

Respiratory Function and Immunological Status in Cocoa and Flour Processing Workers

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Respiratory function and immunological status were studied in 40 cocoa and 53 flour processing workers employed as packers in a confectionery industry and in 65 unexposed control workers in the same industry. A high prevalence of chronic respiratory symptoms was recorded in exposed workers, varying from 5.0% to 30.0% in cocoa workers and from 5.7% to 28.3% in flour workers. Occupational asthma was diagnosed in 2 (5%) of the cocoa workers and in 3 (5.7%) of the flour workers. None of the control workers suffered from occupational asthma. The prevalence of almost all chronic respiratory symptoms was significantly greater in cocoa and flour workers than in control workers. There was also a high prevalence of acute symptoms that developed during the work shift, being highest for cough (cocoa: 57.5%; flour: 50.9%) and eye irritation (cocoa: 50.0%; flour: 54.7%). Significant across-shift reductions of ventilatory capacity were recorded in exposed workers, being largest for flow rates at 50% and the last 25% of the vital capacity on maximum expiratory flow-volume (MEFV) curves (FEF₅₀, FEF₇₅). The prevalence of positive skin tests for cocoa (60.2%) was significantly higher than the prevalence of positive skin tests for flour (25.8%) among the 93 exposed workers ($p < 0.05$). Control workers had significantly lower prevalences of positive skin tests to cocoa (4.6%) and flour (12.3%) than exposed workers ($p < 0.01$). Increased total serum IgE levels were found in 17.5% of cocoa and in 18.7% of flour workers; none of the control workers had increased IgE levels. Bronchoprovocation testing demonstrated significant decreases in lung function following inhalation of cocoa dust extract and flour dust in workers with respiratory symptoms and large across-shift reductions in lung function. Dust concentrations in the working environment were higher than those recommended by Croatian standards. These data suggest that workers employed in the processing of cocoa and flour may be at a high risk for the development of allergic sensitization and respiratory impairment. Am. J. Ind. Med. 33:24-32, 1998 © 1998 Wiley-Liss, Inc.

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INTRODUCTION: EVALUATING RESPIRATORY RISK

Currently, there is great interest in the health problems associated with the food industry and its products. Brooks [1985] has described numerous food ingredients potentially associated with the development of occupational respiratory disease.

Work in the confectionery industry involves the use of different food products. In particular, confectioners are often exposed to the dust of flour, cocoa, and other food products.

Houba et al. [1996] reported that asthma and other respiratory symptoms in bakery workers caused by exposure to wheat proteins are important occupational health problems. Hartman et al. [1985] studied 314 workers in an industrial bakery and found that 74% of the bakers complained of respiratory allergy to flour (rhinitis and/or asthma). Bonahada et al. [1994] showed that despite exposure to relatively low concentrations of respirable flour dust, bakers are at risk for developing both respiratory symptoms and airway hyperresponsiveness. Block et al. [1983] noted that baker's asthma appears to be a form of allergic asthma due to cereal flour, which is mediated by specific IgE antibodies. Gohte et al. [1983] reported work-related symptoms and positive skin and RAST tests against buckwheat in 28% of workers employed in preparing and distributing plant products. Matsumura et al. [1994] reported that atopic mechanisms in bakers contribute to wheat flour sensitization. Fries [1966, 1978] noted that sensitivity to cocoa bean and its derivative, chocolate, is common among those with allergic disease. Ghosh [1977], e.g., reported allergic symptoms in 32.8% of 500 allergic patients after eating chocolate. Morgan [1994] described the cocoa as the most critical raw material for chocolate because of its unique solidification and liquefying properties.

The present study was undertaken to evaluate respiratory function and immunological status in confectionery workers exposed to cocoa and flour dust.

MATERIALS AND METHODS

Subjects

This study included 40 female workers exposed to cocoa and 53 female workers exposed to flour dust. All exposed workers who were present at the time of the study were screened for the investigation. Ninety-three of the 101 workers in this plant (92%) were able to participate. In a previous study, we had studied workers from this industry employed at a separate location [Zuskin et al., 1994, 1994a]. The occupational exposures in the current workers, however, were different from those of the previous study in that, in general, flour and cocoa were not used for the products manufactured by the previously studied workers.

The mean age of cocoa workers was 32 years (range: 19–51 years); their mean height was 156 cm (range: 151–171 cm); and the mean duration of their employment was 11 years (range 1–25 years). Of the cocoa workers, 15% (6/40) were regular mild smokers (defined as individuals smoking on the average 10 cigarettes daily or less). The mean age of flour workers was 33 years (range 19–49 years); their mean height was 167 cm (range 152–175 cm); and their mean duration of employment was 14 years (range 1–22 years). A total of 13% (7/53) were mild smokers.

Cocoa and flour workers were employed in the sorting, milling, and sifting of cocoa or flour. They also processed other ingredients such as sugar, starch, eggs, and milk. Cocoa workers were exposed only to cocoa dust and flour workers only to flour dust. The final products of this manufacture were chocolate bars, chocolate candies, chocolate cookies (mixed or covered with chocolate), chocolate powder for chocolate milk, cakes, cookies, and other miscellaneous confectionery products. In addition, from a group of 85 unexposed female workers employed by the same plant packing these confectionery products, 65 were available for study as a control group for the prevalence of chronic respiratory symptoms. These workers did not move to other occupations within this plant. The age, smoking habits, and duration of employment of the control group were not significantly different from that of the confectionery workers. Their workplace was geographically separate from that of the confectioners, being based in a facility one block away from the main plant.

Respiratory Symptoms

Chronic respiratory symptoms were recorded using the British Medical Research Council questionnaire for respiratory disease [WHO, 1986] with additional questions on occupational asthma [Maestrelli et al., 1992; Godnic-Cvar, 1995]. For all confectionery and control workers, a detailed occupational history as well as data about their smoking habit were recorded. The following definitions for chronic respiratory symptoms were used:

- chronic cough or phlegm: cough and/or phlegm for a minimum of 3 months a year
- chronic bronchitis: cough and phlegm for a minimum of 3 months a year and for not less than 2 successive years
- dyspnea grades: grade 3—shortness of breath when walking with other people at an ordinary pace on level ground; grade 4—shortness of breath when walking at their own pace on level ground
- occupational asthma: recurring attacks of dyspnea, chest tightness, and pulmonary function impairment of the obstructive type diagnosed by physical examination and spirometric measurements during exposure to dust at or following work (decrease of $FEV_1 > 15\%$) and confirmed by medical records

Acute symptoms that developed during the work shift were also recorded in all confectionery workers, but not in controls. These symptoms included cough, dyspnea, irritation or dryness of the throat, secretion, dryness or bleeding of the nose, eye irritation, and headache.

Ventilatory Capacity

Ventilatory capacity measurements were performed by recording maximum expiratory flow-volume (MEFV) curves on a spirometer, the Pneumoscreen (Jaeger, Wurzburg, Germany). The forced vital capacity (FVC), the 1-second forced expiratory volume (FEV₁), and maximum flow rates at 50% and the last 25% of the vital capacity (FEF₅₀, FEF₇₅) were determined on the MEFV curves. The acute effect of exposure to cocoa and flour dust on ventilatory capacity was studied by recording MEFV curves on Monday before and after the daytime work shift. At least three MEFV curves were recorded for each subject, and the best value was used in the analysis. The measured values were compared to predicted values for the Croatian population (including farmers and industrial workers) studied by Mustajbegovic [1992], since lung function was not measured concurrently for control workers. The spirometer was calibrated for volume on a daily basis. Lung function testing and calibration were performed in accordance with standards for spirometric measurements [Quanjer et al., 1993].

Effect of Disodium Cromoglycate (DSCG)

The protective effect of disodium cromoglycate (DSCG) on across-shift lung function reductions in workers was studied in five cocoa workers and in six flour workers (volunteers) who exhibited large across shift reductions of FEF₅₀ and FEF₂₅ (>20%) on two consecutive Mondays. Immediately after an initial lung function test on the first Monday, subjects inhaled 80 mg of DSCG, or a placebo administered by spihaler in a double-blind protocol. At the end of the subsequent 8-hour work shift, these subjects again performed lung function testing. On a second Monday, an identical protocol was performed with the same subject inhaling DSCG or a placebo (whichever had not been inhaled at the first session).

Bronchoprovocation Testing

Bronchoprovocation testing with cocoa dust extract was performed in three cocoa workers with respiratory symptoms, a positive skin prick test reaction to cocoa, and an increased serum total IgE level. We did not use across-shift change in lung function at work as a criterion for performing bronchial provocation testing. However, all three workers had across-shift changes of 15% or more during their work shift. Bronchial inhalation challenge was performed using a cocoa extract and with normal saline as the control. One milliliter of cocoa extract or normal saline was placed in a Heyer Picolo nebulizer (Carl Heyer GmbH, Bad Ems, Germany) with an air flow of 15 L/min, which nebulized the fluid during inspiration only. The subjects continued to inhale the solution (cocoa extract or a placebo) during

normal quiet breathing until the contents of the nebulizer was completely aerosolized. Bronchoprovocation with cocoa extract and normal saline was performed on separate days at least 1 week apart. Lung function was performed using a wedge spirometer (Vitalograph, Buckingham, UK) recording forced expiratory curves on which 1-second forced expiratory volume (FEV₁) and forced expiratory flow rates between 25% and 75% of the vital capacity were calculated. Lung function was measured before and at 1 minute, 10 minutes, and 20 minutes following the inhalation of cocoa extract or saline. A 15% or more drop in baseline FEV₁ was considered significant [Sterk et al., 1993].

Bronchoprovocation with flour dust was performed in three flour workers who complained of respiratory symptoms at work. These workers had positive skin reactions to flour and had positive RAST test (one subject: 11.50 PRU/ml, class +3; the second subject: 18.00 PRU/ml, class +4; the third subject: 19.00 PRU/ml, class +4). Two of these workers were exposed to wheat flour dust, and the other to rye flour dust. The exposure consisted of sifting the flour dust through a fine sieve in an exposure chamber for 30 minutes. Lung function testing was performed before inhalation, and at 5, 10, 15, 20, 25, and 30 minutes during inhalation and again at the same time intervals following bronchoprovocation up to 30 minutes after inhalation of flour dust.

Immunological Studies

Skin prick tests with specific occupational, as well as common allergens were performed in all tested cocoa and flour processing workers. Cocoa and flour extracts were prepared using a standard immunological technique [Sheldon et al., 1957]. Extracts were made with dust collected from the work areas where workers were examined. Skin prick testing was performed with cocoa dust extract and flour dust extract in a concentration of 1:50 w/v. In addition, workers were skin tested with histamine base (1 mg/ml), with *Dermatophagoides pteronnyssinus* (0.2%) as well as with buffer solution used as a control. Skin prick testing was performed according to the recommendations of Kjellman et al. [1988] using a lancet with a 1 mm tip. Skin reactions were read after 20 minutes. A test was considered positive if the diameter of the observed wheal was 3 mm greater than that of the buffer solution.

The serum level of total IgE antibody was measured by the PRIST method (Pharmacia Diagnostics, AB, Upsala, Sweden) using a direct radioimmunological "sandwich" technique based on a paper disc as a solid phase. Levels of IgE below 125 kU/L were considered normal [Johansson, 1968]. Specific IgE (RAST) was determined by radioimmunoassay (Phadebas RAST, Pharmacia Diagnostics AB, Uppsala, Sweden), and expressed as PRU/ml (Phadebas

TABLE I. Prevalence of Chronic Respiratory Symptoms in Confectionery Workers Processing Cocoa and Flour and in Control Workers (Croatia 1995)

Group	Mean age (yr)	Mean employment (yrs)	Chronic cough	Chronic phlegm	Chronic bronchitis	Dyspnea grade 3 and 4	Occupational asthma	Nasal catarrh	Sinusitis
Cocoa N = 40	32 ± 5	11 ± 6	11 ^a 27.5%	8 ^a 20.0%	4 ^a 10.0%	5 ^a 12.5%	2 5.0%	12 ^a 30.0%	8 ^a 20.0%
Flour N = 53	33 ± 4	14 ± 7	15 ^a 28.3%	12 ^a 22.6%	6 ^a 11.3%	8 ^a 15.1%	3 5.7%	13 ^a 24.5%	9 ^a 16.9%
Control N = 65	31 ± 4	10 ± 5	4 6.2%	2 3.1%	2 3.1%	0 0%	0 0%	1 1.5%	1 1.5%

^aDifference between exposed and control worker statistically significant ($p < 0.01$).

TABLE II. Prevalence of Acute Symptoms During Work Shift in Confectionery Workers Processing Cocoa and Flour (Croatia 1995)

Group	Cough	Dyspnea	Throat		Eye	Nose		Headache
			Irritation	Dryness	irritation	Dryness	Bleeding	
Cocoa N = 40	23 57.5%	14 35.0%	17 42.5%	19 47.5%	20 50.0%	18 45.0%	7 17.5%	12 30.0%
Flour N = 53	20 50.9%	18 33.9%	23 43.4%	27 50.9%	29 54.7%	25 47.2%	9 16.9%	15 28.3%

Rast Units/ml). The values of class 0 (<0.035 PRU/ml) are considered normal.

Environmental Measurements

The dust concentrations in the work environment were measured by a two-stage Hexhlet apparatus (Casella, London, UK), which collects total and respirable dust particles by the air flow of 60 L/min. Dust samples were taken during an 8-hour work shift in the areas where workers were working. Five samples of dust were collected in the cocoa processing areas and six samples were collected in the flour processing area. These samples were collected over the entire work shift. Arithmetic means and ranges are presented.

Statistical Analysis

The results of ventilatory capacity measurements were analyzed using the paired t-test to compare across-shift changes. A paired t-test was used to compare baseline lung function (measured before the shift) with predicted values. The chi-square test and Fisher's exact test were used for testing differences in prevalence data of respiratory symptoms between groups; $p < 0.05$ was considered statistically significant.

RESULTS

Respiratory Symptoms

The prevalence of chronic respiratory symptoms are presented in Table I for the 40 cocoa, 53 flour, and 65 control workers. Significantly higher prevalences for all chronic respiratory symptoms (except occupational asthma) were recorded in both exposed groups compared to the control workers ($p < 0.01$). In both of the exposed groups, the highest prevalences were recorded for chronic cough and nasal catarrh. Occupational asthma was recorded in two cocoa (5.0%) workers and in three flour (5.7%) workers. There were no differences in the prevalence of chronic respiratory symptoms between workers with positive and negative skin prick tests to cocoa and flour.

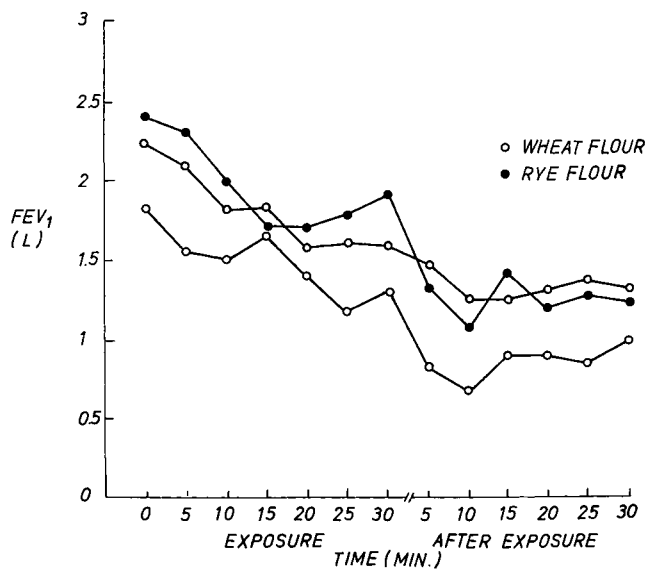
High prevalences of acute symptoms during the work shift were recorded for cocoa and flour workers (Table II). The highest prevalences were recorded for cough (50.9%; 57.5%) and eye irritation (50.0%; 54.7%).

VENTILATORY CAPACITY

Tables III presents across-shift differences and chronic changes in ventilatory capacity among the cocoa and flour workers. There were statistically significant reductions across

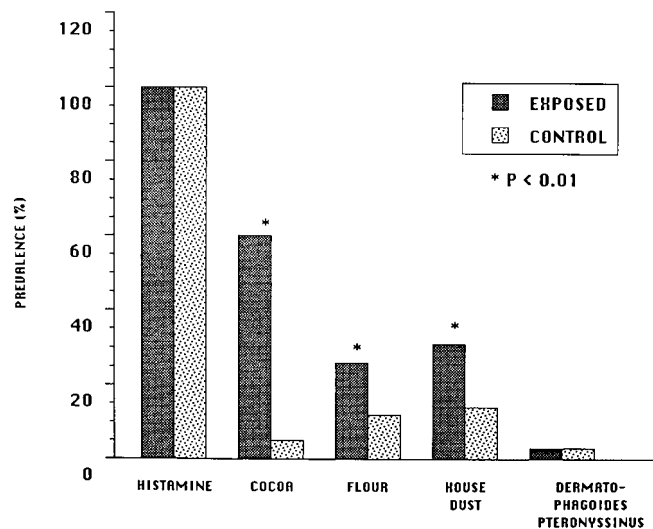
TABLE IV. Ventilatory Capacity in Three Confectionery Workers Following Inhalation of Cocoa Extract

Subject	Test	Before	Time following inhalation						Predicted	% of predicted
			1 min	Difference 1 min-before	10 min	Difference 10 min-before	20 min	Difference 20 min-before		
1	FEV ₁ (ml)	4950	4800	-150	4200	-750	4200	-750	4960	99.8%
	FEF ₂₅₋₇₅ (L/s)	4.55	3.80	-0.75	3.66	-0.89	3.40	-1.15	5.60	81.3%
2	FEV ₁ (ml)	3220	3200	0	2830	-390	2735	-485	3510	91.7%
	FEF ₂₅₋₇₅ (L/s)	4.32	4.17	0.15	3.67	-0.65	3.40	-0.92	5.10	84.7%
3	FEV ₁ (ml)	3150	3040	-110	2950	-200	3130	20	3350	94.0%
	FEF ₂₅₋₇₅ (L/s)	3.20	3.10	-0.10	3.10	-0.10	3.14	-0.06	3.75	85.3%

FIGURE 1. Serial measurements of FEV₁ in three flour workers; two following bronchoprovocation with wheat flour and one with rye flour dust.

Immunological Studies

The prevalence of immediate skin tests sensitivity to cocoa or flour dust extract in exposed and in control workers are presented in Figure 2. Significantly higher prevalences of positive skin tests were seen in the 93 exposed workers for cocoa (56/93; 60.2%) than for flour (24/93; 25.8%) ($p < 0.01$). The prevalences of positive skin tests to cocoa and flour were significantly higher in exposed workers than in control workers (cocoa: 3/65; 4.6% and flour: 8/65; 12.3%) ($p < 0.01$). All tested workers reacted to histamine and none reacted to the buffer control solution.

FIGURE 2. The prevalence of positive skin tests to cocoa and flour dust extract in 93 exposed and 65 control workers. *Difference in the prevalence of positive skin tests between exposed and control workers statistically significant ($P < 0.01$).

A separate analysis of workers with positive skin tests by exposure group demonstrated that among cocoa workers, 29/40 (72.5%) reacted to cocoa and 4/40 (12.5%) to flour. Among the 53 flour workers, 27 (50.9%) had a positive skin test to cocoa and 19 (35.8%) to flour. Cocoa workers had significantly higher prevalences of positive skin tests to cocoa than did flour workers ($p < 0.05$); flour workers had a significantly higher prevalences of positive skin tests to flour than cocoa workers ($p < 0.05$).

An increased total serum IgE level was found in 7 (17.5%) of the cocoa workers and in 10 (18.7%) of the flour

workers. None of the control group workers had increased serum levels of IgE. All exposed workers with increased serum IgE level had positive skin reactions to cocoa or flour dust extract.

The two cocoa workers and the three flour workers with occupational asthma had positive skin tests to cocoa or flour dust extract accompanied by increased serum IgE level (range: 260–470 kU/L).

Environmental Measurements

In the cocoa processing area, the mean total dust level was 9.1 mg/m³ (range: 2–16 mg/m³) and the mean respirable fraction was 2.1 mg/m³ (range: 0.9–3.5 mg/m³). In the flour processing area, the mean total dust was 12.3 mg/m³ (range: 2.4–17.1 mg/m³) and the mean respirable fraction was 1.9 mg/m³ (range: 0.5–2.7 mg/m³). These measured mean total and respirable dust concentrations were higher than those allowed by the Croatian Federal standards for organic dust (total dust: 3 mg/m³; respirable dust fraction: 1 mg/m³). Correlation with health parameters were not found, presumably because workers changed jobs in the factory frequently.

DISCUSSION

Our data demonstrate that work with cocoa and flour dust may be associated with acute and chronic respiratory symptoms, including occupational asthma. Taytard et al. [1988] described a high incidence of cough and chronic bronchitis as well as hypersensitivity in flour workers. Similarly, Jarvinen et al. [1979] and El Karim et al. [1986] reported chronic bronchitis, chronic productive cough, chest tightness, and bronchial asthma in workers exposed to grain and flour dust. Occupational allergy (asthma and rhinitis) was seen in 58% of workers occupationally exposed to flour as reported by Debelic and Sarvan [1982]. A case of baker's asthma due to wheat flour was reported by Walker et al. [1989]. In our study, occupational asthma was determined in 2 (5%) of the cocoa workers and in 3 (5.7%) of the flour workers. Their symptoms started 2–3 years after their employment in the confectionery industry. Brisman and Jarvholm [1995] noted that Swedish bakers exposed over a 10-year period have approximately double the risk of developing asthma than the general population. Musk et al. [1989] reported chest tightness and nasal symptoms in 38% of British bakery workers. Interestingly, in our study we report that a large number of cocoa and flour workers complained of upper respiratory symptoms such as acute dryness of the nose (cocoa workers: 45.0%; flour workers: 47.2%).

Ventilatory capacity testing in our workers demonstrated acute and chronic reductions following exposure to cocoa and flour dust. These changes were particularly

pronounced for FEF₅₀ and FEF₇₅, suggesting obstructive changes located in smaller airways. These data are similar to those found in industrial workers exposed to other dusts, such as those associated with the processing of coffee, tea, spices, soy, and confectionery (Zuskin et al., 1981, 1985, 1988, 1991, 1994). Across-shift changes averaging nearly 10% in FEV₁ were described for this group of workers. These are significant changes, since previous work by Ghio et al. [1991] demonstrated that unexposed blue collar workers do not decrease their lung function (FEV₁) by >2–3% over a shift. Similarly, Zuskin et al. [1994, 1996] demonstrated that unexposed (control) workers demonstrated even a slight across-shift increase of FVC, FEV₁, FEF₅₀, and FEF₇₅. Confectionery workers in our study with more marked across-shift changes are not more likely to have positive skin tests or more symptoms than are their less reactive coworkers.

Pugliese [1973] and Schultze-Werninghaus and Schwartzing [1974] described the inhibitory effect of DSCG in preventing FEV₁ decreases in bakers with occupational asthma. In our study, five cocoa workers and six flour workers with large-across shift FEF₅₀ reductions (range: 25–29%) and FEF₇₅ reductions (range: 26–34%) inhaled DSCG before their work shift. In all tested workers, the reductions were considerably decreased by the DSCG prophylaxis: FEF₅₀: range 5–8%; FEF₂₅: range 7–11%. Such data suggest that mast cell mediator release may play an important role in the bronchoconstrictor effect on airways in cocoa and flour dust exposure.

Bronchoprovocation testing in symptomatic workers demonstrated hyperreactivity to cocoa dust extract in two out of three workers with positive skin tests to cocoa dust allergen. Bronchoprovocation with flour dust (wheat and rye) in three workers caused a marked reduction in FEF₂₅₋₇₅, possibly related to small airway obstruction. Mild late reactions were recorded in all three workers tested with flour. The two workers responding to cocoa extract did not exhibit late reactions. Palczynski et al. [1996] indicated that in patients with bronchial asthma, both neutrophils and eosinophils take part in allergic reaction in the mucosa following bronchial challenge with flour. Hendrick et al. [1976] described that bronchial provocation tests with occupational exposure to flour precipitated dual asthmatic reactions accompanied by rhinitis. Skin tests with aqueous extracts of flour produced positive immediate reactions in these workers. These findings suggest that specific bronchoprovocation may play a role in identifying workers at risk for occupational asthma due to cocoa and flour.

Immunological testing in our workers demonstrated that significantly more of the exposed workers demonstrated positive skin tests to cocoa or flour dust extract than did control workers. Zotti et al. [1994] described atopy in 23.4% of bakers. In their study, many workers complained of work-related asthma, rhinoconjunctivitis, and chronic bronchi-

tis. Skin sensitization to occupational allergens was significantly associated with atopy, smoking habit, and work seniority. Zeitz [1990] suggested that the main mechanism for occupational asthma in bakers is an IgE-mediated immune response. Chocolate allergy was described by Maslansky and Wein [1971]. The authors reported that 2% of allergic individuals manifested specific allergic symptoms after eating chocolate. In our study, 17 of 93 exposed workers (18.3%), including five with occupational asthma, had increased serum IgE level. Increased levels were found only in those with positive skin prick tests to cocoa or flour dust extract.

As in our previous study [Zuskin et al., 1994] of confectionery workers, we have demonstrated frequent respiratory findings in workers exposed to the food products used in this industry. The current study extends our previous findings in that the workers in the present investigation had frequent and consistent exposure to cocoa and flour. These workers had more positive specific skin tests than those in our previous study and these skin tests correlated with the finding of occupational asthma (but not with nonspecific reactivity to these dusts). Interestingly, significant protection from across-shift changes was obtained using DSCG pretreatment in both studies.

This study of cocoa and flour workers suggests frequent lung function and immunological abnormalities in this group. However, despite a high prevalence of positive skin tests in these workers, airway responsiveness was not predicted by skin tests results. In contrast, the five workers with occupational asthma did have positive immunologic findings and DSCG appeared to be effective in protecting workers. These findings suggest that immunologic markers may help to identify workers at risk for occupational asthma caused by cocoa and flour, but not those workers who develop nonspecific reactivity to these dusts. It is most likely that the high dust levels in this plant account for the nonspecific airway findings. We suggest that both environmental control measures and medical surveillance including immunologic testing would be indicated in this industry.

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