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Pulmonary Function Analysis in the Rabbit Following Bronchochallenge to Causative Agents and Mediators of the Acute Byssinotic Response

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New Zealand White rabbits were acutely bronchochallenged for 5 min to ascertain airway responsiveness with six potential byssinogenic agents and mediators: 0.1 g/mL cotton dust extract (CDE), 0.1 g/mL cotton bract extract (CBE), 1 mg/mL endotoxin, 1 mg/mL *n*-formyl methionyl peptide (*n*-fMet), 10 mg/mL 5-hydroxytryptamine (5-HT), and 1 mg/mL prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Methacholine (MC), 10 mg/mL, was used as a control bronchoconstrictor. Clinically objective criteria were established using increases in resistance values compared to those obtained with saline controls. Animals were classified as: mild responders (Mi) = 125–149%; moderate responders (Mo) = 150–199%; or severe responders (S) = greater than 200%. Three of five (2Mo, 1S) rabbits showed increased pulmonary resistance to CDE bronchochallenge, 3/5 (1Mi, 1Mo, 1S) to CBE, 1/5 (Mo) to purified endotoxin, 4/5 (1Mo, 3S) to *n*-fMet, 3/5 (1Mi, 1Mo, 1S) to 5-HT, and 2/5 (1Mo, 1S) to $PGF_{2\alpha}$. All five rabbits (1Mo, 4S) responded to MC bronchochallenge. Rabbits responded minimally to saline, the common solvent of all test agents; however, when challenged with methacholine, a known bronchoconstrictor, rabbits showed significant overt symptoms of acute respiratory distress with immediate and substantial increases in resistance over saline controls. CDE, CBE, and *n*-fMet inhalation challenge resulted in a majority or all animals showing increased resistance. 5-HT contained in CDE and CBE, exhibited similar resistance increases; however, endotoxin, also found in cotton dust, showed little airway reactivity. The rabbit is useful for characterizing changes in pulmonary function parameters seen in the acute byssinotic reaction. This study has demonstrated that bronchochallenge in the rabbit with potential byssinogenic agents (CDE, CBE, endotoxin, and *n*-fMet) and mediators (5-HT and $PGF_{2\alpha}$) result in measurable changes in airway function, particularly increased resistance. Since bronchoconstriction is the major clinical manifestation of the acute byssinotic reaction in man and animals, it is likely that bronchoconstriction observed in cotton mill workers may be in part or totally the result of inherent dust constrictor substances or secondarily released mediators.

Introduction

Byssinosis, an occupational lung disease caused by the inhalation of cotton "dust," affects as many as a quarter of the cotton workers in the United States, and remains the number one health problem facing the textile industry (*1,2*). Acute and chronic byssinosis are differentiated by the severity of symptoms and, more importantly, by the persistence of symptoms during the work week and away from the work environment. The acute symptoms are reversible and particularly evident on Mondays following a weekend's absence from work, but gradually subside over a period of several days in the work environment or immediately when removed from the work environment. The acute byssinotic reaction can be described clinically by the symptoms of chest tightness, dypsnea, coughing, wheezing, and slight elevation in body temperature (*3*); physiologically by a drop in the 1-second forced expiratory volume ($FEV_{1.0}$) caused by respiratory airway constriction (*4*); and hematologically, by peripheral leukocytosis and leukocyte recruitment to air passages (*5*). These acute symptoms gradually develop into chronic irreversible symptoms which persist in a dust-free environment (*1,2*). Elimination of this chronic obstructive disease requires prevention of the acute disease and, thus, the identification of the etiological agents and mediators involved in the pathogenesis.

A number of studies in our laboratory have involved investigations of several proposed etiopathogenic mechanisms of byssinosis including chemotaxins (*6*), hista-

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mine (*7*), 5-HT (*8*), immunoglobulins (*9*), complement components (*10*), and arachidonic acid metabolites (*11*). These investigations have utilized *in vitro* bioassays for detecting smooth muscle constrictors in cotton dust (*11*), histamine release from platelets (*7*), and chemotaxis of polymorphonuclear leukocytes (PMNs) (*6*), as well as *in vivo* studies of bronchopulmonary lavage fluid (*12*) and pulmonary function of rabbits (*13*) and monkeys (*14*) following CDE inhalation.

Recent *in vitro* studies revealed that smooth muscle contracted in response to CDE and CBE. Methysergide (a 5-HT inhibitor) reduced the CBE-induced contraction by 82%. Indomethacin [a prostaglandin (PG) inhibitor] abolished 100% of the contraction. CDE-induced contractions were also totally blocked by indomethacin as well as salicylic acid (PG inhibitor) (*11*). *In vivo* studies of rabbit pulmonary lavage fluid have demonstrated a strong chemotactic response to CDE inhalation with maximum cellular recruitment (68% macrophages and 32% PMNs) occurring at 4 hr post-inhalation. More importantly, the data revealed for the first time, the *in vivo* release of arachidonic acid metabolites and 5-HT in the lung in response to CDE inhalation (*12*). These studies support the *in situ* and *in vitro* investigations by both Elissalde et al. (*15*), who demonstrated $PGF_{2\alpha}$ release by guinea pig lung perfused with CDE, and Fowler et al. (*16*), who demonstrated the release of $PGF_{2\alpha}$ by cultured alveolar macrophages after exposure to CBE.

The results of our studies have led to the development of a potential rabbit animal model of the acute byssinotic reaction. Initial studies of the rabbits' pulmonary function revealed that changes in pulmonary parameters, namely compliance and resistance, could be successfully measured when challenged with histamine and methacholine, known bronchoconstrictors, as well as extracts of cotton dust (*13*).

This paper describes the changes in rabbit pulmonary function parameters following bronchochallenge with MC, CDE, CBE, endotoxin, *n*-fMet, 5-HT, and $PGF_{2\alpha}$ and emphasizes the more important changes of increased pulmonary resistance which accurately reflects bronchoconstriction, the primary clinical symptom indicating airway reactivity.

Materials and Methods

Female New Zealand White rabbits (Rabbits, LTD) were anesthetized with sodium pentobarbital (40 mg/kg) via the marginal ear vein and were intubated with an endotracheal tube (North American Drager).

Experimental protocol and design was followed as outlined in our previous studies with the rabbit (*13*).

Five animals were tested per bronchochallenge agent. Rabbits were first challenged for 5 min with physiological (0.9%) saline at an inspiratory pressure of 15 ± 3 cm H_2O. During the experiment the rabbits' lungs were inflated with 25 cm^3 of room air (approximately 80% inspiratory vital capacity) at 15-min intervals post-exposure to prevent atelectasis. Pulmonary function was evaluated at 5-min intervals post-challenge with the final control values input into the computer at 15 min (if the parameters agree with the readings at 5 and 10 min, i.e., remain basically unchanged). Each animal thus acted as its own control. As an additional control in this study, three animals were tested in the exact same manner as the experimental rabbits, except that saline was used as the "test agent" after the initial saline bronchochallenge which established the baseline parameter levels. Data were collected over a 60-min period. Methacholine, a known bronchoconstricting agent, was used to determine the rabbits' responsiveness. Thirty breath exposures were used rather than 5 min due to severity of reactivity.

Table 1. Tested causative agents and mediators of the acute *byssinotic response.*

Substance	Concn	Source
CDE	0.1 g/mL	See Ainsworth (*17*) for details of preparation
CBE	0.1 g/mL	Prepared as for CDE (*17*)
Endotoxin	1 mg/mL	Standard endotoxin supplied by Cotton, Inc.
n-fMet	1 mg/mL	Sigma Chemical Co., St. Louis
5-HT	10 mg/mL	Sigma Chemical Co., St. Louis
$PGF_{2\alpha}$	1 mg/mL	Sigma Chemical Co., St. Louis
MC	10 mg/mL	Sigma Chemical Co., St. Louis

Bronchochallenge was performed with the potential etiologic agents and mediator substances listed in Table 1 and as described above for saline controls. Post inhalation data were collected at 5 min intervals to 30 min (45+ min if resistance values remained elevated).

Recent acquisition of a computerized pulmonary mechanics analyzer has greatly advanced the precision and accuracy as well as reduced laboratory error in determining pulmonary function parameters [respiratory flow (RF), transpulmonary pressure (TPP), tidal volume (TV), compliance (C), resistance (R), and respiratory rate (RR)]. Pulmonary function parameters were measured by using a computerized pulmonary mechanics analyzer. This computerized technology provided rapid and accurate assessment of airway changes before and after bronchochallenge with proposed etiologic agents and mediators of the actue byssinotic reaction.

Results

Eight proposed etiological agents and/or mediators of bronchoconstriction were tested via bronchochallenge in rabbits. Although several pulmonary function parameters were measured, resistance has been determined to be the most accurate measure of bronchoconstriction in the rabbit and perhaps the best indicator of airway reactivity. Clinically objective criteria were established using increases in resistance values, i.e. percent change from baseline saline controls, to identify responders from nonresponders. Animals were classified as: mild responders (Mi) = 125–149%, moderate responders (Mo) = 150–199%, or severe responders (S) = greater than 200%.

Table 2. Percent of baseline resistance values for saline bronchochallenge controls.

	% of baseline resistence						
Animals	5 min	10 min	15 min	20 min	25 min	30 min	60 min
1	105	100	101	99	108	108	69
2	99	107	107	75	71	65	116
3	69	65	46	58	63	69	82

With this criteria uniformly applied to all animals, the data revealed that saline bronchochallenge caused no substantial increase in resistance over a 60 min period (Table 2). This data supported the fact that any increase in resistance values observed over the 30 min period after the bronchochallenge was due to the bronchochallenge agent, i.e., solute, in the saline solvent.

Table 3 illustrates the number of responders, classification of response, percent change of resistance from saline baseline and the time of maximum response. Of the etiological agents tested, *n*-fMet caused the most consistent ($n = 4/5$) severe responders (1Mo, 3S) (Fig. 4). CDE (Fig. 1) and CBE (Fig. 2) produced increased resistance in 3/5 and 4/5 animals, respectively. CBE resulted in a slightly more delayed reaction than CDE and produced 1Mi, 1Mo, and 2S responders, whereas CDE produced 2Mo and 1S responder. However, one animal reacted to CDE with a 552% increase in R over baseline. Only one rabbit responded to purified endotoxin with a moderate level of reactivity (Fig. 3).

The potential mediator 5-HT produced 3/5 responders (1Mi, 1Mo, 1S) (Fig. 5) and $PGF_{2\alpha}$ produced 2/5 responders (1Mo, 1S) (Fig. 6). The time of maximum reactivity was approximately 10 min (average) for both agents. One animal responded to $PGF_{2\alpha}$ with a 569% increase in R over baseline, a level of reactivity which was similarly recorded for CDE.

All animals (5/5) responded to methacholine bronchochallenge (Fig. 7), a well known and frequently used bronchoactive agent. The reactivity was immediate (5 min) and so severe that data collection proved impossible after one minute post challenge.

All other bronchochallenge agents showed transient airway responses with maximum R as indicated in Table 3. Five animals failed to return to baseline within 30 min. However, in these animals it was felt this was due to delayed responses of 25 min or greater.

Discussion

This study demonstrates that the rabbit can be used successfully for the quantitative measurement of various pulmonary function parameters following bronchoprovocation. Several proposed etiological agents (CDE, CBE, endotoxin, and *n*-fMet) and mediators (5-HT and $PGF_{2\alpha}$) of the bronchoconstrictor response seen in the acute byssinotic reaction of man were tested to evaluate the ability of these agents to elicit bronchoconstriction. Pulmonary resistance was used as the key indicator of airway reactivity (bronchoconstriction).

Two types of controls were used in this study. One control involved bronchochallenge with saline, the common solvent of all bronchochallenge agents, and 3/3 an-

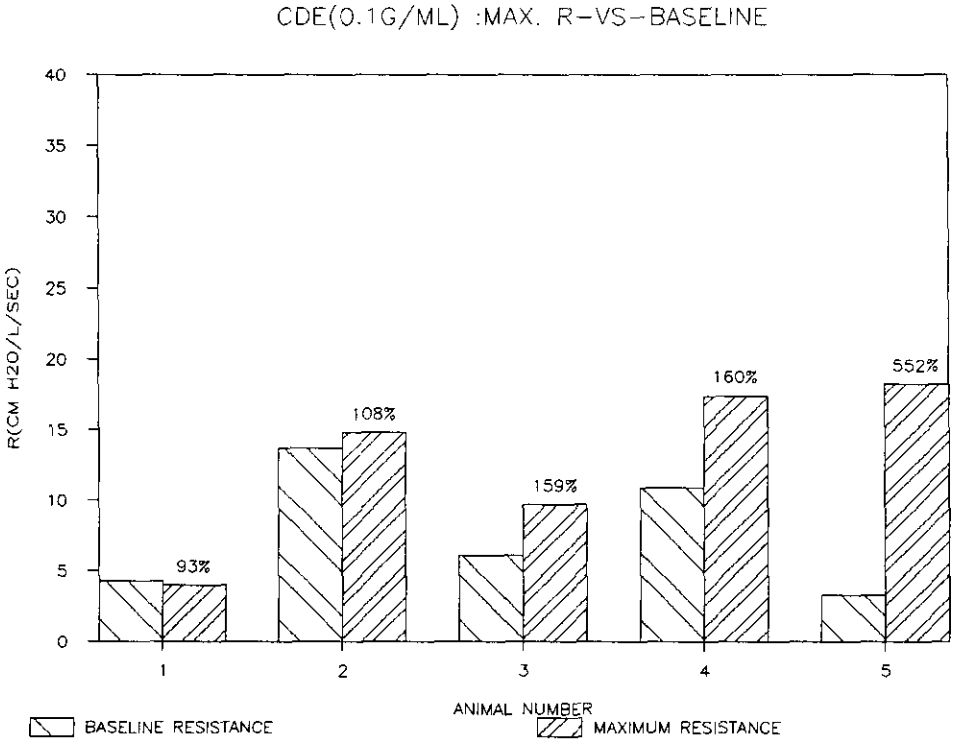

FIGURE 1. Maximum resistance vs. saline baseline resistance for CDE (0.1 g/mL). Animals 1 and 2 are nonresponders; 3 and 4 are moderate responders; and 5 is a severe responder.

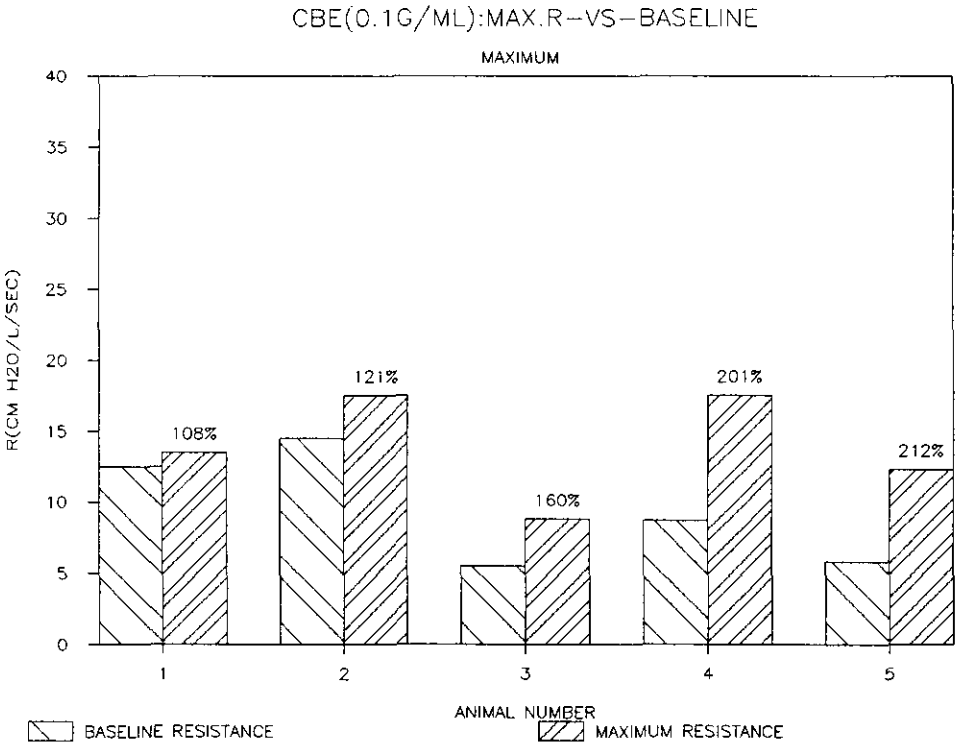

FIGURE 2. Maximum resistance vs. saline baseline resistance for CBE (0.1 g/mL). Animals 1 and 2 are nonresponders; 3 is a moderate responder; and 4 and 5 are severe responders.

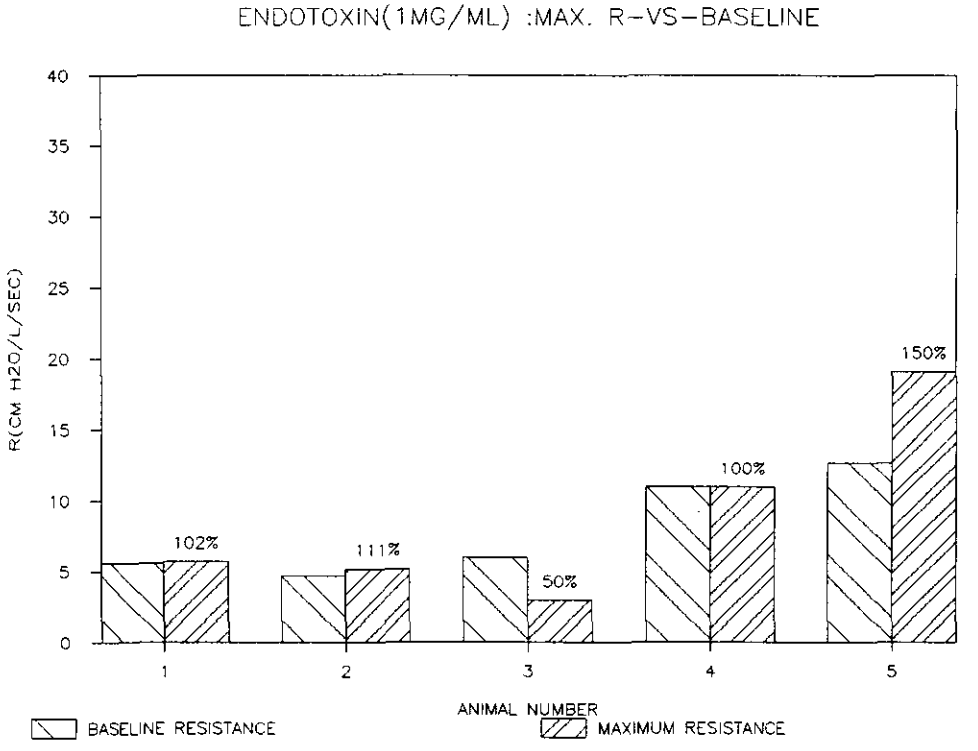

FIGURE 3. Maximum resistance vs. saline baseline resistance for endotoxin (1 mg/mL). Animals 1, 2, 3, and 4 are nonresponders and 5 is a moderate responder.

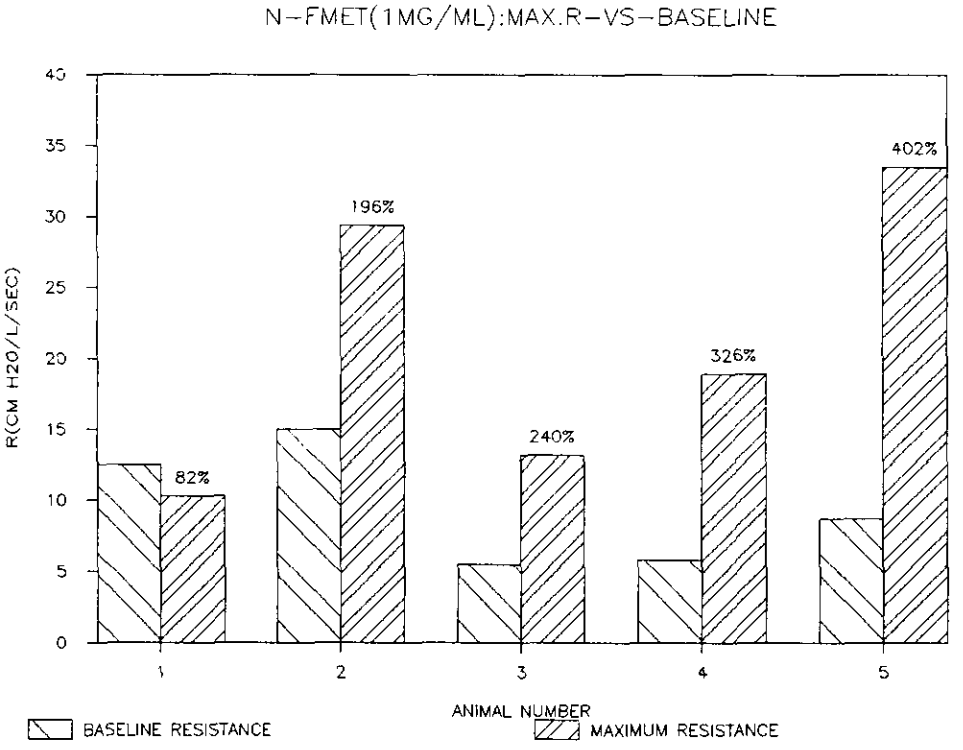

FIGURE 4. Maximum resistance vs. saline baseline resistance for *n*-fMet (1 mg/mL). Animal 1 is a nonresponder; 2 is a moderate responder; and 3, 4, and 5 are severe responders.

imals demonstrated no significant increase in R, i.e., R > 125%. Baseline reactivity was also established using saline prior to bronchochallenge with each test agent, thus allowing each rabbit to serve as its own control. This eliminated difficulties of group comparison due to animal to animal variation.

Methacholine and histamine are known bronchoconstricting agents often used in inhalation challenges in humans to evaluate nonallergic bronchial excitability (*13,18,19*). These agents provide a standard response to which other responses may be compared. In our previous studies with the rabbit histamine (diphosphate, MW = 307.1) at 10 mg/mL was shown to produce 12/14 responders with varying degrees of reactivity (R = 134–423%) (*13*). In the present study, methacholine (chloride, MW = 195.7) at 10 mg/mL resulted in 5/5 responders also with varying degrees of reactivity (R = 164–658%). Methacholine acts on smooth muscle muscarinic receptors (*19*) while histamine acts directly on smooth muscle H_1 receptors (*20,21*). The variation seen in bronchial responsiveness to these pharmacologically dissimilar drugs may be due to the difference in their receptor sites and possibly receptor numbers and activity. Cartier et al. (*22*) showed in studies with human asthmatics that histamine and methacholine have similar times to peak responsiveness. Responsiveness was measured by a decrease in pulmonary conductance and was found to be maximally decreased at 1.55 ± 0.52 min with histamine and 2.0 ± 1.1 min with methacholine. In our studies with histamine bronchochallenge in rabbits, histamine produced an average maximal response at 6.4 ± 4.1 min, whereas methacholine produced a severe response in less than 1 min in all rabbits tested. However, since the severity of the reaction prevented measurement of reactivity over time, it cannot be concluded that "peak" responsiveness occurs in less than 1 min. Nonetheless, the rabbits' responsiveness to methacholine demonstrates that the rabbits react to known bronchoconstricting agents and like humans, show individual variation in reactivity.

Bronchochallenge with aqueous CDE resulted in increased resistance in 3/5 rabbits. This supports our previous studies, in which 9/14 rabbits were responsive (*13*). The percent of responders in the two studies is basically the same, 60% and 64%, respectively. These percentages also correlate well with percentages in our past monkey studies (*14*). Six of eight monkeys (75%) responded acutely to CDE bronchochallenge at 10 min with either a 125% or greater increase in resistance or an 80% or greater decrease in $FEF_{10\text{-}50}$ (S. K. Ainsworth, unpublished data).

Cotton bracts are the major botanical component of cotton mill dust (*23*) and are thought to be the primary source of agent(s) responsible for the development of byssinosis (*24*). Bronchochallenge with CBE induces airway constriction with the same percent (60%) and level of reactivity as noted with CDE, but with an average peak response at 23 min, twice that observed with CDE. Investigations by Buck et al. (*25*) reveal similarities in acute airway constrictor responses of human volunteers to inhaled CBE and those of workers in cotton textile mills. Similar decreases in lung function post

Table 3. Classification of Responders

Bronchochallenge agent	Number of responders (R > 125%)	Classification[a]	Max R, %[b]	Time of max R, min[c]
Etiological agents				
Cotton dust extract	3/5	Mo	160	10
(0.1 g/mL)		Mo	159	20
		S	552	5
Cotton bract extract	3/5	Mo	160	10
(0.1 g/mL)		S	201	25
		S	212	35
Endotoxin (1 mg/mL)	1/5	Mo	150	20
n-Formylmethionyl peptide	4/5	Mo	196	25
(1 mg/mL)		S	240	20
		S	326	10
		S	402	10
Potential mediators				
5-Hydroxytryptamine	2/5	Mo	170	10
(10 mg/mL)		S	314	10
Prostaglandin $F_{2\alpha}$	2/5	Mo	174	5
(1 mg/mL)		S	569	15
Control bronchoconstrictor				
Methacholine (10 mg/mL)	5/5	Mo	164	I
		S	216	I
		S	263	I
		S	320	I
		S	658	I

[a] Mi = mild responder, Mo = moderate responder, S = severe responder.
[b] % = (Maximum increase in R/baseline saline R) × 100.
[c] I = immediate (< 5 min).

inhalation of aqueous CBE and CDE ("reference" samples) were also noted.

It has been well documented that crude CBE elicits strong contractions of airway smooth muscle (*26*), produces platelet aggregation and release of 5-HT (*27*) and TxA_2 (*28*), induces macrophage prostaglandin production (*29*) and produces non-allergic histamine release in lung slices (*30*). However, as suggested by Cloutier et

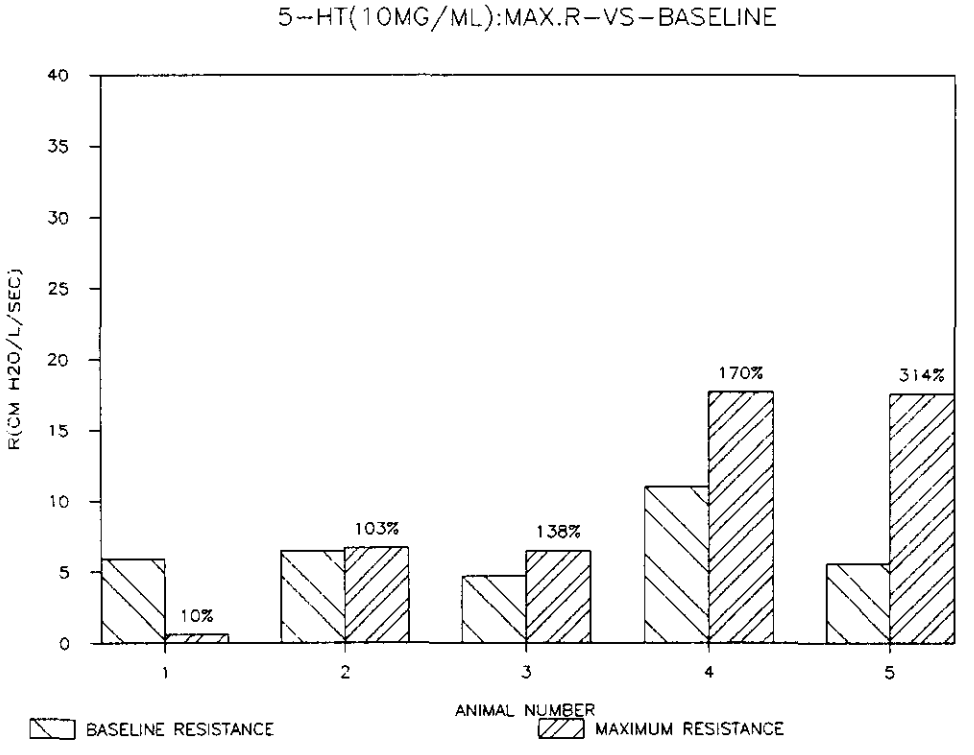

FIGURE 5. Maximum resistance vs. saline baseline resistance for 5-HT (10 mg/mL). Animals 1 and 2 are nonresponders; 3 is a mild responder; 4 is a moderate responder; and 5 is a severe responder.

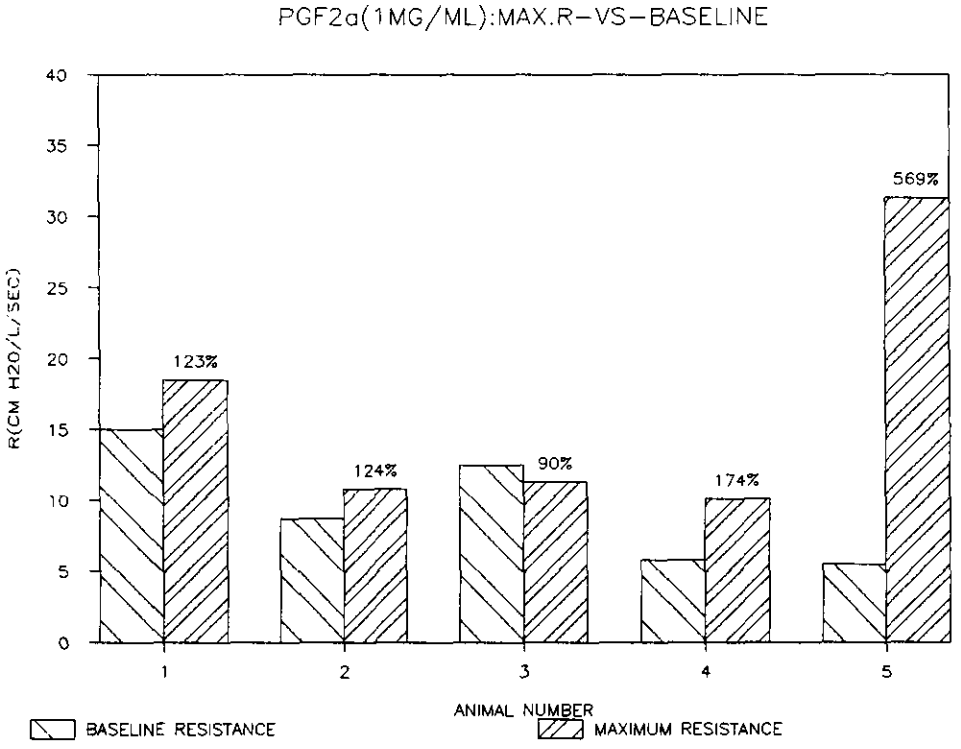

FIGURE 6. Maximum resistance vs. saline baseline resistance for $PGF_{2\alpha}$ (1 mg/mL). Animals 1, 2, and 3 are nonresponders; 4 is a moderate responder; and 5 is a severe responder.

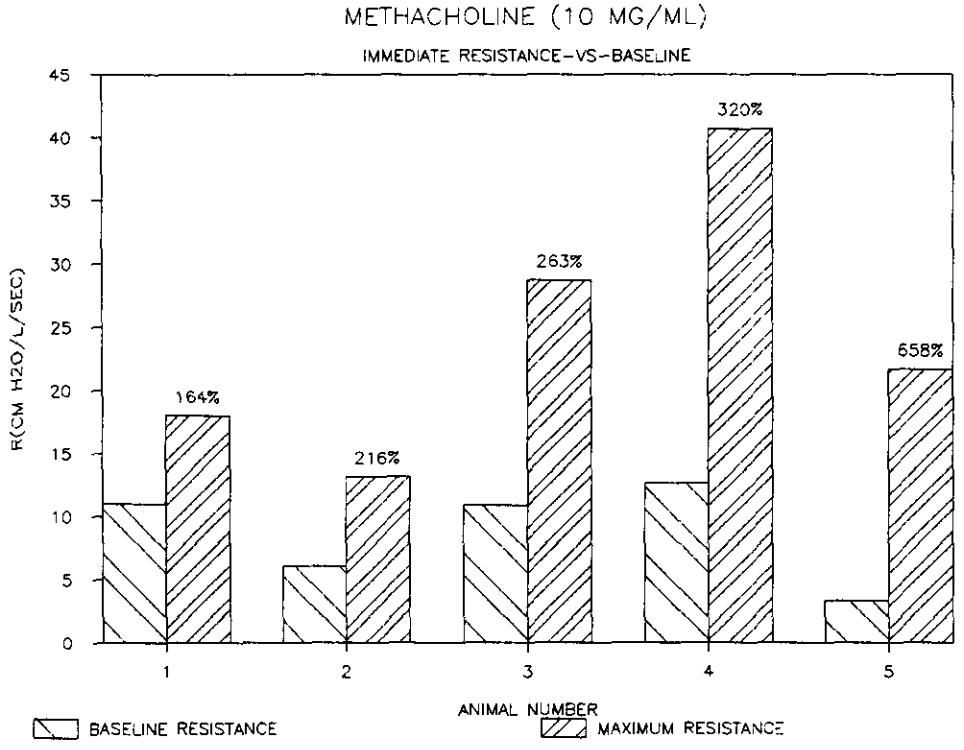

FIGURE 7. Maximum resistance values vs. saline baseline resistance for methacholine (10 mg/mL). Animal 1 is a moderate responder and 2, 3, 4, and 5 are severe responders.

al. (*31*), in order to produce local effects, the responsible compound(s) must be transported across the respiratory airway epithelium. The effects of CBE and one of its components, condensed tannin, was examined using isolated canine airway epithelium. CBE and condensed tannin was found to produce marked changes in the electrophysiologic properties of the tissue. This data revealed an alteration in epithelial function and an inhibition of active ion transport across the epithelium, effects contributed to the condensed tannin at low concentrations of CBE, but to another as yet unidentified compound at high concentrations (*31*). Subsequent studies by Cloutier revealed that CBE inhibited both active sodium absorption and active chloride secretion in canine trachea both of which were reversible (*32*) and that exposure of canine tracheal epithelium to CBE induced an alternative in the paracellular (shunt) pathway (*33*). It was suggested that this disruption in the integrity of the airway epithelium could permit access of compounds which are normally excluded from the interstitium, to airway smooth muscle and to the bloodstream, thus explaining the delay in symptoms of byssinosis after natural and laboratory exposure of CBE. Although these data strongly support tannins as the possible mediator of the alteration in epithelial function, it was stated that more likely this was the result of multiple compounds in CBE. Other possible constituents include endotoxin, a 5-HT agonist and histamine (*32*).

A study of the changes in cotton bract accompanying senescence (*34*) reveal a gradual decrease in the amount of water extractable material, amount of soluble tannins, platelet activity and 5-HT receptor agonist smooth muscle contracting activity. In contrast, both endotoxin content and acetylcholine smooth muscle contracting factor initially increase, but later decrease in the last 3 weeks of senescence. The majority of activity, then, appears to reside in the young, green bract. In fact, it has been shown that extracts from green bracts are more active than extracts from brown bracts in both bronchoactivity and platelet 5-HT release (*35*). However, the majority of the bract found in cotton dust is brown, senescent bract.

Studies by Buck et al. (*36*) show that the active agent in bract is removed with a simple water-extraction procedure. It was demonstrated that the removal of tannins, terpenoids, endotoxins, primary amines, and lacinilenes have no effect on the airway constricting activity of the extract when tested in naive human volunteers (*36*). Studies in our laboratory with *in vitro* bioassays and CBE-induced bronchoconstriction in human volunteers (*37*) suggest that the bronchoconstriction observed in response to semipurified fractions of CBE correlates exceptionally well with the *in vitro* smooth muscle constricting ability of these extracts, but does not correlate with complement activation, endotoxin concentration or chemotaxis.

Endotoxin inhalation has been proposed to explain symptoms of the actue byssinotic reaction (*38,39*) and has been implicated as the etiological agent (*40*). Endotoxins are present in respirable cotton dust (*41*) and are products of the bacterial populations of the cotton bract. It has been shown that the bacterial population increases with bract senescence (*34*) prior to cotton harvest. Thus, there are significant concentrations of bacteria and endotoxin in the air of the card room, concentrations of a magnitude that might be adequate to initiate biological effects (*42*). Russell et al. (*35*) have shown that despite the increased concentration of endotoxin with bract senescence, there exists an inverse relationship between airway responsiveness and endotoxin levels, thus suggesting that endotoxins are not the primary agent involved in airway contractions. Rylander, however, showed a causal role for bacterial endotoxins in byssinosis by demonstrating *in vitro* that pulmonary alveolar macrophages from guinea pigs exposed to endotoxin caused an increased migration of PMNs in a Boyden chamber. However, no migration could be elicited by endotoxin alone (*43*), a finding which agrees with data from our laboratory (*44*). It was also demonstrated that gram-negative bacteria in cotton causes PMNs to release a substance indicative of a platelet-activating factor (PAF-acether) capable of inducing platelets to release serotonin (*45*). Other investigations have revealed that a close relation exists between the amount of lipopolysaccharide (LPS) in cotton dust or inhaled air and the number of neutrophils invading airways after the exposure (*46*) and between $FEV_{1.0}$ changes over shift and the amount of airborne LPS (*47*). Removal of tannins and terpenoid aldehydes from the standard dust, potent agents in CBE, did not influence the dose-response relationship he found between neutrophils and LPS (*48*). Rylander supports the theory that endotoxin-mediated PMN recruitment to the lung subsequently results in lysosomal enzyme release and inflammation, a clogging of pulmonary vasculature and hence a feeling of chest tightness, PMN-induced platelet release of PAF-acether, and leukotriene production, both of which mediate bronchoconstriction (*48*).

In vivo studies with endotoxin, on the other hand, fail to support endotoxin as the etiological agent in byssinosis. Cavagna et al. (*38*) exposed normal subjects to an aerosol containing 40 to 80 μg of purified *E. coli* endotoxin. A significant decrease in $FEV_{1.0}$ was observed in only 2/8 normal subjects inhaling 80 μg of endotoxin. Pernis et al. (*49*) exposed three subjects to 3 mL of saline containing 5, 10, and 20 μg/mL of *E. coli* endotoxin which resulted in a slight reduction in $FEV_{1.0}$. More recently, Van der Zwan et al. (*50*) exposed 19 pulmonary patients to a solution containing 4 mg of *H. influenza* endotoxin and found a biphasic decrease in $FEV_{1.0}$, but only in those patients with a histamine reaction threshold of less than 32 mg/mL. Also in these studies, the amount of LPS required to cause significant respiratory function changes were larger than that found in the experimental card room.

Studies by Buck et al. (*51*) using purified CBE found a retention of up to 70% of the airway constricting activity compared to the crude aqueous CBE, despite the removal of endotoxin with methanol precipitation to concentrations of 1 ng/mL or less, insufficient quantities

to account for the airway constricting activity observed.

In our present study with purified endotoxin bronchochallenge in rabbits, 1/5 rabbits responded with a moderate increase in R over baseline values. These data appear to correlate with the percent reaction rate found in the aforementioned studies in humans.

Peptides, such as *n*-fMet, are potential etiological agents in cotton dust that have potent biological effects in minute concentrations. The structure of *n*-fMet peptides is peculiar to bacterial metabolism as intermediaries in protein biosynthesis. The biologic response to *n*-fMet peptides may be part of a defensive mechanism against bacterial infection, i.e., they have been shown to be strongly chemotactic for human PMNs (*52*) and rabbit PMNs and macrophages (*53*), and to cause the release of histamine in human basophils (*54*). Thus, like the complement-derived C3a and C5a anaphylatoxic (histamine-releasing) fragments, *n*-fMet peptides also possess direct histamine-releasing properties which are unrelated to IgE activation of basophils and tissue mast cells. Also, the histamine-releasing ability of these peptides correlates well with their chemotactic activity (*54*). Snyderman and Pike, however, found *n*-fMet peptides to have no chemotactic activity toward equine PMNs (*55*), even though there are high affinity receptors for these peptides on their surface and the cells were found to respond chemotactically to C5a and zymogen-activated horse plasma (*56*). They did find, however, that equine leukocytes secreted lysosomal enzymes in response to *n*-fMet peptides (*55*). Thus, they suggested that secretion and chemotaxis induced by *n*-fMet peptides are initiated by similar, but independent receptors.

In the present study, we determined that a pure solution of *n*-fMet peptides in saline caused a response in 4/5 rabbits. The response was consistently greater than all the other potential etiological agents tested. This response might be attributed to the histamine-releasing abilities of *n*-fMet peptides as the times to peak response and levels of reactivity are similar to our results with histamine bronchochallenge in the rabbit (*13*). Two animals, however, appear to have delayed peak responses (20 and 25 min), later than any peak response observed with histamine. This may suggest that there is a secondary mechanism extending the bronchoconstrictor response as has been suggested in our exposure studies with the monkey (*14*).

5-HT is found in cotton dust and bract and is therefore considered a potential etiological agent of the acute byssinotic reaction. Analysis of Standard West Texas cotton dust (38–75 μm size) in our laboratory has determined the 5-HT content to be 6.78 ± 0.22 μg 5-HT/g dust (*17*). 5-HT is also considered a potential mediator of the acute byssinotic reaction as it is released from human platelets exposed to extracts of cotton bract (*57*) and the major bacterial contaminant of cotton dust, *Enterobacter agglomerans* (*58*). *In vitro* studies in our laboratory of CDE- and CBE-induced smooth muscle contraction (*11*) have shown CDE and CBE to contain a smooth muscle-contracting agent believed to be 5-HT. CDE and CBE contract smooth muscle with forces equivalent to a 0.32 ± 0.05 μg/mL and 0.7 ± 0.1 μg/mL concentration of 5-HT, respectively. Although the concentration of contractor substance varies, subsequent studies show there is no evidence of a qualitative difference in various samples of cotton dust and bract tested (*8*). Besides 5-HT, no evidence was found for any other direct contractor agent in CDE and CBE (*8*). However, CBE-induced contractions were blocked by methysergide, a 5-HT antagonist, while CDE-induced contractions were not. A methysergide concentration that would block 75 ± 2% of 5-HT activity would block 59 ± 6% of CBE activity and only 12.6 ± 4% of CDE activity (*11*). Therefore it appears that 5-HT content of cotton dust and bract extracts may be an important factor to consider in the acute byssinotic reaction and that the two extracts probably operate at different receptor sites on the smooth muscle.

Bronchochallenge with 5-HT in rabbits produced 3/5 responders (1 Mi, 1 Mo, 1S), all with peak resistance values at 10 min. The level of reactivity is within the range of that observed with CDE and CBE.

Arachidonic acid metabolites [prostaglandins (PG), thromboxanes (Tx), and leukotrienes] have recently been implicated as potential mediators of the acute byssinotic reaction (*11,12,28*). $PGF_{2\alpha}$ and TxA_2 are known potent constrictors of respiratory smooth muscle (*59,60*). $PGF_{2\alpha}$ has been shown to act directly on bronchial smooth muscle (*61*) and to promote bronchial secretions (*62*). Studies in our laboratory have demonstrated the *in vitro* release of $PGF_{2\alpha}$ and TxA_2 from human platelets (*28*). The observation of Bomski et al. (*63*) of peripheral thrombocytopenia in byssinotics over the workshift might indicate that pooling or sequestering of platelets occur in the human lung. This action may occur concomitantly with release of $PGF_{2\alpha}$ and TxA_2 locally in the lung and constriction of respiratory airways. Our studies with smooth muscle-contracting agents (*11*) demonstrated that CDE-induced contractions of rat stomach muscle were blocked 100% by indomethacin and salicylic acid, both PG synthesis inhibitors. Interestingly, the portion of CBE-contracting ability not blocked by methysergide was blocked totally by indomethacin. Thus, it appears that cotton dust and bract contain a substance that causes the release of $PGF_{2\alpha}$, which in turn causes the contraction of smooth muscle. In fact, radioimmunoassay confirmed $PGF_{2\alpha}$ release from rat fundal smooth muscle when exposed to CDE and CBE. In support of these data, our laboratory has demonstrated the *in vivo* release of arachidonic acid metabolites in rabbit lavage fluid in response to CBE (*12*). $PGF_{2\alpha}$ concentrations increased from baseline levels of 1.0 ng/mL to a maximum of 24.6 ng/mL at 4 hr. These data suggest that cotton dust-induced release of arachidonic acid metabolites may be an important mechanism in the pathogenesis of byssinosis.

Bronchochallenge with $PGF_{2\alpha}$ in rabbits results in increased resistance in 2/5 rabbits (1 Mo, 1 S), the same percent and level of reactivity as found with 5-HT. This demonstrates *in vivo* the ability of $PGF_{2\alpha}$ to induce

bronchoconstriction and supports the suggested involvement of arachidonic acid metabolites in the pathogenesis of the acute byssinotic reaction.

The rabbit has proven to be useful for characterizing changes in pulmonary function parameters which occur in the bronchoconstrictor response seen in the acute byssinotic reaction of man. This study has demonstrated that bronchochallenge in the rabbit with several potential etiological agents (CDE, CBE, endotoxin, and *n*-fMet) and mediators (5-HT and $PGF_{2\alpha}$) results in measurable changes in airway function, particularly increased resistance. As bronchoconstriction is the major clinical manifestation of the acute byssinotic reaction in man and animals, it is likely that the bronchoconstriction observed in cotton mill workers may be in part or totally the result of inherent dust constrictor substances or secondarily released mediators.

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