Pulmonary Reaction to Durum Wheat*

A Constituent of Grain Dust

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To identify constituents of grain dust responsible for grain handlers' respiratory symptoms, 11 volunteer grain elevator workers underwent inhalation provocation tests with extracts of durum wheat, durum wheat airborne dust, and grain insects and mites. Factors that might influence the host response to the challenge were assessed. Five of the 11 subjects showed a greater than 20 percent decrement in FEV₁ after inhalation of durum wheat extracts (airway reaction). The bronchial reactions were immediate in 1/5 and late in 4/5. These airway reactions were blocked by sodium cromoglycate. Only one subject showed airway reaction to durum wheat dust extract, and none reacted to mites or insect extracts.

Occupational exposure to grain dust can cause acute and chronic respiratory symptoms and changes in pulmonary functions.⁴ The clinical manifestations of occupational exposure may include cough, expectoration, wheezing, chest tightness, dyspnea, and grain fever, and perhaps allergic alveolitis.⁴ Numerous components of grain dust have been isolated: grain particles, mite and insect fragments, fungi, bacteria, rodent hair, and pesticide residues.⁴,⁵

This study was undertaken to identify the constituent of grain dust responsible for grain handler's symptoms and to determine a site of action in the respiratory tract. Additionally, we assessed factors that may influence or contribute to this process.

Material and Methods

We studied 11 grain handlers who had respiratory symptoms on exposure to durum wheat dust at work. Symptoms included cough, wheezing, chest tightness, and expectoration. We recruited the volunteers for our study from the Superior-Duluth grain elevator working population of 310 grain handlers whom we had studied on another occasion. The workers were all men, with a mean age of 38 years and an age range of 27 to 59 years (Table 1). Each volunteer gave his informed consent according to the guidelines on human experimentation of the University of Wisconsin.

Subjects were tested for atopic diathesis by prick test using six common allergens: ragweed, feathers, eastern oak, cat epithelium, alternaria, and Timothy grass (Greer Laboratories). The subject was considered atopic if a 3 mm or greater wheal developed at 20 minutes to three or more of these allergens. Intradermal tests were used to detect immediate skin test reactivity to extracts of the following: durum wheat, airborne durum wheat dust, Aspergillus fumigatus, grain mites, grain weevils, or grain beetles. These extracts were prepared from material collected from the workers' environment. Subjects were judged to be positive skin reactors if an 8 mm or greater wheal was raised at 10 minutes to an injection of 1,000 PNU/ml or less of extract.

Precipitating serum antibodies against durum wheat, airborne durum wheat dust, molds, and insects were measured by Ouchterlony's immunodiffusion method.

Total C3 complement levels were ascertained by the Mancini immunodiffusion technique.⁶ Activation of C3 complement by the classic or the alternate pathway was ascertained by immunoelectrophoresis using anti-C3 (anti-1A/1C)⁷ and anti-C3 proactivator.⁸ Factor B₃ fragment (C3 activator) and intact C3 proactivator (factor B) indicated activation of C3 by the alternate pathway. A positive reaction was denoted by a precipitin arc in the gamma region (factor B₃) and another in the beta region (factor B) of the electrophoretic field. Detection of C3d (2d fragment would indicate activation of C3 by the classic pathway. A double-humped precipitin arc in the beta region of the electrophoretic field indicated a positive reaction.

Spirometry was performed on an Ohio 840 rolling bar spirometer. The FEV₁ and FEF₂₅-₇₅% were measured from the largest of two acceptable FVC tracings. The instantaneous maximal expiratory flows after exhalation of
Table 1—Characteristics of Grain Handlers Tested*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Job Years</th>
<th>Cigarette Pack-yr</th>
<th>Smoking Status</th>
<th>FEV&lt;sub&gt;1&lt;/sub&gt;</th>
<th>FVC</th>
<th>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC</th>
<th>(\dot{V}_{\text{max50}})</th>
<th>(\dot{V}_{\text{max75}})</th>
<th>DL&lt;sub&gt;co&lt;/sub&gt;</th>
<th>PC&lt;sub&gt;20&lt;/sub&gt;</th>
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<td>13</td>
<td>18.0</td>
<td>Ex</td>
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<td>Nonreactors</td>
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<td>73</td>
<td>68</td>
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* S = smoker; Ex = exsmoker; Ns = nonsmoker. PC<sub>20</sub> = provocation concentration of methacholine (mg/ml) that caused at least a 20% decrease in baseline FEV<sub>1</sub>.

50 and 75 percent of FVC (\(\dot{V}_{\text{max50}}\) and \(\dot{V}_{\text{max75}}\)) were measured from two reproducible maximal expiratory efforts which were displayed on an X-Y recorder and then averaged. Diffusing capacity of the lungs for (DL<sub>co</sub>) was measured by the single-breath method of Ogilvie and associates. We predicted values from published information. Before entering the study, each subject was tested for preexisting airway obstruction. Subjects 40 years of age or younger were termed obstructed if the FEV<sub>1</sub>/FVC<sub>50</sub> was less than 70 percent and subjects over 40 years of age were termed obstructed if the FEV<sub>1</sub>/FVC<sub>50</sub> was less than 75 percent.

Saline extracts of pulverized insects, mites, and durum wheat and airborne durum dust obtained from elevators were prepared by the method of Flaherty and co-workers. Twenty-four hours before the challenge, the lympholyzed extracts were resuspended in sterile, nonpyrogenic Coca buffer with 3.0 percent human serum albumin to effect a final concentration of 100,000 PNU/ml. The resuspended extracts were filtered through a millipore filter (pore size .22 mm), placed in sterile needle vials, and tested for sterility on nutrient agar and Sabouraud’s agar plates incubated at room temperature and 37°C. If the plates showed no growth after 24 hours, the resuspended extracts were diluted into additional needle vials using sterile Coca buffer with 3.0 percent NSA to effect a final concentration of 100,000 PNU/ml, 50,000 PNU/ml, 10,000, 5,000, 1,000, 50, and 1.0 PNU/ml.

Subjects were tested on four or five consecutive days. Each day a challenge was performed using a different extract: durum wheat, durum wheat dust, grain mites, or grain insects. The subject with skin reactivity to A fumigatus was also challenged with an extract of this agent. A Rosenthal dosimeter powering a #42 DeVilbiss nebulizer was used to administer the extracts. Five vital capacity inspirations were taken slowly by the subject and then held for five seconds at each concentration of extract. This maneuver was repeated at gradually increasing concentrations of extracts until either a fall in FEV<sub>1</sub> of at least 20 percent was noted or the maximum concentration (100,000 PNU/ml) of antigen was given. This maximum concentration (100,000 PNU/ml) of antigen was given. This maximum concentration (100,000 PNU/ml) was reached in all challenges. Pulmonary function testing was performed before administration of antigen, ten minutes after administration of antigen at each concentration, and at frequent intervals thereafter up to 24 hours. Oral temperature was measured hourly. Blood samples were drawn for WBC counts and for complement measurements at 10 minutes and at 4, 8, and 24 hours.

Nonspecific bronchial reactivity was measured using inhalation of methacholine by the method recommended by Chai and co-workers. Using the dosimeter technique, methacholine was administered in increasing concentrations from 2.5 mg/ml to a maximum concentration of 25 mg/ml. The test was terminated when at least a 20 percent decrease from the baseline FEV<sub>1</sub> (positive test) was noted or if no response was elicited with the maximum concentration. The results were expressed as the concentration of methacholine producing a 20 percent decrease in FEV<sub>1</sub> (PC<sub>20</sub>) calculated from a dose-response curve. One capsule of sodium cromoglycaye was administered via a spinhaler 10 minutes before the challenge.

RESULTS

Five of the 11 subjects showed a decrease in FEV<sub>1</sub> (> 20 percent) in response to bronchial provocation with extracts of durum wheat (Fig 1). These five were termed airway reactors. The other six showed no significant diminution in FEV<sub>1</sub> when challenged with these extracts and were termed nonreactors.

One subject responded to extracts from both durum wheat and airborne durum wheat dust. In this subject the airway response occurred within 10 to 20 minutes. The other four subjects showed only late responses to durum wheat extract. None
FIGURE 1. Bronchial challenge with durum wheat extract. Airway reactors = FEV₁ decrement 20%.

FIGURE 2. Change in FEV₁ following bronchial challenge with durum wheat extracts after pretreatment with sodium cromoglycate.
responded to extracts of insects or mites, nor was there a response to *A. fumigatus* in the individual tested.

Methacholine inhalation produced a positive test in four of the five airway reactors and in two of the six nonreactors (Table 1). However, the PC_{20} was lower in the positive airway reactors.

Preexisting airway obstruction was present in four of the five airway reactors and three of the six nonreactors (Table 1). However, the obstruction was more severe in the reactors. Four of the five airways reactors were exsmokers, and one subject was a smoker. Of the six nonreactors, the two smokers were obstructed and one of the two exsmokers was obstructed. The four airway reactors who had preexisting airway obstruction responded to methacholine inhalation, while the nonreactors with preexisting airway obstruction showed no response.

Four of the five airway reactors were pretreated with sodium cromoglycate and then challenged with extract of durum wheat. The airway response was blocked by pretreatment in all four subjects (Fig 2).

If a decrement of 35 percent in FEF_{25-75%} V_{max50%}, and V_{max75%} is used as the criterion of detecting airway obstruction, then these tests were no more sensitive than FEV_{1} in detecting the acute airway response induced by durum wheat. However, in two instances decrements in V_{max} revealed an airway response that was not reflected in the FEV_{1}, which suggested a small airway reaction (Fig 3).

One of the five airway reactors and two of the

![Graph](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21267/ on 06/21/2017)

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**Figure 3.** Change from baseline FEV_{1} and V_{max75%} in subject 10 following bronchial challenge with durum wheat extract and in subject 1 following challenge with insect extract.
six nonreactors were atopic. There was no correlation between positive skin tests (common allergens, insects, mites, durum wheat, durum wheat dust, and Aspergillus sp) and a positive bronchial challenge (Table 2).

One of the five airway reactors had serum precipitating antibodies directed to the durum wheat extract and a positive bronchial response to durum wheat. Five subjects showed serum precipitins against extracts which did not induce a bronchial response.

There was no significant change seen in the DLco.

There was no change in leukocyte counts or levels of serum complement in airway or nonairway reactors after challenge, nor was there activation of complement by the classic or alternate pathway.

**DISCUSSION**

Durum wheat has been isolated as a constituent of grain dust that can induce airway response in grain handlers. This response could not be duplicated in these subjects by using extracts of insects, mites, or A fumigatus. The airway response is not always related to the atopic status of the individual. In addition, acquired sensitivity to the specific antigens that induced bronchial response, as indicated by the presence of serum precipitating antibodies, was evidenced in only one of the subjects.

Epidemiologic studies have shown that 40 to 75 percent of grain workers have respiratory symptoms on exposure to grain dust. The effects of inhalation of grain dust into the lungs have been thought to be primarily on the airways and not on the parenchyma. This was based on the observation that diffusing capacity (DLco) and chest roentgenograms remained free of abnormality when a large number of grain handlers was surveyed. The lack of change in the DLco in subjects who were airway reactors in the current study would reaffirm this observation.

The mechanism by which airway response develops, however, has remained unresolved. The reactions had been postulated to be immunologic (type I or type III or both), chemical, or mechanical. Warren and associates demonstrated immediate and late asthmatic reactions to grain dust, compatible with a hypersensitivity reaction. Chan-Yeung and co-workers, in a more recent study, concluded that the mechanism is dual: chronic industrial bronchitis, an allergic asthma, despite the absence of cutaneous reactivity to grain dust.

The lack of specific serum precipitins in the airway reactors in this study suggests that a type III allergic reaction may not play a role in the mediation of these grain handlers' pulmonary response. The lack of fever, and no consistent changes in leukocyte count, as well as the lack of change in DLco and lack of inspiratory crackles by lung examination during the challenges, are inconsistent with the alveolitis that may develop in the type III reaction.

The lack of evidence of complement activation by
the classic or alternate pathway in the subjects' sera does not exclude the probability of local activation of complement in the lungs. Olenchock and co-workers\textsuperscript{17} found that airborne grain dust can activate complement by the alternate pathway in \textit{in vitro} studies. Hence, the role of complement in the pulmonary reaction to grain dust needs further investigation.

One subject's response was consistent with that of a type I, immediate, ICE-mediated reaction. Our data, however, do not allow us to conclude whether the late reactions are immunologically mediated.

The antigen responsible for these bronchial responses may be contained in one or more of the fractions of durum wheat, \textit{eg}, endosperm, bran, or outer layer and germ. The antigen is likely to be in the saline soluble or albumin-globulin fraction of the fractions, but the role of the gliadin and glutenin fraction also deserve further investigation.

Sodium cromoglycate inhibited the bronchial response in all subjects treated. Sodium cromoglycate has been shown to prevent the degranulation of mast cells, and therefore to prevent the release of histamine, SRS-A, and other mediators.\textsuperscript{18} However, it is well known that nonspecific agents, such as cold air, exercise, and toluene di-isocyanate,\textsuperscript{19,20} can cause bronchial response that is also blocked by treatment with sodium cromoglycate. The mechanism involved in these latter cases is unknown, but may be due to direct irritant effect, or to mast cell contained mediator.

The finding that bronchial response is more likely to occur in those with heightened nonspecific bronchial reactivity (low PC\textsubscript{20} methacholine) is consonant with either of the proposed mechanisms, as is the observed increase in the degree of baseline airway obstruction. This may cause the development of asthma by: (1) sensitization of the bronchial mucosa so that local production of IgE occurs and an immediate allergic response develops, or (2) stimulation of the vagal reflex arc. It has been shown that stimulation of irritant receptors can lead to hyperpnea and bronchoconstriction, and nasal stimulation to increased bronchial mucus secretion.\textsuperscript{21} Irritation and inflammation caused by the inhalation of grain dust and its deposition in the bronchi would produce increased baseline obstruction, as our results indicated.

There are more than 500,000 American workers who are exposed to grain dust at their jobs, most of which are related to grain storage, transport, and processing. Our findings that there is no relation between the bronchial response to a specific antigen and the skin response to the same antigen may have a practical consequence in the screening of workers for these jobs. Skin hyperreactivity to grain or grain dust does not by itself detect those workers predisposed to an airway response to grain dust.

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