11.92	11.17	13.04	13.86	14.84	14.17

* The last column lists the baseline (BL) measurement obtained prior to beginning the methacholine challenge.

significant provocation effect was not observed (df 1,12; F = 2.73; p = 0.124). This was due essentially to the small differences seen at the 1 day and 1 week time points, which additionally also give rise to an almost significant interaction of provocation and time (p = 0.052).

Discussion

This study demonstrates that inhalation of an aerosol of aqueous extracts of cotton bract results in a mild and transient increase in responsiveness to methacholine in healthy volunteers. The response to methacholine was independent of the lung function response to the cotton bract itself. All aerosols used were essentially endotoxin-free.

Induction of bronchial hyperresponsiveness after exposure to cotton bract or dust has not been adequately studied. Two recent preliminary reports suggest that such a change in bronchial reactivity may be taking place. Boehleke and associates (28) report that an 8-h exposure to cotton dust resulted in nonspecific airway hyperreactivity. More recently, Rylander and Bake (29) presented preliminary data showing that endotoxin exposure induced airway reactivity to methacholine. In these reports, the relationship between these observations and inflammation of the airway are not supported by studies of cellular responses. Nevertheless, the evidence that cotton dust evokes an inflammatory stimulus is currently accumulating. Both dermal (14) and respiratory challenge in humans (13) result in inflammatory cell influx and visually apparent inflammatory reactions. In addition, it has recently been demonstrated that CBE enhances mucus glycoprotein release from human airways *in vitro* (30) by the release of preformed and *de novo* synthesized mediators, suggesting an additional component of airway inflammation.

The relationship between airway inflammation and the development of airway hyperresponsiveness has been studied most extensively using ozone (O_3) challenge (31). In the dog, the onset of bronchial hyperresponsiveness after O_3 is associated with neutrophil influx (32). Intentional depletion of neutrophils with hydroxyurea inhibits this response (33). In human volunteers after O_3 challenge, a mild increase in nonspecific responsiveness to methacholine has also been related to airway neutrophils recovered in bronchoalveolar lavage (34). Although a causal role for the neutrophil in the development of bronchial hyperresponsiveness has not as yet been established, it clearly is associated with the airway response to environmental insults such as ozone and CBE.

The temporal relationship between airway inflammatory cells and airway responsiveness needs to be explored. The mere presence of these cells may not adequately explain bronchial hyperresponsiveness; alteration or activation of cells may be a prerequisite for such changes. As pointed out by Nadel (11), cell-to-cell interactions may play an important role in the relationship between inflammation and airway hyperreactivity because one type of airway cell (i.e., epithelial cell) may produce mediators that activate or modulate the activities of other cells (e.g., neutrophils). In asthma, airway reactivity persists in the asymptomatic state, but in experimental studies of induced hyperreactivity such as the current one, the hyperresponsiveness quickly subsides. The differences may be due to a chronically modified state with cellular damage in asthma and possibly to a defect in switching off the inflammatory process (12).

The public health importance of examining these relationships becomes evident when considering the possible consequences of cotton dust exposure in textile workers (i.e., chronic airflow obstruction). The possibility that environmentally induced airway hyperresponsiveness can contribute to accelerated lung function loss in cotton textile workers is suggested by the longitudinal observations of Barter and Campbell (7), who found that the rate of deterioration

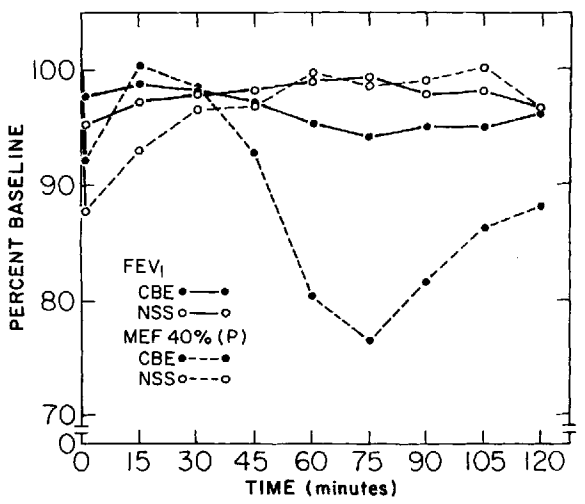

Fig. 3. Mean time-response of FEV_1 and $MEF_{40\%}(P)$ after inhalation of CBE and NSS.

TABLE 3

$PC_{25}MEF_{40\%}(P)$ OVER TIME AFTER PROVOCATION WITH COTTON BRACT EXTRACT AND NORMAL SALINE SOLUTION AEROSOL*

Inhalation Challenge		BL	2 H	8 H	24 H	1 WK
PC_{25}						
CBE	Mean	51.35	25.75	52.18	93.86	55.33
	SD	41.07	30.30	57.53	110.08	49.60
NSS	Mean	50.36	57.58	153.80	81.95	63.99
	SD	43.20	83.82	147.97	105.74	62.70
log (PC_{25}) + 1						
CBE	Mean	2.48	2.04	2.49	2.74	2.62
	SD	0.59	0.69	0.49	0.46	0.33
NSS	Mean	2.54	2.39	2.89	2.63	2.64
	SD	0.41	0.69	0.63	0.54	0.39

* Values are expressed in mg/ml methacholine prior to and after log transformation.

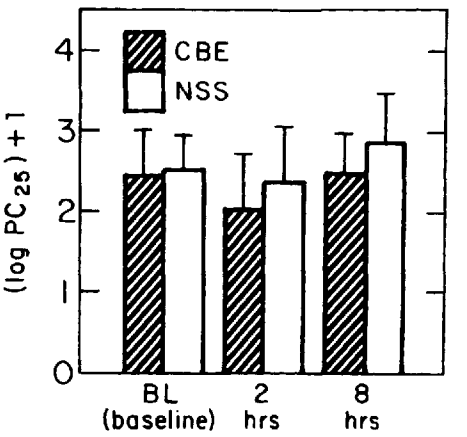

Fig. 4. Mean log $PC_{25}MEF_{40\%}(P)$ (+1) values over time after provocation with both CBE and NSS.

of FEV_1 in bronchitic patients correlated with methacholine reactivity, independently of smoking cigarettes. Subsequent studies of middle-aged smokers with some impairment of lung function have also shown a positive correlation between increased bronchial responsiveness to histamine and accelerated annual decline in FEV_1 (8, 9). These data are suggestive of the "Dutch hypothesis" of chronic obstructive lung disease in that airway hyperreactivity may be an underlying factor in irreversible airflow obstruction (10). Consideration of this hypothesis in the context of lung disease in cotton textile mill workers is important from a public health viewpoint because increased airway reactivity may identify susceptible persons before airflow obstruction becomes irreversible. Although these data currently only allow speculation on their relevance to airway obstruction in byssinosis, the airway hyperreactivity hypothesis remains an intriguing model for the study of chronic airway disease particularly in the context of occupational airway diseases.

Acknowledgment

The writers thank Drs. I. F. Goldstein, P. Brandt-Rauf, G. H. Sewell, A. Patel, and Z. Marom for their assistance during this project. Marilyn Marshall is thanked for word processing, and Dr. J. A. Cooper for reviewing the manuscript.

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