man. In: Kass SH, Wolff SM, eds. Bacterial lipopolysaccharides. Chicago: University of Chicago Press, 1973: 251-56
23 Edwards JH, Evans E, Evans PH, Nicholls PJ, Rajan KT. Effect of a purified cotton dust extract on human lung in culture. Br J Pharmacol 1976;56:394
24 Antweiler J. Histamine liberation by cotton dust extracts: evidence against its causation by bacterial endotoxins. Br J Ind Med 1961; 18:130-32
25 Evans E. Pharmacological investigations of vegetable dusts in relation to byssinosis. Ph.D. thesis. University of Wales, 1978

A Purified Extract from Cotton Bracts Induces Airway Constriction in Humans*

Marion G. Buck, Ph.D.,† and Arend Bouhuys, M.D.‡

When exposed in the laboratory to aqueous extracts of cotton bracts human volunteers respond with a reversible decrease of lung function similar to the acute Monday response of cotton textile workers to mill dust.[1] Bracts (the friable leaf-like structures surrounding the stem of the cotton boll) have been reported by Morey et al[2] to be a principal contaminant of mill dust. Other cotton plant parts, the pericarps or the cotton fiber itself, when tested, did not induce airway constriction in man.[3] We have used this airway constrictor response to cotton bracts as a bioassay method for the isolation and characterization of the causative agent(s) in byssinosis.

Methods and Materials

Preparation of Bract Extracts

For all bioassay challenge studies, the concentration of the bract extract was equivalent to that of our standard crude extract prepared from 1 g bracts per 6 ml water or dilute phosphate buffer (0.01 M, pH 7.2). Bracts were from frost-killed plants picked just prior to harvest from Texas cotton fields. These were pulverized before mixing (2 hr at 23°C) with water. The extract was cleared of leaf debris by squeezing through cheesecloth, followed by centrifugation at 16,000 × g for 10 min, and was sterilized by passage through a 0.45 micron filter.

Factionation

Sephadex G-10 factionation was performed with 75 × 2.5 cm columns equilibrated with 0.01 M phosphate buffer (pH 7.2) and calibrated with blue dextran and NaCl. Four ml of 4 × concentrated standard crude extract were applied to the top of the column and eluted with equilibrating buffer at 23°C.

QAE-Sephadex and SP-Sephadex Ion-exchange Chromatography

QAE-Sephadex was equilibrated with 0.05 M phosphate or tris-HCl buffer, pH 7.2; SP-sephadex with 0.05 M phosphate buffer, pH 7.2. The active fraction from the sephadex G-10 column, 4 × concentrated and adjusted to pH 7.2, was applied to the top of each column (24 × 1.5 cm) and eluted with equilibrating buffer at 23°C. Five milliliter fractions were collected and assayed for protein, carbohydrate, and conductivity.

Successive pooled fractions of the eluates were freeze-dried and then reconstituted to the original volume for inhalation testing. This insured that components were not concentrated as purification progressed.

Protein was determined by the method of Lowry et al[4] and carbohydrate by the phenol sulfuric acid method[5] or the Anthrone assay.[6]

Bio-assay of Airway Constrictor Activity

Airway constrictor effects of the bracts extracts were assayed by comparing lung function values obtained from recordings of partial and maximum expiratory flow-volume (PEFV, MEFV) curves[7,8] before and at 30 min intervals for a 2½-3 hr period following a 10 min inhalation of the aerosolized extract. The aerosol was generated into less than 1 micron diameter droplets with a Dautrebande D30 nebulizer.[9] The curves were recorded with a pneumotachograph-integrator device[10] and an XY recorder; ordinate: expiratory flow rates, abscissa: expired volume. The subject first inspired to about 65 percent of the vital capacity (VC) (the exact level is not crucial and may vary from about 60-75 percent of VC) and the subsequent forced expiration to residual volume yielded the PEFV curve. Without interruption, the subject next inspired maximally, and then again expired forcefully and maximally to residual volume, recording the MEFV curve. Forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV_1), MEF50 percent and MEF40 percent were measured from the MEFV curve. MEF50 percent and MEF40 percent are instantaneous flows at lung volumes corresponding to 50 percent and 40 percent of FVC (maximum inspiration-100 percent FVC). MEF40 percent (P) is the instantaneous flow on the PEFV curve at 40 percent remaining VC. Forty percent VC values were used to avoid peak transients on the PEFV curves at larger lung volumes. To compare responses to bracts extract aerosol we used the MEF40 percent (P) value where the 40 percent VC volume for each subject day was computed as an average from five control (before aerosol inhalation) MEFV curves of that day. This 40 percent volume was used throughout the test period. When airway constriction occurs, any changes in FVC are small relative to changes in MEF40 percent (P) and the 40 percent volume is always measured from the point of maximum inspiration on the MEFV curve.

To compare FVC, FEV_1 and MEF50 percent with predicted values for lung function, we used the average values from the two blows with the highest FEV_1 out of five technically acceptable MEFV curves, in accordance with the protocol used by Schoenberg et al.[11]

Controls inhaling aerosolized normal saline solution replacing bract extract were performed as above.

*From the Department of Internal Medicine, Yale University School of Medicine and the Physiology Laboratory, Utrecht University, The Netherlands.
Supported in part by USPHS-NIOSH Grant OH00752
†Senior Science Faculty Research Fellow, Utrecht University.
‡Professor of Medicine and Epidemiology, Head of Lung Research Center, Yale University. Deceased June 15, 1979.
Reprint requests: Dr. Buck, PO Box 3333, Pulmonary Section, Yale University, New Haven 06510

To compare the responses elicited by the various bract extracts, only subjects responding to the standard crude extract with a 20 percent or greater decrease in MEF40 percent (P) were selected. The response (decrease in MEF40 percent (P)) of treated or purified extracts relative to the standard crude extract was expressed as a percentage of constrictor activity. This was computed by taking the MEF40 percent (P) decrease in response to a purified extract as a percentage of that individual's MEF 40 percent (P) decrease to the standard crude extract.

Subjects

From area universities, we recruited 111 healthy volunteer subjects (no respiratory symptoms, no history of asthma), ages 18-36 yrs (72 men, 39 women; 101 whites, 8 blacks, 2 Orientals; 72 nonsmokers, 39 smokers and ex-smokers). Their lung function (FVC, FEV_1, and MEF50 percent) was normal according to the prediction equations of Schoenberg et al;[11] none of the subjects had values outside 2 standard errors of the estimated values predicted on the basis of sex, race, age, height and weight. In accord with the guidelines of the Yale University Human Investigations Committee, all volunteers were informed of the nature of the study, their role in it, and informed consent was obtained.

Results

Response to Standard Crude Bract Extract

The response of one volunteer to the standard crude extract is shown in Figure 1. At the 90 min post exposure reading, which was the time of maximum response for this subject, the flow as measured by the MEF40 percent (P) value was decreased by 50 percent. Other lung function values were also decreased; FVC by 7 percent, FEV_1 by 11 percent, and MEF40 percent by 41 percent. The more sensitive MEF40 percent (P) value does not require a maximum prior breath which could overcome small constrictor effects.[7,8] We are concerned with the safety of our subjects and wish to eliminate the need to cause a large constriction of the airways and discomfort to our subjects. We never exposed them to a higher concentration than the standard extract. Even our most sensitive subjects experienced only slight symptoms of chest tightness and shortness of breath with this concentration.

In a group of 105 volunteers we found that their average change in MEF40 percent (P) following exposure to the standard crude extract was −20 percent; their average change in FEV_1 was −5 percent. Individual responses varied widely (from a ΔMEF40 percent (P) of + 28 percent to −84 percent; a ΔFEV_1 of +6 percent to −24 percent). Figure 2 shows the MEF40 percent (P) distribution of the response for the 105 subject population. Only 9/105 (8.6 percent) subjects did not respond; their MEF40 percent (P) increased slightly or decreased less than 5 percent over the test period similar to the responses of controls. Controls were 12 subjects who responded to the standard crude extract with a 20 percent or greater decrease in MEF40 percent (P). None of these controls responded to inhalation of saline solution with a more

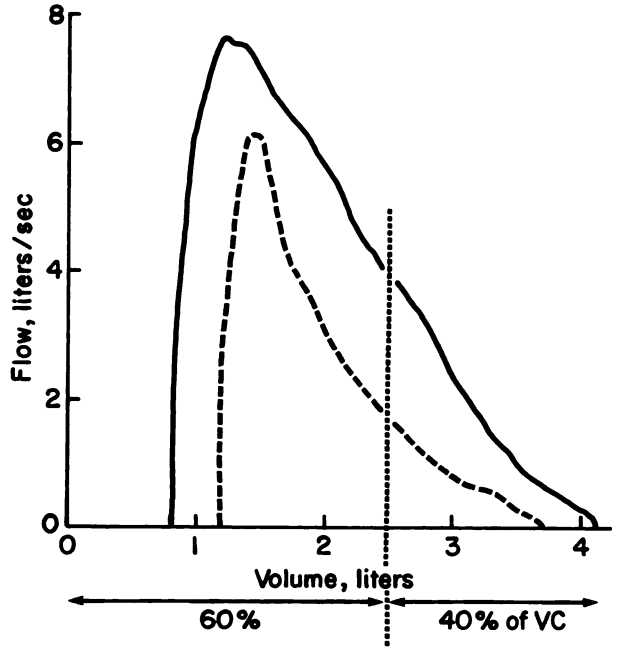

FIGURE 1. Single response to standard crude bract extract. Partial expiratory flow-volume (PEFV) curves before (*solid line*) and 90 min after (*dashed line*) 10 min inhalation of crude bract extract aerosol, by a healthy 26-yr-old woman. The lung volume level for measurement of MEF40 percent and also for MEF40 percent (P) is shown by the dashed vertical line. The point of maximum inspiration is indicated by the zero point on the volume axis. The entire expiration-inspiration-expiration maneuver was recorded without interruption; only expirations are shown. The one second mark for measurement of FEV_1 is not shown.

than 5 percent decrease in MEF40 percent (P) and their average change in MEF40 percent (P) was a slight increase. More than half of the 105 subject group (55/105; 53.3 percent) responded to the standard bract extract with a 25 percent or greater decrease in MEF40 percent (P), and a small number (11/105; 10.5 percent) had flow rate decreases of 45 percent or more.

Ten of the 105 subjects were exposed a second time to the standard crude extract one to eight weeks later. Their response was reproducible. Their average decrease of MEF40 percent (P) was 33.3 percent the first exposure vs 35.5 percent the second exposure. One subject gave similar responses on four occasions, at least one week apart, in an eight-week period.

Volunteers experienced their maximum airway constrictor response about 90-120 min after start of inhalation. This was observed over the entire response distribution; the 40 subjects with average decreases of MEF40 percent (P) from 5-25 percent as well as the 11 subjects with decreases in excess of 45 percent. Previous experience has shown that the effect slowly disappears after 2-3 hours and that lung function returns to control values within 24 hours after inhalation.[12]

The response was influenced significantly by smoking: current cigarette smokers responded less than lifetime nonsmokers (average decrease of MEF40 percent (P): 19.6 percent and 27.3 percent respectively; $P <$

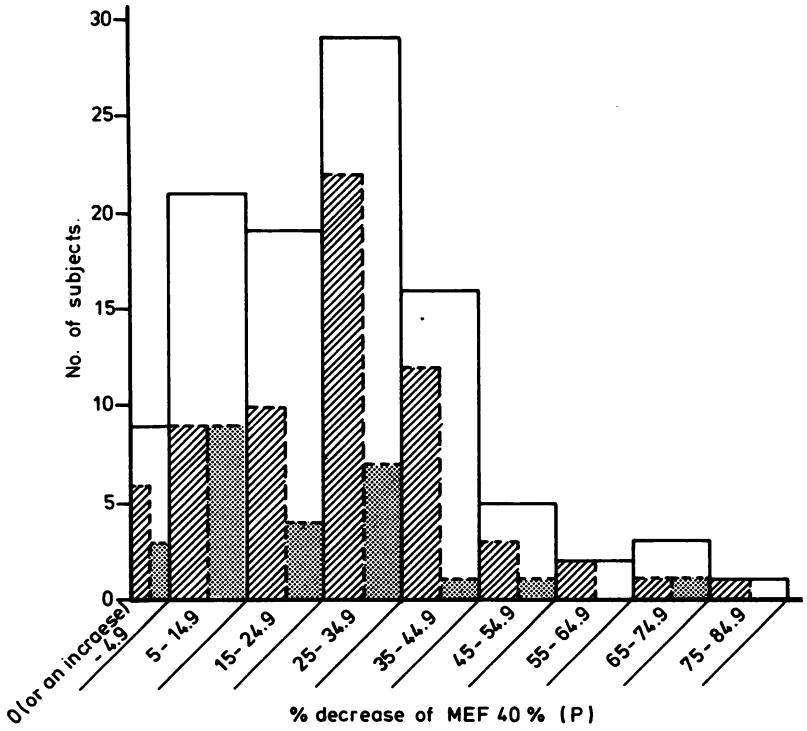

FIGURE 2. Distribution of response of 105 subjects to crude bracts extract. The response is reported as the maximum percent decrease in MEF40 percent (P) over the test period. Bars outlined in solid lines: all 105 subjects; hatched bars inserted within: nonsmokers; stippled bars inserted within: smokers. The 13 ex-smokers have been omitted from this subdivision. For analysis of MEF40 percent (P) values we averaged five technically acceptable PEFV curves at each 30 min post-exposure interval.

0.005). Of the nonsmokers, 62 percent reacted with a 25 percent or more decrease of MEF40 percent (P); only 38 percent of the smokers had a similar response (Fig 2). This difference was not due to differences in initial lung function; average control values were close to predicted values in both smokers and nonsmokers as might be expected since we were using a young population. No difference was found between the responses of men and women nor between those of black, Oriental and white subjects, although there were too few black and Oriental subjects for statistical analysis.

Gossypol Glands and Airway Constrictor Activity

Four out of five subjects did not respond to bracts extracts from a glandless variety of cotton grown in the same geographic area in Texas as our active supply. However, a supply of bracts from Brazil, which had about ten times the number of gossypol glands per leaf, had no more constrictor activity than the Texas supply. In a single preliminary experiment, the gossypol glands from one gram of cotton bracts were removed with the aid of a needle punch apparatus. Each portion (the glands and the deglanded portion) was then subjected to our routine extraction procedure. The extract from the deglanded portion elicited only a 53 percent response in one subject relative to the response set at 100 percent from the same supply of bracts before deglanding. The gland portion, which was only 5 percent of the dry weight of whole bract, elicited a 37 percent response in the same subject.

Table 1—*Effect of Various Treatments on Airway Constrictor Activity of Bract Extracts*

Treatment	% Activity remaining after treatment	(n)
80°C, ½hr	89	(5)
pH 3.0, ½hr, 23°C	89	(5)
pH 14.0, ½hr, 23°C	93	(6)

Solubility Properties of the Constrictor Agent(s)

The single aqueous extraction used for preparation of the standard crude extract removed more than 90 percent of the airway constrictor activity from dried bracts. A second extraction, using the same procedure, yielded an extract with less than 10 percent the constrictor activity of the initial extraction.

Replacing water in the initial extraction procedure with solvents, such as methanol, removed little constrictor activity from dried bracts. Compared with aqueous extraction, only 19 percent of the activity (n = 2) was recovered when methanol was used. However, using a mixture of 80 percent methanol: 20 percent water (v/v) yields an extract with constrictor activity of 100 percent (*ie* equal to the activity of the standard crude extract) (n = 9). Methanol, added to a solution of the standard bracts extract in the final ratio 80:20 (v/v) precipitated about ⅓ the material by volume, including undoubtedly the large proteins. All constrictor activity as tested by bioassay remained in the supernatant solution (n = 8).

Stability Properties

The resistance of the airway constricting agent(s) to various treatments is listed in Table 1. Heating the bract extracts to 80°C for ½ hr diminished the constrictor activity by only 11 percent. Likewise, exposing

the extracts under relatively mild conditions, to extremes of pH (3.0 on the acid side, 14.0 on the alkaline side) had little effect.

Size Characteristics

Components of the standard crude bract extract were separated according to molecular size on a sephadex G-10 column. Three successive fractions were collected from the column; the eluate within the exclusion volume, a ½ volume of eluate after the exclusion volume and prior to salt (NaCl) elution, and a ½ volume collected from start of the salt peak. These were adjusted to the original volume for bioassay. Constrictor activities of the three fractions were 10 percent (n = 3), 69 percent (n = 21) and 21 percent (n = 3) respectively. The fraction eluting immediately after the exclusion volume and containing 69 percent of the activity would be expected to contain components with a molecular weight less than 1,000 daltons.

Charge Characteristics

The active fraction from the sephadex G-10 column was passed through an anion exchange column of QAE-sephadex. The elution pattern is shown in Figure 3. All constrictor activity (n = 4) eluted between 50 and 100 ml of pH 7.2 phosphate buffer, demonstrating that at this pH the active constrictor agent(s) carries no net negative charge. This fraction, which has been adjusted to the original volume, is designated QAE-2. The constrictor activity did not bind to the cation exchanger, SP-sephadex, at pH 7.2 either (n = 2) and thus the agent(s) is not a cation.

Chemical Characteristics

The QAE-2 fraction contained 3.7 mg dry weight/ml. For this determination, buffer salts were largely removed from the QAE-2 fraction by running the QAE-sephadex column in 0.005M phosphate buffer, pH 7.2, desalting on a sephadex G-10 column, adjusting to original volume, and freeze-drying. The original standard crude extract (prepared with water) in comparison contained 63.0 mg/ml solids.

The QAE-2 fraction gave a positive Lowry assay equivalent to 0.07 mg protein/ml when albumin was used as the assay standard. The standard crude extract contained 6.8 mg protein equivalents/ml. Carbohydrate was also present, amounting to 0.54 mg glucose equivalents/ml (phenol-sulfuric acid assay) in the QAE-2 fraction compared to 8.4 mg/ml in the standard crude extract. In the QAE-2 fraction, the presence of carbohydrate was confirmed by the anthrone assay.

The QAE-2 fraction contains two or more fluorescing compounds. There is a band at 365 on excitation at 275 nm and a second broad band at 425 on excitation at 350 nm. In three other extracts which were prepared by different procedures and which were also active in inducing airway constriction, the 365 nm band was almost absent. The compound responsible for this band probably can be eliminated as contributing to constrictor activity.

Mass spectral analysis shows that fraction QAE-2 contains no compounds which volatilize up to 300°C under vacuum. The only compound given off by the dessicated fraction was water, the remainder of the fraction charred on the probe.

As purified, QAE-2 extract contained a minimum of

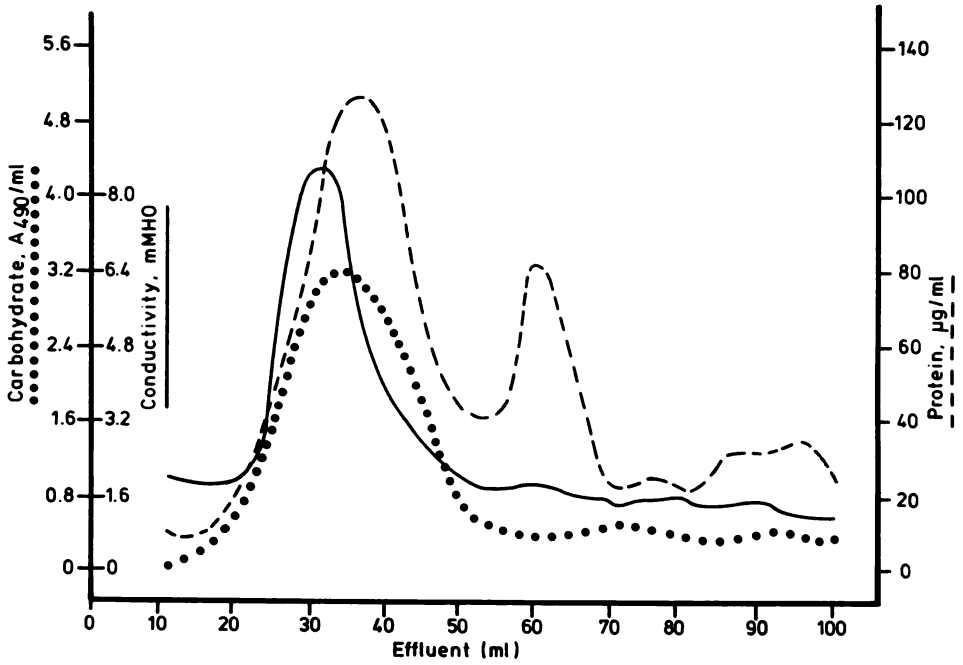

FIGURE 3. Elution pattern of the sephadex G-10 active fraction from the anion exchanger QAE-sephadex. Eluting solvent—0.05M phosphate buffer, pH 7.2. The dashed line is analysis for protein by the Lowry method, the dotted line carbohydrate by the phenol-sulfuric acid method and the solid line conductivity.

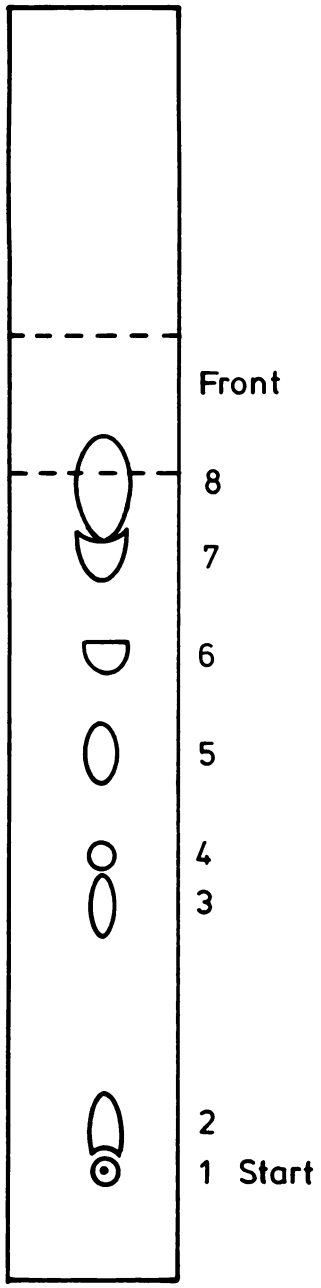

FIGURE 4. TLC tracing of purified fraction QAE-2 from bracts on silica gel G developed with chloroform: methanol: water (60: 40: 20). The dual dashed line solvent front results from the dual nature of the solvent system. All spots are iodine positive; spots 5,6 and 7, in addition, are ninhydrin positive; spot 8, in addition, exhibits UV fluorescence. Spot 1 represents a considerable amount of iodine positive material which remains at the start.

eight different compounds. Eight iodine-positive spots were readily detectable on the thin layer chromatogram of QAE-2 on silica Gel G developed in chloroform-methanol-water (Fig 4). Spots 5, 6, and 7 gave positive ninhydrin reactions[13] and spot 8 exhibited fluorescence under UV light. We were unable to obtain sufficient quantities of these spots for inhalation testing. Incomplete solubility of the QAE-2 extract at high concentrations, due possibly to the presence of buffer salts, prevented the use of preparative thin layer chromatography.

In two preliminary experiments we prepared a buffer-free purified extract. To our standard crude extract, prepared with water, methanol was added to a final ratio 80:20 (v/v). The supernatant solution was passed subsequently through a QAE-sephadex column and the active fraction collected was then chromatographed on a (preparative scale) on Whatman grade 3MM paper in a propranol-ammonia-water (6:3:1) system. Three spots were visible on the chromatogram, only one of which was ninhydrin-positive and it is the one which contained constrictor activity (n = 2).

Airway Constrictor Activity of QAE-2 Purified Extract

The airway constrictor response to our QAE-2 extract, for one subject, is shown in Figure 5. The response was similar to that elicited, for the same subject, (Fig 1) to the standard crude extract. The maximum decrease in lung function occurred at 90 min following exposure and, at that time, all lung function values were decreased: FVC, 4 percent; FEV_1, 3 percent; MEF40 percent, 11 percent; MEF40 percent (P), 31 percent. The apparent lesser response to the QAE-2 extract reflects the loss of activity on the sephadex G-10 column used in preparing the QAE-2 fraction. The QAE-2 extract elicited similar responses in other subjects (n = 4).

DISCUSSION

These studies, as well as previous ones, show that the airway constrictor activity of cotton bracts is highly water soluble. Given the aqueous environment of the lungs this property might help explain the high potency of cotton mill dust on human lung tissue. It is also a

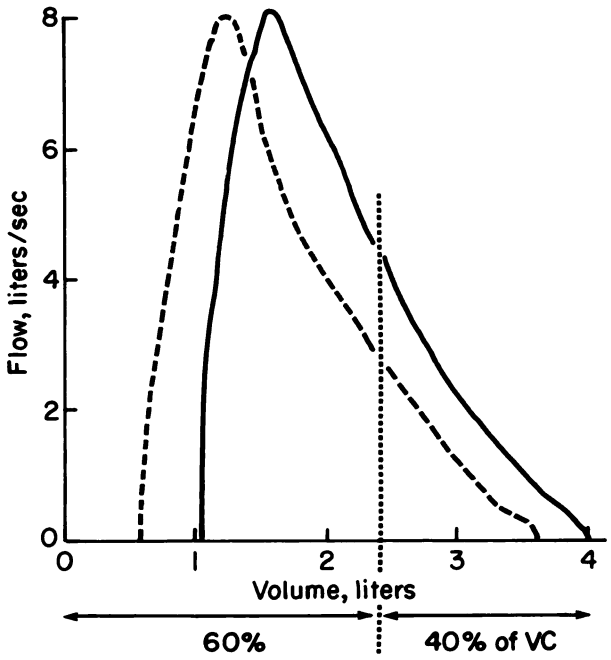

FIGURE 5. Single response to purified fraction QAE-2 from bracts. Partial expiratory flow-volume (PEFV) curves before (*solid line*) and 90 min after (*dashed line*) 10 min inhalation of purified extract aerosol, by a healthy 26-year-old woman. The lung volume level for measurement of MEF40 percent (P) is shown by the dashed vertical line. The point of maximum inspiration is indicated by the zero point on the volume axis. The entire partial expiration–maximum inspiration–maximum expiration maneuver was recorded without interruption; only partial expirations are shown.

property which supports the suggestion that washing cotton before it is processed in the mill might reduce the harmful effects of the dust, provided, of course, such treatment becomes technically feasible. Other properties of the airway constrictor agent(s) are its sparing solubility in methanol and other organic solvents (unpublished observations), its stability to heat and extremes of pH, its small molecular weight (>1,000 daltons) and its neutral charge. In addition, the presence of positive ninhydrin and anthrone assays with our most pure extracts suggests that the constrictor agent(s) may contain amino-nitrogen and hexose residues. Knowledge of these properties may prove useful in the design of new ways to reduce the toxic effects of cotton dust in textile mills.

Several compounds have been suggested as the causative agent in byssinosis. A lacinilene methyl ether compound with leukotactic properties has been isolated from cotton bracts.[14-16] However, its solubility properties (*eg*, soluble in ether but not in water) are quite different from that of the airway constrictor agent(s) in our bract extracts. Also, no volatile compounds such as lacinilene methyl ether were detected by mass spectral analysis of our active QAE-2 fraction. Another compound, bacterial endotoxin, has been detected in cotton dust.[17] Our extracts have been sterilized, removing bacteria, but we cannot, as yet, rule out the presence of endotoxins. Our experiments with gossypol glands are inconclusive. Endotoxin has several properties in common with components of our active purified extracts; however, its molecular weight is much greater than the 1,000 dalton limit of our airway constrictor agent(s). Cotton bracts and cotton dust may contain many compounds, several of which may be biologically active, but only compounds which cause airway constriction in humans (whether or not they have other activities) can be implicated as causative agents in byssinosis.

There is no animal model for byssinosis. Therefore, our bioassay by inhalation testing in humans, offers an appropriate method to reproduce, in the laboratory, some of the symptoms experienced by workers in the cotton dust mills. Our results show that an airway constrictor response on first time exposure to bract extracts is widespread —91 percent among healthy human subjects. These are naive individuals never before in contact with cotton dust. This may be of significance for future studies which attempt to screen workers for their sensitivity to cotton dust by using this type of challenge with bract extracts. Highly sensitive individuals could then be moved out of the dustiest work areas.

We have no explanation why smokers exhibited a lesser response to inhaled cotton bract extracts than nonsmokers. Our finding suggests, however, that at least smokers are not more sensitive to exposure than nonsmokers. Cigarette smoking has been cited as an important contributory factor in the production of byssinosis.[18] Other studies have shown that severe byssinosis occurs among nonsmokers as well as smokers[19] and concluded that the effects of smoking and of cotton dust exposure are roughly additive.[20]

The potency of the bract agent(s) compares favorably with the well known airway constrictor agent histamine. With our most pure bract extract (QAE-2), 3.7 mg dry weight/ml, inhaled for 10 min, are sufficient compared with histamine in which case 10-15 mg/ml (pure base inhaled for 30 seconds) are required to elicit a detectable response in healthy persons.[21] The 90-120 min delay to reach a maximum response to bract extracts is quite different from the response to histamine, which occurs within minutes and disappears in less than one hour. Further, in another study, Schachter et al[22] found no correlation between a subject's response to inhaled histamine and to bract extracts; those with the highest sensitivity to bracts were not necessarily the most sensitive to histamine. Three of these subjects (all responders) were also used in the study described in this paper.

Our present studies are concerned with an acute respiratory response in humans which is similar to the acute symptoms of cotton mill workers. It remains to be proved that elimination of the acute response in workers will prevent also the development of the chronic, debilitating, phase of the disease.

References

1 Buck MG, Bouhuys A. Byssinosis: airway constrictor response to cotton bracts. Lung 1980; 158:25-32

2 Morey PR, Sasser PE, Bethea RM, Kopetzky MT. Botanical trash present in cotton before and after saw-type lint cleaning. Am Ind Hyg Assoc J 1976; 37:407-11

3 Bouhuys A, Nicholls PJ. The effect of cotton dust on respiratory mechanics in man and in guinea pigs. In: Inhaled particles and vapours II, (Davies CN, ed). London: Pergamon Press, 1966:75-84

4 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265-75

5 DuBois M, Gillis KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem 1956; 28:350-56

6 Roe JH. The determination of sugar in blood and spinal fluid with Anthrone reagent. J Biol Chem 1955; 212: 335-43

7 Bouhuys A, Hunt VR, Kim BM, Zapletal A. Maximum expiratory flow rates in induced bronchoconstriction in man. J Clin Invest 1969; 48:1159-68

8 Bouhuys A, Mitchell CA, Schilling RSF, Zuskin E. A physiological study of byssinosis in colonial America. Trans N Y Acad Sci 1973; 35:537-46

9 Dautrebande L. Experimental observation of the participation of alveolar spaces in airway dynamics. In: Airway dynamics (Bouhuys A ed), Springfield, Ill.: Charles C Thomas, 1970:153

10 Virgulto J, Bouhuys A. Electronic circuits for recording of maximum expiratory flow-volume (MEFV) curves. J Appl Physiol 1973; 35:145-47

11 Schoenberg JB, Beck GJ, Bouhuys A. Growth and decay of pulmonary function in healthy blacks and whites. Respir Physiol 1978; 33:367-93

12 Nicholls PJ, Nicholls GR, Bouhuys A. Histamine release by compound 48/80 and textile dusts from lung tissue *in vitro*. In: Inhaled particles and vapours II (Davies

CN, ed), London: Pergamon Press, 1966:69-74
13 Rosen H. A modified ninhydrin colorimetric analysis for amino acids. Arch Biochem Biophys 1957; 67:10-15
14 Stipanovic RO, Wakelyn PJ, Bell AA. Lacinilene C, A revised structure, and lacinilene C 7-methyl ether from *Gossypium* bracts. Phytochem 1975; 14:1041-43
15 Jeffs PW, Lynn DG. Isolation and structure of 1-Hydroxy-7-methoxy-4-isopropyl-1, 6-dimethyl-2(1H)-naphthalenone from cotton. J Org Chem 1975; 40: 2958-60
16 McCormick JP, Pachlatko JP, Schafer TR: Total synthesis of lacinilene C methyl ether, a probable byssinotic agent. Tetrahed Lett 1978; 42:3993-94
17 Rylander R, Imbus HR, Suh MW. Bacterial contamination of cotton as an indicator of respiratory effects among card room workers. Br J Indust Med 1979; 36: 299-304
18 Noweir MH, El-Sadik YM, El-Dakhakhny AA, Osman HA. Dust exposure in manual flax processing in Egypt. Br J Ind Med 1975; 32:147-54
19 Bouhuys A, Schoenberg JB, Beck GJ, Schilling RSF. Epidemiology of chronic lung disease in a cotton mill community. Lung 1977; 154:167-86
20 Bouhuys A, Beck GJ, Schoenberg JB. Priorities in prevention of chronic lung diseases. Lung 1979; 156:129-48
21 Lundin G, Ringquist TR. Effects of histamine on pulmonary ventilation in man. Clin Sci 1960; 19:79-94
22 Schachter EN, Brown S, Zuskin E, Buck M, Kolach B, Bouhuys A. Airway reactivity in cotton bract induced bronchospasm. Am Rev Resp Dis (in press)

Comparative Prevalence and Severity of Emphysema and Bronchitis at Autopsy in Cotton Mill Workers vs Controls*

Philip C. Pratt, M.D., F.C.C.P.

Byssinosis is a clinical syndrome occurring in several industries, the major one of which is cotton textile, best characterized objectively by reduction in FEV_1 during a first workday after a weekend off or a vacation.[1-3] The reduction of FEV_1 is less pronounced on subsequent days of the work week. As a worker with the syndrome continues in his job, the degree of reduction of FEV_1 increases and the effect occurs on more days of the workweek. Eventually in some workers it can be observed every day, and the prework FEV_1 also is reduced. Some workers finally have chronic respiratory failure and are disabled for any form of work.[1-3]

Frequencies of each of these levels of impairment vary in different locations of the textile operation, being greatest in areas where cotton bales are opened and "carding" is carried out, somewhat lower in "spinning," and lowest in "weaving."[1] They also vary within locations according to atmospheric dust concentration.[4] However, it is clear that cases do occur in each area of most cotton mills. It is also apparent that byssinotic symptoms, FEV_1 reduction, and progression to disability are more common in cigarette smokers than in nonsmokers.[5]

Little has been written regarding morphologic correlates of the byssinosis syndrome. Most clinical observers who have considered this point agree that chronic bronchitis and emphysema are present in the lungs of persons with byssinosis, but it has not been shown that any specific lesion separates these cases from the general population.[3] If exposure to cotton dust produces byssinosis by the mechanism of causing the structural changes of bronchitis or emphysema, one should be able to observe increased prevalence or severity of these lesions in textile workers.

With colleagues I have recently reported[6,7] the results of a study of an autopsy population consisting of 659 inflation-fixed lungs, among which were 49 lungs from persons who had stated that they had been cotton mill workers. The present report describes the same data but is directed at different aspects of the interpretations and applicability of the findings.

Materials and Methods

Details of the origin and preparation of the lungs, quantification of lesions, and statistical analysis of the data were presented in the previous report[7] and will not be repeated here. The 659 lungs represented 75 percent of all autopsies done at this hospital over the four-year duration of the project. All subjects were men. The clinical records contained clear statements about smoking history for all but 94 of the cases and about main occupation for all but 54 cases. All quantitative measurements of pulmonary lesions were done without knowledge of the patient background, and clinical information was extracted from the records at a different time and without knowledge of the morphology. Statistical procedures included χ^2 tests of the proportional distribution of centrilobular emphysema (CLE) in the population groups and analysis of covariance[8] for the mean percentages of the measured lesions.

Many different classifications of emphysema exist. The one used in our laboratory includes only four types: centrilobular, panlobular, localized, and paracicatricial. Only centrilobular and panlobular emphysema are distributed diffusely in the lung so as to be compatible with an inhalational origin. Panlobular emphysema is uncommon, often familial, and has been associated with an inherited enzyme defect. Therefore only cases of centrilobular emphysema, by far the most common type, were counted as "emphysema" in this study.

Results

The tables from the previous report[7] are reproduced here (Tables 1 to 4) with permission of the editor of

*From the Departments of Pathology, Durham Veterans Administration and the Duke University Medical Centers, Durham, NC.
Supported in part by research grant HE-10753 from U.S. Public Health Service, Washington, D.C.
Reprint requests: Dr. Pratt, Pathology, Duke University, Durham 27710