Chapter 16. Endotoxin as an Etiologic Agent of Byssinosis: Evidence From Experimental and Epidemiological Studies Involving Human Exposure to Cotton Dust

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During the decade and a half since the promulgation of the OSHA Cotton Dust Standard (OSHA 1978), results of new studies have intensified interest in the hypothesis that inhalation of gram-negative bacterial endotoxin causes byssinosis. This chapter reviews historical and recent clinical and epidemiological research relevant to endotoxin as a possible causative agent of byssinosis.

Historical Findings (Through 1970)

Ramazzini (1713), describing the use of animal dung as an agent used to facilitate retting of flax and hemp, recorded what may be the first observation of direct relevance to the possible etiologic role of endotoxin in byssinosis: ". . . hemp and flax are macerated in stagnant, putrid waters and are first smeared with filth to hasten the necessary maceration when they are submerged under water, and so the particles that the carders breathe in must be poisonous and highly injurious to human beings."

The first clear suggestion that endotoxin may cause respiratory disease associated with the inhalation of cotton dust came as a consequence of a U.S. Public Health Service investigation of an outbreak of acute respiratory illness among individuals making mattresses from a stained low-grade cotton. The severity of illness varied with degree of exposure, and the symptoms started 1 to 6 hr after work with the cotton began and lasted 2 to 5 days. Along with fever, fatigue, generalized aches, anorexia, headache, nausea, and vomiting, many affected individuals experienced "substernal discomfort or pressure, varying in intensity and duration, so that the person was unable to take a deep breath" (Neal et al. 1942).

The cotton associated with these outbreaks was extremely dusty and heavily contaminated by a gram-negative organism referred to by Neal et al. (1942) as the "cotton bacterium." This organism was found in abundance in samples of cotton from a commercial mill in which workers frequently experienced mill fever and was found in varying concentrations on other cotton samples but was not found on samples of high-grade, white cotton. Based on this evidence and results of experimental human inhalation challenges involving samples from the outbreak and other cottons, the authors concluded that the gram-negative cotton bacterium or its product(s) caused the outbreaks of mattress-maker's illness. Furthermore, they hypothesized that this gram-negative bacterium probably causes mill fever and may also be involved in the etiology of chronic lung disease among cotton mill workers.

Airborne Bacteria in Cotton Textile Mills

The presence of large numbers of gram-negative bacteria (GNB) in cotton dust had been reported earlier (Report of the Departmental Committee 1932). However, since cotton workers suffering from byssinosis were not clinically infected, this observation received little attention at the time. Later, Furness and Maitland (1952) studied the microflora in cotton dust and found in excess of 1 billion viable colony-forming units (cfu) of GNB per gram of dust. In a study reported by Drummond and Hamlin (1952) and Hamlin (1952), much higher airborne concentrations of viable bacteria were found in the cardroom than in mill areas associated with later processing stages, and airborne bacteria concentrations were also found to vary by growing region and crop year.

Tuffnell (1960a) observed a predominance of gram-positive *Bacillus* species in the air of cotton and jute mills and an apparent crude association between byssinosis prevalence and the concentration of live *B. pumilis* and *B. subtilis* organisms. A single medium was used to culture both GNB and gram-positive bacteria instead of separate selective media for each of these types of bacteria. Notwithstanding limitations of his preliminary epidemiological approach, Tuffnell (1960b) attempted to experimentally induce typical symptoms in byssinotic patients with aerosolized *Bacillus* organisms. These attempts were unsuccessful.

Roach and Schilling (1960) epidemiologically demonstrated that byssinosis prevalence correlated

with the "protein" fraction of cotton dust rather than with the "cellulose" or "mineral" fractions. Citing Tuffnell's work to discount bacterial involvement in the etiology of byssinosis, they concluded that their own finding "points to some constituent of the plant debris as the active agent." This conclusion ignored the fact that Tuffnell's work focused on gram-positive bacteria and may have served to divert research attention away from the microbiological etiology of byssinosis and toward a plant component (for example, bract) etiology.

Biologically Retted Versus Chemically Retted Flax and Hemp

Bouhuys et al. (1963) noted the absence of Monday chest symptoms and leukocytosis and the absence of a decline in acute forced expiratory volume in one second (FEV_1) among workers at a plant that processed chemically retted flax, an observation that contrasted sharply with findings of a similar survey at a plant that processed biologically retted flax. This observation suggested that the microbes responsible for biological retting may be involved in the etiology of byssinosis. Others have similarly noted that dust from chemically retted hemp is better tolerated by workers than dust from biologically retted hemp (Barbero and Flores 1967) and that dust from chemically retted flax fails to cause the significant acute decline in FEV_1 seen with dust from biologically retted flax (British Occupational Hygiene Society 1980).

The Milan Group: Epidemiological and Experimental Evidence

Pernis et al. (1961) confirmed the presence of endotoxin in cotton dust and performed a variety of animal and human exposure studies that supported endotoxin's causal role. They also briefly described an investigation of a typhoid vaccine factory where substantial concentrations of airborne GNB occurred several times a year during the centrifugation of large quantities of *Salmonella typhi*. Many workers regularly experienced transient low-grade fever following this particular process, and some were excluded from this work area because of a tendency to develop acute respiratory difficulties.

Since various taxonomically different plants (for example, cotton, flax, and hemp) had been implicated in byssinosis and related disorders, Pernis et al. (1961) concluded that a component of the cotton plant was an unlikely candidate for etiologic agent. Acknowledging the likely presence of other cotton dust components that demonstrate activity in vitro, they concluded that gram-negative bacterial endotoxin accounted for a substantial portion of the pharmacologic activities of cotton dust. Furthermore, they suggested that endotoxin tolerance (known to occur after sequential exposures) and the body's loss of this temporary state of resistance to the effects of endotoxin (upon interruption of sequential exposures) might account for the classical reappearance of byssinosis symptoms each Monday. Pernis et al. (1961) clearly favored endotoxin as the cause of mill fever, and suggested that byssinosis may be due to repeated inhalation of smaller amounts of endotoxin.

Later, this same group of researchers published what appears to be the first epidemiological evidence suggesting that the prevalence of byssinosis depends more on airborne endotoxin concentration than on airborne dust concentration (Cavagna et al. 1969). Correlations calculated from results in that report indicated that the prevalence of byssinosis was strongly correlated with airborne endotoxin concentration (r=0.99; p<0.01) but not significantly correlated with airborne dust concentration (r=0.88; p>0.12). Along with results of experimental exposures of animals and humans to *E. coli* lipopolysaccharide (LPS), this evidence provided additional support for endotoxin's role in the etiology of byssinosis (Cavagna et al. 1969). However, the authors concluded that "we are not unaware...that cotton extracts contain other pharmacologically active substances. Therefore, we are far from saying that endotoxins are the sole cause of byssinosis, but they may well be one of the causes."

Recent Experimental Studies Involving Human Exposure to Cotton Dust

For obvious ethical reasons, attempts to elucidate the etiology and pathogenesis of byssinosis by experimentally exposing human volunteers have been limited to the investigation of short-term, reversible effects of dust inhalation. Experimental human exposures that involve conditions typical of the mill industry (for example, exposure to dust

levels typically generated by a card for a period of several hours), particularly exposures to airborne endotoxin, are reviewed in the following paragraphs. Table 67 summarizes these studies. (Experimental studies of human response to inhalation of specific components of cotton dust, including LPS or GNB aerosols, are reviewed elsewhere in this book.)

Exposures to Dust from Washed Cotton

In one type of experiment using human subjects, both endotoxin contamination and potency of card-generated cotton dust (in terms of its ability to induce chest tightness and fever and decrease across-shift FEV_1) have been markedly reduced or eliminated by washing the cotton before carding (Rylander and Haglind 1981, Haglind and Rylander 1984, Castellan 1986, Petsonk et al. 1986). Although prewashing markedly reduces endotoxin content of cotton dust, the concentrations of other components are also reduced. Therefore, results of washing experiments do not by themselves represent strong support of the hypothesis that endotoxin is a causal factor of byssinosis and will not be further discussed.

Exposures to Dust from Untreated Cotton

A second type of experiment involving assessment of human responses to card-generated dust has taken advantage of natural variation in endotoxin contamination of cotton dust. Cottons of different grades and possibly from different growing regions are carded separately. These cottons vary in degree of endotoxin contamination and can therefore be used to produce different concentrations of airborne endotoxin independent of dust concentration and presumably some (but admittedly perhaps not all) other components of the dust. Several such studies have documented an association of fever and symptoms with high endotoxin concentration and an exposure-effect relationship between endotoxin or GNB and a decrease in across-shift FEV_1 (Millner et al. 1983, Olenchock et al. 1983, Rylander and Haglind 1983, Castellan et al. 1984, Rylander et al. 1985, Rylander and Haglind 1986, Castellan et al. 1987). Table 67 summarizes the results of these experimental human exposure studies.

The most clear-cut finding from a study of this type was reported by Castellan et al. (1987). These investigators analyzed results of a series of 108 separate experimental exposures (of groups of 24–35 subjects at a time) to controlled levels of dust generated from carding 32 different cottons. Airborne dust concentration did not correlate with airborne endotoxin concentration (r=0.07, p=0.46). The group mean FEV_1 response did not correlate with dust concentration (r=-0.08, p=0.43). In contrast, a clear exposure-response relationship was observed between group mean FEV_1 response and endotoxin concentration (r=-0.74, p<0.0001).

Logarithmic transformation of endotoxin concentrations clarified the relationship at lower endotoxin levels and resulted in a strengthened correlation between endotoxin concentration and FEV_1 response (r=-0.85, p<0.0001). Whereas all 51 endotoxin exposures above 50 ng/m^3 resulted in significant mean FEV_1 responses, none of the eight endotoxin exposures below 10 ng/m^3 resulted in a significant response, and a linear regression model based on the data predicted a threshold for response at 9 ng/m^3 (Castellan et al. 1987). Although these experimental results do not by themselves prove that endotoxin is causal, they strongly support the hypothesis that endotoxin plays a major role in the cause. In contrast to the washing experiments, concentrations of cotton dust components were not artificially altered in these studies.

The relationship observed by Castellan et al. (1987) between airborne endotoxin concentration and acute decline in FEV_1 is only likely to have been observed if the causal agent is endotoxin or some other cotton dust component with a concentration that closely parallels that of endotoxin. Although the concentrations of many cotton plant products do not parallel the concentration of endotoxin (Bell 1986, Bell et al. 1986), it has been suggested that concentrations of certain phytoalexins (for example, cadelenes and lacinilenes), which are toxic compounds produced by the cotton plant in response to microbial infections, might roughly correlate with endotoxin concentration (Greenblatt and Bell 1986). However, unlike the situation with endotoxin, there is no existing clinical or epidemiological evidence to indicate that these phytoalexins play a causative role.

Table 67. Results of studies in which humans were experimentally exposed to card-generated dust from untreated cotton (limited to studies in which the effects of airborne endotoxin or airborne GNB were evaluated)

Study	Maximal airborne concentrations* Dust (mg/m^3)	Endotoxin (ng/m^3)	GNB (cfu/m^3)	Number of subjects†	Results of study
Millner et al. (1983)	Not reported (VE)	Not tested	22,900 (Andersen; gram-negative selection agar)	56 (Healthy cotton dust "reactors")	Individual FEV_1 change correlated with GNB (r=-0.51) and total bacteria (r=-0.47) (both p<0.001) and dust (r=-0.26, p<0.01) but not with fungi (r=-0.31). Six-hour exposures. Data represent a subset of data analyzed in Castellan et al. (1984).
Olenchock et al. (1983)	Not reported (VE)	Not reported (VE dust; spectrophotometric LAL)	Not tested	56 (Healthy cotton dust "reactors")	Individual FEV_1 change correlated more strongly with endotoxin (r=-0.51, p<0.001) than with dust (r=-0.26, p<0.01). Six-hour exposures. Data represent a subset of data analyzed in Castellan et al. (1984).
Rylander and Haglind (1983)	2.58 (VE)	8,030 (VE dust; gel titration LAL)	Not tested	11 (Smoking cotton workers and workers with byssinosis)	Mean FEV_1 change correlated highly with endotoxin (r=-0.96, p<0.001) but not with dust (r=-0.25). No correlation between dust and endotoxin (r=+0.06). "Threshold" at 1,000 ng/m^3. Four-hour exposures. Data represent a subset of data analyzed in Rylander et al. (1995).
Castellan et al. (1984)	0.55 (VE)	700 (VE dust; spectrophotometric LAL)	Not reported (Andersen; gram-negative selection agar)	54 (Healthy cotton dust "reactors")	Mean FEV_1 change correlated with endotoxin (r=-0.94), GNB (r=-0.91), total bacteria (r=-0.71) (all p<0.00001) and dust (r=-.34, p<0.05) but not with fungi (r=-0.14). Fever occurred after highest endotoxin exposure. Six-hour exposures. Data represent a subset of data analyzed in Castellan et al. (1987).
Rylander et al. (1985)	3.16 (VE)	8,030 (VE dust; gel titration LAL)	Not tested	15 (Smoking cotton workers and workers with byssinosis)	Mean FEV_1 change correlated with endotoxin (r=-0.56, p<0.05) but not with dust (r=0.01). "Threshold" at approximately 33 ng/m^3 endotoxin. No correlation between dust and endotoxin. "Mill fever" occurred after highest endotoxin exposures. Four-hour exposures.
Rylander and Haglind (1986)	3.6 (VE)	8,000 (VE dust; gel titration LAL)	Not tested	13 (cotton workers with and without byssinosis)	Individual and group mean FEV_1 change both correlated with logarithm endotoxin (p<0.01) and not with logarithm dust. No correlation between dust and endotoxin. "Mill fever" occurred after highest endotoxin exposures. Four-hour exposures.
Castellan et al. (1987)	0.55 (VE)	779 (VE dust; spectrophotometric LAL)	Not tested	33–61 (Healthy cotton dust "reactors")	Mean FEV_1 change correlated with endotoxin (r=0.74) and log endotoxin (r=0.85) (both <0.0001) but not with dust (r=0.08). No correlation between dust and endotoxin. "Threshold" at approximately 9 ng/m^3 endotoxin. Six-hour exposures.

*VE, Vertical elutriator.

†Cotton dust "reactors" are individuals selected on the basis of their reaction to cotton dust in preliminary exposures.

In one human study involving exposure to cotton dust, concentrations of several plant products and endotoxin were measured. FEV_1 responses to card-generated cotton dust from glanded cotton (containing gossypol) and to dust from glandless cotton (lacking gossypol) were equivalent at comparable airborne endotoxin concentrations but differing concentrations of dust, tannin, and terpenoid aldehydes (Rylander 1988). In an earlier study, acute FEV_1 responses to dust from cotton from which bracts had been removed prior to boll opening and to dust from cotton grown in an adjacent plot but with bracts left intact were equivalent at comparable airborne dust and endotoxin concentrations (Castellan et al. 1986). These observations support a causal role for endotoxin but not for bracts (Castellan et al. 1986) or the measured plant components (Rylander 1988).

Recent Epidemiological Studies in Cotton and Flax Mills

More than a dozen studies have been conducted and published on the possible association between byssinosis and inhalation of either endotoxin or GNB (the natural source of endotoxin in cotton and flax mills). With one exception discussed briefly above (Cavagna et al. 1969), all have been published since 1977. Table 68 summarizes these epidemiological studies.

Cinkotai's Surveys on Series of Symptoms

In a cross-sectional epidemiological survey, Cinkotai et al. (1977) evaluated prevalence rates of Monday chest symptoms among 720 workers employed in the cardrooms of several British cotton mills with very different dust concentrations and in a wool mill, a tea packing plant, and a tobacco factory. Participation rates were good, ranging from 81 to 100 percent of employees in each surveyed mill. Byssinosis prevalence in the various cotton mills surveyed ranged from 7 to 40 percent, while employees in the other mills lacked such symptoms. Overall analysis revealed no association between byssinosis prevalence and mean dust concentration (which ranged up to 15 mg/m^3 by plant), prompting the authors to comment that "the results clearly demonstrate the weakness of the [dust] standard designed to eradicate byssinosis."

In contrast, a very strong correlation (r>0.95, p<0.001) was observed between byssinosis prevalence and airborne-viable GNB concentration (which ranged up to 3,500 cfu/m^3 as quantified using Andersen impactor sampling and selective culture media). Endotoxin concentration, measured by a tube dilution method of the Limulus amebocyte lysate (LAL) clot test, was not highly correlated with byssinosis prevalence; however, the authors appear to have evaluated the correlation of symptom prevalence with endotoxin concentration in airborne dust (that is, per milligram) rather than with actual airborne endotoxin concentration (that is, per cubic meter). Cinkotai et al. (1977) concluded their report by stating that "the present investigation has demonstrated that airborne GNB in cotton mills are closely linked with the prevalence of byssinotic symptoms."

In a subsequent cross-sectional study, Cinkotai and Whitaker (1978) used the same survey methods in cardrooms of 21 major cotton spinning mills in Lancashire (including the 7 mills studied in their earlier investigation). Overall, 1,057 workers answered the questionnaire—a 93 percent participation rate. As in the earlier study, prevalence of Monday chest symptoms (which ranged from 0 to 40 percent by mill) was not significantly correlated with total dust (which ranged up to 3.85 mg/m^3 by mill) but was correlated (r=0.66, p<0.002) with airborne viable GNB (which ranged up to 6,150 cfu/m^3 by mill).

When interactions of the concentration and the number of years of exposure were evaluated, the strongest correlation also involved GNB (r=0.73, p<0.001). The correlation coefficient for GNB and Monday symptoms, however, was lower than in the previous study (Cinkotai et al. 1977), probably because the earlier study included noncotton facilities, which therefore had very low concentrations of GNB in the air and no occurrences of byssinosis. Endotoxin concentrations were not addressed. Cinkotai and Whitaker (1978) concluded that "the role of inhaled bacteria in the aetiology of byssinosis remains to be clarified."

Ten years later, Cinkotai again cross-sectionally surveyed the Lancashire textile industry (Cinkotai et al. 1988b; Cinkotai et al. 1988c, 1988d). Symptoms questionnaires were completed for 95 percent of the 4,903 workers employed in work areas ranging

Table 68. Results of epidemiological studies on cotton, flax, and hemp workers (limited to studies in which the effects of airborne endotoxin or airborne GNB were evaluated)

Study	Setting/ location	Maximal airborne concentrations* Dust (mg/m³)	Endotoxin (ng/m³)	GNB (cfu/m³)	Number of workers studied	Results of study
Cavagna et al. (1969)	Cotton, textile, and hemp mills/ Milan	5.25 (TD)	8,710 (Soxhlet; TCA extract; rabbit Shwartzman)	Not tested	136	Monday symptoms correlated strongly with airborne endotoxin (r=+0.99, p<0.01) but not with total dust (p>0.12) in the four work areas studied.
Cinkotai et al. (1977)	Cotton, tobacco, tea, and wool mills/ Lancashire	15.00 (TDLF)	Not reported	3,500 (Andersen; endoagar with PCN)	678	Monday symptoms correlated highly with airborne GNB (r=+0.95, p<0.0001) but not with dust. Endotoxin concentration in dust as high as 1,600 ng/mg by LAL gel titration assay.
Cinkotai and Whitaker (1978)	Cotton and textile mills/ Lancashire	3.85 (TDLF)	Not tested	76,900 Andersen; endoagar with PCN)	1,057	Monday symptoms correlated with GNB (r=+0.66, p<0.002) but not with dust (r=+0.11).
Haglind et al. (1981)	Cotton and textile mills/ Sweden	2.00 (TDLF)	Not tested	112,000 (Andersen; Drigalski agar)	248	Monday symptoms correlated with GNB (r=+0.83, p<0.01) and also with dust (r=+0.62; p<0.05). Dust and endotoxin highly intercorrelated.
Diem et al. (1984)	Cotton and synthetic textile mills/ S.E. USA	1.88 (CAM)	1,110 (VE dust; H_2O extract; LAL gel titration)	110,093 (VE dust plated on selective TSA with CH and VM)	123	Monday FEV_1 change correlated weakly with log dust (r=+0.23, p<0.05) but not with log GNB or log endotoxin. However, population was very selected and spirometry and dust sampling were done a week apart. Dust and endotoxin were correlated.
Kawamoto et al. (1987)	Cotton, garnetting, and mattress plants/ California	>1.18 (VE)	100 (VE dust; H_20 extract; chromogenic LAL)	Not tested	128	No significant association of symptoms or of lung function with dust or endotoxin, but endotoxin exposures were very low (median = 5 ng/m³).
Kennedy et al. (1987)	Cotton and textile mills/ Shanghai	2.50 (VE)	920 (VE dust; H_2O extract; chromogenic LAL)	Not tested	404	Stratified analysis showed a relationship between endotoxin (but not dust) and symptoms and baseline and across-shift FEV_1. Regression analysis showed that endotoxin (but not dust) was related to baseline FEV_1 and chronic symptoms (p<0.05).
Cinkotai et al. (1988b, c, and d)	Cotton and synthetic textile mills/ Lancashire	4.17 (TDLF)	580 (VE dust; H_2O extract; LAL gel	15,700 (Andersen; endoagar with PCN titration)	4,656	Many factors associated with Monday symptoms including endotoxin (p<0.001). Generally GNB levels and the prevalence of Monday symptoms were lower than in an earlier survey, but dust levels were not lower (Cinkotai 1978).

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Table 68. Results of epidemiological studies on cotton, flax, and hemp workers (limited to studies in which the effects of airborne endotoxin or airborne GNB were evaluated)—*Continued*

Study	Setting/ location	Maximal airborne concentrations* Dust (mg/m^3)	Endotoxin (ng/m^3)	GNB (cfu/m^3)	Number of workers studied	Results of study
Cinkotai et al. (1988a)	Flax mills/ Normandy	47.10 (TDLF)	Not tested	67,900 (Andersen; endoagar with PCN)	308	Dust and GNB related to chronic bronchitis, but with Monday symptoms. Very high dust and GNB levels and unexpectedly low occurrence of Monday symptoms suggest likely self-selection of workers.
Niven et al. (1991)	Cotton and synthetic textile mills/ Manchester	Not reported (TDLF; TD; personal TDLF)	Not reported (turbidometric LAL)	Not tested	1,893	Monday symptoms most strongly associated with log endotoxin by personal sampling ($p<0.001$).

**CAM*, Continuous aerosol monitor (ppm, Inc., Knoxville, TN). *CH*, Cycloheximide. *PCN*, Penicillin. *TCA*, Trichloroacetic acid. *TD*, Total dust (area). *TDLF*, Total dust, less fly (area). *TSA*, Trypticase soy agar. *VM*, Vancomycin.

from opening areas to weaving areas at the 31 mills surveyed (including four mills that exclusively processed synthetic fibers). Average work area samples of total dust (ranging up to 4.02 mg/m^3) were collected as before. Concentrations of GNB ranging up to 1,150 cfu/m^3 were measured. A portion of these dust samples were assayed for endotoxin (average concentration ranging up to 580 ng/m^3 by work area). Also, personal total dust samples and personal respirable dust samples were collected in workers' breathing zones. In addition to airborne endotoxin concentration ($p<0.001$), numerous other interrelated factors were associated with the prevalence of chest symptoms on Mondays.

Cinkotai et al. (1988c) commented that "the effects of airborne endotoxins...could not be fully evaluated because the available data set was not complete." Although a statistical model of the data developed by stepwise logistic multiple regression did not include endotoxin concentration as a prediction factor, it did include two other factors that are likely to have been related to endotoxin concentration, namely work area (Castellan et al. 1988) and cotton quality (Olenchock et al. 1983). The reduced prevalence of Monday chest symptoms relative to 10 yr earlier and the airborne concentrations of GNB above 300 cfu/m^3 in only three work areas (all cardrooms with among the highest prevalences of byssinotic symptoms) led Cinkotai et al. (1988c) to suggest that "the most important single factor causing the decline in the prevalence of byssinotic symptoms (PBS) may be the disappearance of bacteria from the workroom air. The PBS values peaked in the very three factories where airborne bacteria were observed in significant concentrations . . . . However, since last surveyed about 10 yrs ago . . . their overall decline was quite conspicuous and was not paralleled by a similar decline in airborne dust levels."

This comment was echoed in another report (Cinkotai et al. 1988b) by the statement that "the downward trend in the prevalence of byssinotic symptoms may be associated with the disappearance of much of the bacteria from workroom air." As there had been no specific program to decrease worker exposure to bacteria, the authors suggested that "the quality of dust, however, may have changed, due to the use of cleaner raw cotton and the closure of plants that processed the particularly dirty, coarse fibre." (Cinkotai et al. 1988b)

More recently, Cinkotai et al. (1988a) cross-sectionally surveyed 12 flax mills in Normandy, France, an area noted by the authors as generally having a low prevalence of byssinosis according to previous unpublished reports. Airborne endotoxin was not measured in this study, but mean area total dust

concentrations (up to 47.1 mg/m^3) and mean viable GNB concentrations (up to 67,900 cfu/m^3) were both exceptionally high. Over 90 percent of the 340 people employed in these mills answered a symptoms questionnaire. Overall, the byssinosis prevalence rate was only 12.5 percent, an unexpectedly low figure considering the high levels of dust and GNB exposures. Using a chi-square analysis instead of a correlation analysis (as was done in the previous reports), the authors reported a lack of statistically significant associations between Monday chest symptoms and either airborne dust or GNB concentrations. In addition to difficulty translating the questionnaire, the authors suggested other possible explanations for the unexpectedly low prevalence of Monday symptoms, including self-selection of susceptible workers out of the workplaces with extremely high levels of exposure. Considering these caveats, Cinkotai et al. (1988a) concluded that "it cannot rightly be inferred from the present observations that flax dust or bacteria are not in some way associated with byssinosis."

Bale Bacterial Count and Decreases in Across-Shift FEV_1

Rylander et al. (1979) used results of a company-operated medical monitoring program involving nearly 4,000 cardroom workers in 23 U.S. cotton mills to investigate changes in FEV_1 over a Monday workshift. Three indices of exposure—mean cardroom airborne dust concentration as measured by vertical elutriator (ranging up to 1.35 mg/m^3), mean concentration of GNB cultured from cotton fiber sampled from bales processed in the mills studied, and cotton/synthetic blend ratio—were used as independent variables in various linear and nonlinear statistical models describing the relationship between exposure and across-shift change in FEV_1. A mean across-shift decrement in FEV_1 was determined for each mill, and each mean value was weighted by the number of examined employees at that mill. Although a statistically significant linear relationship between log dust concentration and decline in FEV_1 was found (r=0.50, p<0.01), a stronger correlation (r=0.62, p<0.01) was observed when FEV_1 was correlated with the square root of the product of viable GNB (sampled from bales) times airborne dust concentration adjusted for blend ratio. (In a preliminary report from the first 15 mills studied, Rylander and Lundholm (1978) reported that when across-shift Monday FEV_1 decline was correlated separately with exposure to airborne dust and GNB, the simple correlation coefficients were 0.46 and 0.78, respectively.)

The medical monitoring program from which the data were obtained had been in operation for several years in the studied mills, and Rylander et al. (1979) explained that workers previously found to be affected by dust had been transferred to jobs involving lower dust exposures. In addition, spirometry testing, dust measurements, and bale sampling did not temporally coincide. All these factors were likely to have limited the authors' ability to observe stronger associations between GNB and acute FEV_1 decline even if they had existed. Most limiting, in terms of the overall importance of this study, is the fact that neither airborne GNB nor airborne endotoxin concentrations were measured.

Swedish Symptoms Survey

In a survey of the Swedish cotton textile industry, Haglind et al. (1981) interviewed 86 percent of workers in the opening, carding, and spinning areas. The prevalence of Monday chest symptoms by work area correlated with the concentration of total airborne dust (which ranged up to 2.0 mg/m^3) and the concentration of airborne-viable GNB (which ranged up to 200 million cfu/m^3).

Analyzing all surveyed work sites, Haglind et al. (1981) observed a stronger correlation of Monday chest symptoms with airborne GNB (r=0.73, p<0.01) than with airborne dust (r=0.62, p<0.05). Excluding one work site that processed medical grade cotton, the correlation with dust improved (r=0.81, p<0.001), but the GNB correlation was still reasonably high and statistically significant (r=0.71, p<0.01). Data published in their report indicate that the two measured environmental indices were highly correlated (r=0.68, p<0.008 for all sites; and r=0.90, p<0.001 excluding the medical cotton site), which could explain why both correlated with byssinosis prevalence. Given this correlation between endotoxin and airborne dust, the results of this study cannot be accepted as strong evidence for an endotoxin effect in the absence of a dust effect.

A later study conducted at one of these Swedish mills by Rylander et al. (1983), although an observational study of worker responses to their workplace exposures, must be categorized as clinical rather than epidemiological. Data were collected from only 15 workers (2 of whom were excluded from the analysis), and Rylander et al. (1983) commented that "as the technical capacity of the study was limited, no further attempts were made to recruit additional workers." Thus, as indicated by the authors, little import can be given to the lack of a significant relationship between personal exposures to dust or endotoxin and individual across-shift change in FEV_1. Means of data grouped by work area were more consistent with an endotoxin effect than a dust effect, but low numbers of observations limited analysis of these data.

Endotoxin and Acute FEV_1 Declines in U.S. Textile Mills

Diem et al. (1984) reported a preliminary analysis of some data from the first year of a 5-yr longitudinal respiratory health study of workers employed at selected U.S. cotton textile mills. In a multiple regression analysis of data from 123 male nonsmokers and smokers, acute change in lung function over a Monday workshift was observed to be significantly (though not strongly) correlated with the logarithm of airborne, vertically elutriated dust concentration (r=0.23, p<0.05), but not with either the logarithm of airborne GNB concentration (r=–0.01) or with the logarithm of airborne endotoxin concentration (r=–0.09).

Potential limitations of this study include a highly selected population (having survived a well-established medical monitoring program intended to remove the most susceptible workers), a very strong correlation among the environmental indices (r=0.78 to 0.92), a temporal difference (1 wk) between pulmonary function testing and environmental measurements, and relative imprecision of the titration gel method used as compared with other endotoxin assays (for example, the chromogenic modification of the LAL assay). Thus, the results cannot be accepted as substantial evidence against an endotoxin effect in the absence of a dust effect.

Airborne Endotoxin in a Cotton-Garnetting Mill

Kawamoto et al. (1987) reported on a respiratory health and environmental survey of workers who manufacture padding and mattresses in the 13 cotton-garnetting facilities located in California. The cotton used in these facilities was waste cotton from gins and cottonseed oil mills located in California. Of 193 English- or Spanish-speaking dayshift workers employed, only 66 percent participated in at least some part of the survey. Complete exposure data and before-and-after-shift pulmonary function data were available for only 30 percent of the workers.

Overall, 10 percent of the workers had Monday chest symptoms. Although Kawamoto and associates failed to observe statistically significant associations between acute or chronic chest symptoms or acute or chronic pulmonary function changes and either dust or endotoxin concentration, a trend toward larger decreases in Monday FEV_1 with higher endotoxin exposures is evident in their tabulated data. This trend was not found to be statistically significant (no p value was reported), but the authors used an overly conservative Scheffe method for multiple comparisons (Boardman and Moffitt 1971). Moreover, although the authors claimed that their analysis had adequate statistical power, they seemed to believe that a mean workshift-related change in FEV_1 must be 5.3 or greater before it is worth detecting. In this regard, it is noteworthy that results of the three earlier garnetting and waste cotton studies reviewed by Kawamoto et al. indicate that a mean FEV_1 change this large was unlikely to have been observed.

Bias to the null in the Kawamoto et al. (1987) study may also have resulted from selection factors resulting in the low worker participation rate. Beyond that, only stratified analyses of grouped data were performed, which may have resulted in a potential loss of information from continuous variables (for example, individual measures of endotoxin concentration, dust concentration, and workshift-related change in FEV_1).

The most obvious limitation of this study in terms of its inability to turn up an observable relationship between measures of respiratory morbidity and

endotoxin exposure was that endotoxin exposures were generally very minimal (median level of 5.2 ng/m^3). Very low endotoxin exposures in these California garnetting facilities is consistent with published reports of relatively low GNB and low endotoxin contamination of cotton grown in California (Simpson and Marsh 1985, Castellan et al. 1988). Largely on the basis of this last point, Kawamoto and colleagues appropriately concluded that their negative findings were "not inconsistent with studies that have found dose-response relationships between endotoxin exposure and decreases in FEV_1 during the workshift."

Endotoxin and Respiratory Effects in Shanghai Mills

Kennedy et al. (1987) reported a survey of Chinese cotton mill workers who were more highly exposed (vertically elutriated dust concentrations of up to 2.50 mg/m^3 and vertically elutriated endotoxin concentrations of up to 920 ng/m^3). Acute byssinotic effects could not be assessed in terms of typical Monday chest symptom patterns because of unusual work schedules. A 90 percent participation rate was achieved, but pulmonary function data from an additional 10 percent were excluded from analysis on the basis of nonreproducibility of test results as defined by guidelines of the American Thoracic Society (American Thoracic Society 1979). Use of the 1979 reproducibility criteria are now known to effect substantial study bias by differentially excluding from analysis the more functionally impaired study subjects (American Thoracic Society 1987).

In addition Kennedy et al. (1987) presented evidence that the most heavily exposed group of workers had been influenced by other selection pressures and that remaining workers in this group represented a less affected survivor population. When the highest exposure group was excluded from the analysis, a stratified analysis revealed clear and consistent trends for an endotoxin effect and the absence of a dust effect (on both baseline and decreased across-shift FEV_1, and on both acute and chronic chest symptoms).

In a regression analysis, although the coefficient for airborne endotoxin concentration was not significantly related to a decrease in across-shift FEV_1, it was significantly related to baseline FEV_1 ($p < 0.01$), and both coefficients were substantial. Even in a subgroup of cotton workers who had relatively low endotoxin exposures, Kennedy et al. (1987) observed excesses of acute and chronic chest symptoms and larger decreases in across-shift FEV_1 ($p<0.06$) compared with silk workers. This observation suggested to Kennedy et al. (1987) that "even exposure to endotoxin at 1 to 20 nanograms per cubic meter constitutes an 'adverse respiratory health effect' . . . ." However, because of the cross-sectional nature of the study and because no environmental sampling was done at the silk mill used for comparison, this particular conclusion must be viewed with caution.

A Recent Study of British Textile Workers

A recent preliminary report (Niven et al. 1991) summarized the findings of a survey of 1,093 British textile workers and an environmental survey of the seven cotton and two synthetic fiber mills in which they were employed. Exposures to dust were measured by sampling work areas and personal breathing zones. Exposures to endotoxin were measured using a turbidometric modification of the LAL assay applied to personal dust samples. Byssinosis symptom prevalence was most strongly correlated with log endotoxin ($p<0.001$), suggesting that endotoxin might be an etiological factor.

Beyond Byssinosis

Recent reports from atypical settings add considerable support to the contention that endotoxin inhalation causes byssinosis. For example, 22 percent of the 303 daytime workers at a wool carpet-weaving factory reported Monday symptoms typical of byssinosis (Ozesmi et al. 1987). They were exposed to airborne dust (up to 4.4 mg/m^3) with high endotoxin contamination (up to 31.2 μg/g). Moreover, as in byssinosis, symptomatic individuals were shown to have a decreased across-shift FEV_1 especially on Monday as compared to other weekdays. The entirely different nature of this dust compared to cotton textile mill dust suggested to Ozesmi et al. (1987) that "the finding of endotoxin together with the absence of cotton confirms the theory that

'byssinosis' is due to bacterial endotoxin rather than to cotton per se."

A Monday pattern of symptoms is becoming increasingly observed in cases of "humidifier lung," a condition with many clinical similarities to byssinosis (Ganier et al. 1980). In one reported outbreak at a printing facility with a microbiologically contaminated humidifier, 40 percent of 50 employees experienced fever and 24 percent experienced chest tightness temporally associated with humidifier operation (Rylander and Haglind 1984). Airborne endotoxin concentration after 2 hr of humidifier operation was measured to be as high as 390 ng/m^3.

In another work environment in which acute and chronic airway symptoms were prevalent in employees, a clear relationship between exposure to airborne endotoxin and a decrease in across-shift FEV_1 was observed in data from 57 workers in swine confinement buildings (Donham et al. 1988). Others have also observed a relationship between endotoxin exposure and airflow impairment in swine confinement workers (Zejda et al. 1991).

In animal feed mills, Heederik et al. (1991) have observed a strong negative association of dust and endotoxin with preshift ventilatory function, but "cumulative endotoxin was more strongly related to decreases in lung function than the dust exposure." This suggests that endotoxin exposure was an important factor in the development of chronic impairment of airway function in these workers.

The exposure to airborne endotoxin and associated respiratory syndromes, including decreases in FEV_1, have been documented but not well analyzed among other occupational groups, including poultry farmers (Thelin et al. 1984), silo unloaders (Pratt and May 1984), and grain workers (doPico et al. 1986). Ongoing and future research in these and other areas is likely to shed additional and substantial light on endotoxin as an etiologic agent of occupational respiratory disease.

Published Suggested Limits for Airborne Endotoxin Exposure

As reviewed in other chapters of this book, the existing evidence on the effects of endotoxin has driven a substantial ongoing research effort to identify the factors that influence the contamination of cotton with endotoxin and to pilot intervention strategies to substantially reduce this contamination. In addition, as summarized briefly in the following paragraphs, the existing evidence has resulted in the publication of several suggested levels at which to limit occupational exposure to airborne endotoxin.

Rylander and Lundholm (1978), while recognizing that a definite causal role in the etiology of byssinosis had not yet been established, suggested that "it may be prudent to establish a standard for airborne GNB, to supplement the existing dust standard." A decade later, after endotoxin testing had become more standardized and routine, Rylander (1987) proposed that a standard for endotoxin should be developed for specific application to cotton dust. Rylander (1987) listed endotoxin-based "tentative thresholds for symptoms after exposure to cotton dust," which included "fairly certain" thresholds of 0.5–1.0 μg/m^3 for fever, 0.3–0.5 μg/m^3 for chest tightness, and 0.1–0.2 μg/m^3 for decreases in across-shift FEV_1, [even though Rylander et al. (1985) had previously published a report indicating a lower threshold—33 ng/m^3—for decreases in across-shift FEV_1]. In accord with the interpretation by Kennedy et al. (1987) that chronic respiratory effects may occur at airborne endotoxin exposures in the range of 1–20 ng/m^3, Rylander (1987) listed an "uncertain" threshold of 0.02 μg/m^3 for chronic bronchitis.

Popendorf (1986) proposed a general guideline of 0.1 μg/m^3 as a potentially hazardous airborne concentration for occupational settings. Several years later, while indicating the need for further research before a mandatory Federal standard could be established for airborne endotoxin, Jacobs (1989) called for the establishment of an endotoxin "threshold limit value" to guide voluntary control of this occupational health hazard. In fact, a genetic engineering company has used data from cotton dust exposure studies to establish its own voluntary airborne endotoxin action level at 30 ng/m^3 (Palchak et al. 1988). Concentrations above this level trigger use of personal protective devices, intensified environmental monitoring, medical surveillance, and appropriate additional engineering and administrative controls to protect worker health.

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Cotton and Microorganisms

Janet J. Fischer
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Abstract

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Many microorganisms are found on cotton. When cotton is processed, dust and microorganisms and their associated products (such as endotoxins) become airborne. The airborne dust, microorganisms, and endotoxins generated cause some workers in mills to become ill. The main illness is a respiratory disease called byssinosis. Over the last 20 yr, significant research has gone into studies of byssinosis and mechanisms thought to be important in its etiology. The chapters of this book are written by researchers who have devoted considerable time to these studies. The microorganisms associated with byssinosis are identified by the authors. Each chapter summarizes the results of studies done by the author and others who have done related work.

Keywords: microorganisms, byssinosis, cotton dust, endotoxin, gram-negative bacteria, gram-positive bacteria, fungi, respiratory disease, cotton mills, carding, cotton cultivars, harvesting, storage, bracts, Classer's samples, fiber quality, textile mills

While supplies last, single copies of this publication may be obtained at no cost from Preston E. Sasser, Cotton Incorporated, 4505 Creedmoor Rd., Raleigh, NC 27612.

Copies of this publication may be purchased from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161; telephone (703) 487–4650.

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