ENVIRONMENTAL RESEARCH 59, 350–361 (1992)

Immunological Findings in Hemp Workers[1]

EUGENIJA ZUSKIN,* BOZICA KANCELJAK,† E. NEIL SCHACHTER,‡,[2] THEODORE J. WITEK,‡ SAUL MAAYANI,‡ SATINDRA GOSWAMI,‡ ZVI MAROM,‡ AND NICHOLAS RIENZI‡

Andrija Stampar School of Public Health, Zagreb, Croatia; †Institute for Medical Research and Occupational Medicine, Zagreb, Croatia; and ‡The Mount Sinai School of Medicine, New York, New York

Received July 30, 1991

Immunological status and its relation to respiratory findings were studied in 42 female textile workers occupationally exposed to hemp dust and in 49 female control workers. Skin prick tests with hemp or flax dust extracts from different parts of the mill in hemp workers demonstrated the following frequencies of positive tests to antigens: a mixture of hemp and flax extracts (64%), followed by flax extracts (48%), hemp from combing machines (41%), hemp from carding marchines (38%), hemp from spinning and weaving machines (33%), and hemp from softening machines (20%). The prevalence of positive skin tests to hemp or flax allergens in control workers was consistently lower, ranging from 21 to 5%. Increased total serum IgE was recorded in 35.7% of hemp workers compared to only 5.0% of control workers ($P < 0.05$). Hemp workers with positive skin tests had significantly higher prevalences of chronic respiratory symptoms than those with negative skin tests. There were, however, no differences for acute symptoms between workers with positive and negative skin tests. Across-shift changes and baseline lung function were not different when compared by immunologic status. We showed additionally that a water-soluble extract of hemp dust causes a dose-related contraction of nonsensitized guinea pig tracheal smooth muscle when studied *in vitro*. Our results suggest that frequent immunologic abnormalities can be documented in hemp workers but, with the exception of chronic respiratory symptoms, in general, these do not correlate with respiratory findings.

INTRODUCTION

An asthma-like disease among hemp workers has been described in the Spanish literature under the name of cannabosis (Barbero Carnicero and Flores Marco, 1944). This disease resembles byssinosis, an occupational airway disease known to occur among cotton and flax workers. Velvart and Stavrovska (1963) reported respiratory disease in hemp workers and found that the severity of the disease was related to the amount of dust in the workplace. Bouhuys *et al.* (1967) reported that respiratory findings among hemp workers constitute a serious and disabling problem.

There are only a few studies examining the importance of immunological find-

[1] The research was supported in part by Grant No. YF 733 from the National Institutes of Health, Bethesda, MD; Grant No. RO1-OHO 2593-O1A1 from the National Institute of Occupational Safety and Health, Center for Disease Control, Atlanta, GA; and by the Henry and Catherine Gaissman and the Miller Foundations, New York, NY.

[2] To whom requests for reprints should be addressed at the Mount Sinai School of Medicine, One Gustave L. Levy Place, P.O. Box 1232, New York, NY 10029-6574.

0013-9351/92 $5.00

ings in textile workers. Salvagio *et al.* (1986) in their study raise the possibility that, in some individuals, cotton dust-induced respiratory disease may be due to preexisting or occupationally induced mold allergy. On the other hand, the findings of Mundie *et al.* (1972) do not support an immune etiology for byssinosis. Sepulveda *et al.* (1984) suggested that atopy may be an important determinant reflecting the magnitude of the acute pulmonary responses to textile dust. The allergic properties of cotton and flax dust were studied by Fetisova *et al.* (1970). These authors describe that cotton and flax dust are allergens and that the antigenic properties of these dusts are due to the presence of bacteria and fungi in the dust.

The present study is an extension of our previous study of respiratory impairment in hemp workers (Zuskin *et al.*, 1990). The current study was undertaken to investigate the relationship between immunological status and respiratory function in a group of textile workers occupationally exposed to hemp dust.

SUBJECTS AND METHODS

Immunological Study

Subjects. The study included a group of 42 female textile workers employed in one hemp mill in Croatia. This group represents 70% of all workers employed in the mill. The mean age of the workers was 40 years (range: 23 to 55 years), mean height was 161 cm (range: 155 to 180 cm), and mean duration of exposure to hemp dust was 16 years (range: 2 to 35 years). Ten percent of the workers (4) were light smokers (less than 10 cigarettes per day). Workers were exposed to hemp dust released during the opening of bales and to dust from combing, carding, softening, spinning, and weaving of the hemp. A group of 49 female control workers of similar age and smoking habits, employed in a bottle packing factory, bottling fruit juice, was used as a control. Of these 49 workers, 40 (82%) agreed to have skin tests and blood studies, in addition to the respiratory questionnaire.

Immunological studies. Skin prick tests were performed with aqueous extracts of hemp and flax dust in the 42 exposed hemp workers and in 40 of the control workers. Hemp and flax extracts (1:20 w/v) were prepared using standard immunological techniques employing the dust collected in the workroom where workers were examined (Sheldon *et al.*, 1967). In addition workers were skin-tested with histamine base (0.01 mg/ml), extracts of bacteria, mold, and *Dermatophagoides pteronyssinus* and buffer as a control solution. Bacterial antigen consisted of extracts of *Hemophilus influenzae, Streptococcus pneumoniae, S. viridans, S. pyogenes,* and *Staphylococcus aureus* in a concentration of 60×10^6/ml. Mold antigen was a mixture of *Alternaria, Penicillium, Mucor, Cladosporium, Aspergillus niger,* and *Aspergillus fumigatus* in 0.2% solution. Skin reactions were read after 20 min. A test was considered positive if the diameter of the observed wheal was 3 mm greater than that of the control solution.

The serum level of total IgE immunoglobulin was determined in 42 hemp workers and 40 control workers by a reference laboratory, PRIST (Pharmacia Diagnostics AB, Upsala, Sweden) using the direct radioimmunologic "sandwich" technique. Levels of IgE below 125 IU/ml were considered normal.

Throat smears. In 42 hemp workers throat cultures were collected for bacterial analysis. Throat swabs were obtained during the work shift.

Respiratory symptoms. Chronic respiratory symptoms were recorded in 42 hemp and in 49 control workers using the British Medical Research Council Committee's (1960) questionnaire on respiratory symptoms with additional questions on occupational asthma (WHO, 1986) and on byssinosis (Schilling *et al.*, 1964). In all workers a detailed occupational history and questions about their smoking history were recorded. We defined chronic cough, chronic phlegm, chronic bronchitis, dyspnea grades 3 and 4, occupational asthma, and byssinosis grades as previously outlined (Zuskin *et al.*, 1990).

In all hemp workers, the presence of acute symptoms during the work shift such as cough, dyspnea, chest tightness, irritation or dryness of the throat, secretions or dryness of the nose, and headache were specifically recorded.

Ventilatory capacity. Ventilatory capacity was measured in the 42 hemp workers by recording maximum expiratory flow–volume (MEFV) curves using a portable flow–volume spirometer (Pneumoscreen, Jaeger, Germany). Measurements were recorded as detailed in a previous study (Zuskin *et al.*, 1990).

Environmental measurements. Airborne dust in the hemp mill was sampled during the 8-hr work shift at the worksite of the examined workers. Details of the methods are described in our previous report (Zuskin *et al.*, 1990).

Statistical analysis. The results of ventilatory capacity measurements were analyzed using the t test for differences of paired (acute effects across shift) and unpaired (comparing baseline to predicted values) variables. The χ^2 test (or, where appropriate, Fisher's exact test) was used for testing differences in the prevalence of respiratory symptoms. A $P < 0.05$ was considered statistically significant.

Hemp Dust Extract Assay

In order to investigate the potential of hemp dust to provoke airway smooth muscle contraction we tested the bronchoconstricting potential of hemp dust extract on guinea pig trachea. The extract was obtained from hemp dust collected in the industry. One gram of hemp dust was incubated in 10 ml of sterile water for 24 hr at +4°C. The solution was filtered and centrifuged at 16,000 rpm and the supernatant decanted.

We used the trachea of young Albino Hartly male guinea pigs (300–390 g) purchased from Perfection Breeders (PA). Animals were sacrificed by CO_2 asphyxiation for 2 min and the trachea was removed within 3 min. The animal tissues were trimmed of excess fat and connective tissue. Four segments ("rings" each 4–6 mm wide) were cut from a single trachea, and each was suspended between two L-shaped stainless-steel hooks mounted in a 20-ml organ chamber containing Krebs–Henseleit buffer of the following composition (μM): NaCl, 110.0; KCl, 4.80; $CaCl_2$, 2.35; $MgSO_4$, 1.20; KH_2PO_4, 1.20; $NaHCO_3$, 25.0; dextrose, 11.0, and Na_2EDTA, 0.03, in glass distilled water. Organ chambers were maintained at 36.5 ± 0.5°C and were continuously aerated with 95% O_2 and 5% CO_2 to maintain pH 7.5 ± 0.1. The tissue segments were initially set to 2 g of tension and were allowed to relax for approximately 1.5 hr before the experiment

began. During that period the tissue was washed at 15-min intervals. After the relaxation period, the tension in each tissue segment was adjusted to 2 g for all subsequent assays. Isometric contractions were recorded using Grass FTO3C force displacement transducer attached to a Grass polygraph recorder. Before and after concentration–response assays with our extracts of hemp were performed, a challenge with carbachol 10^{-5} M was run. A dose–response curve with hemp dust extract was obtained by adding progressively increasing volumes of each extract or Krebs (used as a control) into the tissue bath in progressive aliquots of 10, 30, 100, 300, and 1000 μl. The potency of the extracts was determined by comparing the biological activity with the maximal contraction induced by carbachol (10^{-5} M) on the same tissue. The data are expressed as a percentage of the initial maximal carbachol contraction (10^{-5} M).

The protein content in hemp dust extract was determined by the method of Lowry (1951). Endotoxin was measured in the hemp extract using the Limulus Amebocyte Lysate test (Sigma E-Toxate Kit No. 210-A).

RESULTS

Immunological Study

The data on skin tests in hemp and in control workers are presented in Fig. 1. All hemp workers reacted to histamine. The highest prevalence of positive skin tests were found for mixed dust of hemp and flax (64%), followed by flax dust (48%), hemp dust from combing machines (41%), hemp dust from carding machines (38%), hemp dust from spinning machines (33%), hemp dust from weaving machines (33%), and hemp dust from softening machines (20%). These data indicate that the dust collected at the early phase of the hemp processing was the most potent in causing positive skin reactions. Considerably fewer of the hemp

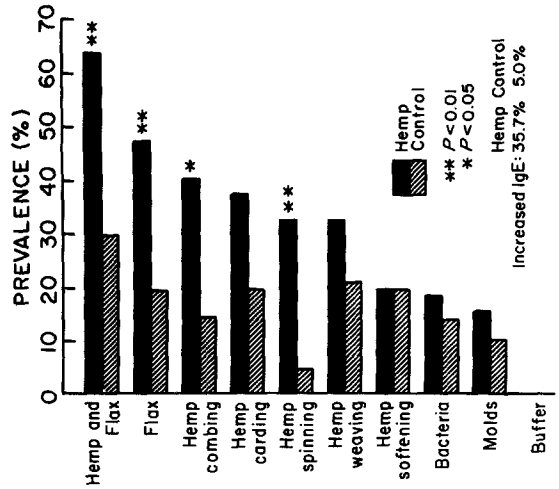

FIG. 1. Prevalence of positive skin prick tests to hemp and flax dust extracts in 42 hemp workers and in 40 control workers.

workers reacted to bacteria (19%) and mold (16%) extracts. None of hemp workers reacted to the buffer solution.

Among 27 hemp workers with positive skin tests, 18 (66.7%) had byssinosis whereas among the 15 hemp workers with negative skin tests only 5 (33.3%) had byssinosis ($P < 0.05$). The workers with byssinosis were employed in hemp mill for 2–30 years. Five workers among those with byssinosis symptoms did not react to any of hemp or flax extracts. They were exposed to hemp dust for 1–5 years.

The prevalence of positive skin tests among control workers was considerably lower than that among hemp workers. The highest prevalence was found with mixed dust of hemp and flax (30%), followed by hemp on weaving machines (21%), flax (20%) hemp on carding machines (20%), hemp on softening machines (20%), hemp on combing machines (15%), and hemp on spinning machines (5%). Among control workers five reacted to bacteria (13%), four to molds (10%), and none to buffer solution. The prevalences of positive skin reactions in hemp and in control workers were significantly different for the mixture of hemp and flax dust ($P < 0.01$), for flax dust ($P < 0.01$), for hemp dust on combing machines ($P < 0.05$), and for hemp dust on spinning machines ($P < 0.01$).

Increased serum levels of total IgE immunoglobulins were found in 15 hemp workers (35.7%) and in 2 control workers (5.0%) ($P < 0.05$). All hemp workers with increased IgE level had positive skin reactions to at least one of the hemp or flax extracts. Among the 2 control workers with increased IgE level both had positive skin test to one of the hemp extracts. Among the 23 hemp workers with byssinosis, 9 (39.1%) had increased serum level of total IgE compared to 6 (31.6%) of the hemp workers without byssinosis (NS).

Throat smears. Bacteriologic cultures of throat smears grew *S. aureus* and *Escherichia coli* in 14.3% of the hemp workers. Considerably fewer workers had positive throat smears for *Proteus mirabilis* (4.8%), *Enterobacter aerogenes* (2.4%), *Enterobacter liquefaciens* (2.4%), *Streptococcus* (beta hemolitic groups A and B) (2.4%), *Proteus morgani* (2.4%), and *Klebsiella* species (2.4%). In 21 (50%) hemp workers normal flora was found.

Respiratory symptoms. Table 1 presents the prevalence of chronic respiratory symptoms in 42 hemp workers and in 49 control workers. For the hemp workers symptoms are recorded by the presence or absence of a positive skin test. Hemp workers had higher prevalences of all chronic respiratory symptoms than did the control workers, being significantly different for chronic cough ($P < 0.01$), chest tightness ($P < 0.01$), asthma ($P < 0.05$), nasal catarrh ($P < 0.01$), sinusitis ($P < 0.01$), and byssinosis ($P < 0.01$). Hemp workers with positive skin tests to one or more of the hemp or flax dust extracts demonstrated generally higher prevalence of all chronic respiratory symptoms, with significant differences for chronic phlegm, chronic bronchitis, nasal catarrh, sinusitis, and byssinosis.

The prevalence of acute symptoms during the work shift in 42 hemp workers is presented in Table 2. The highest prevalence was seen for eye irritation (76.2%), followed by cough and dry throat (73.8%), dyspnea (66.7%), throat irritation (64.3%), headache (61.9%), dry nose (54.8%), secretions from the nose (23.8%), and bleeding of the nose (14.3%). There were no consistent differences in acute symptoms between hemp workers with positive and negative skin tests.

TABLE 1

PREVALENCE OF CHRONIC RESPIRATORY SYMPTOMS IN 42 HEMP WORKERS AND IN 49 CONTROL WORKERS

Group	Mean age (years)	Mean exposure (years)	Chronic cough	Chronic phlegm	Chronic bronchitis	Dyspnea grades 3 and 4	Chest tightness	Asthma	Nasal catarrh	Sinusitis	Byssinosis
Hemp workers ($N = 42$)	40	16	19 45.2%	7 16.7%	7 16.7%	4 9.5%	29 69.0%	8 19.0%	17 40.5%	19 45.2%	23 54.8%
P value			<0.01	NS	NS	NS	<0.01	<0.05	<0.01	<0.01	<0.01
Controls ($N = 49$)	41	17	4 8.2%	2 4.1%	2 4.2%	1 2.0%	0 0%	0 0%	5 10.2%	3 6.1%	0 0%
Hemp workers positive skin test ($N = 27$)	42	18	13 48.1%	8 29.6%	8 29.6%	3 11.1%	19 70.3%	6 22.2%	15 55.6%	14 51.9%	18 66.7%
P value			NS	<0.05	<0.05	NS	NS	NS	<0.01	=0.05	<0.01
Hemp workers negative skin test ($N = 15$)	39	16	6 40.0%	1 6.1%	1 6.1%	1 6.1%	10 66.7%	2 13.3%	2 13.3%	5 33.3%	5 33.3%

Note. NS, difference statistically not significant ($P > 0.05$).

TABLE 2
PREVALENCE OF ACUTE SYMPTOMS DURING THE WORK SHIFT IN HEMP WORKERS

Group	Cough	Dyspnea	Throat		Eye irritation	Nose			Headache
			Irritation	Dryness		Secretion	Dryness	bleeding	
Hemp $N = 42$	31	28	27	31	32	10	23	6	26
	73.8%	66.7%	64.3%	73.8%	76.2%	23.8%	54.8%	14.3%	61.9%
Positive skin	19	17	14	21	20	6	14	3	18
tests $N = 27$	70.4%	62.9%	59.3%	77.8%	74.1%	22.2%	51.9%	11.1%	66.7%
Negative skin	12	11	11	10	12	4	9	3	8
tests $N = 15$	80.0%	73.3%	73.3%	66.7%	80.0%	26.7%	60.0%	20.0%	53.3%

Ventilatory capacity. Table 3 presents the data for ventilatory capacity in hemp workers with positive and negative skin prick tests. Hemp workers had significantly lower respiratory function than predicted values. However, no significant differences were seen between workers with positive and negative skin tests for either across-shift or baseline lung function (expressed as a percentage of predicted).

Environmental dust measurements. The mean total hemp dust concentration was 22.35 (range 3.3–68.5) mg/m^3 with a mean respirable fraction of 9.93 (range 1.3–38.4) mg/m^3. These measurements are much higher than the maximum allowable concentration for vegetable textile dust allowed by Yugoslav occupational standards (total dust 5 mg/m^3; respirable fraction 1 mg/m^3).

Hemp Dust Extract Assay

The mean data for the effect of hemp dust extract on isolated guinea pig tracheal smooth muscle are shown in Fig. 2. A total of 14 guinea pigs was studied and the contractile response to 10, 30, 100, 300, and 1000 μl of hemp dust extract was tested. The results are presented as a percentage of an initial maximal carbachol contraction produced by stimulation with 10^{-5} M carbachol. A typical dose–response relationship between agonist (hemp) and contractile response is demonstrated. The maximal effect was 51% of the maximal carbachol response.

Determination of protein content in hemp dust extract demonstrated that extract used for the *in vitro* study on isolated guinea pig tracheal rings contained 1.575 mg/ml of protein. No endotoxin was measured in the hemp extract.

DISCUSSION

Byssinosis is a clinical syndrome recognized among many textile workers including those processing hemp. The mechanism by which dust in this industry causes acute and chronic respiratory disease is not known, nor is the causal agent well defined. Because of the asthmatic nature of the syndrome, much interest has focused on possible allergic mechanisms.

Our data demonstrate a high prevalence of positive skin tests, particularly to a mixture of hemp and flax dust among the studied hemp workers. The prevalences were considerably higher than those among control workers. Additionally the prevalence of increased total IgE was significantly higher among our hemp (35.7%) than among our control workers (5.0%) ($P < 0.05$).

The relationship between immunologic testing and respiratory symptoms in byssinosis has been extensively studied by many authors. Chen (1986) reported that among hemp–sisal workers there was a correlation between positive skin tests to hemp and increased IgE serum level. Gupta *et al.* (1980) demonstrated an improvement in respiratory symptoms and ventilatory capacity (FVC and FEV_1) following immunotherapy in hemp workers who complained of respiratory symptoms and had positive skin tests to hemp.

Schachter *et al.* (1985), by contrast, injected cotton bract extract intradermally into healthy volunteers never before exposed to cotton dust. They observed an initial wheal-and-flare followed by a delayed induration, characterized by inflammatory cell infiltration. These authors suggested that this skin reaction repre-

TABLE 3
VENTILATORY CAPACITY IN HEMP WORKERS WITH POSITIVE AND WITH NEGATIVE SKIN PRICK TESTS

	Positive skin tests (N = 27)						Negative skin tests (N = 15)					
	Before shift	Difference before–after shift		Expected	Difference measured − expected		Before shift	Difference before–after shift		Expected	Difference measured − expected	
Test	($X \pm$ SD)	%	P	($X \pm$ SD)	%	P	($X \pm$ SD)	%	P	($X \pm$ SD)	%	P
FVC (L)	3.16 ± 0.77	−5.1	NS	3.71 ± 0.84	85.2	NS	3.75 ± 0.59	−8.3	<0.01	4.04 ± 0.57	92.8	NS
FEV_1 (L)	2.56 ± 0.50	−5.5	<0.05	2.25 ± 0.56	96.6	NS	2.87 ± 0.48	−10.8	<0.01	3.14 ± 0.54	91.4	NS
FEF_{50} (L/s)	3.60 ± 0.55	−7.0	<0.05	4.47 ± 0.36	80.5	<0.01	3.85 ± 1.07	−12.7	<0.01	4.71 ± 0.36	81.7	<0.01
FEF_{25} (L/s)	1.65 ± 0.40	−10.9	<0.05	2.25 ± 0.26	73.3	<0.01	1.65 ± 0.54	−15.2	<0.01	2.46 ± 0.41	67.1	<0.01

Note. NS, difference statistically not significant ($P > 0.05$). The data are presented as mean ± SD. FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; FEF_{50}, forced expiratory flow at 50% of the vital capacity above residual volume; FEF_{25}, forced expiratory flow at 25% of the vital capacity above rsidual volume.

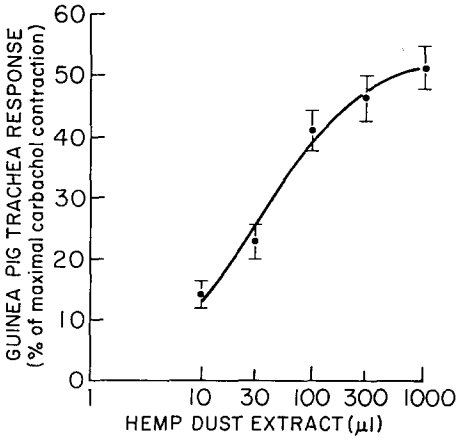

FIG. 2. Contractile response of hemp dust extract in guinea pig trachea, expressed as a percentage of maximal carbachol (10^{-5} M) contraction (mean ± SE).

sented a non-IgE-mediated inflammation initiated by mast cell-derived mediators and may be a model for nonimmune mechanisms in the pathogenesis of byssinotic airway disease.

Popa *et al.* (1969) investigated allergy to hemp, cotton, flax, and jute in textile workers and reported delayed skin reactions to textile allergens. Antibody directed against an antigen present in the cotton plant is described by Massoud and Taylor (1964). They found that the amount of antibody is higher in cardroom workers than in normals and is highest in those workers with byssinosis. Oehling *et al.* (1972) suggested that byssinosis is an immunological reaction due to a specific antigen–antibody reaction, in which the antigen is contained in cotton. Edwards and Jones (1973) by contrast, found that a so-called cotton antigen (a polymer of 5,7,3,4-tetrahydroxyflavan-3-4-diol) extracted from cotton bracts, precipitated 58% IgG, 54% IgM, and 15 IgA in a sample of human serum. On the basis of these results they suggested that human immunoglobulins are nonspecifically precipitated by the tannin-like polymer. Petronio and Bovenzi (1983) reported that in 353 cotton textile workers the average concentration of IgE was higher than that expected for nonatopic subjects. Jones *et al.* (1980) reported a significant interaction between atopy and exposure to cotton dust. Positive skin tests to whole cottonseed and cottonseed linters showed a prevalence of 8% being greatest in the linter-exposed group.

The higher prevalence of skin reactions to hemp extract in our worker group, compared to controls, suggests that with exposure sensitization (over and above any nonspecific inflammatory reaction) may occur. However, these observations do not permit us to quantitate the relative roles of specific and nonspecific inflammatory responses in the pathogenesis of airway disease.

In textile dust there are numerous bacteria and fungi. In the throat cultures of our hemp workers we found different types of bacteria including *S. aureus* (14.3%), *Proteus morgani* and *Klebsiella* species (2.4%). Velvart *et al.* (1964) reported that the bacterial and fungal organisms in hemp dust and in sputum do

not appear to be agents capable of provoking the clinical picture of the disease in hemp workers. Rylander and Haglind (1986) suggest that the amount of airborne endotoxin found in mills determines the risk of developing byssinosis. The presence of these unusual microorganisms in cultures taken from these workers suggests environmental contamination.

Our findings suggest a strong relationship between respiratory symptoms, particularly chronic bronchitis and byssinosis and the presence of positive skin tests to hemp dust (but not total serum IgE). These observations complement those of other investigators particularly Gupta *et al.* (1980) and Chen (1986). By contrast we failed to demonstrate a relationship between skin test reactivity and acute symptoms in the workplace. Moreover, skin test results were not related to objective findings of pulmonary impairment.

Our studies on isolated guinea pig trachea suggest that clinical respiratory findings obtained in humans may have some counterpart in guinea pig tracheal smooth muscle studied *in vitro*. Such data indicate that hemp dust extract has a component(s) that can induce airway constriction by a direct action on guinea pig airway smooth muscle. The guinea pigs used in these experiments were not presensitized to hemp. This contractile activity was thus probably not on an immunoglobulin-mediated basis.

Immunologic abnormalities are common in hemp workers. Both serum IgE and skin test reactivity to hemp extracts were increased in our workers. Our findings suggest a strong relationship between chronic respiratory symptoms and positive skin tests to hemp extract. Nevertheless hemp extract elicited many positive reactions in our controls and hemp extract caused nonspecific airway smooth muscle contraction. The findings of these studies thus suggest that chronic impairment in hemp workers may result from both immunologic and nonspecific inflammatory response.

REFERENCES

Barbero Carnicero, A., and Flores Marco, R. (1944). Enfermedad del canamo. *Rev. Clin. Espanola* **13**, 395–399.

Bouhuys, A., Barbero, A., Lindell, S. E., Roach, S. A., and Schilling, R. S. F. (1967). Byssinosis in hemp workers. *Arch. Environ. Health* **14**, 533–544.

Chen, K. Z. (1986). Occupational asthma induced by sisal hemp. *Chung-Hua I Hsueh Tsa. Chin.* **66**, 282–285.

Edwards, J. H., and Jones, B. M. (1973). Pseudoimmune precipitation by the isolated byssinosis "antigen." *J. Immunol.* **110**, 498–501.

Fetisova, A. A., Titova, S. M., and Alekseeva, O. G. (1970). Allergenic properties of cotton and flax spinning mill dust. *Gig. Truda Prof. Zabol.* **14**, 19–22.

Gupta, B. N., Mehrotra, N. K., Clerk, S. H., Saxena, A. K., George, P. A., Chandra, H., and Mehendra, P. N. (1980). Immunotherapy in hemp workers having respiratory complaints. *Indian J. Med. Sci.* **34**, 72–81.

Jones, R. N., Butcher, B. T., Hammad, Y. Y., Diem, J. E., Glidmeyer, H. W., Lehrer, S. B., Hughes, J. M., and Weil, H. (1980). Interaction of atopy and exposure to cotton dust in the bronchoconstrictor response. *Br. J. Ind. Med.* **37**, 141–146.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randal, R. L. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Massoud, A., and Taylor, G. (1964). Byssinosis: Antibody to cotton antigen in normal subjects and in cotton card-room workers. *Lancet* **2**, 607–611.

Medical Research Council Committee on the Etiology of Chronic Bronchitis (1960). Standardized questionnaire on respiratory symptoms. *Br. Med. J.* **2,** 1665.

Mundie, T. G., Pilla, P. A., and Ainsworth, S. K. (1972). Byssinosis: Serum immunoglobulins and complement concentrations in cotton mill workers. *Arch. Environ. Health* **40,** 326–329.

Oehling, A., Gonzales de la Ruquera, I., and Vines Rueda, J. J. (1972). A contribution to the allergic ethiopathogenity of byssinosis. *Respiration* **29,** 155–160.

Petronio, L., and Bovenzi, M. (1983). Byssinosis and serum IgE concentrations in textile workers in an Italian cottonmill. *Br. J. Ind. Med.* **40,** 39–44.

Popa, V., Gavrilescu, N., Preda, N., Teculescu, D., Plecias, M., and Cirstea, M. (1969). An investigation of allergy in byssinosis: Sensitization to cotton, hemp, flax and jute antigen. *Br. J. Ind. Med.* **26,** 101–108.

Rylander, R., and Haglind, F. (1986). Exposure of cotton workers in an experimental cardroom with reference to airborne endotoxins. *Environ. Health Perspect.* **66,** 83–86.

Salvagio, J. E., O'Neil, C. E., and Butcher, B. T. (1986). Immunologic response to inhaled cotton dust. *Environ. Health Prospect.* **66,** 17–23.

Schachter, E. N., Buck, M. G., Merrill, W. W., Askenase, P., and Witek, T. J. (1985). Skin testing with an aqueous extract of cotton bract. *J. Allergy Clin. Immunol.* **76,** 481–487.

Schilling, R. S. F., Vigliani, E. C., Lammers, R., Valic, F., and Gilson, J. C. (1964). A report on a Conference on Byssinosis. *XIV Int. Congress Occup. Health, Madrid* **2,** 137–145.

Sepulveda, M. J., Castellan, R. M., Hankinson, J. L., and Cocke, J. B. (1984). Acute lung function response to cotton dust in atopic and nonatopic individuals. *Br. J. Ind. Med.* **41,** 487–491.

Sheldon, J. M., Lowel, R. G., and Mathews, K. P. (1967). "A Manual of Clinical Allergy," pp. 507–531. Saunders, Philadelphia/London.

Velvart, J., Plesko, I., and Ditte, L. (1964). The effect of bacteria and fungi on the occurrence and development of rope-maker's disease. *Prac. Lek.* **19,** 304–307.

Velvart, J., and Stavrovska, O. (1963). Health of workers engaged in processing of hemp. *Prac. Lek.* **15,** 153–157.

Zuskin, E., Kanceljak, B., Prokrajac, D., Schachter, E. N., and Witek, T. J. (1990). Respiratory symptoms and lung function in hemp workers. *Br. J. Ind. Med.* 47, 627–632.