When the same data were grouped with respect to age, the following data were obtained: Grain workers between the ages of 31 to 50 years demonstrated elevated IgG levels (Table 50). There was no statistical difference in IgG levels when other age groups were considered. Statistical differences in IgA levels were only observed when grain workers between the ages of 41 to 50 years were compared to controls.

Since it was conceivable that length of employment influenced the data, the test and control immunoglobulin levels were subdivided with respect to length of employment (Table 51). Both IgG and IgA were elevated in the grain workers working in the elevators from 10.6 to 15.5 years. Increased levels of IgA were also observed in grain workers working fewer than 5.5 years in the industry. However, there was no relationship between IgA levels and place of employment. Increased levels of IgG (Table 52) were observed in workers in elevators 1 and 8.

The levels of circulating IgE were also ascertained in serum samples obtained from grain workers and controls using commercially available immunodiffusion plates. The lowest level of sensitivity of this system is 600 I.U. Only four of the 307 grain workers tested had IgE levels above 600 I.U. (1,000-4,000 I.U.) Similarly, only two of the 235 city workers tested had IgE levels above 600 I.U.

Data from the IgE determinations should be interpreted with caution. Recent data from our laboratories, using radioimmunoassays for determination of IgE levels, suggest that the level of IgE in normal serum is below 50 I.U./ml. Serum from highly allergic individuals contains between 300-600 I.U. and, rarely, levels above 900 I.U. Hence, the immunodiffusion method for determining IgE levels is not sensitive enough to detect increases in serum IgE occurring between 100-600 I.U.

CONCLUSION
Grain dust exposure enhances the levels of serum IgA and IgG, an effect which appears to be blunted by smoking (significantly in the case of IgA).

2h. ALPHA$_1$-ANTITRYPSIN (AAT) LEVELS
The levels of alpha$_1$-antitrypsin in serum samples from grain workers and controls were determined by the timed Mancini technique (Appendix XIII). The reproducibility of the system was insured through the use of the protocol outlined under the section on Materials and Methods.

There was no statistical difference when the AAT levels in grain workers (mean $\pm$ SD = 296 $\pm$ 5 mg/dl) were compared to controls (mean $\pm$ SD = 308 $\pm$ 6 mg/dl). When smoker grain workers were compared to smoker controls there was a significant decrease in the AAT levels observed in grain workers (Table 53). No significant differences were observed when other smoking categories were compared.

When the test group and controls were grouped by age, the AAT levels were significantly depressed in grain workers between the ages of 21 to 30 years and 41 to 50 years (Table 54a). Unlike the immunoglobulin levels, there was no relationship between length of employment and AAT levels (Table 54b).

Since it has been shown that subjects heterozygous for the AAT deficiency gene (Pi phenotype MX) have serum levels of AAT that are roughly 60% of the normal levels, we selected sera from the nine grain workers with AAT levels less than 60% of the normal values for phenotyping and trypsin inhibitory capacity (TIC) measurements. These studies were performed by Dr. Richard Talamo of Johns Hopkins University. None of the city workers exhibited intermediate AAT levels by the Mancini test.

The data show that three of the grain workers (#232, #239 and #240) had the heterozygous MZ phenotype and impaired TIC (Table 55). Another three subjects had the MZ phenotype and normal trypsin inhibitory capacity. The remaining three subjects had the MZ phenotype and normal TIC. Because of the small number of heterozygotes found in this study, we did not do statistical correlations with symptoms or lung function, but the review of these six subjects did not reveal any consistent abnormalities. Of the three subjects with MZ phenotype, one (#332) had a slightly decreased D_{LCO} and abnormal CV and N2/L but no evidence of airways obstruction. He had many years of chronic productive cough and wheezing. He also had elevated SGPT and GGT of unknown etiology. One of the three with MS (#52) had an FEV_1/FVC of 74% which may reflect some mild degree of airways obstruction at his age.

2i. MULTIPLE REGRESSION ANALYSIS OF THE EFFECT OF GRAIN DUST EXPOSURE ON IMMUNOGLOBULIN AND ALPHA$_1$-ANTITRYPSIN LEVELS (TABLE 56).

As will be seen in Table 57 grain dust exposure has a highly significant positive effect on the levels of IgA, and IgG ($p < 0.005$) but not on IgM. Length of employment and/or age is also positively related to levels of IgA and IgG with a significant relationship present for smoking ($p < 0.05$). Unfortunately it is not possible to separate the confounding effects of age and length of employment on immunoglobulin levels.

Conversely, grain dust exposure has a significant negative effect ($p < 0.025$) on the level of alpha$_1$-antitrypsin (AAT). Length of employment ($p < 0.0005$) and/or age ($p < 0.001$) and smoking ($p < 0.0005$) on the other hand show a highly significant positive relationship to the level of AAT. In this case age appeared to be a better predictor than length of employment.

Since grain dust exposure appeared to have a significant effect on the immune system which conceivably could in turn be related to the disease syndromes encountered in grain workers, we analyzed the relationship between chronic bronchitis, occupational asthma, grain fever and other symptoms, as well as skin test reactivity, tests of pulmonary function, and the level of immunoglobulins. Using an unpaired t test we found no statistically significant relationships between levels of immunoglobulins on AAT and skin test reactivity or symptoms. However, the levels of IgA, IgG and IgM were consistently higher in subjects who showed skin test reactivity to antigens from airborne grain dust, fungi, insects and mites. A similar trend was seen when comparing symptomatic with non-symptomatic workers for IgA and less consistently for IgG.

The results of a similar analysis of immunoglobulin levels in relation to abnormal tests of pulmonary function are seen in Table 57. Significant association was found between abnormal FVC, $Vmax_{50}$, DL and IgA and between abnormal FVC and IgG. Also a significant association was found between abnormal N2/L, DL and AAT. Once again subjects with abnormal pulmonary function had, with two exceptions, consistently higher levels of IgA and IgG.

CONCLUSIONS

Chronic exposure to grain dust appears to stimulate the immune system as reflected by its positive effect on serum IgA and IgG which increase with age and/or length of employment. The mechanism involved in producing these increases is unclear but may be an adjuvant effect. This may also explain the consistently higher levels of immunoglobulins in subjects who show skin reactivity to antigens found in grain dust. Evidence regarding any relationship of this effect on the immune system to the disease syndromes encountered in grain workers is conflicting. There is no relationship between immunoglobulin levels and acute or chronic symptoms of lung disease. On the other hand, workers with abnormal lung function have significantly higher levels of IgA and/or IgG. These findings merit further study.

Chronic exposure to grain dust appears to be associated with a decrease in the level of AAT. Smoking on the other hand is associated with a decrease in the level of IgA and IgG and an increase in AAT. The significance of the association between abnormal N2/L, DL and AAT is unclear. However, these tests may reflect an inflammatory reaction in the small airways (bronchiolitis) related to chronic grain dust exposure. These findings also merit further investigation.

STUDY II. WORK SHIFT STUDY
Materials and methods
    Population
    We studied 248 grain workers and 192 controls (city services workers). They represented 88% of the 283 grain workers and 80% of the 239 controls previously surveyed (see Study I). The 27 longshoremen were not asked to participate in the work shift study.

    The characteristics of the test and control populations are presented in Table II-Ia and b. The following parameters were evaluated:

    1) Symptoms during the shift and the workers' subjective appraisal of the dust exposure were obtained at the end of the work shift on a standard form.

    2) Pulmonary functions studies before and after the shift included: FEV_1, FEVC, $Vmax_{50}$, $Vmax_{75}$. All of these values were obtained using a rolling bar Ohio 840 spirometer with the techniques described in Appendix VIII.

    3) Blood studies before and after the shift included: a) leukocyte count and differential count, b) serum complement and complement activation measured studied as described in Appendix XIII and XIV.

4) Oral temperature were taken at 700-800 hours, 1100-1200 hours, 1500-1600 hours and 2000 hours.

5) Environmental studies: airborne total and respirable dust levels were measured on each of 209 grain workers and in a sample of 63 controls using personal dust samplers as described in Appendix XV.

6) Mycological studies: Airborne dust from personal samplers was analyzed for fungi as described in a separate report.

Medications taken by subjects during the day of study included: subject #9 - "Tedral," #7 - "Contac," #21 - "Robitusin," #242 - "Cough drops" and #233 - "Dristan."

RESULTS

Dust exposure. Of the 248 grain workers, 197 (79.4%) reported exposure to either wheat, barley or oats. Other exposures included sunflower seeds, corn and rye. Total dust levels in grain workers by elevator and job category are presented in Table 11-2.

Symptoms during work.

Twenty-five % of the grain workers claimed they had worn a mask during at least part of that day. The incidence of respiratory symptoms (cough, expectoration, wheezing and dyspnea), nasal stuffiness and eye irritation (Table 11-3) were higher in grain workers than controls.

The incidence of symptoms during work shifts in grain handlers by smoking categories and subjective appraisal of dust exposure is shown in Table 11-4. Wheezing and/or chest tightness were more common among smokers than nonsmokers, whereas throat symptoms were more common among nonsmokers.

Grain workers who reported a normal or average exposure to dust during the work shift had a higher incidence of cough and phlegm when compared to workers exposed to less than average dust concentrations. Workers exposed to higher dust concentrations (more than average) had a higher incidence of dyspnea, wheezing, eye and nasal symptoms when compared to workers exposed to average or less than average dust concentrations. Most symptoms were more common among workers who reported a heavy exposure to dust some time during that day (p <0.05). Incidence of respiratory symptoms by company and job category are shown on Table 11-5.

Leukocyte count and serum complement

The leukocyte count and serum complement levels before and after work shifts are presented in Tables 11-6 and 11-7. The data show that the total white blood cell counts were not different when pre- and post-samples were compared (Table 11-6). The differential white blood cell count suggests that there were slight shifts in leukocyte subpopulations during a work shift. Grain workers had slight increases in the percentage of segmental neutrophiles and decreases in lymphocytes when compared to controls.

There were no changes in the mean C3 levels during the work shift in either the grain working or control population (Table II-7). Moreover, there was no evidence of classical or alternate complement pathway activation. However, six of 191 controls demonstrated activation of the alternate complement pathway at pre- and post-shift intervals. This may represent faulty on-site specimen handling in Duluth/Superior.

Consideration was also given to individual changes in total C3 levels within the grain workers and city workers. Using the standard deviation from the pre-shift city workers (28 mg %) as the base, we considered a significant increase or decrease in C3 levels to be 2 standard deviations (56 mg %) from the population mean. Using this criterion to analyze the C3 data, seven of 248 grain workers decreased their C3 levels significantly as compared to three of 191 city workers. Conversely, nine of the grain workers increased C3 levels by more than 53 mg%. None of the city workers increased C3 levels by the same value.

There were no correlations between increases or decreases in complement levels and changes in pulmonary function tests, white blood counts or symptoms. Moreover, there was no relationship between changes in complement levels and activation of complement by either the classical or the alternative pathway.

Body temperature
Values are presented in Table II-8. There were no differences in body temperature (800, 1200, 1600 and 2000 hour values) between the grain workers and the controls.

Pulmonary function studies
The pulmonary function studies were performed before and after the work shift (Tables II-9 and II-10). Analysis of the pre- and post-shift values indicated that no significant acute effects on lung function occurred during the work shift (Table 10). On the other hand when the data were expressed as % difference in pre- and post-values (Table 9), the FVC, $Vmax_{50}$ and $Vmax_{75}$ in grain workers were significantly different from controls ($p < 0.05$). The difference was due to a slight increase in function in the controls and an average slight decrease or lesser increase in function in grain workers. Also the actual differences in pre- and post-shift $Vmax_{50}$ and $Vmax_{75}$ were slightly positive in controls and slightly negative in grain workers (Table II-9).

The changes in lung function, before and after a work shift, were also evaluated by multiple regression analyses using the actual pre-post-shift lung function difference or % differences as the dependent variable. The independent variables were grain handling, age, height, current smoking and ex-smoking. This analysis indicated that grain handling had a significant adverse or negative effect on pre-post-shift % differences in lung function independent of the effects of cigarette smoking (Table II-11).

To evaluate the possible clinical significance of changes in pulmonary function, we studied the incidence of post-shift reduction in

function of varying severity that might be considered an abnormal response to the environment (Table II-12a). Although the numbers of workers were small, there was a consistently higher number of grain workers with pre-post reductions in pulmonary functions when compared to city workers having no abnormal lung function changes. The characteristics of the 14 subjects with pre-post shift difference in $FEV_1(>15\%)$ are presented in Table 12b. The mean age (48.4 years) was higher than the population mean, and the mean length of employment was 15.8 years. Only 2 of the 14 were nonsmokers. Four had a history of chronic bronchitis and one subject had asthma. Nine of the 14 had complained of cough or wheezing on exposure. All subjects were exposed to wheat, barley and/or sunflower seeds. The total dust levels varied between .5 and 9.3 mg/m3. Ten of the 14 had pre-existing airways obstruction. Three were atopic and 5 had skin reactivity to grain dust antigens.

These data suggest that a decline in FEV_1 greater than 15% over a work shift can occur at average total dust levels lower than 10 mg/m3 in grain workers with pre-existing airways obstruction, regardless of smoking habits (smokers or ex-smokers).

Relationship between Symptoms during Work and Lung Function Changes

There was no difference (chi square analysis) in the % change of pre-post values when subjects with respiratory symptoms or fever were compared to subjects without symptoms (Table II-13).

Relationship between Symptoms of Pulmonary Function Changes during the Work Shift and Skin Hypersensitivity

The incidence of respiratory or nasal symptoms was not different between atopic (wheal reaction ī 3 mm to one or more common allergens) and non-atopic workers or between grain dust skin reactors and non-reactors.

Also using pre-post mean values for pulmonary function tests, there were no differences when atopic and non-atopic subjects or grain reactors and non-grain reactors were compared.

Relationship between Total Dust Level and Presence or Absence of Symptoms during Work Shift

Workers with respiratory symptoms (cough, expectoration, wheezing or dyspnea) during the work shift were exposed to a higher mean total dust level than workers who did not claim symptoms on the shift studied (Table II-14).

The incidence of symptoms by dust level categories is shown in Table II-15a. Few significant differences between grain workers and controls are seen at dust levels below 10 mg/m3. At levels above 10 mg/m3 there is a significantly higher incidence of cough, dyspnea, fever, eye and throat symptoms among grain workers. It should be noted, however, that relatively low dust levels were encountered during this study. Sixty-seven % of the measured values were below 2 mg/m3 and 86% were below 5 mg/M3. Only 7% of the values were between 5-10 mg/m3 and 7% were above 10 mg/M3. Compared with the conditions that existed prior to 1974, this is a remarkable achievement on the part of the grain companies to control grain dust in their elevators. During

this study only 4% of the measured dust levels exceeded the current nuisance dust standard of 15 mg/m3. On the other hand the relatively small number of workers exposed to grain dust levels above 5 mg/m3 limited our ability to establish a clear cut dose-response relationship. Since control workers could be considered as having zero grain dust exposure, we repeated the analysis using the entire cohort of city workers (N=192). The results are shown in Table 15b. As will be seen in this table, grain workers experience a significant excess of cough and expectoration even at dust levels 5 mg/m3.

Relationship between Total Dust Level and Workers' Subjective Estimation of Dust Exposure

There was a significant relationship between total dust level and workers' subjective estimation of dust levels (Table 11-14).

Relationship between Total Dust Level and Pre-Post Shift Differences in Lung Function Tests

Relationships were studied by multiple regression analyses using pulmonary function (FEV_1, FVC, $Vmax_{50}$ or $Vmax_{75}$) as the dependent variable and dust level, age, height, smoking and ex-smoking habit as independent variables. In the grain workers (Table 11-16) there was a significant negative relation between dust level and pre-post shift % changes in FVC, $Vmax_{50}$ and $Vmax_{75}$ ($P < 0.05$) adjusted for the effects of age, height and smoking habit (Table 11-16). In controls there was no relationship between the dust level and changes in pulmonary function using any test.

The negative effect of grain dust on tests of airways flow and vital capacity appears to be dose related.

Relationship between Dust Levels and Pre-Post Shift Difference in Leukocyte Count and C3 Complement Level

By regression analysis, pre-post shift changes in leukocyte count or in C3 complement level were considered dependent variables and dust level, age and smoking as independent variables. In the controls there was no relationship found between total dust levels and changes in leukocytes or C3 complement levels. In grain workers there was a positive relationship between total dust level and the pre-post shift difference in leukocyte count ($P < 0.05$), but no relation between complement level changes and dust levels. Grain dust exposure thus appears to induce a leukocyte response that is dose related.

Conclusions

Exposure to grain dust during a work shift has a dose related acute adverse effect on the worker. The effects, which are largely on the respiratory system, are seen at relatively low dust concentrations. When compared with city workers, grain workers show a significant excess of cough and expectoration during a work shift at dust concentrations below 5 mg/m3. In addition, the susceptible workers (i.e., those with pre-existing airways obstruction) can experience significant declines in ventilatory function at dust levels below 10 mg/m3. Because of the small proportion of workers (14%) who were exposed to dust concentrations above 5 mg/m3 during this study, it was difficult to establish an exact dose-response relationship between dust concentrations and ventilatory function. There seems to be little

doubt, however, that dust concentrations below the current nuisance dust standard of 15 mg/m3 can have an adverse acute effect on ventilatory function. The grain companies are to be congratulated on the remarkable decrease in dust levels that has been achieved since 1974. During this study 86% of the dust measurements were below 5 mg/m3 and 93% below 10 mg/m3. Further studies should include peak values of dust concentrations as well as time-weighted averages, since workers' symptoms and changes in lung function may be related more to peak concentrations than average concentrations of dust during an 8-hour shift.

STUDY III PROSPECTIVE 3 YEAR FOLLOW-UP (1974-1977)

The purpose was to determine changes in pulmonary function in grain elevator workers who had been previously studied.

Materials and Methods

We studied 172 of the 293 year-round grain workers who were studied in 1974. One hundred and twenty-one subjects were not included in the study or analysis because of the following reasons:

1) Thirty-two subjects were working in the elevators but refused or could not participate.

2) Thirteen subjects were laid off and unavailable.

3) Twelve subjects had retired at ages 62 to 65 except one who had retired earlier because of a stroke residual.

4) Thirteen subjects were on vacation, 8 were on sick leave, 8 had changed jobs and moved away from the area and 3 had been transferred to management.

5) Three had died (2 heart attacks and 1 car accident).

6) The status of 29 workers was unknown.

The characteristics of the population studied are shown in Table III-I.

Pulmonary Function Studies (see appendix VIII)

Pulmonary function studies included forced expiratory volume in I sec (FEV_I), forced vital capacity (FVC), and mean forced expiratory flow during the middle half of the FVC (MMF), all recorded on a 13.5 liter Collins spirometer. The FEV_I and FEF 25-75% were measured from the largest of three acceptable FVC tracings, and all volumes were corrected to BTPS. The instantaneous maximal expiratory flows after exhalation of 50 and 75 % of the FVC ($Vmax_{50}$ and $Vmax_{75}$, respectively) were measured using a rolling bar spirometer and were displayed on an X-Y recorder. The average of 3 reproducible maximal expiratory flow volume curves was used.

Diffusing capacity of the lung for CO (D_{LCO}) was measured by the single-breath method of Ogilvie and associates[14].

Predicted values for FEV_1, FVC, and MMF, $Vmax_{50}$, $Vmax_{75}$ were obtained from the data of Knudson et al.[17]; D_{LCO} from Ogilvie and co-workers[14]. These methods were used in the 1974 study.

The changes in pulmonary function over 3 years were evaluated independently and also by the status of their smoking habit including: smoker who remained smoker; smoker who became ex-smoker; ex-smoker who

remained ex-smoker; nonsmoker who remained nonsmoker. Three ex-smokers who resumed smoking and 4 nonsmokers, who became smokers and then quit, were excluded from analysis.

Other information on these subjects was obtained as described under Material and Methods - Study I.

The current status of their respiratory symptoms was obtained on a standard form. Changes in smoking habit were obtained from the standard questionnaire.

Results
Symptoms. The symptoms reported by workers in 1977 are shown in Table III-2. Most workers (72-84%) reported that their respiratory symptoms remained about the same, but 9-25% were better or had symptoms less often. A small percentage felt their symptoms were worse (Table III-3).

Pulmonary function changes. There were no significant changes in FEV_1 and FVC, but there were significant changes in MMF, $Vmax_{50}$ and $Vmax_{75}$, both actual and when corrected for age by using the changes in % predicted values (Table III-4).

Similar results were detected in the different smoking categories (Tables III-5-8).

The yearly mean decrement (Table III-9) in FEV^1 and FVC was similar to that expected from published data (Knudson, et al.), but the yearly mean decrement in FFM, $Vmax_{50}$ and $Vmax_{75}$ was greater than expected.

There were no differences between the 3 year changes in atopic and non-atopic individuals, between skin reactors to grain dust and non-reactors, between those with chronic bronchitis and those without, or between workers with and without occupational asthma I (Table III-10).

CONCLUSION
This study is seriously faulted by the poor level of participation (59%) of workers previously studied in 1974. However several tests of lung function (MMF, $Vmax_{50}$ and $Vmax_{75}$) showed a yearly mean decrement that was greater than expected, which is probably indicative of the chronic effect of grain dust on ventilatory function.

STUDY IV BRONCHIAL CHALLENGE STUDY
IDENTIFICATION OF GRAIN DUST CONSTITUENTS WHICH CAN INDUCE PULMONARY REACTION
This study was undertaken to identify the constituent of grain dust responsible for grain handlers' symptoms and to determine a site of action in the lung. Host factors which may influence or contribute to this process were also assessed.

Material and Methods
Subjects: The subjects for the study were II grain handlers from northern Wisconsin and Minnesota who had respiratory symptoms on

exposure to durum wheat dust at work. Symptoms included cough, wheezing, chest tightness and expectoration. The workers were all men with a mean age of 38 and an age range of 27-59 years.

Skin tests: Subjects were tested for atopic diathesis by prick test using six common allergens: ragweed, feathers, oak, cat, Alternaria and timothy grass. The subject was considered atopic if he developed a 3 mm or greater wheal at 20 minutes to three or more of these allergens. Intradermal tests were used to detect immediate skin test reactivity to extracts of durum wheat, airborne durum wheat dust, molds, grain mites, grain weevils or grain beetles. These extracts were prepared from material collected from the workers' environment. Subjects were termed positive skin reactors if an 8 mm or greater wheal was raised at 10 minutes to an injection of 1000 PNU per ml or less of extract.

Precipitating antibody: serum precipitating antibodies against durum wheat, airborne durum wheat dust, molds and insects were measured by the immunodiffusion method of Ouchterloney.

Spirometry: Spirometry was performed on an Ohio 840 rolling bar spirometer. The FEV_1 and MMF were measured from the largest of two acceptable FVC tracings, and all volumes were corrected to BTPS. The instantaneous maximal expiratory flows after exhalation of 50 and 75% of FVC ($Vmax_{50}$ and $Vmax_{75}$) were measured from 2 reproducible maximal expiratory efforts which were displayed on an X-Y recorder and then averaged. Diffusing capacity of the lungs for CO (D_{LCO}) was measured by the single breath method of Ogilvie and associates. Before entering the study, each subject was tested for pre-existing airways obstruction. Subjects 40 years of age or under were termed obstructed if the FVC_1/FVC % was less than 70%, and subjects over 40 years of age were termed obstructed if the FEV_1/FVC % was less than 75%.

Preparation of challenge material: The preparation of extracts used for skin tests and bronchial challenge is described in Appendix X. Twenty-four hours prior to the challenge, the lyophilized extracts were resuspended in sterile, non-pyrogenic coca buffer with 3.0% human serum albumin to effect a final concentration of 100,000 PNU/ml. The resuspended extracts were filtered through a millipore filter (pore size .22mm), 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% NSA to effect final concentrations of 100,000 PNU/ml, 50,000 PNU/ml, 10,000, 5,000, 1,000, 50 and 1.0 PNU/ml.

Bronchial challenge: Subjects were tested on 4 or 5 consecutive days. Each day a challenge was performed using a different extract: durum wheat, durum wheat dust, grain mites or grain insects. A Rosenthal dosimeter powering a #42 Devilbis nebulizer was used to administer the extracts. Five vital capacity inspirations were taken slowly by the subject and then held for 5 seconds at each concentration of extract. This maneuver was repeated at gradually increasing concentrations of extracts until either a drop in FEV_1 of at least 20% was noted or the maximum concentration (100,000 PNU/ml) of antigen

was given. Pulmonary function testing was performed before administration of antigen, 10 minutes after administration of antigen at each concentration, and at frequent intervals thereafter up to 24 hours.

Temperature: During each bronchial provocation, oral temperature was measured hourly.

Laboratory tests: Blood samples were drawn for white blood cell counts and for complement (C3) measurements at 20 minutes, 4, 8 and 24 hours. Complement was measured by the method described in Appendix XII.

Methacholine provocation: Non-specific bronchial reactivity was measured using Methacholine inhalation by the method recommended by Chi 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 $\geq$ 20% decrease from the baseline FEV_1 was noted, or if no response was elicited, with the maximum concentration. The results were expressed as the concentration of Methacholine producing a 20% decrease in FEV_1 (Pc20) calculated from a dose-response curve.

Challenge after sodium cromoglycate: One capsule of sodium cromoglycate was administered, via a spinhaler, 10 minutes prior to challenge.

Results

Bronchial provocation challenge: Five of the 11 subjects showed a decrease in FEV_1 ($\geq$ 20%) in response to bronchial provocation with extracts of durum wheat (IV-Fig. 1). These 5 were termed airways reactors. The other 6 showed no significant diminution in FEV_1 when challenged with these extracts and were termed non-reactors (IV-Fig. 2).

Type of response: One subject responded to extracts from both durum wheat and airborne durum wheat dust. In this subject the airways response occurred within 10-20 minutes (IV-Fig. 1). The other 4 subjects showed only late responses to durum wheat extract.

Methacholine response: Methacholine inhalation produced a positive test in 4 of the 5 airways reactors and in two of the 6 non-reactors (Table IV-I). However, the Pc 20 was lower in the positive airways reactors.

Contribution of airways obstruction: Pre-existing airways obstruction was present in 4 of the 5 airways reactors and 3 of the 6 non-reactors (Table IV-I). However, the obstruction was more severe in the reactors. Four of the 5 airways reactors were ex-smokers and one subject was a smoker. Of the 6 non-reactors, the 2 smokers were obstructed and 1 of the 2 ex-smokers was obstructed. The 4 airways reactors who had pre-existing airways obstruction responded to Methacholine inhalation while the non-reactors with pre-existing airways obstruction showed no response.

Effect of sodium cromoglycate: Four of the 5 airways reactors were pre-treated with sodium cromoglycate and challenged with extract of

durum wheat. The n airways response was blocked by pre-treatment in all four subjects (Fig. IV-3).

Skin test: One of the 5 airways reactors and 2 of the 6 non-reactors were atopic. There was no correlation between positive skin tests (CAA, insects, mites, durum wheat, durum wheat dust, and aspergillus species) and a positive bronchial challenge (Table IV-2).

Precipitating antibody: One of the 5 airways reactors had serum precipitating antibodies directed to the durum wheat extract and positive bronchial response to durum wheat. Five subjects showed serum precipitins against extracts which did not induce a bronchial response.

Diffusing capacity: There was no significant change seen in the D_{LCO}.

Blood tests: There was no change in leucocyte counts or levels of serum complement in airways or non-airways reactors after challenge.

Small airways measurement: If a decrement of 35% in MMF, $Vmax_{50}$ and $Vmax_{75}$ is used as the criterion of detecting airways obstruction, then these tests were no more sensitive than FEV_1 in detecting the acute airways response induced by durum wheat. However, in some instances $Vmax_{75}$ revealed an airways response which was not reflected in the FEV_1, which suggested a small airways reaction (Fig. IV-4).

Extracts of durum wheat induced an airways response in grain handlers. This response was not duplicated by extracts of A. fumigatus or grain insects, grain mites or grain weevils. The airways response was not related to either the atopic status of the individual or the presence of precipiting antibodies in the serum.

CONCLUSION
Durum wheat induces an airways response in grain handlers. The effect of inhaled durum wheat appeared to be on the large airways without parenchymal or systemic reactions and without complement consumption. This response can be inhibited by sodium cromoglycate.

Study V – Grain Fever Syndrome
Purpose
This study was designed to delineate the clinical, physiological and immunological events which occurred during episodes of grain fever. It also intended to answer several questions:

1) Does "grain fever" develop during and/or after exposure to grain dust? Can saline extracts of barley induce grain fever?

2) What individuals are more likely to develop "grain fever," grain handlers previously exposed to grain dust or individuals not exposed to grain dust (controls)? Atopic individuals? Skin reactors to grain dust extract of grain, i.e., barley, fungal mite or insect antigens? Bronchial hyperreactors to methacholine? Individuals with pre-existing airways obstruction? Individuals with precipitating antibodies to airborne grain dust or precipitins to fungal antigens?

3) Does fever develop in all subjects with symptoms described as "grain fever?"

4) Do respiratory symptoms occur with "grain fever?"

5) Is there an airways response? Are there parenchymal reactions?

6) Is the complement system activated during these reactions? By the alternate or classical pathway?

7) Is there a blood leukocyte response?

Materials and Methods

Subjects

We studied 6 grain handlers with history of recurrent episodes of "grain fever" detected during the health survey and 6 asymptomatic healthy adults without occupational history of grain handling. The characteristics of these 12 individuals are summarized on Table V-I. The 6 grain handlers complained of respiratory symptoms and eye irritation during exposure to high concentrations of grain dust. Three subjects had a history of productive cough for more than 2 years, 3 were smokers and 3 were nonsmokers. All had worked in the grain industry for I to 30 years with a mean of 13 years. The controls were unemployed, nonsmoking men. One of the controls had some chest tightness on heavy exercise, a second a history of hay fever and a third a history of some wheezing with colds in childhood.

Inhalation Challenge

During the inhalation challenge we studied symptoms, body temperature, blood leukocyte count and differential counts, FEV_1/FVC, MMF, $Vmax_{50}$, $Vmax_{75}$, DLCO and complement changes (total C3) and activation of classical or alternate complement pathway. The Methacholine inhalation tests were administered as recommended by the Asthma and Allergy Disease Center Report for standardization of bronchial challenge procedures. The Pc20 (the provocation concentration which will cause a fall in FEV_1 of 20%) was calculated from the last two points on the log dose-response curve. On the first day, or control day, no inhalation material was given, but measurements were taken at regular intervals. On the second day, baseline values were obtained, and the subjects were exposed to airborne grain with dust in an environmental chamber. The subjects manually created dust aerosols similar to levels which provoked episodes of grain fever. Each subject was tested at frequent intervals for 24-48 hours to provoke an episode of grain fever.

Respirable dust concentrations were determined using a 37 millimeter diameter acrylic filter with an 0.8 micron pore size (DA 800, Gelman). All filters were pre-weighed to the nearest .001 milligram. Prepared filter cassettes with cellulose backup pads were then capped and securely placed into a 10 milliliter nylon cyclone assembly, attached by .75 meter long tygon tubing to personal sampling pumps equipped with pulsation- flow dampers (Model G, MSA). Pumps were periodically monitored over the exposure time to insure a flow-rate of 1.7 liters per minute + .1 liter per minute. Air sampling was stopped when the subjects were removed from the chamber for testing. Based on the actual exposure time, the time-weighted average respirable dust levels were calculated considering the actual time of exposure, which varied between 60 minutes and 120 minutes. Any change in the parameters measured was compared to a baseline established earlier. On

the third day, if the subject was still symptomatic and/or leukocytosis persisted and/or FEV_1 had not returned to the pre-challenge baseline level ± 10%, the subject was tested at regular intervals. Eight of the 12 subjects were observed for 48 hours. If the subject returned to baseline levels, he was tested, on the third day, with saline extract of barley (Appendix X). Each subject was challenged with increased concentrations of barley using a dosimeter-powered Deviblis nebulizer until a decrease of 20% in FEV_1 occurred or a dose of 100,000 PNU/ml was reached. To establish a safe initial dose for inhalation challenge with barley extract, intradermal skin tests were performed. The dilution at which an 8 mm wheal reaction was obtained was used as the initial challenge dilution. No skin test was done with a dose greater than 1000 PNU.

Skin testing (See appendix XI)
Precipitating antibodies (See appendix XII)
Complement (See appendix XIII & XIV)
Grain dust concentration (See appendix XV)

Results
A significant change in experimental parameters was considered to be:
1) Blood leukocyte counts (WBC) above 10,500 per mm3 (leukocytosis).
2) Increased oral temperature 37.8 C (fever).
3) C3 complement levels more than 38 mg % from baseline.
4) A decrease $\geq$ 20% in FEV_1, FVC and DLCO when compared to baseline values. No change greater than 10% was observed during the control day.
5) A decrease $\geq$ 35% in MMF, $Vmax_{50}$ and $Vmax_{75}$ when compared to baseline values.

Precipitins
None of the 12 subjects had precipitins to grain dust or fungal antigens.

Skin tests:
Two of the grain workers and 4 controls had positive prick skin tests to 2 or more common allergens and were considered atopic individuals. Three grain workers and 1 control had positive prick skin test to airborne grain dust antigen. No skin reactivity to barley was observed in the test or control group.

Methocholine Challenge
Methocholine bronchial hyperreactivity was observed in 2 grain workers and 2 controls. Pre-existing airways obstruction defined as an FEV_1/FVC less than 70% was observed in 1 grain worker. The pulmonary function studies done on these subjects before the challenge are presented in Table V-1.

Airborne grain dust challenge
Respirable dust concentrations, time averaged for each individual, are presented in Table V-I. The mean respirable dust concentration was 84 mg/m^3.

Symptoms
All subjects became symptomatic during exposure. In some instances, the symptoms lasted longer than 24 hours but no greater than

36 hours. The most common complaints were "flu-like" symptoms: malaise, myalgias, tiredness, feverish feeling, chills and flushed face.   These symptoms were observed in all grain workers and in 3 of the controls. These symptoms varied in intensity and were particularly marked in 7 of the 12 subjects.   The symptoms were very mild in 2 of the 12 subjects. Seven of the 12 workers had headaches; none complained of eye irritation. The following symptoms were also observed: nasal symptoms (4/12), throat burning (5/12), cough (12/12), wheezing or chest tightness (9/12) and shortness of breath (9/12).   Using baseline comparisons, 10/12 subjects (5/6 controls and 5/6 grain workers) developed leukocytosis (>11,700 per mm3:   range 11,000-24,300).   The average increase in the leukocytes was 11,000 per mm3.   In one additional subject the white count increased by 3,300 but remained below 9,000.   Body temperature rose above 37.8C in 6 of the 12 subjects (5/6 grain workers and 1/6 controls), 2 to 40 hours after exposure.

Pulmonary Function
Airways obstruction (decline in FEV_1 = 20%) developed in 4 of 6 grain workers and 5 of the 6 controls (Table V-1).   Diffusing capacity also decreased in two controls within 24 hours after exposure.   T-test analysis of the changes in FEV_1 between the baseline and immediate post-exposure values revealed a significant (P <0.05) change for grain workers and controls (Fig. V-1).   After challenge, the maximum changes in FEV_1 occurred: 1 hour (1), 2 hours (4), 4 hours (4), 6 hours (1), 8 hours (1) and 24 hours (1) later.   Two of the controls showed a marked decrease in FEV_1 after an improvement from the initial decrease at the 28th hour.   The changes in FVC for the group by t-test analysis was also significant in the grain workers, the controls and both groups together (Fig. V-2).   For the MMF the paired t-test showed significant change for the 12 subjects analyzed together (p <0.001) (Fig. V-3) and for the controls (p <0.01), but it was not significant for the grain workers alone. $Vmax_{50}$ showed significant differences between baseline and control and the grain workers (p <0.01), the controls (p <0.02) and in both groups (p <0.001) (Fig. V-4).

The changes in leukocyte count by t-test on the group was also significant for the grain workers, controls and both groups together (P<0.02), (Table V-1).

The maximum leukocyte change was seen between 4 and 8 hours on most subjects and in 24 hours on 1 subject.

The changes in temperature in the grain workers was significant (P <0.05) by the t-test but was not significant for the controls or grain workers plus controls (Table V-1).

We analyzed the relationship between atopy and the development of airways reaction to dust exposure utilizing x^2 analysis and it was not significant.   Of the 6 atopic individuals, 5 developed a significant change in FEV_1 or airways obstruction (83%) and 1 (17%) did not.   Of the non-atopic individuals 4 of 6 (57%) developed airways obstruction; but 2 of the 6 (33%) did not.

In those individuals with positive skin tests to airborne dust, 3

of 4 (75%) developed airways obstruction, and 1 of the 4 did not. Of those with negative skin tests, 6 of 8 developed airways obstruction (75%); 2 of the 8 did not. Airways obstruction developed in 9 of 12 subjects, 4 of which were non-atopic while 5 were atopic. Airways obstruction developed in 6 of 9 negative skin reactors to airborne grain dust; 3 of 9 reactors were reactors to the airborne dust.

Barley Challenge

Inhalation of barley extracts reproduced grain fever syndrome in 1 of 10. Six of 10 had leukocytosis with left shift, but 1 had fever over 37.8 C. One of 10 had a 20% or greater decline in FEV_1. One of 10 had an increasing C3 level of 44.

Conclusions

Inhalation of high concentrations of airborne dust for 1 to 3 hours induces the grain fever syndrome lasting 24 to 36 hours. This syndrome is characterized by systemic reaction, facial warmth, headache, chills, malaise, myalgias, leukocytosis, left shift fever and is commonly associated with respiratory symptoms, throat and tracheal burning, chest tightness, dyspnea, cough, expectoration and airways obstruction. There is no evidence of parenchymal reaction.

These reactions occur in both grain workers and controls and are independent of previous exposure, atopic status, bronchial hypersensitivity, pre-existing airways obstruction or the presence of precipitins.

Our data do not support the hypothesis that grain fever is a type III allergic reaction since none had precipitins to grain dust, complement was not activated and changes in DLCO were not observed. This data may suggest that grain fever is due to bacterial endotoxin or non-specific release of pharmacologically active substances from the lung after interactions between components of grain dust and lung cells.

Study I.   Health Status of a Cross-section of Grain Handlers in the Twin Ports of Duluth and Superior.

The health status of grain handlers was evaluated by comparing the prevalence of clinical, physiological, immunological, radiological, serological, blood and urine parameters in 310 grain workers (test group) and 239 city service workers (controls) from the same geographic area. The control group was matched to the test group with respect to sex, age, height, weight and smoking habit.

All subjects were studied according to the following protocol:
  1)   a self-administered questionnaire reviewed for completeness by trained interviewers;
  2)   a physical examination performed by physician;
  3)   pulmonary function tests including FEV_1, FVC, MMF, $Vmax_{50}$, $Vmax_{75}$, CV, N2/L, DLCO;
  4)   a chest roentgenograph, postero-anterior view;
  5)   skin prick tests for detection of immediate hypersensitivity to common allergens, fungal antigens, grain mites, grain insects, grain, airborne grain dust and settled grain dust;
  6)   intradermal skin tests for delayed hypersensitivity to PPD, mumps, Candida albicans, Streptokinase-Streptodornase (SK/SD) and

Trichophyton;
    7) detection of serum precipitating antibodies directed toward:
fungal antigens, bacterial antigens, pigeon sera, grain, airborne grain
dust and settled grain dust;
    8) the levels of circulating immunoglobulins (G,A,M,E);
    9) blood hemoglobin and hematocrit;
    10) urinalysis for protein, glucose and blood;
    11) serum creatinine;
    12) serum alanine aminotransferase (SGPT), gamma
glutamyltranspeptidase (GGT) and pseudocholinesterase;
    13) alpha$_1$-antitrypsin levels.

Results and Conclusions from this Study
1) Clinical findings. Grain handlers had a higher prevalence of
respiratory symptoms and signs (ronchi) than comparable non-grain
handling city service workers from the same geographic area (Table 7-9,
12, 24) whether or not they smoked. The effects of grain handling on
prevalence of respiratory symptoms were highly significant, independent
and usually greater than those of smoking (Table 13). The prevalence
of work related respiratory symptoms adjusted for age and smoking habit
was also positively related to place (Tables 19, 20) and length of
employment (Table 15). The data suggested variable environmental
working conditions among elevators and perhaps an accumulative
respiratory effect due to recurring exposures to grain dust.

    Grain workers suffer from:
    a) acute and chronic airways reactions (occupational asthma and
chronic bronchitis) induced by exposure to grain dust with varying
degrees of: cough, expectoration, wheezing and/or chest tightness and
shortness of breath. Durum wheat and barley grain dust were the most
common inducers of symptoms. During the work shift, wheezing and/or
chest tightness occurred immediately after starting work or within 2
hours. In late reactors, wheezing occurred within 2 hours after
leaving work. Very late reactions were not reported.

    Wheezing and dyspnea on exposure were related to length of
employment. This may indicate either increased sensitization to the
allergens in the environment or the bronchial mucosa being
rendered more hyperreactive by the recurrent non-specific inflammatory
reactions of the airways by grain dust. The place of employment was
found to affect the prevalence of symptoms. The highest prevalence of
symptoms were found in 4 companies and the lowest in 2 companies.

    b) A grain fever syndrome (Table 21), characterized by a short-term
febrile illness (flu-like syndrome) that may be associated with
respiratory symptoms. It usually occurs during work or shortly after
work. It is related to exposure to high concentrations of dust any day
of the work week and not necessarily the first day at work or the first
day of the week. There was, however, a small percentage of workers who
had a single episode of grain fever the first time at work and not
again. The workers stated that in the last 3 years, because of the
improvement in the working conditions, grain fever occurred less
frequently. Some workers had grain fever a few hours after work,
compatible with allergic pneumonitis. However, none of these episodes
were severe · enough to require medical attention, and we lack

radiographic proof of allergic pneumonitis. Furthermore, the symptoms tended not to recur unless very high concentrations of dust were again present. Although we cannot deny that in some instances the grain fever syndrome may be a manifestation of allergic alveolitis, we have not found the typical history and radiographic changes of allergic alveolitis in these workers.

    c) Acute recurrent conjunctivitis and rhinitis during exposure to grain dust occurred in most grain workers.
    d) Skin pruritus occurred mostly on exposure to barley dust.
    e) Pesticide exposure caused temporary disabling symptoms
The long-term effects of recurrent symptomatic or asymptomatic exposures to pesticides are unknown, but we have encountered several former grain handlers with chronic neurological defects attributable to pesticide exposure.

2) Pulmonary function status. We concluded that grain dust exposure had an adverse effect on lung function (Tables 25-27, 29-31). The effect of grain dust on lung function was highly significant, and the overall effect was the same or of smaller magnitude than that of smoking. Although there were more grain workers with mild airways obstruction than controls, moderately severe or severe airways obstruction was equally prevalent in both. The effect of grain handling appeared to be mostly on the airways. The high prevalence of abnormal W N2/L, indicating abnormal distribution of ventilation time constants, needs further evaluation. Simple, reproducible spirometric measurements were sufficiently sensitive to detect the effects of grain dust exposure on lung function in the cross-sectional study. Other tests offer little or no advantage, but the potential usefulness in longitudinal studies needs to be further evaluated. There was no correlation between lung function and job category, place or length of employment (Tables 32, 33)[8].

3) Clinico-physiological correlation. Grain workers with symptoms on exposure to dust had lower values of ventilatory function than workers without symptoms on exposure, regardless of smoking habits (Tables 38, 39). This suggests that symptomatic exposure to grain dust results in lower ventilatory function and conceivably leads to non-specific bronchial hyperreactivity. It is also possible that grain workers have a pre-existing lower ventilatory function due to undiagnosed mild or non-symptomatic asthma or non-specific bronchial hyperreactivity, and exposure to grain dusts aggravates this condition. The prevalence of chronic bronchitis with airways obstruction was higher in grain workers than controls, regardless of smoking habits. In addition, chronic bronchitis with airways obstruction was related to length of employment. These findings suggest that chronic grain dust exposure may result in chronic obstructive pulmonary disease.

4) Skin hypersensitivity (allergic). Atopy was more prevalent among controls than grain workers (Table 35). The lower prevalence of atopy in grain workers may imply that the more "allergic" individuals tend to avoid the grain dust environment or leave the industry. This hypothesis could be tested in future longitudinal studies and a cross-sectional study of the "non-survival" population of grain workers.

    The higher prevalence of positive skin test reactivity to insects

and mites in grain workers suggested that the antigens used were more specific for the grain workers because the extracts were prepared with the grain insects and grain mites commonly found in elevators. Hence, grain workers would be more likely to be sensitized. The low prevalence of positive reactions to grain antigens may be due to a low allergenicity of the grain extracts; too low a concentration or loss of the antigenic component of grain during saline extraction. According to previous studies, however, the saline extractable fraction seemed to be the most allergenic of the wheat fractions.

The high prevalence of positive skin tests to airborne grain dusts observed in grain workers suggests that a greater proportion were sensitized to grain dust. Since some city service workers also had positive skin tests to airborne dust, the data suggest that air in the Duluth-Superior area was contaminated with dust from the grain elevators.

The prevalence of skin test reactions to grain dust and insect/mites was significantly higher (by x^2) in atopic grain workers and in atopic control workers than in non-atopic individuals. The data imply that atopic individuals are more likely to become sensitized to grain dust or insect-mite airborne particles than are non-atopic individuals (Table 37).

5) Skin hypersensitivity-symptoms correlation. Overall, there were no significant correlations between symptoms on exposure, chronic symptoms, grain fever, or symptom complexes and skin reactivity to common allergens or specific allergens. The exceptions were: a) dyspnea on exertion was more prevalent among grain workers with positive skin reactivity to fungal antigens and to grain antigens. b) Nasal symptoms on exposure to grain dust were more prevalent among grain workers with positive skin reactivity to grains, barley and oats (Table 42).

6) Skin hypersensitivity-pulmonary function correlation. Grain workers with atopy or skin reactivity to grain dust were more likely to have lower airways function values. The clinical significance of these findings is not clear since abnormal lung function was not more prevalent among atopic individuals or skin reactors to specific grain extracts (Tables 40, 41).

7) Serum precipitating antibodies. City workers had a greater prevalence of precipitins to Trichoderma, T. vulgaris, T. sacchari and to one or more fungi than grain workers (Table 44). Conversely, grain workers had a greater prevalence of precipitins to: durum wheat, rye and airborne dusts of wheat, barley, rye, oats and sunflower to one of the settled dusts than controls. The larger prevalence of precipitins to some grain dusts among grain workers was not surprising, yet they did not correlate with increased prevalence of symptoms or abnormal lung functions. Hence, the data imply that the respiratory reactions to grain dusts are not precipitin-mediated, and that grain fever is not a manifestation of allergic alveolitis type III reaction. Serum precipitins reflect host response to antigens but not necessarily the presence of disease or abnormal pulmonary dysfunction. The reason for the greater prevalence of fungal precipitins among city workers is not clear.

8) Alpha$_1$-Antitrypsin level (AAT). The levels of AAT in grain workers and controls were similar. Pi phenotyping of grain workers with AAT levels less than 60% of the normal values detected 3 MZ's with impaired trypsin inhibitory capacity (TIC) and 3 MZ's with normal TIC. These subjects showed no consistent abnormality in lung function (Tables 53, 55).

9) Chest roentgenograms. The prevalence of abnormal chest roentgenogram findings was small and most changes were of minor clinical significance with a few exceptions (Table 47). There were no cases with diffuse bilateral interstitial infiltration or fibrosis.

10) Liver disease screening (SGPT, GGT, Cholinesterase). We did not detect differences in the frequency of overt liver disease between grain workers and controls (Table 45). Certain findings make us recommend that future prospective morbidity studies include evaluation of liver disease prevalence. In the questionnaire, the grain workers reported exposure to hepatotoxic pesticides. The liver was palpable in a significant number of grain workers. Moreover, the mean values for SGPT were elevated in grain workers. Grain workers had a greater number of abnormal values for GT.

11) Renal disease screening. The results of the renal function screening tests were inconclusive (Tables 45, 46). We would recommend further prospective studies on the potential renal morbidity of pesticide exposure.

12) Immunoglobulins. The levels of IgG and IgA observed in grain workers differed significantly from the city workers, whereas the levels of IgM were similar in both groups (Tables 48, 52). The data suggest that grain dust normally enhances the levels of serum IgA, but that the response was blunted by smoking. Elevated IgG and IgA levels were observed only in grain workers working in the elevators from 10.6 to 15.5 years. Increased levels of IgA were also observed in grain workers working in the industry fewer than 5.5 years.

The place of employment influenced the level of circulating IgG. (Increased levels of IgG in elevators 1 and 8)

Only 4 of the 307 grain workers and 2 of 235 city workers tested had IgE levels above 600 I.U. Data from the IgE determinations should be interpreted with caution. Using radioimmunoassays for determination of IgE, the level of IgE in normal serum is below 50 I.U./ml. Serum from highly allergic individuals contains between 300-600 I.U. Levels above 900 I.U. are rarely observed. Hence, the immunodiffusion method for determining IgE levels is not sensitive enough to detect increases in serum IgE occurring between 100-600 I.U.

Study II - Work Shift Study
Two hundred and forty-eight (248) grain workers and 192 controls (city service workers) were studied. The following parameters were evaluated: 1) symptoms, 2) pulmonary functions (FEV$_1$, FVC, Vmax$_{50}$ and Vmax$_{75}$) before and after the shift, 3) leukocyte count and differential, 4) serum complement C3 level and complement activation

before and after the shift, 5) oral temperature at 7-800 hrs, 1200 hrs, 15-1600 hrs and 2000 hrs, 6) total and respirable time average dust levels by personal sampler, 7) mycological studies described
Whidden et al. "Microbial Flora and Fauna of Respirable Grain Dust from Grain Elevators."

Results and Conclusions from this Study

Exposure to grain dust during an 8 hour work shift appeared to have an adverse, dose-related, acute effect on the workers. This adverse effect is suggested by the following:

1) Grain workers reported more symptoms during work than city service workers of similar age, height and smoking habit (Table II - 3).
2) Grain workers were exposed to a higher concentration of dust than city service workers (Table II - 2).
3) The incidence of respiratory symptoms was positively related to the workers' subjective estimates of dust levels and to time-weighted average total dust concentration (Tables II - 4, 14).
4) Grain workers' subjective estimation of dust level correlated with the measured total dust concentrations (Table II - 14).

The incidence of respiratory symptoms was higher among grain workers exposed to mean total airborne dust (time-weighted average concentration) of 13.9 mg/m3 when compared to grain workers exposed to 4 mg/m3 or less. In the latter group of grain workers the incidence of symptoms was similar to that found among controls.

The negative effect of grain dust exposure on lung function also tends to support the hypothesis that grain dust exposure has an adverse effect on the workers.

The negative effect of grain dust exposure on lung function was suggested by:
1) In a small number of subjects, a greater proportion of grain workers had a significant decrease in FEV_1 (-15%), $Vmax_{50}$ and $Vmax_{75}$ (-25%) (Table II - 12a).
2) There was a negative correlation between $Vmax_{50}$ and $Vmax_{75}$ and total dust level. The higher the dust level the more negative the change in function value (Table II - 11).
3) There was a significant difference between the pre-post-shift lung function changes observed in grain workers and controls. The mean control group values tended to increase slightly during the day, whereas grain workers showed slightly negative changes (Table II-9).

Overall, the acute effects of the dust concentrations found in this study on the lung function did not seem to be of clinical significance since there was no correlation between the presence of symptoms and pre-post-shift changes in function. This small, negative, acute effect may or may not have long-term effects such as a greater than expected yearly loss of function.

At the total dust concentrations these workers were exposed to we found no consistent systemic reaction, i.e., oral temperature, leukocyte count or serum complement level or activation of complement (Tables II - 6-8).

Study III. Prospective 3 Year Follow-up Study

The pulmonary function parameters (FEV_1, FVC, $Vmax_{50}$, $Vmax_{75}$ and DLCO) studied in 1974 were compared with the values obtained in cross-sectional study performed in 1977.

Results and Conclusions

No definite conclusions as to the chronic effects of recurrent grain dust exposure can be derived from this short-term follow-up study using tests of airways flow and diffusing capacity. The non-working grain handlers (left the industry, retired, laid-off) were not included in the evaluation. The results would be affected by those who had to stop working because of respiratory disability and were lost to follow-up.

The mean decline in FEV_1 or FVC was no greater than that expected for aging alone. The results did show, however, a significant mean decline in other tests of airways flow--i.e., MMF, $Vmax_{50}$ and $Vmax_{75}$--which was greater than expected for age alone in any smoking category (Tables III-4-10). Although nonsmokers showed a greater mean decline in flows at low lung volumes than smokers, a very small proportion of nonsmokers showed a decline in function greater than expected. This significant mean function (MMF, $Vmax_{50}$ and $Vmax_{75}$) decline in nonsmokers suggests a grain dust effect independent of age. However, since the majority of those who showed a greater than expected (age related) decline in these functions were smokers, cigarette smoking probably has a greater adverse long-term effect on these functions than grain dust exposure.

Study IV: Pulmonary Reaction to Grain Dust Constituent - Pilot Study in the Identification of Etiologic Agents

The pulmonary and systemic response to extracts of durum wheat, durum wheat airborne dust and insects or mites was studied by inhalation provocation tests on 11 grain workers with symptoms on exposure to grain dust.

Conclusions

Durum wheat, a constituent of grain dust, induced an airways response in grain handlers (Figures IV-1,2). This response was inhibited by sodium cromoglycate (Figure IV-3). The effect of inhaled durum wheat appeared to be on the large airways without parenchymal or systemic reaction and without complement activation. The bronchial reaction was not always related to the atopic status or acquired skin hyperreactivity to grain or grain dust antigens. Our data suggest that a type III allergic reaction does not play a role in the bronchial response to durum wheat extract. Moreover, the data imply a nonimmunologic release of mediators, e.g., histamine, or type I immediate IgE mediated allergic reactions are responsible for the reaction.

Study V: Grain Fever Syndrome

Clinical, physiological and immunological parameters were evaluated in 12 subjects (6 grain workers with grain fever and 6 nonsmoking, asymptomatic controls) after exposure in a chamber to high concentration (>15 mg/m3) of grain dust to reproduce an environment.

Workers considered this concentration of dust similar to that most likely to provoke an episode of grain fever at work. Results after challenge were compared to baseline and to control day results.

Conclusions

Inhalation of high concentrations of airborne grain dust for one to three hours induced the grain fever syndrome manifestation lasting 24 to 36 hours. The syndrome was characterized by systemic reactions: facial warmth, headache, chills, malaise, myalgias, leukocytosis, left shift fever and by respiratory reaction: throat and tracheal burning, chest tightness, dyspnea, cough, expectoration and airways obstruction. There was no evidence of parenchymal reaction.

The reactions occurred in both grain workers and controls and were independent of previous exposure, atopic status, bronchial hypersensitivity, pre-existing airways obstruction or the presence of precipitins.

Our data do not support the hypothesis that grain fever is a type III allergic reaction. None of the test subjects had precipitins to grain dust, complement was not activated and changes in DLCO were not observed. This data may suggest that grain fever is due to non-specific release of pharmacologically active substances from the lung after interactions between components of grain dust and lung cells.

Health Effects of Grain Dust Exposure Summary

Grain dust exposure can induce acute symptomatic reactions of the skin, conjunctiva, upper and lower airways (asthma) and systemic febrile reaction (grain fever syndrome). Grain fever could be induced by inhalation of high concentrations of respirable airborne dust (>20 mg/m3) and was temporarily disabling. Respiratory symptoms were more likely to occur among workers exposed to a total dust time-weighted average concentration of 13.9 mg/m3. They also occurred among workers exposed to a mean total dust time average concentration of 4 mg/m3 and less, but overall the incidence of symptoms in that group of grain workers was similar to that in controls.

Grain dust exposure can induce chronic expectoration (chronic bronchitis) and dyspnea on exertion. The effect of grain dust exposure on symptom prevalence was of greater or the same magnitude as the effect of smoking. The acute physiologic pulmonary changes were significant (e.g., pre-post work shift FEV_1 > 20%) at high (time-weighted average) respirable dust concentrations (> 20 mg) but are infrequent at time average total dust levels below the current TLV of 15 mg/m3.

Recurrent daily exposure to low concentrations (assuming that time-weighted average dust concentrations at the elevator was TLV of 15 mg/m3 or lower) may result in lower ventilatory function than expected for men of the same geographic area not exposed to grain dust. The adverse effect of grain dust exposure on lung function was of equal magnitude or smaller than that of cigarette smoking. The physiologic changes, however, were of small magnitude and may have no significant long-term effects on the worker's sense of well-being, working performance or longevity. On certain occasions, the acute symptoms on

exposure at weighted average total dust levels below accepted TLV nuisance dust (I5 mg/m3) appeared to affect workers' work performance and sense of well-being during and after work, thus affecting their quality of life.

It is reasonable to suspect that acute symptoms on exposure are more related to peak airborne dust concentrations rather than to time-weighted average levels. It is also conceivable that significant transient decreases in ventilatory function may occur during the work shift when high peak airborne dust concentrations occur. Hence, further studies are needed to determine the relationship between peak dust levels and the biological response. In addition, prospective studies are needed to determine the long-term effect of the : I) recurrent adverse acute pulmonary adverse effects of grain dust exposure, 2) the grain fever episodes, and 3) the symptomatic pesticide exposure.

There was no evidence to suggest that grain dust can cause hypersensitivity pneumonitis.

The effects of grain handling and smoking on the lungs were statistically significant, independent and additive. Although allergic predisposition must play an important pathogenetic role in some individuals, acute asthmatic responses can be elicited regardless of the atopic status of the individual.

A component of grain dust, durum wheat, has been identified as an etiologic agent. The mechanism by which durum wheat or grain dust induces asthma is not known. The prevention of asthma to grain dust by pre-treatment with sodium cromolyn suggests an allergic or pharmacologically mediated histamine release. Further studies are needed to identify the fractions of d. wheat and other components of grain dust (e.g., barley, oats, rye, insects, mites or fungi) capable of inducing asthmatic reactions and their mechanism of action.

HEALTH EFFECTS OF GRAIN DUST EXPOSURE
Acute inflammatory reactions
+ skin irritation with pruritus
+ Conjunctiva
I Upper airways: nasal passages, larynx, trachea
I Lower airways: (asthma) + large airways
o Alveoli (no evidence of alveolitis) Small
  airways (probable) but possible under
  certain conditions
Acute systemic reaction (inflammatory? toxic?)
+ Grain fever syndrome
Chronic respiratory effects
+ Chronic bronchitis without airways obstruction
? Hyperreactive airways (probable)
? Chronic bronchitis with airways obstruction (probable)
? Loss of lung function greater than expected for age
Toxic effects of pesticides
+ Acute neurological and gastrointestinal
? Chronic neurological disease from recurrent exposures
  (probable)
o Hepatotoxicity or nephrotoxicity

Other health effects: Unknown
+ There is evidence to support the cause-effect
  relationship with grain dust exposure
? Cause-effect still debatable and more information is needed.   In some
  instances the cause-effect is highly probable.
o No evidence of cause-effect or even that it ever occurred following
  grain dust exposure.
    Based on the results of the studies described above we conclude
that grain dust exposure has an adverse effect on grain handlers.

    We can conclude with certainty that: a) grain dust exposure can
induce acute reactions of the exposed mucosa of acute clinical
significance as indicated by Studies I, II, IV and V, and b) exposure
to high dust concentrations have a definite negative physiologic effect
on the airways and systemic response (leukocytosis, fever) as
demonstrated by Study V.

    At low dust concentrations (Study II) there is a negative
physiologic effect which, except for a few instances, is of small
magnitude. The prognostic significance or long-term effect of this
small negative effect is not clear. The prospective pulmonary function
data (Study III) would suggest that this effect may result in a yearly
loss of airways flow greater than expected for age. However, this may
only be significantly abnormal in smokers.

    Our data (Study II) lend support to the workers' subjective
correlation of acute respiratory response to dust level, since there
was a relationship between prevalence of symptoms on exposure and lung
function changes and total dust levels. The recurrent exposure to
grain dust also appears to have definite chronic clinic effects related
to length of exposure, but the chronic functional effects are less
clear-cut. The higher prevalence of abnormal airways flow in grain
workers and the greater than expected yearly loss of airways flow
suggest a chronic negative functional effect. Whether or not this
effect leads to early disability or decreased performance or affects an
individual's sense of well-being cannot be answered as yet. The
long-term effects of the acute pulmonary recurrent negative effects of
grain dust exposure; grain fever episodes; and symptomatic pesticide
exposure will have to be answered by prospective studies.

    We found no acute or chronic evidence that suggests that grain dust
exposure can cause hypersensitivity pneumonitis. The results of these
studies allow us to make some conclusions about host factors that
affect the response to grain dust and about the mechanism of action of
grain dust.

    Cigarette smoking: Cigarette smoking has an additive effect on
grain handling in regard to the prevalence of acute and chronic
respiratory symptoms, pulmonary function status and prevalence of
pulmonary function abnormalities. Although smokers are more likely to
be affected by chronic grain dust exposure, acute symptoms on exposure
can occur independently from smoking habit.

    Allergic sensitization   (as detected by immediate reaction to
common or specific allergens):   Overall, in this working (survival)

population of grain workers, there was no relation between prevalence of symptoms or abnormal lung function and the atopic status of individuals. In addition, positive bronchial provocation challenge occurred regardless of the atopic status of individuals. There is evidence, however, that allergy may play a role since there was a negative relation between lung function and skin test reactivity in Study I. The bronchial reaction seen in a subject in Study IV can be interpreted as being an immediate IgE type I allergic reaction in a "very atopic" individual. Hence, the lack of overall relation between skin sensitivity and respiratory response should not exclude the probability that in certain individuals the bronchial reaction is mediated via an allergic mechanism. Also, it is likely that very atopic individuals with hyperreactive airways are unlikely to remain in this industry for long. This is suggested by the lower than expected prevalence of atopy to common allergens among the grain workers.

Bronchial hyperreactivity: Bronchial hyperreactivity is likely to predispose to bronchial reaction to grain dust, but Studies IV and V demonstrate that responses to durum wheat or high concentrations of airborne grain dust can occur in subjects without pre-existing hyperreactivity. Further studies on non-specific bronchial hyperreactive airways of the biologic response to airborne dust are needed. To determine if bronchial hyperreactivity is acquired or pre-existing; if it reverses once exposure is discontinued; and to establish the usefulness of Mecholyl test to detect high risk individuals before or during employment.

Pre-existing airways obstruction: Based on Study IV we conclude that airways response to grain is more likely to occur in subjects with severe airways obstruction but according to Study V high concentrations of grain dust can induce bronchial reaction even if there is no pre-existing airways obstruction.

Alpha$_1$-Antitrypsin activity: No apparent relationship was found between level of alpha$_1$-antitrypsin and pulmonary response to grain dust.

Mechanism of action: We have shown that durum wheat saline extract, durum wheat airborne dust saline extract and airborne grain dust from elevators can induce the bronchial reaction by mediator release, since their reaction can be blocked by sodium cromoglycate. An allergic IgE mediated type I reaction was apparent in one subject. We found no evidence that a type III precipitin-mediated reaction or complement activation plays a significant pathogenetic role (Studies II, IV and V).

Further studies are required to identify: the components of grain dust responsible for the pulmonary reaction, their mechanism and site of action; the role of mediator release and complement activation in the pathogenesis of the airways reaction; and the role of host factors which can modify the responses to grain dust, i.e., non-specific bronchial hyperreactivity and allergic predisposition or sensitization.